

# Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by $^{13}\text{C}$ pulse labeling

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## 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*  $^{13}\text{C}$  pulse labeling of (i) a montane *Kobresia* winter pasture of yaks, with moderate grazing regime and (ii) a 7-year-old grazing enclosure plot, both in 3440 m asl. Twenty-seven days after the labeling,  $^{13}\text{C}$  incorporated into shoots did not differ between the grazed (43% of recovered  $^{13}\text{C}$ ) 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  $^{13}\text{C}$  was incorporated into living root tissue of both land use types. In the grazed plots about twice the amount of  $^{13}\text{C}$  remained in soil (18%) and was mineralized to  $\text{CO}_2$  (20%) as compared to the ungrazed plots (soil 10%;  $\text{CO}_2$  9%). Despite the higher contribution of root-derived C to  $\text{CO}_2$  efflux, total  $\text{CO}_2$  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  $\text{CO}_2$  efflux create a positive effect of moderate grazing on soil C input and C sequestration.

**Keywords:**  $^{13}\text{C}$  pulse labeling, C allocation, grazing enclosure 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  $\text{km}^2$ ) 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  $\text{km}^2$  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 (Kuziyakov & 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

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the northeast of the TP to 5960 m asl on the northern slope of Mt Everest, covering an area of 450 000 km<sup>2</sup> (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 CO<sub>2</sub> 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 enclosure 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 CO<sub>2</sub> 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* <sup>13</sup>C 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-ISRIC-FAO, 2006), having an Ah mollic horizon with a thickness of 24 cm and secondary carbonates of pseudomycel type in the lower soil horizon Bkc 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<sup>-1</sup> a<sup>-1</sup> and 0.7 yaks ha<sup>-1</sup> a<sup>-1</sup> and corresponding to 19 livestock units ha<sup>-1</sup> a<sup>-1</sup> (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 <sup>13</sup>CO<sub>2</sub> labeling was conducted on 27 July 2009: three replicate plots were labeled on both the grazed and ungrazed treatment. The <sup>13</sup>CO<sub>2</sub> 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 and additionally sealed with wet soil afterwards. The <sup>13</sup>CO<sub>2</sub>

pulse was produced by injecting 8 mL of 4 M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) into a solution of distilled water containing 0.8 g sodium carbonate (Na<sub>2</sub><sup>13</sup>CO<sub>3</sub>) enriched with <sup>13</sup>C to 99 atom%. Plastic vials containing Na<sub>2</sub><sup>13</sup>CO<sub>3</sub> solution were fixed to the metal bars inside the chamber. The chamber was then closed and the sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added from the outside into the Na<sub>2</sub><sup>13</sup>CO<sub>3</sub> solution, using syringes, to ensure complete evolution of <sup>13</sup>CO<sub>2</sub> into the chamber atmosphere. To guarantee a uniform distribution of <sup>13</sup>CO<sub>2</sub>, a 5-volt fan was used inside the chamber. The progression of the CO<sub>2</sub> concentration inside the chamber was controlled by a CO<sub>2</sub> sensor (GM 70, Vaisala, Helsinki, Finland). As the initial value of the CO<sub>2</sub> 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.

#### Sampling and analyzes

Fluxes of assimilated C were traced as <sup>13</sup>C throughout a 27-day chase period in shoots, roots, soil and soil CO<sub>2</sub> 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 CO<sub>2</sub> efflux, (ii) the turnover of recent assimilates used for root and rhizomicrobial respiration and (iii) root-derived soil CO<sub>2</sub> efflux which comprises rhizomicrobial and root respiration (Kuzyakov, 2006). With this method, CO<sub>2</sub> 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

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

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 CO<sub>2</sub> emission from soil. CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> efflux from soil, CO<sub>2</sub> trapped in NaOH was analyzed by titrating NaOH against 0.1 M hydrochloric acid (HCl). Soil CO<sub>2</sub> efflux  $^{soil}CO_2$  (g C m<sup>-2</sup> day<sup>-1</sup>) was calculated by the following equation:

$$^{soil}CO_2 = \frac{mC}{A \cdot \Delta t}, \quad (1)$$

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 CO<sub>2</sub> efflux, 2 M SrCl<sub>2</sub> was added into NaOH to produce SrCO<sub>3</sub> precipitation for <sup>13</sup>C measurement. After neutralization and drying of SrCO<sub>3</sub> the  $\delta^{13}C$  signature was determined.

