Postprint: Mineralization Characteristics of Soil Organic Carbon Under Long-term Fertilization Practices

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Abstract

Investigating the mineralization characteristics and temperature sensitivity of soil organic carbon under long-term different fertilization practices in dryland farmland can provide a theoretical basis for deepening the understanding of soil carbon cycling processes. This study takes the grain-legume rotation system in the semi-arid Loess Region as the research object, and analyzes the kinetic characteristics and temperature sensitivity of soil organic carbon mineralization under long-term different fertilization practices through indoor incubation experiments at two different temperatures (15 °C and 25 °C). The results show that the soil organic carbon mineralization rate was relatively high in the early stage of incubation, and then decreased slowly. Both fertilization practices and incubation temperature had significant effects on soil organic carbon mineralization. Compared with the no-fertilization control (CK), under the 15 °C incubation condition, the cumulative mineralization amount of soil organic carbon (Cmin) under long-term single phosphorus application (P), nitrogen-phosphorus combined application (NP), and nitrogen-phosphorus-organic fertilizer combined application (NPM) treatments increased by 41%, 85%, and 89%, respectively; under the 25 °C incubation condition, they increased by 7%, 46%, and 77%, respectively. Additionally, compared with the CK treatment, the temperature sensitivity of soil organic carbon mineralization (Q10) under P, NP, and NPM treatments decreased by 25%, 21%, and 6%, respectively. Fertilization altered the kinetic parameters of soil organic carbon mineralization, and the degree of change was related to fertilizer type and incubation temperature. Compared with the CK treatment, under the 15 °C incubation condition, the potentially mineralizable organic carbon amount (Cp) under P, NP, and NPM treatments increased by 29%, 65%, and 48%, respectively; under the 25 °C incubation condition, Cp under NP and NPM treatments increased by 2% and 21%, respectively, while that under P treatment decreased by 36%. The soil organic carbon

mineralization rate constant (k) under different fertilization treatments showed small changes under the 15 °C incubation condition, but increased substantially under the 25 °C incubation condition. Under the 25 °C incubation condition, Cmin and Cp increased significantly with increasing soil organic carbon and total nitrogen contents. It can be seen that long-term fertilization significantly promoted the mineralization of soil organic carbon in the grain-legume rotation system in the semi-arid Loess Region, and weakened the temperature sensitivity of soil organic carbon mineralization.

Full Text

Preamble

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Mineralization Characteristics of Soil Organic Carbon Under Long-Term Fertilization Management*

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Abstract
Investigating the mineralization characteristics and temperature sensitivity of soil organic carbon (SOC) under long-term different fertilization practices in dryland farmland can provide a theoretical basis for deepening our understanding of soil carbon cycling processes. This study examined a grain-legume rotation system in the semiarid Loess Plateau region, analyzing the kinetic characteristics of SOC mineralization and its temperature sensitivity under long-term different fertilization treatments through laboratory incubation experiments at two temperatures (15 °C and 25 °C). The results showed that SOC mineralization rates were initially high and then declined slowly over time. Both fertilization practices and incubation temperature had significant effects on SOC mineralization.

Compared with the no-fertilization control (CK), the cumulative mineralized SOC (Cmin) under long-term phosphorus application alone (P), combined nitrogen and phosphorus application (NP), and combined nitrogen, phosphorus, and manure application (NPM) increased by 41%, 85%, and 89%, respectively, at 15 °C, and by 7%, 46%, and 77%, respectively, at 25 °C. Additionally, compared with CK, the temperature sensitivity of SOC mineralization (Q10) decreased by 25%, 21%, and 6% under P, NP, and NPM treatments, respectively. Fertilization altered the kinetic parameters of SOC mineralization, with the degree of change depending on fertilizer type and incubation temperature. Compared with CK, the potential mineralizable SOC (Cp) under P, NP, and NPM treatments increased by 29%, 65%, and 48%, respectively, at 15 °C. At 25 °C, Cp increased by 2% and 21% under NP and NPM treatments, respectively, while decreasing by 36% under P treatment. The SOC mineralization rate constant (k) showed little variation among fertilization treatments at 15 °C but increased substantially at 25 °C. At 25 °C, both Cmin and Cp increased significantly with increasing SOC and total nitrogen content. These findings demonstrate that long-term fertilization significantly promoted SOC mineralization in the grain-legume rotation system of the semiarid Loess Plateau while reducing the temperature sensitivity of SOC mineralization.

