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Effects of Incubation Temperature and Sludge Addition on Soil Organic Carbon and Nitrogen Mineralization Characteristics in Degraded Grassland Soil

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Abstract: Elucidating the characteristics and underlying mechanisms of soil organic carbon (SOC) and nitrogen mineralization in the context of sludge addition is vital for enhancing soil quality and augmenting the carbon sink capacity of soil. This study examined the chemical properties, enzyme dynamics, and organic carbon and nitrogen mineralization processes of soil from degraded grasslands on the Loess Plateau at various incubation temperatures (5, 15, 25, and 35 °C) and sludge addition rates (0%, 5.0%, 10.0%, and 20.0%) through a laboratory incubation experiment. The results showed that incubation temperature, sludge addition, and their interactive effects significantly altered the soil enzyme C:N, C:P, and N:P stoichiometries. The cumulative mineralization rates of SOC and nitrogen increased significantly with increasing incubation temperature and sludge addition rate. Principal component analysis revealed a significant linear correlation between cumulative SOC and nitrogen mineralization. Random forest analysis indicated that β-1,4-Glucosidase (BG), β-1,4-N-acetyglucosaminidase (NAG), cellobiohydrolase (CBH), ammonium nitrogen (NO_3^-), enzyme C:P ratio, alkaline phosphatase (ALP), and incubation temperature were crucial determinants of cumulative SOC mineralization. Structural equation modeling demonstrated that sludge addition, NO_3^-, NAG, ALP, and enzyme C:P positively impacted SOC mineralization, whereas dissolved organic carbon and BG had negative impacts. Conversely, incubation temperature negatively affected soil nitrogen mineralization, whereas NO_3^-, available phosphorus, and ALP contributed positively. Sludge addition and temperature indirectly modulated soil net nitrogen mineralization by altering soil chemical properties and enzyme activities. These findings underscore the role of SOC and nitrogen mineralization as indicators for evaluating soil nutrient retention capabilities.

Keywords: soil organic carbon mineralization; soil nitrogen mineralization; sludge amendment; temperature; soil enzyme activity

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1. Introduction

Soil degradation is a critical global environmental challenge [1]. The acceleration of soil degradation due to climate change and anthropogenic pressures has reduced agricultural yields, caused biodiversity loss, and diminished ecosystem services [2]. Urbanization and economic growth have led to a substantial increase in the generation of municipal sewage sludge [3]. Sewage sludge, enriched with essential nutrients, such as carbon, nitrogen, and phosphorus, offers a promising avenue for improving soil structure and fertility, thereby contributing to soil restoration and sustainable waste management [4]. The

dynamic processes of soil organic carbon (SOC) and nitrogen mineralization play pivotal roles in the biogeochemical cycles of terrestrial ecosystems. However, the complex interplay between global warming and soil organic matter mineralization complicates the creation of predictive models of soil carbon and nutrient dynamics [5]. Thus, to optimize strategies for nutrient sequestration and enhance soil health, expansive research is required to elucidate the synergistic effects of sludge application and temperature fluctuations on SOC stability and nutrient cycling.

Studies have evaluated the impacts of sludge addition and temperature on soil chemical properties and enzymatic activities [6,7]. The application of sludge to soil matrices significantly modifies soil physicochemical properties, microbial community structure, and diversity. Sastre-Conde et al. [8] demonstrated that composted sewage sludge effectively restored saline-degraded soils, markedly improving plant germination rates and optimizing the cation balance and organic matter content. Similarly, Yue et al. [9] reported that sludge application in unfertilized urban green spaces finely tuned soil nutrient configurations, thereby fostering vegetative growth. Ahmad et al. [10] noted that sludge addition improved soil nutrient levels and microbial and plant biomass and facilitated the sustainable management of solid waste, contributing to reduced greenhouse gas emissions and enhanced nutrient recycling. Geng et al. [11] found that sludge addition markedly increased soil enzyme activity and alleviated carbon and nitrogen constraints within the soil. In a related study, Geng et al. [12] observed that elevated temperatures accelerated the breakdown of total nitrogen and significantly increased the available nitrogen content in Tibetan highland soils. Soil enzyme activity and temperature are positively correlated, with rising temperatures enhancing the enzymatic function [13–15]. Xiao et al. [16] highlighted that soil ecological stoichiometry, which explores the dynamics of various soil chemical elements, is pivotal for understanding ecological interactions and determined that changes in rhizosphere enzyme activity could dictate nutrient dynamics and plant succession patterns. Furthermore, Tan et al. [17] noted that increased temperatures reduced soil carbon-to-phosphorus and nitrogen-to-phosphorus ratios more rapidly than phosphorus alone, thereby hastening the depletion of soil organic matter. Overall, these findings underscore the beneficial role of sludge in augmenting soil nutrient frameworks and enzymatic activity, thereby enriching overall soil health and fertility.

