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# Does Grazing Exclusion Improve Soil Carbon and Nitrogen Stocks in Alpine Grasslands on the Qinghai-Tibetan Plateau? A Meta-Analysis

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**Abstract:** Grazing exclusion has been widely used to restore the degraded alpine grasslands on the Qinghai-Tibetan Plateau (QTP). However, the dynamics of soil organic carbon (SOC) and soil total nitrogen (STN) pools after grazing exclusion and their controlling factors are currently less understood in this region. Here, a meta-analysis was conducted to quantitatively assess the changes in SOC and STN stocks in topsoil (0–30 cm) following grazing exclusion in three major grassland types (alpine meadow, alpine steppe, and alpine desert steppe) on the QTP and to explore the potential factors controlling the effects of grazing exclusion on SOC and STN stocks. The results showed that overall, grazing exclusion significantly increased SOC stock by 16.5% and STN stock by 11.2%. Significant increases in both SOC and STN stocks were observed after grazing exclusion of alpine meadow. In contrast, grazing exclusion did not improve SOC and STN stocks in the other two grassland types. The difference in mean annual precipitation among grassland types was a likely reason for the different dynamics of SOC and STN stocks after grazing exclusion. The effect sizes of both SOC and STN stocks were positively related to the duration of grazing exclusion, and a positive relationship was detected between the effect size of SOC stock and that of STN stock, demonstrating that the dynamics of SOC and STN were closely coupled during the period of grazing exclusion. However, grazing exclusion had no impact on soil C:N ratio for all grassland types, indicating that soil C:N ratio was generally stable after grazing exclusion. Therefore, it is suggested that the increase in STN can support continuous SOC accumulation following grazing exclusion. In conclusion, the findings suggest that the effects of grazing exclusion on SOC and STN stocks differ among grassland types on the QTP, and grazing exclusion of alpine meadows may provide substantial opportunities for improving SOC and STN stocks in this region.

**Keywords:** fencing; soil organic carbon; soil nitrogen; grassland restoration; alpine region; systematic review

## 1. Introduction

Soils are the largest terrestrial reservoir of carbon (C), containing approximately 1500 Pg (1 Pg =  $10^{15}$  g) organic C in the top meter worldwide [1]. The amount is about twice as large as the atmospheric C pool and three times the biotic C pool [2]. Therefore, small changes in the soil organic carbon (SOC) pool may significantly influence the concentration of carbon dioxide ( $\text{CO}_2$ ) in the atmosphere [3]. With rising levels of atmospheric  $\text{CO}_2$ , there has been growing concern on SOC sequestration in recent years [4]. As one of the most widespread terrestrial ecosystems in the world, grasslands play a crucial role in the global C cycle and provide key ecosystem services [5]. It is estimated that grasslands store 10–30% of the global SOC with a C sequestration rate of 0.5 Pg C year<sup>-1</sup> [6]. Due to the strong potential for C sequestration, grassland soils may act as a sink for atmospheric  $\text{CO}_2$  and contribute to the mitigation of climate change [7,8]. Since most of the uncultivated grasslands are grazed by large mammals, the effect of grazing on SOC is vital for C sequestration in grasslands [5]. Unfortunately, large areas of grasslands are experiencing overgrazing worldwide, which not only reduces the plant biodiversity and productivity but also modifies the structure and function of ecosystem, leading to a depletion of SOC stock [6,9]. In the context of climate change, the restoration of overgrazed grasslands is thus urgently needed to recover SOC levels.

Globally, a common practice for restoring overgrazed grasslands is grazing exclusion [10,11]. However, despite the effectiveness of grazing exclusion on grassland restoration, there is currently no consensus on how SOC stock changes following grazing exclusion. Although many studies reported increased SOC stock following grazing exclusion [12,13], some studies found negligible or decreased changes in SOC stock [14–17]. Furthermore, most studies estimated the changes in SOC stock after grazing exclusion by multiplying SOC concentration with soil bulk density (BD) to a fixed soil depth [18,19]. This method may lead to over- or underestimation of SOC stock when soil BD changes after grazing exclusion [13,20]. In fact, the elimination of livestock trampling and the increased accumulation of root biomass after grazing exclusion have considerable impacts on soil BD [14,21]. In this case, SOC stock should better be quantified with equivalent soil mass [20,22]. It has been demonstrated that the equivalent soil mass method produces more accurate results than the fixed depth method when evaluating the changes in SOC stock in response to land-use or management practice [20,23]. Although increasingly used, the equivalent soil mass method is still rarely employed to assess the dynamic of SOC stock after grazing exclusion.

