Short-term grazing exclusion has no impact on soil properties and nutrients of degraded alpine grassland in Tibet, China

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Abstract

Since the 1980s, alpine grasslands have been seriously degraded on the Tibetan Plateau. Grazing exclusion by fencing has been widely adopted to restore degraded grasslands. To clarify the effect of grazing exclusion on soil quality, we investigated soil properties and nutrients by comparing free grazing (FG) and grazing exclusion (GE) grasslands in Tibet. Soil properties, including soil bulk density, pH, particle size distributions, and proportion of aggregates, showed no significant difference bewteen FG and GE plots. Soil organic carbon, soil available nitrogen, available phosphorus contents did not differ with grazing exclusion treatments in both 0-15 cm and 15-30 cm layer. However, soil total nitrogen and total phosphorus contents were remarkably reduced due to grazing exclusion at the 0-15 cm depth. Furthermore, growing season temperature and/or growing season precipitation had significant effects on almost all soil properties and nutrients indicators. This study demonstrates that grazing exclusion had no impact on most soil properties and nutrients in Tibet. Additionally, the potential shift of climate conditions should be considered when recommending any policy designed for alpine grasslands degraded soil restoration in the future. Nevertheless, because the results of the present study come from a short term (6–8 years) grazing exclusion, the

assessments of the ecological effects of the grazing exclusion management strategy on soil quality of degraded alpine grasslands in Tibet still need long term continued research.

1 Introduction

Soil is a key resource that contributes to the Earth System functioning as control and manages the cycles of water, biota and geochemicals (Keesstra et al., 2012; Parras-Alcántara et al., 2013; Brevik et al., 2015). Human unreasonable management of the soil resources is resulting in the land degradation due to the soil erosion, soil organic matter exhaustion, loss of soil structure, pollution, forest fires or deforestations (Cerdà et al., 2009; Novara et al., 2011, 2013; García-Orenes et al., 2012; Pereira et al., 2013; Zhao et al., 2013; Keesstra et al., 2014). This is why there is a need to restore and rehabilitate soils as a source of nutrients and services to the humankind (Bai 2013; Mekonnen et al., 2015a; 2015b; Roa-Fuentes et al., 2015; Tejada and Benitez, 2015). Grazing is one of those human uses of the land that will degrade or not the soils and the land upon the right management (Costa et al., 2015; Papanastasis et al., 2015; Tarhouni et al., 2015).

Grazing exclusion from the creation of large-scale enclosures has become a common management strategy to prevent grassland degradation and sustain grassland ecosystem function by the restoration of degraded vegetation and improvement of soil quality throughout the world in recent decades (Medina-Roldán et al., 2012; Wu et al., 2010; Mofidi et al., 2013). Previous studies examining the effect of grazing exclusion on grassland have primarily investigated the vegetation productivity, plant species and communities (Gonzales and Clements, 2010; Schultz et al., 2011). Nevertheless, soil also plays an important role in supplying organic matter, and cycling nutrients, such as nitrogen and carbon, it could also directly affect vegetation productivity, community composition and plant species richness during the grassland restoration succession process. Information on these aspects is required for a better understanding of the restoration mechanisms and the biological feedback of grassland degradation, and for appropriate management and conservation of grassland (Su et al., 2005; Pulido-Fernández et al., 2013; Mekuria and Aynekulu, 2013).

Some studies have shown grazing exclusion to be associated with several soil physical properties variations (Greenwood and McKenzie, 2001; Hoshino et al., 2009; Medina-Roldán et al., 2012; Mofidi et al., 2013). For instance, soil bulk density was found lower in grazing exclusion grassland compared to free grazed grassland due to the elimination of soil trampling by livestock (Gao et al., 2011), as well as the increase of root biomass accumulation (Yuan et al., 2012). The soil particle size distribution revealed that grazing exclusion led to greater silt and clay content, and lower sand content under non-grazed grasslands (Chen et al., 2012; Mofidi et al., 2013). In addition, grassland with grazing exclusion has higher water holding capacity, total porosity and infiltration rates, consequently, soil moisture is higher in non-grazed grassland (Yuan et al., 2012; Haynes et al., 2014). In general, soil physical properties improved after grazing exclusion due to natural amelioration of the soil structure. Biological activity due to the growth and decay of plant roots, the activity of soil-dwelling animals, and the wetting and drying cycles were the probable mechanisms causing this natural amelioration (Mofidi et al., 2013; Wen et al., 2013a).

Nevertheless, research results with regard to the effect of grazing exclusion on soil nutrients were not consistent. For instance, soil organic carbon in the surface soil under grazing exclusion conditions was reportedly increased in a semi-arid woody rangeland (22 years of grazing exclusion) in the Zagros Mountains, Central Iran (Raiesi and Riahi, 2014), decreased in a montane Kobresia winter pasture (7 years of grazing exclusion) on the north-eastern Tibetan Plateau (Hafner et al., 2012), and showed no change in an upland grassland (7 years of grazing exclusion) in northern England (Medina-Roldán et al., 2012) and in a semi-arid sagebrush steppe (40 years of grazing exclusion) in Fremont County of Wyoming, USA (Shrestha and Stahl, 2008). Soil available phosphorus was significantly greater in grazing exclusion grassland of the Imam Kandi Rangelands, Iran (Mofidi et al., 2013) and the semi-arid rangeland in the northern highlands of Ethiopia (Mekuria and Aynekulu, 2013), but was not significantly changed in the desertified sandy grassland of Inner Mongolia, China (Li et al., 2011) and the subalpine grasslands of the Swiss National Park (Haynes et al., 2014). These results imply a lack of clear relationship between grazing exclusion and soil nutrients which may result from the contributions of different grassland ecosystem types (Luan et al., 2014), inconsistent years of grazing exclusion (Wang et al., 2010; Gao et al., 2011), soil heterogeneity (Mekuria and Aynekulu, 2013), and different environmental conditions (Raiesi and Riahi, 2014).