Keywords: Semiarid agro-ecosystem; Grain-legume rotation; Long-term fertilization; Soil organic carbon mineralization; Temperature sensitivity

Introduction

Soil organic carbon is a crucial component of soil and the core of soil quality, playing a significant role in soil nutrient supply, biological activity, ecosystem material cycling, and structural and functional changes [?, ?]. Mineralization of soil organic carbon represents a vital link in terrestrial ecosystem carbon cycling and substantially influences soil productivity sustainability and CO_2 exchange in the soil-atmosphere system [?, ?]. On one hand, SOC mineralization gradually reduces the content of labile organic carbon, leading to corresponding changes in soil biological, physical properties, and fertility status [?, ?]. On the other hand, the mineralization process increases CO_2 release from soil to the atmosphere, enhancing the greenhouse effect. Rising temperatures, in turn, promote SOC mineralization, creating a positive feedback loop between organic carbon mineralization and temperature increase [?, ?]. Therefore, a deeper understanding of SOC mineralization characteristics and influencing factors across different ecosystems is of great practical significance for soil management and climate change mitigation [?].

SOC mineralization characteristics vary considerably among ecosystems. Wu et al. [?] found that in four typical ecosystems in the central Qilian Mountains, both SOC mineralization rates and the proportion of mineralized SOC were significantly higher in alpine meadows and mountain forests than in desert steppes. Additionally, soil management practices affect organic carbon mineralization. Dai et al. [?] reported that grazing enclosure reduced SOC mineralization rates and temperature sensitivity, while Zhang et al. [?] demonstrated that fertilization significantly increased soil microbial biomass carbon and dissolved organic carbon content, thereby enhancing SOC mineralization rates and amounts. Furthermore, SOC mineralization is influenced by environmental factors and soil properties. Among environmental factors, temperature is the primary factor affecting SOC mineralization [?, ?], as it controls the decomposition of soil organic carbon by influencing root and microbial metabolic activities and various enzyme activities [?, ?]. Within a certain range, both cumulative mineralized SOC and mineralization rates increase with rising temperature. Temperature effects on SOC mineralization are typically expressed as temperature sensitivity (Q10), which represents the factor by which soil respiration rate increases for every 10 °C temperature rise [?, ?].

Numerous studies have investigated the temperature sensitivity of SOC mineralization. For instance, Wu et al. [?] conducted a 61-day laboratory incubation experiment and found that Q10 values for SOC mineralization in spruce forests and meadows on the northern slope of the Qilian Mountains ranged from 1 to 2. Bond-Lamberty et al. [?] reported that global-scale soil respiration Q10 values were approximately 1.5. However, these studies have primarily focused on natural ecosystems, with less attention given to frequently managed dryland farmland ecosystems. As a crucial management practice in farmland ecosystems, fertilization can significantly affect SOC content [?]. For example, Jiao et al. [?] studied the nutrient characteristics of black soil and dark brown soil after 28 years of continuous fertilizer application, finding that all fertilization treatments significantly increased soil organic matter and nutrient contents compared with no-fertilization controls. Moreover, fertilization practices can alter the composition and state of organic carbon [?], thereby affecting mineralization. Therefore, research on SOC mineralization characteristics and temperature sensitivity under long-term different fertilization practices is urgently needed.

The grain-legume rotation system is widely adopted in the semiarid Loess Plateau region. Compared with continuous cropping, grain-legume rotation can enhance soil carbon sequestration function and biodiversity in farmland ecosystems [?], playing a vital role in regional food production and livestock development. This study examined a grain-legume rotation system, collecting soil samples for laboratory mineralization incubation experiments at two temperatures (15 °C and 25 °C) to investigate SOC mineralization characteristics, temperature sensitivity, and relationships between SOC mineralization and soil total nitrogen under long-term different fertilization practices. The objective was to provide insights for SOC management and prediction of SOC changes under future climate change scenarios in this region.