The influences of temperature and sludge addition on the mineralization dynamics of SOC and nitrogen are highly complex [18]. Within the framework of global warming, elevated temperatures are likely to expedite SOC decomposition, thereby augmenting atmospheric carbon dioxide levels and contributing to a positive feedback mechanism in climate warming [19]. The mineralization of SOC and nitrogen constitutes a fundamental process in the decomposition of soil organic matter, profoundly affecting the structural integrity and functional dynamics of terrestrial ecosystems. Pérez-Lomas et al. [20] conducted a detailed investigation of the effects of applying composite compost on the organic carbon profiles of four Mediterranean agricultural soils. Their findings indicated that the incorporation of composite compost markedly enhanced the mineralization rates of SOC, especially in soils characterized by inherently low organic carbon levels. Zhang et al. [21] endeavored to improve soil quality by treating diverse wastes via sludge composting and exploring the equilibrium between the accumulation and degradation of SOC in amended soils. They found that increasing the proportion of composted inputs notably augmented soil respiration and carbon mineralization rates, although municipal sludge compost proved to be more effective as a plant fertilizer. In a three-year field study, Chen et al. [22] observed that biochar-induced competitive interactions among predominant microbial groups significantly fostered bacterial and fungal diversity, consequently diminishing the mineralization of SOC. Ribbons et al. [23] conducted a comprehensive analysis of microbial communities, functionalities, and nitrogen cycling dynamics within four distinct forest soils and determined that fluctuations in soil enzyme activity profoundly influence net nitrogen mineralization. In a comprehensive review of climate dynamics and plant-specific nitrogen preferences, Zhang et al. [24] discovered that net nitrogen mineralization rates in

humid climates substantially exceeded those in semi-arid and arid regions, underscoring a profound linkage between nitrogen mineralization processes and climatic conditions. Wang et al. [25] studied the synergistic effects between soil organic matter and minerals across varied topographical gradients and detailed how long-term climatic conditions sculpt soil attributes. They highlighted that the processes of SOC and nitrogen mineralization are predominantly governed by climatic variables and the composition of soil organic matter. However, the intrinsic associations and interactive mechanisms underlying the mineralization processes of SOC and nitrogen remain only partially understood, necessitating more exhaustive investigative efforts.

The Loess Plateau of China is a dry and semi-arid region characterized by an exceptionally fragile ecological environment. Prolonged and improper pastoral and agricultural practices, coupled with unsuitable land use, have led to intense soil erosion and aggravated land degradation [26]. The loess soils on the Loess Plateau are characterized by low organic matter content and inadequate nitrogen and phosphorus levels, which, along with notable daily temperature fluctuations, substantially influence the quality of vegetation restoration [27]. However, despite the high nutrient content of sludge, there is a dearth of research exploring its impact as a soil amendment in loess areas. Therefore, this study evaluated the effects of sludge addition and incubation temperature on the dynamics of SOC and nitrogen mineralization in degraded grassland soils sampled from the Loess Plateau. The hypotheses of this study were as follows: (1) Different temperatures and sludge additions significantly affect soil chemical properties, enzyme activities, and ecological stoichiometry. (2) Elevated temperature has a stronger effect on soil carbon and nitrogen mineralization than that of sludge addition. (3) A level of synergy exists between net soil carbon mineralization and net nitrogen mineralization. The findings of this study are expected to offer a robust theoretical foundation for both restoration of degraded soils on the Loess Plateau and strategic planning of municipal sludge disposal.

