Response of dominant grassland species in the temperate steppe of Inner Mongolia to different land uses at leaf and ecosystem levels

M. LIU, J.R. GONG+, Y. PAN, Q.P. LUO, Z.W. ZHAI, L.L. YANG, and S. XU

State Key Laboratory of Surface Processes and Resource Ecology, College of Resources Science and Technology, Faculty of Geographical Science, Beijing Normal University, Beijing, China

Abstract

In order to study the responses of dominant species to different land uses in the semiarid temperate grassland of Inner Mongolia, we tested the physiological responses of *Stipa grandis*, *Leymus chinensis*, and *Artemisia frigida* to mowing, grazing exclusion, and grazing land uses at the leaf and ecosystem levels. The grazing-exclusion and mowing sites released CO_2, but the grazing site was a net carbon sink. *L. chinensis* and *S. grandis* contributed more to the ecosystem CO_2 exchange than *A. frigida*. At the grazing-exclusion and mowing sites, *Leymus chinensis* and *Stipa grandis* both exhibited a higher light-saturation point and higher maximum photosynthetic rate than that at the grazing site, which increased photosynthesis and growth compared to those at the grazing site. In contrast, *A. frigida* possessed a higher nitrogen content than the other species, and more of the light energy used for photosynthesis, particularly at the grazing site.

Additional key words: gas exchange; osmoregulation; water-use efficiency.

Introduction

Grasslands in China cover nearly 4×10^6 km^2, which is more than 40% of the total land area. The temperate arid and semiarid grasslands of Inner Mongolia comprise the main temperate grassland in northern China, and play an important role in both livestock farming and environmental conservation (Akiyama and Kawamura 2007, Jia *et al.* 2016). However, the region's expanding human population and changing life styles have led to ecosystem degradation due to excessively intensive grassland use (Zhou *et al.* 2007). These changes have undoubtedly greatly affected ecosystem processes, including leaf- and canopy-scale photosynthesis, as a result of changes in vegetation cover, community species composition, ecosystem productivity, and nitrogen utilization (Houghton *et al.* 1999, Zeller *et al.* 2000, Wohlfahrt *et al.* 2003, Robson *et al.* 2007, Ingram *et al.* 2008, Ciais *et al.* 2011). Grazing and mowing are two of the most important land-use types that affect ecosystem processes and alter CO_2 exchange in managed grassland ecosystems (Han *et al.* 2012, Zhang *et al.* 2012, Chen *et al.* 2015). These disturbances put ecosystems under considerable stress, making it critical to understand the mechanisms that underpin ecosystem structure, functioning, and stability under different land uses (Ives and Carpenter 2007). In particular, it is still not clear how the environmental differences between land uses affect CO_2 exchange in grasslands (Chimner and Welker 2011).

The dominant species in natural communities play a key role in conferring short-term resistance to reductions in ecosystem functioning (Smith and Knapp 2003). Shifts in cover by the dominant species are therefore expected to have large impacts on net CO_2 exchange by altering the

---

Received 9 June 2016, *accepted* 10 February 2017, *published as online-first* 13 March 2017.

+Corresponding author; e-mail: jrgong@bnu.edu.cn

Abbreviations: AQY – apparent quantum yield; Chl – chlorophyll; D – thermal dissipation; *E* – transpiration rate; EE – excess excitation energy; ET – evapotranspiration; F_m – maximum fluorescence yield in the dark-adapted state; F_m' – maximum fluorescence yield in the light-adapted state; F_o – minimum fluorescence yield in the dark-adapted state; F_o' – minimum fluorescence yield in the light-adapted state; F_s – steady-state fluorescence; F_v' – variable fluorescence in the light-adapted state; GPP – gross primary production; LCP – light-compensation point; LSP – light-saturation point; NEE – net ecosystem exchange; PET – photosynthetic electron transport; P_N – net photosynthetic rate; P_{Nmax} – maximum photosynthetic rate; q_P – photochemical quenching coefficient; R_{eco} – ecosystem respiration; S1 – grazing-exclusion site; S2 – mowing site; S3 – grazing site; WUE – water-use efficiency.

Acknowledgements: We thank Professor Baoyin of Inner Mongolia University for field assistance and Inner Mongolia University for its substantial support during our experiment. This study was supported by the National Natural Science Foundation of China (Grant No. 41571048), the Key National R & D program of China (Grant No. 2016YFC0500502), and the State Key Basic Research and Development Plan of China (Grant No. 2014CB138803).

CO_2 fluxes associated with decomposition, respiration, and photosynthesis (Sun *et al.* 2010). The ecophysiological response mechanisms of the dominant species play a key role in an ecosystem's responses to environmental change (Liu *et al.* 2012), so understanding how these mechanisms respond to different land-use types is important for understanding community and ecosystem processes.

Chlorophyll (Chl) fluorescence analysis and CO_2-exchange measurements have been combined to study light adaptations and detect changes in the photosynthetic apparatus in a response to stress (Poskuta *et al.* 1998, Larcher 2003, Bellasio *et al.* 2016). To protect themselves against stress, plants produce more compatible solutes, including proteins, soluble sugars, and proline, in order to facilitate osmotic adjustment and help stabilize the activities of key enzymes including antioxidant enzymes that scavenge reactive oxygen species (Liu *et al.* 2011).

Nitrogen is a limiting factor for plant growth in many types of grassland. N enrichment generally increased gross primary production (GPP) and net primary production by stimulating plant growth and biomass production (LeBauer and Treseder 2008, Xia and Wan 2008, Niu *et al.* 2010). However, the majority of ecosystem carbon uptake by photosynthesis is eventually released back into the atmosphere by ecosystem respiration (R_{eco}), which can also be stimulated by N enrichment (Xu and Wan 2008). Other key elements, such as phosphorus, may be limited, and this can also effect plant growth and contribute to variations in C exchange. The balances among multiple elements are integrated over a range of scales, from individuals to ecosystems. They can reflect the outcome of interactions among many underlying physiological and biochemical adjustments as organisms respond to their surroundings (Agren and Weih 2012). Therefore, understanding how ecosystems achieve stoichiometric homeostasis provides important insights into the major mechanisms responsible for determining the structure, functioning, and stability of grassland ecosystems (Yu *et al.* 2010).

The effects of land-use change on plant species diversity and community composition are relatively well understood, but the effects on plant tissue quality, energy distribution among components of the photosynthetic system, and osmoregulation physiology are poorly documented. Therefore, in the present study, we investigated plant- and community-level ecophysiological responses of the dominant vegetation in temperate grassland of northern China under different land-use types. Our objectives were to determine whether the leaf-level response to different land uses was consistent with the ecosystem-level response in terms of gas-exchange physiology, vegetation cover, and productivity, and to determine the physiological mechanisms that govern the response of the three dominant species to the different land-use types.

Materials and methods

Study site: Our study was carried out during the 2011 growing season at a long-term experimental site managed by the Grassland Ecosystem Research Station of Inner Mongolia University (116°02′E to 116°30′E, 44°48′N to 44°49′N). The station is located in relatively flat land in the middle reaches of the Xilin River at an average elevation of 1,505 m. The region has a semiarid continental temperate steppe climate with a dry spring and a moist summer. Based on data obtained from 1950 to 2011 at the Xilingol weather station (about 15 km from our study site), this region has a mean annual temperature ranging from –42.4 to 38.3°C. The total annual precipitation ranges from 300 to 360 mm (of which 70% falls from June to August). In 2011, the total precipitation was 188 mm during the growing season (about 43 mm drier compared to a normal year). The mean daily air temperature showed a typical seasonal pattern for the study area, with the peak appearing from July to August (Fig. 1S, *supplement available online*).

