Effects of Rainfall Frequency on Soil Carbon, Nitrogen, and Phosphorus Stoichiometric Characteristics in the Gahai Wet Meadow, Gannan (Postprint)

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Abstract

Rainfall is a critical disturbance factor for wetland water resource recharge and soil respiration, and the projected increase in extreme rainfall variability due to global climate change exerts significant influences on wetland ecosystems. To examine the variation characteristics of soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP) contents and their stoichiometric ratios in Tibetan Plateau wet meadows under extreme rainfall frequencies, this study selected wet meadow soils in the Gahai-Zecha Nature Reserve in Luqu County on the northeastern edge of the Tibetan Plateau as the research subject, establishing five treatments: blank control (CK: 0 mm), watering once per week (DF1: 25 mm $\times$ 19 times), watering once every two weeks (DF2: 25 mm $\times$ 9 times), watering once every three weeks (DF3: 25 mm $\times$ 6 times), and watering once every four weeks (DF4: 25 mm $\times$ 4 times), to analyze the variation patterns of SOC, TN, and TP stoichiometric characteristics in the 0–40 cm soil layer under extreme rainfall frequencies. The results indicated that under different rainfall frequencies, soil SOC content increased with increasing rainfall frequency, while TN and TP contents exhibited contrasting trends. Within the soil vertical profile, both SOC and TN contents decreased with increasing soil depth, whereas TP content showed no significant change with soil depth; C:P and N:P both decreased with soil depth, while C:N showed no significant change with soil depth; C:N, C:P, and N:P showed no significant differences among different rainfall frequencies. Furthermore, over time, soil SOC content displayed an initial increase followed by a decrease during different months of the growing season, TN content exhibited an initial decrease followed by an increase, while TP content showed an "M"-shaped pattern. Therefore, as alterations in global rainfall patterns continue to intensify, higher rainfall frequencies will aggravate the loss of nitrogen

and phosphorus contents in shallow soils of alpine wet meadows, exacerbating eutrophication hazards in the water environment of alpine wet meadows.

Full Text

Preamble

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Abstract: Rainfall is a critical disturbance factor affecting water resource replenishment and soil respiration in wetlands. Increased variability in extreme rainfall events driven by global climate change will significantly impact wetland ecosystems. To investigate changes in soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and their stoichiometric ratios under extreme rainfall frequencies, we conducted a study on wet meadow soils in the Gahai-Zecha Nature Reserve on the northeastern edge of the Tibetan Plateau. We established five treatments: a control (natural rainfall only), weekly irrigation (25 mm × 19 events), fortnightly irrigation (25 mm × 9 events), three-weekly irrigation (25 mm × 6 events), and four-weekly irrigation (25 mm × 4 events) to analyze changes in soil stoichiometric characteristics under extreme rainfall frequencies. Results showed that under different rainfall frequencies, soil SOC content increased with increasing rainfall frequency, while TN and TP content showed the opposite trend. In the soil vertical profile, SOC and TN contents decreased significantly with increasing soil depth, whereas TP content showed no significant change with depth. The C:N ratio showed no significant change with soil depth, while both C:P and N:P ratios decreased with depth. Additionally, as the growing season progressed, SOC content exhibited an initial increase followed by a decrease, TN content showed a decrease followed by an increase, and TP content displayed an "M"-shaped trend. Therefore, as global rainfall patterns continue to change, higher rainfall frequencies will intensify the loss of nitrogen and phosphorus in shallow soils of alpine wet meadows, exacerbating eutrophication risks in alpine wet meadow water environments.

Keywords: Tibetan Plateau; wet meadow; soil stoichiometric characteristics; rainfall frequency

Introduction

Ecological stoichiometry (ES) is the science investigating the balance of multiple chemical elements (primarily carbon, nitrogen, and phosphorus) in ecological interactions and processes []. It provides an effective approach for studying the coupling relationships of C, N, and P elements in ecosystems and offers new insights for understanding ecosystem nutrient cycling and biogeochemical mechanisms []. Carbon, nitrogen, and phosphorus are essential nutrients in terrestrial ecosystems and critical elements for plant growth, directly affecting soil microbial biomass and long-term nutrient accumulation. Changes in external environmental conditions alter soil nutrient contents, and when C, N, and P elements are deficient or excessive in soil, the relative limitation of elements on plant growth can be determined based on stoichiometric ratios. Therefore, investigating changes in soil nutrient content and ecological stoichiometric characteristics is crucial for clarifying ecosystem nutrient balance processes and their response mechanisms to environmental change [].

