

## Effects of Moisture on Soil Nitrogen Mineralization During Degradation Succession in Gahai Wetland (Postprint)

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### Abstract

Moisture is the primary controlling factor of the nitrogen mineralization process in wetland soils and plays an important role in nitrogen cycling processes in wetland soils. Taking the Gannan Gahai Wetland as the study area, four degradation levels (Undegraded UD, Lightly Degraded LD, Moderately Degraded MD, and Heavily Degraded HD) and four field water capacities (20% FC, 40% FC, 60% FC, and 80% FC) were established. Through a 49-day aerobic incubation experiment under laboratory conditions, the soil nitrogen mineralization characteristics in the 0–10 cm soil layer of wetland soils under each treatment condition were determined. The results showed that: (1) Under all moisture conditions, with increasing incubation time, the ammonification rate, nitrification rate, and net nitrogen mineralization rate of soils across the four degradation levels all exhibited an initial increase followed by a decrease. (2) The mean net nitrogen mineralization amount of soil first increased and then decreased with increasing moisture. Under 60% FC conditions, the mean net nitrogen mineralization amount of soils across various degradation levels ranged from 34.91 to 44.94  $\text{mg} \cdot \text{kg}^{-1}$ , which was 22.31–30.29  $\text{mg} \cdot \text{kg}^{-1}$ , 10.91–19.84  $\text{mg} \cdot \text{kg}^{-1}$ , and 8.57–19.50  $\text{mg} \cdot \text{kg}^{-1}$  higher than that under 20% FC, 40% FC, and 80% FC, respectively. (3) Both the mean net nitrogen mineralization amount and the mean net nitrogen mineralization rate of soil decreased with increasing wetland degradation level. Appropriate moisture is beneficial to soil nitrogen mineralization, whereas excessively high moisture is detrimental to soil nitrogen mineralization; meanwhile, wetland degradation reduces soil nitrogen mineralization.

## Full Text

### Effect of Water on Nitrogen Mineralization in Degraded Succession of Gahai Wetland

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#### Abstract

Moisture is the primary regulatory factor of nitrogen mineralization processes in wetland soils and plays a crucial role in soil nitrogen cycling. This study investigated the Gannan Gahai wetland, establishing four degradation gradients (non-degraded, UD; lightly degraded, LD; moderately degraded, MD; and heavily degraded, HD) and four field water holding capacity levels (20% FC, 40% FC, 60% FC, and 80% FC). Through 49 days of aerobic incubation under controlled conditions, we measured the nitrogen mineralization characteristics of wetland soil in the 0-10 cm layer. The results showed that: (1) Under various moisture conditions, the ammonification rate, nitrification rate, and net nitrogen mineralization rate of soils across all four degradation gradients exhibited a trend of initially increasing then decreasing with prolonged incubation time. (2) The average net nitrogen mineralization increased initially then decreased with increasing water content; at 60% FC, the value ranged from 34.91 to 44.94  $\text{mg} \cdot \text{kg}^{-1}$ , representing increases of 22.31-30.29  $\text{mg} \cdot \text{kg}^{-1}$ , 10.91-19.84  $\text{mg} \cdot \text{kg}^{-1}$ , and 8.57-19.50  $\text{mg} \cdot \text{kg}^{-1}$  compared to 20% FC, 40% FC, and 80% FC, respectively. (3) Both the average net nitrogen mineralization amount and rate decreased with increasing wetland degradation. Appropriate moisture favors soil nitrogen mineralization, while excessive moisture is unfavorable; simultaneously, wetland degradation reduces soil nitrogen mineralization.

**Keywords:** nitrogen mineralization; soil moisture content; Gahai Wetland; moisture; degree of degradation

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## Introduction

Nitrogen plays a vital role in plant growth as one of the essential elements required for plant development and serves as a fundamental component for plants to synthesize proteins, nucleic acids, and other substances. In wetland soils, nitrogen must undergo microbial mineralization to be converted into inorganic forms of available nitrogen that plants can absorb and utilize. Therefore, studying nitrogen mineralization in wetland soils is significant for understanding nitrogen utilization by wetland plants and improving soil fertility.