Three measurement sites were established in the study area: a grazing-exclusion site (S1, 44°14.91′N, 116° 23.2′E), a mowing site (S2, 44°15.21′N, 116°23.1′E), and a grazing site (S3, 44°14.27′N, 116°25.0′E). All three sites were adjacent to each other. The 15-ha grazing-exclusion site has been fenced since 2008 and protected under natural conditions. The mowing site, which covers about 14 ha, has been mowed annually in August (to within 5 cm from the soil surface) since grazing was excluded in 2008. The long-term grazing site, which has been grazed since 1956, has an area of 15 ha and was grazed by 11 sheep throughout the year, which is equivalent to light grazing. The long-term grazing, mowed, and enclosed areas had the same grazing intensity and vegetation cover before 2008. The area is dominated by *Stipa grandis*, *Leymus chinensis*, and *Artemisia frigida* (Fig. 1), with other species accounting for no more than 33% of the aboveground biomass.

Plant biomass: We randomly selected eight 1,000 m^2 sample plots at each site, but with the constraint that the plots were located at least 5 m from the edge of the site to avoid edge effects. The aboveground biomass was harvested by clipping all plants just above the soil surface in five randomly selected 1 m × 1 m quadrats in each plot. We determined the peak plant aboveground biomass production in middle of August in 2011, when the pasture vegetation was mature. After removing the aboveground biomass, all living plants were separated by species (into the three dominant species and "other" for all other plants) and then stored separately in paper bags. The samples were then oven-dried at 65°C to constant mass to determine the oven-dry biomass. The total net dry mass for the harvested aboveground biomass equaled the sum of the biomass amounts of four species categories. The dried samples

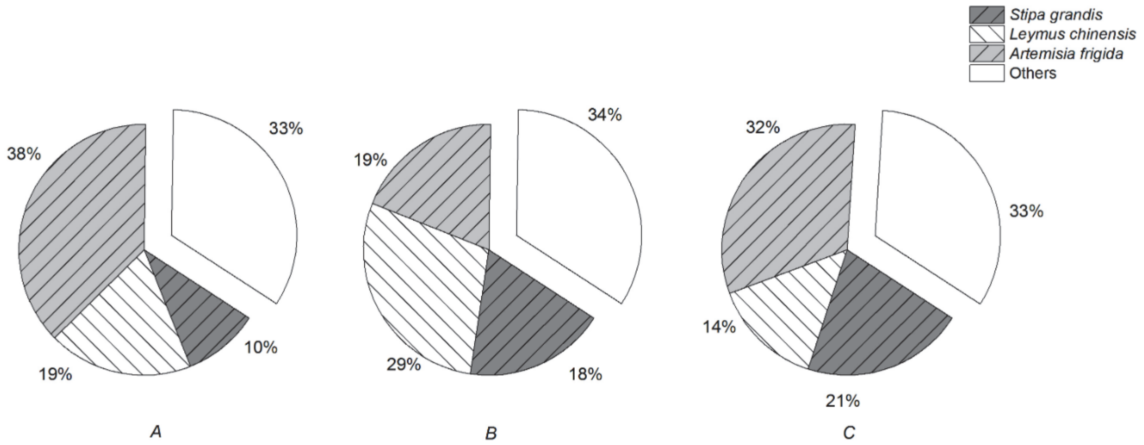

Fig. 1. Relative biomass of the dominant species under the three land-use types: (*A*) mowing site, (*B*) grazing-exclusion site, and (*C*) grazing site.

were ground to pass through a 0.5-mm mesh using a grinding mill (*XA-1A*, Nanjing, China) and were then homogenized before analysis of the C, N, and P contents. The total C, N, and P contents were measured using the Walkley–Black wet oxidation technique (Nelson and Sommers 1982) with an automatic elemental analyzer (*Vario EL*, *Elementar*, Germany), the Kjeldahl method (Bremner 1960) with an automated Kjeldahl analyzer (*K-06*, Shanghai Shengsheng, China), and automated colorimetry (Soon and Kalra 1995) with the ultraviolet-visible spectrophotometer (*756PC*, Jinghua, China), respectively. Based on these values, we calculated the C/N, C/P and N/P ratios.

Leaf gas exchange: Measurements of gas exchange were performed in August 2011 in three plots per site, which had not been sampled for biomass determination. We randomly chose 10 individual plants of the three dominant species in each plot for each measurement period. The measurements were performed on clear, cloudless days between 08:00 and 20:00 h using a *Li-6400* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA). Five mature leaves from the current-year foliage were measured for each individual. For these individuals, we measured the net photosynthetic rate (P_N) and transpiration (E). Water-use efficiency (WUE) of each species was then calculated using the following equation: WUE = P_N/E.

Chl fluorescence parameters: Variable Chl *a* fluorescence was measured with a portable *LI-6400-40* pulse-amplitude-modulation fluorometer (*Li-Cor*, USA). For these measurements, the mature, fully expanded leaves were maintained in darkness overnight, still attached to the plant. Before each measurement, samples were kept in clip cuvettes for more than 30 min at ambient temperature to allow dark adaptation. The maximum fluorescence yield in the dark (F_m) and minimum fluorescence yield in the dark (F_0) were measured simultaneously. The leaves were then exposed to a PPFD of 1,000 μmol m^{-2} s^{-1} for more than 3 min to measure the maximum fluorescence (F_m'), the minimum fluorescence (F_o'), and the steady-state fluorescence (F_s) in the light-adapted state after P_N stabilized. F_v' represents variable fluorescence in the light-adapted state: $F_v' = F_m' - F_o'$. These measurements were performed every 2 h from 08:00 to 20:00 h on sunny days.

Photochemical quenching (q_P) was calculated using the following formula (Demmig-Adams *et al*. 1996):

$$q_P = (F_m' - F_s)/(F_m' - F_o')$$

We calculated the proportion of the light absorbed by the PSII antennae and utilized in PSII photochemistry, and used this data to calculate the photosynthetic electron transport (PET), the fraction of light absorbed by the PSII antennae that was dissipated thermally (D), and the fraction of absorbed light that was not accounted for by either PET or D, which represents excess excitation energy (EE), using the following formulas (Genty *et al*. 1989, Demmig-Adams *et al*. 1996):

$$\mathrm{PET} = (F_m' - F_s)/F_m'$$
$$D = 1 - (F_v'/F_m')$$
$$\mathrm{EE} = (F_v'/F_m') \times (1 - q_P)$$

The light energy absorbed by Chl molecules is released *via* these three mechanisms, and their total equals the total energy captured. Based on the proportions of the total energy:

$$\mathrm{PET} + D + \mathrm{EE} = 1$$

Ecosystem gas exchange: After finishing the leaf gas-exchange and Chl fluorescence measurements, ecosystem gas exchange was measured using a transparent chamber (0.5 m × 0.5 m × 0.5 m) attached to an infrared gas analyzer (*Li-6400*, *Li-Cor*, USA) with a closed-cell foam gasket at the base to form a seal with the frame. Five frames were installed at a level of soil surface at the sites for each land use, and the soil was allowed to recover from this disturbance for at least 1 d before measurements were obtained. The air in the chamber was mixed by two small fans. The method was similar to that of Niu *et al.* (2007). CO_2 and H_2O fluxes were determined from the time-courses of the concentrations, starting 30 s after closure of the chamber and lasting for an additional 60 s, to calculate net ecosystem exchange (NEE, based on the CO_2 flux) and evapotranspiration (ET, based on the H_2O flux). After finishing the NEE measurements, the chamber was ventilated and covered with an opaque cloth. Then the CO_2-exchange measurements were repeated. Because the second set of measurements eliminated light (and hence photosynthesis), the values represented R_{eco}. Gross primary production (GPP) was calculated as the difference between NEE and R_{eco}. Ecosystem WUE was calculated as NEE/ET.