Wetlands are highly dynamic and complex multifunctional ecosystems that serve as critical transitional zones buffering and stabilizing coastal, riparian, and lacustrine interfaces between land and water []. In recent years, extreme rainfall events caused by climate change have increased in frequency, disrupting wetland soil nutrient cycling, reducing vegetation diversity, and hindering the sustainable development of wetland ecosystems []. Studies have shown that increased rainfall reduces soil organic carbon and total nitrogen contents and the N:P ratio in wetlands [], while high-frequency rainfall promotes the decomposition and transformation of dissolved organic carbon and ammonium nitrogen, enhances nitrate nitrogen production [], and affects soil organic carbon mineralization processes and soil organic matter decomposition rates []. Other studies have found that soil organic carbon, total nitrogen, and total phosphorus contents do not respond significantly to changes in rainfall frequency []. These inconsistent findings suggest that changes in soil nutrient and stoichiometric characteristics depend not only on leaching intensity after rainfall changes [] but also on inputs from litter and root exudates []. Most research on precipitation pattern changes has focused on single factors of rainfall amount or frequency, yielding inconsistent results. Therefore, further studies on the effects of different rainfall frequencies and amounts on the spatial and temporal distribution characteristics of soil nutrients and stoichiometry are needed to accurately assess and predict trends in alpine meadow soil stoichiometric characteristics under future rainfall pattern changes, propose relevant protection and management measures, and provide theoretical support for sustainable development of alpine meadow ecosystems.

Currently, scholars have conducted numerous studies on ecological stoichiometry, but most have focused on laboratory incubation experiments or simple soil moisture regulation to investigate effects of different soil moisture levels on soil stoichiometric characteristics. The Tibetan Plateau, as a sensitive region to global change and an ecological security barrier for Asia [], has rarely been studied regarding soil stoichiometric characteristics of alpine wet meadows under extreme rainfall backgrounds. There is a severe lack of in-depth understanding and data support for soil nutrient cycling in Tibetan Plateau wet meadows. Therefore, this study focuses on wet meadows in the Gahai Nature Reserve on the northeastern edge of the Tibetan Plateau. Through extreme rainfall simulation experiments, we investigated the response mechanisms of soil stoichiometric characteristics to extreme rainfall frequencies and their relationships with other environmental factors, providing a scientific basis for sustainable development of alpine wet meadow ecosystems on the Tibetan Plateau.

1.1 Study Area Description

The study area is located in the Gahai-Zecha National Nature Reserve in Luqu County on the northeastern edge of the Tibetan Plateau (33°58 ~34°30 N, 102°05 ~102°29 E). The reserve covers a wetland area of 57,846 hm^2, including 51,160 hm^2 of wet meadows []. The climate is characterized as alpine humid climate on the Tibetan Plateau, with an average annual temperature of 1.2°C, maximum monthly temperature of 10.5°C in July, maximum annual temperature variation of 52.5°C, and average daily temperature variation of 13.7°C. Annual precipitation is 781.8 mm, concentrated in July-September. Soil types are primarily dark meadow soil, swamp soil, and peat soil with high organic matter content []. The area is rich in plant resources with 313 species, dominated by perennial herbs including Carex meyeriana, Artemisia subulata, Poa subfastigiata, Kobresia kansuensis, Carex enervis, and Carex moorcroftii [].

