Relationships of plant species richness, grazing, and aridity with soil organic carbon in Xinjiang (China) grassland

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Abstract

Soil carbon is a critical ecosystem function in drylands. In these ecosystems, positive relationships between plant species richness (SR) and soil carbon storage (SOC) that have been found in biodiversity experiments and observational studies may be reduced by grazing and aridity. However, studies about the extent to which SR, grazing intensity, and aridity are interactively and directly or indirectly related with SOC so far provided mixed results. Using a network of 199 grassland sites across a large aridity gradient in western China, selected to represent low, medium, and high grazing intensity, we found that SOC at the depth of 0–30 cm was positively related with SR and, to a lesser degree, with grazing intensity. Aridity had no direct relationship with SOC but affected it indirectly and negatively via its negative relationships with both SR and grazing intensity and via its positive relationship with soil pH. There were no indications that grazing intensity could modify the positive SR–SOC relationship, possibly because very high grazing intensities did not occur in the study region. We conclude that current levels of SR and grazing intensity should be maintained to avoid SOC-loss and CO2 release form grassland under predicted aridity increases in the study region.

Introduction

Most of the carbon (C) in the Earth’s terrestrial ecosystems is stored in soil and a large part of this soil C can be found in grasslands (Davidson and Janssens 2006). Biotic communities are the fundamental drivers of carbon fixation, and of its later incorporation into soil organic matter. Multiple experimental studies in grassland (and more recently forest) ecosystems have demonstrated that reductions in plant species richness (SR) lead to reductions in soil organic C (SOC) via decreased plant productivity, reduced soil C inputs, decreased soil microbial activity, and further pathways (Balvanera et al. 2006, Fornara and Tilman 2009, Eisenhauer et al. 2010, Lange et al. 2015, Li et al. 2019). However, there are few comparative studies reporting relationships between SR and SOC storage in natural grasslands across large spatial scales, where gradients of aridity and grazing intensity may modify SR, SOC, and the relationship between the two (Milchunas et al. 1988, Derner et al. 2006, Klumpp et al. 2009, Deng et al. 2014, Joubert et al. 2017, Chen et al. 2018). Here we asked if the positive relationship between SR and SOC that has been found in grassland biodiversity experiments and observational studies is reduced by grazing and aridity, as has been suggested by recent work (Maestre et al. 2022, Sanaei et al. 2023, Zhang et al. 2023), across the large grassland region in Xinjiang, western China.

In addition to these potential interactive relationships between grazing, aridity, and SR on SOC, aridity may affect SOC directly or indirectly via relationship with grazing and SR. However, whether aridity effects on
SOC are fully indirectly driven via such changes in biotic communities, along with the contribution of aridity-independent relationships of grazing and SR with SOC, remains less understood in this context. Thus, in addition to testing for interactions between aridity, grazing, and SR in their association with SOC, we also tested for direct and indirect effects of aridity on SOC, using grazing and SR as intermediary variables in path analyses.

We selected 199 grassland sites with low, intermediate, or high grazing intensity in equal proportions within nine grassland vegetation types (Xu 1993) of different aridity for our comparative observational study and measured SR and SOC at each site across 0–30 cm of soil depth in replicated plots. We examined the main and interactive relationships of aridity, grazing intensity, and SR with SOC using linear mixed models with grassland type as random term. We then combined all these analyses into structural equation models (SEMs) to assess potential direct and indirect relationships between variables, in particular aridity and SOC.

Methods

Study sites and data collection

Xinjiang is in the northwest of China and far away from the sea, thus forming a typical temperate continental climate characterized by low rainfall and abundant light. Xinjiang has a special landform of two basins lying between three mountain ranges. These are Altay Mountains in the north, Kunlun Mountains in the South and Tianshan Mountains in the middle. These three mountain ranges together are a major distribution area of natural grassland covering 3.32 $\times$ 10$^7$ ha and accounting for 58% of the total natural grasslands in Xinjiang. They also represent the main livestock husbandry area in Xinjiang.

