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Original Research

Density and Stoichiometric Characteristics of Carbon, Nitrogen, and Phosphorus in Surface Soil of Alpine Grassland in Sanjiangyuan

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Abstract

The balance and stoichiometry of carbon (C), nitrogen (N), and phosphorus (P) can reflect the quality and content of organic matter and is very important for understanding ecological processes and ecosystem response to climate change and disturbance. To explore the spatial distribution characteristics of C, N, P, and their stoichiometry of topsoil in Sanjiangyuan (SJY), we sampled 0-20 cm soil of alpine grassland in SJY, measured soil C, N, and P, and analyzed their correlation with latitude, longitude, altitude, and vegetation characteristics (diversity, height, coverage, biomass, etc.). The results showed that the average densities of soil organic C (SOC), total N (TN), total P (TP), and soil inorganic C (SIC) in SJY were 7.56 kg·m^{-2}, 0.71 kg·m^{-2}, 151.57 g·m^{-2}, and 1.77 kg·m^{-2}, respectively. The spatial distribution of SOC, TN, and TP in SJY showed a pattern of high in the east and low in the west, while SIC showed the opposite pattern. The heterogeneity of soil TP in the SJY region was small, which indicated that there may be little difference in soil parent materials in the whole region. The soil nutrients were mainly affected by longitude, soil water content, pH, and vegetation height and coverage. The C: N: P ratio of the 0-20 cm soil in the study area was approximately 48:5:1, and the C: N, C: P, and N: P ratios were significantly affected by soil water content and vegetation height. Soil C: N ratio (10.45) and N:P ratio (4.51) were lower than the national level, which indicated that the soil N mineralization capacity of SJY was higher than that of other regions in China, and the degree of soil N limit was small.

Keywords: Qinghai-Tibetan Plateau, Sanjiangyuan, soil nutrients, stoichiometry

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Introduction

Ecosystem element balance is the focus of global change ecology and biogeochemical cycles [1]. The cycles of C, N, and P among ecosystems are coupled, the variation of one element will affect the turnover cycle of relevant elements in the ecosystem [2-5]. Ecological stoichiometry is a science that studies the energy balance of biological systems and the balance of multiple chemical elements (mainly carbon, nitrogen, and phosphorus), as well as the effect of element balance on ecological interaction, which makes the research theories of different levels (molecular, cell, organism, population, ecosystem and global scale) of biological discipline organically unified [1]. By studying C, N, and P contents and their stoichiometry, the nutritional status of soil and plants can be determined to improve the understanding of the nutrient cycle and biological processes in the ecosystem [6].

As the link of atmosphere, hydrosphere, biosphere, and lithosphere in the ecosystem, the soil is the substrate of plant survival and an indispensable part of the ecosystem [7], and the largest pool of organic carbon (C) in the global terrestrial ecosystem and soil C, nitrogen (N) and phosphorus (P) are usually closely related to it [8-9]. In the nutrient cycling of terrestrial ecosystems, soil organic matter, nitrogen, and other nutrients control the structure, function, and productivity of the ecosystems, while assimilation and mineralization determine the cycling rate [10]. Meanwhile, soil C, N, and P are affected by climate, geography, and other reasons, so they have high variability [5].

The stoichiometry of C, N, and P has long been considered as a useful signal of their coupling relationship with nutrient constraints in different ecosystems [8-9, 11]. Globally, the C: N: P ratio of the 0-10 cm soil layer is well limited [8], and land cover/vegetation type has an important impact on the C: N: P ratio of soil [11]. The soil C: N: P ratio is the main indicator reflecting the internal C, N, and P cycles in soil, which integrates the variability of ecosystem functions, is easy to measure, and helps to control the response of ecological processes to global change, so it becomes an important parameter to determine the characteristics of soil C, N, and P balance [1]. Studying the balance of C, N, and P is of great significance to understand the potential of ecosystem carbon sinks and how ecosystems respond to future climate warming. The application of ecological stoichiometry could provide useful criteria for tracking the nutrient cycle among compartments in ecosystems, since not only the concentration of C, N, and P but also the relationships among them (C: N: P) drive most of the ecological processes [6].