Photosynthetic light-response curves: We obtained photosynthetic light-response curves at 2-h intervals from 09:00 to 11:00 h at a range of light intensities from 0 to 2,500 μmol(photon) m^{-2} s^{-1} using the *Li-6400* instrument. The irradiance response was measured at 25°C and a relative humidity of 50%, with the CO_2 concentration at 400 μmol mol^{-1}. The PPFD was set at 2,500; 2,000; 1,500; 1,000; 500, 200, 150, 100, 50, 20, and 0 μmol m^{-2} s^{-1}. The maximum photosynthetic rate (P_{Nmax}), light-compensation point (LCP), light-saturation point (LSP), and apparent quantum yield (AQY) of each species were obtained using the software provided with the *Li-6400* instrument from the light-response data.

Substances involved in osmoregulation: After measuring the photosynthetic characteristics, we randomly collected 10 mature healthy leaves per species from each plot and froze them in liquid nitrogen for subsequent biochemical analyses. Samples (1 g) were then ground in liquid nitrogen. The protein content was determined by the *Coomassie Brilliant Blue G250* method (Sedmak and Grossberg 1977). The proline content was determined according to the method of Bates *et al.* (1973). The soluble sugar content was determined using the method of Buysse and Merckx (1993).

Data and statistical analysis: We used analysis of variance (*ANOVA*) to test for significant differences between the land uses. For the leaf nutrient contents, we used two-way *ANOVA*, with species and land use as the two levels. Unless otherwise noted, significance was defined at $p<0.05$. When the *ANOVA* results revealed a significant difference between the land uses, we used the least-significant-difference (LSD) test to identify which pairs of land uses differed significantly. All analyses were conducted using *version 13.0* of the *SPSS* software (www.ibm.com/software/analytics/spss/). All graphs were created using *version 7.5* of the *Origin* software (http://www.originlab.com/).

Results

Aboveground biomass: The three dominant species accounted for more than 66% of the total community aboveground biomass in each of the three land-use types, although the proportions for the three dominant species differed significantly between the land-use types (Fig. 1). The total aboveground biomass values at the S2, S3, and S1 were 79.62, 72.46, and 76.73 g m^{-2}, respectively. At S2, *A. frigida* and *L. chinensis* accounted for the highest proportions of the three dominant species (38 and 19%, respectively). At S3, *A. frigida* accounted for the largest proportion (32%), followed by *S. grandis* (21%). In contrast, *L. chinensis* accounted for the largest proportion (29%) at S1, with the other species accounting for roughly equal amounts (*ca*. 18%), but the proportion of *A. frigida* (19%) was significantly lower than at the grazing site.

CO_2 exchange of the dominant plants and the ecosystem: Comparing the CO_2 exchange by the dominant species at the ecosystem scale revealed that CO_2 exchange differed between the land uses (Fig. 2). At the S1 and S2, the diurnal NEE was in total 16.74×10^3 and 16.50×10^3 μmol m^{-2} per d, respectively (*i.e.*, net carbon emission). In contrast, at S3, the diurnal NEE was -58.23×10^3 μmol m^{-2} per d (*i.e.*, net carbon sequestration). GPP and R_{eco} were significantly negatively correlated under all three land-use types (Fig. 3). GPP differed marginally significantly between the S2 and S1 sites, but both these sites had significantly lower GPP than the S3. GPP under the S1 and S2 land uses decreased slowly throughout the day (Fig. 3*A,B*), whereas GPP at the S3 decreased much more rapidly (Fig. 3*C*).

At the S2 and S1 sites, P_N of *L. chinensis* and *S. grandis* both showed a diurnal trend similar to that of NEE (Fig. 2). P_N of *L. chinensis* and *S. grandis* decreased to values near 0 by noon, followed by relatively stable values, and both values were higher than that of *A. frigida* at the two sites (Fig. 2*A,B,D,E*). At the S3, *L. chinensis* and *S. grandis* both had low P_N and P_N decreased to values well below 0 by midday to early afternoon (Fig. 2*C,F*). In contrast, *A. frigida* showed similar P_N trends in all three land-use types, decreasing rapidly to reach highly negative values by noon (Fig. 2*G–I*). The three species all had negative P_N values or values near 0 after midday in all three land-use types.

Water-use efficiency of the dominant plants and the ecosystem: There was no significant difference in WUE between the land-use types at the ecosystem scale, but ET showed different trends (Fig. 4). WUE of the three land uses increased gradually throughout the day, reaching positive values by midday. ET differed significantly between the S3 site (which showed a sharp peak by early afternoon) and the other two sites, which showed either a much smaller increase (S2) or a steady decrease after reaching a mid-morning peak (S1). ET was the largest one at the S3, followed by the S2, and was the lowest one at the S1.

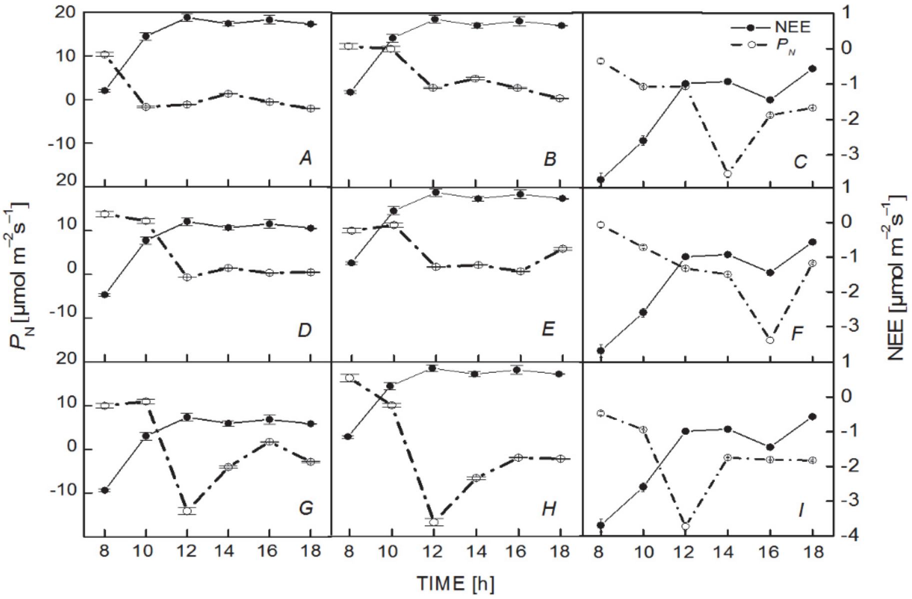

Fig. 2. Diurnal patterns of CO2 exchange (P_N – net photosynthetic rate; NEE – net ecosystem exchange) by plants of the three dominant species and by the whole ecosystem. (*A*) *Stipa grandis* in mowing site; (*B*) *Stipa grandis* in grazing-exclusion site; (*C*) *Stipa grandis* in grazing site; (*D*) *Leymus chinensis* in mowing site; (*E*) *Leymus chinensis* in grazing-exclusion site; (*F*) *Leymus chinensis* in grazing site; (*G*) *Artemisia frigida* in mowing site; (*H*) *Artemisia frigida* in grazing-exclusion site; (*I*) *Artemisia frigida* in grazing site. Values are means ± SD (n = 10).