2. Materials and Methods

2.1. Soil and Sludge Samples

Soil samples were collected from the top 20 cm layer of degraded grasslands within the Wangmaogou watershed in Shaanxi Province, China, and were sieved through a 2 mm mesh to exclude litter and roots. This region has a continental monsoon climate, with an average annual precipitation of 513 mm and average temperature of 10.2 °C. This region is situated on the northern Loess Plateau and is dominated by loess soil. The low organic matter content of this soil contributes significantly to extensive soil erosion and creates a delicately fragile ecosystem. The chemical properties of the soil samples are listed in Table 1.

Table 1. Basic chemical properties of grassland soil and sludge.

Treatment	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	DOC ($mg \cdot kg^{-1}$)	NH_4^+ ($mg \cdot kg^{-1}$)	NO_3^- ($mg \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)
Composted sludge	237.54 ± 15.70	22.23 ± 1.54	17.10 ± 0.32	4391.11 ± 331.07	1154.33 ± 54.85	199.68 ± 7.03	1070.40 ± 162.24
Grassland soil	4.89 ± 0.12	0.38 ± 0.02	0.49 ± 0.04	58.97 ± 5.20	17.65 ± 1.93	18.36 ± 4.38	10.41 ± 1.62

Note: SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; DOC: dissolved organic carbon; NH_4^+: ammonium nitrogen; NO_3^-: nitrate nitrogen; AP: available phosphorus.

Sludge samples were collected from a wastewater treatment facility in Xi'an, China. The collected sludge was aerobically composted within an experimental hall and allowed to mature naturally, yielding dry composted sludge. The main chemical characteristics of the dry sludge are listed in Table 1. The dry sludge was pulverized, passed through a 2 mm mesh, and mixed with soil samples to conduct mineralization incubation experiments.

2.2. Experimental Design

The experimental design included four sludge addition levels and four incubation temperatures, resulting in 16 treatment configurations. The soil samples were homoge-

neously blended with sludge at ratios of 0.0%, 5.0%, 10.0%, and 20.0% dry weight (labeled CK, S5, S10, and S20, respectively). Each 60.0 g aliquot of the blended soil sample was carefully allocated into 250 mL culture bottles, maintaining the soil moisture content at approximately 60% of its field capacity to optimize the conditions for microbial activity. Prior to the main incubation phase, the bottles underwent a 7-day pre-incubation at a constant 25 °C to catalyze and stabilize microbial activity within the soil matrix. Following this initial phase, the bottles were subject to a 50-day incubation period at temperatures of 5, 15, 25, and 35 °C. Soil moisture levels within the culture bottles were maintained at approximately 60% of the field capacity throughout the incubation period by employing a gravimetric method for precise hydration control every two days.

2.3. Soil Carbon and Nitrogen Mineralization Determination

To measure net carbon (Net-C) mineralization, gas samples were collected on days 1, 3, 7, 10, 15, 20, 25, 30, 35, 40, 45, and 50 to monitor the progression of carbon mineralization. Prior to gas sample collection, the culture bottle was hermetically sealed using a rubber stopper, followed by the injection of 30 mL of fresh air to ensure a consistent testing environment. The injected air was mixed thoroughly, and a 30 mL sample of the equilibrated gas mixture was withdrawn from the culture bottle and transferred into a vacuum gas collection bottle. The culture bottle was resealed and placed in a constant temperature incubator for 8 h, after which a second gas collection was performed. The concentration of CO_2 in both gas samples was quantified using a gas chromatograph (Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA).

The rate of SOC mineralization was determined using Equation (1), written as follows:

$$R_c = \beta \times \frac{V}{m} \times \frac{1}{C} \times \frac{\Delta c}{\Delta t} \times \frac{273}{273+T} \times \alpha \quad (1)$$

where R_c: SOC mineralization rate ($mgC \cdot g^{-1} \cdot SOC \cdot d^{-1}$); "$\beta$": conversion coefficient for CO_2 gas to standard units, 1.964 ($kg \cdot m^3$); V: total gas volume inside the culture bottle (m^3); m: dry mass of the sample in the culture bottle (kg); C: organic carbon content of each sample ($g \cdot kg^{-1}$); Δc: change in CO_2 concentration over the incubation period ($mg \cdot kg^{-1}$); ΔT: incubation time (d); T: incubation temperature (°C); and α: mass conversion coefficient for converting CO_2 to C, 12/44.

