Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by $^{13}$CO$_2$ pulse labeling

SILKE HAFNER*, SEBASTIAN UNTEREGELSBACHER*, ELKE SEEBER†, BECKER LENA‡, XINGLIANG XU§, XIAOGANG LI¶, GEORG GUGGENBERGER‡, GEORG MIEHE** and YAKOV KUZYAKOV***††

*Department of Agroecosystem Research, BayCEER, University of Bayreuth, Bayreuth, Germany, †Institute of Geobotany, University of Halle, Halle, Germany, ‡Institute of Soil Science, Leibniz Universität Hannover, Hannover, Germany, §Key Laboratory of Ecosystem Network Observation and Modelling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China, ¶MOE Key Laboratory of Arid and Grassland Ecology, School of Life Sciences, Lanzhou University, Lanzhou, Gansu Province 730000, China, **Faculty of Geography, Philipps-Universität Marburg, Marburg, Germany, ††Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany

Abstract

Since the late 1950s, governmental rangeland policies have changed the grazing management on the Tibetan Plateau (TP). Increasing grazing pressure and, since the 1980s, the privatization and fencing of pastures near villages has led to land degradation, whereas remote pastures have recovered from stronger overgrazing. To clarify the effect of moderate grazing on the carbon (C) cycle of the TP, we investigated differences in below-ground C stocks and C allocation using in situ $^{13}$CO$_2$ pulse labeling of (i) a montane Kobresia winter pasture of yaks, with moderate grazing regime and (ii) a 7-year-old grazing exclosure plot, both in 3440 m asl. Twenty-seven days after the labeling, $^{13}$C incorporated into shoots did not differ between the grazed (43% of recovered $^{13}$C) and ungrazed (38%) plots. In the grazed plots, however, less C was lost by shoot respiration (17% vs. 42%), and more was translocated below-ground (40% vs. 20%). Within the below-ground pools, <2% of $^{13}$C was incorporated into living root tissue of both land use types. In the grazed plots about twice the amount of $^{13}$C remained in soil (18%) and was mineralized to CO$_2$ (20%) as compared to the ungrazed plots (soil 10%; CO$_2$ 9%). Despite the higher contribution of root-derived C to CO$_2$ efflux, total CO$_2$ efflux did not differ between the two land use types. C stocks in the soil layers 0–5 and 5–15 cm under grazed grassland were significantly larger than in the ungrazed grassland. However, C stocks below 15 cm were not affected after 7 years without grazing. We conclude that the larger below-ground C allocation of plants, the larger amount of recently assimilated C remaining in the soil, and less soil organic matter-derived CO$_2$ efflux create a positive effect of moderate grazing on soil C input and C sequestration.

Keywords: $^{13}$C pulse labeling, C allocation, grazing exclosure experiment, grazing intensity, montane Kobresia pasture, Qinghai-Tibetan Plateau, soil organic carbon

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Introduction

The importance of grasslands for global carbon (C) cycling and C sequestration in soil is highlighted by two facts: first, the extension of grassland area, covering nearly one-fifth (24 million km$^2$) of the world’s land surface (Lieth, 1978); second, the large quantity of C stored in grassland soils (200–300 Pg C) (Scurlock & Hall, 1998). This corresponds to 10% (Eswaran et al., 1993) to 30% (Anderson, 1991) of the C stock in soils on a global scale (1500 Pg C) (Wang et al., 2002). On the Tibetan Plateau (TP) grasslands cover 1.5 million km$^2$ and represent one-third of the total plateau area (Sun & Zheng, 1998). The high C sequestration here results from very high below-ground allocation of assimilates by grasses (Kuzyakov & Domanski, 2000) and from stabilization of root-derived C in soil due to dry periods in summer and long cold winters.

These grasslands are dominated by Kobresia species that belong to the Cyperaceae family. Kobresia pastures are characterized by a closed vegetation cover of Cyperaceae turf and a very dense root system (Miehe et al., 2008b). The Kobresia biome extends from 3000 m asl in...
the northeast of the TP to 5960 m asl on the northern slope of Mt Everest, covering an area of 450 000 km² (Miehe et al., 2008b).

The grasslands of the TP represent the world’s largest high altitude pasture area (Wang et al., 2005), where animal husbandry represents the traditional land use. The rapid increase in livestock during recent decades raised concern about the ongoing ecological and environmental impacts (Zhou et al., 2006). The spatial arrangement of the pasture land was altered after sedentarization programmes were instated in 1959 (Gad, 2005). Since then, grazing pressure around the villages has increased (Zhao & Zhou, 1999), leading to overgrazing and land degradation, whereas in remote areas vegetation succession has been initiated.

Kobresia pastures were found to have a neutral net ecosystem CO₂ exchange (Shi et al., 2006) or may even represent a moderate C sink (Ni, 2002), but changes in land use and grassland management could be a decisive factor for a C sink and source switch (Wang et al., 2005). Therefore, determining the grassland C dynamics of the TP and the influence of changing grazing activity is crucial to understand the regional and global C budget.

