Variation of livestock grazing intensity modified the magnitude of carbon sequestration and flow within the plant-soil system of a meadow steppe ecosystem

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Author contributions

XP Xin, RR Yan and DY Jin designed the study; DY Jin and HB Xu performed the experiments; DY Jin analyzed the data, and wrote the manuscript; JQ Chen, JG Qi, LH Li and YC Yan provided important and valuable suggestions for designing the study and manuscript writing.
**Abstract**

**Aims:** Livestock grazing, one of the principal utilization patterns, usually exerts a substantial effect on the carbon allocations between the above- and belowground components of a grassland ecosystem. The major aims of this study were to evaluate the proportions of $^{13}$C allocation to various C pools of the plant-soil system of a meadow steppe ecosystem in response to livestock grazing intensity.

**Methods:** *In situ* stable $^{13}$C isotope pulse labeling was conducted in the plots of a long-term grazing experiment with 4 levels of grazing intensities. Plant and soil materials were sampled at on eight occasions (0, 3, 10, 18, 31, 56 and 100 days after labeling) to analyze the decline in $^{13}$C over time, and their composition signature of $^{13}$C were analyzed by the isotope ratio mass spectrometer technique.

**Results:** We found a significantly larger decline in assimilated $^{13}$C for the heavily grazed swards compared to other grazing intensities, with the relocation rate of $^{13}$C from shoots to belowground C pool being the highest. In contrast, light grazing significantly allocated $^{13}$C assimilates in the belowground pool, especially in the live root and topsoil C-pools.

**Conclusions:** The effects of livestock grazing on the carbon transfers and stocks within the plant-soil system of the meadow steppe were highly intensity dependent, and different carbon pools differed in response to gradient changes in grazing intensity.

**Keywords:** grazing intensity, $^{13}$C pulse labeling, carbon translocation, temperate grassland
Introduction

Grasslands play an important role in the carbon cycle of the global ecosphere, and the impacts of human activities on the carbon cycling of natural grassland ecosystems have been the frontier and core topics in ecological studies of terrestrial ecosystems worldwide (Cao and Woodward 1998; Piao et al. 2012). Since more than 80% of the grassland carbon is stored in the soil (Fan et al. 2008; Ni 2002; Post et al. 1982; Yang et al. 2012), any changes in the belowground carbon stock may affect the overall carbon cycling compartments and the associated processes of the grassland ecosystems, leading to relevant changes in the atmospheric CO\(_2\) concentration and soil-derived ecosystem services to varying degrees (Davidson and Janssens 2006; Jin et al. 2018; Trumbore and Czimczik 2008).

Grazing is the most widely adopted land use of grassland ecosystems, being among the most critical factors affecting their phytomass production and matter circulation (Zhou et al. 2019). In terms of scale, the degradation of approximately 35% of the world’s total area of grasslands can be attributed by overgrazing, the impacts of which far exceed any other land use type (WRI 2018). To date, the impacts of grazing on the carbon cycling of grassland ecosystems have mostly focused on the aspects of net primary production, root biomass, soil stock, and soil respiration, whereas the effects of grazing on the carbon flow transfers within the plant-soil system, especially among the belowground components or sub-pools, have been much less adequately elaborated upon (Zhou et al. 2017). Currently, most of the studies have been conducted by comparing only grazing vs. no-grazing cases, irrespective of the historical context, grazing intensity and the types of grazing livestock (Bagchi and Ritchie 2010; Bai et al. 2012; Hafner et al. 2012; Liu et al. 2015). This explains why contradictory conclusions have been reached by different authors in analogous studies (He et al. 2011; Ojima et al. 1993; Zou et al. 2007).

The variations in the sizes of above- and below-ground C pools actually reflect the changes in the C influx
transported from shoots to roots and to the soil, which are generally difficult to accurately assess or quantify by regular methods. In stark contrast, the stable $^{13}$C isotope labeling technique furnishes an ideal available tool to trace the destinations of the C fluxes among the various carbon components of terrestrial ecosystems (Brüggemann et al. 2011; Kuzyakov and Domanski 2000; Kuzyakov and Schneckenberger 2004). A retrieval of relevant literature shows that only a few studies have employed this approach to examine the effects of grazing on the transfers of carbon in grassland ecosystems, and additionally, they mostly only dealt with certain pathways but not the associated pools (Hafner et al. 2012; Han et al. 2008; Zhao et al. 2015; Klumpp et al. 2009).

In this study, a stable $^{13}$C isotope pulse labeling experiment was conducted in a meadow steppe ecosystem of Inner Mongolia, which included 4 grazing intensities. Our objectives were to examine the effects of gradient grazing on 1) the dynamics of fixed carbon in shoots; 2) allocations of recovered $^{13}$C from the canopy to the belowground pools; and 3) changes in the carbon stock of all the components with increasing grazing intensity on an annual basis.