The cumulative *Net-C* mineralization was calculated according to Equation (2), written as follows:

$$Net - C = \sum_{i=1}^{n-1} (Ri \times Ti) \quad (2)$$

where *Net-C*: unit organic carbon cumulative mineralization ($mg \cdot g^{-1} \cdot SOC^{-1}$); i: index number for the measurement occasion; n: total number of measurements; Ri: average organic carbon mineralization rate between two sampling times ($mg \cdot g^{-1} \cdot SOC \cdot d^{-1}$); and Ti: number of days between these intervals (d).

To measure net nitrogen (Net-N) mineralization, destructive sampling was conducted on days 1, 3, 7, 15, 25, 35, and 50 of incubation, with each sampling consisting of three replicates, resulting in 336 samples. Soil ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) contents were determined using a fully automated discrete chemical analyzer with flow injection analysis [28]. The net nitrogen mineralization rate was calculated using Equation (3).

$$R_N = \frac{1}{C} \times \frac{\left[\left(NH_4^+N\right)_{t_2} + \left(NO_3^- - N\right)_{t_2}\right] - \left[\left(NH_4^+ - N\right)_{t_1} + \left(NO_3^- - N\right)_{t_1}\right]}{t_2 - t_1} \quad (3)$$

where R_N: net nitrogen mineralization rate between times t_1 and t_2 ($mg \cdot g^{-1} \cdot SOC^{-1} \cdot d^{-1}$); C: organic carbon content of each sample ($g \cdot kg^{-1}$); (NH_4^+)t_2 and (NO_3^-)t_2: ammonium nitrogen and nitrate nitrogen content in the soil at time t_2, respectively ($mg \cdot kg^{-1}$); and

$(NH_4^+)t_1$ and $(NO_3^-)t_1$: ammonium nitrogen and nitrate nitrogen content in the soil at time t_1, respectively ($mg \cdot kg^{-1}$).

The cumulative Net-N mineralization was calculated according to Equation (4), written as follows:

$$Net - N = \sum_{i=1}^{n-1} (Ri \times Ti) \quad (4)$$

where *Net-N*: unit organic carbon cumulative mineralization ($mg \cdot g^{-1} \cdot SOC^{-1}$); *i*: index number for the measurement occasion; *n*: total number of measurements; *Ri*: average net nitrogen mineralization rate between two sampling times ($mg \cdot g^{-1} \cdot SOC \cdot d^{-1}$); and *Ti*: number of days between these intervals (d).

2.4. Soil Nutrients and Enzyme Activity Determination

Following a 50-day incubation period, SOC, dissolved organic carbon (DOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), β-1,4-Glucosidase (BG), cellulobiohydrolase (CBH), β-1,4-N-acetyglucosaminidase (NAG), and alkaline phosphatase (ALP) were quantified. SOC concentrations were determined using the dichromate oxidation–ferrous sulfate titration method. Soil NH_4^+, NO_3^-, TP, and AP concentrations were determined using an automated discontinuous chemical analyzer (Clever Chem 200, Germany). Soil TN concentrations were determined using a Foss 8400 automatic Kjeldahl nitrogen analyzer. The stoichiometric ratios of the SOC to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) were calculated as SOC:TN, SOC:TP, and TN:TP, respectively.

The potential activities of four soil enzymes associated with soil carbon (C), nitrogen (N), and phosphorus (P) cycling were assayed. Soil BG and CBH enzymes facilitate carbon acquisition; soil NAG catalyzes nitrogen acquisition; and soil ALP promotes phosphorus acquisition. The different soil enzyme functions and the substrates required are shown in Table 2. Enzymatic activity was determined using a 96-well plate fluorometric assay, following the protocol outlined by Saiya-Cork et al. [29]. Enzyme activities were standardized against SOC concentration, with the results expressed as $nmol \cdot h^{-1} \cdot g^{-1} \cdot SOC^{-1}$. The logarithmic ratios of soil enzyme activities were calculated as ln(BG + CBH):ln(NAG), ln(BG + CBH):ln(ALP), and ln(NAG):ln(ALP) [30].

Table 2. Different soil enzyme functions and required substrates.