The soil nitrogen mineralization process is influenced by numerous factors, including land use change, soil temperature, moisture, and grazing disturbance.

Many scholars have conducted research on soil nitrogen mineralization in grasslands under grazing activities. For instance, some studies in central Inner Mongolia grasslands suggest that grazing can increase soil net nitrogen mineralization rates and facilitate soil nitrogen transformation processes. However, other research in the Xilin River Basin grasslands of Inner Mongolia found that grazing reduces mineral nitrogen content and inhibits soil nitrogen mineralization. These conflicting results indicate that different grazing intensities have varying effects on grassland soil nitrogen mineralization. During grazing, animal trampling and digging disturbances can affect wetland vegetation, soil physicochemical properties, and the spatial distribution patterns of carbon and nitrogen, thereby influencing the wetland soil nitrogen mineralization process.

Moisture is a key factor affecting soil nitrogen mineralization, as soil water availability regulates microbial quantity and activity, significantly impacting the mineralization process. Research on purple soil in southwestern China found that under constant temperature incubation, both cumulative mineralized nitrogen and net mineralization rates gradually increased with soil water content. Stanford and Smith also noted that within certain soil moisture conditions, nitrogen mineralization is significantly positively correlated with soil moisture, while exceeding a certain water content becomes unfavorable for soil nitrogen mineralization. However, studies on the response of wetland soil nitrogen mineralization to moisture are relatively scarce, particularly regarding how soil nitrogen mineralization responds to moisture during wetland degradation processes. It is evident that both moisture and grazing intensity significantly influence soil nitrogen mineralization, yet most current research has not comprehensively considered the combined effects of these two factors.

Located on the southeastern edge of the Qinghai-Tibet Plateau, the Gahai Wetland is an important component of the plateau's wetland ecosystem and one of the regions most severely affected by human activities. In recent years, due to overgrazing and wetland drainage, the Gahai Wetland has experienced serious degradation. Current research in this area has primarily focused on biodiversity, soil organic carbon, and greenhouse gases, while indoor simulation studies on soil nitrogen mineralization during wetland degradation have not been reported. Meanwhile, domestic and international research on soil nitrogen mineralization has concentrated on farmland, forests, grasslands, and lakeside wetlands, with relatively few studies examining how nitrogen mineralization in alpine wetland soils of different degradation degrees responds to moisture changes. Therefore, this study conducted indoor moisture control experiments using swamp meadow soils from different degradation degrees in the Gahai Wetland to investigate the effects of moisture variation on the nitrogen mineralization process in alpine wetland soils under varying degradation levels. The findings aim to provide a theoretical basis for rational restoration of degraded alpine wetland areas based on moisture conditions and soil characteristics, as well as for regulating soil nitrogen transformation processes through optimized moisture management practices in this region.

## Materials and Methods

### 1.1 Study Soil

The test soil was collected in November 2020 (non-growing season) from the Gahai swamp meadow area (33°58' ~34°32' N, 102°09' ~102°46' E). The region has an average annual temperature of 1.2°C, with the warmest month in July (average 12.4°C) and the coldest month in January (average -9.1°C). Annual precipitation is 781.8 mm, unevenly distributed throughout the year and concentrated in July–September, with annual evaporation of 1150.5 mm. Soil samples were collected from four degradation gradients: non-degraded (UD), lightly degraded (LD), moderately degraded (MD), and heavily degraded (HD) plots. Detailed plot information is provided in Table 1. A “snake-shaped” sampling method was used to collect soil cores from 10 points at 0–10 cm depth in each plot. The samples were mixed to form composite samples, with plant residues and stones removed. Collected samples were placed in fresh-keeping bags, transported to the laboratory, and air-dried naturally before being ground and passed through a 2 mm sieve. Each mixed soil sample was replicated three times for nitrogen mineralization measurements. Basic soil physicochemical properties are shown in Table 2.