1. Materials and Methods

1.1 Study Area

The experiment was conducted at the Changwu Agro-Ecological Experimental Station on the Loess Plateau, Chinese Academy of Sciences. Established in 1984, the station is located in Changwu County, Shaanxi Province, at an altitude of 1,200 m. The study area is typical of the gully region of the Loess Plateau, with soil type classified as yellow-capped black loess (Huang Gai Hei Lutu) developed from Malan loess parent material. The climate is warm temperate semi-humid continental monsoon, with annual sunshine duration of 2,226.5 h (51% sunshine percentage), total annual radiation of 483.7 kJ $\cdot$ cm^{-3}, mean annual temperature of 9.2 °C, and frost-free period of 171 days. Annual precipitation is 578 mm, concentrated mainly in July-September (53% of annual rainfall). The accumulated temperature above 0 °C is 3,029 °C. Initial soil properties at the experiment establishment were: organic carbon 6.09 g $\cdot$ kg^{-1}, total nitrogen 0.8 g $\cdot$ kg^{-1}, and total phosphorus 0.7 g $\cdot$ kg^{-1}.

1.2 Experimental Design

The long-term fertilization experiment was established in 1984, including different cropping systems (continuous maize, continuous wheat, continuous alfalfa, and grain-legume rotation) and fertilization treatments. This study focused on the 3-year grain-legume rotation system. Four fertilization treatments were selected: (1) no fertilization (CK), (2) phosphorus application alone (P), (3) combined nitrogen and phosphorus application (NP), and (4) combined nitrogen, phosphorus, and manure application (NPM). Each treatment had three replicates, totaling 12 plots. Each plot measured 10.3 m × 6.5 m. The crop rotation sequence was pea-wheat-wheat+millet. Nitrogen fertilizer was urea, phosphorus fertilizer was superphosphate, and organic manure was cattle manure. The P treatment received 26.2 kg(P_2O_5) $\cdot$ hm^{-2} $\cdot$ a^{-1}. The NP treatment received 120 kg(N) $\cdot$ hm^{-2} $\cdot$ a^{-1} + 26.2 kg(P_2O_5) $\cdot$ hm^{-2} $\cdot$ a^{-1}. The NPM treatment received 120 kg(N) $\cdot$ hm^{-2} $\cdot$ a^{-1} + 26.2 kg(P_2O_5) $\cdot$ hm^{-2} $\cdot$ a^{-1} + 75 t $\cdot$ hm^{-2} $\cdot$ a^{-1} (cattle manure). The cattle manure contained 17.68 g $\cdot$ kg^{-1} organic carbon, 1.97 g $\cdot$ kg^{-1} total nitrogen, and 0.97 g $\cdot$ kg^{-1} total phosphorus. Fertilizers were broadcast on the soil surface before sowing and then incorporated by tillage. Basic physicochemical properties of soils under different fertilization treatments are shown in Table 1 .

1.3 Sample Collection and Processing

In September 2011, soil samples were collected from the 0–20 cm layer in each plot using an "S" pattern to randomly select five points, which were combined into a composite sample. Samples were air-dried in the laboratory, and debris such as litter and plant roots were removed. One portion of each sample was ground to pass through a 2.0 mm sieve for mineralization incubation, while another portion was ground to pass through a 0.25 mm sieve for determination of soil organic carbon and total nitrogen. Soil organic carbon was determined by the potassium dichromate external heating method, and total nitrogen by the Kjeldahl method [**?**].

1.4 Soil Organic Carbon Mineralization Incubation Experiment

This experiment employed indoor constant-temperature incubation with alkali absorption to measure SOC mineralization. Specifically, 10.0 g of soil sample was placed in a 250 mL glass culture bottle and evenly distributed at the bottom. Distilled water was added to adjust moisture content to 60% of field water-holding capacity. A vial containing 5 mL of 0.5 mol • L^{-1} NaOH solution was carefully placed inside the culture bottle, which was then sealed. Bottles were incubated at constant temperatures of 15 °C and 25 °C in biochemical incubators (three replicates per treatment). Absorption vials were removed on days 2, 4, 8, 15, 22, 29, 36, 43, 50, and 57 after incubation, and the solution was completely transferred to 250 mL Erlenmeyer flasks. Then, 2 mL of 1 mol • L^{-1} $BaCl_2$ solution and 2 drops of phenolphthalein indicator were added, and the solution was titrated with 0.5 mol • L^{-1} HCl until the red color disappeared. SOC mineralization amounts for different treatments during the incubation period were calculated based on CO_2 release. Soil water content was periodically corrected using the weighing method during incubation. Six culture bottles without soil samples were set up at each temperature as blanks.

