Title: EFFECTS OF RODENT-INDUCED LAND DEGRADATION ON ECOSYTEM CARBON FLUXES IN ALPINE MEADOW IN THE QINGHAI-TIBET PLATEAU, CHINA

Short title: EFFECTS OF LAND DEGRADATION ON ECOSYTEM CARBON BALANCE

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Abstract The widespread land degradation in alpine meadow ecosystem would affect ecosystem carbon (C) balance. Six levels of degraded lands (D1-D6, according to the number of rodent holes and coverage) were set to investigate effects of rodent-induced land degradation on ecosystem CO_2 fluxes, biomass and soil chemical properties. Soil organic carbon (SOC), labile soil carbon (LC), total nitrogen (TN) and inorganic nitrogen (N) were obtained by chemical analysis. Soil respiration (Rs), net ecosystem exchange (NEE) and ecosystem respiration (ER) were measured by Li-Cor 6400 *xt*. Gross ecosystem production (GEP) was the sum of NEE and ER. Aboveground biomass (AGB) was based on a linear regression with coverage and plant height as independent variables. Root biomass (RB) was obtained by using the core method. Rs, ER, GEP and AGB were significantly higher in slightly degraded (D3 and D6, group I) than in severely degraded land (D1, D2, D4 and D5, group II). Positive averages of NEE in the growing season indicate alpine meadow ecosystem is a weak C sink during the growing season. Only significant difference was observed in ER among different degradation levels. Rs, ER, GEP were 38.2%, 44.3% and 46.5% lower in group I than in group II. The parallel changes of ER and GEP resulted in insignificant difference of NEE between the two groups. Positive correlations of AGB with ER, NEE and GEP and the relative small AGB in Group II suggest the control of AGB on ecosystem CO_2 fluxes. Correlations of RB with SOC, LC, TN and inorganic N indicate the regulation of RB on SOC, LC and TN with increasing

number of rodent holes in alpine meadow ecosystem in permafrost region of QTP.

Keywords: rodent activities, alpine meadow, soil respiration, net ecosystem exchange, ecosystem respiration

Introduction

Soil contains the largest ecosystem carbon (C) stock (Batjes, 1996). The widespread land degradation (Dregne, 2002) including land use change, soil and vegetation degradation has resulted in severe soil C and nitrogen (N) loss (Wang et al., 2009; Parras-Alcántara et al., 2013), which is estimated to be 19-29 Pg worldwide (Lal, 2001). Restoration of the degraded ecosystems, therefore, had a great potential to sequestrate C from the atmosphere (Lal, 2004), with an annual rate of 0.9 to 1.9 Pg C for a 25 to 50 year period in drylands (Lal, 2001).

Grassland stores about 15.2% of the terrestrial ecosystem C stock that is primarily in the form of soil C (Ajtay, 1979). Either the aboveground vegetation (Fan et al., 2007) or the top one meter soil and root C stock (Yang et al., 2008) in alpine meadow in the Qinghai-Tibet Plateau (QTP) account for a large proportion of those in grassland ecosystem in China (Ni, 2002). However, over one thirds of grassland in the QTP has been severely degraded due to climate change, grazing and road constructing in 1990s (Ma et al., 1999), which has led to 1.8 Gg C loss in aboveground C stock from 1986 to 2000 (Wang et al., 2008). In addition to the vegetation C loss, land degradation could also result in decline in soil C and N (Wang et al., 2008;Wen et al., 2013), and consequently might alter C role of the alpine meadow (Li et al., 2012).

The primary causing factor for “black soil type” degradation over the QTP is rodent grazing and burrowing (Ma et al., 1999). Rodent grazing activities trigger decline in biomass, change in below-ground biomass distribution, soil structure, microclimate, soil erosion, nutrient loss and imbalance of nutrient cycling, and finally affect the ecosystem C balance (Li et al., 2011). Current studies concerning ecosystem C balance in alpine meadow focus on net ecosystem exchange (NEE) (Kato et al., 2004), inter-annual variation in NEE (Kato et al., 2006), soil respiration (Rs) and ecosystem respiration (ER) responses to experimental warming (Peng et al., 2014b; Luo et al., 2010; Lin et al., 2011). Effects of rodent-induced land degradation on ecosystem CO_2 fluxes are rarely investigated. To our knowledge, there are two studies examining the responses of Rs to land degradation (Zhang et al., 2010; Wang et al., 2007b). However, Rs cannot provide solid evidence for determining the ecosystem C balance. No field experiment has been conducted in the permafrost region of the QTP to investigate the effect of land degradation on NEE, a direct measure of the ecosystem C balance, and on its components: ER and gross ecosystem production (GEP). We conducted a field study to investigate (1) how the NEE and its components respond to rodent-induced land degradation, and (2) how biotic and abiotic factors affect those CO_2 fluxes with land degradation process in *Kobresia pygmaea* dominated alpine meadow in a permafrost area of the QTP.