### 1.2 Experimental Design

We employed indoor constant-temperature incubation to determine nitrogen mineralization characteristics of wetland soils under different moisture conditions. The experiment included four wetland degradation degrees (non-degraded, lightly degraded, moderately degraded, and heavily degraded) and four field water holding capacity levels (20% FC, 40% FC, 60% FC, and 80% FC). For each degradation degree, soil samples from the 0–10 cm layer were collected. Based on changes in field water holding capacity during wetland degradation (Table 3), we established four moisture treatments for each degradation level, with three replicates per treatment, totaling 48 soil samples. Samples were incubated in a constant-temperature incubator at 25°C.

The specific experimental procedure was as follows: 100 g of air-dried soil passed through a 2 mm sieve was weighed into a 300 mL plastic beaker. Based on the field water holding capacity of each degradation degree, distilled water was added to adjust the moisture content to the designed levels. The beakers were sealed with plastic wrap, punctured with 5–6 small holes to maintain moderate aeration, and placed in a 25°C incubator. During the incubation period, moisture loss was replenished every 2–3 days using the weighing method. Destructive sampling was performed at 3, 7, 14, 28, and 49 days. For each sampling, 20 g of fresh soil was weighed from each beaker, extracted with 2 mol · L<sup>-1</sup> potassium chloride solution (soil:extractant ratio of 1:5), and shaken for 1 hour. After extraction, 20 mL of the filtrate was pipetted into a digestion tube, and 0.5 g of magnesium oxide was added. Ammonium nitrogen content was determined by Kjeldahl distillation with 20 g · L<sup>-1</sup> boric acid absorption, followed by back-

titration with  $0.005 \text{ mol} \cdot \text{L}^{-1}$  sulfuric acid. After distillation, 0.5 g of Devarda's alloy was added to the same solution to determine nitrate nitrogen content.

### 1.3 Calculation of Soil Nitrogen Mineralization Amount and Rates

Cumulative nitrogen mineralization amount equals the sum of net nitrogen mineralization at each sampling time throughout the incubation period ( $\text{mg} \cdot \text{kg}^{-1}$ ). Net nitrogen mineralization amount is the difference between mineralized nitrogen before and after incubation ( $\text{mg} \cdot \text{kg}^{-1}$ ). The calculation formulas for soil nitrogen mineralization rates are as follows:

Ammonification rate:

$$R_{amm} = \frac{c(NH_4^+ - N)_t - c(NH_4^+ - N)_0}{\Delta t}$$

Nitrification rate:

$$R_{nit} = \frac{c(NO_3^- - N)_t - c(NO_3^- - N)_0}{\Delta t}$$

Net nitrogen mineralization rate:

$$R_{min} = \frac{[c(NH_4^+ - N)_t + c(NO_3^- - N)_t] - [c(NH_4^+ - N)_0 + c(NO_3^- - N)_0]}{\Delta t}$$

where  $c(NH_4^+ - N)_0$  and  $c(NH_4^+ - N)_t$  represent ammonium nitrogen content before and after incubation ( $\text{mg} \cdot \text{kg}^{-1}$ ),  $c(NO_3^- - N)_0$  and  $c(NO_3^- - N)_t$  represent nitrate nitrogen content before and after incubation ( $\text{mg} \cdot \text{kg}^{-1}$ ), and  $\Delta t$  represents the time interval.

### 1.4 Data Analysis

Statistical analysis was performed using Excel 2010 and SPSS 25.0 software. One-way ANOVA and multiple comparison methods (LSD) were used to analyze significant differences among variables across different degradation degrees ( $P < 0.05$ ). Two-way ANOVA was employed to analyze and compare the effects of wetland degradation degree, moisture, and their interactions.

## Results

### 2.1 Effects of Moisture Variation on Soil Ammonification Rate at Different Degradation Stages

During the incubation period, the ammonification rate of soils across all four degradation gradients showed an initial increase followed by a decrease over time. All treatments peaked at 3–7 days, then gradually declined, with the fastest decrease occurring at 3–14 days, after which they stabilized (Figure 1). In terms of degradation degree, significant differences in soil ammonification rates

were observed among degradation gradients at 3–14 days, following the pattern UD > LD > MD > HD ( $P < 0.05$ ), with no significant differences thereafter ( $P > 0.05$ ). The ammonification rate of each degradation gradient increased initially then decreased with increasing moisture, peaking at 7 days under 80% FC, while other treatments peaked at 3 days. The ammonification rate at 60% FC increased by  $2.25 \text{ mg} \cdot \text{kg}^{-1}$  compared to 20% FC, 40% FC, and 80% FC, with significant differences ( $P < 0.05$ ). After 3–14 days, the ammonification rate decreased rapidly and stabilized, with no significant differences ( $P > 0.05$ ).