In addition to SOC, soil N in grasslands has also received much attention because it plays a key role in global biogeochemical cycles [6,24]. Moreover, the interactions of C and N in soils are of great importance for regulating the main ecological processes such as nutrient cycling and energy flow [25]. The relationship between soil total nitrogen (STN) status and SOC sequestration has been discussed in previous studies, some of which pointed out that the dynamic of STN determined whether the C sink in terrestrial ecosystems could be sustained over the long-term [26,27]. Other studies claimed that N might be a major limitation for the formation of soil organic matter in grasslands, and then influenced SOC sequestration [28,29]. Consequently, the impact of grazing exclusion on STN stock should also be quantitatively assessed to better understand the effectiveness of grazing exclusion on improving SOC stock.

The Qinghai-Tibetan Plateau (QTP), known as the third pole of the world, is the highest, largest, and most unique type of plateau in the world with an area of 2.5 million km<sup>2</sup> [13,30]. Over 85% of the QTP is covered by alpine grasslands, which are principally used for yak and Tibetan sheep grazing [18]. However, it is estimated that almost 30% of alpine grasslands in the QTP have been severely degraded due to the impacts of overgrazing, rodent activities, and climate change in recent decades [31,32]. Grassland degradation in this region has not only led to a decline in forage yield for livestock grazing but also induced a reduction in ecosystem services [18,33]. In 2003, China's state and local authorities initiated a national ecological project named "Returning Grazing Land to Grassland" to recover the degraded grasslands [34]. From then on, grazing exclusion has become a commonly used management practice for restoring the degraded alpine grasslands on the QTP [11,19]. Nevertheless, the dynamics

of SOC and STN stocks after grazing exclusion and their controlling factors are currently not well understood in this region because of the inconsistent results in individual studies [13,14,16,35].

Based on the dataset compiled from published studies, a meta-analysis was carried out to quantitatively assess the changes in SOC and STN stocks in topsoil (0–30 cm) after grazing exclusion in three major grassland types (alpine meadow, alpine steppe, and alpine desert steppe) on the QTP. Potential factors influencing the dynamics of SOC and STN stocks after grazing exclusion were also explored. The following hypotheses were tested: (1) grazing exclusion would improve both SOC and STN stocks because vegetation restoration could increase the inputs while reducing the outputs of soil organic matter; (2) the change in SOC stock was closely correlated to that in STN during the period of grazing exclusion.

## 2. Materials and Methods

### 2.1. Data Compilation

This meta-analysis was based on studies that investigated the impacts of grazing exclusion on SOC and STN dynamics in alpine grasslands of the QTP. Several databases including Web of Science, Google Scholar and China National Knowledge Infrastructure were employed to search peer-reviewed studies published before October 2019. The keywords used in the literature search including “grazing exclusion or fencing”, “soil carbon or soil nitrogen or soil properties”, “grassland or pasture”, and “Qinghai-Tibetan Plateau or Qinghai or Tibet”. To be included in this analysis, a study had to meet the following criteria: (1) studies should be conducted in natural grazed grasslands rather than in virgin grasslands or cultivated grasslands; (2) studies were carried out using paired-site chronosequence, making similar climatic and soil conditions for the grazing and grazing exclusion sites; (3) the duration of grazing exclusion should be at least one year; (4) SOC stock, STN stock, or both of them (0–30 cm soil layer) were presented or could be calculated based on concentrations of SOC and STN, BD, and sampling depth for both grazing and grazing exclusion sites. Since soil equivalent mass correction was performed to estimate changes in SOC and STN stocks after grazing exclusion in this study, soil BD of both grazing and grazing exclusion sites had to be presented or could be calculated according to stock and concentration of SOC or STN; (5) studies needed to provide sample sizes for both grazing and grazing exclusion sites. Applying these criteria, a total of 110 paired observations reported by 40 peer-reviewed publications were selected for further analyses (Figure 1 and Supplementary Table S1).

The raw data were either obtained from tables or extracted from digitizing graphs using the GetData Graph Digitizer (version 2.25, Sergei Fedorov, Moscow, Russian Federation). For each publication, the following information was also compiled when it was available: location (county, latitude, and longitude), elevation (m), mean annual temperature (MAT, °C) mean annual precipitation (MAP, mm), grassland type, the duration of grazing exclusion (year), sampling depth (cm), aboveground biomass (AGB,  $\text{g m}^{-2}$ ), and belowground biomass (BGB,  $\text{g m}^{-2}$ ). Soil C:N ratio (molar ratio) was calculated based on the concentrations of SOC and STN. The collected dataset was grouped into three categories according to grassland types (alpine meadow, alpine steppe, and alpine desert steppe) to assess whether the impacts of grazing exclusion on SOC and STN stocks could be different among grassland types.