Materials and methods

Study site

The study site is located in the central part of the Hulun Buir meadow steppe region (49°19'N, 119°57'E) occupying the northeastern fraction of Inner Mongolia. The elevations generally vary between 666 and 680 m, characterized by a gentle rolling relief. The climate is continental temperate and semiarid, with an annual frost-free period of approximately 110 days. The mean annual air temperature of the region varies between -5 and -2°C, with the mean annual maximum of 36.2°C occurring in July and the minimum of -48.5°C in January. The mean annual
precipitation mostly ranges from 350 to 400 mm, of which approximately 80% falls between July and September. The most commonly associated soil type is chernozem, characterized by a loam or sandy-loam texture, with pH values generally between 5.82 and 6.39. The *Leymus chinensis*, *Stipa baicalensis*, and *Filifolium sibiricum* meadow steppe communities are most characteristic of the vegetation, with *Scutellaria baicalensis*, *Carex pediformis*, *Galium verum* and *Bupleurum scorzonerifolium* as important accompanying species. Free-ranging livestock is a major land use pattern. However, due to the much shorter growing season compared with other steppe regions of the country, grazing can occur only from June to October in this grassland region.

**Grazing treatment design**

This study was conducted in a permanent grazing experimental plot set up by the Hulun Buir Grassland Ecosystem Observation and Research Station with the Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, which is located in the Xiertala Pastoral Farm. This grazing platform was established in 2009 and contains a total of 18 subplots (3 blocks with 6 plots each 300 × 167 m) in total 5 ha in size (Fig. 1). The three replicate blocks were performed under a specific grazing intensity, resulting in 3 block sets of 6 treatments, with stocking levels of 0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 Animal Units (AU) ha⁻¹, respectively (1 AU = 500 kg body weight of adult cattle), which correspond to stocking rates of 0, 2, 3, 4, 6 or 8 young beef (250~300 kg) per plot. The blocks were arranged in light of the randomized block design procedure (Fig. 1). Continuous grazing usually takes place for 120 days between June and October on an annual basis that started in 2009. The study site had previously been under long-term free-range cattle for a long period. Baseline measurements were made prior to the installation of the field treatment facilities, which showed
that the amount of aboveground biomass (AGB) did not differ before the grazing treatments installation, and AGB ranged from 800 to 850 kg ha\(^{-1}\), with a canopy coverage of 36% to 42% and a canopy height of 7 cm to 9 cm.

Chernozem soil was prominent, with a total soil nitrogen (TN) content of 3.75-4.08 g kg\(^{-1}\) and an organic carbon concentration of the topsoil of 36.4-39.5 g kg\(^{-1}\) (Yan et al. 2015).

\( ^{13}\text{CO}_2 \) pulse labeling

To obtain a highly efficient and representative experimental effect, 4 of 6 grazing intensity treatments (0.00, 0.23, 0.46 and 0.92 AU ha\(^{-1}\)), in total 4×3 plots were selected to carry out the \(^{13}\text{CO}_2 \) pulse labeling. The \(^{13}\text{CO}_2 \) labeling was continuously conducted on July 4~6, 2015, i.e., days that were sunny and without wind. The \(^{13}\text{CO}_2 \) pulse was applied simultaneously into chambers that were separately inserted into 3 spots of each plot. The chamber was 1 m × 1 m in size, 60 cm high, and fabricated with pieces of polymethyl methacrylate that allowed more than 95% transmittance of photosynthetically active radiation. To avoid gas losses, the bottom edges of a chamber were tightly buried in the soil and sealed with wet soil. The \(^{13}\text{CO}_2 \) pulse materials were prepared by injecting 60 ml of 2 M hydrochloric acid (HCl) into a solution of distilled water (100 ml) containing 6 g barium carbonate (Ba\(^{13}\text{CO}_3\)) enriched with \(^{13}\text{C} \) to 99 atoms%. Glass beakers, each with a 100 ml volume and containing Ba\(^{13}\text{CO}_3\) solution, were fixed on a small platform inside the chamber. Before \(^{13}\text{CO}_2 \) labeling was carried out, the chamber was first closed, and then the vegetation could equilibrate for half an hour to reduce the concentration of CO\(_2\) remained in the chamber and to improve the \(^{13}\text{CO}_2 \) assimilation efficiency thereafter. After these steps were completed, hydrochloric acid (HCl) was carefully added four times (at 8:00, 9:00, 10:00 and 11:00 am, each with 15 ml HCl) from the outside into the Ba\(^{13}\text{CO}_3\) solution by a syringe to ensure complete evolution of \(^{13}\text{CO}_2 \) in the chamber’s atmosphere.
To guarantee a uniform distribution of $^{13}\text{CO}_2$, four 5-volt fans were installed in each upper corner of the chamber.

Consequently, the plants assimilated the labeled $^{13}\text{CO}_2$ for 4 hours during the 3 days of treatments, generally from 8:00 am to 12:00 am, after which the chamber was removed. In addition, a cooling system was run during the time when $^{13}\text{CO}_2$ labeling was conducted to prevent the plants from being affected by high temperatures that may inhibit photosynthesis inside the chamber.

Tissue sampling

After the pulse labeling had been done, plant and soil samples were collected on eight occasions (0, 3, 10, 18, 31, 56 and 100 days after the treatment) during the entire study period. To obtain the combined mean residence time (MRT) of carbon in the aboveground vegetal tissues, living shoots were additionally sampled two more times (316 and 394 days). At each sampling time, the plant-soil system was separated into the following components: shoots (live and standing dead), roots (living and dead, separated by different specific gravity in water), and rhizospheric soil (fine soil, bulk soil sieved at 2mm). One sample point was collected in each chamber at every sampling time, and there were 3 chambers in each plot. Above-ground tissues were cut from a small round area with a diameter of 10 cm, and the litter in the spot was collected after above-ground tissues were removed, and then rhizospheric soil and root samples were immediately collected with the 10cm diameter soil core from three soil layers (0-10, 10-20 and 20-30 cm) that were sampled separately. Carbonate in the rhizospheric soil was removed by adding 10 M HCl for 3 days, then neutralized by adding deionized water and dried (Harris et al. 2001). All plant materials were dried, weighed and ball milled. Three replicate plant samples (unlabeled spots) were taken from each block and were used as a natural reference. The C isotope composition ($\delta^{13}\text{C}$, ‰) signature and the carbon contents of shoot, root, soil
and control samples were measured by an isotope ratio mass spectrometer (IsoPrime 100, Isoprime, UK) coupled with an elemental analyzer (Elementar, Vario Pyro Cube, Germany).

