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Short communication

# Grazing exclusion decreases soil organic C storage at an alpine grassland of the Qinghai–Tibetan Plateau





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#### ABSTRACT

Grazing exclusion has been proposed as a choice for restoring degraded grasslands on the Qinghai-Tibetan Plateau, but its effects on soil properties are not clear. The present study was designed to investigate whether various soil organic carbon (OC) and nitrogen (N) pools and enzymatic activities were changed through grazing exclusion. A paddock of grassland was fenced in May 2002 for exclusion of livestock grazing, while the surrounding grassland continued conventional grazing by yak (Bos grunniens) and sheep (Ovis aries). Eight years after grazing exclusion, besides a reduction in plant species, the root biomass and soil bulk density in the top 15-cm depth were reduced by 34% and 26%, respectively, compared to the grazed grassland. Grazing exclusion enhanced the C/N ratios of shoots and roots by 18-19%, indicating a quality reduction in the shoot and root litters compared with the non-exclusion. Grazing exclusion also lowered stocks of total soil OC and N, microbial biomass C and N, and acid-extracted carbohydrate C and soil enzymatic activities (per area) of  $\beta$ -glucosidase, urase, and phosphatase in the 0–15 cm soil layer. Under grazing exclusion, less C input from the root-associated sources and possibly greater C output through heterotrophic respiration might have reduced various soil OC storages. However, a significant increase in soil mineral N pool was found under no grazing compared to grazing, possibly due to less plant N demand and uptake and change in N mineralization and/or immobilization. In conclusion, grazing exclusion is not beneficial to soil OC sequestration on the northeastern Qinghai-Tibetan Plateau.

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#### 1. Introduction

It is generally considered that overgrazing is largely responsible for grassland degradation in the Qinghai–Tibetan Plateau (Han et al., 2008; Miehe et al., 2009; Harris, 2010). Grazing exclusion has been recommended as one of measures for restoring degraded grasslands in this region (Han et al., 2008). The restoration strategy is expected to improve degraded primary productivity and soil quality. However, grazing exclusion may induce the replacement of grazing weeds that highly adapted to grazing by other functional types (Miehe et al., 2009), and alter energy flow and material cycling due to the absence of defoliation, trampling, and dung and urine depositions (Altesor et al., 2005). Above- and below-ground productivity and soil organic matter (OM) pool usually display variable effects in response to grazing exclusion (Milchunas and Lauenroth, 1993; McNaughton et al., 1998; Shrestha and Stahl, 2008; Rueda et al., 2010). On the Qinghai–Tibetan Plateau, Wu et al. (2010a) reported that ungrazed grassland had higher soil OC storage than grazed grassland, but Hafner et al. (2012) found a significant reduction in soil OC pool.

Soil OC content is often used as a measure of soil quality (Bodlák et al., 2012). Labile fractions of soil OM such as light OM, carbohydrates, and microbial biomass and soil enzyme activities change more rapidly than the total soil OM in response to varying land use and management (Gregorich et al., 1994), and thus are good indictors of changes in soil quality (Holt, 1997; Vasconcellos et al., 2013). In the present study, a grazing exclusion experiment was established in 2002 on the north eastern fringe of Qinghai–Tibetan Plateau to assess changes in soil quality. Total OC and N, light OC and N, acid-extracted carbohydrates, mineral N, microbial biomass, and enzymatic activities were compared between the grasslands of grazing exclusion and non-exclusion for eight years.

#### 2. Materials and methods

The study site  $(37^{\circ}17' \text{ N}, 102^{\circ}31' \text{ E}, 3600 \text{ m} \text{ a.s.l.})$  was close to Gansu Agricultural University alpine Grassland Experimental Station  $(37^{\circ}40' \text{ N}, 102^{\circ}32' \text{ E}, 2960 \text{ m} \text{ a.s.l.})$  where mean annual

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Grazed grassland

**Fig. 1.** Soils were sampled 2 m away from the rectangular fence: inside for grazing exclusion and outside for grazing inclusion.

temperature and precipitation are  $-0.1 \,^{\circ}$ C and 416 mm, with a plant growth period of <120 days. The vegetation was dominated by grasses (*Kobresia humilis, Carex scabrirostris, Stipa aliena, Elymus nutans, Poa crymophila, Festuca ovina*), along with some forbs and legumes. Soil is similar to Cryrendoll developed from homogeneous loess (>1 m in depth), and has a particle-size composition of sand 1–2%, silt 65–67% and clay 31–32%, with a pH range of 6.7–7.1 (soil: water = 1: 2.5).

The site and neighboring areas were open grasslands and extensively grazed for a long time by yak (*Bos grunniens*) and sheep (*Ovis aries*). In May 2002, a paddock ( $200 \text{ m} \times 50 \text{ m}$ ) of grassland was fenced out at the center of a large south-facing slope (about 15°) that had a uniform vegetation cover, plant species composition, and soil properties. Since its establishment, the fenced plot has completely been excluded from livestock grazing, while the surrounding grassland continued conventional grazing by yak and sheep (about 1.64 animals per ha) around the year. This grazing intensity was relatively heavy, as shown by vegetation performance in the field (Fig. S1). It was estimated that more than 90% of the aboveground biomass was consumed by the animals.

