

Vertical Distribution Characteristics of Soil Organic Carbon Isotopes in the Upper Reaches of the Shule River

Authors: Guo Xinlei, Guo Xinlei

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Abstract

Abstract: The profile distribution of soil organic carbon (SOC) content and its $\delta^{13}\text{C}$ values is of great significance for revealing SOC cycling processes and their patterns. This study investigated soil profiles under different alpine grassland types in the upper reaches of the Shule River on the northeastern margin of the Tibetan Plateau, measuring and analyzing SOC content, soil pH, carbon-to-nitrogen ratio, and organic carbon $\delta^{13}\text{C}$ values of alpine steppe, steppe meadow, and alpine meadow soils. The results showed: 1) SOC content ranges were 4.7-36.1 g · kg⁻¹, 2.4-21.7 g · kg⁻¹, and 4.0-26.1 g · kg⁻¹ for alpine meadow, steppe meadow, and alpine steppe soils, respectively; SOC content in all three soils significantly decreased with soil depth ($P < 0.05$); 2) $\delta^{13}\text{C}_{\text{SOC}}$ values in all three soils exhibited a pattern of initial increase, subsequent decrease, and final stabilization with depth, reaching maxima within the 10-30 cm layer, then gradually decreasing and essentially stabilizing below 60 cm; variation amplitude of $\delta^{13}\text{C}_{\text{SOC}}$ differed among soil profiles, with alpine meadow soil showing the largest variation; 3) Carbon-to-nitrogen ratios in all three soils significantly decreased with soil profile depth ($P < 0.01$), with surface soils having lower decomposition degrees than deep soils, and steppe meadow soil exhibiting the highest decomposition degree.

Full Text

Vertical Distribution of Soil Organic Carbon Isotopes in the Upper Reaches of the Shule River

Guo Xinlei^{1*}, Yi Shuhua¹, Qin Yu¹, Chen Jianjun^{12}

¹State Key Laboratory of Cryospheric Sciences, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000,

China

²University of Chinese Academy of Sciences, Beijing 101408, China

Abstract

The vertical distribution of soil organic carbon (SOC) content and its $\delta^{13}\text{C}$ values in soil profiles provides crucial insights into SOC cycling processes and mechanisms. This study examined soil profiles under different alpine grassland types in the upper reaches of the Shule River on the northeastern edge of the Tibetan Plateau, analyzing SOC content, soil pH, carbon-to-nitrogen ratios, and organic carbon $\delta^{13}\text{C}$ values in alpine steppe, steppe meadow, and alpine meadow soils. The results revealed three key patterns: (1) SOC content in alpine meadow soil, steppe meadow soil, and alpine steppe soil ranged from $4.7\text{--}36.1\text{ g}\cdot\text{kg}^{-1}$, $2.4\text{--}21.7\text{ g}\cdot\text{kg}^{-1}$, and $4.0\text{--}26.1\text{ g}\cdot\text{kg}^{-1}$, respectively, with all three soil types showing significant decreases in SOC content with increasing soil depth ($P < 0.05$). (2) $\delta^{13}\text{C}$ values in all three soil types exhibited an initial increase, followed by a decrease and eventual stabilization with depth, reaching maximum values within the 10–30 cm depth interval before gradually decreasing and stabilizing below 60 cm. The magnitude of $\delta^{13}\text{C}$ variation differed among soil profiles, with alpine meadow soil showing the greatest amplitude of change. (3) Carbon-to-nitrogen ratios in all three soil types decreased significantly with depth ($P < 0.01$), indicating lower decomposition degrees in surface soils compared to deeper layers, with steppe meadow soil exhibiting the highest decomposition degree.

Keywords: Soil organic carbon; Stable carbon isotope; Shule River Basin

Introduction

Grasslands represent one of the most widely distributed ecosystem types on Earth, covering approximately 20% of the land surface and playing a significant role in the global carbon cycle [?]. In China, grasslands are primarily distributed across the Tibetan Plateau, Inner Mongolia Plateau, Northeast Plain, Loess Plateau, and Xinjiang region [?]. The Tibetan Plateau grassland area spans approximately $1.65 \times 10^6\text{ km}^2$ [?], constituting one of China's and the world's important pastoral regions. Changes in the alpine grassland ecosystem of the Tibetan Plateau directly affect local and regional ecological resources, environmental conditions, socioeconomic development, and the functional effectiveness of the national ecological security barrier [?]. As a massive carbon reservoir, grasslands play an extremely important role in China's terrestrial ecosystem carbon cycle [?]. Yang et al. [?] estimated that carbon storage within alpine grasslands of the Tibetan Plateau reaches approximately 7.4 Pg in the top 1 m depth, with an average organic carbon density of 6.5 kg m^{-2} . In recent years, climate warming and irrational human socioeconomic activities have caused varying degrees of grassland degradation across many regions of the Tibetan Plateau, with soil degradation representing the core component of

grassland degradation [?].

