Contents lists available at ScienceDirect





## **Ecological Engineering**

journal homepage: www.elsevier.com/locate/ecoleng

# Effects of grazing exclusion on CO<sub>2</sub> fluxes in a steppe grassland on the Loess Plateau (China)



### Wang Dong<sup>a,c</sup>, Wu Gao-Lin<sup>a,b,c,\*</sup>, Liu Yu<sup>a,c</sup>, Yang Zheng<sup>a,c</sup>, Hao Hong-Min<sup>a</sup>

<sup>a</sup> State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, China

<sup>b</sup> State Key Laboratory of Grassland Agro-Ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu 730020, China

<sup>c</sup> Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, China

#### ARTICLE INFO

Article history: Received 3 March 2015 Accepted 20 June 2015 Available online 3 July 2015

Keywords: Carbon flux Grazing exclusion Plant-soil interface The loess plateau

#### ABSTRACT

Understanding how grazing exclusion affects carbon exchange by the plant and soil features is essential for clarifying the contribution of grassland management to the carbon budget. This paper studied effects of grazing exclusion on soil respiration, ecosystem respiration, net ecosystem carbon exchange, plant and soil characterizes for two growing-seasons (2012 and 2013) in a steppe grassland on the Loess Plateau, China. Grazing exclusion markedly increased soil respiration, ecosystem respiration and net ecosystem exchange about 0.36, 0.65 and  $-0.10 \text{ g C m}^{-2} \text{ d}^{-1}$ , respectively. Grazing exclusion enhanced aboveground biomass, belowground biomass and cover, and which were positive correlated with CO<sub>2</sub> flux. Soil water content, rather than soil temperature, was the major environmental factor controlling soil respiration and ecosystem respiration by partial least-squares regression. The ratios of soil respiration, ecosystem grassland were similarly with them at grazed grassland. Results suggested that grazing exclusion was a positive management practice to help maintain or increase carbon stocks in a steppe grassland on the Loess Plateau.

© 2015 Elsevier B.V. All rights reserved.

#### 1. Introduction

Carbon (C) exchange research has become a focal topic around the world because of observe increasing in levels of atmospheric carbon dioxide (CO<sub>2</sub>). Several terrestrial ecologists have been researching on C exchange in different ecosystems, such as tropical forests, prairie grassland, farmland, etc. (Richter et al., 1999; Li et al., 2010; Gomez-Casanovas et al., 2012). Grassland covers about 30% of land surface all over the world and stores 28–37% of the terrestrial soil organic carbon (SOC) (Lal, 2004). Understanding grassland CO<sub>2</sub> fluxes is essential for clarifying the contribution of grassland ecosystems to the global C cycle (Frank et al., 2002).

Globally, overgrazing by livestock is one of the most important human induced causes of arid and semiarid grasslands degradation, such as lower grass yields, carrying capacity, soil nutrient

\* Corresponding author

E-mail address: gaolinwu@gmail.com (G.-L. Wu).

http://dx.doi.org/10.1016/j.ecoleng.2015.06.017 0925-8574/© 2015 Elsevier B.V. All rights reserved. content (Gass and Binkley, 2011; Li et al., 2013; McSherry and Ritchie, 2013). The effect of overgrazing on the plant community and on soil resources are considered destructive because it reduces vegetation cover (Gao et al., 2011; Wu et al., 2013; Wang et al., 2014) and compacts soil as a direct result of trampling (Shi et al., 2013a). Seeking a rational grassland regime is an urgent issue for professionals, herders and the government to achieve sustainable animal production to maintain the health of the grassland ecosystem (Conant et al., 2001).

Degraded grasslands have the capacity for self-recovery if the disturbance ceases for an extended length of time allowing for natural succession (Deng et al., 2014). Grazing exclusion by fencing was conducted as an effective restoration and management regime, for grazing exclusion restored soil structure, soil nutrients and returned grazing potential (Gao et al., 2011; Gass and Binkley, 2011; Wu et al., 2013; Wang et al., 2014). Some research works have been conducted to study the effects of grazing exclusion on carbon sequestration (Qiu et al., 2013; Wang et al., 2014), soil microbial respiration and net ecosystem exchange (Du et al., 2012). Owensby et al. (2006) concluded that both grazing exclusion and grazing tallgrass prairie appeared to be carbon-storage neutral and grazing

Abbreviations: SR, soil respiration; NEE, net ecosystem carbon dioxide exchange; ER, ecosystem respiration; ST, soil temperature; GPP, gross primary productivity; FG, fenced grassland with grazing exclusion; GG, grazed grassland; SWC, soil water content; AGB, aboveground biomass; BGB, belowground biomass.

was not a viable option to increase carbon sequestration. Polley et al. (2008) showed that grazing exclusion significantly influenced C exchange on northern mixed-grass prairie. Such discrepancies between results illustrate the need for more research attention in the effects of grazing exclusion on ecosystem carbon exchange.