Alpine grasslands of the Tibetan Plateau, which are the most expansive areas of alpine grassland in the world, have undergone serious regional degradation in the past three decades due to a combination of global climate change, rapidly increasing grazing pressure, rodent damage and other factors (Harris, 2010). In response to the problem of grassland degradation in the Tibetan Plateau, China's state and local authorities initiated a program in 2004 called the 'retire livestock and restore grassland' policy. This campaign has focused mostly on grazing exclusion by fencing as an approach to recover the degraded rangelands and to prevent new degradation (Wei et al., 2012). This program has been in progress for more than ten years, although, with an increasing number of studies of grazing exclusion effects on soil properties of alpine grassland ecosystems, greater emphasis has been placed on a single alpine grassland type: the alpine meadow (Wu et al., 2010; Dong et al., 2012; Li et al., 2013), and usually at one experimental or investigation site (Gao et al., 2011; Hafner et al., 2012; Shi et al., 2013).

In the present study, three alpine grassland types in nine counties were selected to investigate the effects of grazing exclusion on the soil quality of degraded alpine grasslands in Tibet. We contrast free grazing and grazing exclusion treatments to address the following questions: (1) How does grazing exclusion affect the soil quality, evaluated by soil properties and nutrients, in alpine grassland of Tibet? and (2) Does the soil properties and nutrients response to grazing exclusion differ among different alpine grassland types? On the basis of the removal of soil trampling by livestock and the probable increase of litter biomass accumulation with grazing exclusion (Wang et al., 2010), we hypothesized that soil properties and nutrients would improve in the absence of grazing. Based on different plant species diversity and community structure, vegetation productivity and cover, and environmental conditions (Wu et al., 2014a), we further hypothesized that soil properties and nutrients responses to the absence of grazing would differ among different alpine grassland types.

2 Materials and Methods

2.1 Study area

Tibet is located between 26°50′ and 36°29′ N and 78°15′ and 99°07′ E and covers a total area of more than 1.2 million km^2, which is approximately one-eighth of the total land surface of

China. Tibet is an important ecological security shelter zone that acts as an integral water reservoir, regulating climate change and water resources in China and eastern Asia. Solar radiation is strong with annual radiation varying between 140 and 190 kcal cm^{-2} in different parts of the region and long sunshine hours with annual sunshine ranging from 1800 to 3200 h, increasing from the east to the west. Due to geographical conditions and atmospheric circulation, the average annual temperature is rather low with a large diurnal range, and the temperature varies from 18 °C to −4 °C, and decreases gradually from the southeast to the northwest. The average annual precipitation is less than 1000 mm in most areas of Tibet, reaching 2817 mm in the east and decreasing to approximately 70 mm in the west (Dai et al., 2011).

Alpine grasslands are the most dominant ecosystems in Tibet, covering more than 70% of the whole plateau's area. Alpine steppe is the most common grassland type in Tibet; it is composed of drought tolerant perennial herbs or small shrubs under cold and arid or semiarid climate conditions, which represents approximately 38.9% of the total Tibetan grassland area. Alpine meadow is the second largest grassland type and is composed of perennial mesic and mesoxeric herbs under cold and wet climate conditions, occupying approximately 31.3% of the total grassland area of Tibet. Alpine desert steppe occupies approximately 10.7% of the total grassland area and is composed by xeric small shrubs and small grasses under cold and arid climate conditions; it is a transitional type of alpine grassland from the steppe to the desert in Tibet (Land Management Bureau of Tibet, 1994).

2.2 Survey design and sampling

Since the 'retire livestock and restore pastures' ecological program started in 2004, more than 2.4×10^6 ha of alpine grasslands in Tibet have been fenced to exclude livestock grazing (Yan and Lu, 2015). We conducted a multi-site survey during the peak growing season from late July to mid-August in 2013 in nine counties which represented three of the main natural grassland vegetation types in Tibet, including alpine meadow, alpine steppe and alpine desert steppe (Fig. 1). In these nine counties, grazing exclusion areas, which have been excluded from livestock with metal fences, were established during the years of 2005-2007. Since fencing establishment, the fenced grasslands were excluded livestock all year-round and the metal enclosures were also effective to exclude large wildlife herbivores, such as *Pantholops* *hodgsoni*, *Procapra picticaudata*, and *Equus kiang*. The adjacently open grassland outside the enclosures were still traditionally grazed by yak and sheep around the year, which the actual averaged stocking rate approximate ranges from 0.16 sheep units ha^{-1} of the western counties to 2.05 sheep units ha^{-1} in the eastern counties for the study region (Wu et al., 2013, 2014a). In the present study, the enclosed areas inside the fencing were defined as grazing exclusion (GE) plots and the areas outside of the fencing nearby were defined as free grazing (FG) plots.

At each sample location, three pairs of 0.5 m × 0.5 m quadrats at each GE and FG treatment sample plots were laid out collinearly at intervals of approximately 20 m. In total, 54 quadrats of alpine grassland in Tibet were sampled with 27 quadrats (9 plots × 3 quadrats) for FG treatments and 27 quadrats for GE treatments, respectively. The quadrats of FG plots chosen in this study were well matched with the adjacent GE plots, and both quadrats in GE and FG plots are within 800 m from the enclosure edges to make sure that each pair sites were as similar as possible in slope, aspect, and soils. At each quadrat, all aboveground plants and litter were removed from the soil surface before the sampling. Five soil samples were obtained for each quadrat from FG plots and GE plots by bucket auger at two different depths: 0-15 cm and 15-30 cm, and five soil samples were mixed as a soil sample for the soil property and nutrient analysis. For the determination of soil bulk density, soil cores (5.4 cm in diameter) were also taken from each layer using a stainless-steel cylinder. In addition, the location and elevation of each site were measured using GPS (Garmin MAP62CSX made in Garmin Ltd, USA).