Grazing exclosure experiments by fencing were conducted in south Tibet and Qinghai to investigate the effects of overgrazing and decreasing grazing pressure on biomass production, species composition by succession and pasture degradation. One year after enclosure, Kobresia species were overgrown with graminoids, i.e. Poaceae (Miehe et al., 2003, 2006, 2008a). However, the effect of replacing Kobresia with taller grasses on C cycling remains unclear, particularly if the below-ground part of the C cycling is addressed. To elucidate how grazing affects C sequestration in the Kobresia pasture of the TP, the below-ground C allocation by plants and sources of CO₂ efflux need to be related to the response of storage and distribution of soil organic carbon (SOC).

We hypothesize that, in the absence of grazing, the allocation of assimilates below-ground decreases. The reduced grazing pressure causes plants to invest more in above-ground biomass and reduce root biomass and rhizodeposition. Based on a 2–2.5 times higher contribution of root-derived C to stabilized SOC pools as compared to shoot-derived C (Rasse et al., 2005), we further hypothesize that the lower root biomass in the absence of grazing and the consequent decrease in Kobresia density has a negative effect on C sequestration. Therefore, our study is focused on the effect of grazing on (i) short-term allocation of recent assimilates below-ground, (ii) medium-term effects on the living and dead root biomass and (iii) long-term effects on stabilized SOC pools.

To test these hypotheses we carried out an in situ ¹³C labeling experiment on a montane pasture on the TP, which is used as winter pasture for yaks, and on a fenced plot, where yaks and sheep have been excluded since 2002. This was designed to (i) determine the partitioning of recently fixed C among pools and fluxes in the plant–soil system, (ii) evaluate differences in the partitioning pattern of recent assimilates between the grazed and ungrazed grassland and (iii) estimate the effect of grazing on C input into soil. C stocks were evaluated to estimate the medium-term (dead roots) and long-term (stabilized SOC pools) effects of grazing on C sequestration in soil.

Material and methods

Site description

The experimental site (99°51'E, 35°32'N, 3440 m asl) is located near Xinghai in the province of Qinghai on the north-eastern TP. The montane pasture is situated at 3440 m asl on a loess-covered gravel terrace of the Yellow River. In the north-east of the study site, the terrace elevates 360 m above the Da Heba River and is bordered in the south-west by mountains rising 800 m above the terrace (Miehe et al., 2008a). The area is influenced by the East Asian monsoon. Annual precipitation at the study site, obtained by the project rain gauges (Miehe et al., 2008a), averaged 582 mm from 2002 to 2010. This exceeds the 40-year mean annual precipitation obtained by the Xinghai climate station of 353 mm (1961–2001) (Miehe et al., 2008a). Most of the precipitation falls in summer during the growing season from May to September. Mean monthly temperatures (1961–2001) in Xinghai are below −10 °C in December and January, above 5 °C between May and September, and above 10 °C during June and August (Miehe et al., 2008a).

Soils developed from unconsolidated aeolian silts (mainly loess) and are classified as Haplic Kastanozems (WRB IUSS-ISR-B FAO, 2006), having an Ah mollic horizon with a thickness of 24 cm and secondary carbonates of pseudomycel type in the lower soil horizon Bk from 30 cm to more than 90 cm soil depth. The topsoil of the grazed plots (0–5 cm) consisted of undecomposed felty remains of fine sedge-roots, amorphous humus and minerogenic matter. This phenomenon is termed 'Kobresia turf' (Miehe, 1988; Kaiser, 2004).

Governmental rangeland policies have involved denationalization of pasture land. In 1995, the site under investigation has been fenced by the owner and has been used as winter pasture for yaks and sheep for 6- to 7-month per year (Miehe et al., 2008a). The pasture land covers 24 ha (Koch, 2003). According to an official census of livestock in 2002, 82 sheep and 30 yaks graze here (Koch, 2003), which is equivalent to an average grazing intensity of 2 sheep ha⁻¹ a⁻¹ and 0.7 yaks ha⁻¹ a⁻¹ and corresponding to 19 livestock units ha⁻¹ a⁻¹ (Food and Agricultural Organisation, 2005). In addition to stocking, the relative cover of weeds, classified according to the China Weed Information System (CWIS), is low (9% and 13% on grazed and ungrazed plots). The relative cover of...
Stellera chamaejasme – one of the most serious grazing weeds in China (Liu et al., 2004) – is also low (1% and 6%). The grazing regime of the pasture is therefore classified as moderate.

On the pasture a grazing enclosure plot covering an area of 20 × 20 m was created by fencing in 2002 in cooperation with the Northwest Institute of Plateau Biology, CAS, Xining (Miehe et al., 2008a). The fences exclude yaks and sheep, initiating vegetation succession. The site provides the opportunity to study differences in C partitioning between moderate grazing and the influence of absent grazing as the basic conditions of both sites (land use, species composition and environmental conditions) were the same before the exclusion.