Whether grassland ecosystem acts as a sink or source for atmospheric CO<sub>2</sub> depends on harvesting, mowing, grazing and other grassland disturbances (Frank, 2002; Wohlfahrt et al., 2008; McSherry and Ritchie, 2013). The factors that driving ecosystem carbon exchange rate included soil water content, soil temperature, soil type and other soil properties (Flanagan and Johnson, 2005; Potts et al., 2006 Zhao et al., 2011); aboveground biomass, belowground biomass, litter and other vegetation characteristics (Frank, 2002; Risch and Frank, 2006). However, the main factors that driving variations in soil respiration, ecosystem respiration and net ecosystem exchange between grazed and fenced grasslands have not been conclusive. Therefore, it's necessary to clarify the interactions between soil factors, plant characteristic and carbon exchanges after grazing exclusion (Cao et al., 2004).

The Loess Plateau in China, with an area of  $6.4 \times 10^5$  km<sup>2</sup>, is well known for its complex terrain, extreme drought conditions and severe soil erosion, due to a combination of overgrazing and intensification of cultivation (Deng et al., 2014). The Chinese government has instituted various erosion mitigation measures on the Loess Plateau, especially the convert cropland to forest or grassland, grazing exclusion by fencing, etc. (Chang et al., 2011; Wang et al., 2014; Zhang et al., 2015). Grazing exclusion by fencing on the Loess Plateau has made it possible to understand the effect of grazing exclusion on ecosystem carbon exchange. The objectives of this study were: (1) to analyze the effects of grazing exclusion onplant and soil characteristics; (2) to determine how grazing exclusion influence CO<sub>2</sub> fluxes during growing-season; and (3) to analyze the carbon exchanges in terms of functional relationships to the major soil environmental and biologic driversin a temperate steppe on the Loess Plateau, China.

#### 2. Materials and methods

#### 2.1. Study site and experiment design

The study site was located at Lanzhou city, Gansu province, China ( $104^{\circ}09'E$ ,  $35^{\circ}57'N$ , 1966 m.a.s.l). It belonged to a semi-arid continental temperate monsoon climate. According to data available for the period 2001–2013 at the study site from the National Meteorological Information Center of China, the mean annual air temperature was 7.4 °C, the coldest being -7.2 °C in January and the warmest being 19.8 °C in July (Fig. 1). The average annual precipitation was 383 mm, approximately 80% of which fell between May and September (Fig. 1). The soil belonged to the Calcic Cambisol group according to Food and Agriculture Organization and United Nations Education Scientific and Cultural Organization soil classification system (FAO-UNESCO, 2009).

The experiments conducted at fenced grassland with grazing exclusion (FG) and grazed grassland (GG) for two growing-seasons (2012 and 2013). The dominant species in the study area were Stipa bungeana Trin., Artemisia frigida Willd. and Leymus secalinus Tzvel. both at fenced grassland and grazed grassland. Before grazing exclusion, the permanent grasslands used as grazing land. Both FG and GG had similar initial conditions (slope degree, slope direction, topography and altitude). Fenced grassland was established in 2005 and covered a total area of about 8 ha. Outside of fenced grassland, grassland remained under grazing intensity of 2-3.5 sheep  $ha^{-1}$  from May to September, and 1–2 sheep  $ha^{-1}$  from October to the following April at the next year. No fertilizer or herbicides was applied to the grasslands prior to the experiment. Within both fenced grassland (FG) and an area of equivalent size outside fenced grassland (GG), five  $50 \times 50 \text{ m}^2$  plots established randomly in 2005. Three specially designed Stainless Steel collars with dimensions of  $0.5 \times 0.5 \times 0.1 \text{ m}^3$  were placed in the central part of each plot for measurements of soil respiration, ecosystem respiration and net ecosystem carbon exchange. Care was taken when inserting the collars to limit the severing of roots and the disturbance of soil structure.



Fig. 1. Monthly precipitation (P) and mean air temperature (T) between 2001 and 2011 and in 2012 and 2013 at the study site.

## 2.2. Measurement of $CO_2$ fluxes, soil temperature and soil water content

Ecosystem carbon dioxide  $(CO_2)$  exchange was measured with a transparent chamber  $(0.5 \times 0.5 \times 0.5 \text{ m}^3)$  connected to a LI-6400 (LI-COR, Lincoln, NE) following Bubier et al. (2007). To prevent pressure differences at the time of sampling and minimize kinetic fractionation by soil CO<sub>2</sub> advection, the extracted gas volume was replaced by a similar volume of air transferred to an inflatable balloon inside the chamber (Gomez-Casanovas et al., 2012). Steady-state conditions were achieved inside the chamber after 2 min, and nine consecutive logs of CO<sub>2</sub> concentrations were subsequently recorded at 10-s intervals on each frame during a 90s period (Jia et al., 2007). The increases in air temperature within the chamber were less than 0.2 °C during the 90-s period. Soil respiration was measured by a transparent climate-controlled chamber. Living vegetation in the chamber was removed by hand at least one day before soil respiration measurement. Net ecosystem exchange was measured by a transparent climatecontrolled chamber. Ecosystem respiration was measured by a dark climate-controlled chamber as light was eliminated (hence photosynthesis). The living vegetation in the chamber left intact, when ecosystem respiration and net ecosystem carbon exchange measured.