2 Materials and Methods

2.1 Site description

The study site is situated in the source region of the Yangtze River, inland of the QTP near the Beiluhe observational station (Fig.1, 34°49′N, 92°56′E) at an altitude of 4635 m. This area has a typical alpine climate: mean annual temperature is −3.8°C and monthly air temperature ranges from

−27.9 ℃ in January to 19.2 ℃ in July. Mean annual precipitation is 290.9 mm, of which over 95% falls during the warm growing season (May to October). Mean annual potential evaporation is 1316.9 mm, mean annual relative humidity is 57%, and mean annual wind velocity is 4.1 m s^{-1} (Lu et al., 2006). The study site is a winter-grazed range, dominated by alpine meadow vegetation: *Kobresia capillifolia*, *K. pygmaea*, and *Carex moorcroftii*, with a mean plant height of 5 cm. Plant roots occur mainly within the 0-20 cm soil layer, and average soil organic carbon (SOC) is 1.5%. The soil development is weak, and the soil belongs to alpine meadow soil (Chinese soil taxonomy), or is classified as a Cryosol according to World Reference Base for Soil Resources, 2006, with a Mattic Epipedon at a depth of approximately 0-10 cm, and an organic-rich layer at a depth of 20-30 cm (Wang et al., 2007a). The parent soil material is of fluvio-glacial origin and is composed of 99% sand. Permafrost thickness observed near the experimental site is 30-70 m and the depth of the active layer is 1.5-3.5 m (Wu and Liu, 2004). However, the thickness of the active layer has been increasing at a rate of 3.1 cm y^{-1} since 1995 due to climatic warming (Wu and Liu, 2004).

2.2 Experimental design and measurement protocol

2.2.1 Experimental design

We selected six habitats with different number of rodent holes (NRHs) and community coverage in a mountain slope based on our filed observation. The habitats were sequenced D1-D6 from east to northeast. The distance between each habitat was about 200-300 m. In each site, two sub-plots (2m×4m) were set up. The NRHS, coverage, plant height and major species in D1-D6 were shown in Table 1.

2.2.2 Measurement protocol

Soil temperature: Soil temperature at the depth of 5cm was monitored by a thermo-probe attached to Li-Cor 6400 (Lincoln, NE, USA.) when measurements of Rs, NEE and ER were conducted.

CO_2 fluxes: Rs was measured using 5-cm tall PVC collars, which were permanently inserted 2-3 cm into the soil in the center of each plot. The measuring procedure of Rs was similar to that reported in former studies (Peng et al., 2014b; Zhou et al., 2007). ER and NEE were measured with a transparent chamber (0.5 × 0.5 × 0.5 m) attached to an infrared gas analyzer (Li-Cor 6400, Lincoln, NE, USA). The method used was similar to that reported by (Steduto et al., 2002) and (Niu et al., 2008). GEP was the calculated as the sum of NEE and ER. Rs, NEE and ER were measured once a month from June to September in each plot.

Soil sampling: One soil sample was collected at the soil depth of 0-30 cm in each plot in June, 2012.

AGB and RB: AGB was obtained from a step-wise linear regression with AGB as the dependent variable, and coverage and plant height as independent variables (Peng et al., 2014b;Xu et al., 2015). Coverage of each plot was measured using a 10 cm × 10 cm frame in four diagonally divided subplots replicated eight times in D1-D6 in June 2012. Plant height was measured 40 times by a ruler, and averaged for each plot. RB was obtained from soil samples that were air-dried for one week and passed through a sieve (Φ=2 mm) to remove large particles. Roots were separated from the soil by washing, and fine roots was retrieved by sieve (Φ=0.25 mm). Living roots were separated from dead roots by their color and consistency (Yang et al., 2007). And the separated roots were dried at 75 ℃ for 48 h.