In studies of soil erosion, land degradation, and global carbon cycling, soil organic matter serves as both the core of soil quality and one of the most dynamic ecosystem carbon pools in the Earth's surface, exerting major influences on soil quality and climate change [?, ?]. Research has demonstrated that stable isotope tracing technology can effectively reveal soil organic matter decomposition degrees, trace minor migrations and transformations of soil carbon forms and reserves, and quantitatively evaluate the relative contributions of new versus old soil organic carbon to carbon storage [?]. Although numerous studies have investigated soil greenhouse gas emissions [?], soil carbon budgets [?], and biodiversity [?] in the Shule River region, few have addressed the distribution characteristics and composition of soil organic carbon isotopes in this area. Therefore, this study selected soils under different alpine grassland types in the upper Shule River basin as research objects, collecting soil samples from various profile layers to examine vertical distribution characteristics of soil organic matter and differences in stable carbon isotope composition based on SOC content and $\delta^{13}\text{C}$ values. The research aims to provide a theoretical basis for studying the biogeochemical cycling of soil organic matter and restoring degraded grasslands in the upper Shule River region.

1.1 Study Area Overview

The study area is located in the upper reaches of the Shule River, on the western segment of the Qilian Mountains at the northeastern edge of the Tibetan Plateau (38°25' -38°29' N, 98°12' -98°29' E) [?]. The climate is cold and dry with strong winds, featuring a mean annual temperature of -2.7°C and mean annual precipitation of 349.2 mm [?]. The region covers an area of 11,000 km², administratively belonging to the border area between Tianjun County in Haixi Mongolian Autonomous Prefecture, Qinghai Province, and Subei Mongolian Autonomous County in Jiuquan City, Gansu Province [?]. Dominant plant species include *Carex tristachya*, *Kobresia pygmaea*, *Aster tataricus*, *Stipa purpurea*, *Leontopodium alpinum*, and *Polygonum sibiricum* [?]. The permafrost type in this region belongs to the Altun-Qilian high-altitude mountain permafrost zone, dominated by mountain permafrost [?]. Soil types primarily include alpine cold desert soil, alpine meadow steppe soil, light chestnut soil, chestnut soil, and mountain sierozem [?]. The active layer in the permafrost zone experiences an annual freezing period of approximately seven months (October to April of the following year) [?].

Description of sample sites

During July-August 2014, three sampling sites were selected within the study area, representing three vegetation types: alpine meadow, alpine steppe, and steppe meadow. At each site, three soil profiles were randomly selected, totaling nine profiles. Basic characteristics of the profiles and dominant plant species are presented in Table 1. Each profile was excavated to a depth of 1 m, and

soil samples were collected at ten depth intervals (0-10 cm, 10-20 cm, up to 90-100 cm) using a ring knife method, with three replicates per depth. After transport to the laboratory, samples were sieved to remove stones and debris, then air-dried, yielding a total of 270 soil samples.

1.3.1 Soil pH Determination

Soil pH was measured using deionized water as an extractant at a soil-to-water ratio of 1:2. Ten grams of air-dried soil sieved through a 10-mesh screen was placed in a 50 ml beaker, and 20 ml of deionized water was added. The mixture was vigorously stirred with a glass rod for 1-2 minutes, covered with aluminum foil, allowed to stand for 30 minutes, and then measured using a pH meter (UB-7, Sartorius, Germany). Average values were calculated from replicate measurements.

1.3.2 Soil Organic Carbon Content and Carbon-to-Nitrogen Ratio Determination

Two 5.0 g portions of soil sieved through a 100-mesh screen were prepared (total 10.0 g). One portion was placed in a 50 ml centrifuge tube, soaked in 2 mol/L hydrochloric acid solution for 24 hours to remove carbonates, covered with aluminum foil, and shaken every six hours. The sample was then washed with deionized water until neutral, dried at 60°C for 24 hours, cooled to room temperature in a desiccator, and ground. The second portion was sent to the College of Pastoral Agriculture Science and Technology, Lanzhou University, for total soil organic carbon and nitrogen analysis.