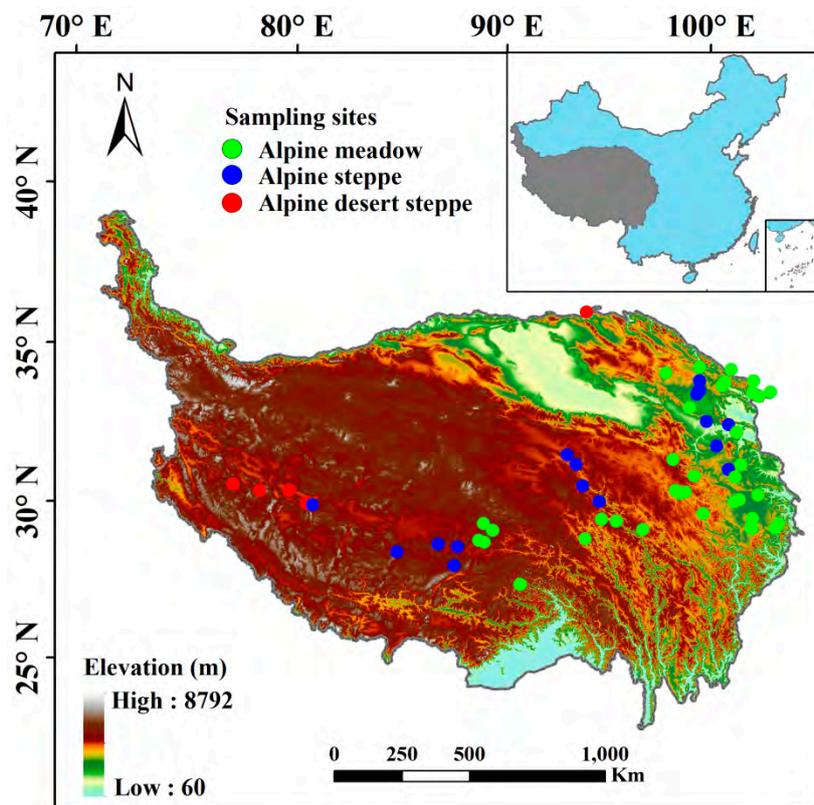


Figure 1. Sampling sites distribution of the individual studies included in this meta-analysis.

## 2.2. Data Calculation

In the present study, the minimum equivalent soil mass method, which adjusted soil mass to the lowest soil mass within each pair of sites, was employed to perform mass correction for both SOC and STN stocks. This method has been demonstrated as a better choice than the maximum equivalent soil mass method in native and restored grassland ecosystems where SOC concentrations decrease through the soil profile [20]. As described by Lee et al. [20] and Bárcena et al. [36], the first step of the minimum equivalent soil mass method was to calculate the mass per unit area of the soil according to a fixed depth in both grazing and grazing exclusion sites:

$$M_{fd} = BD \times h \times 100, \quad (1)$$

where  $M_{fd}$  is the dry soil mass to a fixed depth ( $\text{Mg ha}^{-1}$ );  $BD$  is the bulk density ( $\text{g cm}^{-3}$ ); and  $h$  is the thickness of soil layer (cm). Second, the  $M_{fd}$  in grazing and grazing exclusion sites was compared to select the site with the lightest soil mass, which was considered as the reference soil. A certain amount of soil mass ( $M_{sub}$ ,  $\text{Mg ha}^{-1}$ ) needed to be subtracted from the heavier soil, which had higher soil mass compared to the reference soil to obtain equivalent soil mass:

$$M_{sub} = M_{fd} - M_{equiv}, \quad (2)$$

where  $M_{equiv}$  is the equivalent soil mass ( $\text{Mg ha}^{-1}$ ). Finally, the SOC and STN stocks in equivalent soil mass were calculated using the following equations:

$$Stock = Con \times M \times 0.001, \quad (3)$$

$$Stock_{equiv} = Stock_{fd} - Stock_{sub}, \quad (4)$$

where  $Stock$  is the SOC or STN stock ( $Mg\ ha^{-1}$ );  $Con$  is the concentration of SOC or STN ( $g\ kg^{-1}$ );  $M$  is the soil mass ( $Mg\ ha^{-1}$ );  $Stock_{equiv}$  is the SOC or STN stock in equivalent soil mass ( $Mg\ ha^{-1}$ );  $Stock_{fd}$  is the SOC or STN stock to a fixed depth ( $Mg\ ha^{-1}$ ); and  $Stock_{sub}$  is the SOC or STN stock calculated for  $M_{sub}$  ( $Mg\ ha^{-1}$ ).

### 2.3. Data Analysis

To assess the differences in SOC stock, STN stocks, or soil C:N ratio between grazing and grazing exclusion sites, the natural log-transformed response ratio ( $\ln RR$ ) was used as the effect size:

$$\ln(RR) = \ln\left(\frac{X_{GE}}{X_G}\right), \quad (5)$$

where  $X_{GE}$  and  $X_G$  represent the mean SOC stock, STN stock, or soil C:N ratio at grazing exclusion sites and grazing sites, respectively. The results were transformed to the percentage changes  $((RR-1) \times 100)$  to present the impacts of grazing exclusion on SOC stock, STN stock, and soil C:N ratio. Positive percentage changes indicated grazing exclusion increased SOC stock, STN stock, or soil C:N ratio, whereas negative values denoted reductions in SOC stock, STN stock, or soil C:N ratio after grazing exclusion [37,38].