2.3 Soil samples analysis

Soil bulk density (BD) was sampled from 0–15 cm and 15–30 cm depths using soil cutting ring of 5.3 cm in diameter, then was determined as the moisture-corrected (oven-dried at 105 °C) mass of each sample divided by the measured volume of the excavated soil core (Campbell et al., 2014). Soil samples for soil property and nutrient analyses were first removed roots and litter by hand then air-dried, crushed, and passed through a 2 mm-mesh sieve. Soil particle size distributions (PSD) were determined by the pipette method following H_2O_2 treatment to destroy organic matter and dispersion of soil suspensions by sodium hexametaphosphate (Su et al., 2010). The proportion of soil aggregates (PM) was also measured by using a pipette method with five aggregate-size classes (2-0.25 mm, 0.25-0.05

mm, 0.05-0.02 mm, 0.02-0.002 mm, < 0.002 mm) (Liu, 1996). Soil pH was determined in soil–water suspensions (1:2.5, *v*/*v*) (Alvarenga et al., 2012). Soil organic carbon (SOC) and soil total nitrogen (TN) contents were determined by using a vario MACRO cube elemental analyzer (Elementar Analysensysteme GmbH, German) (Qu et al., 2014). To remove inorganic carbon, all samples for SOC analysis were acid treated with hydrochloric acid (10% HCl) prior to analysis. Total phosphorus (TP) content was determined using the $NaHCO_3$ alkali digestion method and by molybdenum antimony colorimetry (Cao et al., 2013). Available nitrogen (AN) was determined by using the continuous alkali-hydrolyzed reduction diffusion method (Wang et al., 2013) and Available phosphorus (AP) was determined using the Olsen method (Olsen et al., 1954).

2.4 Climate data

Monthly meteorological datasets were derived from the China Meteorological Data Sharing Service System (CMDSSS, http://data.cma.gov.cn) with spatial resolutions of 0.5° from 2005–2013. The data sources include monthly mean temperature and monthly precipitation data from more than 2400 well distributed climate stations across China, as well as digital elevation model (DEM) data. The meteorological gridded datasets were generated by CMDSSS through Thin Plate Spline (TPS) method using ANUSPLIN software (ERSI, Redlands, California, U.S.A.) and a goodness of fit of the interpolated values were validated by CMDSSS (Shi et al., 2014). The growing season temperature (GST) and growing season precipitation (GSP) were defined as the average air temperature and the accumulated precipitation during the growing season of alpine grasslands from May to September. The GST and GSP from 2005 to 2013 matched with nine sites' locations were extracted from these meteorological raster surfaces in ArcGIS 10.0 (ERSI, Redlands, California, U.S.A.) for further analyses.

2.5 Statistical analysis

A paired difference *t*-test was used to test the potential effect of grazing exclusion on each soil property and nutrient indicator. Analysis of covariance (ANCOVA) by the general linear model (GLM) was employed to evaluate the effects of grazing exclusion treatment, soil depth, and climatic factors on each soil property and nutrient indicator of alpine grasslands. In the

ANCOVA analysis, the fixed factor was alpine grassland grazing treatments (FG and GE) and soil depth, while the covariates were GST and GSP. Homogeneity of variances and normal distribution of residuals were verified by examining plots of the distribution of residuals and of the residuals against fitted values to fulfill statistical assumptions of ANCOVA. The two covariates growing season temperature and growing season precipitation that were used to fit the linear ANCOVA models were not highly interacted with the fixed factor ($P > 0.05$). Pearson correlation analysis was used to test the relationships among soil properties and nutrients indices. The least significant difference test was used to compare the means at $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics 19 software (SPSS/IBM, Chicago, IL, USA).

3 Results

3.1 Soil properties

Soil bulk density (BD) of alpine grasslands (alpine meadow + alpine steppe + alpine desert steppe) in the 0-15 cm and 15-30 cm soil layers were lower, whereas soil pH in both soil layers were higher in grazing exclusion (GE) plots than in the free grazing (FG) plots, but the differences were all not significant between GE and FG plots ($P > 0.05$) (Table 1). Among three alpine grassland types, no significant differences in soil BD were observed with GE treatments ($P > 0.05$), except for significantly decreased soil BD in the 0-15 cm soil layer of alpine meadow ($P < 0.05$). Soil pH was significantly altered by the grazing exclusion treatment in the 0-15 cm layer of the alpine meadow ($P < 0.05$), but was not significantly altered at the 15-30 cm depth in alpine meadow and at both soil layers in other two alpine grasslands ($P > 0.05$).

Soil particle size distributions (PSD) indicated the alpine grassland soil texture was sandy loam, consisting primarily of sand (2–0.05 mm). The soil proportion of aggregates (PM) mainly showed aggregates compositions sizes of 2-0.25 mm and 0.25-0.05 mm sizes in alpine grassland (Table 1). However, for both PSD and PM, the mean values of almost all indicators in both soil layers did not differ significantly between GE and FG grasslands ($P > 0.05$). The results from a ANCOVA demonstrate that grazing exclusion, soil depth, and their interaction has no effect on most of soil properties, nevertheless, almost all soil properties indicators were significantly impacted by climate factors, GST and/or GSP (Table 2).

3.2 Soil nutrients

Grazing exclusion did not significantly affect the soil organic carbon (SOC), soil available nitrogen (AN), and soil available phosphorus (AP) contents in both soil layers ($P > 0.05$), but soil total nitrogen (TN) and total phosphorus (TP) at the 0-15 cm depth significantly decreased 15.63% and 12.50%, respectively, due to grazing exclusion treatments ($P < 0.05$) (Fig. 2). Among the three alpine grassland types, grazing exclusion significantly increased SOC and TN contents in the 15-30 cm layer of the alpine desert steppe, and grazing exclusion significantly decreased soil TP and AP at the 0-15 cm depth in the alpine meadow. Statistical analyses from ANCOVA showed that all soil nutrients, including SOC, TN, TP, AN, and AP, were not significantly impacted by grazing exclusion and soil depth. For the climatic factors, GST had a significant effect on soil TP contents, whereas GSP had a significant effect on SOC, soil TN, and soil AN contents (Table 2).