To study the relationships of grazing intensity and SR with SOC along the aridity gradient in these grasslands, we used a similar comparative study design as in Liu et al. (2023), but here we selected 199 sampling sites according to grazing level rather than level of wood encroachment. Of the 284 sites used in that previous study four were also used in the present study. The 199 sampling sites covered nine grassland types from alpine meadow to temperate meadow steppe, mountain meadow, temperate steppe, alpine steppe, temperate desert steppe, temperate desert, temperate steppe desert (Xu 1993) (Supporting information).

The local grassland station in charge of sampling sites provided the level of grazing intensity of each sampling site. They determined grazing intensity by measuring the consumed percentage of the height of dominant palatable grass species compared with surrounding plots with grazing exclusion. The sampling sites were grouped into three classes of grazing from low (mean value of consumed height over the last 10 years before sampling < 25%, 68 sites) to medium (mean value of consumed height over the last 10 years before sampling 25–75%, 96 sites) to high (mean value of consumed height over the last 10 years before sampling > 75%, 35 sites). The grazing history of all sampling sites was [?] 50 years. The main gazing livestock were sheep.

From 2011–2013 we conducted field surveys between mid-July and early August, when standing aboveground biomass reached its maximum. Year was used as blocking factor because it would not have been feasible to survey all sites in one year. At each site, a 100 x 100 m sampling plot was established and then five 1 x 1 m quadrats were evenly arranged along a diagonal of the sampling plot. The average number of plant species of the five quadrats was used as the species richness (SR) of a sampling site. A stainless-steel corer (100 cm$^3$, 5 cm diameter) was used to sample the soil for determining bulk densities at 0–5, 5–10, 10–20 and 20–30 cm depth. At sampling sites where there were many stones, a soil column excavation method was used. Soil samples for C-content analysis were also taken at depths of 0–5, 5–10, 10–20 and 20–30 cm.

In the laboratory, all soil samples were air-dried and then sieved through a 2-mm mesh to remove gravel and roots. The obtained fine earth (< 2 mm) was used for further analysis. Soil pH of each soil depth was determined using a 1:5 soil:water mixture. The obtained fine earth was ground in a ball mill (Retsch MM20, Germany) and its total carbon content (TC, g kg$^{-1}$) was determined using an elemental analyzer (Euro Vector EA3000, Germany). The soil inorganic carbon content (SIC, g kg$^{-1}$) was analyzed using a carbonate analyzer (Eijkelkamp, Netherlands). The soil organic carbon content (SOC, g kg$^{-1}$) was calculated as the difference between TC and SIC (Lange et al. 2015).
The mean annual precipitation (MAP; 1961–2011) and mean annual temperature (MAT; 1961–2011) of each sampling site were obtained from a geographic information system-based multiple regression using the China Meteorological Administration climate database (http://www.cma.gov.cn/) (Ninyerola et al. 2000). The potential evapotranspiration (PET) of each sampling site was derived from the Consortium for Spatial Information (http://www.cgiar-csi.org/). An aridity index (hereafter referred to as aridity) was calculated as one minus the ratio of MAP to PET. In this study, we used aridity instead of MAP, because it involves both MAP and potential evapotranspiration, and is therefore a more accurate variable for measuring water availability.

**Statistical analyses**

We used general linear mixed models to analyze main and interactive effects of continuous (altitude, aridity index, MAT, soil pH, soil bulk density, SR) and categorical explanatory variables (3-level factor grazing intensity) on SOC. Year (3-level factor), grassland type (9-level factor), and site (199-level factor) were used as random-effect terms. Using the first two of these is equivalent to correcting site-level data for differences between years and grassland types, i.e., working with residuals after fitting the two terms as fixed effects, but maintains the correct number of residual degrees of freedom (Schmid et al. 2017). Therefore, to plot results of the mixed models, we used corrected values by adding the overall mean of dependent variables to these residuals. In addition, we also plot raw data in figures to show how dependent variables varied across years and grassland types. Note that we did not use grassland type as fixed-effect term because some grassland types had few replicate sites (Supporting information) and it would therefore have been problematic to look at them separately. SR was log-2 transformed and bulk soil density and SOC were log-10 transformed to achieve homoscedasticity and normally distributed residuals for these variables when used as dependent variables. We selected final models using AIC values, starting with the most complex models, removing terms when this resulted in an AIC reduction > 2 (Burnham and Anderson 2002). For the final models we used type-II sum of squares to assess significance, that is each main effect was estimated as if fitted last in the sequence of main effects (Langsrud 2003).