The study site represents a semihumid Kobresia grassland which is characteristic for the upper montane belt of the Qinghai TP. To obtain the total vegetation cover for both land use types the total cover of every single species per plot was estimated and summed up thereafter. At the time the experiment was carried out, the plots were dominated by perennial herbs, grasses and Cyperaceae with a total cover of 56%, 29% and 12% at the grazed and 42%, 13% and 9% at the ungrazed site, respectively (Table 1). During the experimental setup, most plant species were at the developmental stage of flowering or fruiting. All of the plant species were perennials, except within the genus Gentianella, where annual species occurred as well (Table 1). After 7 years of grazing exclusion, total cover was reduced due to the negative impact of litter accumulation (Table 1). The mean vegetation height increased from 15 cm to more than 30 cm. Within the experimental procedure, no significant change in plant species composition or abundance was observed.

**Pulse labeling**

The 13CO2 labeling was conducted on 27 July 2009: three replicate plots were labeled on both the grazed and ungrazed treatment. The 13CO2 pulse was applied simultaneously (with 2–3 min difference) into each chamber. The chambers were 50 cm long, 50 cm wide and 20 cm high and consisted of four metal bars covered with transparent polyethylene foil with more than 90% transmittance of photosynthetically active radiation. To avoid gas losses, the foil was buried into the soil 50 cm deep, metal bars inside the chamber. The chamber was then closed and the sulphuric acid (H2SO4) was carefully added from the outside into the Na213CO3 solution, using syringes, to ensure complete evolution of 13CO2 into the chamber atmosphere. To guarantee a uniform distribution of 13CO2, a 5-volt fan was used inside the chamber. The progression of the CO2 concentration inside the chamber was reached, complete assimilation of the label was assumed. Consequently, plants assimilated the label for 1 h before the chamber was removed.

Fluxes of assimilated C were traced as 13C throughout a 27-day chase period in shoots, roots and soil CO2 efflux. Samples were taken at increasing time intervals: 1, 5, 12, 18 and 27 days after labeling. To ensure homogeneity, at each date two samples of each compartment were taken from one plot and combined thereafter. Shoots were cut on a small area with a diameter of 6.4 cm. Soil and root samples were taken from three layers: 0–5, 5–15 and 15–30 cm. Roots were separated into living and dead roots before drying. Roots were cracked and defined as living roots when the inside was white and moist, which indicated intact transport tissue. All samples were dried, weighed and ball milled. Five replicate natural abundance samples (unlabeled plots) were taken from each treatment and compartment and were used as a reference.

The static alkali absorption method (Lundegardh, 1921; Kirita, 1971; Singh & Gupta, 1977) was used to determine (i) total CO2 efflux, (ii) the turnover of recent assimilates used for root and rhizomicrobial respiration and (iii) root-derived soil CO2 efflux which comprises rhizomicrobial and root respiration (Kuzmakov, 2006). With this method, CO2 originating from soil is absorbed in alkali (NaOH) within a closed chamber over a specific time period. Therefore, directly after the cutting of the shoots, an opaque chamber (6.4 cm diameter, 10 cm high) was installed on the uncovered soil surface to avoid photosynthesis.

### Table 1 Dominant plant species classified based on the belonging to a plant functional group occurring in both land use types

<table>
<thead>
<tr>
<th>Plant functional group</th>
<th>Dominant species</th>
<th>Vegetation cover</th>
<th>Live cycle and reproductive strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grazed</td>
<td>Ungrazed</td>
</tr>
<tr>
<td>Perennial herbs</td>
<td>Tanacetum spec. Thalictrum alpinum Trigonella ruthenica</td>
<td>56</td>
<td>42</td>
</tr>
<tr>
<td>Poaceae</td>
<td>Festuca forrestii Leymus secalinus Stipa aliena</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>Cyperaceae</td>
<td>Kobresia filifolia Kobresia pygmaea</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Weeds</td>
<td>Gentiana straminea Lancea tibetica Stellera chamaejasme</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Annual herbs</td>
<td>Gentianella spec.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total plant cover</td>
<td></td>
<td>107</td>
<td>76</td>
</tr>
</tbody>
</table>

Total and individual vegetation cover (%), life cycle and reproductive strategy are indicated for every plant functional groups.

of regrown shoots. A graduated beaker containing 1 m NaOH was placed inside the chamber to trap CO₂ emission from soil. CO₂ was absorbed in NaOH between the samplings for periods of 1, 4, 7, 6 and 9 days. The amount of NaOH was adjusted to the duration of the CO₂ trapping periods (20, 20, 30, 40 and 50 mL) to ensure that the neutralization does not exceed one-third of the NaOH inside the chamber (Gupta & Singh, 1977). To quantify the total CO₂ efflux from soil, CO₂ trapped in NaOH was analyzed by titrating NaOH against 0.1M hydrochloric acid (HCl). Soil CO₂ efflux soilCO₂ (g C m⁻² day⁻¹) was calculated by the following equation:

\[
\text{soilCO}_2 = \frac{mC}{A \cdot \Delta t}
\]

where \(mC\) represents the amount of C absorbed in NaOH within the time period \(\Delta t\) after the chamber was closed. \(A\) is the surface area covered by the chamber.