To evaluate the CO<sub>2</sub> fluxes from the <sup>13</sup>C labeled plots determined by the static alkali absorption method, CO<sub>2</sub> efflux from adjacent unlabeled plots was determined with a CO<sub>2</sub> sensor (GM 70, Vaisala) installed in closed chambers (area 144 cm<sup>2</sup>). The CO<sub>2</sub> increase in the closed chambers was determined 10 times in 5 min and the CO<sub>2</sub> efflux rate was calculated based on linear regression. To obtain a daily mean for CO<sub>2</sub> efflux within the chase period of both land use types, measurements of the CO<sub>2</sub> efflux rate were repeated 27 times within 24 h on both plots on 14 August 2009.

For  $\delta^{13}C$  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  $\delta^{13}C$  signature and the total C content of shoots, roots, soil and SrCO<sub>3</sub> 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.** <sup>13</sup>C incorporation into plant and soil C pools derived from pulse labeling is presented as percentage of <sup>13</sup>C recovery. As a reference, <sup>13</sup>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% <sup>13</sup>C of total added in grazed and ungrazed treatment, respectively). All calculations were done using <sup>13</sup>C atom%. The obtained data of  $\delta^{13}C$  (‰) by element analyzer - isotope ratio mass spectrometer were recalculated as follows. First, the isotopic ratio (<sup>13</sup>C/<sup>12</sup>C) of each sample  $R_{sample}$  was calculated:

$$R_{sample} = \left( \frac{\delta^{13}C}{1000} \cdot R_{PDB} \right) + R_{PDB}. \quad (2)$$

$R_{PDB} = 0.011237$  is the isotopic ratio of <sup>13</sup>C/<sup>12</sup>C in Pee Dee Belemnite.

Second, <sup>13</sup>C<sub>atom%</sub> (% of total C atoms) of the sample was calculated:

$$^{13}C_{atom\%} = \left( \frac{R_{sample}}{R_{sample} + 1} \right) \cdot 100. \quad (3)$$

The enrichment of <sup>13</sup>C in a C pool (<sup>13</sup>C<sub>atom% excess</sub>, % of total C atoms) derived from the pulse labeling was calculated by subtracting the amount of <sup>13</sup>C in the natural abundance sample (<sup>13</sup>C<sub>atom% of NA</sub>, % of total C atoms) from the amount of <sup>13</sup>C in the sample (<sup>13</sup>C<sub>atom% of sample</sub>, % of total C atoms)

$$^{13}C_{atom\% \text{ excess}} = ^{13}C_{atom\% \text{ of sample}} - ^{13}C_{atom\% \text{ of NA}}. \quad (4)$$

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

$$^{13}C_t = \frac{^{13}C_{t \text{ atom\% excess}}}{100} \cdot C_{pool}. \quad (5)$$

To obtain the weighted <sup>13</sup>C (<sup>13</sup>C<sub>rec</sub>, % of recovered <sup>13</sup>C) recovered in a C pool <sup>13</sup>C<sub>t pool</sub> (% <sup>13</sup>C of total added) at time  $t$  after the labeling, it was related to the reference recovery of day 1 (<sup>13</sup>C<sub>t 1 rec</sub>, % <sup>13</sup>C of total added) of the corresponding plot.

$$^{13}C_{rec} = \frac{^{13}C_{t \text{ pool}}}{^{13}C_{t 1 \text{ rec}}} \cdot 100. \quad (6)$$

**Shoot respiration.** Losses of assimilated <sup>13</sup>C by shoot respiration were not measured. Therefore, shoot respiration (<sup>shoot</sup>CO<sub>2</sub>, % of recovered <sup>13</sup>C) was estimated by the following equation:

$$^{shoot}CO_2 = 100 - (^{13}C_{shoot} + ^{13}C_{belowground}). \quad (7)$$

The sum of recovered <sup>13</sup>C in shoots (<sup>13</sup>C<sub>shoot</sub>, % of recovered <sup>13</sup>C) and in below-ground C pools (<sup>13</sup>C<sub>belowground</sub>, % of recovered <sup>13</sup>C) (including <sup>13</sup>CO<sub>2</sub> efflux from soil) was subtracted from 100. This approach assumes that shoot respiration is the only missing sink for <sup>13</sup>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 <sup>13</sup>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<sup>-1</sup>) 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:

$$C_{stock} = z \cdot \rho \cdot C \cdot 1000, \quad (8)$$

where  $z$  (cm) is the thickness of the considered soil layer,  $\rho$  (g cm<sup>-3</sup>) is the bulk density and  $C$  (%) is the C content (Table 2).