In previous meta-analyses, the effect sizes were generally weighted by the inverse of pooled variance [11] or replication [37–39]. Since not all the collected studies reported the standard deviations of the mean values, and extreme weights might be induced by variance-based weighting function [40], the effect sizes were thus weighted by a function of replication [37–39]:

$$weight = \frac{n_{GE} \times n_G}{n_{GE} + n_G}, \quad (6)$$

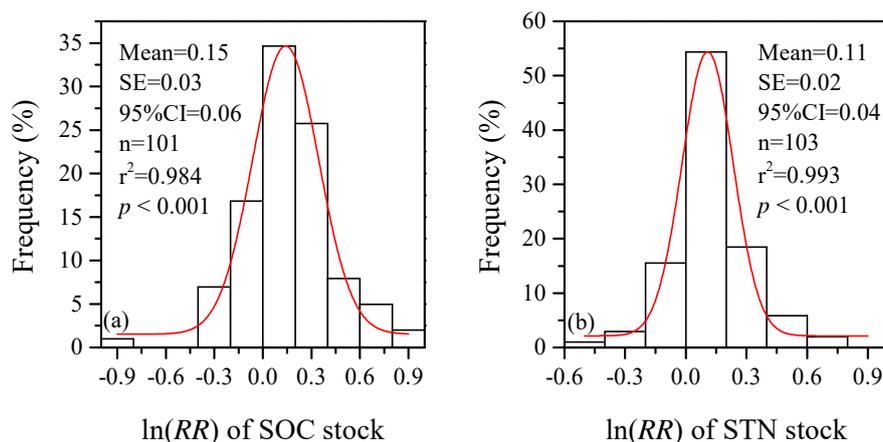
where  $n_{GE}$  and  $n_G$  are the numbers of replications of the treatment group (grazing exclusion) and control group (grazing), respectively.

Mean effect sizes and the 95% confidence intervals (CIs) were generated by a bootstrapping procedure based on 4999 iterations permutations using MetaWin 2.1 (Sinauer Associates, Sunderland, UK) [41]. It should be noted that a mixed-effects model or a fixed-effects model was technically not appropriate for non-parametric meta-analytic procedures based on weighting by replication. Nevertheless, a fixed-effects model had to be selected when running a correct bootstrapping using MetaWin [38]. The effects of grazing exclusion on SOC and STN stocks were deemed as significant if the 95% CIs did not overlap with zero. Means of different categorical variables were considered to be significantly different from one another if their 95% CIs were non-overlapping.

## 3. Results

### 3.1. Frequency Distribution of Effect Sizes of SOC and STN Stocks

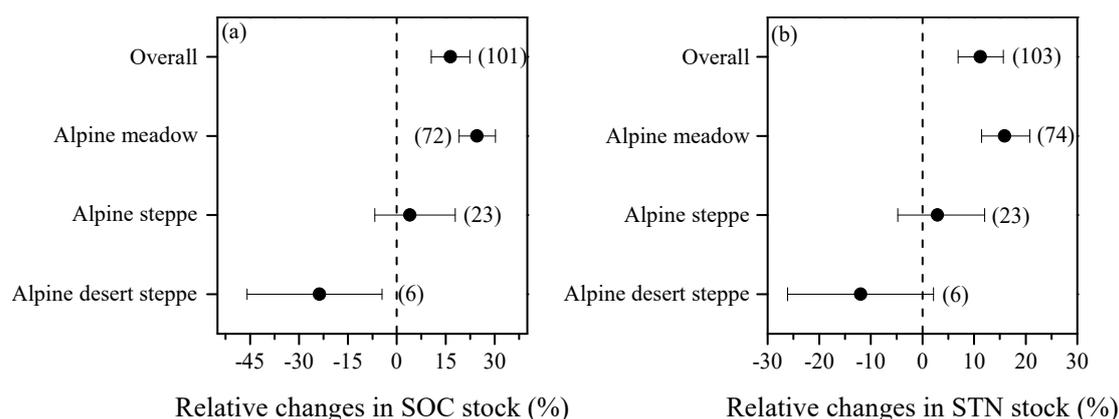
As shown in Figure 2, the effect sizes of both SOC and STN stocks varied greatly among different observations. For both SOC and STN stocks, the frequency distribution of the effect sizes could be characterized by a Gaussian distribution. The mean effect sizes of SOC and STN stocks were 0.15 and 0.11, respectively. Among the 101 observations, 75 observations showed increases in SOC stock after grazing exclusion. In most cases, the effect sizes of SOC stock varied between  $-0.10$  and  $0.30$ . Similarly, grazing exclusion led to increases in STN stock in most observations (83 of the total 103 observations). The effect sizes of STN stock were mainly distributed between  $-0.10$  and  $0.30$  as well.