## 2.2 Effects of Moisture Variation on Soil Nitrification Rate at Different Degradation Stages

During the incubation period, the nitrification rate of soils across all four degradation gradients exhibited an initial increase followed by a decrease over time. All treatments peaked at 3–7 days, then gradually declined and stabilized (Figure 2). Regarding degradation degree, significant differences in soil nitrification rates were observed among degradation gradients at 3–14 days ( $P < 0.05$ ), following the pattern HD > MD > LD > UD, with no significant differences at other times ( $P > 0.05$ ). Under each moisture condition, soil nitrification rates increased initially then decreased with moisture gradient. During 0–3 days, nitrification rates increased gradually, while during 0–49 days they decreased rapidly after 7 days and stabilized, with no significant differences ( $P > 0.05$ ). The mean nitrification rate was highest at 60% FC, showing no significant difference with 40% FC ( $P > 0.05$ ) but significant differences with 20% FC and 80% FC ( $P < 0.05$ ).

## 2.3 Effects of Moisture Variation on Soil Net Nitrogen Mineralization Rate at Different Degradation Stages

During the incubation period, the net nitrogen mineralization rate of soils across all four degradation gradients showed an initial increase followed by a decrease over time. All treatments peaked at 3–7 days, then gradually declined, with the fastest decrease at 3–14 days, after which they stabilized (Figure 3). Under each moisture condition, the net nitrogen mineralization rate followed the pattern UD > LD > MD > HD. The net nitrogen mineralization rate of each degradation gradient increased initially then decreased with increasing moisture, peaking at 7 days under 80% FC and at 3 days for other treatments. The differences reached significant levels ( $P < 0.05$ ). The net nitrogen mineralization rate increased gradually during 0–3 days, then decreased rapidly after 7 days and stabilized, with no significant differences ( $P > 0.05$ ).

## 2.4 Effects of Moisture Variation on Soil Net Nitrogen Mineralization Amount at Different Degradation Stages

The net nitrogen mineralization amount of each degradation gradient under the four moisture conditions showed an initial increase followed by a decrease over the incubation period (Figure 4). Within each time period, the net nitrogen

mineralization amount across the four degradation gradients generally increased initially then decreased with moisture variation, following the pattern 60% FC > 40% FC > 80% FC > 20% FC. During 0–3 days, the net nitrogen mineralization amount ranged from 6.22 to 31.20 mg · kg<sup>-1</sup>, with a maximum of 31.20 mg · kg<sup>-1</sup> under 60% FC and a minimum of 6.22 mg · kg<sup>-1</sup> under 20% FC. During 0–7 days, the range was 13.69–49.34 mg · kg<sup>-1</sup>, with a maximum of 49.34 mg · kg<sup>-1</sup> under 60% FC and a minimum of 13.69 mg · kg<sup>-1</sup> under 20% FC. During 0–28 days, the range was 15.35–52.21 mg · kg<sup>-1</sup>, with the maximum under 60% FC (52.21 mg · kg<sup>-1</sup>) and the minimum under 20% FC (15.35 mg · kg<sup>-1</sup>). During 0–49 days, the range was 12.58–44.63 mg · kg<sup>-1</sup>, with the maximum under 60% FC (44.63 mg · kg<sup>-1</sup>) and the minimum under 20% FC (12.58 mg · kg<sup>-1</sup>).

Two-way ANOVA results showed that moisture, degradation degree, and their interaction had significant effects on soil ammonification rate, nitrification rate, and net nitrogen mineralization amount ( $P < 0.01$ ). Degradation degree had no significant effect on net nitrogen mineralization rate ( $P > 0.05$ ), while moisture and the interaction between degradation degree and moisture had significant effects on net nitrogen mineralization rate ( $P < 0.01$ ).