1.5 Data Analysis

The cumulative process of soil CO_2-C follows a first-order reaction kinetic equation. Therefore, a first-order kinetic equation was used to fit cumulative mineralized SOC:

$$C_{min} = C_p(1 - e^{-kt})$$

where C_{min} is cumulative mineralized SOC (g • kg^{-1}) after time t (days), C_p is potential mineralizable SOC (g • kg^{-1}), k is the SOC mineralization rate constant (d^{-1}), and t is incubation time (days). Variance analysis and graphing were performed using JMP software (v10.0) and SigmaPlot 12.5.

2. Results

2.1 Mineralization Characteristics of Soil Organic Carbon Under Different Fertilization Treatments

The dynamic changes in SOC mineralization rate (v) over incubation time under different fertilization treatments at two temperatures are shown in Figure 1 [Figure 1: see original paper]. During the initial incubation stage, SOC mineralization rates increased rapidly, peaking around day 2, then declined slowly with prolonged incubation. By the end of the 57-day incubation, the SOC mineralization rate under NPM treatment at 15 °C was 0.028 g • kg^{-1} • d^{-1}, which was 1.02, 1.34, and 1.89 times that of NP, P, and CK treatments, respectively. At 25 °C, the rate was 0.041 g • kg^{-1} • d^{-1}, which was 1.21, 1.66, and 1.77 times that of NP, P, and CK treatments, respectively. SOC mineralization rates at both temperatures followed the trend NPM > NP > P > CK, indicating that fertilization enhanced SOC mineralization rates.

As shown in Figure 1 [Figure 1: see original paper], cumulative mineralized SOC (C_{min}) increased rapidly during the first 22 days and more slowly thereafter until the end of incubation at both temperatures (15 °C and 25 °C). Additionally,

C_{min} followed the trend NPM > NP > P > CK at both temperatures. ANOVA results indicated that at 15 °C, both SOC mineralization rate (v) and C_{min} under NPM and NP treatments were significantly higher than CK ($P < 0.05$), but not significantly different from each other ($P > 0.05$). At 25 °C, both v and C_{min} under NPM treatment were significantly higher than P and CK treatments ($P < 0.05$), with no significant difference between P and CK ($P > 0.05$). Meanwhile, v and C_{min} under NP treatment were also significantly higher than CK ($P < 0.05$).

2.2 Cumulative Mineralization of Soil Organic Carbon Under Different Fertilization Treatments

During the 57-day incubation period, cumulative mineralized SOC (C_{min}) ranged from 1.0 to 2.8 $g \cdot kg^{-1}$, and the proportion of mineralized carbon to total organic carbon (C_{min}/C_0) ranged from 0.11 to 0.23. Both C_{min} and C_{min}/C_0 at 25 °C were significantly higher than at 15 °C ($P < 0.05$). Compared with 15 °C, C_{min} at 25 °C increased by 57%, 19%, 24%, and 47% for CK, P, NP, and NPM treatments, respectively, while C_{min}/C_0 increased by 73%, 20%, 22%, and 47%, respectively. Compared with CK, long-term P, NP, and NPM applications increased C_{min} by 41%, 85%, and 89% at 15 °C ($P < 0.05$), and by 7%, 46%, and 77% at 25 °C ($P < 0.05$), respectively. ANOVA showed no significant differences in C_{min}/C_0 among fertilization treatments at either temperature ($P > 0.05$).