To determine the amount of recent assimilates partitioned to soil CO₂ efflux, 2 m SrCl₂ was added into NaOH to produce SrCO₃ precipitation for \(^{13}\)C measurement. After neutralization and drying of SrCO₃ the \(^{13}\)C signature was determined.

To evaluate the CO₂ fluxes from the \(^{13}\)C labeled plots determined by the static alkali absorption method, CO₂ efflux from adjacent unlabeled plots was determined with a CO₂ sensor (GM 70, Vaisala) installed in closed chambers (area 144 cm²). The CO₂ increase in the closed chambers was determined based on linear regression. To obtain a daily mean for CO₂ efflux within the chase period of both land use types, measurements of the CO₂ efflux rate were repeated 27 times 10 times in 5 min and the CO₂ efflux rate was calculated based on linear regression. To obtain a daily mean for CO₂ efflux within the chase period of both land use types, measurements of the CO₂ efflux rate were repeated 27 times within 24 h on both plots on 14 August 2009.

For \(^{13}\)C analyzes in soil organic C, carbonates were removed from the soil samples (Harris et al., 2001) during 3 days in a desiccator that contained 10 m HCl. Thereafter, the samples were neutralized by adding deionized water and dried thereafter.

The \(^{13}\)C signature and the total C content of shoots, roots, soil and SrCO₃ and of natural abundance control samples were determined by the isotope ratio mass spectrometer (Delta Plus; Thermo Fisher Scientific, Bremen, Germany) coupled with an elemental analyzer (NC 2500; CE Instruments, Milano, Italy).

Calculations

Stable isotope calculation. \(^{13}\)C incorporation into plant and soil C pools derived from pulse labeling is presented as percentage of \(^{13}\)C recovery. As a reference, \(^{13}\)C recovered in every replicate plot and all considered C pools 1 day after the labeling was used (27%, 39%, 40% and 44%, 87%, 79% \(^{13}\)C of total added in grazed and ungrazed treatment, respectively). All calculations were done using \(^{13}\)C atom%. The obtained data of \(^{13}\)C (%c) by element analyzer - isotope ratio mass spectrometer were recalculated as follows. First, the isotopic ratio \((^{13}\text{C}/^{12}\text{C})\) of each sample \(R_{\text{sample}}\) was calculated:

\[
R_{\text{sample}} = \left(\frac{^{13}\text{C}}{^{12}\text{C}}\right)_{\text{sample}} = \frac{R_{\text{PDB}}}{100} + R_{\text{PDB}}.
\]

\(R_{\text{PDB}} = 0.011237\) is the isotopic ratio of \(^{13}\text{C}/^{12}\text{C}\) in Pee Dee Belemnite.

Second, \(^{13}\text{C}_{\text{atom}}\%\) (% of total C atoms) of the sample was calculated:

\[
^{13}\text{C}_{\text{atom}}\% = \left(\frac{R_{\text{sample}}}{R_{\text{sample}} + 1}\right) \cdot 100.
\]

The enrichment of \(^{13}\text{C}\) in a C pool \((^{13}\text{C}_{\text{atom}}\%\) excess, % of total C atoms\) derived from the pulse labeling was calculated by subtracting the amount of \(^{13}\text{C}\) in the natural abundance sample \((^{13}\text{C}_{\text{atom}}\%\) of NA, % of total C atoms\) from the amount of \(^{13}\text{C}\) in the sample \((^{13}\text{C}_{\text{atom}}\%\) of sample, % of total C atoms\)

\[
^{13}\text{C}_{\text{atom}}\% \text{ excess} = ^{13}\text{C}_{\text{atom}}\% \text{ of sample} - ^{13}\text{C}_{\text{atom}}\% \text{ of NA}.
\]

To determine the amount of \(^{13}\text{C}\) incorporated into C pools at a specific time \(t\) after the labeling \((^{13}\text{C}_{t}, \text{ g m}^{-2})\), the increment in \(^{13}\text{C}\) at that time \((^{13}\text{C}_{\text{atom}}\% \text{ excess, % of total C atoms})\) was multiplied by the mass of C in the considered pool \((C_{\text{pool}}, \text{ g m}^{-2})\).

\[
^{13}\text{C}_{t} = \frac{^{13}\text{C}_{\text{atom}}\% \text{ excess, } t}{100} \cdot C_{\text{pool}}.
\]

To obtain the weighted \(^{13}\text{C}\) (\(^{13}\text{C}_{\text{rec, %}}\) of recovered \(^{13}\text{C}\)) recovered in a C pool \((^{13}\text{C}_{\text{pool, %}}\) of total added \()\) at time \(t\) after the labeling, it was related to the reference recovery of day 1 \((^{13}\text{C}_{\text{rec, %}}\) of total added of the corresponding plot.

\[
^{13}\text{C}_{\text{rec}} = \frac{^{13}\text{C}_{\text{pool}}}{^{13}\text{C}_{\text{rec, %}}} \cdot 100.
\]