## 2.5 Effects of Moisture Variation on Cumulative Nitrogen Mineralization in Wetland Soils at Different Degradation Degrees

As shown in Figure 5, cumulative nitrogen mineralization in soils at different degradation stages increased gradually with incubation time. Under the same incubation time, cumulative nitrogen mineralization for the same degradation degree increased initially then decreased with increasing moisture, with significant differences among the four moisture conditions ( $P < 0.05$ ), following the pattern 60% FC > 40% FC > 80% FC > 20% FC. From the perspective of degradation degree, under the same moisture condition, cumulative nitrogen mineralization decreased gradually with increasing degradation degree at each incubation time, following the pattern UD > LD > MD > HD.

## Discussion

### 3.1 Temporal Changes in Soil Nitrogen Mineralization Process

This study found that mineralization across all degradation degrees was characterized by rapid early-stage and slower late-stage processes, similar to findings by Tian et al. The primary reason may be that abundant labile organic matter in the early incubation period provided sufficient substrate for microbial survival. When moisture content was appropriate, enhanced microbial activity promoted the nitrogen mineralization process, resulting in rapid early-stage mineralization and high nitrogen mineralization rates. As incubation time extended, reduced soil substrate and nutrient supply limited microbial activity, causing nitrogen mineralization rates to gradually decline.

### 3.2 Effects of Moisture on Soil Nitrogen Mineralization Process

Moisture conditions can alter soil aeration status, affect the diffusion of soluble substrates, change microbial community structure and activity, and consequently influence ammonification, nitrification, and denitrification processes in soil nitrogen mineralization. Our results showed that within a certain moisture range, both net nitrogen mineralization amount and rate of soils across all degradation degrees increased with increasing water content, reaching maximum values at 60% FC, then decreasing with further moisture increase. This is consistent with findings by Gui et al. The possible reason is that within an appropriate moisture range, increased soil water content facilitates nutrient release and creates more favorable conditions for microorganisms involved in mineralization and nitrification, thereby promoting nitrogen mineralization. However, when moisture increases beyond a certain threshold, soil oxygen content decreases, reducing soil permeability and causing declines in microbial quantity and activity. At this point, microbial immobilization exceeds nitrification, and denitrifying bacteria activity strengthens, causing nitrate nitrogen to be lost through denitrification, which inhibits the nitrification process and reduces nitrogen mineralization amount and rate. Therefore, regulating appropriate soil moisture content is beneficial for improving soil nitrogen supply.

Our study primarily used indoor incubation methods, excluding external environmental influences to more accurately understand how soil nitrogen mineralization responds to moisture. We found that within the 20% FC–60% FC range, increased moisture promoted soil nitrogen mineralization rates, while moisture exceeding 60% FC inhibited the nitrogen mineralization process. The heavily degraded wetland soil had a water content of 21.56%, and moisture above 60% FC reduced soil nitrogen mineralization rates. Therefore, appropriate measures such as dam construction and water retention are needed to increase soil moisture in degraded wetlands and restore wetland ecosystem functions. These findings provide a theoretical basis for regulating soil nitrogen transformation processes through optimized moisture management in alpine wetlands. Meanwhile, wetland degradation inhibits soil nitrogen mineralization, making it urgent to implement grassland improvement, fencing, and grazing prohibition measures to restore degraded wetlands and improve nitrogen utilization efficiency while reducing nitrogen loss.

### 3.3 Effects of Different Degradation Levels on Soil Nitrogen Mineralization Process

Different degradation levels have varying effects on soil nitrogen mineralization. This study found that under all moisture conditions, the mean soil nitrification rate was highest in the heavily degraded gradient, consistent with findings by Wang et al. This may be because when wetland degradation exceeds a certain threshold, reduced aboveground biomass decreases plant uptake of soil nitrogen, favoring nitrifying microorganisms to obtain nitrogen from soil and thus enhancing the nitrification process. Additionally, plateau rodent damage in heavily



degraded plots altered soil layer distribution, enhanced soil aeration, and facilitated the conversion of ammonium nitrogen to nitrate nitrogen, promoting nitrification and increasing nitrification rates in heavily degraded soils.