Soil temperature and soil moisture were measured near each collar at the time of  $CO_2$  flux measurement. Soil water content measured at 9:00 am on each observation date, and was calculated by oven-dried the surface 5 cm fresh soil at 105 °C for 48 h. Soil temperature was measured by soil temperature probes (REBS STP1, LI-COR Inc., Nebraska, USA) inserted at a depth of 5 cm adjacent to each collar. Soil temperature and  $CO_2$  fluxes rates were measured eight times per day with 3 h interval from 6 o'clock to 6 o'clock the next day on each observation date (Gomez-Casanovas et al., 2012). Atmospheric  $CO_2$  assimilated by photosynthesis (GPP) equals the ecosystem respiration (ER) minus net ecosystem respiration, net

ecosystem exchange, soil water content and soil temperature were measured in each plot two times per month during May– September in 2012 and 2013, and the values of those five factors in the following paper were the mean of the two times value.

#### 2.3. Plant and soil samplings

Aboveground biomass (AGB), litter and cover were estimated from harvesting squares  $(1 \text{ m}^2)$  located close to the chambers in each plot. Three belowground biomass (BGB, 0-50 cm) core samples were collected from each harvesting square using a cylinder auger of 9 cm in diameter. Roots were separated from the soil by washing over a 0.2-mm mesh. Litter, AGB and BGB samples were oven-dried at 80 °C for 72 h and then weighed as dry matter. Litter, AGB, and BGB were collected monthly during the growing seasons in 2012 and 2013. We collected five soil samples at depth of 0–30 cm by a cylinder auger of 9 cm in diameter in each harvesting square at the end of growing season once per year. Then mixed five homogenized soil samples to one sample and analyzed soil properties. Soil carbon content was assayed by dichromate oxidation. Soil bulk density (BD) was calculated depending on the inner diameter of the core sampler, sampling depth and oven dried weight of the composite soil samples. Soil organic carbon storage calculated by multiplying soil carbon content by soil bulk density. The soil samples were only collected once at the end of the growing seasons in 2012, 2013 respectively.

#### 2.4. Data analysis

The statistical comparisons were conducted using two-way analysis of variance (ANOVA) for the effects with grassland management and month of measuring period on soil respiration, ecosystem respiration, net ecosystem exchange, aboveground biomass belowground biomass, soil water content, soil temperature, litter, and cover by SPSS 11.5 for Windows software (Chicago,



Fig. 2. Variation of averaged aboveground biomass (AGB), belowground biomass (BGB), litter and cover during the growing seasons from 2012 to 2013 at fenced grassland (FG) and grazed grassland (GG). Vertical bars indicate standard error of mean of five quadrats for each month.

Illinois, USA). Also, partial least-squares regression (PLSR) procedure was implemented in SIMCA-P (Umetrics AB, Sweden).

In order to evaluate the response of soil respiration, ecosystem respiration, net ecosystem exchange to grassland management (grazing exclusion = 1, grazing = 0), soil microhabitat and community features changes, PLSR was applied to the results obtained. PLSR describes variation in both the independent and dependent variables by determining latent factors, which are linear-weighted combinations of the input variables (Yu et al., 2010). In the PLSR model,  $Q^2$  is the fraction of the total variation of the dependent variables that can be predicted. When  $Q^2$  is greater than 0.5, the model is considered to exhibit good predictive ability (Shi et al., 2013b). The importance of a predictor for both the independent and the dependent variables is given by the variable importance for the projection (VIP). Terms with large VIP values are the most relevant for explaining the dependent variable. Regression coefficients reveal the direction and strength of the impact of each variable in the PLSR model. In addition, the root-meansquared error-of-prediction (RMSEP) provides useful information for calibrating the regression model.

#### 3. Results

#### 3.1. Soil and plant characteristic response

Aboveground biomass increased rapidly at the beginning of May and reached a maximum in August for both fenced and grazed grasslands (Fig. 2a). Belowground biomass and cover peaked in September in 2012 and in October in 2013 (Fig. 2b and d). Soil organic carbon contents of FG and GG at the depth of 0–20 cm were  $6.68 \pm 0.08$  and  $6.32 \pm 0.17$  g kg<sup>-1</sup>, respectively. Soil bulk density of FG and GG were  $1.26 \pm 0.01$  and  $1.29 \pm 0.01$  g cm<sup>-3</sup>, respectively. Cover of FG and GG were  $59.72 \pm 1.56\%$  and  $43.46 \pm 1.26\%$  during two years experiment, respectively.

Two-way ANOVAs indicated grassland management significant effects on soil carbon storage, soil bulk density. Aboveground biomass, belowground biomass, litter, cover and soil temperature were significant with grassland management (P < 0.01, n = 100) and month of measuring period (P < 0.01, n = 100) over two years experiment (Table 1). Soil water content was only influenced by the month of measuring period (P < 0.01, n = 100). Significant interactive effects on ecosystem respiration, aboveground biomass and litter were found with grassland management and month of measuring period (P < 0.01, n = 100).