Enzyme	Abbreviation	Substrate	Function
Cellobiohydrolase	CBH	4-MUB-N-acetyl-β-D-glucosaminide	Releases disaccharides from cellulose
β-1,4-Glucosidase	BG	4-MUB-β-D-glucoside	Releases glucose from cellulose
β-1,4-N-acetyglucosaminidase	NAG	4-MUB-β-D-cellobioside	Releases N-acetyl glucosamine from oligosaccharides
Alkaline Phosphatase	ALP	4- MUB-phosphate	Hydrolysis of organophosphates to phosphates

2.5. Statistical Analysis

A two-way analysis of variance was used to assess the influence of incubation temperature, sludge addition, and their interactive effects on soil chemical properties, enzyme activities, and stoichiometric ratios. Means were compared using Duncan's multiple range test for detailed analysis. Principal component analysis (PCA) was conducted to elucidate the similarities in soil chemical properties, enzyme activities, and cumulative mineralization across various treatments. Correlation analysis delineated the associations among soil chemical properties, enzyme activities, and cumulative mineralization rates. A random forest model quantitatively identified the key predictors influencing the cumulative mineralization of net soil organic carbon and nitrogen. The significance of the model and its predictors were validated using the A3 and rfPermute packages [31]. A structural equation model (SEM) was constructed to elucidate the direct and indirect influences of significant

predictors on the cumulative mineralization of net soil organic carbon and nitrogen. The model fit was evaluated using a non-significant chi-square test ($p > 0.05$), goodness of fit index (GFI), and root mean square error of approximation. The PCA, random forest model, and SEM were implemented using the Vegan, randomForest, lavaan, and ggplot 2 packages in R [32,33].

3. Results

3.1. Soil Chemical Properties

The incubation temperature significantly influenced the concentrations of SOC, DOC, NH_4^+, NO_3^-, and AP (Figure 1) ($p < 0.05$, $p < 0.01$, $p < 0.001$). With increasing incubation temperature, there was a significant reduction in SOC and NH_4^+ concentrations. Specifically, compared to the concentrations at 5 °C, at 15 to 35 °C, the SOC concentration declined by 1.64% to 4.93% and the NH_4^+ concentration declined by 5.43% to 24.42%, respectively. The concentrations of DOC and NO_3^- escalated significantly as the incubation temperature increased. Compared to at 5 °C, at 15 to 35 °C, the DOC concentration increased by 3.19% to 13.23% and the NO_3^- concentration increased by 101.57% to 285.22%. The content of soil AP increased significantly with increasing temperature, showing notable increases of 5.44 to 10.65% under incubation temperatures of 15 °C to 35 °C compared with that at 5 °C.