Our study also found that under all moisture conditions, soil net nitrogen mineralization amount decreased with increasing wetland degradation degree, which differs somewhat from results by Ma et al. This discrepancy may be because non-degraded wetlands have relatively abundant vegetation, and the presence of plant stems and leaves provides rich organic matter for microbial activity. Under microbial decomposition, this can regulate the priming effect of soil organic matter, improve nutrient utilization efficiency, and maintain long-term high soil fertility under appropriate moisture conditions, thereby accelerating the nitrogen mineralization process. However, as wetland degradation increases, severe vegetation destruction reduces litter quantity, decreasing available nitrogen sources for microorganisms. Microorganisms then need to absorb more inorganic nitrogen from soil to meet their growth requirements, promoting nitrogen assimilation and reducing ammonifying bacterial activity, which decreases ammonium nitrogen content and ammonification rate, consequently reducing nitrogen mineralization amount and rate. In heavily degraded wetlands, animal digging activities alter soil layer distribution, increase soil water evaporation, reduce soil moisture content, and enhance soil aeration, causing ammonium nitrogen to be converted to nitrate nitrogen under nitrifying microorganisms, which promotes nitrification and increases nitrification rates in heavily degraded wetland soils.

## Conclusion

- (1) Under all moisture conditions, the ammonification rate, nitrification rate, and net nitrogen mineralization rate of soils across the four degradation degrees showed a trend of initially increasing then decreasing with incubation time. All treatments peaked at 3–7 days, decreased rapidly thereafter, and stabilized after 14 days, with no significant differences among degradation degrees ( $P > 0.05$ ).
- (2) The average net nitrogen mineralization amount and rate of soils across the four degradation degrees increased initially then decreased with increasing moisture. Therefore, within an appropriate moisture range, increasing moisture promotes the nitrogen mineralization process in soils at different degradation degrees, but moisture exceeding 60% FC inhibits nitrogen mineralization.
- (3) Under the same moisture condition, the average ammonification rate decreased with increasing wetland degradation degree, while the average nitrification rate increased with degradation degree. The average net nitrogen mineralization amount and rate showed the same trend as the ammonification rate.