1.3.3 Soil Organic Matter Stable Carbon Isotope Analysis

Pretreated soil samples were packed into 5 ml centrifuge tubes and analyzed for stable carbon isotopes using a stable isotope mass spectrometer (MAT 253, Thermo Finnigan, California, USA). Each sample was analyzed in at least three replicates and compared against the PDB (Pee Dee Belemnite) standard. The $\delta^{13}\text{C}$ value was calculated using the formula: $\delta^{13}\text{C}(\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$, where R_{sample} is the isotope ratio ($^{13}\text{C}/^{12}\text{C}$) of the sample and $\text{R}_{\text{standard}}$ is the isotope ratio of the reference standard (PDB). Analytical error was less than $\pm 0.1\text{‰}$.

Statistical analysis was performed using SPSS software for one-way ANOVA and correlation analysis, while Origin 9.0 software was used for graph preparation.

Results

2.1 Soil pH, Organic Carbon Content, and Carbon-to-Nitrogen Ratio Profile Distribution Characteristics

[Figure 1: see original paper] Variations of pH through the soil profiles

Soils under all three vegetation types were weakly alkaline, with pH values ranging from 7.1 to 7.8. With increasing depth, pH values initially decreased, then increased, and finally decreased again. All three soil types reached minimum pH values within the 10–20 cm depth interval. As soil depth continued to increase, pH values rose accordingly. Soils under meadow and steppe meadow vegetation reached maximum pH values within the 70–80 cm depth interval, while alpine steppe soils peaked at 60–70 cm depth. Subsequently, pH values decreased in all three soil types. Overall, pH differences among the three soil types were relatively minor.

[Figure 2: see original paper] Variations of SOC contents through the soil profiles

Soil organic carbon content in all three vegetation types decreased with depth. SOC was concentrated primarily in the 0–20 cm depth interval, where alpine meadow soil, steppe meadow soil, and alpine steppe soil contained 62.73 g/kg, 41.48 g/kg, and 48.11 g/kg of organic carbon, respectively, accounting for 47.44%, 44.12%, and 45.13% of total profile SOC content. All three soil types showed sharp decreases in SOC content with depth: alpine meadow soil within 0–30 cm, alpine steppe soil within 0–40 cm, and steppe meadow soil within 0–50 cm. Below 70 cm depth, SOC content varied only slightly across profiles.

[Figure 3: see original paper] Variations of C/N mass ratios through the soil profiles

Carbon-to-nitrogen ratios differed significantly among the three vegetation types, following the order: alpine meadow soil > steppe meadow soil > alpine steppe soil, and all decreased with depth. Maximum C/N ratios occurred in surface soils across all three types. Within the 0–40 cm depth interval, all three soils showed decreasing C/N ratios with depth. In the 40–60 cm interval, alpine meadow soil C/N ratios increased while steppe meadow and alpine steppe soils showed slight decreases. Within 60–80 cm, both alpine meadow and alpine steppe soils decreased, whereas steppe meadow soil increased slightly. In the 80–100 cm interval, alpine meadow and steppe meadow soils increased slightly while alpine steppe soil decreased. From 0–30 cm depth, C/N ratios decreased by 4.0, 2.5, and 1.8 for alpine meadow, steppe meadow, and alpine steppe soils, respectively, while from 70–100 cm they decreased by 1.9, 0.8, and 1.2.

2.2 Soil Organic Carbon Isotope Profile Distribution Characteristics

[Figure 4: see original paper] Variations of the $\delta^{13}\text{C}$ values of soil organic matter through the soil profiles

Soil organic carbon isotopes under all three vegetation types showed ^{13}C enrichment with depth, with enrichment degrees of 1–2‰. The $\delta^{13}\text{C}$ values exhibited a pattern of rapid initial increase, gradual decrease to a certain level, and subsequent stabilization. Minimum $\delta^{13}\text{C}$ values occurred in surface soils across all types. With increasing depth, alpine meadow soil reached maximum $\delta^{13}\text{C}$ values within 10–20 cm, while alpine steppe and steppe meadow soils peaked at 20–