**Figure 2.** Frequency distributions of the effect sizes of soil organic carbon (SOC) (a) and soil total nitrogen (STN) (b) stocks among all observations. The curves were fitted by a Gaussian function.  $\ln(RR)$  indicates response ratio.

### 3.2. Changes in SOC and STN Stocks after Grazing Exclusion

Across all the studies compiled in this meta-analysis, SOC stock significantly increased by 16.5% after grazing exclusion. For alpine meadow, grazing exclusion significantly increased SOC stock by 24.6%, which was significantly higher than that after grazing exclusion of alpine steppe (4.0%). Conversely, grazing exclusion led to a significant reduction in SOC stock (−23.7%) in alpine desert steppe. The changes in SOC stock after grazing exclusion also differed significantly between alpine meadow and alpine desert steppe (Figure 3a). Overall, STN stock significantly increased by 11.2% after grazing exclusion. Grazing exclusion enhanced STN stock by 15.9% and 2.9% in alpine meadow and alpine steppe, respectively. Nevertheless, the increase was only significant in alpine meadow. Similar to SOC stock, STN stock showed a decreasing trend after grazing exclusion of alpine desert steppe, but the decrease (−12.0%) was not significant. The changes in STN stock after grazing exclusion only differed significantly between alpine meadow and alpine desert steppe (Figure 3b).

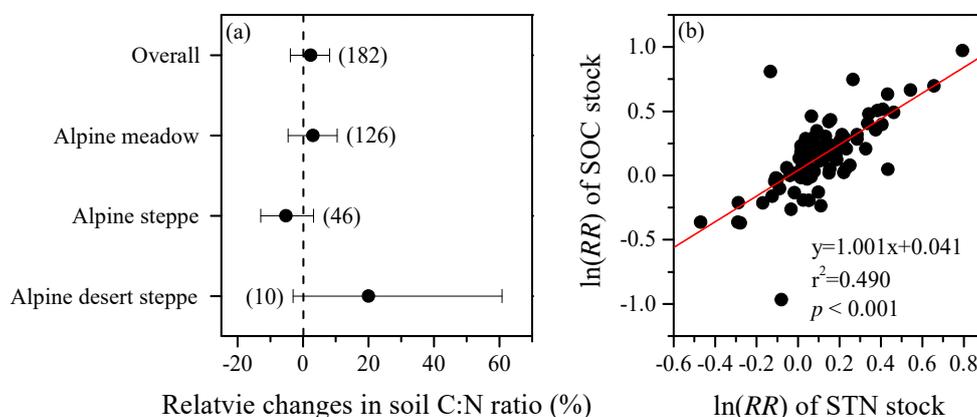


**Figure 3.** Relative changes in soil organic carbon (SOC) (a) and soil total nitrogen (STN) (b) stocks after grazing exclusion. Points are means with 95% confidence intervals. Numbers of experimental observations are in parentheses.

### 3.3. Effects of Grazing Exclusion on Soil C-N Coupling Relationship

Overall, grazing exclusion increased soil C:N ratio by 2.3%. For alpine meadow and alpine desert steppe, soil C:N ratio increased by 3.0% and 20.0% after grazing exclusion, respectively. In contrast, grazing exclusion of alpine steppe reduced soil C:N ratio by 5.2%. Nevertheless, none of the changes were significant (Figure 4a). As illustrated in Figure 4b, significantly positive relationship was detected

between the effect size of SOC stock and that of STN stock ( $r^2 = 0.490$ ,  $p < 0.001$ ). In 73% observations, both SOC and STN stocks showed increasing trends after grazing exclusion. In contrast, nearly 14% observations found that grazing exclusion led to reductions in both SOC and STN stocks. The rest of observations found that grazing exclusion had opposite effects on SOC and STN stocks.



**Figure 4.** Effects of grazing exclusion on soil carbon-nitrogen (C-N) coupling relationship: (a) relative changes in soil C:N ratio after grazing exclusion; (b) relationship between the effect size of soil organic carbon (SOC) stock and that of soil total nitrogen (STN) stock. ln(RR) indicates response ratio.