To explore potential causal relationships between aridity, grazing intensity, SR, and SOC we constructed structural equation models (SEMs) combining linear mixed models for SR, bulk soil density and SOC, using year, grassland type and site as random-effect terms (function “piecewiseSEM” in R) (Lefcheck 2016). All analyses were performed using R version 4.3.1 (R Core Team 2021).

**3 Results**

The sampling sites covered a wide range of altitude (230 m to 4232 m), MAT (–1.56 to 13.73 ), aridity (0.53 to 0.97) and soil pH (6.06 to 9.70). Mean plant species richness per m² (SR) ranged from 1–16, and SOC at 0–5, 5–10, 10–20, and 20–30 cm from 1.82 –171.81, 0.96–143.25, 0.65–87.93, and 0.35–94.32 g kg⁻¹, respectively.

SOC had a positive relationship with SR and grazing intensity, both with and without the covariate soil bulk density in the mixed models (Table 1). SOC decreased with soil depth, soil pH, and with the covariate soil bulk density. There was only one significant interaction included in the best model, namely that between the covariate soil bulk density and soil pH. However, the interaction between SR and grazing intensity, if added to the best model (thus making the model worse), was far from significant (F₂,183 = 0.68, P = 0.51 with covariate bulk density and F₂,186 = 0.67, P = 0.51 without covariate bulk density). Environmental variables such as altitude, MAT, and aridity were not included in the best model. However, when aridity and its interaction with SR were added to the best model (thus making the model worse), the interaction was just significant (F₁,187 = 4.18, P = 0.042 with covariate bulk density and F₁,189 = 3.71, P = 0.055 without covariate bulk density). The mixed-model analysis corrected for variation among sites, grassland types and year. If this correction was not applied, effects of SR and to a lesser degree effects of grazing level were even stronger (Figs. 1, 2), because grassland types differed in average SR (Supporting information) and grazing treatments were not perfectly orthogonal to grassland type and year.

In the mixed-model analysis with SR as dependent variable and grassland type and year as random-effects
terms, SR was reduced under more arid conditions (Table 2) but unrelated to grazing level and other site variables. The effect of aridity on SR was stronger if no correction for grassland type and year was applied (Fig. 3), because aridity varied between grassland types (Supporting information). Used as dependent variable, soil bulk density slightly decreased with soil depth and more strongly with SR, with an anti-synergistic interaction between the two (Supporting information).

The above results are summarized in the path analyses from SEMs in Fig. 4. As shown in the mixed-model analysis (Table 1), SR and grazing intensity (in the SEM the three-level factor was converted to a continuous variable with values 0, 1, and 2) were positively associated with SOC while soil pH, soil depth, and soil bulk density were negatively associated with SOC. Besides the direct relationship of SR with SOC there was also a positive but small indirect relationship of SR with SOC via bulk density (multiplication of two negative path coefficients). Aridity had indirect negative effects on SOC via its negative effects on SR and grazing intensity and via its positive effect on soil pH.

4 Discussion

Positive effects of plant species richness (here SR) on ecosystem functions, including levels of aboveground carbon stock and belowground SOC, have been observed in multiple experiments simulating plant species loss from grassland or forest ecosystems (Balvanera et al. 2006, Tilman et al. 2014, Weisser et al. 2017, Huang et al. 2018, Li et al. 2019, Lange et al. 2021). However, when different real-world ecosystems without simulated species loss are compared, relationships between SR and ecosystem functioning are more varied and in part often due to confounding influences of third variables (Schmid 2002, Ma et al. 2010, Dee et al. 2023). In the present study, the positive relationship between SR and SOC remained unchanged after accounting for multiple environmental variables across different grassland types (fitting separate slopes for the different grassland types in the linear mixed model only produced a very small variance component for the interaction SR x vegetation type). The direct positive relationship between SR and SOC was accompanied by an indirect positive one via soil bulk density (see Fig. 4).