3.3 Relationships among soil properties and nutrients

*T*he relationships among different soil properties and nutrients are shown in Table 3. In general, correlation analyses showed that soil BD was positively correlated with soil sand content ($P < 0.01$) and negatively correlated with soil silt content and most soil nutrient contents ($P < 0.01$). The 2-0.25 mm and 0.25-0.05 mm sized soil aggregates were significantly correlated with soil PSD and soil pH ($P < 0.01$). SOC, soil TN and AN contents were significantly positively correlated with soil silt content, and significantly negatively correlated with soil sand content ($P < 0.01$). However, no correlations were found between soil TP, AP contents and any of the soil PSD ($P > 0.05$). In addition, SOC, soil TN, TP, AN, and AP contents were significantly positively correlated with each other in the alpine grassland.

4 Discussion

4.1 Effect of grazing exclusion on soil properties

Fencing to exclude livestock has been reported to cause reductions in soil BD in different types of grasslands in the world, such as the upland grassland in northern England (Medina-Roldán et al., 2012), and a semi-arid sandy grassland in northern China (Su et al., 2005). Soil BD was slightly lower in the GE plots compared to FG plots of the alpine grassland in Tibet. The elimination of soil trampling by livestock, as well as the high organic matter content, high soil silt and clay content, and the presence of extensive shallow root systems in the grazing exclusion areas, contributed to a decrease in soil BD (Su et al., 2005; Yuan et al., 2012).

It was found that the soil pH was lower in non-grazed rangelands compared with grazed rangelands probably because of the addition of livestock urine which increased soil pH largely due to the hydrolysis of urine-urea in grazed grassland (Raiesi and Riahi, 2014). However, soil pH was not significantly different between FG and GE grasslands in Tibet (Table 2). This was probably due to the relatively low effect of livestock on soil pH in this region, which was due to low livestock distributions. The actual averaged stocking rate approximate ranges from 0.16 sheep units ha^{-1} of the western counties to 2.05 sheep units ha^{-1} in the eastern counties (Wu et al., 2013, 2014a).

Grazing exclusion had no significant influence on soil PSD in the alpine grassland, and soil sand, silt and clay contents did not differ significantly between FG and GE grasslands. This result was not consistent with the results from the Imam Kandi Rangelands, Iran (Mofidi et al., 2013) and in the sandy rangeland of Inner Mongolia, northern China (Li et al., 2011; Chen et al., 2012), in which grazing exclusion led to greater fine soil particle content and lower coarse sand content due to an increased ability of vegetation to prevent soil erosion and trap windblown fine particles (Chen et al., 2012; Wen et al., 2013b). This non-consistent result in alpine grassland of Tibet was maybe due to the sparse and dwarf vegetation status in the alpine environment and relatively short grazing exclusion period

Soil aggregates play a key role in protecting soil organic matter from microbial decomposition (Leifeld and Kögel-Knabner, 2003). They are dynamic soil properties that tend to respond rapidly to environmental changes; for instance, different land use types would exercise their effects on soil aggregate formation and stabilization in various ways and magnitudes (Bongiovanni and Lobartini, 2006). In the alpine grasslands of Tibet, grazing exclusion had no effect on small sized soil aggregates (< 0.05 mm). However, soil aggregate fractions with 2-0.25 mm and 0.25-0.05 mm were significantly affected by grazing exclusion (Table 2).

4.2 Effect of grazing exclusion on soil nutrients

In the present study, SOC concentrations at both 0-15 cm and 15-30 cm depth were not affected by grazing exclusion treatment, indicating that changes in grazing regime had little effect on soil organic matter quality in alpine grasslands. Nevertheless, the effects of grazing exclusion on SOC of alpine grassland in the Tibetan Plateau from different studies were shown to be contradictory; in various cases, they have demonstrated a positive effect (Wu et al., 2010; Gao et al., 2011), a negative effect (Hafner et al., 2012; Shi et al., 2013) and a neutral effect (Dong et al., 2012). In fact, these controversies were also reported from different studies on grassland ecosystems restoration in other region (Mekuria and Aynekulu, 2013; Raiesi and Riahi, 2014). These differences may partly be due to whether grazing pressure exceeds carrying capacity of a site and whether it is sufficiently far beyond that capacity to reach the ecological threshold (Sasaki et al., 2011, Wu et al. 2014b). Additionally, differences among sites in climatic conditions and/or in grazing seasonality and intensity may be, at least in part, responsible for the observed results (Speed et al. 2014).

SOC contents were significantly positively correlated with soil silt contents and significantly negatively correlated with soil sand content (Table 3). This is because of the amount of soil organic matter associated with silt and clay due to their higher capacity for holding water and nutrients compared to sand (Plante et al., 2006). Thus, soil particle size distributions play an important role in regulating the capacity of a soil to preserve organic matter; for instance, SOC content significantly increased due to grazing exclusion with both higher clay and silt contents and lower sand content in a desert steppe in northwestern China (Wen et al., 2013b). However, in the present study, both soil particle size distribution and SOC content were unchanged by grazing exclusion treatment in the alpine grasslands.

Grazers can alter N stocks by both increasing or decreasing N inputs and N outputs. Regarding outputs, grazers promote higher N losses from urine and dung patches but can also stimulate N retention by decreasing N losses through greater root allocation. Regarding inputs, grazing can decrease N inputs by decreasing legume biomass or cover but can also increase N redeposition from the atmosphere, partially compensating for N losses (Andrioli et al. 2010, Piñeiro et al. 2010). Significant differences were observed in soil TN concentrations between the GE plots and FG plots in the 0-15 cm soil layer, indicating that the N nutrients in the soil surface layer were reduced due to grazing exclusion (Fig. 2). The decrease in soil surface layer TN contents due to grazing exclusion was also found in previous studies in the Tibetan Plateau (Shi et al., 2013). These responses are likely to happen in grazing treatments that maintained a higher carbon input from root, litter and excreta while an ungrazed treatment would strongly decrease this input and promote aboveground allocation (Kelly et al. 1996).

Grazing exclusion substantially improved soil N availability in the temperate steppe in northern China which suggests that there are positive effects of ecological restoration on soil N availability (Wang et al. 2010, Chen et al. 2012). However, this improvement was not found in alpine grasslands with ecological restoration by grazing exclusion (Fig. 2), which an earlier research also showed no significant effect of grazing exclusion on soil N availability in tundra ecosystem (Stark et al. 2015). This is maybe because that soil N availability is the balance of multiple ecological processes, such as nitrification, mineralization, denitrification, nitrate leaching, plant uptake, etc. and relative short grazing exclusion time in alpine grasslands did not change this balance.