On the Qinghai–Tibetan Plateau, since the majority of the roots in meadows grow in the top 15-cm soil depth (Li et al., 2011; Wu et al., 2011), grazing exclusion induced changes in soil quality would mainly happen in this horizon. Soils in grazing exclusion and inclusion were sampled in September (the end of growth season) 2008 and 2010, respectively, and in June (the beginning of growth season) 2010. For grazing inclusion, soil samples were taken 2 m away from the rectangular fence, with one composite sample taken on each of width sides and two composite samples on each of length sides (Fig. 1). Each composite sample was pooled in the 0–15 cm depth from 5 to 7 random soil cores (taken using an auger, 38 mm in inner diameter). Similarly, soil samples for grazing exclusion were taken inside the fence and 2 m away from the rectangular fence (Fig. 1). All together, 6 composite samples were collected for inclusion and 6 for exclusion, with each treatment pseudo replicated, a common procedure also used in other studies (*cf.*, Hafner et al., 2012; Medina-Roldán et al., 2012). With a big area, homogeneous topography and soil texture, and random sampling, the obtained information would certainly reflect the changes in soil biological attributes induced by exclusion.

After removal of roots, each composite soil sample was sieved to <2 mm and split into two sub-samples. One sub-sample was kept at 4 °C for analyses of soil microbial biomass C and N, soil enzymatic activities, acid-extracted carbohydrates, and mineral N. The other sub-sample was air-dried at room temperature for measuring basic soil properties and total and light soil OC and N.

At soil sampling in September 2010, we measured vegetation composition and covers of species, total above- and below-ground (15 cm in depth) biomasses, and soil bulk density. Vegetation composition and above- and below-ground biomass were measured on randomly selected quadrates ( $50 \text{ cm} \times 50 \text{ cm}$ ) (Zhang, 2006), with six quadrates outside and six inside the fence, where soils were sampled. Shannon–Wiener index (*H*) was calculated accord-

ing to the formula 
$$H = -\sum_{i=1}^{3} P_i \ln(P_i)$$
, where  $P_i$  is the proportion

of species *i* relative to the total number of species; *S* is the total number of species in the community (richness). Roots in the composite soil samples (taken using an auger, 38 mm in inner diameter) were carefully collected after washing off the soil on a 2-mm sieve. Above- and below-ground biomasses were weighted after ovendrying at 80 °C, and ground for C and N analyses. Soil bulk densities were determined in the 15-cm depth using a cutting ring (volume  $100 \text{ cm}^3$ , inner diameter 3.8 mm) (Institute of Soil Science, 1978). For estimation of animal excrement input in the grazed area, feces were collected on 4 selected plots (5 m × 5 m) around the fence and the weights were recorded after oven-drying at 80 °C.

The separation of light OM was performed accordingly to the method modified by Six et al. (1998). Thirty grams of air dried soil (<2 mm) was suspended in 50 mL sodium iodide (1.8 g cm<sup>-3</sup>) by shaking with a reciprocal shaker for 1 h. The suspension was allowed to stand for 20 min before centrifugation at 5000 × g for 10 min. After centrifugation, the floating material (light OM) was collected on a 0.45-µm hydrophilic polyvinylidene fluoride filter under a vacuum and rinsed thoroughly with distilled water to remove sodium iodide. The remaining fraction was re-suspended in another 50 mL sodium iodide (1.8 g cm<sup>-3</sup>) to further recover light OM fraction following the same procedure. The successively separated portions of light OM were pooled together and dried at 50 °C.

OC and N of the bulk soil and light OM fraction were determined by the Walkley-Black method (Nelson and Sommers, 1982) and a Kjeldahl method (Institute of Soil Science, 1978), respectively. C contents of above- and below-ground biomasses were determined by a Multi C/N 3100 (Analytik Jena, Jena, Germany) and N by a Kjeldahl method (Institute of Soil Science, 1978). Carbohydrates were extracted by 0.5 M  $H_2SO_4$  at 80  $^\circ$ C and total concentration in extract was determined by the colorimetric anthrone method at a wavelength of 625 nm (Puget et al., 1999). NH<sub>4</sub><sup>+</sup>–N and NO<sub>3</sub><sup>-</sup>–N were extracted with 2 M KCl (soil:solution ratio of 1:5) and determined using a San<sup>++</sup> Automated Wet Chemistry Analyzer (Skalar, Breda, Netherlands). Soil microbial biomass was extracted by the chloroform fumigation-extraction method (Vance et al., 1987). Contents of organic C and N in filtered extracts were analyzed with a Multi C/N 3100 (Analytik Jena, Jena, Germany). Microbial biomass C or N was estimated by the difference between C or N concentration in the fumigated and non-fumigated extracts, then by dividing the difference by 0.45 for C (Beck et al., 1997) and 0.54 for N (Brookes et al., 1985). Activities of soil  $\beta$ -glucosidase and phosphatase were assayed according to Tabatabai (1982), and urease activity was

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