#### 3.2. Carbon exchange dynamics

The minimum values of soil respiration, ecosystem respiration and net ecosystem exchange appeared in June 2012, similar to soil

#### Table 1

Two-way ANOVA results of grassland management (Management) and month of measuring period (Month) effect on biotic and abiotic characteristics and  $CO_2$  effluxes. *F* = *F* statistic and df = degree of freedom.

| Factors | Management |         |         | Month |         |         | $Month \times Management$ |       |         |
|---------|------------|---------|---------|-------|---------|---------|---------------------------|-------|---------|
|         | df         | F       | Р       | df    | F       | Р       | df                        | F     | Р       |
| SR      | 1          | 50.186  | < 0.001 | 4     | 10.289  | < 0.001 | 4                         | 0.826 | 0.512   |
| ER      | 1          | 49.959  | < 0.001 | 4     | 17.373  | < 0.001 | 4                         | 3.977 | 0.005   |
| NEE     | 1          | 7.767   | 0.006   | 4     | 12.886  | < 0.001 | 4                         | 0.297 | 0.879   |
| ST      | 1          | 9.598   | 0.003   | 4     | 107.164 | < 0.001 | 4                         | 1.084 | 0.369   |
| SWC     | 1          | 0.289   | 0.592   | 4     | 7.553   | < 0.001 | 4                         | 0.045 | 0.996   |
| Cover   | 1          | 125.197 | < 0.001 | 4     | 22.366  | < 0.001 | 4                         | 1.704 | 0.156   |
| AGB     | 1          | 132.262 | < 0.001 | 4     | 32.77   | < 0.001 | 4                         | 5.067 | 0.001   |
| BGB     | 1          | 14.767  | < 0.001 | 4     | 25.301  | < 0.001 | 4                         | 0.125 | 0.973   |
| Litter  | 1          | 194.832 | < 0.001 | 4     | 27.812  | < 0.001 | 4                         | 19.96 | < 0.001 |
| SOCS    | 1          | 61.044  | < 0.001 | 1     | 1       | 1       | 1                         | 1     | /       |
| BD      | 1          | 28.839  | < 0.001 | /     | /       | /       | 1                         | /     | 1       |



**Fig. 3.** Variation of soil temperature (ST, a), soil water content (SWC, b), soil respiration (SR, c), ecosystem respiration (ER, d) and net ecosystem carbon

(FG) and grazed grassland (GG). Vertical bars indicate standard error of mean of five quadrats for each month. The bar chart was the mean value for the two years experiment. \*\*, \* were significant differences between FG and GG at the 0.01 and 0.05 level, respectively.

water content (Fig. 3). Grazing exclusion significantly increased soil respiration (P < 0.01), ecosystem respiration (P < 0.01) by 0.36 and 0.65 g C m<sup>-2</sup> d<sup>-1</sup>, respectively (Fig. 3). Grassland with grazing exclusion fixed significantly more about  $0.10 \text{ g C m}^{-2} d^{-1}$  from the atmosphere to the ecosystem than grazed grassland (P < 0.05). Total, atmospheric CO<sub>2</sub> assimilated by photosynthesis (GPP) at fenced grassland were larger about  $0.75 \text{ g C m}^{-2} d^{-1}$  than at grazed grassland in this study (Fig. 4). The CO<sub>2</sub> fluxes of soil respiration, ecosystem respiration and net ecosystem exchange at fenced grassland and grazed grassland were about 43%, 85% 15% and 40%, 84% 16% of the atmospheric CO<sub>2</sub> assimilated by photosynthesis, respectively (Fig. 4).



Fig. 4. Mean soil respiration, ecosystem respiration and net ecosystem exchange rate at grazed grassland and fenced grassland over the two years experiment.

#### 3.3. Main factors controlling the carbon exchange

Soil respiration and ecosystem respiration were significantly positive correlated with cover, aboveground biomass, belowground biomass, litter and soil water content according to the partial correlation analysis (Table 2). Net ecosystem exchange was significantly negative correlated with cover, aboveground biomass, belowground biomass, positive correlated with soil bulk density.

The preliminary analysis indicated many biotic/abiotic factors and three CO<sub>2</sub> effluxes were highly correlations. To identify the dominant drivers control the carbon exchanges, the PLSR was useful for highly multicollinearity variables, such as the carbon exchange rate and biotic/abiotic factors used here. The biggest  $Q^2$  values for soil respiration and ecosystem respiration models were 0.73 and 0.79, respectively, indicated the good predictive ability and robustness of the two models. However, the biggest  $Q^2$  values for NEE was only 0.28, which was below than 0.5 and considered to exhibit bad predictive ability. The PLSR prediction models were also illustrated by the low RMSEP, SR with 0.164  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> and ER with 0.280  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. All of the considered factors were related to soil respiration and ecosystem respiration to some extent, yet only some of them have VIP > 1 (Table 4). Factors with VIP values below 1 are of minor importance for soil respiration and ecosystem respiration.

For the soil respiration, the first component accounted for 52.3% of the variance in the dataset (Table 3). The addition of two components cumulatively accounted for 77.3% of the total variance in the soil respiration. Adding more components to the PLSR models did not substantially improve the description of the contributions to the variance. In the case of soil respiration, the highest VIP value was obtained for soil water content (VIP = 1.60; RCs = 0.438), followed by the grassland management (VIP = 1.07; RCs = 0.253) (Table 4). Soil respiration appeared to increase with grazing exclusion and soil water content increasing (due to the positive regression coefficients).

