Original Research

Restoration Measures Supported Surface Soil Carbon and Nitrogen Density in Alpine Grassland of Sanjiangyuan Region, China

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Abstract

Soil carbon (C) and nitrogen (N) have different responses to different restoration measures. In this paper, surface (0-30 cm) soil C and N densities under different restoration measures in the Sanjiangyuan region were investigated and analyzed. The results showed that although there was no significant difference between rest-grazing (RG) and normal grazing (NG) grassland, SOCD in RG increased by 10.15%, and total N density (TND) increased by 8.56% in the year of the experiment. Compared with cropland, 8 years after Grain for Green (GFG), SOCD increased by 10.49-19.31%, SICD increased by 22.47-54.20%, and TND increased by 1.3-17.45%. Compared with HTT (extreme degradation-black soil beach), 12 years after planting artificial grassland, SOCD increased by 43.97-77.21%, SICD increased by 89.19-716.22%, and TND increased by 49.16-71.40%. Conclusions: Differences in soil types and climatic zones were responsible for the differences in soil C and N in different regions. Short-term grazing rest in Sanjiangyuan region has a certain effect on soil fertility restoration. Implementing the GFG project in the agro-pastoral ecotone improved soil fertility and, concurrently, moderate disturbance should be carried out on the basis of increasing vegetation diversity. Artificial grassland planting on black soil beach can improve soil quality, and moderate grazing disturbance in winter had the best immobilization effect on SIC, while no disturbance in the whole year was beneficial to the accumulation of organic matter. Both biological and abiotic factors have affected soil C and N content and density, but the degree of the influence varied with geographic location.

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Introduction

Located in the southern part of Qinghai Province and the hinterland of the Qinghai-Tibetan Plateau (QTP), Sanjiangyuan is the birthplace of the Yangtze River, the Yellow River and the Lancang River. It is known as the "Chinese Water Tower" and is also a region with high biodiversity and sensitive ecological environment [1]. In recent years, due to the impact of climate change and human activities, the ecosystem of Sanjiangyuan has been deteriorating, which has an important impact on the QTP. In order to protect the fragile eco-climatic environment of Sanjiangyuan, effectively curb grassland degradation and maintain species diversity [2], the State Council launched the "General Plan for Ecological Protection and Construction of Sanjiangyuan Nature Reserve in Qinghai Province" in 2005, and implemented a series of ecological protection and construction projects such as Grain for Green (GFG), which transforms the cultivated land into natural vegetation or perennial artificial grassland/woodland. The aim is to protect and restore the ecological function, improve the regional ecological environment and promote the harmonious development of humans and nature [3]. At the same time, in order to protect the grassland ecological environment, the CPC Central Committee and the State Council proposed in 2011 to further improve the project of "returning grazing land to grassland". According to local conditions and technology, we should focus on rotational grazing and seasonal grazing rest, i.e., forbidding grazing policy for severely degraded grasslands or ecologically fragile areas with slow vegetation restoration, and grazing rest policy for areas with degradation, desertification and overgrazing but good grassland environment and vegetation condition [1, 4]. According to the actual situation in different areas, the local government has taken different measures to restore grassland. For example, in the agro-pastoral ecotone (like Tongde County), the long-term cultivated land is converted to forest and/or grassland. The measures of planting artificial grassland in pastoral areas (such as Maqin County) were adopted [1]. In order to prevent grassland deterioration and restore grassland ecology, the grazing-rest policy should be implemented in areas with better grassland environment and vegetation conditions (Guinan County) [4].

Soil carbon (C) and nitrogen (N) are important components of soil nutrients, they are major nutrient sources for plant growth, and also play an important role in global C and N cycle and balance [5-6]. There are many studies on soil C and N, mainly involving the effects of land use [7-9], management measures [10-12], restoration measures [13-19] on soil C and N, and the relationship between plant functional groups and soil factors [20-22]. However, most related research in China has been concentrated in the Loess Plateau area [10, 13-14, 17, 19, 22], but few studies have focused on alpine regions – especially the Sanjiangyuan region [12, 18, 23-25]. Based on the above grassland restoration background, we investigated the changes of C and N in grassland soil under different restoration measures in the Sanjiangyuan region. The purpose of this study is to clarify: 1) the level of soil C and N in different research areas and 2) the response degree of soil C and N to different restoration measures, and which measures lead to faster recovery of soil C and N.

Materials and Methods

Description of the Study Sites and Experiments

Rest-Grazing and Grazing Experiment

Guinan County (35°09'-36°08'N, 100°13'-101°33'E), Hainan Prefecture, Qinghai Province, is located in the northeastern edge of the QTP, with an average elevation of 3100 m. The area is a typical plateau continental climate with an average annual temperature of 2.4°C, precipitation of 415.8 mm, evaporation of 1378.5 mm and 2712.7 sunshine hours [26-27]. The experimental site is located in Taxiu Township and Sendo Town, with an average elevation of about 3300 m. The interval between the two sampling sites is about 20 km. Half of the grasslands selected at each sampling site are rest-grazing (RG) during the green-returning period (which starts at the end of April 2016), and half are grazed normally (NG). The grassland environment and vegetation conditions in the experimental area were good. The main species were *Kobresia humilis*, *Stipa capillata* L., *Festuca ovina*, *Medicago archiducis-nicolai*, *Astragalus propinquus* Schischkin and *Saussurea hieracioides* Hook. F., *Taraxacum mongolicum* Hand.- Mazz. Soil samples were taken in mid-October 2016.

'Grain for Green' Measures

The experiment was carried out on Tongde Ranch (34°09'N, 100°09'E) located in the Batan area, northeastern Tongde County, Qinghai Province, with an elevation of 3200 m. The climate is typical plateau continental climate, with an average annual temperature of 0.2ºC, annual precipitation of 429.8 mm, annual evaporation of 1466.4 mm, and an annual sunshine duration of 2745.8 h. The soil is dark chestnut calcareous soil [28].