Grassland, which accounts for about 40% of the global surface area, is an important terrestrial ecosystem and plays a key role in regulating biogeochemical C, N, and P cycles [12]. Sanjiangyuan (SJY), located in the hinterland of the Qinghai-Tibetan Plateau (QTP), is the birthplace of the Yangtze River, Yellow River, and Lancang River, and is a vital ecological environment protection area in Qinghai Province and even in the whole country. As its main ecosystem, grassland is a unique ecosystem formed by long-term evolution and development under the unique climate, geography, geology, and other environmental conditions of the QTP [13]. There are few studies on the spatial distribution of soil nutrients and their stoichiometry in SJY [7, 14-15], and limited work has mainly focused on the effects of altitude, grazing intensity, and land use on soil organic matter, enzyme activities, and heavy metals [16-20]. Caused by the difference in hydrothermal conditions and biomass productivity and quality allocation patterns [21-22], and the difference in grassland utilization in different regions caused by human interference [23-24] in SJY, the soil input of organic matter from vegetation [25] and nutrients from herbivore excreta [26] are affected. Based on this, we speculate that there are great differences in soil C, N, and P densities and their stoichiometric ratios in SJY, and they will decrease with the worsening of hydrothermal conditions from east to west. Thus, we aim to obtain the soil C, N, and P contents and their stoichiometric ratios by collecting and analyzing soil samples, and to analyze the relationship between them and environmental factors in alpine grasslands in SJY, to reveal the change law of soil nutrients and their stoichiometry at the regional scale, to provide a basis for understanding the nutrient cycling and functional changes of alpine grassland soil under the influence of global climate change and human activities.

Material and Methods

Study Area

Sanjiangyuan (SJY) is located in the south of Qinghai Province and the hinterland of the QTP, and the geographical location is between 89°24′-102°23′E and 31°39′-36°16′N, with a total area of 39.5 × 10^4 km^2 and an average altitude of more than 4000 m. The study area is a typical plateau continental climate, with alternating cold and hot seasons, distinct dry and wet conditions, a small annual temperature range, a large daily temperature range, strong solar radiation, and no obvious distinction between the four seasons. The main grassland types in SJY are alpine meadow and alpine steppe, and the plant species include *Kobresia*, *Carex*, *Stipa*, *Poa*, *Elymus*, etc. [27-28]. The main soil types in SJY are alpine meadow soil, alpine steppe soil, alpine desert soil, mountain meadow soil, gray cinnamon soil, chestnut soil, swamp soil, tidal soil, peat soil, etc. [29].

Sampling and Analyzing

In late August 2017, 15 sampling regions (Table 1) were selected in the alpine grassland of SJY.

Table 1. Sampling sites information.

Site	Latitude (°)	Longitude (°)	Altitude (m)	MAT (ºC)	MAP (mm)	AGB (g·m^{-2})	VH (cm)	VC (%)	Shannon-Weiner	Soil pH (KCl/soil = 2.5:1)	SWC (%)
Site 1	34.3556	100.4839	3817	0.6	515	399.56±23.30	13.41±1.82	93.75±2.31	1.72±0.37	6.21±0.33	71.65±12.99
Site 2	34.3565	100.4889	3911	0.6	515	247.73±58.44	5.28±1.63	95.63±3.20	2.49±0.19	6.09±0.61	48.28±11.37
Site 3	33.1022	100.7094	3683	2.6	657	389.97±54.88	27.82±3.31	94.38±3.2	1.99±0.34	5.84±0.55	43.36±5.23
Site 4	33.455	101.4942	3595	1.6	746	275.91±33.55	16.67±1.93	91.88±7.04	2.60±0.48	5.34±0.77	56.80±9.29
Site 5	34.7542	98.1311	4250	-2.5	321	71.44±16.53	4.36±2.35	54.76±9.81	1.51±0.16	7.71±0.05	16.36±0.74
Site 6	34.7147	101.5964	3519	0.6	589	387.89±137.76	14.14±4.85	89.38±7.76	2.83±0.41	6.92±0.19	46.07±3.56
Site 7	35.2528	100.6692	3300	1.5	432	144.38±43.90	16.64±1.64	74.38±10.16	1.98±0.14	7.66±0.09	28.93±1.95
Site 8	33.3503	97.1528	3926	-2.5	517	175.61±58.85	12.32±2.45	85.00±13.69	2.36±0.28	7.09±0.33	28.41±3.92
Site 9	32.2928	96.4575	3642	3.5	583	160.54±33.55	14.90±3.58	76.00±6.52	1.76±0.26	8.00±0.10	20.15±5.24
Site 10	33.6858	95.8736	4097	-0.5	430	253.78±48.69	6.01±0.99	95.63±1.77	1.95±0.25	7.65±0.09	42.03±7.61
Site 11	34.9933	94.4889	4329	-0.5	419	93.59±40.29	3.04±0.51	61.50±18.64	1.41±0.29	7.50±0.09	32.97±7.93
Site 12	34.9935	94.4895	4338	-0.5	419	122.24±60.69	5.13±1.43	68.75±19.55	1.24±0.35	7.64±0.15	54.86±20.73
Site 13	34.2094	92.4389	4474	-3.5	290	26.77±24.96	5.82±1.23	18.81±5.84	1.86±0.26	8.20±0.22	7.65±1.72
Site 14	34.5978	102.2733	3209	3.5	600	342.56±109.56	21.53±4.90	75.00±7.56	2.94±0.11	6.04±0.26	38.11±1.22
Site 15	33.6692	101.8617	3466	1.1	616	325.77±35.10	16.46±2.56	85.63±7.76	2.81±0.44	5.12±0.14	52.69±3.23