30 cm depth. Notably, surface soil $\delta^{13}\text{C}$ values followed the order: alpine steppe soil > steppe meadow soil > alpine meadow soil, whereas at maximum values the order reversed: alpine steppe soil < steppe meadow soil < alpine meadow soil. Consequently, across the entire profile, alpine steppe soil showed the smallest $\delta^{13}\text{C}$ enrichment (0.9‰), steppe meadow soil intermediate (1.24‰), and alpine meadow soil the greatest (2.05‰). All three soils showed rapid $\delta^{13}\text{C}$ decreases within the 20–40 cm depth interval. In the 40–60 cm interval, alpine steppe and alpine meadow soils showed slight $\delta^{13}\text{C}$ increases while steppe meadow soil exhibited more complex patterns. Below 60 cm, alpine steppe and steppe meadow soils maintained relatively stable $\delta^{13}\text{C}$ values, whereas alpine meadow soil showed more pronounced increases.

Discussion

Soil organic matter represents the most critical soil attribute and the core of soil quality, serving as both a key component of soil structure and an essential energy source for soil biological activity [?]. Significant differences in soil organic matter exist among different alpine grassland types, with steppe meadow and alpine steppe soils being substantially lower than alpine meadow soil, particularly within the 0–20 cm depth interval where differences are most pronounced. Consistent with previous research findings [?], soil profile SOC content decreased progressively from the surface downward. In natural systems, SOC balance is controlled by both carbon input from plant production and carbon output through decomposition [?]. Jobbágy and Jackson [?] analyzed 2,721 soil samples and 117 root biomass samples from a global soil database, representing all major ecosystem types including tundra, desert, cropland, temperate evergreen forest, temperate deciduous forest, temperate grassland, tropical deciduous forest, and boreal forest. Their results indicated that, globally, 40% of soil profile carbon is stored within the 0–20 cm depth interval, with a gradually decreasing trend, though distribution patterns differ significantly among ecosystem types.

In the upper Shule River region, SOC content was concentrated primarily within the 0–20 cm depth interval, where alpine meadow soil contained 62.73 g/kg SOC (47.44% of total profile carbon), alpine steppe soil contained 48.11 g/kg (45.13%), and steppe meadow soil contained 41.48 g/kg (44.12%). SOC content increased progressively from steppe meadow soil to alpine steppe soil to alpine meadow soil, likely because alpine meadow vegetation has the highest above-ground biomass [?], providing greater carbon input to the soil. However, the three soil types exhibited different SOC decrease patterns with depth: alpine meadow soil showed sharp decreases within 0–30 cm, alpine steppe soil within 0–40 cm, and steppe meadow soil within 0–50 cm. Below 60 cm, SOC content varied only slightly across profiles, yet the absolute quantity of deep soil carbon remains an important component of total profile carbon storage, with approximately 18% of alpine meadow SOC, 13% of steppe meadow SOC, and 17% of alpine steppe SOC stored below 60 cm. The relatively small variation in deep soil SOC likely reflects similar parent materials and comparable organic

matter decomposition degrees at depth. In summary, plant litter strongly influences surface soil SOC content, while parent material and soil factors may exert greater influence on deep soil SOC.

Soil organic matter $\delta^{13}\text{C}$ typically enriches by 1–3‰ with increasing profile depth, a phenomenon widely reported in the literature [?], though no unified explanation has been established. Primary mechanisms include: (1) Fossil fuel combustion and enhanced soil organic matter mineralization over the past 200+ years have depleted atmospheric CO_2 carbon isotope ratios by approximately 1.3‰. Plants assimilating this atmospheric CO_2 may consequently lower surface soil organic matter carbon isotope ratios [?]. Plant photosynthates and litter integrate this atmospheric CO_2 depletion signal, which may produce a time-lag effect as this carbon moves down the soil profile. Thus, deeper soil organic matter is less affected by the depletion signal, resulting in carbon isotope ratio enrichment from surface to depth [?]. (2) Soil organic matter components exhibit varying degradability. Plant litter primarily comprises monosaccharides, polysaccharides (e.g., cellulose), and lignin [?]. With increasing depth, readily degradable compounds with lower carbon isotope ratios are preferentially decomposed, leaving relatively higher proportions of recalcitrant compounds with higher carbon isotope ratios, thus enriching ^{13}C [?, ?]. (3) During microbial degradation and biosynthesis of organic matter, anaerobic reactions fix soil-derived CO_2 with higher carbon isotope ratios, causing significant shifts in microbial carbon isotope composition (1–1.5‰). Microbial products gradually become important soil organic matter components through selective preservation [?].