Since 2002, according to local conditions, Tongde County began to implement large-scale measures of

returning farmland to forest and grass. In forest belts, *Caragana korshinskii* Kom is mainly planted; *Elymus breviaristatus* (Keng) Keng f. is planted in grass belts [29]. Grass seeds are harvested at the end of August every year, and straw is used for feeding cattle and sheep. Throughout the winter, cattle and sheep are allowed to feed in grass and forest belts. Grass belts are renewed/plowed every four years. The cultivated land in this area has a history of more than 60 years, mainly for rape (*Brassica napus*) and barley (*Hordeum vulgare* Linn. var. nudum Hook. f.) rotation. The main species in natural grazing grassland are *Kobresia humilis*, *Stipa capillata* L., *Poa pratensis* L., *Oxytropis kansuensi*s Bunge, and *Artemisia gmelinii* Weber ex Stechmann, etc.

The land conversion began in 2008. And in this study, five treatments were chosen: cultivated land (cropland), grass belt (GRG1), forest belt (GFG2), enclosure (GRG3, a 25 × 25 m fence for setting up flux equipment in the experimental area, which was built in 2008 without grazing interference), and natural grassland (NG, normal grazing was carried on). By October 2016 the sampling area had been restored for 8 years.

Measures of Planting Artificial Grassland

The experimental site is located in Maqin County, Guoluo Tibetan Autonomous Prefecture, Qinghai Province (33°43'-35°16'N, 98°48'-100°56'E). The area has a continental cold and humid climate, with an average altitude of over 4100 m, an annual average temperature of -3.8-3.5ºC, and a large temperature difference between day and night. Annual precipitation ranges from 423 mm to 565 mm, mostly in June-September. The annual sunshine time is 2313-2607 h, with strong light radiation. Vegetation types are rich, mainly alpine meadows, which are the main natural grasslands for grazing. Soil types are mainly alpine meadow soil [30].

In this area, artificial grassland planting measures were implemented for the degraded grassland of "Black Soil Beach". *Elymus nutans* (upper grass) and *Poa pratensis* (lower grass) were mainly planted. The sampling area was established in 2002, and the pasture was fenced in the growing season, and the vegetation on the ground was used for winter grazing after seeds were collected. At the same time, a small fence of 25 × 25 m was built in the experimental area, and the fence was for setting up flux equipment. There was no interference in the fence all year except for data collection. The sampling time was October 2014. The selected treatments were degenerated grassland (HTT), winter grazing pasture (WL), year-round forbidden grazing pasture (WF), and natural grazing (NG, normal grazing, the main species were *Kobresia pygmaea*, *Kobresia humilis*, *Kobresia capillifolia*, *Stipa capillata* L., *Festuca ovina*, *Leontopodium leontopodioides*, etc).

Sample Collection and Processing

In each sampling site, 4-6 sampling plots (about 10 m × 5 m) were set up, and the interval between the sites was more than 50 m. According to the "S" sampling method, soil samples were collected by drilling layers (0-10, 10-20, 20-30) with a diameter of 3 cm, and a mixed sample was formed by 5-6 drills per layer in each plot. Meanwhile, the soil bulk density was collected by a 3.8 cm ring knife.

After the soil samples were brought back to the laboratory, impurities were removed and part of them was used to determine soil water content. According to the quartile method, some soil samples were preserved at 4ºC for the determination of inorganic nitrogen (SIN) and microbial biomass C and N, while the others were air-dried and divided into two parts – one for determining soil pH and the other for determining SOC, TN and SIC after 0.15 mm screening.

Soil C and N Analysis

The bulk density and water content were determined by drying method, soil pH was determined in 1:2.5 (w/v) soil/KCl extracts using a combination glass electrode, SIN was determined in 2 M KCl extracts with a Skalar San++ continuous flow analyzer (Skalar Analytical, Breda, The Netherlands), TN by Kjeldahl N analyzer, SOC by potassium dichromate-concentrated sulfuric acid oxidation, SIC by acid titration, and microbial biomass by chloroform fumigation and extraction [31].

Calculating C and N density [32]:

$$SOCD = \sum_{i=1}^{n} Di \times BDi \times SOCi$$

$$SICD = \sum_{i=1}^{n} Di \times BDi \times SICi$$

$$STND = \sum_{i=1}^{n} Di \times BDi \times STNi$$

$$TCD = SOCD + SICD$$

Among them, n is the soil layer, i is 0-10 cm, 10-20 cm or 20-30 cm. *Di* denotes the depth of the soil layer (cm), *BDi* denotes the bulk density of the layer (g cm^{-3}), *SOCi*, *SICi* and *STNi* denote the contents of SOC, SIC and STN (g kg^{-1}) of each layer, respectively.

Data Processing

All statistical analyses were performed by SPSS ver. 16.0 for Windows （SPSS Inc., Chicago, IL, USA）. Significant differences of measurement variables between different land use types were detected by one-way ANOVA or t-test ($P<0.05$). When the variance is uniform, the minimum standard deviation (LSD) is used for multiple comparisons. When variance is inconsistent, the games-Howell test is used for

post-test. Stepwise regression analysis was used to screen the main influencing factors of soil C and N change. The critical value of partial regression square sum of significance test was 0.05.

Results

Response of C and N Density to Rest-Grazing

The average SOCD of 0-30 cm soil layer in the study area was 9.103 (8.292-9.914) kg m^{-2}, SICD was 1.338 (1.162-1.514) kg m^{-2}, TND was 1.014 (0.940-1.088) kg m^{-2} and TCD was 10.442 (9.806-11.077) kg m^{-2} (Fig. 1, Table 2).

Changes of C and N densities in 0-30 cm soil layer: although there was no significant difference in C and N densities between the two treatments, SOCD, TND, TCD and SICD in RG increased by 10.15%, 8.56%, 4.88% and 21.43%, respectively, compared with the general grazing land.

Response of C and N Density to 'Grain for Green'

The average SOCD of 0-30 cm soil layer was 7.375 (6.539-7.785) kg m^{-2}, SICD was 3.561 (2.430-5.022) kg m^{-2}, TND was 0.786 (0.745-0.875) kg m^{-2} and TCD was 10.93 (8.970-12.807) kg m^{-2} (Fig. 2, Table 3).

SOCD in 0-30 cm soil layer: Compared with cultivated land, after 8 years of restoration, GFG1 increased by 10.49%, GFG2 increased by 15.09%, and GFG3 increased by 19.31%.