**Table 2** Above- and below-ground C stocks (Mg C ha<sup>-1</sup>) of the grazed and ungrazed grassland

	Depth (cm)	Grazed	Ungrazed
Shoot		2.35 ± 0.15	7.28 ± 0.05**
Dead roots	0–5	1.61 ± 0.11	1.17 ± 0.27
Living roots	0–5	1.01 ± 0.01	0.30 ± 0.01**
	5–15	0.33 ± 0.01	0.15 ± 0.01**
	15–30	0.54 ± 0.01	0.31 ± 0.02**
Soil	0–5	26.1 ± 1.30	20.5 ± 1.40**
	5–15	41.8 ± 1.70	29.1 ± 3.60**
	15–30	33.8 ± 1.52	34.5 ± 0.66
Total below-ground C stocks	0–30	103.6 ± 4.04	84.9 ± 3.35**

\*\*Indicates highly significant differences at  $P < 0.01$  between the grazed and ungrazed plots.

To determine redistribution of C between above- and below-ground biomass the ratio between living roots and shoots was calculated.

*Turnover rate and mean residence time of rhizodeposits and plant assimilates used for root and rhizomicrobial respiration.* To determine the combined turnover rate (TR) of rhizodeposits and plant assimilates used for root and rhizomicrobial respiration, an exponential decay function was fitted to the <sup>13</sup>C<sub>2</sub> efflux rate from soil:

$$C_t = C_0 \cdot e^{-kt} \quad (9)$$

Here,  $C_t$  (% of recovered <sup>13</sup>C day<sup>-1</sup>) is the <sup>13</sup>C<sub>2</sub> efflux rate at time  $t$  and  $C_0$  (% of recovered <sup>13</sup>C day<sup>-1</sup>) is the initial <sup>13</sup>C<sub>2</sub> efflux rate at the beginning of the labeling.  $t$  (day) represents the ongoing time after the labeling in days and  $k$  is the decay constant that corresponds to the TR (day<sup>-1</sup>):

$$TR = k. \quad (10)$$

Additionally, the combined mean residence time, MRT (day) of C in rhizodeposits and plant assimilates used for root respiration and rhizodeposition is calculated as the reciprocal value of the TR:

$$MRT = \frac{1}{TR}. \quad (11)$$

*Partitioning of total CO<sub>2</sub> efflux from soil into SOM- and root-derived CO<sub>2</sub>.* The partitioning of total CO<sub>2</sub> efflux from soil into SOM-derived CO<sub>2</sub> (in this study SOM-derived CO<sub>2</sub> and CO<sub>2</sub> originating from the decomposition of dead plant remains) and root-derived CO<sub>2</sub> (the sum of root respiration and rhizomicrobial respiration) (Kuzaykov, 2006) was done by the root-regression method based on a positive linear correlation between root biomass and CO<sub>2</sub> efflux from soil (Kucera & Kirkham, 1971). The  $y$ -intercept (total CO<sub>2</sub> efflux rate) of the extrapolated regression line corresponds to SOM-derived CO<sub>2</sub>. Root-derived CO<sub>2</sub> was revealed by subtracting SOM-

derived CO<sub>2</sub> from the total CO<sub>2</sub> efflux. Linear regression was done by correlating average root biomass (0–30 cm) for every single plot vs. the related total CO<sub>2</sub> efflux rate, determined by the alkali absorption method. The average total CO<sub>2</sub> efflux rate within the chase period for the grazed and ungrazed sites was used to reveal differences in the portion of root-derived CO<sub>2</sub> between the two plots.

### Statistics

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 affiliated. The non-parametric Mann–Whitney  $U$ -test was applied to reveal significant differences between the grazed and ungrazed grassland in <sup>13</sup>C partitioning during the chase period, after 27 days and for every time step of <sup>13</sup>C 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<sup>-1</sup> 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<sup>-1</sup>) (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<sup>-1</sup> at the grazed and 0.3 Mg C ha<sup>-1</sup> 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<sup>-1</sup> in the soil under the grazed grasses to 20.5 Mg C ha<sup>-1</sup> 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<sup>-1</sup>. 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.

