Effects of Soil Enzyme Activity on Organic Carbon Mineralization in Dam Land Soil under Simulated Wetting-Drying Conditions: Postprint

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Date: 2024-12-16T00:00:00+00:00

Abstract

Climate change leads to increased frequency of prolonged droughts and heavy rainfall events, thereby affecting ecosystem carbon cycling. Therefore, understanding how soil enzyme activities under different dry-wet conditions affect organic carbon mineralization can help deepen our understanding of carbon cycling mechanisms and advance the achievement of global carbon neutrality goals. Accordingly, this study selected check dams (a unique erosion control structure on the Loess Plateau) as the research object, and designed three treatments—flooding stress, drought stress, and dry-wet cycles—to monitor soil enzyme activities and organic carbon mineralization processes. The results showed that dry-wet cycles had an instantaneous priming effect on organic carbon mineralization, and the cumulative mineralization of organic carbon was intermediate between drought stress and flooding stress. Simultaneously, dry-wet cycles increased both carbon- and nitrogen-cycling related enzyme activities, but enzyme activities gradually decreased and stabilized with increasing cycle number. Enzyme activities under all three moisture treatments were limited by carbon and phosphorus, with carbon limitation becoming more intense with increasing dry-wet cycle number; after the fourth dry-wet alternation, phosphorus limitation under dry-wet alternation exceeded that under flooding stress and drought stress. The enzyme activity factors limiting organic carbon mineralization also differed when soils were under drought, flooding stress, and dry-wet cycles. Under drought stress, the direct effect of phosphatase factor was 99%; under flooding stress, the direct effect of EAAC/N (carbon-cycling related enzyme/nitrogen-cycling related enzyme) and xylosidase factor was 87%; under drought stress and flooding stress, the interaction effects between factors were only 1% and 13%, respectively. Under dry-wet cycle conditions, the direct effect of phosphatase and β-N-acetylglucosaminidase factors was 75%, with interaction effects between factors accounting for 25%; dry-wet cycles could significantly increase interconnections among factors. This study can provide theoretical support for clarifying the role of check dam construction on the Loess Plateau in achieving national carbon neutrality goals.

Full Text

Abstract

Climate change has increased the frequency of long-term droughts and heavy rainfall events, impacting ecosystem carbon cycling. Understanding how soil enzyme activity under different moisture conditions affects organic carbon mineralization can deepen our comprehension of carbon cycle mechanisms and advance global carbon neutrality goals. This study selected the unique check dams built for erosion control on the Loess Plateau as the research subject and designed three treatments—flooding stress, drought stress, and wet-dry cycles—to monitor soil enzyme activity and organic carbon mineralization processes. The results indicate that wet-dry cycles exert a transient priming effect on organic carbon mineralization, with cumulative organic carbon mineralization occurring between that of drought and flooding stress. Wet-dry cycles also increased carbon and nitrogen cycle-related enzyme activities, though enzyme activity gradually decreased and stabilized with increasing cycle frequency. Enzyme activity under all three moisture treatments was limited by carbon and phosphorus, with carbon limitation becoming more pronounced as wet-dry cycles increased. After the fourth wet-dry cycle, phosphorus limitation under the wet-dry treatment exceeded that under flooding and drought stress. The enzyme activity factors limiting organic carbon mineralization varied under drought stress, flooding stress, and wet-dry cycles. Under drought stress, the direct effect of phosphatase was 99%; under flooding stress, the direct effect of EAAC/N (carbon cycle-related enzyme/nitrogen cycle-related enzyme) and xyloglucosidase was 87%; and the interactive effects between factors under drought and flooding stress were only 1% and 13%, respectively. Under wet-dry cycle conditions, the direct effect of phosphatase and N-acetylglucosaminidase was 75%, while interactive effects accounted for 25%, demonstrating that wet-dry cycles significantly increase interactions among factors. This study provides theoretical support for clarifying the role of check dam construction on the Loess Plateau in achieving national carbon neutrality goals.