Note: MAT = Mean annual temperature, MAP = Mean annual precipitation, VH = Vegetation height, VC = Vegetation cover, SWC = Soil water content, AGB = Aboveground biomass. Values are means±SD.

The spatial scale of each sample region was approximately 1 km, 5-8 sampling points (the interval distance of each sample point was about 100 m) were randomly selected in each sample region, and 5 points were randomly selected to form a mixed sample with a depth of 0-20 cm (80-90% root distribution area) within the range of 50 m × 50 m. All samples were taken back to the laboratory immediately, and after passing the 2 mm sieve, the roots and other impurities were removed and divided into two parts. One part was dried by air, and one was kept for soil pH measurement, the other part was screened by 0.15 mm for soil nutrient determination; one part was kept fresh in a 4ºC refrigerator for the determination of inorganic nitrogen (SIN, sum of ammonium nitrogen and nitrate nitrogen) and microbial biomass (microbial biomass carbon, MBC; microbial biomass nitrogen, MBN).

The soil bulk density (BD) and water content (SWC) 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), soil total N (TN) by Kjeldahl N analyzer, soil organic C (SOC) by potassium dichromate-concentrated sulfuric acid oxidation, soil inorganic C (SIC) by acid titration, soil total phosphorus (TP) was determined with Skalar San++ continuous flow analyzer after digestion by double acid method [30]. Soil microbial biomass was determined by chloroform fumigation and extraction [31-32]. Briefly, the fumigated and non-fumigated samples (10 g dry weight equivalent) were extracted with 50 ml of 0.5 M K_2SO_4 for 30 min on a shaker. The extracts were filtered through 0.45 μm filters and determined for extracted C by the potassium dichromate-vitriol oxidation method and N by Kjeldahl digestion [30]. MBC and MBN were calculated from the differences between the extracted C and N contents in the fumigated and non-fumigated samples using conversion factors of 0.38 and 0.45 [31-32], respectively.

Calculating C, N, and P density [33]:

$$S = C \times \rho \times h \times 10$$

Among them, C denotes the contents of SOC, SIC, TC, TN, STN, and TP ($g\cdot kg^{-1}$), h denotes the depth of the soil layer (cm), and ρ denotes the BD of the layer ($g\cdot cm^{-3}$), respectively.

Data Analysis

The statistical analysis was performed by SPSS ver. 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Pearson's correlation was used to detect the relationships between soil C, N, P, and C: N: P and environmental factors. Stepwise regression analysis was used to screen the main influencing factors of the soil C, N, P, and C: N: P ratio. The critical value of the partial regression square sum of significance test was 0.05.

Results and Discussion

Spatial Variation in Soil C, N, and P, and Their Influencing Factors

The soil is much more complex than other terrestrial systems and has high heterogeneity in horizontal and vertical directions [34]. The relative instability of soil tends to promote and preserve the spatial heterogeneity of nutrient cycling. This heterogeneity is caused by local-scale disturbances, such as land-use change and human disturbance, as well as regional-scale differences in Glacier history, climate, geological parent material, topography, and biodiversity. Nutrients are continuously redistributed in terrestrial ecosystems through various ways, including plant litter, soil water flow, and plant atmosphere exchange [34].