Shoot respiration. Losses of assimilated \(^{13}\text{C}\) by shoot respiration were not measured. Therefore, shoot respiration \((^{13}\text{C}_{\text{shoot, %}}\) of recovered \(^{13}\text{C}\)) was estimated by the following equation:

\[
^{13}\text{C}_{\text{shoot}} = 100 - \left(^{13}\text{C}_{\text{shoot, %}} + ^{13}\text{C}_{\text{belowground}}\right).
\]

The sum of recovered \(^{13}\text{C}\) in shoots \((^{13}\text{C}_{\text{shoot, %}}\) of recovered \(^{13}\text{C}\)) and in below-ground C pools \((^{13}\text{C}_{\text{belowground, %}}\) of recovered \(^{13}\text{C}\)) (including \(^{13}\text{CO}_2\) efflux from soil) was subtracted from 100. This approach assumes that shoot respiration is the only missing sink for \(^{13}\text{C}\) in the considered system. Losses by leaching of dissolved organic matter were accepted to be negligible, as there was only one rainfall within the chase period and the soil was not affected by groundwater. As \(^{13}\text{C}\) was referred to the recovery 1 day after the labeling, shoot respiration is presented for the first time 5 days after the labeling.

C stocks and biomass ratio. For the comparison of C sequestration between the grazed and ungrazed grassland, C stocks (kg C ha⁻¹) of the above- and below-ground biomass and of the soil were calculated. Carbon stocks in the soil layers 0–5, 5–15 and 15–30 cm were calculated using the following equation:

\[
\text{Cstock} = z \cdot \rho \cdot C \cdot 1000,
\]

where \(z\) (cm) is the thickness of the considered soil layer, \(\rho\) (g cm⁻³) is the bulk density and \(C\) (%) is the C content (Table 2).
The significance of differences between grazed and ungrazed plots considering the above- and below-ground plant biomass and SOC stocks was obtained by using one-way ANOVA, which was calculated separately for every layer. Where homogeneity of variances was confirmed, a post hoc Tukey HSD test was applied. The non-parametric Mann–Whitney U-test was applied to reveal significant differences between the grazed and ungrazed grassland in 13C partitioning during the chase period, after 27 days and for every time step of 13C dynamics. To reveal significant differences between the sampling steps of either the grazed or the ungrazed grassland, the non-parametric Wilcoxon matched pairs test was applied. Means and standard errors are presented in the figures and tables. Statistical analysis was carried out using STATISTICA for Windows (version 7.0; StatSoft Inc., Tulsa, OK, USA).

### Results

#### Above- and below-ground carbon stocks

Above-ground C stocks differed significantly between the grazed and ungrazed plots after 7 years (Table 2). 2.4 Mg C ha\(^{-1}\) was stored in shoots at the plot used for winter grazing, corresponding to one-third of the above-ground C stocks of the ungrazed plot (7.3 Mg C ha\(^{-1}\)) (Table 2). In contrast, root C stock was significantly decreased due to the absence of grazing. These changes are indicated by the below- to above-ground biomass ratio, which was significantly smaller in the ungrazed grassland (0.1) than in the grazed grassland (0.5). The major differences in root C stocks appeared in the upper 5 cm of the soil profile, where 1 Mg C ha\(^{-1}\) at the grazed and 0.3 Mg C ha\(^{-1}\) at the ungrazed plots were stored in roots. In this layer, the absence of grazing for 7 years decreased root C stocks by 70% (Table 2). In the deeper layers (5–15 and 15–30 cm) the root C stocks were only 50% smaller in the ungrazed vs. grazed plots.

A comparison of the SOC stocks in the upper 5 cm and the underlying 10 cm between the plots revealed a significantly smaller (P < 0.01) SOC stock in the ungrazed plots (Table 2). The absence of grazing for 7 years reduced SOC stocks in the upper 5 cm from 26 Mg C ha\(^{-1}\) in the soil under the grazed grasses to 20.5 Mg C ha\(^{-1}\) in the ungrazed treatment (Table 2).
The same effect was observed in the underlying 10 cm, where SOC stocks were reduced by 12 Mg C ha$^{-1}$. No effect of the grazing absence was revealed in soil deeper than 15 cm.

In conclusion, the absence of grazing during 7 years led to significant redistribution of C stocks from below- to above-ground, which is reflected not only in an increase of shoot and decrease of root biomass, but also in a decrease of SOC stocks in the soil.

$^{13}$C dynamics in the plant–soil system

After the labeling, $^{13}$C in shoots of both land use types followed an exponential decrease within the chase period (Fig. 1). The decline reflects C loss by shoot respiration and below-ground allocation of assimilated $^{13}$C. The dynamics of $^{13}$C allocation in shoots were similar for both land use types. This is confirmed by the recovery of $^{13}$C in shoots that did not differ significantly in any sampling between the grazed and ungrazed plots.