Keywords: wet-dry alternation; soil enzyme activity; soil organic carbon mineralization; Loess Plateau

Introduction

Climate warming is altering global precipitation patterns, significantly increasing the frequency of extreme rainfall and drought events. Consequently, soil wet-dry alternation phenomena are becoming more common, particularly in arid and semi-arid regions. As soil surfaces experience prolonged drought followed by relatively rapid rewetting, carbon cycling processes are profoundly affected, accelerating organic carbon release and exacerbating global warming. Soil enzyme activity serves as a critical component of organic matter decomposition and biogeochemical cycling, and is widely used to assess microbial nutrient demand in biochemical processes.

Sinsabaugh et al. found that microbial resource allocation can be expressed through the ratio of carbon, nitrogen, and phosphorus hydrolase activities, with stoichiometric ratios indicating microbial metabolic limitations. Organic carbon mineralization is a microbial process that decomposes active organic components and releases CO_2. Soil microbial activity is closely linked to moisture changes, decreasing as soil water content declines. During prolonged drought, soil microorganisms accumulate solutes such as proteins and polysaccharides to prevent cellular dehydration. Upon rewetting, active microorganisms utilize these accumulated solutes to accelerate reproduction, thereby increasing microbial respiration and soil CO_2 release.

The six hydrolases studied—β-glucosidase, cellulase, and xylosidase (which dissolve cellulose and sugars into available organic carbon), leucine aminopeptidase and N-acetylglucosaminidase (which degrade proteins and chitin to acquire nitrogen), and phosphatase (which hydrolyzes organic phosphorus to release inorganic phosphorus)—provide metabolic power for soil ecosystems and directly participate in soil organic carbon decomposition and accumulation. Zhao et al. studied the relationship between soil enzyme activity and cumulative organic carbon mineralization, finding that β-glucosidase activity positively correlated with mineralization amount. Yu et al. discovered that soil enzyme activity increases with soil moisture content but follows a hump-shaped pattern under wet-dry alternation conditions. Further investigation into how soil enzyme activity changes under different moisture conditions is crucial for clearly understanding the mechanisms of organic carbon mineralization.

Check dams are important gully erosion control structures on the Loess Plateau. According to statistics, approximately 57,000 check dams have been constructed in the region as of 2020, forming extensive dam land suitable for agriculture and intercepting 2.1$×10^{9}$ tons of sediment. These areas are considered significant carbon sinks. Through erosion processes, sediment is continuously transported to gullies and undergoes deposition, compaction, and deep burial within check dams, altering the hydrothermal conditions and affecting organic carbon mineralization and storage. Rainfall in the Loess Plateau is concentrated from July to September, accounting for approximately 70% of annual precipitation, often in the form of heavy storms. This causes dam land surface soils to remain relatively dry for extended periods while experiencing rapid wetting and slow drying during flood seasons. Therefore, investigating the effects of soil enzyme activity on organic carbon mineralization in Loess Plateau dam land under varying moisture conditions is essential for understanding carbon cycling processes in soil ecosystems.

Currently, comparative studies on organic carbon mineralization characteristics in dam land soils under drought stress, flooding stress, and wet-dry cycles are relatively scarce, and the regulatory mechanisms of soil enzyme activity on organic carbon mineralization require further clarification. This study selected the unique check dams of the Loess Plateau as the research subject to investigate soil enzyme activity and organic carbon mineralization characteristics under different moisture conditions through laboratory simulation experiments, quantify the contribution of soil enzyme activity to organic carbon mineralization, and provide theoretical support for clarifying the role of check dam construction in achieving national carbon neutrality goals.

Materials and Methods

1.1 Study Area Overview

The study area is located in the Zhenggou small watershed in Zizhou County, Shaanxi Province, on the left bank of the lower Dali River (a secondary tributary of the Yellow River). The watershed covers 1.9 km^2 with coordinates 37°43 00 N, 109°58 29 E. It represents a typical loess hilly-gully region with loess soil, elevation ranging from 950–1200 m, mean annual precipitation of approximately 520 mm (concentrated in heavy storms), and mean annual temperature of 10.2 °C. The sampling site was located on dam land at a key check dam (Zhenggou Key Dam) within the watershed' s catchment area, with no other check dams present in the upstream or downstream gullies.

1.2 Soil Collection

Soil collection was conducted in October 2021. Due to the sediment retention and discharge functions of check dams, dam land becomes submerged during floods. After flood events, water is gradually discharged through drainage facilities, causing the soil to dry and creating wet-dry cycles on the dam land surface. Based on this reality, the dam was divided into three zones: front, middle, and back [Figure 2: see original paper]. In each zone, soil samples were collected from the 0–20 cm depth using a five-point method. Samples from the three zones were combined into a single composite sample and refrigerated at 4 °C for soil enzyme activity measurement. Basic physicochemical properties are shown in .