Chemical analysis: SOC was analyzed using the Walkley-Black method (Walkley, 1947). TN was measured via the Kjeldahl method. Ammonia and nitrate N were measured colorimetrically through a spectrometer. Labile soil carbon (LC) measurement was carried out by the procedure advocated by (Moscatelli et al., 2007).

2.3 Data analysis

The statistical significance of soil temperature, Rs, NEE, ER and GEP in D1-D6 were tested by the one way-ANOVA analysis. Monthly data measured in each subplots from June to September were used in the analysis. Based on the total NHRs, CO_2 fluxes in D3 and D6 were ranked as group Ⅰ and those in D1, D2, D4 and D5 were the group Ⅱ. The statistical significance of CO_2 fluxes between the two groups were also tested by one-way ANOVA analysis. The monthly differences in CO_2 fluxes were analyzed by repeated ANOVA. Relationships of Rs, NEE and ER with soil temperature, ABG, RB, and TN or inorganic N were analyzed by linear regression analyses. Pearson correlation analyses were employed to investigate the relationships of NRHs with soil chemical properties and biomass. The linear regression and Pearson correlation were considered significant with $P<0.05$. Rs, NEE and ER data were the averages of four months in each degrade level when conducting the correlation analyses. All the analyses were conducted in SPSS 16.0 for windows.

3 Results

3.1 Soil temperature

Soil temperature at the depth of 5 cm maximized in July and monthly average soil temperature had no significant change ($P>0.05$) among different degradation levels (Fig. 2). The monthly average soil temperature was about 9.6-12.4 °C from D1 to D6. Soil temperature also had no significant difference between group Ⅰ and Ⅱ.

3.2 Soil chemical properties and biomass

SOC, LC, TN, ammonia N and RB were the highest in D2 than in other habitats (Table 3). AGB was higher in D3 and D6 than in others. SOC, LC, TN, and inorganic N (NH_4^+-N and NO_3^--N) had no obvious trend with the increasing NRHs, whereas AGB (r=-0.89, $P<0.05$) negatively correlated with the NRHs.

3.3 Rs, NEE, ER and GEP

Repeated one-way ANOVA showed the significant seasonal change in Rs ($P<0.01$), ER ($P<0.05$), NEE ($P<0.01$) and GEP ($P<0.01$). The maximum Rs and ER were in July (Figs. 3a, b), whereas the maximum NEE and GEP were in June (Figs. 3c, d). Growing seasonal average Rs and NEE had no significant difference in D1-D6 while ER and GEP were marginally higher in D3 and D6 than in others (Table 4). Rs, ER and GEP were higher in group Ⅰ than in group Ⅱ ($P<0.05$). Insignificant but higher NEE was observed in group Ⅰ (2.13 μ mol m^{-2} s^{-1}) than in group Ⅱ (1.09 μ mol m^{-2} s^{-1}).

3.4 Relationship of Rs, ER, NEE and GEP with affecting factors

Ecosystem CO_2 fluxes had no obvious relationship with soil temperature (Fig. 4a), soil inorganic N (Fig. 4b) and RB (Fig. 4d), while they positively correlated with AGB with the steepest regression slope in Rs, followed by ER and NEE (Fig. 4c).

4 Discussion

4.1 C and N loss

Land degradation would cause the decline in SOC and TN density, and reduction in C and N stocks either in temperate grassland (Zhang et al., 2010) or in alpine meadow ecosystem (Xue et al., 2009). Insignificant difference of SOC, LC, TN and inorganic N among D1, D3, D4, D5 and D6 indicate (Table 3) nutrient loss associated with land degradation induced by rodent activities differs with the C and N loss resulted from land degradation caused by other factors, such as desertification and wind erosion. Soil loses C and N during the degradation process by (1) reducing vegetative growth and exposing the soil surface to wind and water erosion, and (2) reducing the return of litter to soil (Nunes et al., 2012). Higher AGB in D3 and D6 (Table 3) suggesting more litter returning to the soil, but more ecosystem CO_2 emission from soil in terms of higher Rs could be the reason for lower SOC, LC and TN in D3 and D6. Positive correlation between AGB and Rs indicates decomposition of fresh litter from AGB might be the major component of Rs in alpine meadow. The highest RB in D2 in spite of lower AGB comparing with that in D3 and D6 (Table 3) proves RB is the major source of soil C and N in the alpine meadow ecosystem.