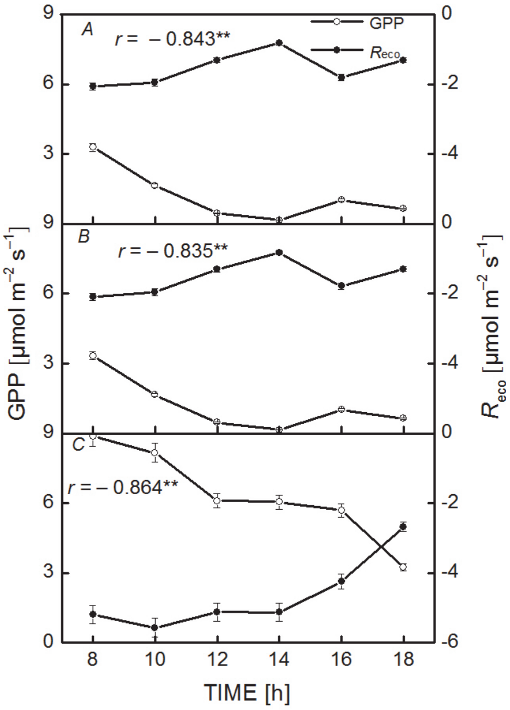

Fig. 3. Diurnal changes of ecosystem-level gross primary production (GPP) and ecosystem respiration (R_{eco}) for the three land uses: (*A*) mowing site; (*B*) grazing-exclusion site; (*C*) grazing site. Values are means ± SD (n = 10). ** , p<0.05.

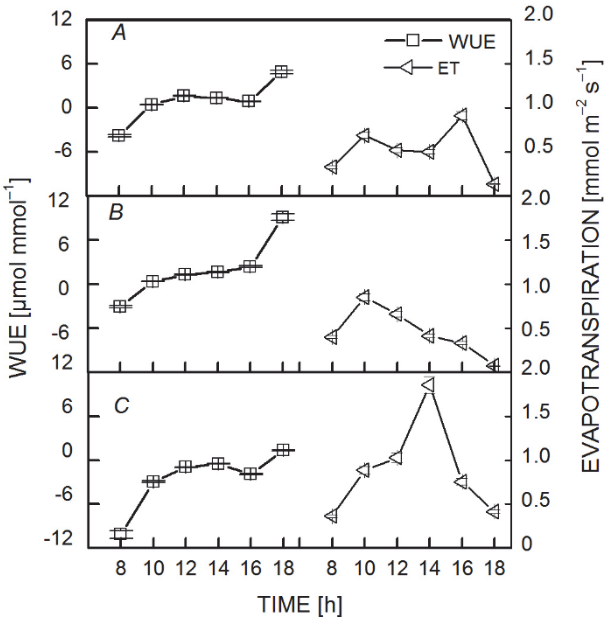

Fig. 4. Diurnal changes of ecosystem-scale water-use efficiency (WUE) and evapotranspiration (ET). (*A*) mowing site; (*B*) grazing-exclusion site; (*C*) grazing site. Values are means ± SD (n = 10).

WUE of the three dominant species showed similar trends at the S1 and S2 sites (Fig. 5), with an overall slow decrease to values slightly below 0 over the course of the day. However, at the S3, all three species showed a decrease to moderately large or large negative values by midday (*A. frigida*), early afternoon (*S. grandis*), and late afternoon (*L. chinensis*). The difference between the S3 and the other sites was significant, but the two other sites did not differ significantly.

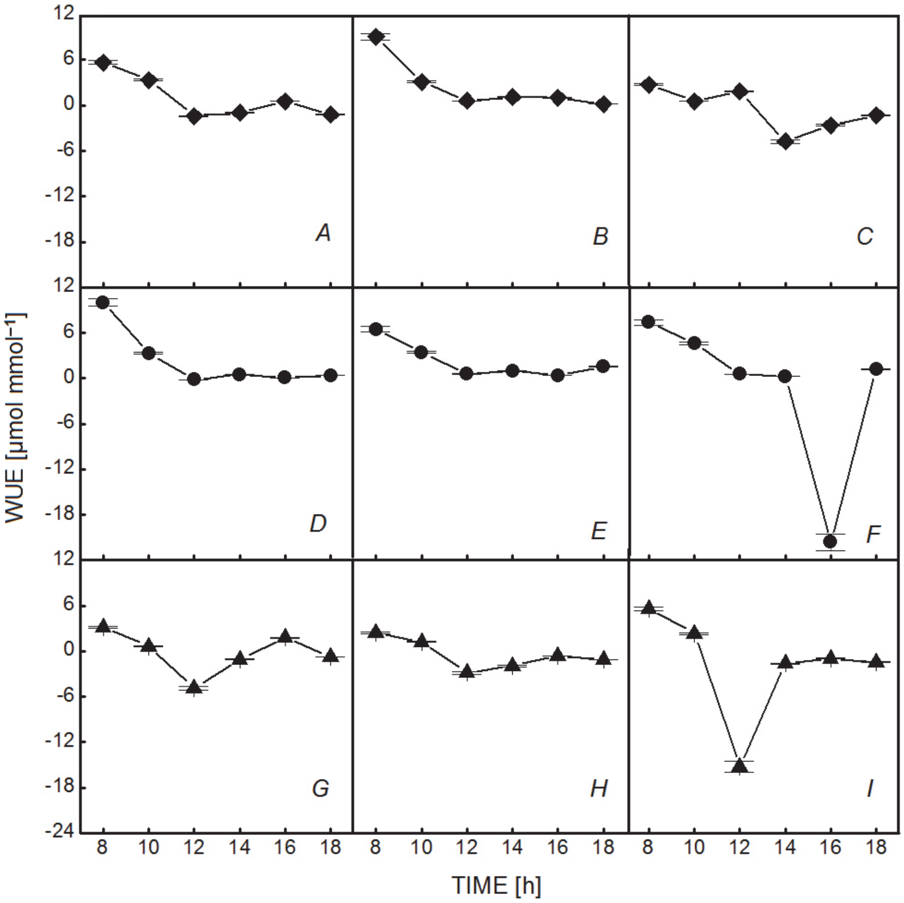

Fig. 5. Diurnal changes of water-use efficiency (WUE) per leaf in the three dominant plant species. (*A*) *Stipa grandis* in mowing site; (*B*) *Stipa grandis* in grazing-exclusion site; (*C*) *Stipa grandis* in grazing site; (*D*) *Leymus chinensis* in mowing site; (*E*) *Leymus chinensis* in grazing-exclusion site; (*F*) *Leymus chinensis* in grazing site; (*G*) *Artemisia frigida* in mowing site; (*H*) *Artemisia frigida* in grazing-exclusion site; (*I*) *Artemisia frigida* in grazing site. Values are means ± SD (n = 10).

Light-response curves: The photosynthetic characteristics of the three dominant species differed significantly between the three land uses (Table 1). For *S. grandis*, the light-saturation point (LSP), apparent quantum yield (AQY), and maximum photosynthetic rate (P_{Nmax}) were significantly higher at the S1 and S2 sites than that at the S3. In contrast, *L. chinensis* had significantly higher LSP, LCP, and P_{Nmax}, but significantly lower AQY at the S1 and S2 sites compared to that at S3. *A. frigida* had significantly higher LSP, LCP, AQY, and P_{Nmax} at the S2 and S3 sites than those at the S1 site.

Energy partitioning of the dominant species: The photosynthetic energy utilization and its distribution in the three dominant plant species differed between the three land uses (Fig. 6). At the S1, *S. grandis* and *L. chinensis* allocated more of the absorbed light to D throughout most of the day (Fig. 6*B,E*), whereas *A. frigida* allocated more of the light to excess energy (EE) throughout most of the day (Fig. 6*H*). At the S2, *S. grandis* and *L. chinensis* showed trends similar to that at the S1 site because more light was allocated to D (Fig. 6*A,D*). In contrast, *A. frigida* allocated more light than the other species to PET at the S3 (Fig. 6*I*).