### <sup>13</sup>C dynamics in the plant–soil system

After the labeling, <sup>13</sup>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 <sup>13</sup>C. The dynamics of <sup>13</sup>C allocation in shoots were similar for both land use types. This is confirmed by the recovery of <sup>13</sup>C in shoots that did not differ significantly in any sampling between the grazed and ungrazed plots.

Total relocation of <sup>13</sup>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 <sup>13</sup>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 <sup>13</sup>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 <sup>13</sup>C dynamics were similar for both land use types. One month after the labeling 17% of recovered <sup>13</sup>C was used for shoot respiration at the grazed site, which is less than half that of the ungrazed site (42% of recovered <sup>13</sup>C) (Fig. 3).

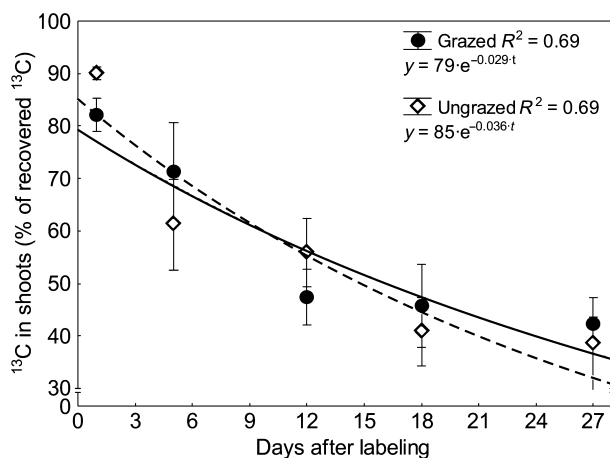


Fig. 1 <sup>13</sup>C dynamics in shoots during the chase period.

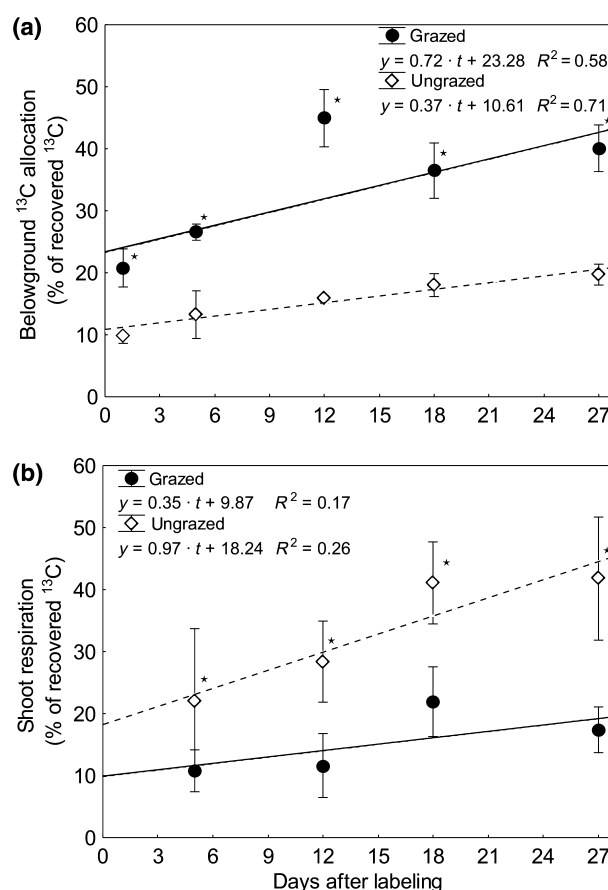
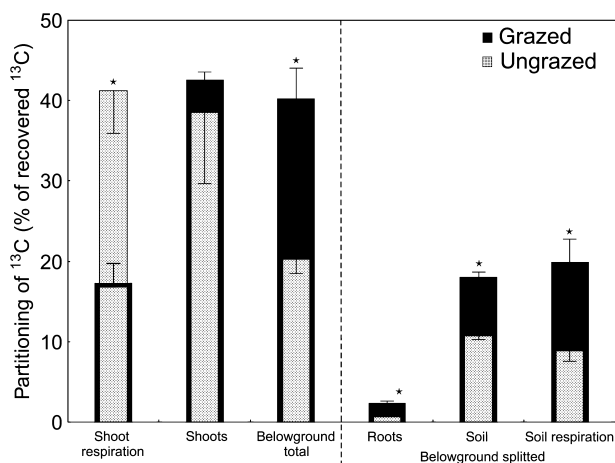