By analyzing the C, N, and P contents of the soil surface in SJY, it was found that there was a large spatial variation in nutrient density. The densities of SOC and TC varied from 0.72 to 19.83 $kg\cdot m^{-2}$ and from 3.22 to 19.88 $kg\cdot m^{-2}$ in the 0-20 cm layer, respectively. SOC and TC had a positive relationship with longitude ($P<0.001$), while both decreased significantly with increasing altitude ($P<0.001$). There was no significant difference in SOC density between the SJY (7.56 $kg\cdot m^{-2}$) and Qinghai-Tibetan Plateau (7.2 $kg\cdot m^{-2}$) estimated by Tian et al. [35]. The SIC at the 0-20 cm depth ranged from 0.01 to 5.85 $kg\cdot m^{-2}$, and the spatial heterogeneity of the SIC was large (the coefficient of variation reached 0.86), which indicates that the index is unstable and vulnerable to external disturbance. Contrary to the spatial variation in SOC and TC, soil SIC increased significantly with the increase of altitude but decreased significantly with increasing longitude ($P<0.001$). Soil TN density varied from 0.08 to 1.92 $kg\cdot m^{-2}$, and TP was from 103.82 to 267.34 $g\cdot m^{-2}$. Consistent with the SOC and TC, TN and TP were also positively affected by longitude ($P<0.001$) and decreased with the increase of altitude ($P<0.05$) (Figs 1, 2, Table 2). SWC (varied from 7.65% to 71.65%) increased significantly with the increasing longitude ($P<0.001$). The soil pH (ranging from 5.34 to 8.20) was negatively correlated with longitude ($P<0.001$), the soil in Jiuzhi, Banma, Dari, Maqin, and other eastern and southern regions is acidic, while the soil in Hoh Xil, Nangqian, Qumalai, and other western regions is neutral or alkaline.

Soil C and N mainly come from litter return and soil microbial decomposition [36]. The results showed that the soil water and fertilizer status in the grassland with poor water holding capacity and water storage capacity was poor, while the vegetation biomass of grassland with good water holding capacity increased, plant litter and rotten roots were rich, and soil nutrients were naturally on the high side [37]. Our study also showed that soil C, N, and P were significantly affected by soil water content, pH, and vegetation coverage. This is not only related to the geographical differences of the sampling sites [13] but also to the grazing