SICD in 0-30 cm soil layer: Compared with cultivated land, after 8 years of restoration, GFG1 increased by 49.42%, GFG2 increased by 54.20%, and GFG3 increased by 22.47%.

TND in 0-30 cm soil layer: Compared with cultivated land, after 8 years of restoration, GFG1 increased by 1.3%, GFG2 increased by 17.45%, and GFG3 increased by 8.32%.

TCD (SOCD+SICD) in 0-30 cm soil layer: Compared with cultivated land, after 8 years of restoration, GFG1 increased by 21.03%, GFG2 increased by 25.67%, and GFG3 increased by 20.14%.

Response of C and N Density to the Establishment of Artificial Grassland

The average SOCD in 0-30 cm soil layer was 10.896 (6.491-16.246) kg m^{-2}, SICD was 0.135 (0.037-0.302) kg m^{-2}, TND was 1.005 (0.598-1.504) kg m^{-2} and TCD was 11.032 (6.528-16.378) kg m^{-2} (Fig. 3, Table 4).

SOCD in 0-30 cm soil layer: Compared with HTT, after 12 years of recovery, WL increased by 43.97% and WF increased by 77.21%.

Fig. 1. Soil organic carbon density (SOCD, a), soil inorganic carbon density (SICD, b), total nitrogen density (TND, c) and total carbon density (TCD, d) in surface (0-30 cm) soil of RG (rest-grazing in returning green period) and NG (normal grazing grassland). There were no significant differences between RG and NG at *P*<0.05 for all the indicators. Bars indicate mean±SD (standard deviation).
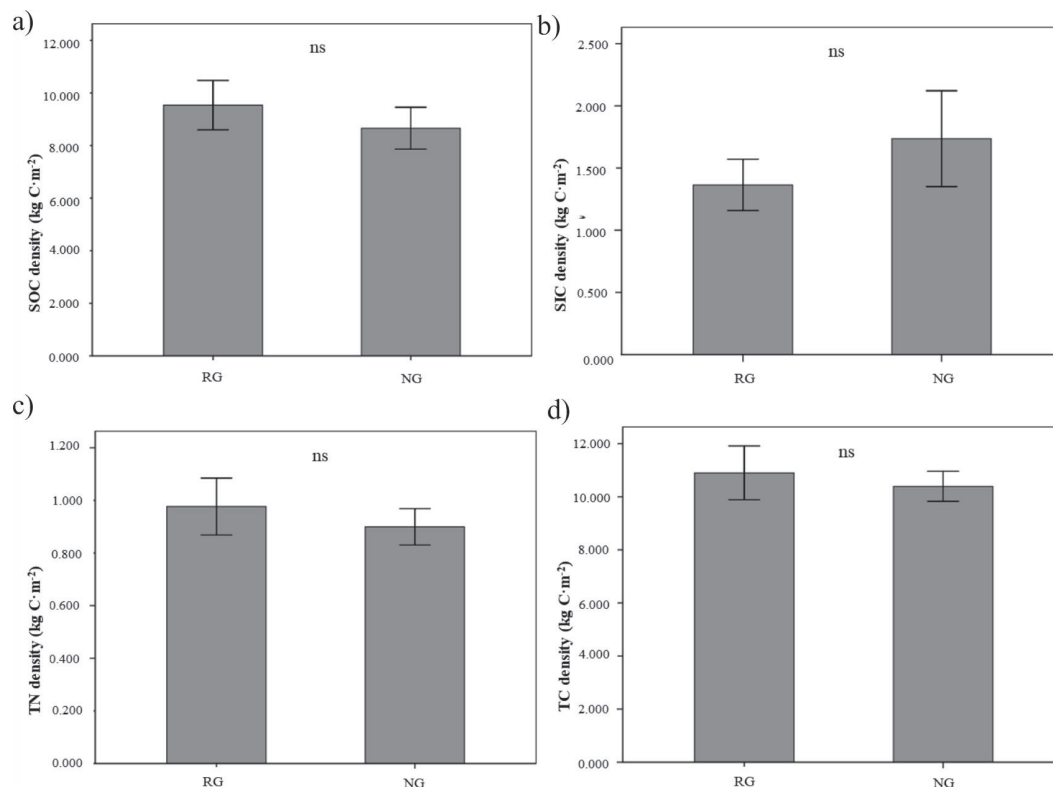
Fig. 2. Soil organic carbon density (SOCD, a), soil inorganic carbon density (SICD, b), total nitrogen density (TND, c) and total carbon density (TCD, d) in surface (0-30 cm) soil of cropland, GFG1 (grass belt), GFG2 (forest belt), GFG3 (enclosure) and NG (normal grazing grassland). Different lowercase letters indicate significant differences between different treatments at $P<0.05$. Bars indicate mean±SD.
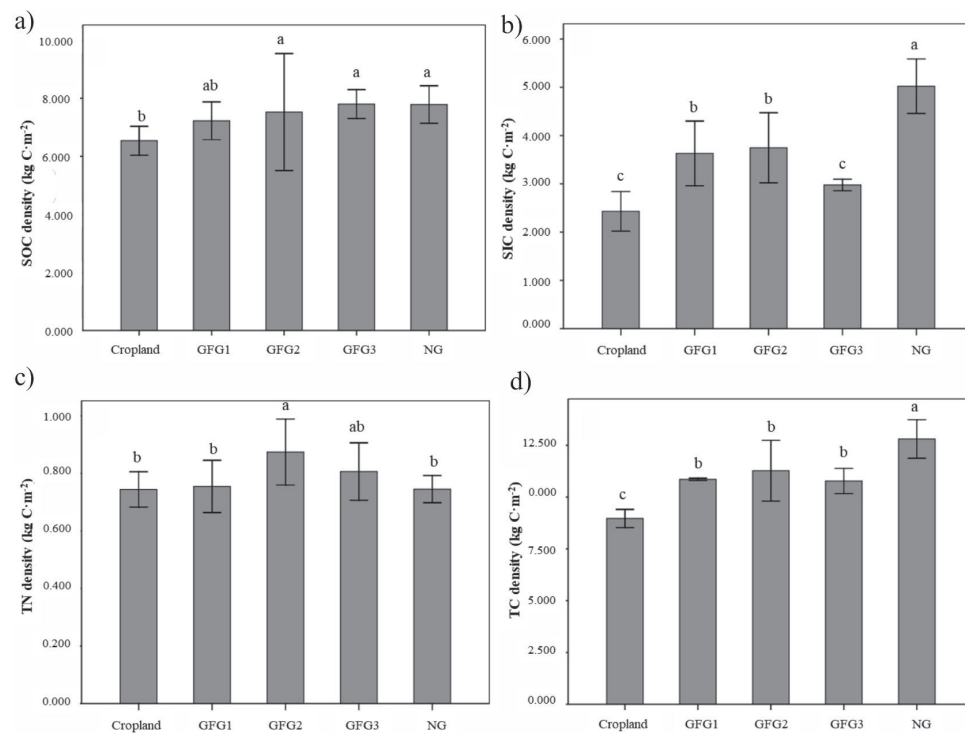