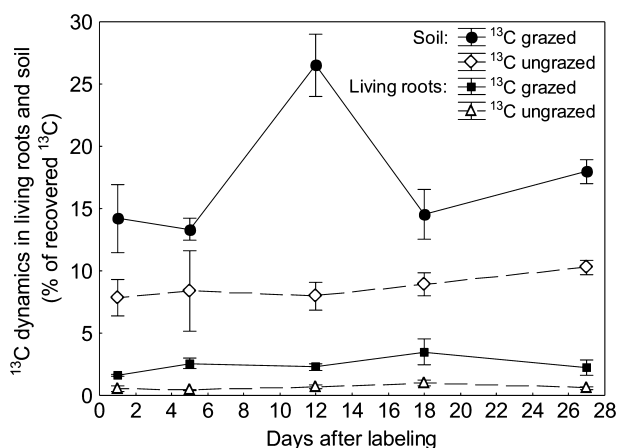
Fig. 2 <sup>13</sup>C allocation to below-ground C pools (roots, soil and soil respiration) (a) and <sup>13</sup>C losses by shoot respiration (b) during 27 days after labeling. \*Denotes significant differences at  $P < 0.05$  between the grazed and ungrazed plots.

<sup>13</sup>C relocation to below-ground pools was significantly larger at the grazed site (Fig. 2). Maximum recovery of <sup>13</sup>C in below-ground pools was 12 days after the labeling in the grazed treatment. This peak <sup>13</sup>C recovery was induced by the maximum allocation rate in shoots between day 1 and 12 after labeling. In contrast, <sup>13</sup>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 <sup>13</sup>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 <sup>13</sup>C was incorporated into root tissue at both grasslands (Fig. 3). Only 1.6% and 0.5% of <sup>13</sup>C was recovered in living roots of the grazed and ungrazed plots 1 day after labeling, respectively. The recovery in roots did



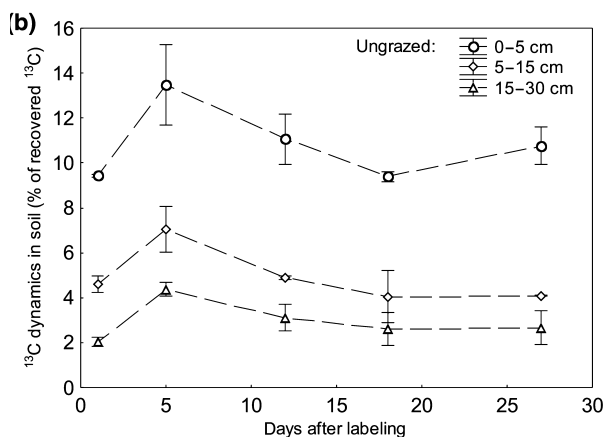
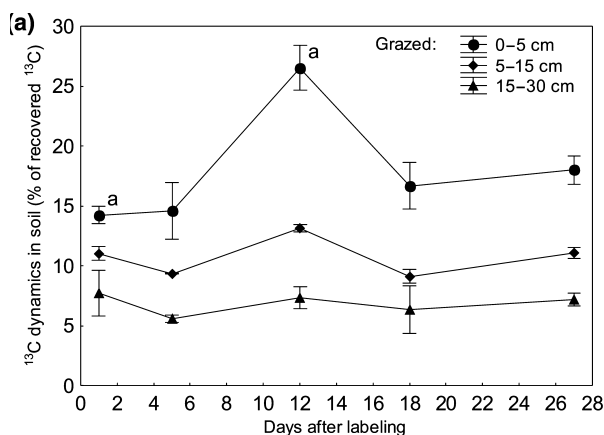
**Fig. 3** Partitioning of  $^{13}\text{C}$  27 days after the assimilation. \*Denotes significant differences at  $P < 0.05$  between the grazed and ungrazed plots.



**Fig. 4**  $^{13}\text{C}$  dynamics during 27 days in roots and soil (0–30 cm).

not vary within the following samplings (Fig. 4). However,  $^{13}\text{C}$  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  $^{13}\text{C}$  was found in dead roots (0–5 cm) during the chase period (in grazed and ungrazed treatment, respectively).