[**Figure 1: see original paper**] The geographical location map of the study area

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1.2 Experimental Design

In May 2021, we selected a flat wet meadow area with consistent vegetation type within the Gahai-Zecha Nature Reserve as the study site. Using a randomized block design, we established 15 plots (2 m × 2 m each) with 2 m spacing between plots. Each plot received natural rainfall, and we applied supplemental irrigation equivalent to 25 mm of natural rainfall per event. Five rainfall frequency treatments were established to simulate extreme rainfall inputs in the region []: control (natural rainfall only), weekly irrigation (25 mm × 19 events), fortnightly irrigation (25 mm × 9 events), three-weekly irrigation (25 mm × 6 events), and four-weekly irrigation (25 mm × 4 events). Each treatment had three replicates. During the May-September growing season, we controlled rainfall intensity during irrigation to avoid scouring effects. The total supplemental irrigation amounts were: 0 mm for control, 475 mm for weekly (25 mm × 19), 225 mm for fortnightly (25 mm × 9), 150 mm for three-weekly (25 mm × 6), and 100 mm for four-weekly (25 mm × 4).

1.3 Sample Collection and Measurement

In mid-June, July, August, and September 2021, we collected soil samples from 0-10 cm, 10-20 cm, and 20-40 cm layers using a soil auger with an "S" pattern in each plot. Soil samples from the same layer and treatment were combined into composite samples (three replicates per treatment). Within 24 hours, we removed stones, roots, and other debris, passed samples through a 2 mm sieve, and stored them at 4°C. Soil organic carbon content was measured using the potassium dichromate volumetric method, total nitrogen using the Kjeldahl method, and total phosphorus using the molybdenum-antimony colorimetric method []. Aboveground biomass was collected monthly using the traditional harvest method. All plants in each plot were cut at the base, returned to the laboratory, heated in an oven at 105°C for 30 minutes to kill enzymes, then dried at 65°C to constant weight to obtain dry mass.

1.4 Data Processing

Data were organized using Microsoft Excel 2021 and analyzed using SPSS 26.0 with three-way ANOVA to test the effects of rainfall frequency, soil layer, and month on soil stoichiometric characteristics. Multiple comparisons were performed using Duncan's method at $\alpha = 0.05$ significance level. Figures were created using Origin 2021.

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2 Results and Analysis

2.1 Effects of Extreme Rainfall Frequency on Soil Stoichiometric Characteristics

Analysis showed that under the same rainfall frequency, SOC, TN, and TP contents differed significantly among soil layers ($P < 0.05$). Both SOC and TN exhibited surface accumulation characteristics, with the highest values in the 0-10 cm layer, decreasing with soil depth. Across different rainfall frequencies, SOC content in the 0-10 cm layer increased with rainfall frequency, while TN and TP contents decreased. In the 0-10 cm layer, SOC content was highest under the weekly irrigation treatment (63.4 $g \cdot kg^{-1}$), followed by fortnightly (58.9 $g \cdot kg^{-1}$), three-weekly (54.9 $g \cdot kg^{-1}$), four-weekly (38.8 $g \cdot kg^{-1}$), and control (20.6 $g \cdot kg^{-1}$) treatments. For TN content, the 0-10 cm layer showed the highest values, followed by 10-20 cm and 20-40 cm layers, with no significant difference between the latter two. Under the same rainfall frequency gradient, SOC content in the 0-10 cm layer was significantly higher than in 10-20 cm and 20-40 cm layers ($P < 0.05$). The C:N ratio ranged from 10.02 to 23.53, C:P ratio from 344.72 to 797.28, and N:P ratio from 38.52 to 80.24 across all treatments.

**** Analysis of variance for the effect of treatments, soil layer, and month on soil ecological chemometric characteristics

Three-way ANOVA showed that SOC, TN, and TP contents differed significantly among rainfall frequencies, soil layers, and months ($P < 0.01$). Under single-factor effects of rainfall frequency, soil layer, or month, SOC, TN, and TP showed different levels of significant differences ($P < 0.01$). Under the combined effects of rainfall frequency, soil layer, and month, SOC, TN, and TP showed extremely significant differences ($P < 0.01$). Two-way interactions among the three factors and their interactions with SOC, TN, and TP were also extremely significant ($P < 0.01$).