Fig. 3. Soil organic carbon density (SOCD, a), soil inorganic carbon density (SICD, b), total nitrogen density (TND, c) and total carbon density (TCD, d) in surface (0-30 cm) soil of HTT (extreme degradation-black soil beach), WL (winter grazing pasture), WF (year-round forbidden grazing pasture) and NG (normal grazing grassland). Different lowercase letters indicate significant differences between different treatments at $P<0.05$. Bars indicate mean±SD.
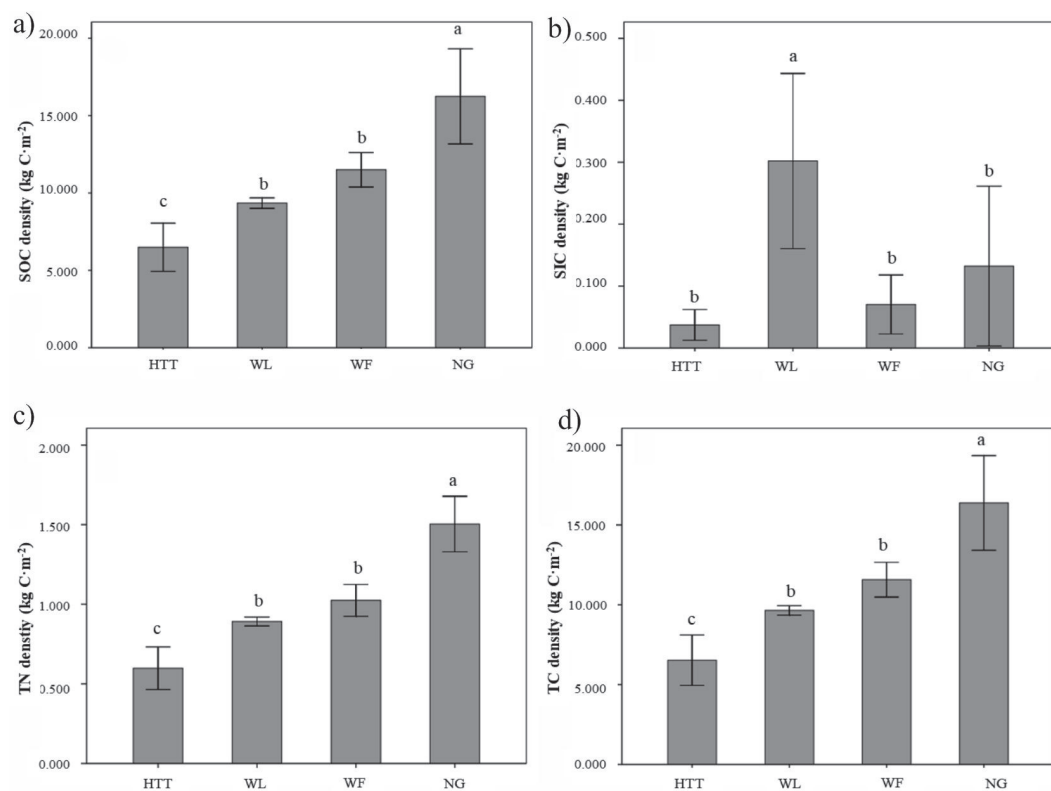
Table 1. Regression models relating soil C and N with soil properties and belowground biomass with soil depth under all and different treatments.

Sites	Treatment	Linear Models	R²
Tongde County	Total	SOC = 7.472 + 0.012MBC + 4.459TN	0.847***
		TN = 1.014 + 0.066SOC + 0.532WACD-0.024SM-0.021SIC	0.848***
		SIC = -90.361 + 13.594pH - 8.815AWCD + 0.007MBC	0.581***
	Cropland	SOC = 2.893 + 7.482TN	0.997***
		TN = 0.881 + 0.068SOC	0.863***
		SIC = -182.579 + 24.796pH	0.998***
	GRG1	SOC = 9.472 + 0.050MBN + 2.734TN-0.033SIN + 0.143SM + 0.011DOC	0.999***
		TN = -0.423 + 0.171SOC - 0.055SM	0.863***
		SIC = 16.809 - 2.334TN	0.696***
	GRG2	SOC = 1.719 + 22.265AWCD	0.778***
		TN = 8.998-0.164SIC- 0.579pH	0.975***
		SIC = 50.209-5.717TN-3.068pH	0.957***
	GRG3	SOC = 259.958 - 30.268pH + 0.283BGB	0.977***
		TN = 26.676 - 3.077pH	0.886***
		SIC = -102.219 + 14.714pH-0.211SIN + 0.051BGB	0.999***
	NG	SOC = 585.446 - 72.878pH	0.932***
		TN = 1.832 + 5.736AWCD-0.191DON	0.989***
		SIC = 24.453 - 4.550TN	0.865***
Maqin County	Total	SOC = 0.991 + 10.875TN + 0.641BGB - 0.017DOC	0.989***
		TN = 0.088SOC-0.050BGB + 0.002DOC	0.985***
	HTT	SOC = -1.569 + 11.561TN	0.988***
		TN = 0.159 + 0.085SOC	0.988***
		SIC = -0.001 + 0.120TN	0.850***
	WL	SOC = -1.575 + 10.980TN	0.977***
		TN = -0.484 + 0.071SOC + 1.284AWCD	0.991***
		SIC = 8.864 - 0.252SOC	0.836***
	WF	SOC = 16.766 + 1.175SIN	0.918***
		TN = 2.900 + 0.223BGB	0.898***
		SIC = 0.828-0.002MBC	0.862***
	NG	SOC = -2.773 + 9.293TN + 0.683BGB + 8.873AWCD	0.999***
		TN = 0.296 + 0.107SOC - 0.073BGB - 0.923AWCD	0.998***
		SIC = 0.280 + 0.004SOC - 0.119AWCD	0.936***
Guinan County	Total	SOC = -2.412 + 9.440TN + 0.145BGB	0.950***
		TN = 0.802 + 0.086SOC	0.926***
		SIC = 19.838-0.381SOC	0.858***
	RG	SOC = -2.135 + 8.093TN + 0.324DON	0.964***
		TN = 0.840 + 0.084SOC	0.939***
		SIC = 25.999-3.807TN-10.306AWCD + 0.004MBC	0.921***
	NG	SOC = -5.529 + 10.881TN	0.944***
		TN = 0.909 + 0.065SOC + 0.027DON	0.970***
		SIC = -197.466 + 26.929pH	0.795***