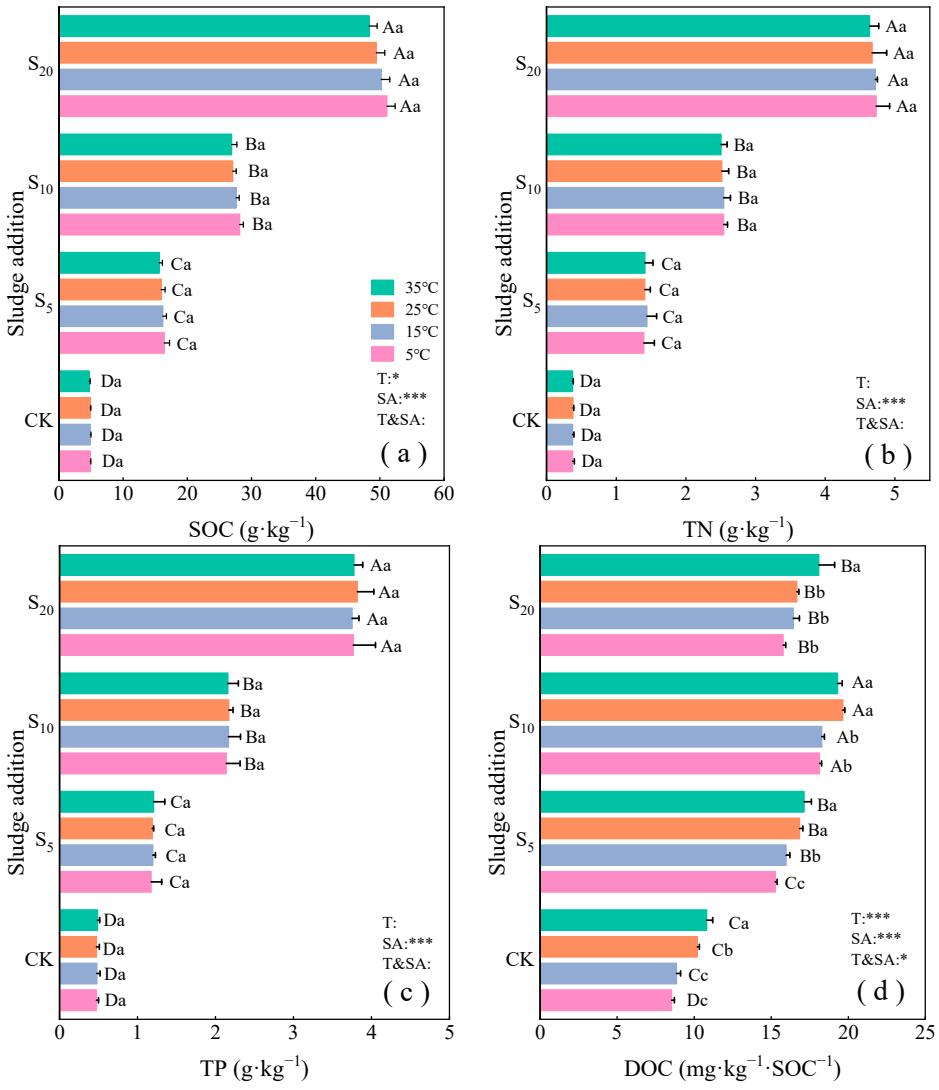


Figure 1. *Cont.*

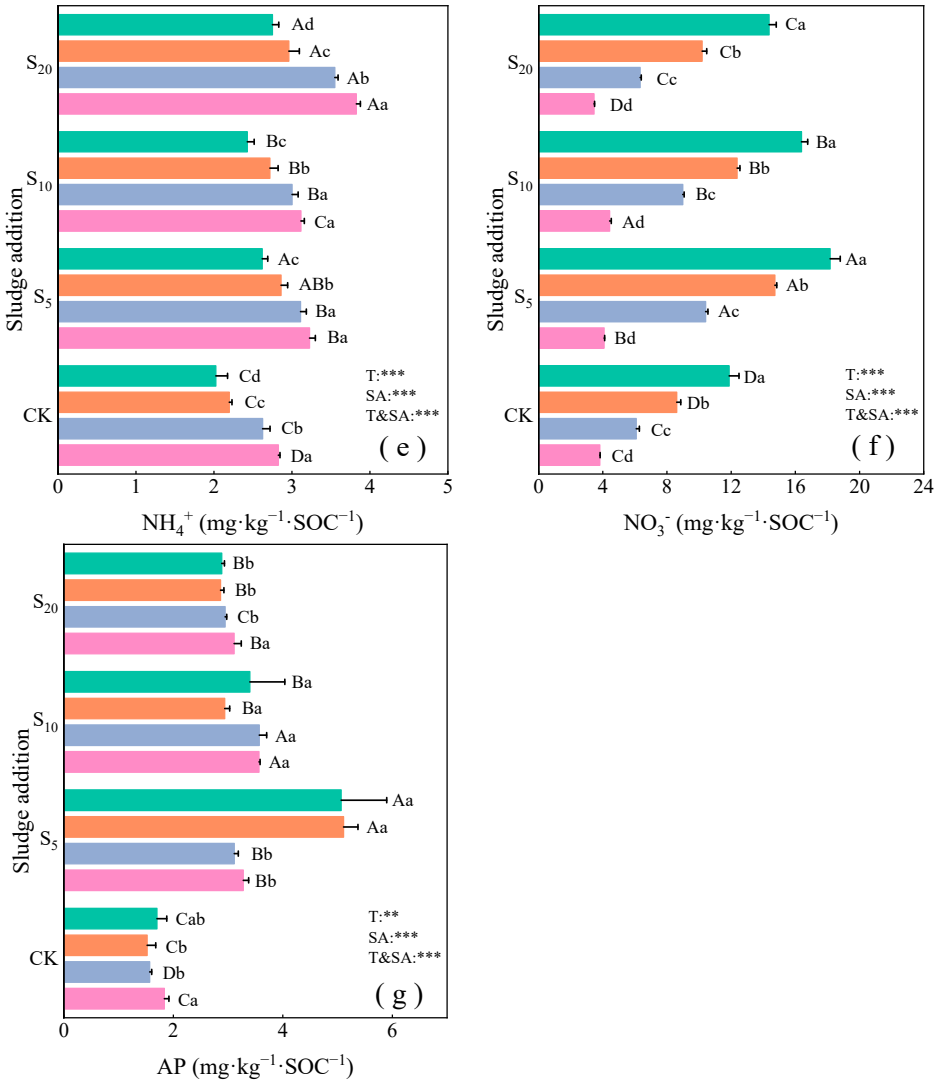


Figure 1. Effect of incubation temperature and sludge addition on soil nutrient properties. (**a**) Soil organic carbon. (**b**) Soil total nitrogen. (**c**) Soil total phosphorus. (**d**) Soil soluble organic carbon. (**e**) Soil ammonium nitrogen. (**f**) Soil nitrate nitrogen. (**g**) Soil available phosphorus. Note: Different uppercase letters indicate significant differences among sludge addition treatments at the same incubation temperatures ($p < 0.05$). Different lowercase letters indicate significant differences among different incubation temperatures at the same sludge addition treatment ($p < 0.05$). T: incubation temperature, SA: sludge addition, T&SA: interactive effect of incubation temperature and sludge addition. Statistical significance is indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The same as below.

Sludge addition significantly influenced the concentrations of SOC, TN, TP, DOC, NH_4^+, NO_3^-, and AP in the soil (Figure 1) ($p < 0.001$). The concentrations of SOC, TN, TP, and NH_4^+ increased markedly with increasing proportions of sludge, peaking under S20. Relative to the control (CK), the SOC, TN, and TP levels under S5 to S20 increased 233% to 932%, 2.78% to 911%, and 149% to 