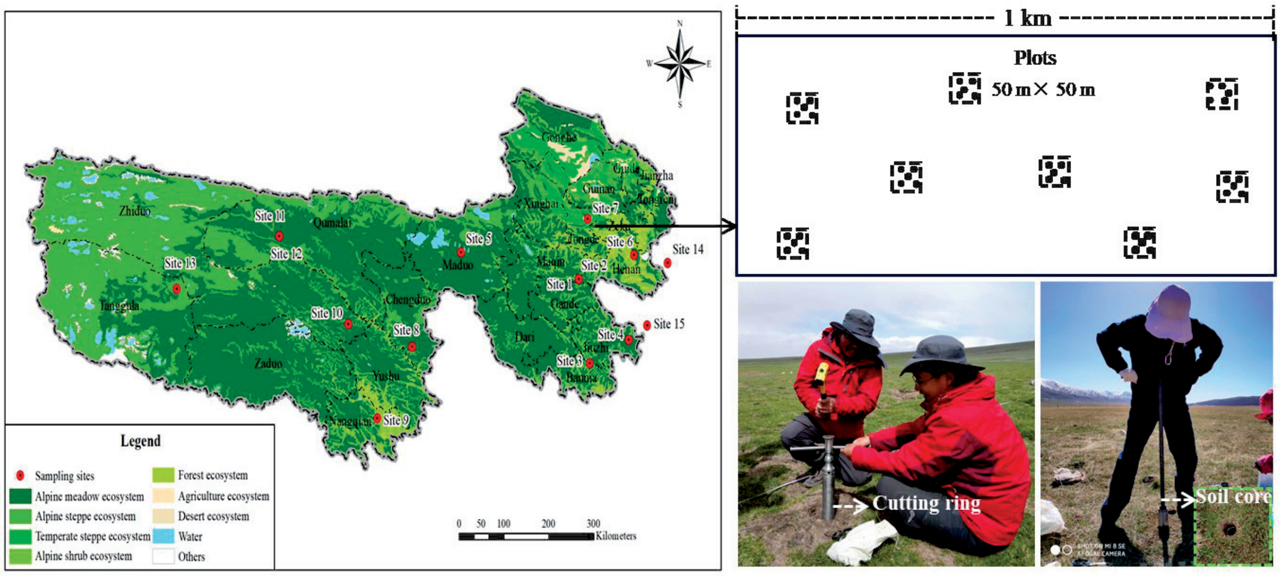

Fig. 1. Sampling sites and samples collection in Sanjiangyuan.