In this study, Q10 values for SOC mineralization ranged from 1.19 to 1.57. Q10 values under P, NP, and NPM treatments decreased by 25%, 21%, and 6%, respectively, compared with CK. These results demonstrate that long-term fertilization reduced the temperature sensitivity of SOC mineralization in the grain-legume rotation system of the semiarid Loess Plateau.

2.3 Kinetic Characteristics of Soil Organic Carbon Mineralization Under Different Fertilization Treatments

Fertilization altered the kinetic parameters of SOC mineralization, with the degree of change depending on fertilizer type and incubation temperature (Table 2). At 15 °C, potential mineralizable SOC (C_p) ranged from 1.50 to 2.48 $g \cdot kg^{-1}$, accounting for 17% to 25% of total SOC (C_0). Compared with CK, C_p under P, NP, and NPM treatments increased by 29%, 65%, and 48%, respectively, though differences were not significant ($P > 0.05$). At 25 °C, C_p ranged from 1.73 to 3.26 $g \cdot kg^{-1}$, accounting for 19% to 34% of C_0. Compared with CK, C_p increased by 2% and 21% under NP and NPM treatments ($P > 0.05$), respectively, while decreasing by 36% under P treatment ($P > 0.05$). Additionally, C_p under CK, NP, and NPM treatments at 25 °C increased by 80%, 12%, and 46%, respectively, compared with 15 °C, while P treatment decreased by 11%. The C_p/C_0 ratio showed similar trends to C_p. Fertilization treatments had no significant effect on C_p/C_0 ($P > 0.05$), while incubation temperature significantly affected both C_p and C_p/C_0 ($P < 0.05$). The SOC mineralization rate constant k showed little variation among fertilization treatments at 15 °C but increased substantially at 25 °C, though differences were not significant ($P > 0.05$). Incubation temperature had no significant effect on k ($P > 0.05$).

2.4 Dependence of Organic Carbon Mineralization on Soil Carbon and Nitrogen Contents

Analysis of relationships between SOC mineralization characteristics, kinetic parameters, and soil carbon and nitrogen contents revealed that C_{min}/C_0, C_p/C_0, and k were not affected by SOC content, total nitrogen content, or C/N ratio at either temperature ($P > 0.05$). C_{min} and C_p were also not affected by C/N ratio ($P > 0.05$). However, relationships between C_{min} and C_p with SOC and total nitrogen contents were temperature-dependent (Table 3). Both C_{min} and C_p increased with increasing SOC and total nitrogen contents. These increases were not significant at 15 °C ($P > 0.05$) but reached highly significant ($P < 0.01$) and significant levels ($P < 0.05$) at 25 °C, respectively. This indicates that SOC mineralization was not limited by SOC and total nitrogen contents at low temperature but became limited at higher temperature.

3. Discussion and Conclusion

The grain-legume rotation system is widely adopted in the semiarid Loess Plateau region. Compared with continuous cropping, grain-legume rotation can enhance soil carbon sequestration function and biodiversity in farmland ecosystems [?], playing a significant role in regional food production and livestock development. This study found that SOC mineralization amounts under all fertilization treatments increased with temperature, consistent with other researchers' reports. Zak et al. [?] found that SOC mineralization in a U.S. hardwood forest soil at 25 °C was significantly higher than at 15 °C and 5 °C. Huang et al. [?] reported that when incubation temperature increased from 12 °C to 24 °C, SOC decomposition during a 30-day period increased by 1.83 to 2.09 times. Liu et al. [?] found that when temperature increased from 15 °C to 25 °C, cumulative CO_2-C released from SOC mineralization in paddy and forest soils increased by 157.8% and 135.8%, respectively. Researchers have provided consistent explanations for temperature' s promoting effect on SOC mineralization. Kirschbaum [?] suggested that increased SOC mineralization with temperature within a certain range may be related to enhanced soil microbial and enzyme activities. Rustad et al. [?] found that temperature increases within a certain range favored microbial community growth and reproduction, increasing microbial activity. Feng et al. [?] showed that temperature increases significantly enhanced enzyme activities in both organic and mineral soil layers, with more pronounced increases in invertase, nitrate reductase, and urease activities in the organic layer. Enhanced microbial and enzyme activities intensify organic matter decomposition, increasing SOC mineralization rates and amounts.