Table 1. Continued.

Note: NG = natural grazing grassland, GFG = Grain for Green, GFG1 = grass belt, GFG2 = forest belt, GFG3 = enclosure, HTT = extreme degradation-black soil beach, WL = winter grazing pasture, WF = year-round forbidden grazing pasture, RG = rest-grazing, SOC = soil organic carbon, SIC = soil inorganic C, TN = soil total nitrogen, SIN = soil inorganic N, MBC = soil microbial biomass C, DOC = dissolved organic C, MBN = soil microbial biomass N, DON = dissolved organic N, AWCD = average well color development (microbial metabolic activities based on the use of different C sources), pH = soil pH, BGB = belowground biomass, SM = soil moisture. Significance of the linear regression was marked with three asterisks ($P<0.001$).

SICD in 0-30 cm soil layer: Compared with HTT, WL increased 716.22% and WF increased by 89.19%, 12 years after restoration.

TND in 0-30 cm soil layer: Compared with HTT, after 12 years of recovery, WL increased by 49.16% and WF increased by 71.40%.

TCD (SOCD+SICD) in 0-30 cm soil layer: Compared with HTT, WL increased by 47.78% and WF increased by 77.28% after 12 years of restoration.

Factors Influencing Soil C and N

The results of stepwise regression analysis (Table 1) showed that the factors affecting soil C and N content were slightly different under different grassland restoration measures in different areas. Generally, in the rest-grazing pasture of Guinan, root biomass, microbial activity and soil pH are the main factors. In the Tongde area of 'Grain for Green', physical factors (soil moisture, pH), biomass factor (root biomass) and microbial activity (e.g., AWCD, MBC, MBN) have significant effects on soil C and N. In the restored area of artificial grassland in Maqin, root biomass and microbial activity are the main factors affecting soil C and N content. On the whole, both biological and abiotic factors have effects on soil C and N content and density, but the degree of influence varies with geographical location.

Discussion

Response of Soil C and N Densities to Rest-Grazing

Through the role of natural forces, rest-grazing can enhance community productivity and improve the vegetation and soil condition of degraded grassland, which is one of the means of restoration and management of degraded grassland [33]. The results showed that long-term rest-grazing (8 years) increased aboveground and underground biomass [34], while short-term grazing increased grassland community height, coverage and biomass [35], as well as soil C, N and phosphorus contents (rest grazing for one year) also increased in varying degrees [36]. This is similar to our results (Table 2), except for SIC. The relatively high SIC in grazing land may be due to the increased degradation of organic matter caused by grazing disturbance, which increases the partial pressure of CO_2 and facilitates the formation of SIC [30, 37]. The overall impact of grazing rest is to stabilize the grassland ecosystem [35]. Rest-grazing can alleviate the adverse effects of grazing on vegetation, increase vegetation diversity, aboveground and underground biomass, and make the vegetation trampled by cattle and sheep enter the stage of natural restoration [38].

Response of Soil C and N Density to 'Grain for Green'

Returning farmland to forest/grass is an effective measure to restore soil fertility and improve soil quality. Farmland abandonment and vegetation restoration reduced the tillage pressure on soil. With the growth, development and succession of vegetation, there will be a large number of herbaceous plants, plant roots and surface litter that can increase the content of soil organic matter (SOM), slow down the decomposition of organic matter, and then affect the quality of soil and nutrient changes, which will have positive significance for regional C emission reduction in the long run [39-40]. Zhao et al. (2015) compared photosynthetic C fixation between natural grassland and artificial grassland (annual oat and perennial elymus) by the isotope ^{13}C labeling method [41]. We found that natural grassland with high species richness and root/shoot ratio could preserve more C, followed by perennial artificial grassland, and annual artificial grassland (single species) was the least. This is similar to the results of this paper, after farmland conversion, the C, N recovery of single planting *Elymus nutans* (less tillage, trampling disturbance in winter) is slower, while the C and N recovery of forest belt (with high species diversity, no tillage, trampling disturbance in winter) is the fastest, followed by GFG3 (no disturbance). The results also show that no-tillage and perennial forage systems (corresponding to NG in this paper) are conducive to the generation of physically stable macroaggregates, thus helping to protect soil organic matter from microbial degradation in different grain sizes [42]. At the same time, a higher organic C returning system (or lower loss of organic C) and lower soil disturbance are more conducive to the high stability of soil structure [42]. Farming destroys large aggregates and increases the proportion of small aggregates and silt + clay components, which promotes microbial degradation of exposed SOM. This reduces stability and makes the soil more vulnerable to wind or water erosion [43], which accelerates the loss of soil C and N. There was

Table 2. Soil physiochemical properties in rest-grazing area.