4.2 CO_2 fluxes

Soil temperature explains most of the temporal (Peng et al., 2014a) but RB determines the spatial variation in Rs over the QTP (Geng et al., 2012). No obvious relationship between Rs and soil temperature (Fig. 4a) suggests other factors might involve in controlling the temporal variation in Rs with land degradation processes. Rs decline is observed with intensification of land degradation in an alpine meadow ecosystem, and the significant reduction only appears in severely degraded level (Zhang et al., 2010). Lower Rs in group Ⅱ than in group Ⅰ (1) supports the above finding because community coverage in D1, D2, D4 and D5 (Table 1) conforms to the standard of the severe land degradation for alpine meadow ecosystem (Xue et al., 2009), (2) indicates the controlling effect of biomass on Rs in degraded land induced by rodent activities. Rs is composed of autotrophic respiration from plant roots and their symbionts, and heterotrophic respiration from litter and SOC decomposition (Hanson et al., 2000). AGB and dead roots, the major sources of litter in alpine meadow all reduce (Sun and Wang, 2008), and SOC also abates due to the shrunk litter input into soil as a result of lower AGB and plant detritus (Wang et al., 2009;Wen et al., 2013). Lower RB, SOC and LC yet higher AGB in D3 and D6 than in D1 and D2 (Table 3) implies AGB is the major controlling factor of Rs with the development of land degradation, which is consolidated by the positive correlation between Rs and AGB (Fig. 4c). In disturbed ecosystems microorganisms competition induce the microbes to sue more C energy for cell integrity and maintenance (Moscatelli et al., 2007), and the consequent higher respiration quotient (Nunes et al., 2012) could contribute to the insignificant change in Rs with development of land degradation.

ER comprises of respiration of AGB and Rs (Zhang *et al.*, 2009). Higher Rs therefore could be one reason for the higher ER in D3 and D6. Lower relative difference in Rs (38.2%) than in ER (44.5%) between the two groups suggests the influence other factors like AGB on ER difference, which is supported by the positive correlation relationship between ER and AGB (Fig. 4c).

The highest net photosynthesis in June in alpine meadow ecosystem (Yi et al., 2000) justifies the maximum GEP in June (Fig. 3d). Sedge percentage will decrease, and forbs percentage increase with the development of land degradation (Liu et al., 2008). The relative higher net photosynthetic rate of forbs species (*Polygonum viviparum Linn.*) than that of sedge species (*Carex atrofusca Schkuhr*, our unpublished data) and higher AGB might compensate for the effect of species composition change on GEP due to the positive correlation between GEP and AGB (Fig. 4c) in the current study.

The maximum NEE in June is a result of the highest GEP and lower ER in this time (Fig. 2). Positive seasonal average NEE indicates alpine meadow is weak C sink in the growing season, and lower NEE in group Ⅱ suggests the decline of C sequestration ability of alpine meadow ecosystem in degraded land. The non-significant difference of NEE in the two groups might be the result of the corresponding change of ER (44.5% higher in group Ⅰ than in group Ⅱ) and GEP (46.5% higher in group Ⅰ than in group Ⅱ).

4.3 Implication of the soil C dynamics

The non-significant difference in NEE among different degradation levels suggest that SOC loss (in D1, D4 and D5) with land degradation is not directly resulted from changes in net C uptake and emission. Higher SOC, LC, TN in D2 with more NRHs, and the positive correlation between RB and SOC suggests that other dynamics associated with land degradation, like species composition and C allocation between AGB and RB change might involve in the soil C dynamic in degraded land in alpine meadow ecosystem.

Conclusion

Rs, ER and GEP all decreased with increasing NRHs. The corresponding change in ER and

GEP leads to insignificant change in NEE. All the ecosystem CO_2 fluxes are primarily affected by AGB. SOC and soil nutrients change in degraded land is not directly resulted from response of net ecosystem C balance to land degradation. Other processes like species composition and above- and belowground biomass allocation might play a role in the soil C dynamic with development of land degradation.