Alpine meadow, alpine steppe, and steppe meadow soils in the Shule River basin showed consistent $\delta^{13}\text{C}$ patterns with depth, characterized by enrichment-depletion-stabilization trends. However, the magnitude of $\delta^{13}\text{C}$ variation differed significantly among grassland types, with enrichments of 2.05‰, 1.24‰, and 0.9‰ for alpine meadow, steppe meadow, and alpine steppe soils, respectively. Within the 0–10 cm depth interval, alpine meadow soil exhibited the lowest $\delta^{13}\text{C}$ values, likely due to its highest aboveground biomass and concentrated root biomass distribution within 0–10 cm [?], resulting in higher contents of readily decomposable carbon compounds and stronger influence from atmospheric CO_2 depletion effects, thus enriching ^{12}C . All three soils reached maximum $\delta^{13}\text{C}$ values within the 10–20 cm depth interval, consistent with findings from multiple studies [?, ?]. This peak may be explained by rapid, extensive decomposition of readily degradable litter components at this depth, causing rapid ^{13}C enrichment in soil organic matter. Within the 20–30 cm interval, new plant carbon input from root distribution [?] and the strong correlation between root biomass distribution and soil carbon distribution [?] caused rapid $\delta^{13}\text{C}$ decreases. Below 30 cm, where plant roots are rarely distributed in the study area, $\delta^{13}\text{C}$ values likely stabilized due to combined effects of atmospheric CO_2 depletion, microbial anaerobic effects, and soil factors (pH, temperature).

The soil carbon-to-nitrogen ratio represents the ratio of carbon to to-

tal nitrogen content in soil organic matter. Since 95-98% of soil nitrogen originates from soil organic matter mineralization, and nitrogen mineralization loss correlates well with organic matter mineralization loss, the C/N ratio is frequently used as an indicator of organic matter decomposition [?]. Microbial biomass maintains a C/N ratio of 10 ± 2 , while suitable plant litter for decomposition has a C/N ratio of 30 ± 15 .

All three alpine grassland soil types showed gradually decreasing C/N ratios from the surface downward, consistent with general soil accumulation patterns. Alpine meadow soil C/N ratios ranged from 12-19, alpine steppe soil from 11-16, and steppe meadow soil from 6-15. The magnitude of C/N ratio change varied among soil types and depth intervals: from 0-30 cm, ratios decreased by 4.0, 2.5, and 1.8 for alpine meadow, steppe meadow, and alpine steppe soils, respectively, while from 70-100 cm they decreased by 1.9, 0.8, and 1.2. Li et al. [?] used high-throughput sequencing to investigate effects of long-term fertilizer application on soil microbial community structure in oasis farmland, finding that SOC content was the most important factor influencing deep soil microbial communities, while total nitrogen content most strongly affected surface soil microbial community structure. This corroborates the vertical distribution pattern of C/N ratios. High C/N ratios indicate nitrogen limitation for microbial decomposition, whereas low ratios promote available nitrogen increase, making organic carbon content the controlling factor. Thus, small C/N ratio variations in deep soils likely result from combined effects of higher recalcitrant substance content and lower microbial biomass. Our results demonstrate that surface soil organic matter decomposition degrees are significantly lower than those of deep soils, with steppe meadow soil showing the highest decomposition degree.

Conclusion

Significant differences exist in SOC content and stable isotope composition among soil profiles under the three alpine grassland types in the Shule River basin. Across entire profiles, SOC content showed a decreasing trend, though the rate of change varied at different depths. With increasing depth, $\delta^{13}\text{C}$ values in all three grassland types exhibited an increase-decrease-stabilization pattern, reaching maximum values within the 10-30 cm depth interval. The magnitude of $\delta^{13}\text{C}$ variation differed significantly among grassland types ($P < 0.05$), with alpine meadow soil showing the greatest amplitude (ranging from -26.37% to -24.32%). Surface soil decomposition degrees were significantly lower than those of deep soils across all three grassland types, with steppe meadow soil exhibiting the highest decomposition degree.

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*Corresponding author: Guo Xinlei, E-mail: 18810988626@163.com

Author Contributions:

Guo Xinlei, Yi Shuhua, and Qin Yu: Conceived the research idea and designed the study;

Guo Xinlei: Performed the experiments;

Guo Xinlei, Qin Yu, and Chen Jianjun: Collected, processed, and analyzed the data;

Guo Xinlei: Drafted the manuscript;

Yi Shuhua, Guo Xinlei, and Chen Jianjun: Revised the final version of the paper.

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