Element concentrations and their ratios in the dominant species: The land use significantly affected the N content, P content, C:N ratio, C:P ratio, and N:P ratio (Fig. 7). The leaf C content did not differ significantly between the three land uses for any species. The N and P contents were generally higher at the S3 site than those at the S1 site. In contrast, the C:N and C:P ratios were generally the highest at the S1, except for a higher C:P ratio in *L. chinensis* at the S2. The interactions between the land-use type and species were significant for all parameters except the C content.

Table 1. Photosynthetic parameters and osmoregulation substances of the dominant species in the three land uses. For a given species, values in a column labeled with *different letters* differ significantly (*ANOVA* followed by LSD test, $p<0.05$). S1 – grazing-exclusion site; S2 – mowing site; S3 – grazing site. LSP – light-saturation point; LCP – light-compensation point; AQY – apparent quantum yield; P_{Nmax} – maximum photosynthetic rate.

Plant	Type	LSP [μmol m^{-2} s^{-1}]	LCP [μmol m^{-2} s^{-1}]	AQY [μmol μmol^{-1}]	P_{Nmax} [μmol m^{-2} s^{-1}]	Protein [mg g^{-1}]	Proline [mg g^{-1}]	Soluble sugar [mg g^{-1}]
S. grandis	S1	1,237.4 ± 35.7^a	92.0 ± 2.6^c	0.045 ± 0.013^a	21.6 ± 0.6^a	9.5 ± 0.3^b	0.4 ± 0.1^c	116.5 ± 3.4^a
	S2	1,251.2 ± 36.1^a	188.6 ± 5.4^a	0.025 ± 0.007^b	15.7 ± 0.4^b	10.9 ± 0.3^a	1.1 ± 0.3^a	81.1 ± 2.3^b
	S3	957.2 ± 27.6^b	161.0 ± 4.6^b	0.021 ± 0.006^c	10.9 ± 0.3^c	6.6 ± 0.2^c	0.6 ± 0.2^b	113.1 ± 3.3^a
A. frigida	S1	1,293.0 ± 37.3^b	60.0 ± 1.7^c	0.007 ± 0.002^c	20.8 ± 0.6^c	11.3 ± 0.3^b	3.7 ± 0.1^a	97.6 ± 2.8^a
	S2	1,932.0 ± 55.7^a	174.8 ± 5.0^b	0.043 ± 0.012^a	37.6 ± 1.1^a	12.4 ± 0.4^b	1.9 ± 0.5^c	89.1 ± 2.6^a
	S3	1,959.0 ± 56.5^a	202.4 ± 5.8^a	0.033 ± 0.009^b	27.0 ± 0.7^b	13.9 ± 0.4^a	2.9 ± 0.9^b	96.2 ± 2.8^a
L. chinensis	S1	952.2 ± 27.4^b	101.2 ± 2.9^a	0.027 ± 0.001^b	10.3 ± 0.2^b	12.9 ± 0.4^a	0.8 ± 0.1^b	80.2 ± 2.3^a
	S2	1,048.8 ± 30.2^b	92.1 ± 2.6^c	0.025 ± 0.001^b	13.4 ± 0.3^a	10.9 ± 0.3^b	1.2 ± 0.1^a	0.6 ± 2.3^a
	S3	700.2 ± 15.7^c	64.4 ± 3.2^b	0.045 ± 0.013^a	6.1 ± 0.1^c	9.6 ± 0.3^c	0.8 ± 0.1^b	73.2 ± 2.1^a

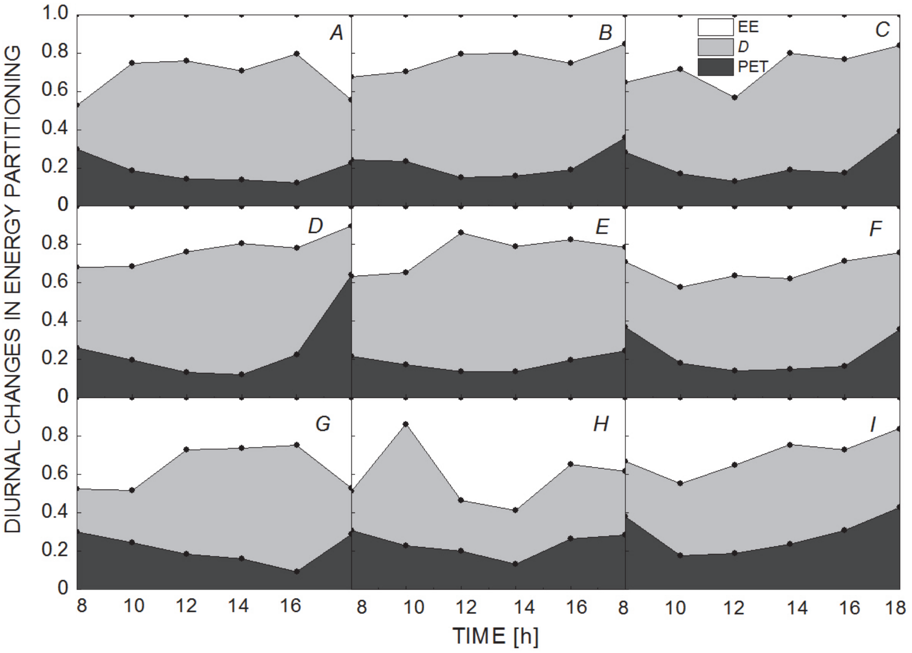

Fig. 6. Diurnal changes in energy partitioning for the three dominant plant species. (*A*) *Stipa grandis* in mowing site; (*B*) *Stipa grandis* in grazing-exclusion site; (*C*) *Stipa grandis* in grazing site; (*D*) *Leymus chinensis* in mowing site; (*E*) *Leymus chinensis* in grazing-exclusion site; (*F*) *Leymus chinensis* in grazing site; (*G*) *Artemisia frigida* in mowing site; (*H*) *Artemisia frigida* in grazing-exclusion site; (*I*) *Artemisia frigida* in grazing site. PET – photosynthetic electron transport; *D* – thermal dissipation; EE – excess energy.

Leaf osmoregulation substances: The contents of osmoregulation substances in the three dominant species differed significantly between the three land uses (Table 1). The soluble sugar content did not differ significantly between the land uses for any species, except for a significantly lower content at the S2 site for *S. grandis*. For *A. frigida*, the protein content was significantly higher at the S3 site than at the other sites, whereas the proline content was significantly higher at the S1 site. For *L. chinensis*, the protein content was significantly higher at the S1 site, but the proline content was significantly higher at the S2. For *S. grandis*, the protein and proline contents were significantly higher at the S2 site.

Discussion

Aboveground biomass: The dominant species in natural communities play an important role in ecosystem functioning, and the ecosystem productivity is typically maintained by a few dominant species (Smith and Knapp 2003). In the present study, the aboveground biomass of the dominant species accounted for more than 66% of the total community biomass at all three land uses, which confirms the results of previous studies, where the dominant species provided most of the community's production (Bai *et al.* 2004, Mariotte *et al.* 2012). Both *L. chinensis* and *S. grandis* have relatively high palatability to livestock, whereas *A. frigida* has relatively low palatability; as a result, the latter species tends to increase in abundance at grazed sites, as it did in the present study. In the present study, the palatable *L. chinensis* accounted for the highest proportion of biomass at the S1 site. With increasing grazing intensity, *L. chinensis* is preferentially eaten by livestock (Liu *et al.* 2016), so it therefore accounted for the lowest proportion of biomass among the dominant species at the S3 site. At the S2 and S1 sites, *A. frigida* gradually became the dominant species, suggesting that its dominance at a site may be a sign of grassland degeneration (Wang and Li 1999). In a previous study of mowing, clipping the vegetation at 6 to 10 cm above the soil surface had lesser impact on the growth of species with a low canopy, such as *L. chinensis* and *A. frigida*, but had a greater impact on *S. grandis*, which is taller than the other species (Niu *et al.* 2010). Therefore, *L. chinensis* and *A. frigida* accounted for a higher proportion of the biomass than that of *S. grandis* at the S2 site. Overall, the different management regimes resulted in a significant difference in the species dominance based on aboveground biomass in our study plots.