[**Figure 2: see original paper**] Soil stoichiometry characteristics under different rainfall frequencies

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2.2 Aboveground Biomass Under Different Rainfall Frequencies

All treatments showed significant differences in aboveground biomass ($P < 0.05$). Under different rainfall frequencies, aboveground biomass showed clear temporal variation. SOC content showed an initial increase followed by a decrease over time, with peak values in August: weekly (42.74 $g \cdot kg^{-1}$) > fortnightly (38.91 $g \cdot kg^{-1}$) > three-weekly (30.24 $g \cdot kg^{-1}$) > four-weekly (30.22 $g \cdot kg^{-1}$) > control (29.21 $g \cdot kg^{-1}$). Minimum values occurred in September: weekly (22.31 $g \cdot kg^{-1}$) > fortnightly (20.77 $g \cdot kg^{-1}$) > three-weekly (16.37 $g \cdot kg^{-1}$) > four-weekly (15.65 $g \cdot kg^{-1}$) > control (14.02 $g \cdot kg^{-1}$). TN content showed a decreasing then increasing seasonal trend, with minimum values in July: weekly (0.98 $g \cdot kg^{-1}$) > fortnightly (0.95 $g \cdot kg^{-1}$) > three-weekly (0.93 $g \cdot kg^{-1}$) > four-weekly (0.91 $g \cdot kg^{-1}$) > control (0.89 $g \cdot kg^{-1}$). TP content showed an "M"-shaped trend, increasing from June to July, decreasing from July to August, and reaching its peak in September.

[**Figure 3: see original paper**] Aboveground biomass under different rainfall frequencies

[**Figure 4: see original paper**] Seasonal variation of soil nutrient content with different rainfall frequencies

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2.3 Correlations Between Soil Nutrients and Stoichiometric Ratios Under Different Rainfall Frequencies

Correlation analysis revealed extremely significant negative correlations between SOC and TN ($P < 0.01$), SOC and TP ($P < 0.01$), and TN and TP ($P < 0.01$). SOC showed extremely significant positive correlations with C:N ratio ($P < 0.01$) and C:P ratio ($P < 0.01$), and extremely significant negative correlations with N:P ratio ($P < 0.01$). TN showed extremely significant negative correlations with C:P ratio ($P < 0.01$) and N:P ratio ($P < 0.01$). TP showed extremely significant positive correlations with C:P ratio ($P < 0.01$) and N:P ratio ($P < 0.01$), and a significant positive correlation with C:N ratio ($P < 0.01$). C:N ratio showed extremely significant negative correlations with C:P ratio ($P < 0.01$) and N:P ratio ($P < 0.01$), while C:P and N:P ratios showed extremely significant positive correlations ($P < 0.01$).

Regression analysis between aboveground biomass and SOC, TN, and TP revealed that aboveground biomass had a low explanatory power for SOC ($R^2 = 0.0301$) with the regression equation: $y = 0.0183x + 21.793$. Aboveground biomass had high explanatory power for TN ($R^2 = 0.5112$) with the equation: $y = 0.0059x + 23.761$. Aboveground biomass had moderate explanatory power for TP ($R^2 = 0.0859$) with the equation: $y = 0.5115x + 51.057$.

[**Figure 5: see original paper**] Correlation analysis of soil nutrients and stoichiometric ratios with different rainfall frequencies

[**Figure 6: see original paper**] Linear relationship between aboveground biomass and SOC, TN and TP

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3 Discussion

3.1 Differences in Soil Nutrient Stoichiometric Characteristics Under Different Rainfall Frequencies

Soil organic carbon is a crucial component affecting terrestrial ecosystem productivity and global climate change [], while soil nitrogen and phosphorus are essential mineral nutrients and key limiting elements for plant growth []. Their interactions play vital roles in maintaining ecosystem balance []. This study found that SOC and TN contents decreased with increasing soil depth, consistent with previous research [], while TP content remained stable vertically. This is because SOC and TN primarily originate from aboveground litter decomposition, animal and microbial remains [], with additional nitrogen from atmospheric deposition. The study area is dominated by herbaceous plants with minimal disturbance from herbivores or human activities, resulting in vigorous vegetation growth. Herbaceous plant roots concentrate in surface soil layers, providing thick litter layers and dense decaying root systems that supply abundant raw materials for humus decomposition and organic matter synthesis [].