Soil TP contents at a depth of 0-15 cm significant decreased by 12.5% in GE grasslands. The reduction of total P in soil surface layer due to grazing exclusion maybe contributed by the absence inputs of animal excreta, which has long been recognized as an important pathway in the P cycle in grazed pasture, and higher soil P uptake by vegetation (Chaneton and Lavado, 1996). Soil AP was not affected by grazing exclusion in alpine grasslands, which is consistent with research in a temperate subhumid grassland in Argentina that grazing did not affect soil available nutrients, although it did accelerate soil phosphorus cycling rates (Chaneton and Lavado, 1996).

4.3 The effect of climate factors

Our results from ANCOVA analysis indicated that grazing exclusion almost had no effect on soil properties and nutrients. However, climate conditions during the growing season played an important role in controlling the soil quality status of alpine grasslands in Tibet because GST and/or GSP were found to have significant effects on almost all soil properties and nutrients indicators (Table 2). Therefore, the soil properties and nutrients of alpine grasslands in Tibet were primarily driven by the climate gradients distributions but not by grazing exclusion treatments. Climatic factors, including temperature and precipitation, can directly or indirectly impact soil quality status by controlling soil environmental conditions, soil weathering process, soil microbes and enzymes activities, substrate availability, translocation of dissolved ions, and so on (Barthold et al., 2013; Clarholm and Skyllberg, 2013; Chen et al., 2015).

Soil BD was significantly impacted by both temperature and precipitation in this alpine region, which maybe as a result of the expansion and compression of the soil matrix due to changing of freezing and thawing processes caused by climate (Henry, 2007; Yang et al. 2010). Soil pH affected by the climate factors was found in many natural ecosystems (Barton et al., 1994), which is also approved in alpine grasslands in Tibet in the present study. Soil aggregate is a dynamic soil property, which varies over time, partially depending on climatic processes (Dimoyiannis, 2009). In alpine grasslands, proportions of soil aggregates were generally influenced by both GST and GSP. Similar findings were also reported by Rillig et al. (2002) which found increasing temperature could decrease soil aggregate water stability by stimulating the role of arbuscular mycorrhizal fungi in soil aggregation in an annual grassland in northern California, USA; and by Dimoyiannis (2009) which reported total monthly precipitation and mean monthly air temperature strongly correlated with seasonal soil aggregate stability in the Thessaly plain, central Greece.

We found soil nutrients, including SOC, soil TN and AN contents, were significantly affected by GSP (Table 2). Therefore, precipitation during the growing season played an important role in controlling the soil C and N contents of alpine grasslands in Tibet. The potential changes in precipitation are identified as vital aspects of regional climate change, which can alter the distribution and dynamics of water availability and subsequently alter soil biogeochemical processes at the ecosystem level (Cerdà and Lavée, 1999; Hao et al., 2013). The precipitation could play the most prominent role in grassland ecosystem C and N dynamics, especially for arid and semi-arid ecosystems, through their influence on plant productivity (Robertson et al., 2009), soil carbon cycle processes (Hao et al., 2013), soil N transformations (Cregger et al., 2014). There are increasing evidences to show that, the total amount of precipitation and the altered precipitation patterns, control the dynamics of net primary production, soil organic carbon storage, carbon dioxide fluxes, and soil N cycling and transformations of alpine grassland ecosystems in Tibetan Plateau (Zhuang et al., 2010; Zhang et al., 2012; Shen et al., 2015).

5 Conclusions

In an attempt to alleviate the problem of grassland degradation on the Tibetan Plateau, China's state and local authorities have recently initiated a program called the 'retire livestock and restore grassland' project, in which fencing to exclude grazers has been used as an approach for restoring degraded grasslands. In the present study, we conducted a field survey to evaluate the effectiveness of the grazing exclusion on soil properties and nutrients in restoring degraded alpine grasslands in Tibet. In general, grazing exclusion by fencing had no impact on most soil properties and nutrients, and even caused a considerable decrease in soil TN and TP in the soil surface layer of alpine grassland ecosystems, including alpine meadow, alpine steppe, and alpine desert steppe. Nevertheless, climate conditions during the growing season played an important role in controlling the soil quality status of alpine grasslands.

Therefore, at present, the restoration policy is not effective for improving the soil quality of degraded alpine grassland in Tibet. It is noted that the results of the present study come from short term (6–8 years) grazing exclusion, while the restoration of soil quality status of degraded grassland is a long term evolutionary process. Thus, it is still uncertain whether grazing exclusion will improve soil properties and nutrients or not if this policy is continuously implemented for decades. Long term observations and continued research are still necessary to assess the ecological effects of the grazing exclusion management strategy on soil quality of degraded alpine grasslands in Tibet. In addition, because the soil properties and nutrients of alpine grasslands in Tibet were primarily driven by the climate factors, the potential shift of climate conditions should be considered when recommending any policy designed for the degraded soil restoration of alpine grasslands in the future.

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Table 1 Statistical comparison of overall mean values of soil properties ± standard error (S.E.) at 0-15 and 15- 30 cm depth using paired difference *t*-test (α = 0.05) between free grazing (FG) plots and grazing exclusion (GE) plots. *P*-values below 0.05 are in bold.