**Calculations and statistical analyses**

The carbon isotope abundance ratio ($^{13}$C/$^{12}$C) of a given sample is expressed as $R_{sample}$ relative to the $\delta^{13}$C and international standard Pee Dee Belemnite (PDB). The carbon isotopic abundance ratio of any sample is thus expressed as shown in Eq. (1):

$$R_{sample} = \left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{PDB}$$  

where $R_{PDB}$ is the isotope abundance ratio of PDB ($R_{PDB} = 0.01112333$). The enrichment value of $^{13}$C as $^{13}$C$_{atom\%}$, excess (% of total C atoms) is shown in Eq. (2-3):

$$^{13}$C$_{atom\%}$ = $\left(\frac{R_{sample}}{R_{sample} + 1}\right) \times 100$$  

$$^{13}$C$_{atom\%, excess} = ^{13}$C$_{atom\%, sample} - ^{13}$C$_{atom\%, nature}$$

where $^{13}$C$_{atom\%, sample}$ (% of total C atoms) and $^{13}$C$_{atom\%, nature}$ (% of total C atoms) are the amounts of $^{13}$C in the sample and natural abundance, respectively. To determine the amount of $^{13}$C incorporated into various plant and soil pools, equation (4) was used:

$$^{13}$C$_{t, pool} = \frac{^{13}$C$_{atom\%, excess}}{100} \times C_{pool}$$

where $^{13}$C$_{t, pool}$ is the mass of $^{13}$C (g/m$^2$) in the considered pool at time $t$ after labeling, $^{13}$C$_{t, atom\%}$, excess is the increase in $^{13}$C at $t$ time in the considered pool. The percentage of $^{13}$C recovered in each pool ($^{13}$C$_{t, rec}$) is defined by the sum of the total assimilated $^{13}$C mass of day 1 in the plant-soil system pools (see Eq. (5)) and we also assume that the decreased amount of assimilated $^{13}$C mass in plant-soil system over time is attributed to ecosystem
respiration.

\[ \frac{13C_{\text{t,rec}}}{\sum 13C_{0,subpool}} = \frac{13C_{t,subpool}}{\sum 13C_{0,subpool}} \]  

The first-order exponential decay function was used to simulate assimilated $^{13}$C decreased in shoot C pool, and the expressed was shown in Eq. (6):

\[ ^{13}C_{t,\text{shoot}} = a + ^{13}C_{0,\text{shoot}} \times e^{-kt} \]  

where $^{13}C_{t,\text{shoot}}$ is the percentage of $^{13}$C recovered at t time in shoot, $^{13}C_{0,\text{shoot}}$ is the percentage of $^{13}$C recovered at day 1 in shoot, a is the intercept of the model, and k is the coefficient of decay rate. The MRT was calculated by reciprocal of k.

For the comparison of C sequestration among the four grazed treatments, C stocks (Mg C ha$^{-1}$) of the above- and below-ground mixed vegetation biomass and of the soil were calculated. Carbon stocks in the soil layers 0–10, 10–20 and 20–30 cm were calculated using the following equation:

\[ C_{\text{stock},i} = T_i \times BD_i \times SOC_i \]  

where $T_i$ (cm) is the thickness, BD$_i$ (g cm$^{-3}$) is the bulk density and SOC$_i$ (%) is the C content of the $i$th soil layer.

The significance test of differences among grazing treatments with respect to biomass, C pool, and $^{13}$C mass was carried out by a linear mixed model, in which block and sampling time were considered random effects. To conduct a multiple comparison between every two grazing intensity levels, a post hoc Tukey HSD test was used. The first-order exponential decay function was fitted by the nonlinear simulation model (NLS). All graphics and statistical analyses were carried out by R software (R Core Team 2019).
Results

Dynamics of recovered $^{13}$C in shoots

Recovered $^{13}$C in the shoots under all grazing intensities followed a first-order exponential decreasing trend with the time of labeling (Fig. 2), with the MRT of $^{13}$C in shoots averaging 19 days. Among the treatments, a significantly larger decline trajectory in $^{13}$C recovery was detected only in the heavily grazed swards (G0.92), which occurred most notably during the period of the 10th to the 56th days after labeling. The allocation rates of $^{13}$C from shoots to the belowground C pools and shoot respiration as of the 56th day after labeling were calculated as 66.43%, 52.81%, 50.03% and 63.32% of the assimilated $^{13}$C for the 4 treatments, respectively, with that of the G0.00 and G0.92 treatments being significantly higher than the rest of the treatments. The remaining amounts of $^{13}$C were largely retained in the standing dead and litter pools.