Total relocation of $^{13}$C from shoots to below-ground C pools and shoot respiration between the 1st day and the 27th day after labeling amounted to 36% and 52% of recovered $^{13}$C in the grazed and ungrazed treatment, respectively (Fig. 1). Different portions were used for below-ground C allocation and for shoot respiration (Fig. 2). The amount of $^{13}$C lost by shoot respiration was significantly larger in the ungrazed treatment in every sampling. The fact that C utilization for shoot respiration of the ungrazed grassland was higher at the beginning of the chase period is indicated because $^{13}$C dynamics were similar for both land use types. One month after the labeling 17% of recovered $^{13}$C was used for shoot respiration at the grazed site, which is less than half that of the ungrazed site (42% of recovered $^{13}$C) (Fig. 3).

$^{13}$C relocation to below-ground pools was significantly larger at the grazed site (Fig. 2). Maximum recovery of $^{13}$C in below-ground pools was 12 days after the labeling in the grazed treatment. This peak $^{13}$C recovery was induced by the maximum allocation rate in shoots between day 1 and 12 after labeling. In contrast, $^{13}$C recovery in all below-ground pools remained constant in the ungrazed treatment during the chase period. At the end of the chase period, 40% of $^{13}$C was recovered in all below-ground C pools in the grazed grassland, which was twice as much as in the ungrazed grassland (Fig. 3). The difference for below-ground relocation corresponded to the distribution of above- and below-ground biomass. Thus, the absence of grazing led to reduced below-ground C fluxes and increased C losses by shoot respiration.

Within the below-ground C pools, the least portion of $^{13}$C was incorporated into root tissue at both grasslands (Fig. 3). Only 1.6% and 0.5% of $^{13}$C was recovered in living roots of the grazed and ungrazed plots 1 day after labeling, respectively. The recovery in roots did
not vary within the following samplings (Fig. 4). However, $^{13}$C recovered in roots of the grasses under grazing was always significantly larger compared to the ungrazed grassland. Additionally, on average 0.7% and 0.2% of recovered $^{13}$C was found in dead roots (0–5 cm) during the chase period (in grazed and ungrazed treatment, respectively).

In soil the $^{13}$C recovery was much larger than in the roots of both land use types and at any time during the chase period (Fig. 4). One day after labeling, 14% and 7% of recovered $^{13}$C was found in the soil of the grazed and ungrazed grassland, respectively. Although $^{13}$C recovery in soil was higher than in the roots, the dynamics in the ungrazed treatment was similar to that in roots because the recovery remained constant. Furthermore, differences in the dynamics between the soil layers were not observed (Fig. 5). In contrast, a maximum in $^{13}$C recovery (26%) occurred 12 days after the labeling in soil at the grazed treatment. This maximum is related to an increase in $^{13}$C in the topsoil in 0–5 cm (Fig. 5). The observed increase between the 1st and 12th day after labeling was significant and resulted from the maximum relocation rate from shoots to below-ground pools at the beginning of the chase period (Fig. 1). In the underlying soil layers, $^{13}$C recovery remained constant during the chase period. The $^{13}$C amount that remained in soil at the end of the chase period was significantly higher under grazing and amounted to 18% of recovered $^{13}$C, compared to 10.4% in the ungrazed grassland (Fig. 3).

Similarly to the $^{13}$C in soil, $^{13}$C released from soil as CO$_2$ in the grazed grassland was always significantly larger in all samplings compared to the ungrazed site (Fig. 6). The highest mineralization rate of root assimilates and rhizodeposits in the grazed site (first until 12th day after labeling) corresponded to the maximum $^{13}$C recovery in soil. Within the chase period, only
half the $^{13}$C amount was mineralized to CO$_2$ at the ungrazed plots (9% of recovered $^{13}$C) vs. grazed plots (20% of recovered $^{13}$C) (Fig. 3).

In summary, grazing led to increased C input into soil and a higher contribution of assimilates to CO$_2$ efflux from soil.

Contribution of assimilates to CO$_2$ efflux from soil and CO$_2$ sources

Linear regression between CO$_2$ efflux and living root biomass identified the portions of root-derived CO$_2$ and SOM-derived CO$_2$ (Fig. 7). The SOM-derived CO$_2$ efflux amounted to 4.8 g C m$^{-2}$ day$^{-1}$, based on the y-intercept of the linear regression. On average 0.9 and 0.3 g C m$^{-2}$ day$^{-1}$ are derived from living roots (grazed and ungrazed variant, respectively), which corresponded to a percentage of 16% and 6% of total soil CO$_2$. These portions of root-derived CO$_2$ differed significantly. In contrast, average total CO$_2$ efflux of the grazed and ungrazed grassland (5.8 ± 0.5 and 5.0 ± 0.5 g C m$^{-2}$ day$^{-1}$) did not differ significantly. The contribution of recently assimilated C to root-derived CO$_2$ efflux as determined by $^{13}$C labeling, amounted to 20% and 9% of recovered $^{13}$C (grazed and ungrazed case, respectively).

To evaluate the lifetime of assimilates in roots and soil, the TR of assimilate C in below-ground pools was determined (Fig. 6). The TR refers to assimilates that were directly respired by roots and to rhizodeposits that were released to soil and mineralized by microorganisms. The TR of assimilates at the grazed plots (0.24 ± 0.06 day$^{-1}$) was higher than the TR of the ungrazed plots (0.18 ± 0.04 day$^{-1}$). Correspondingly, the MRT of assimilate C below-ground for the grazed plots was 4.2 days, which is shorter than the value under the ungrazed plots (5.5 days). This shows faster below-ground turnover of C under the grazed grassland.