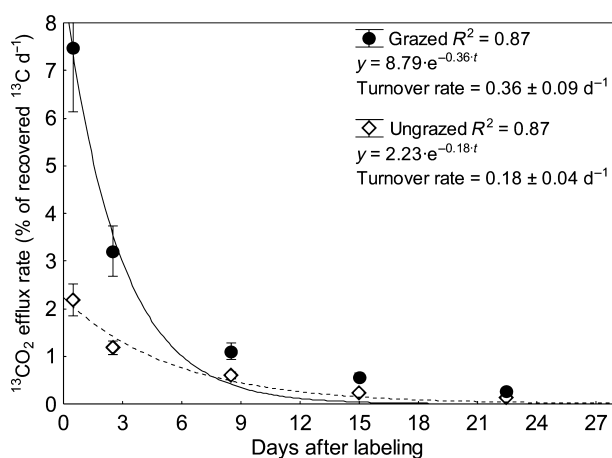
In soil the  $^{13}\text{C}$  recovery was much larger than in the roots of both land use types and at any time during the chase period (Fig. 4). One day after labeling, 14% and 7% of recovered  $^{13}\text{C}$  was found in the soil of the grazed and ungrazed grassland, respectively. Although  $^{13}\text{C}$  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  $^{13}\text{C}$  recovery (26%) occurred 12 days after the



**Fig. 5**  $^{13}\text{C}$  recovered in soil during 27 days in the soil layers 0–5, 5–15 and 15–30 cm of the grazed (a) and ungrazed (b) plots. (a) denotes significant differences at  $P < 0.05$  between the  $^{13}\text{C}$  recovery of day 1 and 12 after labeling in the soil layer 0–5 cm of the grazed plots.

labeling in soil at the grazed treatment. This maximum is related to an increase in  $^{13}\text{C}$  in the topsoil 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,  $^{13}\text{C}$  recovery remained constant during the chase period. The  $^{13}\text{C}$  amount that remained in soil at the end of the chase period was significantly higher under grazing and amounted to 18% of recovered  $^{13}\text{C}$ , compared to 10.4% in the ungrazed grassland (Fig. 3).

Similarly to the  $^{13}\text{C}$  in soil,  $^{13}\text{C}$  released from soil as  $\text{CO}_2$  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  $^{13}\text{C}$  recovery in soil. Within the chase period, only



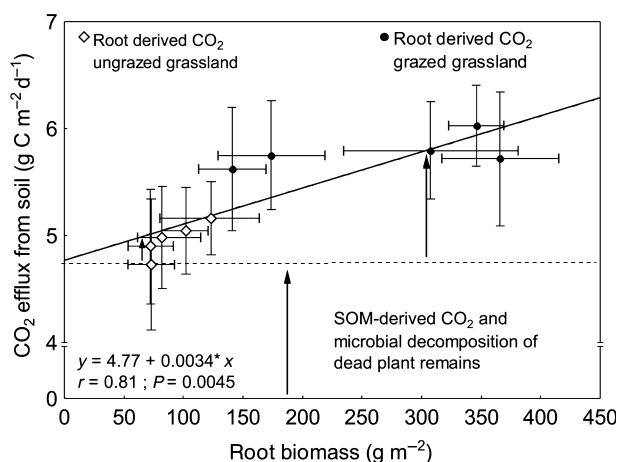
**Fig. 6**  $^{13}\text{CO}_2$  efflux rate from soil of the grazed and ungrazed grasslands, curve fitted by first-order kinetics and calculated turnover rates of non-structural C used for root and rhizomicrobial respiration.

half the  $^{13}\text{C}$  amount was mineralized to  $\text{CO}_2$  at the ungrazed plots (9% of recovered  $^{13}\text{C}$ ) vs. grazed plots (20% of recovered  $^{13}\text{C}$ ) (Fig. 3).

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

#### Contribution of assimilates to $\text{CO}_2$ efflux from soil and $\text{CO}_2$ sources

Linear regression between  $\text{CO}_2$  efflux and living root biomass identified the portions of root-derived  $\text{CO}_2$  and SOM-derived  $\text{CO}_2$  (Fig. 7). The SOM-derived  $\text{CO}_2$



**Fig. 7** Linear regression between living root biomass and total  $\text{CO}_2$  efflux from soil ( $n = 5$ ) for the grazed and ungrazed plots. Arrows indicate the portion of SOM-derived  $\text{CO}_2$  and the differences in root-derived  $\text{CO}_2$  for the grazed and ungrazed grassland.