Recovered $^{13}$C allocated to below-ground pools

The recovery rates of $^{13}$C to the belowground pools were much less temporally variable compared with those in the vegetation during the first 100 days for all the individual pools of each treatment (Fig. 3).

Of the individual below-ground components, approximately 5.04% to 18.08% of $^{13}$C was allocated to the live root pools of all treatments after pulse labeling. Of special note, significantly lower proportions were measured in the heavily grazed subplots on most of the sampling occasions, whereas significantly higher values were measured in the lightly grazed subplots (G0.23) on three sampling occasions out of the total seven occasions.

In contrast, a range of 0.84% to 4.73% of recovered $^{13}$C was measured in dead roots of all treatments during the
chase period. The temporal patterns were much less variable and highly comparable among the treatments, and no significant differences in either the trend or the bulk were found (Fig. 3).

The $^{13}$C recovery in the 0-30 cm soil pools was basically comparable to that in the live roots but much higher than that in the dead roots of all treatments and on any sampling occasion during the chase period (Fig. 3). In addition, the temporal trajectories were fairly level and comparable among the treatments. Overall, compared to ungrazed, grazing led to a lower $^{13}$C recovery rate in all individual soil layers, and at the entire soil profile. On the whole soil $^{13}$C recovery rate was highest under light grazing (G0.23) followed be high grazing (G0.92) and moderate grazing (G0.46).

When all measurements on all sampling occasions were taken together until 31$^{st}$ day after labeling, the relationships between grazing intensity and recovered $^{13}$C in live roots, soil and the total belowground pool, displayed bump-shaped curves where recovered $^{13}$C increased with grazing intensity until a maximal point and then declined. This maximal point was reached under moderate grazing (G0.46) which had the largest enhancing effects on recovered $^{13}$C in soil and total belowground pools, but under light grazing (G0.23) in live root pool (Fig. 4).

When considering the relation between recovered $^{13}$C in live roots to soil at all sampling dates, we found that more percentage of assimilated $^{13}$C was found in soil compared to roots under heavy grazing, where more $^{13}$C was found in roots compared to soil in the ungrazed and lightly grazed subplots, whereas almost equal % of recovered $^{13}$C was found under moderately and lightly grazed subplots (Fig. 5).

Effects of grazing on carbon allocation

Fig. 6 shows that grazing substantially lowered the total carbon fixation rate (9.8% compared to 17.4%), the
decreased degree of which apparently increased with grazing intensity, whereas the efficiency of conversion from photosynthesis to shoot biomass as well as from shoot biomass to standing dead and litter was reduced by heavy grazing to variable extents. However, light and moderate grazing indeed slightly enhanced the conversion rates from photosynthesis to shoot biomass. In addition, the efficiency of conversion values from shoots to live roots was enhanced by light grazing but lowered by moderate and heavy grazing, while those for roots and aboveground litter to the soils were promoted to certain degrees in all grazing treatments. Concerning transfer to soil pool, grazing treatments were comparable, whereas ungrazed treatment was the lowest.

Changes in carbon stocks with increasing grazing intensity

An analysis of the plant and soil C stock data showed that the majority of the total carbon stock was contained in the belowground pool, varying between 95.73% and 98.80% among the grazing intensity treatments. Of the belowground carbon stock (live root, dead root and soil), the soil component accounted for a much higher proportion than the root compartment, which ranged from 93.70% to 96.11% among the treatments. However, significant changes indeed occurred in standing dead material and living root carbon pool (0-30 cm soil layer), showing for both variables the lowest amount under heavy grazing followed by moderate and light grazing and grazing exclusion, respectively (Table 1). In regard to soil C pool, no significant changes in the total carbon stock (0-30 cm depth) were detected with increasing grazing intensity except for heavy grazing which had a higher soil C stock than light grazing (Table 1).

Among the components of the above-ground carbon stock, the live shoots contained the largest fraction in all the treatments, followed by the litter and standing dead vegetation. In stark contrast to the case for the
belowground carbon stock, significant decreases in both the total aboveground carbon stock and its components were engendered by grazing, all of which displayed a decreasing trends with increasing grazing intensity.

Discussion

Effects of grazing on carbon fixation and allocation

The $^{13}$C fixed by shoots has several destinations. It may be emitted through shoot respiration directly to the atmosphere, used in new shoot growth, temporarily stored (e.g., in starch), or allocated into roots (Brüggemann et al. 2011; Wu et al. 2010). Grass species usually have the ability to adapt their C allocation patterns to grazing activity (Fig. 6). The partitioning of $^{13}$C was almost completed in shoots within 19 days after labeling. Thus, measurements of $^{13}$C lasting for 31 days after labeling should have reflected a steady state of the recovered $^{13}$C in the plants (Wu et al. 2010). It is crucial to discriminate the meanings of $^{13}$C content and $^{13}$C proportion. The former represents the amount of $^{13}$C mass remaining in the considered pools, while the latter mainly indicates the $^{13}$C allocation amount.

Grazing reduced the quantities of total C fixation, and the amounts of carbon in the plant-soil C pools exhibited obvious decreases to varying degrees with increasing grazing intensity, except for the SOC of the light grazing treatment (Fig. 6), while heavy grazing exhibited an opposite trend, especially the topsoil pool. The increased above- and below-ground biomasses induced by light grazing may have stimulated photosynthetically fixed C inputs into roots, leading to increased root exudates and root biomass (Liu et al. 2015; McSherry and Ritchie 2013; Schönbach et al. 2011; Zhang et al. 2015) as also reported by the meta-analysis results by Zhou et al. (2017).