689%, respectively, whereas the NH_4^+ levels increased by 16.49% to 35.35%. The most notable increase in soil DOC content occurred under S10, with significant increases ranging from 69.79% to 96.26% under S5 to S20, relative to CK. Under S5 to S20, NO_3^- and AP concentrations in the soil increased by 13.08% to 55.96% for NO_3^- and 78.17% to 150.02% for AP relative to CK, with the most substantial enhancements under S5. Furthermore, the interaction between sludge addition and temperature significantly influenced the concentrations of DOC, NH_4^+, NO_3^-, and AP ($p < 0.05$ or $p < 0.001$).

3.2. Soil Enzyme Activities

The incubation temperature, sludge addition, and their combined effects markedly influenced the activities of soil enzymes, such as CBH, BG, NAG, and ALP (Figure 2) ($p < 0.001$). The enzymatic activities of CBH, BG, NAG, and ALP significantly increased with increasing incubation temperature. Relative to the baseline at 5 °C, enzyme activities at temperatures from 15 to 35 °C exhibited substantial elevations: CBH (81.35% to 426.68%), BG (80.03% to 225.80%), NAG (95.49% to 215.10%), and ALP (116.91% to 204.61%). Likewise, relative to the CK, under S5 to S20, the activities of CBH (47.72% to 54.18%), BG (71.55% to 91.81%), NAG (69.59% to 128.33%), and ALP (13.75% to 17.49%) notably increased.