In conclusion, grazing had no effect on total CO$_2$ efflux from soil, but root-derived CO$_2$ efflux and the contribution of assimilates to CO$_2$ efflux from soil and the turnover of root-derived C was increased.

Discussion

Effect of grazing on below-ground C allocation by plants

Below-ground C fluxes are lowered when grazing is absent: a significantly larger portion of recently assimilated $^{13}$C was found in below-ground C pools (40%) under moderate grazing compared to the exclosure plots (20%) (Fig. 3). An even greater percentage of photosynthetic $^{13}$C was translocated below-ground (58.7%) on a similar alpine Kobresia winter pasture at Haibei Research Station (Wu et al., 2010).

The lower below-ground C allocation induced a redistribution of C to above-ground biomass, as indicated by the living below- to above-ground biomass ratio: it decreased from 0.5 at the grazed plots to 0.1 at the ungrazed plots. Those ratios are valid for relative differences between the grazed and ungrazed plots because they were determined similarly. Nonetheless, the actual ratio of below- to above-ground biomass for grasslands is higher. For temperate grassland an average root : shoot ratio of 4.2 was determined (Mokany et al., 2006). In our study, smaller root : shoot ratios resulted from considering living roots only.
The higher nutrient requirement of the grasses under grazing increases C relocation to below-ground C pools. Therefore, altered interactions between roots and soil form the basis for an improved nutrient supply. Defoliation, for example, increases root exudation (Paterson & Sim, 1999, 2000; Hamilton & Frank, 2001; Kuzyakov et al., 2002), which increases the abundance and activity of microorganisms in the rhizosphere (Uhlírová et al., 2005; Blagodatskaya et al., 2009). This in turn accelerates SOM turnover (Blagodatskaya et al., 2007) and improves nutrient acquisition (Bardgett et al., 1998). Comparing the allocation pattern of both land use types, the main differences at the current development stage of the grasses occur in the photosynthetic C input into soil and CO2 efflux (Fig. 3). The pattern illustrates increased rhizodeposition under moderate grazing and subsequent increase in SOM turnover. Note, however, that defoliation of grasses grown under dry conditions leads to opposite effects, namely less microbial activity and consequently less CO2 efflux from soil (Gavrichkova et al., 2010). Furthermore, the necessity for grazed plants to allocate C below-ground as storage for regrowth after grazing (Lieth, 1978) explains the larger below-ground C fluxes, especially the higher C recovery in living roots.

Roots have been determined as the major C sink within the below-ground pools in several studies (Domanski et al., 2001; Wu et al., 2010). In contrast, the amount of 13C recovered in living roots was the smallest within the below-ground pools (Fig. 3) and much less than the amount of 13C remaining in soil. These differences reflect the plant development stage, which influences C incorporation into roots (Swinnen et al., 1994; Palta & Gregory, 1997; Kuzyakov et al., 1999). At the time of the labeling, most plants had been flowering (e.g. S. chamaesjasme, Trigonella rutheonica) or even fruiting (e.g. Leymus secalinus, Kobresia pygmaea) (Table 1). Therefore, low 13C incorporation can firstly be attributed to the investment of assimilates for building up generative organs and shoot tissue, and secondly to less need for root growth because the root system was already well developed. The period for root growth and storage is at the beginning and end of the growing season. Furthermore, the low incorporation of 13C into root tissue indicates direct utilization of non-structural C (starch, organic acids and soluble sugars such as glucose, fructose and sucrose) (Xu et al., 2008) for root respiration (Hall & Scurlock, 1991; Xu et al., 2008) and for rhizodeposition, which is illustrated by subsequent maximum 13C recovery in soil (Fig. 5). During the experiment no significant changes of 13C in roots were detected, but maximum 13C recovery in roots was 18 days after labeling (Fig. 4). This is in accordance with previous studies reporting that maximum recovery of recently assimilated C in roots occurred after several weeks (Rangel-Castro et al., 2004; Wu et al., 2010).

The used method of separating living from dead roots by color may contain errors (Wu et al., 2010). The insufficient separation and assignment of living roots as dead is partly indicated by the 13C recovery in dead roots in the first samplings after labeling. Although the 13C recovery was significantly lower than in living roots, the 13C incorporation into living roots was underestimated. In addition, the dominant plants growing on both plots are perennials (Table 1), and a large quantity of the already existing roots was not considered by this method.

Effect of grazing on SOC stocks

Land use changes on the TP affect grass biomass production and C sequestration in soil. Moderate grazing (2 yaks ha−1) increased root biomass and the below-ground C input and therefore had a positive effect on SOC storage in alpine meadows on the eastern TP (Gao et al., 2007).