During the 31-day chase period, approximately 35.16% of the total assimilated carbon remained in the total...
below-ground carbon pool under the light grazing intensity (G0.23), which was 12.02% higher than that under the no-grazing treatment (G0.00), and approximately 14.48% lower in C loss compared with G0.00. In contrast, only 25.53% of the total assimilated carbon remained in the total belowground carbon pool in the G0.92 treatment, indicating that heavy grazing accelerated C turnover in the soil. Kuzyakov et al. (2001) reported that the average long-term C sequestration proportion under grass vegetation was approximately 13% of assimilated C; these findings basically match our results. It should be pointed out that the partitioning patterns revealed in this study were obtained in light of single C isotope pulse treatments and thus only presented short-term trends of C allocation in the plant-soil systems.

**Effects of grazing intensity on the dynamics of $^{13}$C recovered in shoots**

The dynamics of recovered $^{13}$C in shoots generally reflect transfers of assimilated C into belowground pools and C loss by shoot respiration (Pausch and Kuzyakov 2018; Zhao et al. 2015). Compared with those reported by other analogous studies, the decline of $^{13}$C over time produced in our study are much steeper, indicating that the turnover time of shoot carbon in this meadow steppe ecosystem is much faster, and may be attributed to the specific plant species composition and the relatively hospitable climate conditions of this meadow steppe ecosystem (Tate et al. 2000; Wang et al. 2015).

Several studies indicated that the dynamics of recovered $^{13}$C in shoots did not vary significantly among land use patterns or intensities (Hafner et al. 2012; Zhao et al. 2015). However, a significant faster decline in assimilated $^{13}$C was detected at G0.92 (the highest grazing intensity), pointing to the shortest MRT of $^{13}$C in shoots of the plants under this grazing intensity. A further analysis revealed that this increased turnover was due mainly to shifts in the plant species composition of the sward at this grazing intensity. Indeed intense grazing was characterized by
significant increases in the abundance of annuals and a few forbs or C4 plant species. Zhao et al. (2015, 2017) found that C lost by shoot respiration was significantly greater for annual than perennial herbs.

Effects of grazing intensity on carbon transfers to below-ground pools

We observed significant increases in the $^{13}$C allocation proportion from shoots to the live root pool at G0.23 and slight increases at G0.46 but significant decreases at G0.92 compared with the allocation proportions in the grazed plots over time (Fig. 3). The enhanced nutrient requirements of grasses under moderate grazing may often increase C allocation from the canopy to the roots. In addition, the necessity for defoliated plants to allocate more C belowground to maintain enhanced activities of live roots or as storage for regrowth after grazing may also be partially responsible for the larger belowground C transfer from the canopy to the roots in this study. This was consistent with the results reported by Hafner et al. (2012), who found that $^{13}$C allocation to the below-ground pool was significantly larger at the moderately grazed site than heavy grazed in Tibetan meadow grassland. Furthermore, several studies reported that light grazing stimulated more photosynthetically fixed C inputs to roots, leading to increased compensatory growth and root biomass (Liu et al. 2015; McSherry and Ritchie 2013; Zhang et al. 2015).

By stark contrast, serious defoliation under heavy grazing usually increases root exudation (Hamilton and Frank 2001; Kuzyakov et al. 2001), which may increase the carbon decomposed by microorganisms in the rhizosphere. A study of the same ecosystem revealed that heavy grazing may largely lead to an increase in the proportion of root respiration relative to total soil respiration (Yan et al. 2017). These results may collectively explain the significantly lowered retention of $^{13}$C transferred from the canopy to the roots under heavy grazing. Pausch and Kuzyakov (2018) noted that heavy grazing tended to stimulate C transfer from the roots to the soil.
Of special note, we observed consistently higher $^{13}$C content in both the dead root and the soil pool under grazing intensities compared with the ungrazed plots. Of these effects, heavy grazing on the dead root pool appeared the most pronounced. A major mechanism lies in the fact that trampling by cattle in this study increased the bulk density but decreased the total soil porosity under varying degrees of grazing intensity (e.g. Ludvíková et al. 2014), leading to corresponding decreases in soil respiration and decomposition of dead roots (Yan et al. 2017).

Impacts of grazing intensity on below-ground carbon stocks

A number of studies have been conducted to examine the effects of livestock grazing on the belowground carbon pools, particularly the root and topsoil components, the results of which are quite variable and inconclusive. These differences are largely due to the differences in grassland type, grazing history, and site-specific conditions among different studies (Semmartin et al. 2008; Zhao et al. 2007).

Our study showed significant decreases in the live root biomass under heavy grazing intensity but a slight increase under light grazing intensity (Table 1), which is consistent with other studies (Gao et al. 2008). The increase in live root biomass under light grazing intensity was mainly related to increases in canopy growth due to reduced defoliation, enhanced by light grazing, which might be well explained by the intermediate disturbance hypothesis (Li et al. 2011). In stark contrast, the low living root biomass in heavy grazing might have resulted from combined a negative effect of grazing on the soil physical and chemical conditions, regrowth capacity of vegetation and shifts in the species composition engendered by heavy grazing. Annual and biennial herbs are generally characterized by a shallow distribution and low root biomass as well as short longevity (Barger et al. 2004; Dorrough et al. 2004), which may explain part of the results. The slight decrease in the standing biomass of dead roots under heavy grazing
intensity was apparently connected to the decrease in that of the live roots.