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## Figures

*Source: ChinaXiv –Machine translation. Verify with original.*

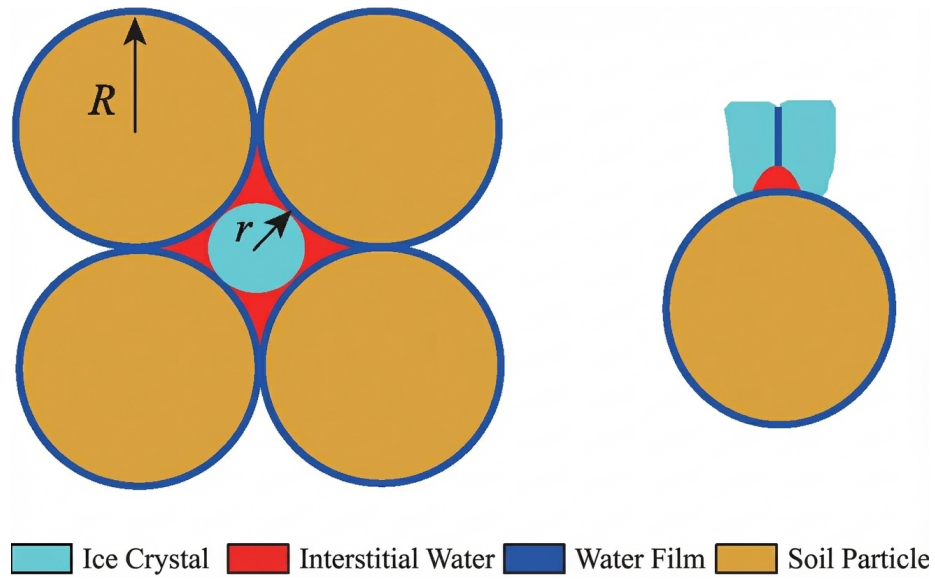


Figure 1: Figure 1

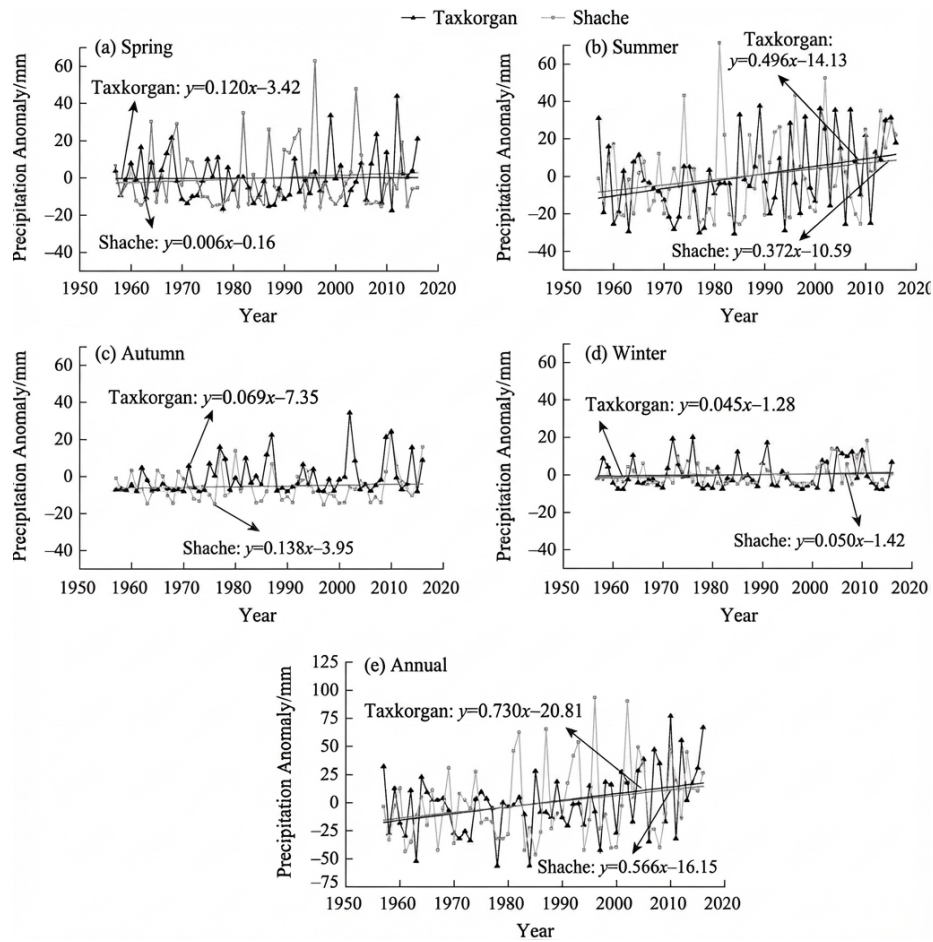


Figure 2: Figure 3

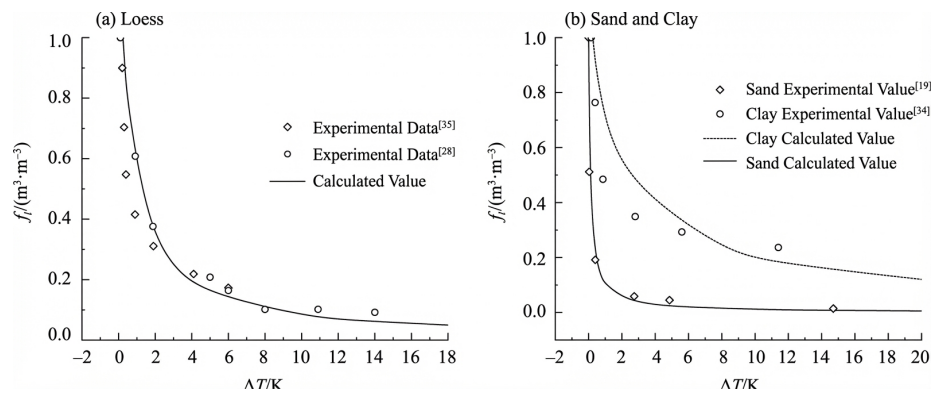


Figure 3: Figure 7