### 3.4. Relationships between MAT, MAP, the Duration of Grazing Exclusion, and the Effect Sizes of SOC and STN Stocks

The Person correlation coefficients between MAT, MAP, the duration of grazing exclusion, and the effect sizes of SOC and STN stocks were presented in Table 1. The effect sizes of both SOC and STN stocks showed no relationship with MAT ( $p > 0.05$ ). There was no relationship between the effect size of STN stock and MAP ( $p > 0.05$ ), which was positively correlated to the effect size of SOC stock ( $p < 0.05$ ). By comparison, the duration of grazing exclusion showed positive relationships with the effect sizes of both SOC ( $p < 0.01$ ) and STN stocks ( $p < 0.05$ ). Although the relationships were statistically significant, it should be noted that the relationships were generally low as indicated by the low  $r$  values (0.242–0.310).

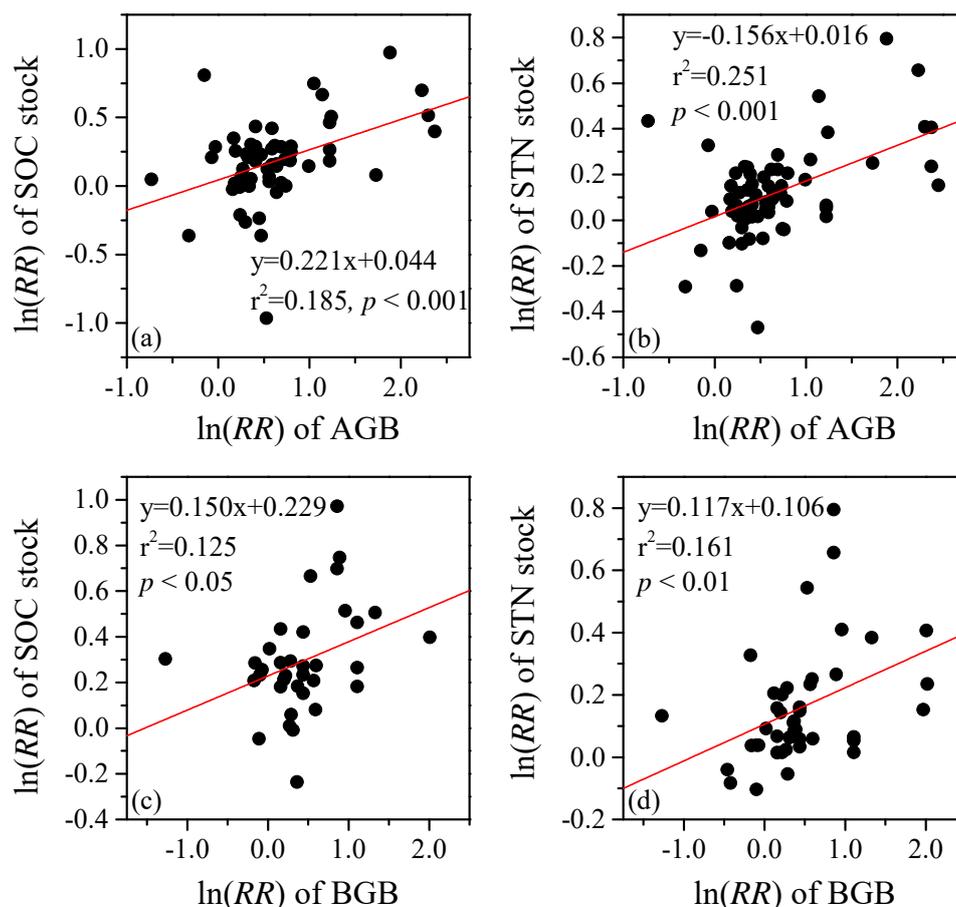
**Table 1.** Person correlation coefficients ( $r$ ) between mean annual temperature (MAT), mean annual precipitation (MAP), the duration of grazing exclusion and the effect sizes of soil organic carbon (SOC) and soil total nitrogen (STN) stocks.

	ln(RR) of SOC Stock		ln(RR) of STN Stock	
	$r$	$n$	$r$	$n$
MAT ( $^{\circ}$ C)	0.081	97	0.132	100
MAP (mm)	<b>0.247 *</b>	98	0.193	100
Duration (year)	<b>0.310 **</b>	98	<b>0.242 *</b>	101

Bold values indicate correlations are significant ( $p < 0.05$  or  $0.01$ ); ln(RR) indicates response ratio; \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

### 3.5. Relationships between the Effect Sizes of SOC Stock, STN Stock, AGB and BGB

As shown in Figure 5a,b, the effect sizes of both SOC ( $r^2 = 0.185$ ,  $p < 0.001$ ) and STN stocks ( $r^2 = 0.251$ ,  $p < 0.001$ ) were positively correlated to that of AGB. Positive relationship was also observed between the effect size of SOC stock and that of BGB ( $r^2 = 0.125$ ,  $p < 0.05$ ) (Figure 5c). Similarly, the effect size of STN stock showed a positive relationship with that of BGB ( $r^2 = 0.161$ ,  $p < 0.01$ ) (Figure 5d). However, the relationships between the effect sizes of SOC stock, STN stock, AGB, and BGB were generally weak because the  $r^2$  values were low.