Layer	Treatment	pH	BD	SM	SOC	SIC	TN	SIN	MBC	DOC	MBN	DON	AWCD	BGB
0–10 cm	RG	7.445(0.122)	0.748(0.172)	13.453(4.294)	43.654(8.163)	3.490(1.929)	4.323(0.867)	9.034(2.142)	1197.471(218.670)	232.650(95.687)	142.033(45.968)	34.533(19.293)	1.015(0.068)	20.870(9.923)
	NG	7.458(0.141)	0.739(0.067)	11.992(1.819)	42.036(7.919)	4.204(2.718)	4.277(0.733)	10.255(4.596)	1150.285(240.268)	195.283(50.382)	131.840(26.553)	23.425(8.612)	1.080(0.152)	25.771(13.357)
10–20 cm	RG	7.576(0.062)	0.877(0.113)	13.517(3.392)	39.552(6.443)	4.674(1.483)	3.959(0.698)	6.940(1.573)	869.695(114.691)	169.723(33.593)	118.355(27.460)	20.612(6.319)	0.959(0.059)	3.596(1.744)
	NG	7.608(0.073)	0.878(0.123)	12.881(1.707)	36.888(6.255)	6.194(2.861)	3.761(0.581)	9.381(2.490)	950.208(207.107)	156.802(32.245)	104.508(15.617)	17.545(4.532)	0.930(0.030)	5.142(3.818)
20–30 cm	RG	7.646(0.079)b	0.936(0.134)	13.932(3.509)	30.059(3.566)	7.266(2.963)b	3.270(0.445)	7.220(3.127)	659.392(68.937)	137.747(18.649)	97.317(23.661)	15.671(3.610)a	0.808(0.194)	1.901(0.899)
	NG	7.727(0.073)a	0.878(0.095)	13.014(2.552)	26.963(6.402)	9.885(2.370)a	2.961(0.506)	6.880(1.970)	667.842(134.227)	126.372(30.072)	81.290(21.014)	12.307(3.710)b	0.750(0.102)	1.829(0.750)

Note: BD = bulk density, other word abbreviations were as shown in Table 1. Values are means±SD. Means followed the different letter (s) are significantly different at $P<0.05$. (n = 6).

no significant difference in SOCD and TND between cropland and grass belt. The results were similar to those of Sarker et al. (2018), which may be related to annual fertility input of cultivated land [42]. However, the results were different from those of Li et al. (2016) in the Qinghai Lake area, and the higher SIC in cultivated land in Qinghai Lake area was also different from ours [32]. The SIC of NG was higher than that of cropland. The impact of land use on SIC and SOC is not entirely consistent. After farmland conversion, SOC under GFG3 was relatively high, but there was no significant difference with GFG1 and GFG2. The slowest increase of SIC in GFG3 may be due to the lack of moderate disturbance such as animal trampling, which slows down the decomposition of organic matter and plant roots, thus reducing the source of CO_2 that generates SIC, and weakening the transfer of SOC to SIC [44]. Therefore, at the same time of implementing the GFG project, moderate interference is helpful to restore soil fertility.

Response of Soil C and N density to Measures of Artificial Grassland Planting

For extremely degraded grassland, the quality of which is very poor, its primary vegetation species basically disappeared and evolved into a large area of secondary bare land – "black soil beach" – accompanied by toxic and harmful plants, litter disappeared, the proportion of edible forage decreased to almost zero, soil erosion, organic matter reduced by more than one-fold [1], which is in line with our results (Table 4). For the restoration of this kind of grassland, the measures of artificial community rebuilding were adopted, which included the mixed planting of the upper grass (*Elymus nutans*) and the lower grass (*Poa pratensis*) [1], which not only restored the grassland vegetation quickly, but also provided high-quality pasture for grazing livestock [45]. The trampling of livestock increased litter and soil C, N input [1] and improved soil quality. Liu et al. (2015) found that SOC decreased and SIC content increased with the degree of degradation [24], which was different from the change of SIC in our results. In our study area, SIC, like SOC, is also the lowest in HTT, which may be related to the lack of litter in HTT and the decrease of soil microbial activity. At the same time, the degree of soil acidification in HTT is more serious [30] (Table 4). The SICD of WL treatment was significantly higher than that of other treatments, even 128.79% higher than that of NG. This may be due to the change of acidic environment of original soil after 12 years of planting artificial grassland, which is conducive to SIC accumulation [30].

Comparison of Soil C and N Densities in Natural Grassland in Different Areas

Comparing the C and N densities of natural grasslands in three areas, we found that TCD and

Table 3. Soil physiochemical properties in GFG area.