As soil depth increases, litter input and root systems decrease, microbial quantity and activity decline, decomposition rates slow, and available organic matter decreases, leading to reduced SOC and TN contents.

Phosphorus in soil is primarily a sedimentary mineral derived from parent rock weathering and organic matter input []. Weathering differences are minimal in shallow soils [], resulting in relatively stable vertical distribution of TP. The C:N:P ratio is an important indicator for elucidating nutrient limitations and characterizing ecological processes []. Our results showed that C:N and C:P ratios decreased with soil depth, while N:P ratio remained stable across layers. This pattern occurs because SOC and TN decrease with depth while TP remains relatively constant. Compared with national soil stoichiometric averages [], our C:P and N:P ratios were excessively high, indicating phosphorus deficiency and phosphorus limitation on plant growth in the study area.

This study demonstrated significant differences in SOC, TN, and TP contents among rainfall frequency treatments, indicating that rainfall frequency changes affect soil stoichiometric characteristics. SOC content increased with rainfall frequency, while TN and TP decreased. Increased rainfall frequency intensifies leaching of nitrogen and phosphorus [], with phosphorus loss primarily caused by leaching []. Increased soil water content enhances phosphorus leaching losses, and some phosphorus is absorbed by plant roots [], increasing plant phosphorus consumption within certain water content ranges. Therefore, increased rainfall frequency intensifies losses of nitrogen and phosphorus in soil.

3.2 Seasonal Dynamics of Soil Nutrients

Soil C:N:P stoichiometric characteristics are influenced by climate conditions, parent material, topography, soil type, biological conditions, and hydrothermal conditions []. This study found clear seasonal variations in SOC, TN, and TP contents during the growing season. In June, undecomposed leaf, branch, and root residues from the previous year entered the soil through humification processes under favorable moisture and temperature conditions [], while growing plants released root exudates and litter [], increasing SOC content. In July, large diurnal temperature differences caused frequent freeze-thaw cycles, mineralizing and releasing organic matter, and precipitation leaching caused substantial organic carbon loss [], reducing SOC content. TN content reached its annual minimum in July because plants entered their rapid growth phase with high nutrient demand. Increased temperature enhanced soil respiration rates [], accelerated root physiological metabolism, and increased microbial nitrogen utilization. As plants entered senescence (September), accumulated litter decomposition products returned to soil, gradually increasing nitrogen content.

TP content showed an "M"-shaped trend with a turning point in July-August. Plants absorb and assimilate soil phosphorus through roots, converting it into organic components such as nucleic acids and phospholipids. When aboveground parts grow rapidly, roots and rhizomes absorb large amounts of phosphorus from soil to support growth, reducing soil TP content. In late growing season, decreased natural precipitation and lower temperatures slowed plant growth, and nutrients from aboveground parts returned to soil, gradually increasing TP content.

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4 Conclusion

Based on analysis of soil nutrient contents and stoichiometric ratios under extreme rainfall frequencies in Tibetan Plateau wet meadows, we studied the response patterns of soil C, N, and P to extreme rainfall frequencies. The conclusions are:

1) Increased rainfall frequency benefits SOC accumulation but causes nitrogen and phosphorus loss. SOC and TN contents decrease with increasing soil profile depth, while TP shows minimal vertical change and stable distribution.
2) The vertical distribution of C:N:P ratios shows consistency: C:N and C:P ratios decrease with soil depth, while N:P ratio remains stable across layers. Soil stoichiometric ratios indicate phosphorus deficiency in the study area, with plant growth primarily limited by phosphorus.
3) SOC, TN, and TP contents show obvious seasonal dynamics during the growing season: SOC shows an initial increase then decrease, TN shows a decrease then increase, and TP shows an "M"-shaped trend.

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Note: Figure translations are in progress. See original paper for figures.