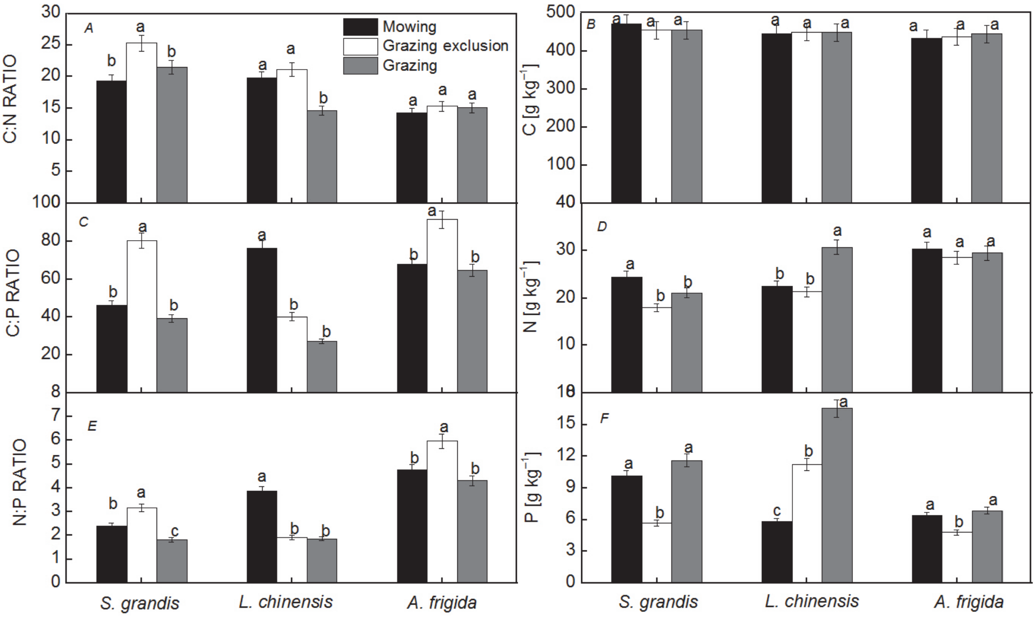

Fig. 7. The ecological stoichiometry results for key nutrients (C, N, and P) for the three dominant plant species. For a given parameter, bars labeled with different letters differ significantly ($p<0.05$). Values are means ± SD ($n = 10$). *S. grandis* – *Stipa grandis*, *L. chinensis* – *Leymus chinensis*, *A. frigida* – *Artemisia frigida.*

CO_2 exchange by the ecosystem and the dominant species: Land management and land use change are thought to offer opportunities to slow the rate of increase of atmospheric CO_2 and mitigate the potential damaging effects of climate change (Jones *et al.* 2006). Different land-use types must have different effects on the ecosystem carbon budget. At the S1 and S2 sites, the diurnal NEE was positive, suggesting that the sites both exhibited net release of C. In contrast, at the S3 site, the diurnal NEE was negative, which indicated C sequestration. At the S3 site, ecosystem C uptake (GPP) was greater than C release (R_{eco}), creating a net C sink. Plants in plots with moderate grazing should exhibit compensatory growth, which is a positive response in terms of C sequestration (Belsky 1986, Gong *et al.* 2015). At the same time, grazing can increase soil microorganism biomass and N deposition, thereby increasing soil organic matter and total soil N (Li *et al.* 2008). Therefore, the S3 site became the C sink in our study. However, with increasing grazing intensity, the root system tends to become concentrated in the surface soil, where C released from the roots is easily oxidized by soil microbes, so grazing sites are likely to release more CO_2 with a high grazing intensity (Neff *et al.* 2005). In the present study, the S1 and S2 sites both had high R_{eco}, making both sites the net C source. NEE stopped increasing by noon in all land uses, probably due to photoinhibition and stomatal regulation based on our observation of decreased P_N at this time (Fu *et al.* 2006). Near midday, plants close their stomata as a result of water stress, and must therefore undergo adaptations to avoid damage of the photosynthetic apparatus caused by high temperatures and excess light energy (Demmig-Adams and Adams 1992, Krause and Winter 1996). In the present study, P_N of *L. chinensis* and *S. grandis* was the highest one at the S1 site, followed by the S2, and was the lowest at the S3 site. The trend was similar for NEE, which suggests that *L. chinensis* and *S. grandis* contributed most to the ecosystem CO_2 exchange. Though *A. frigida* accounted for a higher proportion of aboveground biomass than the other species at the S2 site, its P_N was lower. Therefore, *A. frigida* contributed less to the ecosystem's photosynthetic carbon fixation at the S1 and S2 sites.

The ecosystem WUE did not differ significantly between the S1 and S2 sites. This may be because the duration of grazing exclusion was short (only eight years) and the cutting frequency was low (*i.e.*, annual), thereby decreasing the environmental difference between the two ecosystems. Mowing causes the removal of tall, tussock-forming species and can reduce litter accumulation and improve canopy exposure to solar radiation, which improves the growth and recruitment of small plants (Collins *et al.* 1998, Wahlman and Milberg 2002, Yang *et al.* 2011). Livestock exclusion has been shown to be a good method to restore vegetation and mitigate the soil loss caused by wind erosion in grasslands (Su and Zhao 2003, Su *et al.* 2004). The relatively high vegetation at the S1 and S2 sites would reduce evaporation of soil water (Wilsey *et al.* 2002). In contrast, at higher grazing intensities, the vegetation may be trampled by the livestock and this, combined with high consumption of aboveground biomass, can cause the vegetation cover to decrease significantly (Zhao *et al.* 2009). This may lead to higher evaporation of soil water at the S3 site. In arid and semiarid grasslands, grazing generally increases the heterogeneity of soil water and nutrient conditions in terms of both temporal patterns and differences between spatial scales (Cross and Schlesinger 1999, Zhao *et al.* 2009). Because our study was conducted in an unusually dry year, this higher evaporation might increase water stress for plants at the S3 site. Therefore, WUE of the plants in our study fluctuated during the day and ecosystem WUE became the lowest at the S3 site. Therefore, to some extent, the dominant species determined the functioning of the grassland ecosystem with respect to water use, which was consistent with the results of previous studies (Bai *et al.* 2004, Liu *et al.* 2012).

Energy partitioning by the three dominant species: Photosynthesis consumes large quantities of energy, but the efficiency of energy capture depends on how the energy is allocated among the components of a plant's photosynthetic system. At the S1 and S2 sites, *L. chinensis* and *S. grandis* lost most of the energy absorbed by antenna pigments to thermal dissipation, which can protect the photosynthetic apparatus (Demmig-Adams *et al.* 1996). At the same time, *L. chinensis* and *S. grandis* both had a higher LSP and P_{Nmax} at the S1 site than that at the S3 site. This suggests that they had the ability to utilize light more efficiently to support photosynthesis and promote growth (Li and Chen 2009). In contrast, *A. frigida* had the high P_{Nmax} at all three sites; it also had high EE, but absorbed more light as excess excitation energy at the S1 and S2 sites, which is likely to damage the plant's photosynthetic apparatus. This may explain why P_N of *L. chinensis* and *S. grandis* was higher than that of *A. frigida* at the S1 and S2 sites. In addition, *A. frigida* had higher PET than the other species at the S3 site, which indicates that *A. frigida* was able to capture more of the available energy to support photosynthesis and promote its growth (Demmig-Adams *et al.* 1996). This may be why *A. frigida*, which increases in relative abundance at grazed sites, can become a quantitative indicator of grazing pressure (Li 1994). The three dominant species all had negative P_N values late in the day indicating that respiration exceeded photosynthesis. In previous research in northern China, high temperatures and irradiance led to the serious depression of P_N (Jiang and Zhu 2001). In this study, the high excess energy indicated that the photosynthetic system was likely to be damaged by the high light levels which decreased the photosynthetic capacity and led to respiration exceeding photosynthesis.