Layer	Treatment	pH	BD	SM	SOC	SIC	TN	SIN	MBC	DOC	MBN	DON	AWCD	BGB
0~10 cm	Cropland	7.655(0.035)b	1.068(0.112)	15.133(1.041)b	19.592(1.001)c	7.109(1.127)c	2.225(0.060)c	30.056(5.835)b	260.685(55.639)d	144.367(18.741)b	54.159(4.288)c	15.626(1.862)bc	0.643(0.050)c	/
	GFG1	7.875(0.124)a	1.022(0.223)	19.172(1.897)a	24.020(2.571)b	10.830(0.701)ab	2.586(0.177)b	44.520(15.243)a	401.034(69.793)c	145.771(12.957)b	83.093(24.480)b	18.321(2.025)abc	1.017(0.065)b	3.105(1.319)
	GFG2	7.825(0.097)a	0.943(0.444)	13.686(1.387)b	24.339(3.884)b	11.101(1.796)a	2.928(0.346)a	10.988(6.377)c	441.313(133.180)c	99.143(17.515)c	82.597(31.532)b	13.429(3.641)c	1.120(0.096)ab	3.588(2.288)
	GFG3	7.650(0.024)b	0.930(0.265)	15.089(1.007)b	30.288(2.737)a	8.576(0.586)bc	3.042(0.142)a	12.091(1.541)c	836.931(140.397)a	199.482(44.904)a	155.881(31.901)a	20.868(3.538)a	1.170(0.090)a	9.955(2.126)
	NG	7.605(0.027)b	1.073(0.046)	14.740(1.177)b	30.616(2.748)a	10.694(2.496)ab	3.011(0.149)a	35.871(7.979)ab	701.980(48.028)b	135.172(29.164)bc	107.815(7.147)b	15.252(2.138)b	0.764(0.012)c	6.558(1.356)
10~20 cm	Cropland	7.633(0.029)c	1.165(0.061)	15.926(1.033)b	19.511(1.284)c	7.031(1.244)c	2.239(0.108)c	18.607(3.982)b	263.728(47.975)d	129.360(9.312)b	52.564(9.745)c	16.981(1.697)a	0.798(0.019)c	/
	GFG1	7.845(0.081)a	1.089(0.337)	21.308(1.653)a	24.363(1.964)b	10.928(1.459)b	2.583(0.268)b	42.487(11.332)a	502.195(31.766)b	131.090(16.305)b	96.053(22.762)b	19.618(4.351)a	1.108(0.064)a	1.813(1.011)
	GFG2	7.850(0.100)a	1.227(0.424)	14.531(0.993)bc	24.888(2.724)ab	10.993(1.147)b	2.808(0.323)ab	6.896(2.882)c	360.303(97.869)c	99.683(21.467)bc	75.665(28.403)b	12.740(2.829)b	1.013(0.052)b	2.097(1.313)
	GFG3	7.700(0.014)bc	0.943(0.224)	13.474(0.614)c	27.515(1.515)a	9.183(0.502)b	2.983(0.207)a	9.057(3.017)c	607.210(102.551)a	169.958(46.797)a	121.030(14.241)a	16.762(2.174)b	1.046(0.056)ab	4.019(0.980)
	NG	7.720(0.026)bc	1.165(0.061)	14.974(1.410)bc	22.740(1.677)b	15.467(1.690)a	2.095(0.315)c	24.002(4.749)b	379.568(38.354)c	99.952(16.786)c	52.626(4.000)c	11.154(1.416)b	0.413(0.054)d	0.757(0.199)
20~30 cm	Cropland	7.678(0.048)c	1.127(0.077)	15.797(1.324)b	19.267(1.000)ab	7.615(1.502)c	2.177(0.127)c	21.596(1.879)a	243.180(88.455)b	118.925(9.514)b	45.604(7.926)b	15.112(1.412)a	0.785(0.113)	/
	GFG1	8.025(0.066)a	1.015(0.172)	22.459(1.538)a	20.892(4.314)a	13.156(3.038)b	2.064(0.547)ab	25.024(7.482)a	361.954(140.893)a	107.532(14.567)bc	61.964(27.165)ab	14.555(1.800)ab	0.791(0.085)	1.117(0.828)
	GFG2	7.985(0.221)ab	1.086(0.253)	15.416(2.224)bc	20.065(0.783)a	12.452(2.032)b	2.341(0.421)a	6.573(2.940)b	208.410(121.742)b	97.920(25.816)bc	45.004(26.085)bc	11.388(3.665)bc	0.849(0.066)	0.956(0.869)
	GFG3	7.868(0.068)bc	1.077(0.085)	13.458(0.541)c	22.196(2.183)a	12.185(1.356)b	2.253(0.370)a	5.679(0.431)b	398.811(90.321)a	150.990(35.712)a	71.985(5.689)a	13.479(2.759)ab	0.958(0.076)	2.187(0.494)
	NG	7.842(0.035)ab	1.098(0.040)	15.657(1.462)b	16.848(1.860)b	18.630(0.968)a	1.630(0.085)b	20.147(2.714)a	195.795(48.138)b	85.186(15.973)c	24.968(7.123)c	8.900(1.097)c	0.223(0.027)	/

Note: Word abbreviations were as shown in Table 1. Values are means±SD. Means followed the different letter (s) are significantly different at $P<0.05$. (n = 4/6).

Table 4. Soil physiochemical properties in the area of planting artificial grassland.

Layer	Treatment	pH	BD	SM	SOC	SIC	TN	SIN	MBC	DOC	MBN	DON	AWCD	BGB
0~10 cm	HTT	5.978(0.169)b	0.840(0.102)ab	28.044(1.755)b	24.526(4.472)c	0.131(0.059)	2.247(0.368)c	27.727(8.824)ab	169.056(65.069)c	96.521(16.889)b	61.113(16.302)c	6.506(1.526)c	0.916(0.063)b	0.324(0.356)c
	WL	6.684(0.164)a	1.009(0.122)a	28.098(1.324)b	35.529(1.353)bc	0.112(0.058)	3.307(0.156)b	13.184(2.550)b	732.172(208.430)ab	105.241(69.981)b	116.455(12.814)b	12.642(1.944)b	1.059(0.050)b	4.765(0.886)b
	WF	6.279(0.115)b	0.842(0.068)ab	36.512(3.146)b	47.148(2.506)b	0.088(0.063)	4.146(0.234)b	34.273(16.470)a	451.988(59.481)bc	121.807(61.240)ab	121.856(25.779)b	14.412(2.528)b	1.277(0.102)a	5.204(2.318)b
	NG	6.189(0.471)b	0.832(0.201)b	48.138(10.877)a	75.821(15.523)a	0.164(0.061)	6.057(1.158)a	34.773(14.230)a	973.113(385.921)a	194.960(68.285)a	183.591(34.840)a	20.297(6.059)a	1.253(0.159)a	21.722(4.161)a
10~20 cm	HTT	6.000(0.132)c	1.005(0.103)	28.355(1.459)b	24.531(4.289)c	0.150(0.125)b	2.249(0.369)c	24.892(8.562)a	166.035(100.401)c	54.192(18.282)b	32.669(10.315)c	4.582(0.990)b	0.976(0.035)b	0.454(0.350)b
	WL	7.276(0.193)a	1.075(0.127)	27.153(1.207)b	30.414(2.059)bc	0.425(0.155)a	2.910(0.167)bc	8.876(1.301)b	503.436(60.265)ab	112.976(69.223)ab	68.117(7.883)b	13.022(2.106)a	0.925(0.023)b	0.850(0.858)b
	WF	6.549(0.193)b	1.043(0.027)	32.911(1.721)b	40.018(2.596)ab	0.162(0.085)b	3.528(0.441)b	22.469(3.761)a	315.254(22.796)bc	102.066(22.522)ab	75.316(11.239)b	13.487(1.059)a	1.184(0.121)a	1.296(0.900)ab
	NG	6.505(0.583)b	1.089(0.127)	40.385(8.156)a	50.334(13.700)a	0.268(0.226)ab	5.416(1.192)a	20.151(5.477)a	565.341(250.383)a	179.374(89.482)a	120.057(36.460)a	15.816(3.320)a	1.086(0.074)ab	2.111(0.720)a
20~30 cm	HTT	6.258(0.218)c	1.079(0.159)	24.678(0.667)b	18.204(1.813)c	0.104(0.023)b	1.696(0.177)c	25.401(7.638)a	58.640(82.040)	56.405(18.731)b	15.046(7.285)c	3.548(1.342)b	0.889(0.037)b	0.225(0.175)
	WL	7.551(0.057)a	1.022(0.089)	24.601(0.835)b	24.397(0.886)b	2.398(1.194)a	2.402(0.091)b	5.782(1.594)c	331.713(185.577)	122.399(66.149)a	36.623(3.801)b	13.019(3.018)a	0.880(0.006)b	0.237(0.164)
	WF	7.294(0.089)a	1.046(0.139)	27.313(2.277)b	32.125(2.808)a	0.500(0.191)b	2.941(0.127)ab	12.955(2.244)b	186.019(75.426)	127.796(43.175)a	48.038(7.490)a	13.335(2.742)a	1.075(0.020)a	0.482(0.220)
	NG	6.886(0.421)b	1.149(0.221)	31.002(3.166)a	35.405(6.619)a	0.772(0.839)b	3.298(0.743)a	14.971(4.618)b	337.105(309.203)	131.125(23.629)a	41.426(5.894)ab	12.710(2.730)a	0.885(0.046)b	0.529(0.333)