In this study, cumulative mineralized SOC under all treatments at both incubation temperatures showed the trend NPM > NP > P > CK. This may be because long-term fertilization promotes plant growth, increasing root biomass and the types and quantities of root exudates, thereby increasing the amount of labile organic carbon available for mineralization [?]. Additionally, long-term fertilization can increase soil nitrogen content, which promotes mineralization of existing SOC [?]. Although nitrogen is the main nutrient limiting factor in semiarid Loess Plateau agro-ecosystems [?], phosphorus application alone rarely promotes plant growth and may even reduce crop yield [?]. However, the cropping system in this study includes legume crops, and phosphorus application can promote nitrogen fixation in legumes [?], thereby increasing soil nitrogen content. In this grain-legume rotation system, phosphorus application alone increased soil nitrogen by 12%, thus promoting SOC mineralization. Furthermore, organic manure contains large amounts of organic carbon and diverse biologically active substances (microorganisms, enzymes, etc.) [?], which not only provide more mineralizable carbon but also accelerate mineralization of both newly input and existing SOC. Considering the significant relationship between aboveground and belowground biomass [?], the NPM treatment likely had a greater effect on increasing root biomass and exudates than the NP treatment. This is consistent with Tang et al. [?] and explains why SOC mineralization under NPM treatment was significantly higher than other treatments in this study. However, it should be noted that organic carbon from applied manure also participated in the mineralization process, so future studies should distinguish between mineralization of original SOC and newly input organic carbon.

SOC mineralization is influenced by soil management practices, environmental factors, and soil properties. The temperature sensitivity of SOC mineralization has become a research hotspot in recent years. Q10 represents the factor by which soil respiration rate increases for every 10 °C temperature rise [?, ?]. Hamdi et al. [?] reported that Q10 values for SOC mineralization in a wheat-sorghum-forage rotation system ranged from 1.7 to 1.8. Wu et al. [?] found through a 61-day incubation experiment that Q10 values for SOC mineralization in spruce forests and meadows on the northern slope of the Qilian Mountains ranged from 1 to 2. In this study, Q10 values ranged from 1.19 to 1.57, similar to results from other studies. The Q10 values for different fertilization treatments followed the order CK > NPM > NP > P. Thus, for the grain-legume rotation system in the semiarid Loess Plateau, long-term fertilization reduced the temperature sensitivity of SOC mineralization, thereby mitigating the positive feedback effect between soil CO_2 release and temperature increase [?].

The effects of NP and NPM treatments on SOC mineralization kinetic characteristics in this study are consistent with other research [?, ?], while the P treatment effect was opposite to other studies, possibly due to substrate supply for SOC mineralization. NP and NPM treatments significantly increased labile organic carbon content in soil [?], enhancing substrate supply for mineralization. The effect of P treatment on microbial carbon source utilization and functional diversity is the result of multiple factors, including soil sampling methods, phosphorus application duration, and experimental conditions.

Previous studies have shown that short-term phosphorus application increased microbial carbon source utilization and functional diversity, while long-term phosphorus application largely reduced them [?]. This study was conducted under long-term fertilization conditions, so the P treatment had a smaller effect on increasing labile organic carbon. At higher temperatures, this newly added labile organic carbon had stronger binding with fine soil particles [?], reducing its availability and making substrate limitation more apparent. Additionally, changes in the SOC mineralization rate constant also demonstrated substrate supply effects. In this experiment, the mineralization rate constant (k) under P treatment increased with temperature, while other treatments decreased with temperature. Furthermore, k values showed little variation among fertilization treatments at 15 °C but increased substantially at 25 °C. These results indicate that SOC mineralization was not substrate-limited at low temperature but became substrate-limited at high temperature. In this study, cumulative mineralized SOC under NPM, NP, and P treatments was higher than CK at all incubation temperatures and time periods, indicating that chemical or organic fertilizer application promoted SOC mineralization. However, long-term fertilization significantly increased SOC pool capacity without significantly increasing the proportion of mineralized carbon to total organic carbon (C_{min}/C_0). Therefore, fertilizer application is more conducive to SOC sequestration.

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