Soil physical properties	**Depth**	**Alpine meadow**		**Alpine steppe**		**Alpine desert steppe**		**Alpine grassland**	
		FG	**GE**	**FG**	**GE**	**FG**	**GE**	**FG**	**GE**
BD (g cm^{-3})	0-15 cm	**1.35 ± 0.09**	**1.13 ± 0.10**	1.58 ± 0.03	1.61 ± 0.05	1.52 ± 0.11	1.37 ± 0.10	1.47 ± 0.05	1.37 ± 0.06
	15-30 cm	1.47 ± 0.06	1.38 ± 0.10	1.53 ± 0.06	1.60 ±0.06	1.39 ±0.01	1.38 ± 0.06	1.49 ± 0.04	1.48 ± 0.06
pH	0-15 cm	**7.27 ± 0.18**	**7.71 ± 0.14**	7.87 ± 0.23	7.83 ± 0.19	8.41 ± 0.08	8.48 ± 0.01	7.66 ± 0.15	7.84 ± 0.11
	15-30 cm	7.51 ± 0.16	7.69 ± 0.16	8.16 ± 0.18	8.06 ± 0.14	8.48 ± 0.03	8.46 ± 0.04	7.91 ± 0.13	7.94 ± 0.11
PSD (%)									
Sand (2-0.05 mm)	0-15 cm	67.90 ± 4.50	68.39 ± 2.13	77.93 ± 2.42	80.11 ± 1.64	79.99 ± 1.18	78.44 ± 2.66	73.70 ± 2.44	74.71 ± 1.63
	15-30 cm	70.74 ± 4.00	67.96 ± 2.53	78.48 ± 3.26	82.53 ± 2.20	83.43 ± 2.16	80.56 ± 2.19	75.60 ± 2.42	75.84 ± 2.02
Silt (0.05-0.02 mm)	0-15 cm	12.55 ± 2.64	11.11 ± 1.32	5.22 ± 1.12	4.93 ± 0.51	3.61 ± 0.55	3.93 ± 0.63	8.30 ± 1.46	7.57 ± 0.88
	15-30 cm	10.00 ± 1.93	9.99 ± 1.41	3.03 ± 0.44	3.23 ± 0.40	2.83 ± 0.84	2.04 ± 0.06	6.10 ± 1.10	6.10 ± 0.94
Silt (0.02-0.002 mm)	0-15 cm	11.21 ± 2.01	11.08 ± 0.99	5.82 ± 1.06	4.56 ± 0.76	7.79 ± 0.68	8.69 ± 0.71	8.44 ± 1.11	7.92 ± 0.81
	15-30 cm	10.36 ± 1.80	10.41 ± 1.30	6.79 ± 1.77	3.72 ± 0.58	6.94 ± 1.45	9.11 ± 1.88	8.39 ± 1.16	7.20 ± 0.90
Clay (<0.002 mm)	0-15 cm	8.34 ± 0.58	9.42 ± 0.88	11.03 ± 1.14	10.40 ± 0.98	8.60 ± 1.14	8.95 ± 2.87	9.56 ± 0.62	9.80 ± 0.64
	15-30 cm	8.89 ± 0.93	11.64 ± 1.25	11.69 ± 1.93	10.51 ± 1.77	6.80 ± 0.74	8.29 ± 2.33	9.90 ± 0.99	10.77 ± 0.99
PM (%)									
2-0.25 mm	0-15 cm	42.68 ± 1.55	38.60 ± 0.86	38.28 ± 4.78	35.27 ± 4.24	30.76 ± 3.23	31.38 ± 4.62	39.40 ± 2.32	36.32 ± 1.98
	15-30 cm	44.14 ± 2.41	39.42 ± 1.84	48.71 ± 5.66	42.97 ± 5.11	43.92 ± 4.76	33.52 ± 6.35	46.15 ± 2.74	40.34 ± 2.50
0.25-0.05 mm	0-15 cm	56.83 ± 1.48	60.91 ± 0.85	61.06 ± 4.77	64.03 ± 4.29	65.63 ± 3.23	65.40 ± 4.50	59.69 ± 2.26	62.79 ± 1.97
	15-30 cm	55.42 ± 2.37	60.11 ± 1.83	50.70 ± 5.65	56.30 ± 5.14	53.89 ± 5.12	64.47 ± 7.18	53.15 ± 2.74	58.90 ± 3.06
0.05-0.02 mm	0-15 cm	0.36 ± 0.07	0.35 ± 0.05	0.40 ± 0.06	0.38 ± 0.09	1.44 ± 0.05	1.36 ± 0.07	0.50 ± 0.08	0.48 ± 0.08
	15-30 cm	0.30 ± 0.03	0.33 ± 0.03	0.33 ± 0.07	0.38 ± 0.12	0.92 ± 0.36	0.90 ± 0.35	0.38 ± 0.06	0.41 ± 0.07
0.02-0.002 mm	0-15 cm	0.11 ± 0.02	0.12 ± 0.02	0.21 ± 0.04	0.22 ± 0.08	**1.28 ± 0.04**	**1.12 ± 0.07**	0.28 ± 0.07	0.27 ± 0.07
	15-30 cm	0.12 ± 0.02	0.12 ± 0.01	0.18 ± 0.04	0.24 ± 0.10	0.80 ± 0.32	0.70 ± 0.31	0.22 ± 0.05	0.24 ± 0.06
<0.002 mm	0-15 cm	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.09 ± 0.04	**0.90 ± 0.05**	**0.74 ± 0.01**	0.13 ± 0.05	0.14 ± 0.05
	15-30 cm	0.03 ± 0.01	0.03 ± 0.01	0.08 ± 0.02	0.11 ± 0.07	0.46 ± 0.23	0.42 ± 0.20	0.10 ± 0.03	0.11 ± 0.04

BD: Bulk density, PSD: Particle size distributions, PM: Proportion of aggregates

Table 2 Results from analysis of covariance (ANCOVA) by the general linear model (GLM) showing *F* values and *P* values of soil properties and nutrients, which the fixed factor was grazing treatments (G: free grazing and grazing exclusion) and soil depth (D: 0-15 cm and 15-30 cm), while the covariates were growing season temperature (GST) and growing season precipitation (GSP). *P*-values below 0.05 are in bold.