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Table 1 Features of different habitats, which are represented by different number of rodents holes (NRHs, deep and shallow), coverage, plant height (H) and major plant species

DD	NRHs (deep)	NRHs (shallow)	Coverage	H (cm)	Major species
D1	19	7	0.18	9.5	*Carex Moorcroftii*
D2	5	13	0.35	7	*Kobresia Humilis, K. Pygmaea*
D3	0	3	0.8	6.5	*K. Pygmaea*
D4	12	15	0.42	8	*C. Moorcroftii, K. Pygmaea*
D5	17	13	0.3	7.5	*C. Moorcroftii, K. Pygmaea*
D6	2	0	0.6	12	*C. Moorcroftii*

Table 2 Major devices, measuring procedure, specific feature of methods and equipments to conduct the measurement of soil chemical properties and ecosystem CO_2 fluxes

Items	**Devices or procedure**	**Specific feature**	**Literature**
T	6000-09TC, Li-Cor, Utah, USA	A thermo-probe	
Rs	6400-09, Li-Cor, Utah, USA	A collar 5 cm in depth	Zhou et al., 2007
ER	6400-*xt*, Li-Cor, Utah, USA	A collar 50 cm in depth	Steduo et al., 2002, Niu et al., 2008
NEE	6400-*xt*, Li-Cor, Utah, USA	A transparent chamber CO_2 gradient	Steduo et al., 2002, Niu et al., 2008
AGB	A frame and a ruler	Linear regression	Xu et al., 2015
RB	An auger		Xu et al., 2015
SOC	Walkley-Black method		Walkley et al., 1947
TN	Kjeldahl nitrogen method		
NH_4^+, NO_3^-	Spectrometer		
LC	Spectrometer		Blair et al., 1995

T is the soil temperature at 5cm depth; Rs, soil respiration; ER, ecosystem respiration; NEE, net ecosystem exchange; AGB, aboveground biomass; RB, root biomass; SOC, soil organic carbon; TN, total nitrogen, NH_4^+, NO_3^-, ammonia and nitrate nitrogen; LC, labile carbon.

Table 3 Soil organic carbon (SOC), labile soil carbon (LC), total nitrogen (TN), inorganic nitrogen ($NH4^+$-N and $NO3^-$-N), above-ground biomass (AGB) and root biomass (RB) in different sites (D1-D6). The values in the table were the average and standard error of soil samples in each site.

DD	SOC ($g\ kg^{-1}$)	LC ($g\ kg^{-1}$)	TN ($mg\ kg^{-1}$)	NH_4^+_N ($mg\ kg^{-1}$)	NO_3^-_N ($mg\ kg^{-1}$)	AGB ($g\ m^{-2}$)	RB ($kg\ m^{-2}$)	C:N
D1	4.91±0.13b	1.21±0.13b	0.44±0.02b	8.21±0.32b	4.16±0.62a	149	3.8±0.06c	11.2±0.7ab
D2	8.70±1.19a	2.12±0.31a	0.75±0.10a	13.11±1.23a	3.81±0.51ab	145	13.8±3.5a	11.5±0.2ab
D3	5.02±1.01b	1.23±0.29b	0.46±0.11ab	8.54±1.00b	2.31±0.38bc	272	11.3±1.3a	10.9±0.3a
D4	3.95±0.62b	1.28±0.34b	0.36±0.05ab	7.56±1.39b	2.62±0.24bc	189	6.0±1.5b	10.8±0.3b
D5	3.77±0.32b	0.9±0.09b	0.38±0.03b	9.38±1.33b	1.98±0.21c	141	6.1±0.9b	10.1±0.2b
D6	3.41±0.35b	0.83±0.04b	0.34±0.02b	8.08±0.76b	2.64±0.10bc	336	5.7±0.3b	9.9±0.4b

Different letters in each column stands for significant difference of at $P<0.05$ level.

Table 4 Results (F values) of ANOVA on the effect of land degradation on soil respiration (Rs), ER (ecosystem respiration), NEE (net ecosystem exchange) and GEP (gross ecosystem respiration)

	D1-D6				Group Ⅰ and group Ⅱ			
	Rs	ER	NEE	GEP	Rs	ER	NEE	GEP
F	1.69	2.64	1.35	2.27	7.41	8.21	1.59	6.01
P	0.12	**0.04**	0.26	0.06	**0.01**	**0.006**	0.21	**0.02**

Group Ⅰ includes D3 and D6 while group Ⅱ includes D1, D2, D4, and D5

Numbers in bold stands for the statistical significance at P<0.05 level

Figure captions

Fig. 1. Location of the study area

Fig. 2. Soil temperature in each degradation level (D1-D6) from June to September. Error bars was the standard error for D1-D6 in each month.

Fig. 3. Monthly soil respiration (Rs, a), ecosystem respiration (ER, b), net ecosystem exchange (NEE, c) and gross ecosystem production (GEP, d) among different degradation levels from June to September. Values in the bars were the average of four replicates (two replicates in two subplots), and error bars are standard errors.