In addition to SR, grazing intensity was also positively associated with SOC in our study, although with a smaller path coefficient in SEMs (see Fig. 4). Other studies reported negative (Steffens et al. 2008, Han et al. 2008, He et al. 2011, McSherry and Ritchie 2013), neutral (Shrestha and Stahl 2008), or positive (Reeder et al. 2004, Abdalla et al. 2018) impacts of higher levels of grazing on SOC storage in arid regions. A potential explanation for the positive relationships is that grazing increases nutrient cycling and biomass allocation to roots (de Mazancourt et al. 1998, Belovsky and Slade 2000, Ingram et al. 2008, Abdalla et al. 2018). Furthermore, as in all observational studies, we cannot exclude the possibility of reversed causality, i.e., that sites with higher SOC were preferentially used for more intense grazing.

SR and to a lesser extent grazing intensity were negatively related to aridity, leading to negative indirect effects of aridity on SOC via these two variables. In addition, aridity had a further indirect negative effect on SOC via increased soil pH (see Fig. 4). The three indirect effects explained all aridity effects on SOC, leaving no direct effect and suggesting that among our 199 study sites across western China effects of aridity are fully driven via changes in SR, grazing intensity, and soil pH. Other environmental variables (altitude, MAT) had neither direct nor indirect effects on SOC, except that it decreased with soil depth. Water availability is a limiting factor for SR in arid areas (Delgado-Baquerizo et al. 2013) and more intense grazing is less likely applied at more arid sites within grassland types (between grassland types we had avoided a correlation between aridity and grazing by equal assignment of levels of grazing intensity). With predicted increases in aridity due to global warming (Feng and Fu 2013, Hu et al. 2021), SOC in Xingjian grasslands may decline due to the described indirect effects, contributing to increased release of CO$_2$ from soils. To mitigate this danger as far as possible, grassland management should try to maintain SR under increased aridity. As we show in our study, this could protect SOC levels because we did not find any direct negative effects of aridity on SOC.

Despite the large variation in explanatory variables among our study sites, we did not find interactive relationships of SR, grazing, and aridity with SOC in our best linear mixed model and a just significant
interaction between aridity and SR with SOC in a second-best model (see Results section). In a recent multi-site study across three regions representing three vegetation types in northern China, Zhang et al. (2023) found that experimental exclusion of grazing animals after 10 years led to increased plant diversity and ecosystem multifunctionality in the two more arid regions. Similarly, Maestre et al. (2022), using a standardized survey at 98 sites across six continents, found interactive relationships of grazing, climate, soil, and biodiversity with ecosystem functions, including carbon storage. However, these authors also found that positive relationships between grazing and soil carbon were observed in colder and species-rich areas. In another study from arid grassland on the Qinghai-Tibetan Plateau, grazing intensity and SR, as in our case, were independently and positively associated with SOC (Ganjurjav et al. 2015). It is conceivable that grazing intensity in western China and the Qinghai-Tibetan Plateau was not as high as in the study regions in northern China (Zhang et al. 2023) or elsewhere (Donkor et al. 2002, Gao et al. 2008) and thus could not exert negative effects on SR and SOC even under the most arid conditions. In a recent global meta-analysis of experimental results, biodiversity–ecosystem functioning relationships were found to be largely unchanged by a range of global change factors tested (Hong et al. 2022). Future experiments should manipulate grazing intensity at multiple levels to account for possible hump-shaped effects (Deng et al. 2014, Joubert et al. 2017). Furthermore, experimental results may differ between adding and removing grazers because grassland systems with a long evolutionary history of high vs. low grazing may respond differently to such manipulation (Milchunas et al. 1998, Cingolani et al. 2005). In addition to maintaining SR, we need additional research to develop appropriate grazing management for the protection of SOC under increased grassland aridity.