Soil Properties	**G**		**D**		**G × D**		**GST**		**GSP**	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
BD	1.31	0.255	1.73	0.192	0.69	0.41	**12.84**	**0.001**	19.24	< 0.001
pH	1.93	0.168	**4.68**	**0.033**	0.9	0.346	**83.73**	**< 0.001**	24.85	< 0.001
PSD										
Sand (2-0.05 mm)	0.1	0.756	0.56	0.455	0.04	0.849	0.31	0.578	**13.26**	**< 0.001**
Silt (0.05-0.02 mm)	0.15	0.701	3.68	0.058	0.15	0.704	2.05	0.155	**28.6**	**< 0.001**
Silt (0.02-0.002 mm)	0.67	0.414	0.11	0.737	0.09	0.769	0.06	0.801	**4.41**	**0.038**
Clay (<0.002 mm)	0.43	0.511	0.61	0.438	0.14	0.71	0.35	0.557	0.04	0.847
PM										
2-0.25 mm	**4.18**	**0.043**	**6.15**	**0.015**	0.39	0.533	**22.36**	**< 0.001**	0.01	0.944
0.25-0.05 mm	**4.05**	**0.047**	**5.62**	**0.02**	0.36	0.55	**19.03**	**< 0.001**	0.126	0.723
0.05-0.02 mm	0.01	0.947	2.26	0.136	0.16	0.691	**18.92**	**< 0.001**	**11.17**	**0.001**
0.02-0.002 mm	0.01	0.935	0.93	0.337	0.05	0.829	**21.88**	**< 0.001**	**24.4**	**< 0.001**
<0.002 mm	0.04	0.851	0.82	0.367	0.02	0.896	**23.28**	**< 0.001**	**26.18**	**< 0.001**
SOC	0.41	0.524	0.22	0.64	1.38	0.243	0.09	0.764	**12.75**	**0.001**
TN	0.05	0.818	0.53	0.467	2.46	0.12	0.83	0.364	**19.18**	**< 0.001**
TP	1.89	0.172	0.29	0.59	0.53	0.469	**11.98**	**0.001**	2.44	0.121
AN	0.02	0.904	0.02	0.892	1.99	0.161	0.1	0.758	**43.26**	**< 0.001**
AP	0.92	0.34	3.06	0.08	0.34	0.56	0.69	0.41	0.09	0.77

BD: Bulk density, PSD: Particle size distributions, PM: Proportion of aggregates, SOC: Soil organic carbon, TN: Total nitrogen, TP: Total phosphorus, AN: Available nitrogen, AP: Available phosphorus

Table 3 Pearson's correlation coefficients among soil property and nutrient indicators of alpine grasslands and their significance levels. [a] $P < 0.05$, [b] $P < 0.01$

Soil properties	BD	Sand	Silt1	Silt2	Clay	PM1	PM2	PM3	PM4	PM5	pH	SOC	TN	TP	AN
Sand	0.42[b]														
Silt1	-0.36[b]	-0.83[b]													
Silt2	-0.43[b]	-0.92[b]	-0.77[b]												
Clay	-0.06	-0.37[b]	-0.15	0.12											
PM1	0.25[a]	0.31[b]	-0.14	-0.28[b]	-0.29[b]										
PM2	-0.25[b]	-0.32[b]	0.14	0.28[b]	0.30[b]	-0.99[b]									
PM3	-0.06	-0.05	0.02	0.14	-0.06	-0.17	0.10								
PM4	0.04	0.16	-0.20[a]	-0.04	-0.08	-0.09	0.02	0.95[b]							
PM5	0.04	0.20[a]	-0.22[a]	-0.06	-0.14	-0.06	-0.01	0.91[b]	0.98[b]						
pH	0.05	0.31[b]	-0.54[b]	-0.25[b]	0.22[a]	-0.34[b]	0.32[b]	0.20[a]	0.26[b]	0.27[b]					
SOC	-0.68[b]	-0.33[b]	0.35[b]	0.35[b]	-0.06	-0.06	0.07	-0.22	-0.12	-0.12	-0.15				
TN	-0.69[b]	-0.38[b]	0.39[b]	0.39[b]	-0.03	-0.08	0.08	-0.01	-0.13	-0.13	-0.14	0.97[b]			
TP	-0.16	-0.10	0.07	0.05	0.10	0.22[a]	-0.22[a]	-0.04	-0.06	-0.04	-0.16	0.19[a]	0.25[b]		
AN	-0.62[b]	-0.37[b]	0.46[b]	0.39[b]	-0.13	0.11	-0.10	-0.11	-0.21	-0.21	-0.35[b]	0.78[b]	0.79[b]	0.26[b]	
AP	-0.39[b]	-0.16	0.08	0.17	0.08	0.05	-0.05	0.03	-0.02	-0.01	-0.06	0.49[b]	0.51[b]	0.46[b]	0.50[b]

BD: Bulk density, Sand: Sand (2-0.05 mm), Silt1: Silt (0.05-0.02 mm), Silt2: Silt (0.02-0.002 mm), Clay: Clay (<0.002 mm), PSD: particle size distributions, PM: Proportion of aggregates (PM1: 2-0.25 mm, PM2: 0.25-0.05 mm, PM3: 0.05-0.02 mm, PM4: 0.02-0.002 mm, PM5: <0.002 mm), SOC: Soil organic carbon, TN: Total nitrogen, TP: Total phosphorus, AN: Available nitrogen, AP: Available phosphorus