efflux amounted to  $4.8 \text{ g C m}^{-2} \text{ day}^{-1}$ , based on the y-intercept of the linear regression. On average  $0.9$  and  $0.3 \text{ g C m}^{-2} \text{ day}^{-1}$  are derived from living roots (grazed and ungrazed variant, respectively), which corresponded to a percentage of 16% and 6% of total soil  $\text{CO}_2$ . These portions of root-derived  $\text{CO}_2$  differed significantly. In contrast, average total  $\text{CO}_2$  efflux of the grazed and ungrazed grassland ( $5.8 \pm 0.5$  and  $5.0 \pm 0.5 \text{ g C m}^{-2} \text{ day}^{-1}$ ) did not differ significantly. The contribution of recently assimilated C to root-derived  $\text{CO}_2$  efflux as determined by  $^{13}\text{C}$  labeling, amounted to 20% and 9% of recovered  $^{13}\text{C}$  (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 ( $0.24 \pm 0.06 \text{ day}^{-1}$ ) was higher than the TR of the ungrazed plots ( $0.18 \pm 0.04 \text{ day}^{-1}$ ). 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  $\text{CO}_2$  efflux from soil, but root-derived  $\text{CO}_2$  efflux and the contribution of assimilates to  $\text{CO}_2$  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  $^{13}\text{C}$  was found in below-ground C pools (40%) under moderate grazing compared to the enclosure plots (20%) (Fig. 3). An even greater percentage of photosynthetic  $^{13}\text{C}$  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 (Patterson & Sim, 1999, 2000; Hamilton & Frank, 2001; Kuzyakov *et al.*, 2002), which increases the abundance and activity of microorganisms in the rhizosphere (Uhlířová *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 CO<sub>2</sub> 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 CO<sub>2</sub> 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) explain 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 <sup>13</sup>C recovered in living roots was the smallest within the below-ground pools (Fig. 3) and much less than the amount of <sup>13</sup>C 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. chamaejasme*, *Trigonella ruthenica*) or even fruiting (e.g. *Leymus secalinus*, *Kobresia pygmaea*) (Table 1). Therefore, low <sup>13</sup>C 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 <sup>13</sup>C 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 <sup>13</sup>C recovery in soil (Fig. 5). During the experiment no significant changes of <sup>13</sup>C in roots were detected, but maximum <sup>13</sup>C 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 <sup>13</sup>C recovery in dead roots in the first samplings after labeling. Although the <sup>13</sup>C recovery was significantly lower than in living roots, the <sup>13</sup>C 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<sup>-1</sup>) 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

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<sub>2</sub> 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<sub>2</sub> efflux into root-derived and SOM-derived components because root-derived CO<sub>2</sub> does not refer to C lost from soil (Werth & Kuzyakov, 2008). Based on the linear regression between root biomass and total CO<sub>2</sub> efflux from soil, we showed that the proportion of root-derived CO<sub>2</sub> was significantly lower in the ungrazed (4%) vs. grazed plots (18%). The inaccuracy of the linear regression ( $R = 0.81$ ) and the CO<sub>2</sub> efflux can be traced back to the variation of environmental parameters among the sampling steps (Kucera & Kirkham, 1971) that influence root-derived CO<sub>2</sub>, as temperature (Kucera & Kirkham, 1971), soil moisture (Kucera & Kirkham, 1971) and photosynthetic activity (Kuzyakov & Gavrichkova, 2010). The <sup>13</sup>C recovery in soil CO<sub>2</sub> of the ungrazed (9% of recovered <sup>13</sup>C) and grazed site (20%) (Fig. 3) showed similar pattern than the share of root-derived C of total CO<sub>2</sub> efflux. This illustrates that a higher share of root-derived CO<sub>2</sub> of total CO<sub>2</sub> efflux is attended by a higher amount of recent assimilates partitioned to soil CO<sub>2</sub> efflux in the grazed plots. The significantly higher <sup>13</sup>C amount in CO<sub>2</sub> 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 <sup>13</sup>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<sub>2</sub> efflux from soil.

As the CO<sub>2</sub> efflux of the grazed and ungrazed grassland is similar, but the share of root-derived C of total CO<sub>2</sub> efflux is smaller and the turnover of recent assimilates partitioned to soil CO<sub>2</sub> 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.

<sup>13</sup>C labeling experiments combined with the evaluation of C stocks demonstrated a negative effect of grazing exclosure 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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