Note: Word abbreviations were as shown in Table 1. Values are means±SD. Means followed the different letter (s) are significantly different at $P<0.05$. (n = 4/6).
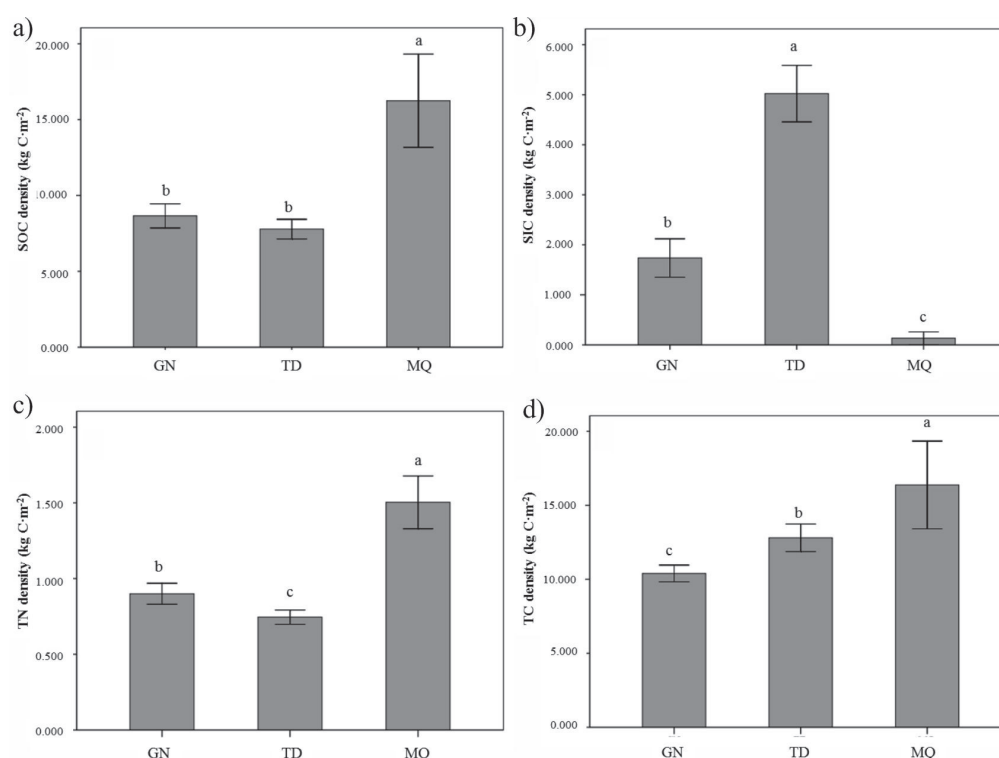
Fig. 4. Surface (0-30 cm) soil organic carbon density (SOCD, a), soil inorganic carbon density (SICD, b), total nitrogen density (TND, c) and total carbon density (TCD, d) of normal grazing grassland in GN (Guinan County), TD (Tongde County), and MQ (Maqin County). Different lowercase letters indicate significant differences between different areas at *P*<0.05. Bars indicate mean±SD.

SOCD in 0-30 cm soil of Maqin were significantly higher than those in Guinan and Tongde, while SIC in Tongde was significantly higher than those in Guinan and Maqin. Differences in soil types and climatic zones may be responsible for the differences in soil C and N in different regions [42]. 1) The growth of plants in Tongde and Guinan was restricted by relatively low precipitation and high temperature, resulting in a decrease in C entering the soil system [46-47]. Geographically, the south of Maqin has higher elevation relatively more precipitation and lower temperature, relatively higher productivity and higher C input to soil [48]. 2) According to the soil characteristics, the soil in Tongde sampling area is dark chestnut calcareous soil with relatively high inorganic C content and strong lime reaction [27, 49], and the soil in the Maqin area is alpine meadow soil with no lime reaction or lime reaction under A/B layer, which is rich in OM and humus. The soil of Guinan sampling area is carbonate alpine meadow soil, and the content of OM and humus is slightly lower than that of alpine meadow soil, which has a certain calcification process [50-51].

Conclusions

Short-term grazing rest in the Sanjiangyuan area has a certain effect on soil fertility restoration. The implementation of the "Grain for Green" project in the agro-pastoral ecotone improves soil fertility. Concurrently, moderate disturbance should be carried out on the basis of increasing vegetation diversity. Artificial grassland planting on black soil beach can improve soil quality, and moderate grazing disturbance in winter had the best immobilization effect on SIC, while no disturbance in the whole year was beneficial to the accumulation of organic matter. Both biological and abiotic factors have effects on soil C and N content and density, but the degree of influence varies with geographical location.

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Conflict of Interest

The authors declare no conflict of interest.

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