1.3 Wet-Dry Cycle Mineralization Experiment

Based on field monitoring and previous research, soil moisture content in dam land ranges from 10.8%–31.2% during dry seasons and often reaches waterlogged conditions during rainy seasons. The experiment established three moisture treatments: wet-dry cycles (100%WHC–30%WHC), flooding stress (100%WHC), and drought stress (30%WHC). The wet-dry cycle treatment consisted of four consecutive cycles, each including a slow drying process and rapid wetting process. The wetting process used rapid spraying to reach 100% water holding capacity (WHC) within minutes, while drying reduced moisture to 30% WHC. Preliminary experiments monitoring soil water content showed that reducing from 100% to 30% WHC required approximately 7 days. Therefore, each wet-dry cycle was set to 14 days, totaling 56 days of incubation. Flooding and drought stress treatments lasted the same duration but without alternation, with daily moisture monitoring and supplementation to maintain 100% WHC for flooding stress and 30% WHC for drought stress.

For the experiment, 500 g of sieved soil (<2 mm) was placed in incubation vessels. Each treatment had three replicates and was incubated at 25$±1°Cinaclimatechamber.A20mLsolutionof0.1mol · L^{-1}$ NaOH was added to plastic bottles to absorb released CO_2 [Figure 3: see original paper]. After incubation, the collected solution was filtered (0.45 m filter head) and titrated with 0.5 $\mathrm{mol} \cdot \mathrm{L}^{-1}$ $BaCl_2$ (using methyl orange as indicator) to calculate CO_2 release. Soil samples were collected during the incubation period for enzyme activity measurement.

1.4 Analytical Methods

1.4.1 Soil CO_2 Release Calculation The CO_2 release calculation formula is:

$$C_i = \frac{(V_0 - V_i) \times M \times M_c}{m}$$

where C_i is the moles of H^+ consumed in the ith titration, V_0 and V_i are the volumes of $BaCl_2$ solution consumed before and after incubation (L), M is the molar concentration of $BaCl_2$ ($\mathrm{mol} \cdot \mathrm{L}^{-1}$), M_c is the atomic mass of carbon, and m is the mass of the soil sample (g).

1.4.2 Soil Enzyme Activity Measurement Six hydrolytic enzymes were measured using microplate fluorometry: β-glucosidase (BG), cellulase (EC), xylosidase (EG), N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and phosphatase (PHO) . Fresh soil (1 g) was added to 125 mL of 0.05 $\mathrm{mol} \cdot \mathrm{L}^{-1}$ sodium acetate buffer (pH 5.0), shaken for 30 minutes, then 0.2 mL of soil suspension was pipetted into centrifuge tubes with 0.25 mL of fluorescent substrate. After 4 hours of dark incubation at 25 °C, 0.25 mL of the sample was transferred to a 96-well plate for fluorescence measurement at 365 nm excitation and 450 nm emission. Enzyme activity was calculated as:

$$A_b = \frac{(f - f_b - q) \times V \times e}{(f_s - f_r) \times V_i \times m \times t}$$

where A_b is enzyme activity ($\mathrm{mol} \cdot \mathrm{g}^{-1} \cdot \mathrm{h}^{-1}$), f is sample fluorescence, f_b is blank fluorescence, q is quenching coefficient, V is total suspension volume (125 mL), e is fluorescence release coefficient, V_i is sample volume (0.2 mL), m is dry soil mass, and t is incubation time (4 h).