In regard to the soil carbon stock, no significant changes engendered by grazing in the entire soil layer as a whole, which presumably was attributed to the relatively short history (about 10 years) of livestock. However, we detected significant increases in the carbon content of the topsoil that were caused by heavy grazing. Heavy grazing decreased the C pools in soils, due to decreased root production and exudates due to plant removal by livestock grazing (e.g. Bagchi and Ritchie 2010; Frank and Groffman 1998). These different results arise largely from differences in the historical context of grazing, consecutive duration of grazing, species of livestock (sheep, goat, cattle), vegetation, and soil. In the present study, we found that heavy grazing physically increased the soil bulk density due to frequent trampling of the soil by livestock, which would substantially constrain root and soil respiration. Trampling also enhanced the entry and mixture of stand dead and ground detritus into the soil. In addition, shifts in species composition engendered by heavy grazing favoring annuals and forbs could lead to the concentration of roots in the topsoil, increasing carbon input to this soil layer.

Conclusions

In this study, we found a significantly larger decline in $^{13}$C recovery in the heavily grazed swards, with the relocation rate of $^{13}$C from shoots to the belowground C pool being the highest. In contrast, light grazing significantly promoted the recovered $^{13}$C in the belowground pool, especially in the live root and topsoil sub-pools. Whereas less $^{13}$C was allocated to live roots in the ungrazed and lightly grazed subplots compared to moderately grazed subplots. The opposite was observed under heavy grazing, here the living root to carbon stock ratio was highest. This study highlights the beneficial effect of moderate and light grazing on shoot biomass and living roots. However, there is
necessity of gradient treatments in grazing-related research works, which may provide further insight into the effects of land use on the carbon cycling of grassland ecosystems.

Compliance with ethical standards

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Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

Data are available on request from the corresponding author.
References

Bagchi S, Ritchie ME (2010) Introduced grazers can restrict potential soil carbon sequestration through impacts on plant community composition. Ecol Lett 13:959-968. https://doi.org/10.1111/j.1461-0248.2010.01486.x

Bai Y, Wu J, Clark CM, Pan Q, Zhang L, Chen S, Wang Q, Han X (2012) Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient. J Appl Ecol 49:1204-1215. https://doi.org/10.1111/j.1365-2664.2012.02205.x

Barger N, Ojima D, Belnap J, Wang S, Yanfen W, Chen Z (2004) Changes in plant functional groups, litter quality, and soil carbon and nitrogen mineralization with sheep grazing in an Inner Mongolian grassland. Rangel Ecol Manag 57:613-619. https://doi.org/10.2111/1551-5028(2004)057[0613:CIPFGL]2.0.CO;2

Brüggemann N, Gessler A, Kayler Z et al (2011) Carbon allocation and carbon isotope fluxes in the plant- soil-atmosphere continuum: a review. Biogeosciences 8:3457-3489. https://doi.org/10.5194/bg-8-3457-2011

Cao M, Woodward FI (1998) Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. Nature 393:249-252. https://doi.org/10.1038/30460

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165-173. https://doi.org/10.1038/nature04514

Dorrough J, Ash J, McIntyre S (2004) Plant responses to livestock grazing frequency in an Australian temperate grassland. Ecography 27:798-810. https://doi.org/10.1111/j.0906-7590.2004.04004.x

Fan J, Zhong H, Harris W, Yu G, Wang S, Hu Z, Yue Y (2008) Carbon storage in the grasslands of China based on
field measurements of above- and below-ground biomass. Clim Change 86:375-396.

https://doi.org/10.1007/s10584-007-9316-6

Frank DA, Groffman PM (1998) Ungulate vs. landscape control of soil C and N processes in grasslands of Yellowstone national park. Ecology 79:2229-2241. https://doi.org/10.1890/0012-9658(1998)079[2229:UVLCOS]2.0.CO;2

Gao YZ, Giese M, Lin S, Sattelmacher B, Zhao Y, Brueck H (2008) Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. Plant Soil 307:41-50. https://doi.org/10.1007/s11104-008-9579-3

Hafner S, Unteregelsbacher S, Seeber E, Lena B, Xu X, Li X, Guggenberger G, Miehe G, Kuzyakov Y (2012) Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by $^{13}$CO$_2$ pulse labeling. Glob Change Biol 18:528-538. https://doi.org/10.1111/j.1365-2486.2011.02557.x

Hamilton EW, Frank DA (2001) Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. Ecology 82: 2397-2402.