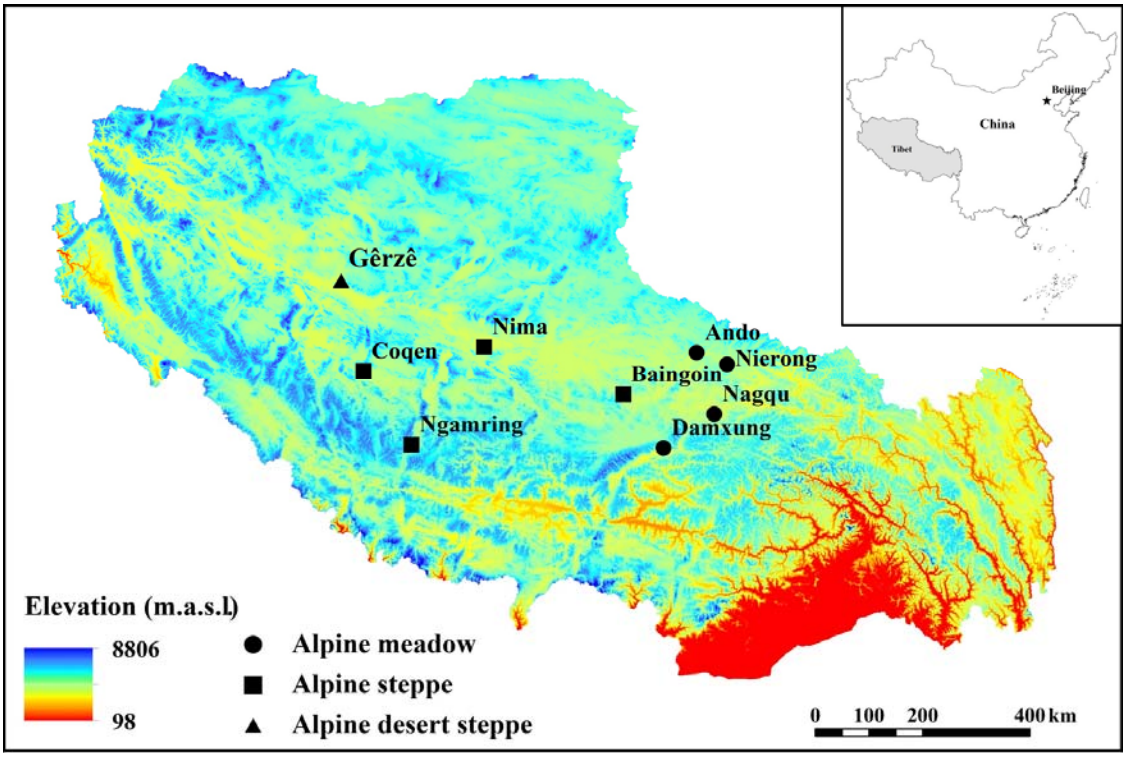

Figure 1. Location of study area and distribution of sampling sites of alpine grassland.

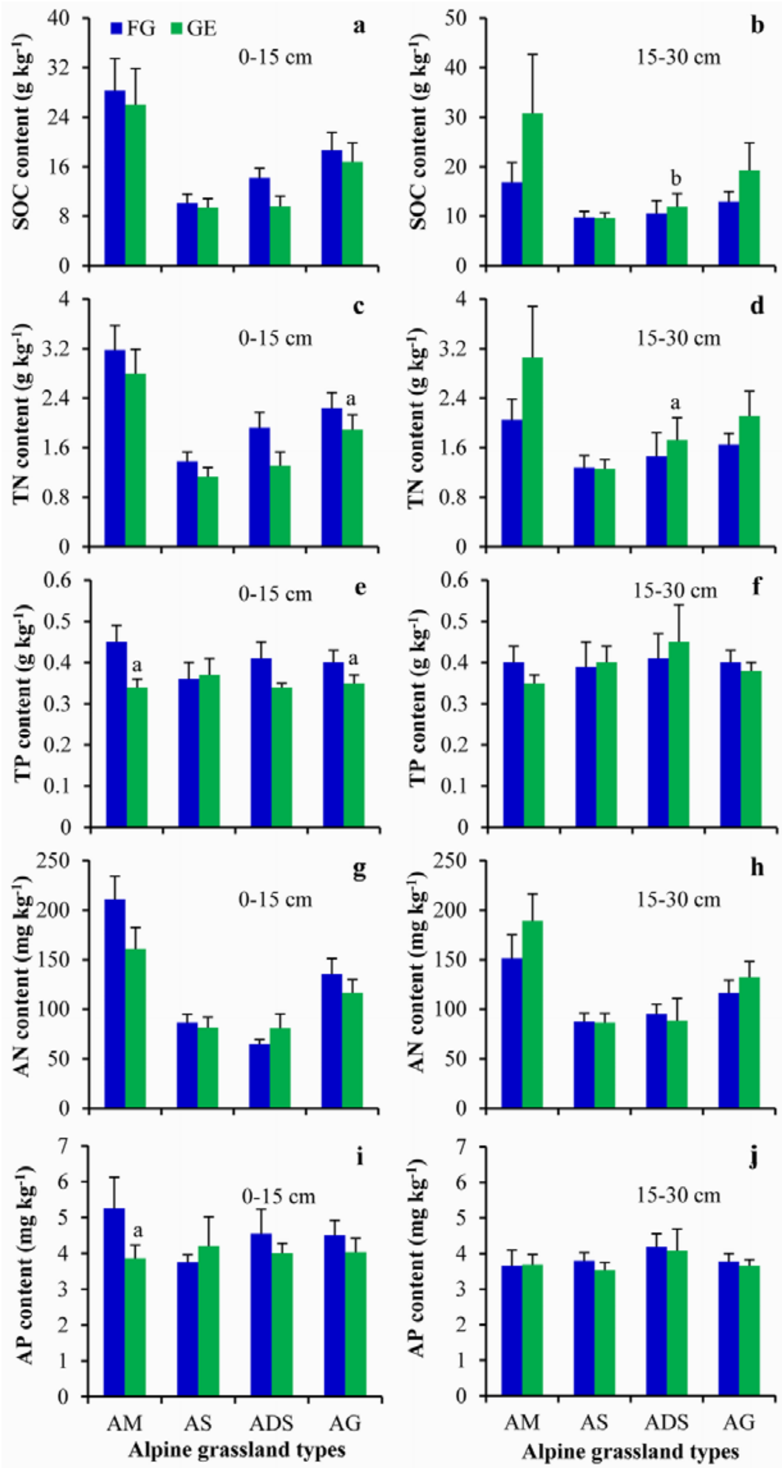

Figure 2. Statistical comparison of soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), and available phosphorus (AP) contents at 0-15 cm and 15-30 cm depth using paired difference t-test ($\alpha = 0.05$) between free grazing (FG) plots and grazing exclusion (GE) plots. Error bars represent standard errors, AM, AS, ADS, and AG represent alpine meadow, alpine steppe, alpine desert steppe, and alpine grasslands (AM + AS +ADS), respectively, $^{a}P < 0.05$, $^{b}P < 0.01$.