1.4.3 Soil Enzyme Stoichiometry and Vector Characteristics Soil enzyme stoichiometric ratios include EAAC/N (carbon cycle-related enzyme/nitrogen cycle-related enzyme), EAAC/P (carbon cycle-related enzyme/phosphorus cycle-related enzyme), and EAAN/P (nitrogen cycle-related enzyme/phosphorus cycle-related enzyme), calculated as:

$$EAA_{C/N} = \frac{BG + EC + EG}{NAG + LAP}$$

$$EAA_{C/P} = \frac{BG + EC + EG}{PHO}$$

$$EAA_{N/P} = \frac{NAG + LAP}{PHO}$$

Vector length (Vector L) and angle (Vector A) indicate microbial carbon and phosphorus limitation. Longer vector length indicates stronger carbon limitation; vector angle <45° indicates nitrogen limitation, while >45° indicates phosphorus limitation:

$$\text{Vector L} = \sqrt{(BG + EC + EG)^2 + (NAG + LAP)^2}$$

$$\text{Vector A (Degrees)} = \arctan 2\left(\frac{NAG + LAP}{BG + EC + EG}\right) \times \frac{180}{\pi}$$

1.4.4 Data Processing and Statistical Analysis Experimental data were organized in Excel and charts were produced using OriginPro 2021. SPSS 26.0 was used for variance and correlation analyses. Stepwise regression identified significant factors (first-order factors) affecting organic carbon mineralization under different moisture treatments. Linear regression then identified four second-order factors that directly affected first-order factors and indirectly affected mineralization. Finally, JMP 10.0 was used to quantify the contribution of each factor' s direct, indirect, and interactive effects. The contribution calculation formula is:

$$FDI_{st} = 1 - F_{CDF}(F_{Hyp}, df_{Model} - 1, \lambda)$$

where FDI_{st} is the distribution function, F_{CDF} is the cumulative distribution function, F_{Hyp} is the hypothesis F-value, df_{Model} is model degrees of freedom, and λ is the non-central parameter.

Results

2.1 Soil Organic Carbon Mineralization Characteristics

Soil organic carbon mineralization varied significantly among treatments [Figure 4: see original paper]. During the first wet-dry cycle, drought stress and wet-dry alternation showed the greatest decline in mineralization (85.7%), while flooding stress declined more steadily (only 66.6%). Both flooding stress and wet-dry cycles showed stage-wise declines, but with different patterns: drought stress declined steadily, while wet-dry cycles exhibited brief increases during each wetting period. After five wet-dry cycles, the priming effect increased soil organic carbon mineralization by 66.6% compared to drought stress, confirming the transient priming effect of wet-dry alternation.

Cumulative mineralization under wet-dry cycles was higher than under drought stress but lower than under flooding stress [Figure 5: see original paper]. Although each rewetting increased mineralization compared to drought stress due to the priming effect, the magnitude decreased with increasing cycles. The cumulative mineralization under wet-dry cycles remained below that under flooding stress, indicating that the increased mineralization from priming could not offset the reduced mineralization during dry periods.

2.2 Soil Enzyme Activity Variation

Soil enzyme activity generally decreased then stabilized with increasing wet-dry cycles [Figure 6: see original paper]. β-glucosidase, xylosidase, and phosphatase showed no significant changes with cycle frequency ($P>0.05$), while cellulase and N-acetylglucosaminidase decreased significantly ($P<0.05$). Within each cycle, enzyme activities under drought stress were consistently lower than under flooding stress and wet-dry cycles. Phosphatase showed no significant differences among moisture treatments. Notably, N-acetylglucosaminidase activity under wet-dry cycles shifted from significantly higher than drought and flooding stress to significantly lower after multiple cycles ($P<0.05$).

2.3 Soil Enzymatic Stoichiometry Characteristics

Enzyme stoichiometric ratios varied with treatment and cycle frequency [Figure 7: see original paper]. EAAC/N ranged from 1.81-0.64 and EAAC/P from 3.46-1.04, both decreasing with wet-dry cycles. EAAN/P showed a decreasing then increasing trend (2.16-1.14). Vector length increased with wet-dry cycles and was consistently higher under wet-dry treatment than under drought or flooding stress [Figure 8: see original paper]. All three treatments showed carbon limitation, which intensified with increasing wet-dry cycles. Phosphorus limitation was observed throughout the experiment, but after the fourth wet-dry cycle, phosphorus limitation under wet-dry treatment exceeded that under drought and flooding stress.

2.4 Contribution of Soil Enzyme Activity to Mineralization

Stepwise regression identified different limiting factors under each moisture condition . Phosphatase was the primary factor under drought stress ($R^2=0.923$), EAAC/N and xylosidase were most significant under flooding stress ($R^2=0.923$), and N-acetylglucosaminidase significantly affected mineralization under wet-dry cycles ($R^2=0.923$).