Han G, Hao X, Zhao M, Wang M, Ellert BH, Willms W, Wang M (2008) Effect of grazing intensity on carbon and nitrogen in soil and vegetation in a meadow steppe in Inner Mongolia. Agric Ecosyst Environ 125:21-32. https://doi.org/10.1016/j.agee.2007.11.009

Harris D, Horwath W, Kessel C (2001) Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci Soc Am J 65:1853-1856. https://doi.org/10.2136/sssaj2001.1853

He NP, Zhang YH, Yu Q, Chen QS, Pan QM, Zhang GM, Han XG (2011) Grazing intensity impacts soil carbon and nitrogen storage of continental steppe. Ecosphere 2:art8. https://doi.org/10.1890/ES10-00017.1
Jin D, Murray PJ, Xin X, Qin Y, Chen B, Qing G, Zhang Z, Yan R (2018) Attribution of explanatory factors for change in soil organic carbon density in the native grasslands of Inner Mongolia, China. J Arid Land 10:375-387. https://doi.org/10.1007/s40333-018-0056-4

Klumpp K, Fontaine S, Attard E, Le Roux X, Gleixner G, Soussana JF (2009) Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. J Ecol 97: 876-885. https://doi.org/10.1111/j.1365-2745.2009.01549.x

Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. Review. J Plant Nutr Soil Sci 163:421-431. https://doi.org/10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R

Kuzyakov Y, Ehrensberger H, Stahr K (2001) Carbon partitioning and below-ground translocation by Lolium perenne. Soil Biol Biochem 33:61-74. https://doi.org/10.1016/S0038-0717(00)00115-2

Kuzyakov Y, Schneckenberger K (2004) Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. Arch Agron Soil Sci 50:115-132. https://doi.org/10.1080/03650340310001627658

Li W, Huang H, Zhang Z, Wu G (2011) Effects of grazing on the soil properties and C and N storage in relation to biomass allocation in an alpine meadow. Journal of Soil Science & Plant Nutrition 11: 27-39.

Liu N, Kan HM, Yang GW, Zhang YJ (2015) Changes in plant, soil, and microbes in a typical steppe from simulated grazing: explaining potential change in soil C. Ecol Monogr 85:269-286. https://doi.org/10.1890/14-1368.1

Ludvíková V, Pavlů VV, Gaisler J, Hejcman M, Pavlů L (2014) Long term defoliation by cattle grazing with and without trampling differently affects soil penetration resistance and plant species composition in Agrostis capillaris grassland. Agric Ecosyst Environ 197:204-211. https://doi.org/10.1016/j.agee.2014.07.017
McSherry ME, Ritchie ME (2013) Effects of grazing on grassland soil carbon: a global review. Glob Change Biol 19:1347-1357. https://doi.org/10.1111/gcb.12144

Ni J (2002) Carbon storage in grasslands of China. J Arid Environ 50:205-218. https://doi.org/10.1006/jare.2001.0902

Ojima DS, Dirks BOM, Glenn EP, Owensby CE, Scurlock JO (1993) Assessment of C budget for grasslands and drylands of the world. Water Air Soil Pollut 70:95-109. https://doi.org/10.1007/BF01104990

Pausch J, Kuzyakov Y (2018) Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Glob Change Biol 24:1-12. https://doi.org/10.1111/gcb.13850

Piao SL, Ito A, Li SG et al (2012) The carbon budget of terrestrial ecosystems in East Asia over the last two decades. Biogeosciences 9:3571-3586. https://doi.org/10.5194/bg-9-3571-2012

Post WM, Emanuel WR, Zinke PJ, Stangenberger AG (1982) Soil carbon pools and world life zones. Nature 298:156-159. https://doi.org/10.1038/298156a0

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Schönbach P, Wan H, Gierus M, Bai Y, Müller K, Lin L, Susenbeth A, Taube F (2011) Grassland responses to grazing: effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem. Plant Soil 340:103-115. https://doi.org/10.1007/s11104-010-0366-6

Semmartin M, Garibaldi LA, Chaneton EJ (2008) Grazing history effects on above- and below-ground litter decomposition and nutrient cycling in two co-occurring grasses. Plant Soil 303:177-189. https://doi.org/10.1007/s11104-007-9497-9
Tate KR, Scott N, Ross D, Parshotam A, Claydon J (2000) Plant effects on soil carbon storage and turnover in a montane beech (Nothofagus) forest and adjacent tussock grassland in New Zealand. Aust J Soil Res 38:685-697. https://doi.org/10.1071/SR99092

Trumbore SE, Czimczik CI (2008) Geology. An uncertain future for soil carbon. Science 321:1455-1456. https://doi.org/10.1126/science.1160232

Wang X, Yan Y, Zhao S, Xin X, Yang G, Yan R (2015) Variation of soil respiration and its environmental factors in Hulunber meadow steppe. Acta Ecol Sin 35:1-4. https://doi.org/10.1016/j.chnaes.2014.12.001

WRI (2018) World resource report.

Wu Y, Tan H, Deng Y, Wu J, Xu X, Wang Y, Tang Y, Higashi T, Cui X (2010) Partitioning pattern of carbon flux in a Kobresia grassland on the Qinghai-Tibetan plateau revealed by field 13C pulse-labeling. Glob Change Biol 16:2322-2333. https://doi.org/10.1111/j.1365-2486.2009.02069.x

Yan R, Xin X, Yan Y, Wang X, Zhang B, Yang G, Liu S, Deng Y, Li L (2015) Impacts of differing grazing rates on canopy structure and species composition in Hulunber meadow steppe. Rangel Ecol Manag 68:54-64. https://doi.org/10.1016/j.rama.2014.12.001

Yan RR, Tang HJ, Lv SH et al (2017) Response of ecosystem CO2 fluxes to grazing intensities - a five-year experiment in the Hulunber meadow steppe of China. Sci Rep 7:9491. https://doi.org/10.1038/s41598-017-09855-1

Yang T, Li P, Liu P, Wu X (2012) Distribution of grassland biomass carbon storage in china. Adv Mater Res 518-523:183-188. https://doi.org/10.4028/www.scientific.net/AMR.518-523.183