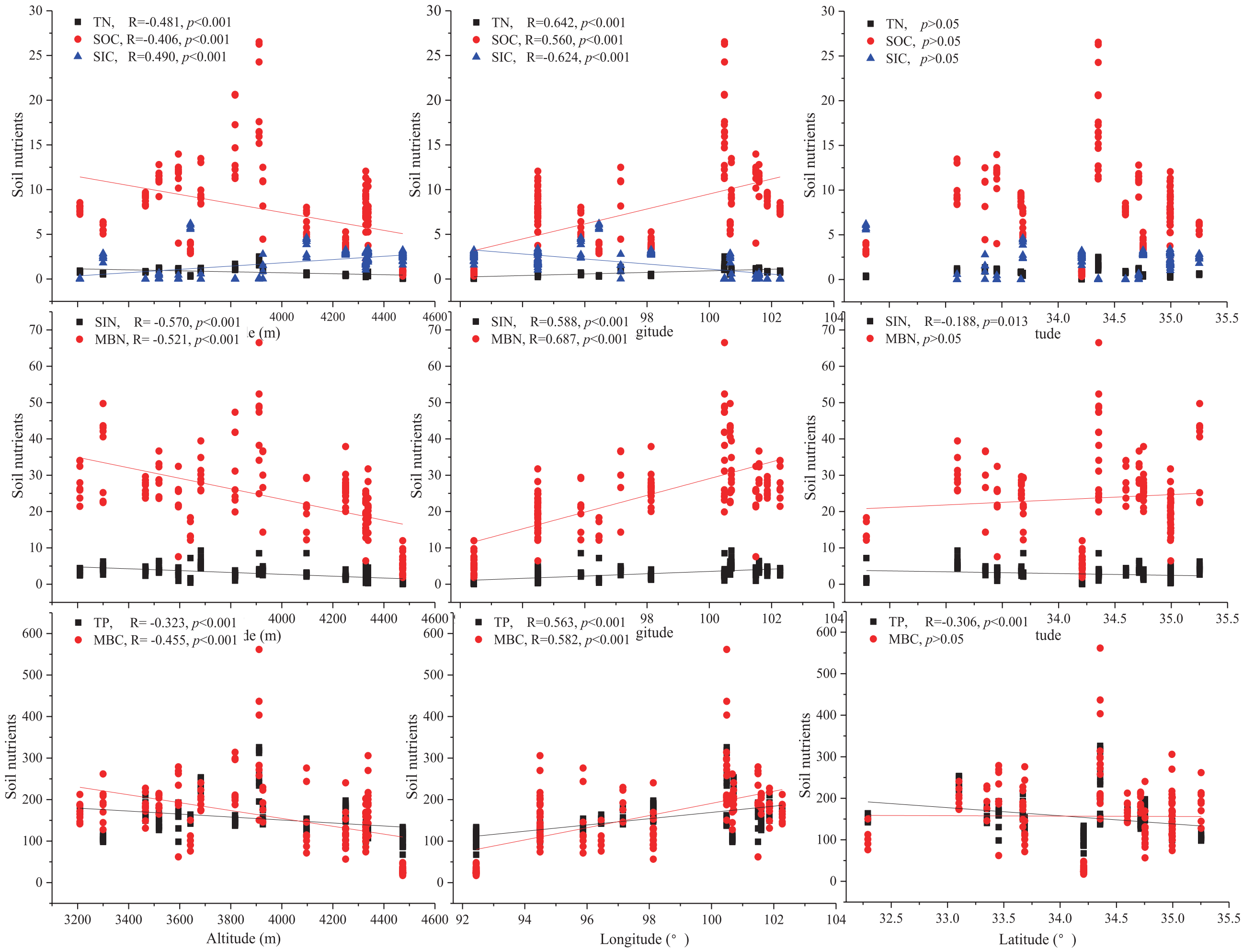

Fig. 2. Variation of soil nutrients with altitude, longitude, and latitude in Sanjiangyuan. TN = soil total nitrogen, SOC = soil organic carbon, SIC = soil inorganic C, TP = soil total phosphorus, SIN = soil inorganic nitrogen, MBN = microbial biomass nitrogen, MBC = microbial biomass carbon.

Table 2. Statistics of carbon, nitrogen, phosphorus density, and their stoichiometric characteristics in Sanjiangyuan.

Indicators	Average	Maximum	Minimum	Standard deviation	Coefficient of variation
SOC ($kg \cdot m^{-2}$)	7.56	19.83	0.72	5.04	0.67
SIC ($kg \cdot m^{-2}$)	1.77	5.85	0.01	1.52	0.86
TC ($kg \cdot m^{-2}$)	9.33	19.88	3.22	4.22	0.45
TN ($kg \cdot m^{-2}$)	0.71	1.92	0.08	0.46	0.65
SIN ($g \cdot m^{-2}$)	2.74	5.96	0.43	1.87	0.68
TP ($g \cdot m^{-2}$)	151.57	267.34	103.82	45.24	0.30
MBC ($g \cdot m^{-2}$)	156.79	3330.70	28.17	84.93	0.54
MBN ($g \cdot m^{-2}$)	23.71	45.14	5.63	11.31	0.48
Soil C: N	10.45	12.74	8.76	1.72	0.16
Soil C: P	48.29	101.04	6.96	27.18	0.56
Soil N: P	4.51	8.84	0.81	2.37	0.53

activities experienced by the grassland in the SJY region, because grazing will affect the accumulation of soil nutrients [6], and the productivity and carrying capacity of different regions are different [22]. Soil P is a sedimentary element, related to parent materials, and mainly comes from the weathering of rocks. Rock weathering is a long and relatively stable process, so it is not easily affected by biological and abiotic conditions [38]. The weathering degree has little difference in the 0-60 cm soil layer, so the spatial variability of soil P is small [5]. The heterogeneity of soil TP in the whole region is the smallest, which indicates that there may be little difference in the parent material of grassland in SJY [39], because P is the only element that must be provided by the parent material of C, N, S, and P [40]. The spatial variation trend of soil P was similar at the national scale [34] and small scale [12].

Stoichiometric Characteristics and Their Influencing Factors of Soil C, N, and P

Studies have shown that the C: N ratio, C: P ratio, and N: P ratio of surface soil (0-10 cm mineral soil) worldwide are 14.31, 186, and 13, respectively [8]. The C: N, C: P, and N: P ratios of soil in China were 12, 61, and 5, respectively, and the C: N: P ratio of all soil layers was 60:5:1 based on the data of the second national soil survey [34]. Our survey results showed that the average C: N: P ratio of the 0-20 cm soil layer in SJY area was 48:5:1, and the C: N, C: P, and N: P ratios were respective 10.45 (with a range of 8.76-12.74), 48.29 (with a range of 6.96-101.04), and 4.51 (with a range of 0.81-8.84) (Table 3), which were far lower than the global and national levels. Spatially, the C: N ratio had no significant change in latitude,

Table 3. Regression models relating soil C, N, and P with biotic and abiotic factors in Sanjiangyuan.