Fig. 4. Linear regressions of CO_2 fluxes (soil respiration [Rs], ecosystem respiration [ER], net ecosystem exchange [NEE]) with soil temperature (a), inorganic nitrogen (b), aboveground biomass (c) and root biomass (d). Rs, ER and NEE data were the average of four measurements from June to September within two subplots; inorganic nitrogen and root biomass (0-30cm) was derived from soil samples at the 0-30cm depth in June.

Figure 1

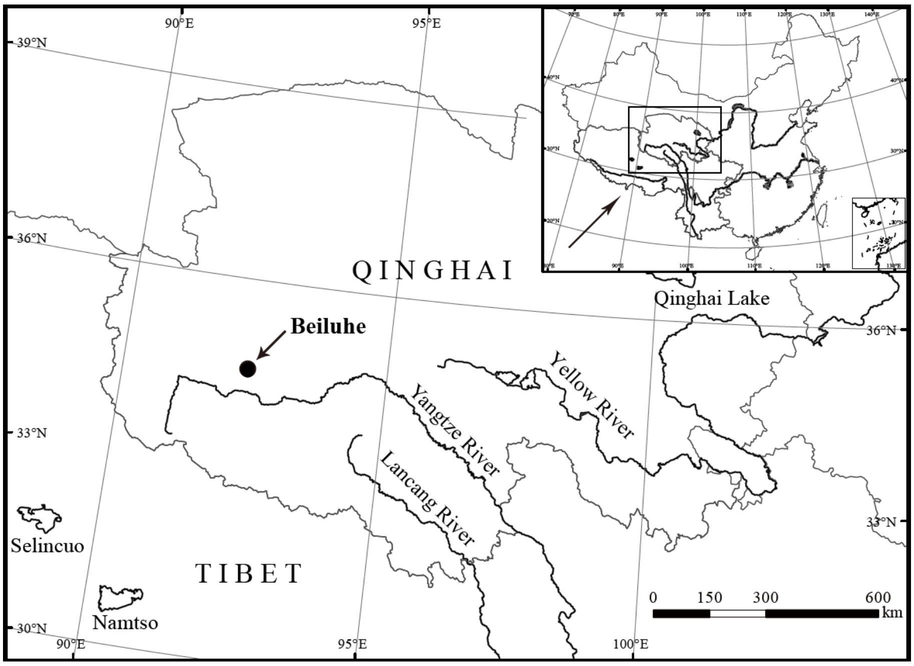
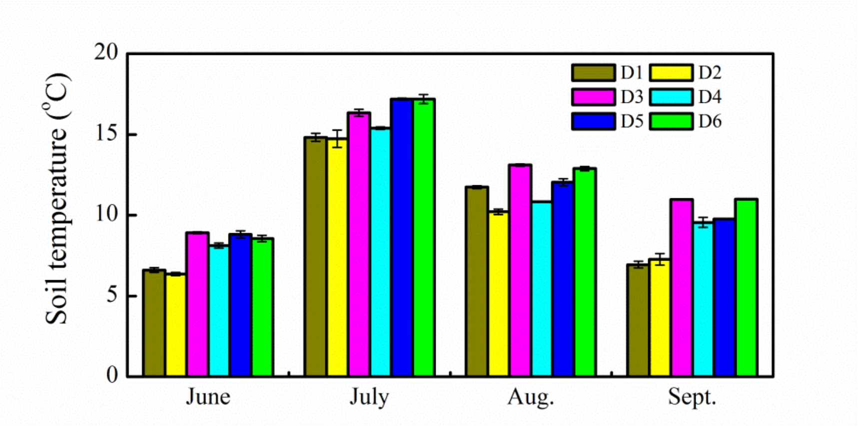

90°E
95°E
39°N
36°N
33°N
30°N
QINGHAI
Qinghai Lake
Beiluhe
Yellow River
Yangtze River
Lancang River
Selincuo
Namtso
TIBET
0
150
300
600
km
90°E
95°E
100°E
36°N
33°N