For ecosystem respiration, three components accounted for 95.9% of the variance (Table 4). The addition of further components did not significantly improve the accounting of the factors contributing to the variance. The highest VIP value was obtained for soil water content (VIP=1.52; RCs=0.752), followed by the aboveground biomass (VIP=1.05; RCs=0.281), grassland management (VIP=1.02; RCs=0.057) (Table 4). Aboveground biomass and soil water content were the two main factors that affected ecosystem respiration.

Table 2

Table 3

Partial correlation matrix of the biotic/abiotic factors and CO<sub>2</sub> effluxes with the fixed factor that month of measuring period used in the PLSR models.

| Factors | SR       | ER       | NEE      | ST     | SWC    | Cover         | AGB    | BGB    | Litter | SOCS   |
|---------|----------|----------|----------|--------|--------|---------------|--------|--------|--------|--------|
| ER      | 0.89**   |          |          |        |        |               |        |        |        |        |
| NEE     | -0.515** | -0.455** |          |        |        |               |        |        |        |        |
| ST      | 0.029    | 0.136    | -0.167   |        |        |               |        |        |        |        |
| SWC     | 0.567    | 0.535    | -0.133   | -0.525 |        |               |        |        |        |        |
| Cover   | 0.459    | 0.436    | -0.427** | 0.194  | -0.095 |               |        |        |        |        |
| AGB     | 0.437    | 0.492    | -0.274*  | 0.213  | -0.064 | 0.655         |        |        |        |        |
| BGB     | 0.276    | 0.289    | -0.342** | 0.177  | -0.06  | 0.4           | 0.372  |        |        |        |
| Litter  | 0.431    | 0.499    | -0.01    | 0.133  | 0.069  | 0.455         | 0.652  | 0.189  |        |        |
| SOCS    | 0.214    | 0.19     | -0.168   | -0.129 | -0.017 | 0.443         | 0.543  | 0.162  | 0.425  |        |
| BD      | -0.446   | -0.5     | 0.281    | 0.019  | -0.128 | $-0.299^{**}$ | -0.163 | -0.193 | -0.31  | -0.077 |

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

| Summary of the PLSR of soil respiration (SR), ecosystem respiratio | on (ER) and net ecosystem carbon exchange (NEE |
|--|--|
|--|--|

| Response variable Y | R <sup>2</sup> | Q <sup>2</sup> | Component   | % of explained variability in Y | Cumulative explained variability in $Y(\%)$ | $\begin{array}{l} RMSEP \\ (\mu molm^{-2}s^{-1}) \end{array}$ | Q <sup>2</sup> cum      |
|---------------------|----------------|----------------|-------------|---------------------------------|---|---|-------------------------|
| SR                  | 0.773          | 0.731          | 1<br>2      | 52.3<br>18.7                    | 52.3<br>71                                  | 0.235<br>0.184  | 0.508<br>0.682          |
|                     |                |                | 3<br>4      | 6.3<br>2 1                      | 77.3<br>79 4                                | 0.164<br>0.157  | 0.731<br>0.730          |
| ER                  | 0.832          | 0.792          | 1           | 68.6                            | 68.6  | 0.449   | 0.548                   |
|                     |                |                | 2<br>3      | 22.6<br>4.7                     | 91.2<br>95.9                                | 0.316<br>0.28   | 0.76<br>0.792           |
|                     |                |                | 4           | 1.6                             | 97.5  | 0.268   | 0.788                   |
| NEE                 | 0.347          | 0.281          | 1<br>2<br>3 | 18.7<br>16<br>4.5               | 18.7<br>34.7<br>39.2                        |   | 0.166<br>0.281<br>0.263 |

 Table 4

 VIP values and PLSR weights to soil respiration (SR), ecosystem respiration (ER).

| Factors    | SR               |      | ER               | ER   |  |  |
|------------|------------------|------|------------------|------|--|--|
|            | RCs <sup>a</sup> | VIP  | RCs <sup>a</sup> | VIP  |  |  |
| Management | 0.253            | 1.07 | 0.057            | 1.02 |  |  |
| ST         | 0.415            | 0.84 | 0.433            | 0.78 |  |  |
| SWC        | 0.793            | 1.60 | 0.752            | 1.52 |  |  |
| Cover      | 0.212            | 0.99 | 0.125            | 0.97 |  |  |
| AGB        | 0.135            | 0.97 | 0.281            | 1.05 |  |  |
| BGB        | 0.044            | 0.67 | 0.087            | 0.72 |  |  |
| Litter     | -0.027           | 0.89 | 0.079            | 0.93 |  |  |
| SOCS       | -0.025           | 0.79 | -0.027           | 0.84 |  |  |
| BD         | -0.121           | 0.91 | -0.232           | 0.97 |  |  |

<sup>a</sup> Regresssion coefficients.