References


Table 1: Relationships of soil variables, plant species richness (SR, log-2 transformed), and grazing intensity (GI) with SOC (log-10 transformed). Analysis of variance results of linear mixed models with random-effect terms year, grassland type, and site. Upper part analysis without soil bulk density, lower part analysis with soil bulk density (log-10 transformed). Variance components (VC) of random-effect terms are listed below the fixed-effect terms. DF: degrees of freedom of numerator; DDF: degrees of freedom of denominator; SS: type-II sum of squares; F: variance ratio; P: P value. + and – in last column indicate direction of relationships.

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<td>Soil pH</td>
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Table 2: Effects of aridity on plant species richness (SR, log-2 transformed). Analysis of variance results of linear mixed model with random-effect terms year and grassland type (residual = site). Variance components (VC) of the latter are listed below the fixed-effect term aridity. DF: degree of freedom of numerator; DDF: degree of freedom of denominator; SS: sum of squares; F: variance ratio; P: P-value. – in last column indicates direction of effect.

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<td>Year (n = 3)</td>
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<td>Residual (n = 796)</td>
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Table 2: Effects of aridity on plant species richness (SR, log-2 transformed). Analysis of variance results of linear mixed model with random-effect terms year and grassland type (residual = site). Variance components (VC) of the latter are listed below the fixed-effect term aridity. DF: degree of freedom of numerator; DDF: degree of freedom of denominator; SS: sum of squares; F: variance ratio; P: P-value. – in last column indicates direction of effect.

Figure legends

Figure 1: Relationship between soil organic carbon (SOC, log-10 transformed) and plant species richness (SR, log-2 transformed). a) shows the overall relationship and b) the relationship after adjusting for differences between sampling years and grassland types (see Table 1). Both relationships are highly significant (P < 0.001), but the first relationship is stronger because differences potentially due to effects of year and grassland type are not excluded. Adjusted values in b) were calculated by adding the overall mean to residuals obtained in a fit with the explanatory terms year and grassland type.

Figure 2: Relationship between soil organic carbon (SOC, log-10 transformed) and gazing intensity (3-level factor). a) shows the overall relationship and b) the relationship after adjusting for differences between sampling years and grassland types (see Table 1). Both relationships are highly significant (P < 0.001), but the first relationship is stronger because differences potentially due to effects of year and grassland type are not excluded. Adjusted values in b) were calculated by adding the overall mean to residuals obtained in a fit with the explanatory terms year and grassland type.
Figure 3: Relationships between plant species richness (SR, log-2 transformed) and aridity index (1–MAT/PET). a) shows the overall relationship and b) the relationship after adjusting for differences between sampling years and grassland types (see Table 2). Both relationships are highly significant ($P < 0.001$), but the first relationship is stronger because differences potentially due to effects of year and grassland type are not excluded. Adjusted values in b) were calculated by adding the overall mean to residuals obtained in a fit with the explanatory terms year and grassland type.

Figure 4: Structural equation models (SEMs) relating soil organic carbon (SOC, log-10 transformed) to aridity index (1–MAT/PET), plant species richness (log-2 transformed), grazing intensity (continuous variable with levels 1, 2, and 3), soil depth, soil pH, and soil bulk density (log-10 transformed). Arrows are shown with standardized path coefficients (dashed arrows for negative path coefficients), indicating by how much the standard deviation of a variable at the end of an arrow would change if the variable at the beginning of the arrow would change by one standard deviation. All path coefficients were highly significant ($P < 0.001$). The vertical arrows above or below variables indicate error variation (1–$R^2$), that is the proportion of variance of the corresponding variable that is not explained by the model. Statistics for the overall model fit are given in the lower left of the figure. See text for further explanations.

Fig. 1a

Fig. 1b
Fig. 2a

Fig. 2b
Fig. 3a

Fig. 3b
Fig. 4

Fisher’s $C = 25.43$, $P = 0.185$, df = 20, n= 796