**Figure 5.** Relationships between: (a) the effect size of soil organic carbon (SOC) stock and that of aboveground biomass (AGB); (b) the effect size of soil organic nitrogen (STN) stock and that of AGB; (c) the effect size of SOC stock and that of belowground biomass (BGB); (d) the effect size of STN stock and that of BGB.  $\ln(RR)$  indicates response ratio.

## 4. Discussion

### 4.1. Overall Effects of Grazing Exclusion on SOC and STN Stocks

Overall, the results of this meta-analysis showed that grazing exclusion significantly enhanced both SOC (16.5%) and STN (11.2%) stocks in topsoil, supporting the first hypothesis that grazing exclusion would improve both SOC and STN stocks in alpine grasslands of the QTP. The results were in agreement with those of previous syntheses [10,34,42]. For example, Xiong et al. [10] found that SOC stock in the upper 30 cm soil layer significantly increased by 14.4% after grazing exclusion in grasslands across China. Since C and N dynamics in soils are primarily determined by the balance between the inputs (e.g., litter and dead roots) and the outputs (e.g., decomposition) of soil organic matter [37,43], there are several underlying mechanisms responsible for the increased SOC and STN stocks after grazing exclusion. First, the removal of grazing pressure reduced the outputs of C and N from the ecosystem to livestock and increased the net primary productivity of grasslands (Figure 5) [34,44,45]. In this case, the inputs of organic matter into the soils might increase. Second, grazing exclusion improved the capacity of soil water conservation by reducing bare soil water evaporation because of the increased vegetation height, coverage, and mulch. The improved soil moisture further led to a higher plant productivity and soil organic matter inputs [34]. Third, the exclusion of livestock trampling might promote the physical protection of soil organic matter by increasing soil aggregate, slowing down the decomposition of soil organic matter [46,47]. Fourth, the increased proportion of leguminous species in plant community after grazing exclusion might contribute to STN accumulation

through biological N fixation [48]. However, the relative importance of these mechanisms in improving SOC and STN stocks during the period of grazing exclusion remains unclear and needs to be focused on in future studies.

#### 4.2. Factors Controlling the Effects of Grazing Exclusion on SOC and STN Stocks

In alpine regions, both temperature and evapotranspiration are low because of the high elevation. Hence, SOC is difficult to decompose and mostly remains in soils for a long time in alpine grasslands [49]. It was estimated that the C stored in the soils of alpine grasslands accounted for more than half of the total SOC stock in grasslands of China, indicating a significant role of alpine grassland soils in regulating climate change [50]. Previous studies have pointed out that SOC and STN stocks differ considerably among different grassland types on the QTP [50,51]. Nevertheless, whether the effectiveness of grazing exclusion on improving SOC and STN stocks can be different in alpine meadow, alpine steppe, and alpine desert steppe—three major grassland types on the QTP—remains unclear. In this study, significant increases in both SOC (24.6%) and STN stocks (15.9%) were observed after grazing exclusion of alpine meadow (Figure 3), demonstrating that grazing exclusion was an effective management practice for enhancing SOC and STN stocks in alpine meadow of the QTP. In contrast, grazing exclusion of alpine steppe had no impact on both SOC (4.0%) and STN stocks (2.9%), and grazing exclusion of alpine desert steppe only significantly altered SOC stock (−23.7%) (Figure 3). The results implied that grazing exclusion might not be a promising way to recover SOC and STN levels in alpine steppe and alpine desert steppe on the QTP. Climatic conditions are common factors used for the classification of grassland type [52]. The relationships between SOC stock, MAT, and MAP in grasslands were reported in previous studies, most of which found that SOC or STN stock was positively correlated to MAP [10,53,54]. In contrast, there is currently no consensus on the relationship between SOC or STN stock and MAT because of the inconsistent results in individual studies [10,34,53]. In the present study, the effect sizes of both SOC and STN stocks were unrelated to MAT (Table 1), suggesting that MAT was not a key factor regulating the impacts of grazing exclusion on SOC and STN stocks in alpine grasslands of the QTP. By comparison, a positive relationship was observed between the effect size of SOC stock and MAP (Table 1), although the relationship was weak. The result indicated that the effectiveness of grazing exclusion on improving SOC stock might increase with MAP, which was generally higher in alpine meadow than in alpine steppe and alpine desert steppe [55]. This was possibly due to the fact that plant productivity usually increased with MAP in alpine grasslands of the QTP, leading to higher inputs of soil organic matter in alpine meadow than those in other two grassland types [55,56]. Since nearly 60% of the grasslands on the QTP are alpine meadows, it is suggested that grazing exclusion of alpine meadow may provide substantial opportunities for improving SOC and STN levels on the QTP.