#### 4. Discussions

Grazing exclusion enhanced above and below ground biomass, litter, cover, soil carbon storage, soil respiration and ecosystem respiration in steppe grassland on the Loess Plateau. Soil respiration and ecosystem respiration were positive correlated with plant biomass, cover, and soil water content. Rather than soil temperature, soil water content was the key soil microhabitat factor that controlled soil respiration and ecosystem respiration. Furthermore, grassland with grazing exclusion fixed more CO<sub>2</sub> than grazing grassland.

Grazing exclusion enhanced aboveground biomass, belowground biomass and litter mass, and conducive to soil organic matter formation and accumulation, thus significant increased soil carbon storage and decreased bulk density (Cao et al., 2004; Garcia-Pausas et al., 2011; McSherry and Ritchie, 2013). Aboveground biomass, belowground biomass and litter at fenced grassland were about 1.84, 1.17 and 2.25 times than were at grazed grassland.Aboveground and belowground biomasses, litter and cover were exhibited positive relationships with soil respiration and ecosystem respiration (Table 2). The higher aboveground biomass and belowground biomasses lead to increase photosynthesis, a higher translocation rate of carbon to the rhizosphere, and increase decomposition rates (Bremer et al., 1998; Högberg et al., 2001; Jia et al., 2007; Polley et al., 2008; Klumpp et al., 2009 Zhao et al., 2011).

The dependences of soil respiration and ecosystem respiration on SWC and ST were reported in many ecosystems, but their relative importance is controversial (Huxman et al., 2004; Liu et al., 2009; Gomez-Casanovas et al., 2012). Jia et al. (2007) found that the effect of temperature on carbon exchange rate manifested only when there was sufficient soil water to permit significant root and microbial respiration in arid and semi-arid regions. Soil respiration and ecosystem respiration were lowest when soil water content was lowest both at FG and at GG in June 2012 (Fig. 3b). Soil water stress would not only directly suppress microbial and root activities, but also indirectly decrease respiration via inhibition of plant growth and substrate availability (Huxman et al., 2004; Suseela et al., 2012). Moreover, soil water content was positively correlated with leaf area index, which controlled the gross primary productivity and net ecosystem exchange in steppe (Flanagan et al., 2002). Soil water content, controlled by amount and timing of precipitation, affected carbon exchange processes for grassland ecosystem in semi-arid area (Du et al., 2012). Soil respiration and ecosystem respiration increased after grazing exclusion, attributed to soil water content increased from 9.14% to 9.40% (Fig. 3b). Soil water content was the main factor controlled the season pattern of SR and ER during the two years experiment in the study region.

The  $CO_2$  fluxes proportions of soil respiration, ecosystem respiration and net ecosystem exchange for the atmospheric  $CO_2$ 

assimilated by photosynthesis at fenced grassland were similar with grazed grassland (Fig. 4). There was a positive correlation between soil respiration, ecosystem respiration and net primary productivity (Raich and Schlesinger, 1992; Raich and Potter, 1995; Gomez-Casanovas et al., 2012) or gross primary productivity (Janssens et al., 2001; Flanagan et al., 2002). Plant photosynthetic activity was an important factor determining rates of soil respiration, ecosystem respiration and net ecosystem exchange. and highly influenced by biome type and climate (Gomez-Casanovas et al., 2012). In this study, both fenced grassland and grazed grassland had same climate factors and dominant species, so the plant photosynthetic activity might be similarly. Atmospheric CO<sub>2</sub> assimilated by photosynthesis, which was bigger at fenced grassland, depended on soil water content and plant biomass in the semi-arid region (Chou et al., 2008; Jia et al., 2007; Du et al., 2012). The regrowth leaves following defoliation at grazed grassland often are more physiologically active than the older leaves that contribute much of leaf area at fenced grassland (Owensby et al., 2006). The more physiologically active could neutralize the influence of lower biomass and soil water content on ecosystem exchange ratios at grazed grassland. Fenced grassland fixed significant more CO<sub>2</sub> than grazed grassland over the two years experiment. Grazing exclusion should be conducted as a positive management practices to maintain C exchange balance for long-term fenced grassland.

#### 5. Conclusion

Grazing exclusion markedly increased soil respiration, ecosystem respiration and net ecosystem exchange about 0.36, 0.65 and -0.10 g C m<sup>-2</sup> d<sup>-1</sup>, respectively. Fenced grassland had significantly larger aboveground biomass, belowground biomass, litter and cover than grazed grassland. Plant biomasses, litter and cover were significantly positive correlated with soil respiration and ecosystem respiration. Soil water content, rather than soil temperature, was the major soil environmental factor controlling soil respiration and ecosystem respiration during the growing-season on the Loess Plateau. The ratios of soil respiration, ecosystem respiration and net ecosystem exchange to atmospheric CO<sub>2</sub> assimilated by photosynthesis at grazed grassland were similar with grazed grassland. The value of NEE was significantly smaller at fenced grassland, indicated greater net CO<sub>2</sub> uptake in the fenced area relative to grazed grassland. These results suggested that grazing exclusion was a positive management practice to maintain or increase C stocks in grassland ecosystems on the Loess Plateau.