Concentrations of nutrient elements and osmoregulation substances: In plants, nutrient elements fulfil structural, functional, and storage roles. C is predominantly a structural component, whereas N and P determine plant functions. In general, structural matter is stable, but functional matter changes in response to environmental variation (Sterner and Elser 2002). This may explain why the C contents of the three dominant species in our study did not differ significantly between the three land uses. Sardans and Peñuelas (2008) noted that when the N:P ratio was lower than 14, the ecosystem was limited by N availability, whereas when N:P was greater than 16, the ecosystem was limited by P. In the present study, the N:P ratios were all lower than 7, suggesting that plant growth was strongly limited by N. The high leaf N content in *A. frigida* usually results in an increased number of thylakoids and decreased thylakoid protein content in chloroplasts (Evans 1989, Xu 2013). This would increase the ability of this species to utilize strong light at the S3 site, where light intensity would increase due to removal of the other species by grazing. On the other hand, the high proline and protein contents of *A. frigida* compared to the other species would protect this species from drought or grazing damage. *L. chinensis* and *S. grandis* had significantly higher protein contents at the S1 and S2 sites than that at the S3 site. This suggests that *A. frigida* can be an important species for resisting grassland degradation at the S3 site. Furthermore, under conditions of limited nutrients, soil N may be mostly absorbed by plants, with limited microbial growth in the soil, leading to decreased respiration by soil microbes (Wang and Bakken 1997). This may explain why R_{eco} was low at the S3 site and why this site became a net C sink. Many studies have demonstrated that Chinese grasslands have a high potential for increasing C storage through improved management (He *et al.* 2012, Ma *et al.* 2016). Therefore, based on the results of the present study, light grazing appears to be a suitable land use under the region's semiarid conditions.

Conclusion: The dominant species in the ecosystem accounted for the highest proportion of biomass and therefore exerted a powerful influence on the ecosystem's CO_2 exchange. The grazing-exclusion and mowing sites were both net sources of CO_2, whereas the grazing site was a net C sink. Therefore, in our study area, light grazing can be a beneficial land use in terms of C sequestration. At the grazing-exclusion and mowing sites, *L. chinensis* and *S. grandis* had high photosynthetic capacity, and their high protein contents promoted plant growth. At all three sites, *A. frigida* had a high N content, which supported a high maximum carboxylation rate and capture more energy to support photosynthesis. Its high proline and protein contents can also help to protect the plants from damage. This may explain why *A. frigida* became an important species and may be suitable for resisting grassland degradation, while also serving as an indicator species for grazing pressure (*i.e.*, because it is not a preferred forage species, it increases in abundance when more palatable species are consumed by livestock). However, in the present study, the weather was considerably drier than normal, so the responses of the dominant species and ecosystem to the three land uses should be studied even under more average precipitation amount.

References

Agren G.I., Weih M.: Plant stoichiometry at different scales: element concentration patterns reflect environment more than genotype. – New Phytol. **194**: 944-952, 2012.

Akiyama T., Kawamura K.: Grassland degradation in China: methods of monitoring, management and restoration. – Grassl. Sci. **53**: 1-17, 2007.

Bai Y.F., Han X.G., Wu J.G. *et al.*: Ecosystem stability and compensatory effects in the Inner Mongolia grassland. – Nature **431**: 181-184, 2004.

Bates L.S., Waldren R.P., Teare I.D.: Rapid determination of free proline for water-stress studies. – Plant Soil **39**: 205-207, 1973.

Bellasio C., Beerling D.J., Griffiths H.: An Excel tool for deriving key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence: theory and practice. – Plant Cell Environ. **39**: 1180-1197, 2016.

Belsky A.J.: Does herbivory benefit plants? A review of the evidence. – Am. Nat. **127**: 870-892, 1986.

Bremner J.M.: Determination of nitrogen in soil by the Kjeldahl method. – J. Agr. Sci. **55**: 11-33, 1960.

Buysse J., Merckx R.: An improved colorimetric method to quantify sugar content of plant tissue. – J. Exp. Bot. **44**: 1627-1629, 1993.

Chen J., Shi W.Y., Cao J.J.: Effects of grazing on ecosystem CO_2 exchange in a meadow grassland on the Tibetan Plateau during the growing season. – Environ. Manage. **55**: 347-359, 2015.

Chimner R.A., Welker J.M.: Influence of grazing and precipitation on ecosystem carbon cycling in a mixed-grass prairie. – Pastoralism **1**: 20, 2011.

Ciais P., Gervois S., Vuichard N. *et al.*: Effects of land use change and management on the European cropland carbon balance. – Glob. Change Biol. **17**: 320-338, 2011.

Collins S.L., Knapp A.K., Briggs J.M. *et al.*: Modulation of diversity by grazing and mowing in native tallgrass prairie. – Science **280**: 745-747, 1998.

Cross A.F., Schlesinger W.H.: Plant regulation of soil nutrient distribution in the northern Chihuahuan Desert. – Plant Ecol. **145**: 11-25, 1999.

Demmig-Adams B., Adams W.W.: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Phys. **43**: 599-626, 1992.

Demmig-Adams B., Adams W.W., Barker D.H. *et al.*: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Evans J.R.: Photosynthesis and nitrogen relationships in leaves of C3 plants. – Oecologia **78**: 9-19, 1989.

Fu Y.L., Yu G.R., Sun X.M. *et al.*: Depression of net ecosystem CO_2 exchange in semi-arid *Leymus chinensis* steppe and alpine shrub. – Agr. Forest Meteorol. **137**: 234-244, 2006.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Gong X.F., Fanselow N., Dittert K. *et al.*: Response of primary production and biomass allocation to nitrogen and water supplementation along a grazing intensity gradient in semiarid grassland. – Eur. J. Agron. **63**: 27-35, 2015.

Han Y., Zhang Z., Wang C.H. *et al.*: Effects of mowing and nitrogen addition on soil respiration in three patches in an old field grassland in Inner Mongolia. – J. Plant Ecol. **5**: 219-228, 2012.

He N., Zhang Y.H., Dai J.Z. *et al.*: Land-use impact on soil carbon and nitrogen sequestration in typical steppe ecosystems, Inner Mongolia. – J. Geogr. Sci. **22**: 859-873, 2012.

Houghton R.A., Hackler J.L., Lawrence K.T.: The U.S. carbon budget: contributions from land-use change. – Science **285**: 574-578, 1999.

Ingram L.J., Stahl P.D., Schuman G.E., *et al.*: Grazing impacts on soil carbon and microbial communities in a mixed-grass ecosystem. – Soil Sci. Soc. Am. J. **72**: 939-948, 2008.

Ives A.R., Carpenter S.R.: Stability and diversity of ecosystems. – Science **317**: 58-62, 2007.

Jia W.X., Liu M., Yang Y.H. *et al.*: Estimation and uncertainty analyses of grassland biomass in Northern China: comparison of multiple remote sensing data sources and modeling approaches. – Ecol. Indic. **60**: 1031-1040, 2016.

Jiang G.M., Zhu G.J.: Different patterns of gas exchange and photochemical efficiency in three desert shrub species under two natural temperatures and irradiances in Mu Us sandy area of China. – Photosynthetica **39**: 257-262, 2001.