Linear Models	R^2
TN = -10.249 + 0.10SWC + 0.078longitude + 0.001altitude + 0.180Shannon + 0.005VC	0.692***
SOC = -13.752 + 0.122SWC - 1.445pH-0.206VH + 0.035VC + 0.276longitude	0.720***
SIC = -6.870 + 1.147pH + 0.017VC - 0.003AGB	0.662***
TC = -20.837 + 0.114SWC + 0.051VC-0.203VH + 0.253longitude	0.655***
TP = -1061.374- 10.382pH + 15.513longitude + 0.102altitude - 18.540latitude	0.568***
MBN = -210.489 + 2.361longitude - 0.48VH + 0.122VC	0.561***
MBC = -725.620 + 1.934SWC + 0.819VC + 7.991longitude - 2.249VH	0.625***
C: N = 10.068 + 0.056SWC - 0.077VH - 0.419Sahnnon	0.398***
C: P = 94.711 + 1.031SWC - 1.566VH - 0.018altitude + 0.035AGB	0.759***
N: P = -13.811 + 0.078SWC-0.106VH + 0.184longitude + 0.502pH + 0.004AGB-0.001altitude	0.718***

SOC = soil organic carbon, SIC = soil inorganic C, TN = soil total nitrogen, MBC = soil microbial biomass C, MBN = soil microbial biomass N, pH = soil pH, SWC, Soil water content, AGB = Aboveground biomass, VH = Vegetation height, VC = Vegetation cover, Shannon = Shannon-Weiner. Significance of the linear regression was marked with three asterisks ($P<0.001$).

longitude, or altitude, while the C: P ratio and N: P ratio increased with increasing longitude and decreased with increasing altitude (Fig. 3). The distribution pattern of C: N, C: P, and N: P ratios is similar to that of Tian et al. [34], and the law of C: N: P ratio inversely related to soil pH (Pearson's correlation analysis) is similar to that of Walker & Adams [40]. This is expected to decrease precipitation in the west and north of the study area, resulting in a decrease in the soil water content, a decrease in decomposable hydrogen ions leads to an increase of pH value, and the soil pH determines the degree of mineralization and decomposition of soil nutrients. Generally, under the same external conditions, the decomposition of neutral soil is faster than that of acidic soil [7]. In our study area, the nutrient content of acidic soil in the southeast was higher than that of neutral alkali soil in the northwest, and the positive effect of soil water content on the C: N: P ratio also confirms this rule.

The ecological stoichiometry of soil C, N, and P can be used not only as an important index of soil organic matter composition, soil quality, and nutrient supply capacity, but also as an evaluation index of soil C, N, and P mineralization and fixation [39]. Moreover, soil C: N is an important indicator of soil N mineralization ability, indicating the decomposition rate of organic matter in soil [41]. Grasslands with a high soil C: N ratio show that the soil N mineralization ability is weak, and microorganisms may be limited by N, thus causing the decrease of organic matter decomposition rate, and resulting in an increase in soil C storage [42]. Compared to the study on the topsoil C: N ratio at the national scale, the C: N ratio at 0-20 cm in the SJY region was lower, indicating that the soil N mineralization ability was higher than that in other regions of China, and soil N was limited to a small extent. Different from the large variation of SOC and TN in the study area, the spatial heterogeneity of the C: N ratio is small, which shows that C and N have the same change trend with the change of environmental factors, resulting in a relatively stable ratio, which is in line with the characteristics of stoichiometry [5]. Our results are consistent with other reports of different ecosystem types and different soil sampling times, which usually occur in soils with high biodiversity and spatial heterogeneity [7, 12, 43]. Soil N and P are important elements for plant growth and development, and as indicators of soil nutrient supply to plants, their stoichiometric characteristics have a certain impact on plant growth [44]. According to the data of the second national soil survey, the N: P ratio of soil in China is 5 [34], while the N: P ratio of the study area is 4.51, which is slightly lower than the national level but significantly higher than that in the Loess Hilly and Gully area (0.86) [45], which also shows that compared with other regions, the vegetation growth in SJY is less limited by soil N.

Future Directions

Due to the limitations of some external conditions, we have only preliminarily analyzed the content and stoichiometry of C, N, and P in the surface soil of SJY. Although the contents of soil C, N, and P usually decrease vertically with the increase of soil depth, the vertical change pattern of soil C: N: P is more complex [46]. In addition, due to the differences in hydrothermal