Quantitative analysis revealed that direct effects consistently exceeded interactive effects across all moisture conditions [Figure 9: see original paper]. Under drought stress, phosphatase contributed 99% directly to mineralization, with total single-factor contributions at 87% and interactive contributions at 13%. Under flooding stress, single-factor contributions totaled 13% while interactive contributions reached 87%—the opposite pattern. Under wet-dry cycles, phosphatase and N-acetylglucosaminidase contributed 75% directly, with interactive effects at 25%. Second-order factors showed the highest contribution from enzyme stoichiometry (up to 99% under drought stress), indicating that when soil is dry, enzymes interact less and act primarily through direct effects, whereas under wetter conditions, enzyme interactions increase substantially.

Discussion

3.1 Patterns of Soil Organic Carbon Mineralization in Check Dam Land Under Different Moisture Conditions

This study confirms that flooding stress produced the highest soil organic carbon mineralization rates, validating that moisture is a dominant factor controlling mineralization. In semi-arid regions, soil organic carbon mineralization is more sensitive to moisture increases. Drought stress produced the lowest mineralization rates because moisture levels could not sustain the minimum water requirements of some microorganisms, causing massive microbial death and reducing organic carbon consumption by decomposers. Research shows that when soil moisture decreases from 30% to 5% WHC, microbial biomass decreases by 19.1% and heterotrophic respiration declines, ultimately reducing organic carbon output.

Under wet-dry cycles, mineralization rates fell between those of flooding and drought stress. Upon rewetting, mineralization surged due to the "Birch effect" —a transient priming effect caused by rapid moisture changes disrupting soil structure. Soil aggregates, as both structural units and primary carriers of soil organic carbon, undergo fragmentation during wet-dry cycles through combined physical, chemical, and biological processes. This destroys physical protection of organic carbon, increases microbial accessibility, and enhances mineralization. With increasing cycle frequency, this priming effect weakened as the pool of readily available organic matter became depleted.

Cumulative mineralization was highest under flooding stress, followed by wet-dry cycles, then drought stress. While flooding can cause organic carbon loss through surface water flow, check dams regulate flood duration through spillways and drainage structures, increasing the time soils remain at high moisture rather than fully saturated. This promotes nutrient release and microbial activity while reducing prolonged anaerobic conditions that impair nutrient transformation. Thus, check dams achieve long-term carbon sequestration and short-term emission reduction while enhancing soil fertility.

3.2 Effects of Soil Enzyme Activity on Organic Carbon Mineralization Under Different Moisture Conditions

Soil enzyme activity patterns mirrored instantaneous organic carbon mineralization changes across moisture treatments. Since mineralization is primarily a microbial process, stronger microbial activity corresponds to higher mineralization capacity. Minor moisture changes affect enzyme activity, thereby influencing mineralization. However, phosphatase showed minimal response to moisture variations, likely because phosphorus availability is more influenced by parent material than moisture conditions. Under non-optimal moisture, the conversion of organic phosphorus to inorganic phosphorus is restricted, preventing soil moisture from becoming a limiting factor for phosphatase.

N-acetylglucosaminidase showed contrasting responses to flooding stress versus wet-dry cycles. Its resistance to flooding stress was significantly higher than to wet-dry alternation, as stable waterlogged conditions allowed enzyme adaptation, whereas fluctuating moisture prevented acclimation. Under drought and wet-dry treatments, carbon and nitrogen cycling enzymes were moisture-stressed and could not effectively utilize soil organic matter, making phosphatase (under drought) and N-acetylglucosaminidase (under wet-dry cycles) the primary influencing factors.

Direct effects of enzyme activity on mineralization consistently exceeded interactive effects, likely due to inherent limitations in carbon, nitrogen, and phosphorus availability from parent material, which constrained microbial efficiency and weakened enzyme linkages when demands were unmet. However, as soil moisture increased, enzyme interactions intensified. Under wetter conditions, microbial populations increased, promoting enzyme connectivity and synergistic effects. Carbon and nitrogen cycling enzymes frequently interacted during organic matter decomposition, with β-glucosidase, xylosidase, and N-acetylglucosaminidase identified as primary limiting factors. This study quantifies the contribution of each factor to organic carbon mineralization under different moisture conditions, providing essential insights for carbon cycle research in check dam ecosystems.

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