Jones S.K., Rees R.M., Kosmas D. *et al.*: Carbon sequestration in a temperate grassland; management and climate controls. – Soil Use Manage. **22**: 132-142, 2006.

Krause G.H., Winter K.: Photoinhibition of photosynthesis in plants growing in natural tropical forest gaps. A chlorophyll fluorescence study. – Plant Biol. **109**: 456-462, 1996.

Larcher W.: Carbon utilization and dry matter production. – In: Larcher W. (ed.): Physiological Plant Ecology 4th ed. Pp. 69-173. Springer, New York 2003.

LeBauer D.S., Treseder K.K.: Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. – Ecology **89**: 371-379, 2008.

Li C.L., Hao X.Y., Zhao M.L. *et al.*: Influence of historic sheep grazing on vegetation and soil properties of a Desert Steppe in Inner Mongolia. – Agr. Ecosyst. Environ. **128**: 109-116, 2008.

Li X.W., Chen S.L.: Diurnal changes in gas exchange and chlorophyll fluorescence parameters of *Fritillaria cirrhosa* and *F. delavayi* under field conditions. – Photosynthetica **47**: 191-198, 2009.

Li Y.H.: [Research on the grazing degradation model of the main steppe rangelands in Inner Mongolia and some considerations for the establishment of a computerized rangeland monitoring system.] – Acta Phytoecol. Sin. **18**: 68-79, 1994. [In Chinese]

Liu C., Liu Y., Guo K. *et al.*: Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. – Environ. Exp. Bot. **71**: 174-183, 2011.

Liu C., Song X.X., Wang L. *et al.*: Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore assemblage and pre-grazing plant diversity. – J. Appl. Ecol. **53**: 242-250, 2016.

Liu Y., Pan Q, Zheng S.: Intra-seasonal precipitation amount and pattern differentially affect primary production of two dominant species of Inner Mongolia grassland. – Acta Oecol. **44**: 2-10, 2012.

Ma A.N., He N.P., Yu G.R. *et al.*: Carbon storage in Chinese grassland ecosystems: influence of different integrative methods. – Sci. Rep. **6**: 21378, 2016.

Mariotte P., Buttler A., Johnson D.: Exclusion of root competition increases competitive abilities of subordinate plant species through root-shoot interactions. – J. Veg. Sci. **23**: 1148-1158, 2012.

Neff J.C., Reynolds R.L., Belnap J. *et al.*: Multi-decadal impacts of grazing on soil physical and biogeochemical properties in southeast Utah. – Ecol. Appl. **15**: 87-95, 2005.

Nelson D.W., Sommers L.E.: Total carbon, organic carbon and organic matter. – In: Page A.L., Miller R.H., Keeney D.R. (ed.): Methods of Soil Analysis. Pp. 961-1010. American Society of Agronomy, Madison 1982.

Niu S.L., Wu M.Y., Han Y. *et al.*: Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. – New Phytol. 177: 209-219, 2007.

Niu S.L., Wu M.Y., Han Y. *et al.*: Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. – Glob. Change Biol. **16**: 144-155, 2010.

Poskuta J.W., McAdam J.H., Waciawczyk-Lach E. *et al.*: Photosynthetic and respiratory characteristics of *Cortaderia pilosa* (D'Urv.) Hack., the dominant component of Falkland Islands. – J. Plant Physiol. **153**: 517-519, 1998.

Robson T.M., Lavorel S., Clement J.C. *et al.*: Neglect of mowing and manuring leads to slower nitrogen cycling in subalpine grasslands. – Soil Biol. Biochem. **39**: 930-941, 2007.

Sardans J., Peñuelas J.: Drought changes nutrient sources, content and stoichiometry in the bryophyte *Hypnum cupressiforme* Hedw. growing in a Mediterranean forest. – J. Bryol. **30**: 59-65, 2008.

Sedmak J.J., Grossberg S.E.: A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. – Anal. Biochem. **79**: 544-552, 1977.

Smith M.D., Knapp A.K.: Dominant species maintain ecosystem function with non-random species loss. – Ecol. Lett. **6**: 509-517, 2003.

Soon Y.K., Kalra Y.P.: A comparison of plant tissue digestion methods for nitrogen and phosphorus analyses. – Can. J. Soil Sci. **75**: 243-245, 1995.

Sterner R.W., Elser J.J.: Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere. Pp. 1-43. Princeton University Press, Princeton 2002.

Su Y.Z., Li Y.L., Cui J.Y. *et al.*: Influences of continuous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, northern China. – Catena **59**: 267-278, 2004.

Su Y.Z., Zhao H.L.: Soil properties and plant species in an age sequence of *Caragana microphylla* plantations in the Horqin Sandy Land, north China. – Ecol. Eng. **20**: 223-235, 2003.

Sun W., Resco V., Williams D.G.: Nocturnal and seasonal patterns of carbon isotope composition of leaf dark respired carbon dioxide differ among dominant species in semiarid savanna. – Oecologia **164**: 297-310, 2010.

Wahlman H., Milberg P.: Management of semi-natural grassland vegetation: evaluation of a long-term experiment in southern Sweden. – Ann. Bot. Fenn. **39**: 159-166, 2002.

Wang J.G., Bakken L.R.: Competition for nitrogen during mineralization of plant residues in soil: microbial response to C and N availability. – Soil Biol. Biochem. **29**: 163-170, 1997.

Wang S.P., Li Y.H.: [Degradation mechanism of typical grassland in Inner Mongolia.] – Chin. J. Appl. Ecol. **10**: 437-447, 1999. [In Chinese]

Wilsey B.J., Parent G., Roulet N.T. *et al.*: Tropical pasture carbon cycling: relationships between C source/sink strength, above-ground biomass and grazing. – Ecol. Lett. **5**: 367-376, 2002.

Wohlfahrt G., Bahn M., Newesely C. *et al.*: Canopy structure versus physiology effects on net photosynthesis of mountain grasslands differing in land use. – Ecol. Model. **170**: 407-426, 2003.

Xia J., Wan S.: Global response patterns of terrestrial plant species to nitrogen enrichment. – New Phytol. **179**: 428-439, 2008.

Xu D.Q.: [The Science of Photosynthesis.] Pp. 256-258. Science Press, Beijing 2013. [In Chinese]

Xu W., Wan S.: Water- and plant-mediated responses of soil respiration to topography, fire, and nitrogen fertilization in a semiarid grassland in northern China. – Soil Biol. Biochem. **40**: 679-687, 2008.

Yang H.J., Li Y., Wu M.Y. *et al.*: Plant community responses to nitrogen addition and increased precipitation: the importance of water availability and species traits. – Glob. Change Biol. **17**: 2936-2944, 2011.

Yu Q., Chen Q.S., Elser J.J. *et al.*: Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability. – Ecol. Lett. **13**: 1390-1399, 2010.

Zeller V., Bahn M., Aichner M., Tappeiner U.: Impact of land-use change on nitrogen mineralization in the Southern Alps. – Biol. Fert. Soils **31**: 441-448, 2000.

Zhang Z.H., Duan J.H., Wang S.P. *et al.*: Effects of land use and management on ecosystem respiration in alpine meadow on the Tibetan plateau. – Soil Till. Res. **124**: 161-169, 2012.

Zhao W., Chen S.P., Han X.G., Lin G.H.: Effects of long-term grazing on the morphological and functional traits of *Leymus chinensis* in the semiarid grassland of Inner Mongolia, China. – Ecol. Res. **24**: 99-108, 2009.

Zhou X.H., Wan S.Q., Luo Y.Q.: Source components and interannual variability of soil CO_2 efflux under experimental warming and clipping in a grassland ecosystem. – Glob. Change Biol. 13: 761-775, 2007.