Figure 2



Soil temperature (°C)
0
5
10
15
20
June
July
Aug.
Sept.
D1
D2
D3
D4
D5
D6

Figure 3

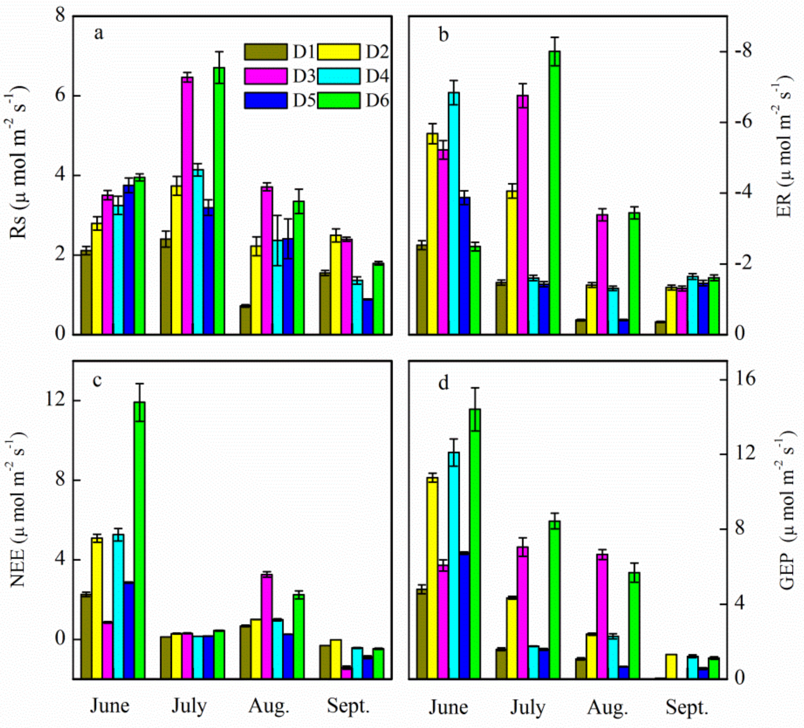
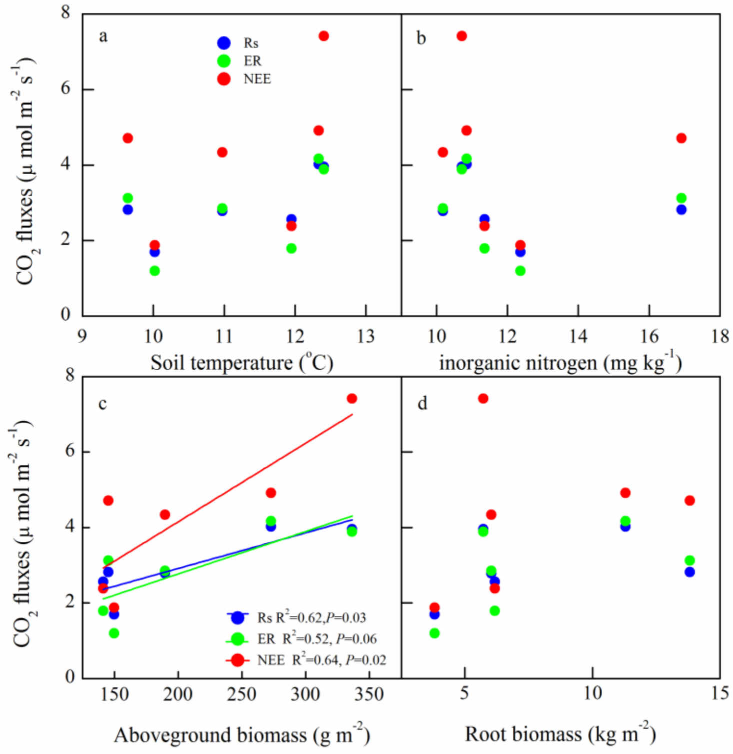

a
b
c
d
D1
D2
D3
D4
D5
D6
Rs (μ mol m^{-2} s^{-1})
ER (μ mol m^{-2} s^{-1})
NEE (μ mol m^{-2} s^{-1})
GEP (μ mol m^{-2} s^{-1})
0
2
4
6
8
-2
-4
-6
-8
12
16
June
July
Aug.
Sept.

Figure 4


a
Rs
ER
NEE
b
c
d
CO$_2$ fluxes (μ mol m^{-2} s^{-1})
0
2
4
6
8
9
10
11
12
13
Soil temperature (^{o}C)
10
12
14
16
18
inorganic nitrogen (mg kg^{-1})
Rs R^2=0.62, P=0.03
ER R^2=0.52, P=0.06
NEE R^2=0.64, P=0.02
150
200
250
300
350
Aboveground biomass (g m^{-2})
5
10
15
Root biomass (kg m^{-2})