#### Acknowledgments

We thank editors and two anonymous reviewers for their valuable comments and suggestions on the manuscript. This study was funded by Projects of Natural Science Foundation of China (NSFC41371282), the Strategic Priority Research Program - Climate Change: Carbon Budget and Related Issues of the Chinese Academy of Sciences (CAS) (Grant No. XDA05050403), the Action Plan for West Development Project of CAS (KZCX2-XB3-13) and Project of Natural Science Foundation of Shaanxi Province (2014KJXX-15), Lanzhou Institute of Animal and Veterinary Pharmaceutics Sciences, Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2014-LIHPS-08).

#### References

Bremer, D.J., Ham, J.M., Owensby, C.E., Knapp, A.K., 1998. Responses of soil respiration to clipping and grazing in a tallgrass prairie. J. Environ. Qual. 27, 1539–1548. Bubier, J.L., Moore, T.R., Bledzki, L.A., 2007. Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. Global Change Biol. 13, 1168–1186.

- Cao, G.M., Tang, Y.H., Mo, W.H., Wang, Y.A., Li, Y.N., Zhao, X.Q., 2004. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. Soil Biol. Biochem. 36, 237–243.
- Chang, R.Y., Fu, B.J., Liu, G.H., Liu, S.G., 2011. Soil carbon sequestration potential for Grain for Green Project in Loess Plateau. China Environ. Manage. 48, 1158–1172.
- Chen, W.W., Wolf, B., Zheng, X.H., Yao, Z.S., Butterbach-Bahl, K., Bruggemann, N., Han, S.H., Liu, C.Y., Han, X.G., 2013. Carbon dioxide emission from temperate semiarid steppe during the non-growing season. Atmos. Environ. 64, 141–149.
- Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W., Allen-Diaz, B., 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. Global Change Biol. 14, 1382–1394.
- Conant, R.T., Paustian, K., Elliott, E.T., 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecol. Appl. 11, 343–355.
- Deng, L., Zhang, Z.N., Shangguan, Z.P., 2014. Long-term fencing effects on plant diversity and soil properties in China. Soil Tillage Res. 137, 7–15.
- Du, Q., Liu, H.Z., Feng, J.W., Wang, L., Huang, J.P., Zhang, W., Bernhofer, C., 2012. Carbon dioxide exchange processes over the grassland ecosystems in semiarid areas of China. Sci. China Earth Sci. 55, 644–655.
- FAO-UNESCO, 2009. Guidelines for soil description. FAO, Rome, Italy.
- Flanagan, L.B., Johnson, B.G., 2005. Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. Agric. For. Meteorol. 130, 237–253.
- Flanagan, L.B., Wever, L.A., Carlson, P.J., 2002. Seasonal and interannual variation in carbon dioxide exchange and carbon balance in a northern temperate grassland. Global Change Biol. 8, 599–615.
- Frank, A.B., 2002. Carbon dioxide fluxes over a grazed prairie and seeded pasture in the Northern Great Plains. Environ. Pollut. 116, 397–403.
- Frank, A.B., Liebig, M.A., Hanson, J.D., 2002. Soil carbon dioxide fluxes in northern semiarid grasslands. Soil Biol. Biochem. 34, 1235–1241.
- Gao, Y.H., Zeng, X.Y., Schumann, M., Chen, H., 2011. Effectiveness of exclosures on restoration of degraded alpine meadow in the eastern Tibetan Plateau. Arid Land Res. Manage. 25, 164–175.
- Garcia-Pausas, J., Casals, P., Romanya, J., Vallecillo, S., Sebastia, M.T., 2011. Seasonal patterns of belowground biomass and productivity in mountain grasslands in the Pyrenees. Plant Soil 340, 315–326.
- Gass, T.M., Binkley, D., 2011. Soil nutrient losses in an altered ecosystem are associated with native ungulate grazing. J. Appl. Ecol. 48, 952–960. Gomez-Casanovas, N., Matamala, R., Cook, D.R., Gonzalez-Meler, M.A., 2012. Net
- Gomez-Casanovas, N., Matamala, R., Cook, D.R., Gonzalez-Meler, M.A., 2012. Net ecosystem exchange modifies the relationship between the autotrophic and heterotrophic components of soil respiration with abiotic factors in prairie grasslands. Global Change Biol. 18, 2532–2545.
- Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
- Huxman, T.E., Snyder, K.A., Tissue, D., Leffler, A.J., Ogle, K., Pockman, W.T., Sandquist, D.R., Potts, D.L., Schwinning, S., 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141, 254–268.
- Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, Ü., Morgenstern, K., Oltchev, S., Clement, R., GuĐmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biol. 7, 269–278.
- Jia, B.R., Zhou, G.S., Wang, F.Y., Wang, Y.H., Weng, E.S., 2007. Effects of grazing on soil respiration Of Leymus chinensis steppe. Clim. Change 82, 211–223.
- Klumpp, K., Fontaine, S., Attard, E., Le Roux, X., Gleixner, G., Soussana, J.F., 2009. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. J. Ecol. 97, 876–885.
- Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123, 1–22.