Empirical evidence has indicated that the duration of grazing exclusion plays a crucial role in determining the dynamics of SOC and STN stocks after grazing exclusion [10,42,57]. The results of this meta-analysis showed that the effect sizes of both SOC and STN stocks were positively correlated to the duration of grazing exclusion (Table 1), demonstrating that both SOC and STN stocks increased with the duration of grazing exclusion. In the compiled studies, the duration of grazing exclusion varied from 1 year to 11 years. Compared to short-term grazing exclusion, grazing exclusion with longer years often leads to higher plant productivity and coverage because the exclusion of livestock grazing creates a suitable environment for plant growth [58]. In this case, the inputs of soil organic matter (e.g., litter and dead roots) may increase with the duration of grazing exclusion and contribute to continuous accumulations in both SOC and STN stocks. Moreover, similar to the findings of Deng et al. [34], the results of this study showed that there was no relationship between the annual change in SOC or STN stock (% year<sup>−1</sup>) and the duration of grazing exclusion (Supplementary Table S2), suggesting that the duration of grazing exclusion did not affect the variation rate of SOC or STN stock. Therefore, it is suggested that the duration of grazing exclusion may not be a key factor determining the effectiveness of grazing exclusion on improving SOC and STN stocks in alpine grasslands of the QTP, at least within

the first decade following grazing exclusion. Nevertheless, some studies found that long-term grazing exclusion could induce a decline of plant biodiversity because plant species with weaker competitive ability might disappear from the plant community [48,57,59]. Consequently, the balance between SOC recovery and plant biodiversity maintenance should be paid closer attention to in the future to explore the optimum exclusion duration in alpine grasslands of the QTP.

#### 4.3. C-N Interactions

Since additional N is required to support terrestrial C sequestration due to stoichiometric relationship in soils, the availability of N is thus crucial for determining whether the C sink in terrestrial ecosystems can be sustained over the long-term [26,27]. As illustrated above, both SOC and STN stocks showed increasing trends with the duration of grazing exclusion (Table 1), and a positive relationship was detected between the effect size of SOC stock and that of STN stock (Figure 3b). The results implied that the dynamics of SOC and STN during the period of grazing exclusion were strongly coupled in alpine grasslands of the QTP. Moreover, the results of this study showed that grazing exclusion had no impact on soil C:N ratio for all grassland types on the QTP (Figure 4a). The results were in agreement with those of previous syntheses [11,34,42]. For example, Hu et al. [42] pointed out that there was no significant change in soil C:N ratio if the duration of grazing exclusion was less than 15 years. Yu et al. [11] observed that the differences in soil C:N ratio between grazing sites and grazing exclusion sites were not significant at all stages of grazing exclusion. The stable soil C:N ratio after grazing exclusion indicated that N might not be a limiting factor of SOC accumulation during the period of grazing exclusion in alpine grasslands of the QTP. Hence, it is suggested that the increase in STN may support continuous SOC accumulation following grazing exclusion in alpine grasslands of the QTP.

## 5. Conclusions

In this meta-analysis, it was observed that grazing exclusion significantly increased SOC (16.5%) and STN (11.2%) stocks in the upper 30 cm soil layer in alpine grasslands of the QTP. The impacts of grazing exclusion on SOC and STN stocks differed considerably among different grassland types. For alpine meadow, grazing exclusion significantly enhanced SOC and STN stocks by 24.6% and 15.9%, respectively. However, neither SOC nor STN stocks showed increasing trends after grazing exclusion of alpine steppe and alpine desert steppe. The difference in MAP among the three grassland types was a likely reason for the different dynamics of SOC and STN stocks following grazing exclusion. Hence, it is suggested that grazing exclusion is an effective management practice for recovering SOC and STN levels in alpine meadow on the QTP. Both SOC and STN stocks increased with the duration of grazing exclusion, as indicated by the positive relationship between the effect size of SOC or STN stock and the duration of grazing exclusion. However, the variation rates of both SOC and STN stocks were unrelated to the duration of grazing exclusion, suggesting that the duration of grazing exclusion did not influence the effectiveness of grazing exclusion on improving SOC and STN stocks. A positive relationship was detected between the effect size of SOC stock and that of STN stock. Moreover, grazing exclusion had no impact on soil C:N ratio for all grassland types. The results indicated that the dynamics of SOC and STN were strongly coupled and the increase in STN could support continuous SOC accumulation during the period of grazing exclusion. The trade-off between SOC accumulation and plant biodiversity maintenance should be paid more attention in further studies to explore the optimum exclusion duration in this region.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2071-1050/12/3/977/s1>, Table S1. List of studies included in this meta-analysis.; Table S2. Person correlation coefficients ( $r$ ) between the annual soil organic carbon (SOC) stock change, the annual soil total nitrogen (STN) change, mean annual temperature (MAT), mean annual precipitation (MAP), and the duration of grazing exclusion.

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