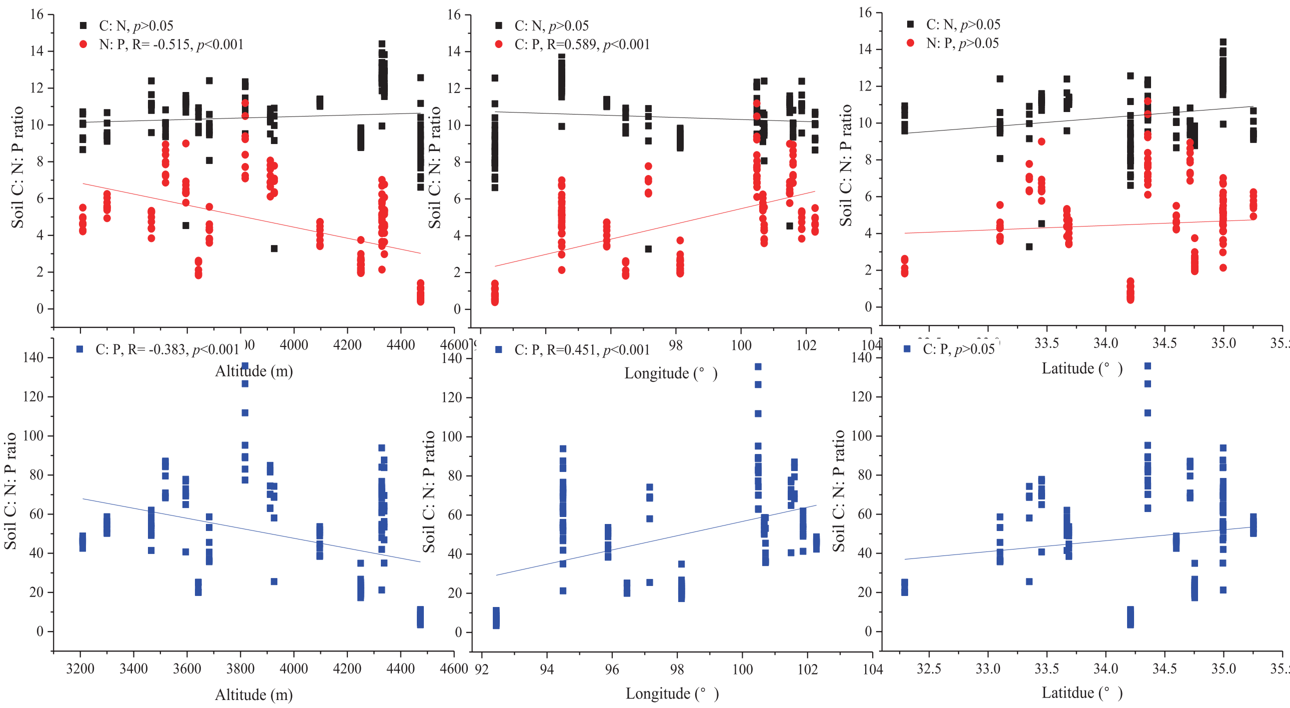

Fig. 3. Variation of soil C:N:P ratio with altitude, longitude, and latitude in Sanjiangyuan.

conditions and biomass allocation patterns on the spatial scale, the regulation of C, N, and P contents and ratios has also changed, so the latter has a more complex change pattern in space. At the same time, climate change and human disturbance activities (such as land use change, grazing, fencing) will significantly change the distribution pattern and change rate of soil C, N, and P nutrients. Due to the sensitivity of the QTP to climate change and human disturbance activities, there is still much room for the study of soil-vegetation C, N, and P in the SJY region. In future work, we will continue to pay attention to the carbon, nitrogen, and phosphorus of the Sanjiangyuan ecosystem, and combine the aboveground (plant community, animal excreta) - underground (soil vertical space - microorganism) ecosystems for research.

Conclusions

For the first issue, we conclude that the heterogeneity of soil carbon and nitrogen in Sanjiangyuan is high, especially the soil inorganic carbon. The mean values of SOC, TN, and SIC in the surface soil of Sanjiangyuan were 7.56 $kg \cdot m^{-2}$, 0.71 $kg \cdot m^{-2}$, and 1.77 $kg \cdot m^{-2}$, respectively. The phosphorus mainly from parent material (regional mean value is 151.57 $g \cdot m^{-2}$) is relatively stable. The C: N: P ratio of topsoil in Sanjiangyuan is 48:5:1. For question two, we found that the soil carbon, nitrogen, and phosphorus density and their stoichiometric ratio (C: P ratio and N: P ratio) fluctuate regularly with changes in altitude and longitude in the Sanjiangyuan region, which also shows that these indicators are controlled by hydrothermal conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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