- Li, X.D., Fu, H., Guo, D., Li, X.D., Wan, C.G., 2010. Partitioning soil respiration and assessing the carbon balance in a Setaria italica (L.) Beauv. Cropland on the Loess Plateau, Northern China. Soil Biol. Biochem. 42, 337–346.
- Li, X.D., Zhang, C.P., Fu, H., Guo, D., Song, X.R., Wan, C.G., Ren, J.Z., 2013. Grazing exclusion alters soil microbial respiration, root respiration and the soil carbon balance in grasslands of the Loess Plateau, northern China. Soil Sci. Plant Nutr. 59, 877–887.
- Liu, W.X., Zhang, Z., Wan, S.Q., 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Global Change Biol. 15, 184–195.
- McSherry, M.E., Ritchie, M.E., 2013. Effects of grazing on grassland soil carbon: a global review. Global Change Biol. 19, 1347–1357.
- Owensby, C.E., Ham, J.M., Auen, L.M., 2006. Fluxes of CO<sub>2</sub> from grazed and ungrazed tallgrass prairie. Rangeland Ecol. Manage. 59, 111–127.
- Polley, H.W., Frank, A.B., Sanabria, J., Phillips, R.L., 2008. Interannual variability in carbon dioxide fluxes and flux-climate relationships on grazed and ungrazed northern mixed-grass prairie. Global Change Biol. 14, 1620–1632.
- Potts, D.L., Huxman, T.E., Cable, J.M., English, N.B., Ignace, D.D., Eilts, J.A., Mason, M.J., Weltzin, J.F., Williams, D.G., 2006. Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. New Phytol. 170, 849–860.
- Qiu, L.P., Wei, X.R., Zhang, X.C., Cheng, J.M., 2013. Ecosystem carbon and nitrogen accumulation after grazing exclusion in semiarid grassland. PLoS ONE 8, e55433.
- Raich, J.W., Potter, C.S., 1995. Global patterns of carbon-dioxide emissions from soils. Global Biogeochem. Cycles 9, 23–36.
- Raich, J.W., Schlesinger, W.H., 1992. The global carbon-dioxide flux in soil
- respiration and its relationship to vegetation and climate. Tellus B 44, 81–99. Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation
- and turnover of soil carbon in a re-establishing forest. Nature 400, 56–58. Risch, A.C., Frank, D.A., 2006. Carbon dioxide fluxes in a spatially and temporally heterogeneous temperate grassland. Oecologia 147, 291–302.
- Shi, X.M., Li, X.G., Li, C.T., Zhao, Y., Shang, Z.H., Ma, Q.F., 2013a. Grazing exclusion decreases soil organic C storage at an alpine grassland of the Qinghai-Tibetan Plateau. Ecol. Eng. 57, 183–187.
- Shi, Z.H., Ai, L., Li, X., Huang, X.D., Wu, G.L., Liao, W., 2013b. Partial least-squares regression for linking land-cover patterns to soil erosion and sediment yield in watersheds. J. Hydrol. 498, 165–176.
- Suseela, V., Conant, R.T., Wallenstein, M.D., Dukes, J.S., 2012. Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. Global Change Biol. 18, 336–348.
- Wang, D., Wu, G.L., Zhu, Y.J., Shi, Z.H., 2014. Grazing exclusion effects on above-and below-ground C and N pools of typical grassland on the Loess Plateau (China). Catena 123, 113-120.
- Wohlfahrt, G., Anderson-Dunn, M., Bahn, M., Balzarolo, M., Berninger, F., Campbell, C., Carrara, A., Cescatti, A., Christensen, T., Dore, S., Eugster, W., Friborg, T., Furger, M., Gianelle, D., Gimeno, C., Hargreaves, K., Hari, P., Haslwanter, A., Johansson, T., Marcolla, B., Milford, C., Nagy, Z., Nemitz, E., Rogiers, N., Sanz, M.J., Siegwolf, R.T. W., Susiluoto, S., Sutton, M., Tuba, Z., Ugolini, F., Valentini, R., Zorer, R., Cernusca, A., 2008. Biotic, abiotic, and management controls on the net ecosystem CO<sub>2</sub> exchange of European mountain grassland ecosystems. Ecosystems 11, 1338–1351.
- Wu, T.N., Wu, G.L., Wang, D., Shi, Z.H., 2013. Soil-hydrological properties response to grazing exclusion in a steppe grassland of the Loess Plateau. Environ. Earth Sci. 71, 745–752.
- Yu, H., Luedeling, E., Xu, J., 2010. Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. Proc. Natl. Acad. Sci. U.S.A. 107, 22151–22156.
- Zhang, Y.J., Guo, S.L., Liu, Q.F., Jiang, J.S., Wang, R., Li, N.N., 2015. Responses of soil respiration to land use conversions in degraded ecosystem of the semi-arid Lores Plateau Ecol. Eng. 74, 196–205.
- Loess Plateau. Ecol. Eng. 74, 196–205.
   Zhao, Y., Peth, S., Hallett, P., Wang, X.Y., Giese, M., Gao, Y.Z., Horn, R., 2011. Factors controlling the spatial patterns of soil moisture in a grazed semi-arid steppe investigated by multivariate geostatistics. Ecohydrology 4, 36–48.