By establishing exclosure plots, we simulated two contrasting grazing regimes. Seven years after the exclosure of grazing by yaks and sheep, the plants allocated less C below-ground and the SOC stocks in the upper 15 cm of the soil were reduced significantly. The effect on long-term C stock (SOC) was confirmed by the partitioning of recent assimilates, showing that the portion of plant-derived C remaining in soil was larger in the grazed (18%) vs. ungrazed grassland (10.4%), especially in the upper 15 cm (Fig. 3). Kuzyakov (2001) showed by evaluating various studies that the average long-term C sequestration under grasses is about 13% of assimilated C which matches our results. Relating the observed reduction of SOC stocks to the partitioning pattern of assimilates, we conclude that the incorporation of C into stable soil C pools decreased with the absence of grazing. Combining various studies, Rasse et al. (2005) showed that in soil the MRT of C originating from roots is 2.4 times longer than that of C originating from above-ground litter. This indicates that root litter and the transformation products are more resistant to degradation than shoot litter (Oades, 1988), enhancing SOM stabilization. The mechanisms for the higher contribution of root-C to stable soil C pools are (Puget & Drinkwater, 2001; Rasse et al., 2005) (i) higher chemical recalcitrance due to a higher lignin/N ratio in roots and higher tannin content compared to shoots, (ii) direct input of particulate organic matter at a scale of physically protected C and (iii) stabilization of rhizodeposits through binding on the mineral phase. As a consequence, the significantly higher SOC stocks in the upper 5 cm and underlying 10 cm due to grazing

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can be attributed to higher root biomass and subsequently enhanced C sequestration in soil. In contrast to the root residues, shoot residues undergo a long decomposition process above soil until reaching physically stabilized sites. Additionally, in the absence of grazing, above-ground litter accumulates on the soil surface and results in C immobilization (Schuman et al., 1999), as the decomposition of above-ground litter and therefore C incorporation into soil is decreased due to missing physical breakdown (Naeth et al., 1991).

Measurement of total CO$_2$ efflux from soil showed no significant differences between the land use types. With regard to changes in C stocks, it is necessary to partition CO$_2$ efflux into root-derived and SOM-derived components because root-derived CO$_2$ does not refer to C lost from soil (Wерh & Kuzyakov, 2008). Based on the linear regression between root biomass and total CO$_2$ efflux from soil, we showed that the proportion of root-derived CO$_2$ was significantly lower in the ungrazed (4%) vs. grazed plots (18%). The inaccuracy of the linear regression ($R = 0.81$) and the CO$_2$ efflux can be traced back to the variation of environmental parameters among the sampling steps (Kucera & Kirkham, 1971) that influence root-derived CO$_2$ as temperature (Kucera & Kirkham, 1971), soil moisture (Kucera & Kirkham, 1971) and photosynthetic activity (Kuzyakov & Gavrichkova, 2010). The $^{13}$C recovery in soil CO$_2$ of the ungrazed (9% of recovered $^{13}$C) and grazed site (20%) (Fig. 3) showed similar pattern than the share of root-derived C of total CO$_2$ efflux. This illustrates that a higher share of root-derived CO$_2$ of total CO$_2$ efflux is attended by a higher amount of recent assimilates partitioned to soil CO$_2$ efflux in the grazed plots. The significantly higher $^{13}$C amount in CO$_2$ efflux from soil of the grazed site is verified by the calculated TR of C in rhizodeposits and assimilates translocated to roots (Fig. 6). The TR estimated here by $^{13}$C pulse labeling were similar to those in experiments that artificially injected labeled substrates into soil (Jones et al., 2005). Higher TR in the grazed plot indicates a higher relative contribution of assimilates to CO$_2$ efflux from soil.

As the CO$_2$ efflux of the grazed and ungrazed grassland is similar, but the share of root-derived C of total CO$_2$ efflux is smaller and the turnover of recent assimilates partitioned to soil CO$_2$ efflux is slower in the ungrazed grassland we conclude that the missing portion is compensated by the decomposition of medium-term (living and dead roots) to long-term C stocks (SOC).

Conclusions
Seven years without grazing reduced SOC stocks in the layers 0–5 and 5–15 cm by three processes: (i) lower total C input into the soil by plants due to decreased allocation of assimilates belowground, including (a) the reduction of total root amounts, and (b) reduction of rhizodeposition, (ii) less incorporation of root-derived C into stable soil C pools compared to the grazed grassland and (iii) the decomposition of medium-term (living and dead roots) to long-term C stocks (SOC) accompanied by decreased input.

$^{13}$C labeling experiments combined with the evaluation of C stocks demonstrated a negative effect of grazing exclusion on short-term (rhizodeposition including exudation), medium-term and long-term C stock in the upper 15 cm of the soil profile. We conclude that the absence of grazing in remote areas leads to a decrease in C sequestration and that sustainable moderate grazing is a suitable tool to preserve the high ability of the montane pasture land to store C.

It should be noted, however, that the results cannot be extrapolated to heavy grazed pastures because of very strong degradation of the vegetation cover, disruption of the Kobresia turf with subsequent fatal consequences for soils not only concerning the C sequestration, but also concerning complete loss of the whole soil profile by wind and water erosion.

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