Zhang B, Zhou X, Zhou L, Ju R (2015) A global synthesis of below-ground carbon responses to biotic disturbance: a
Zhao L, Chen D, Zhao N, Li Q, Cheng Q, Xu S, Wang S, Zhao X (2015) Responses of carbon transfer, partitioning, and residence time to land use in the plant–soil system of an alpine meadow on the Qinghai-Tibetan plateau. Biol Fertil Soils 51:781-790. https://doi.org/10.1007/s00374-015-1024-1

Zhao Y, Peth S, Krümmelbein J, Horn R, Wang Z, Steffens M, Hoffmann C, Peng X (2007) Spatial variability of soil properties affected by grazing intensity in Inner Mongolia grassland. Ecol Model 205:241-254. https://doi.org/10.1016/j.ecolmodel.2007.02.019

Zhou G, Luo Q, Chen Y, He M, Zhou L, Frank D, He Y, Fu Y, Zhang B, Zhou X (2019) Effects of livestock grazing on grassland carbon storage and release override impacts associated with global climate change. Glob Chang Biol 25:1119-1132. https://doi.org/10.1111/gcb.14533

Zhou G, Zhou X, He Y, Shao J, Hu Z, Liu H, Zhou H, Hosseinibai S (2017) Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. Glob Change Biol 23:1167-1179. https://doi.org/10.1111/gcb.13431

Zou C, Wang K, Wang T, Xu W (2007) Overgrazing and soil carbon dynamics in eastern Inner Mongolia of China. Ecol Res 22:135-142. https://doi.org/10.1007/s11284-006-0009-9
Table 1. Above- and belowground carbon stocks (Mg C ha\(^{-1}\)) in response to grazing intensity.

| Components          | Depth (cm) | G0.00       | G0.23       | G0.46       | G0.92       |
|---------------------|------------|-------------|-------------|-------------|-------------|
| Live shoots         | 0.00       | 2.01±0.15d\(^{†}\) | 1.67±0.10c  | 1.23±0.09b  | 0.72±0.04a  |
| Standing dead       | 0.00       | 0.83±0.10d  | 0.58±0.07c  | 0.27±0.04b  | 0.15±0.03a  |
| Litter              | 0.00       | 1.47±0.19c  | 0.84±0.08b  | 0.60±0.03ab | 0.38±0.06a  |
| Total aboveground   | 0.00       | 4.30±0.41d  | 3.08±0.07c  | 2.11±0.13b  | 1.26±0.05a  |
| Living roots        | 0-30       | 4.24±0.13c  | 4.45±0.15c  | 3.18±0.11b  | 2.66±0.13a  |
| Dead roots          | 0-30       | 2.01±0.15a  | 2.19±0.18ab | 2.42±0.18b  | 1.86±0.13a  |
| Soil                | 0-10       | 35.61±0.84a | 35.68±0.65a | 37.83±0.86ab| 40.17±0.90b |
|                     | 10-20      | 29.40±0.78a | 29.05±0.62a | 31.37±0.85ab| 32.64±1.22b |
|                     | 20-30      | 25.50±0.99a | 24.41±0.51a | 25.06±0.67a | 26.51±0.46a |
|                     | 0-30       | 90.52±1.91ab| 89.14±1.37a | 94.27±1.84ab| 99.32±1.75b |
| Total belowground   | 0.00       | 96.24±1.91a | 95.14±1.27a | 99.34±1.89ab| 103.36±1.81b|
| Sum of total        | 0.00       | 100.55±1.45a| 98.22±0.56a | 101.45±0.65ab| 104.62±0.96b|

\(^{†}\)Within rows, values followed by different letters are significantly different (P≤0.05) based on post hoc Tukey HSD tests in R. Values are presented as the mean ± se.
Figure captions

Fig. 1 Experimental design and plot layout: 0.00, 0.23, 0.34, 0.46, 0.69 and 0.92 AU ha\(^{-1}\) represent relevant stocking rates for different treatments.

Fig. 2 Dynamics of recovered \(^{13}\)C in shoots during the chase period. Mean values (n = 3) and standard errors are presented.

Fig. 3 Dynamics of recovered \(^{13}\)C in the root and soil components during 100 days after labeling. Mean values (n = 3) and standard errors are presented.

Fig. 4 Partitioning of recovered \(^{13}\)C to living root (a), soil (b) and living roots + soil (c)) in the soil depth of 0–30 cm during 31 days after labeling under increased grazing intensities. The whiskers of the box-and-whisker panels denote the maximum values that are less than or equal to the 3\(^{rd}\) quartile + 1.5*IQR (interquartile range) and the minimum values that are greater than or equal to the 1\(^{st}\) quartile - 1.5*IQR.

Fig 5 Relationship between \(^{13}\)C allocated to living roots and soil organic carbon (SOC) as a % of the total assimilated carbon. The dotted line is the 1:1 diagonal line, the cross-bars denote the standard errors, and the cross-points indicate the mean values.

Fig. 6 Schematic diagram of \(^{13}\)C partitioning in diverse C pools under different grazing intensities in the meadow grassland plant-soil system. The absolute estimates of the carbon pool sizes (upper numbers in Mg C ha\(^{-1}\)) and their percentages (lower numbers) of the total \(^{13}\)C fixation rates are indicated.