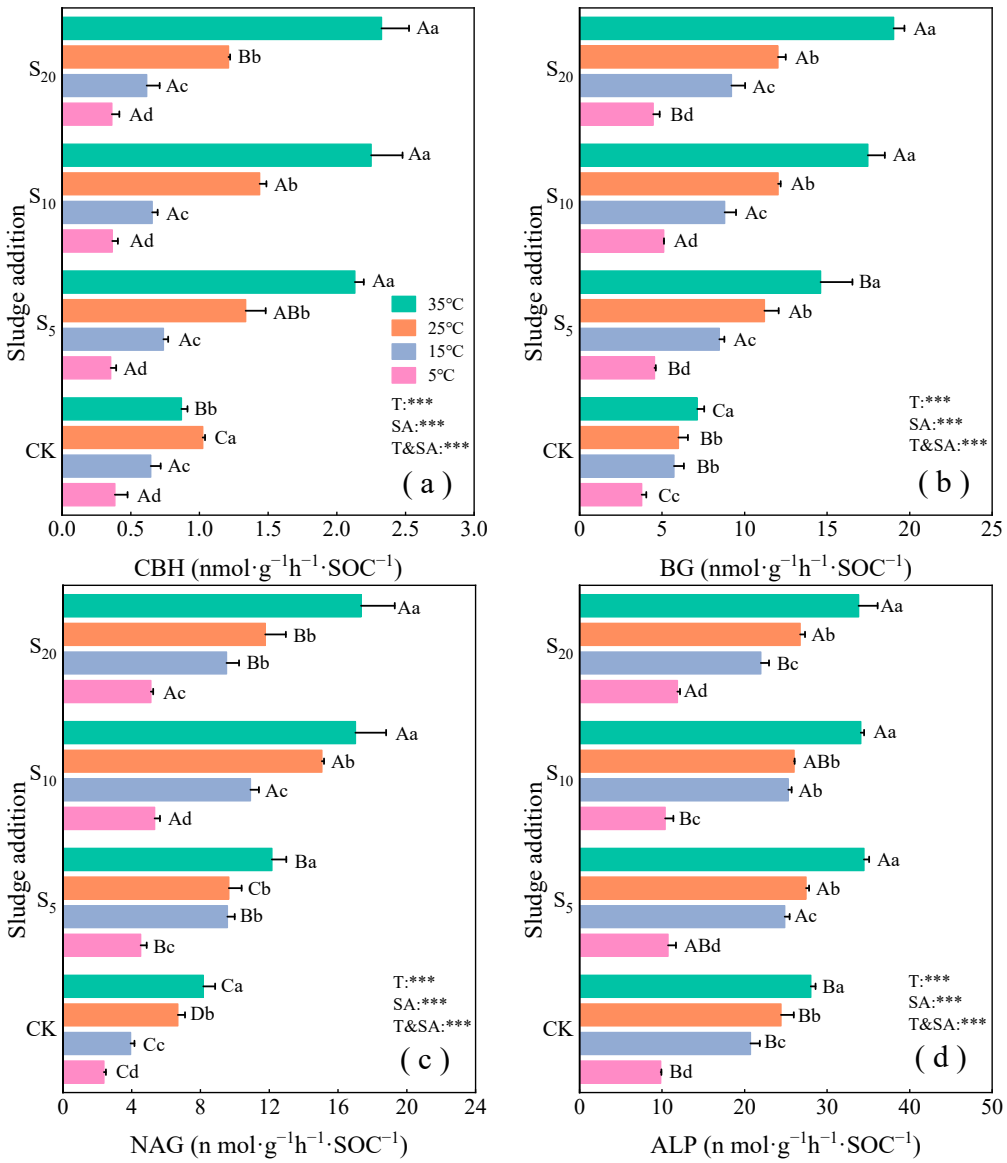


Figure 2. Effect of incubation temperature and sludge addition on soil enzyme activities. (**a**) Cellobiohydrolase. (**b**) β-1,4-Glucosidase. (**c**) β-1,4-N-acetyglucosaminidase. (**d**) Alkaline Phosphatase. Note: Different uppercase letters indicate significant differences among sludge addition treatments at the same incubation temperatures ($p < 0.05$). Different lowercase letters indicate significant differences among different incubation temperatures at the same sludge addition treatment ($p < 0.05$). T: incubation temperature, SA: sludge addition, T&SA: interactive effect of incubation tem-perature and sludge addition. Statistical significance is indicated as follows: ***, $p < 0.001$.

3.3. Stoichiometric Characteristics of Soil Chemical Properties and Enzyme Activities

The addition of sludge significantly influenced the soil C:N, C:P, and N:P ratios (Figure 3) ($p < 0.001$). The soil C:N ratio exhibited a notable reduction, ranging from 11.81% to 17.57% under S5 to S20 relative to the CK, correlating with the increased proportions of sludge addition. Concurrently, the soil C:P and N:P ratios significantly increased, increasing by 26.19% to 34.06% and 49.70% to 58.48%, respectively, under S5 to S20 compared with the CK.

The incubation temperature, sludge addition, and their interactions had pronounced impacts on the ratios of soil enzymes C:N, C:P, and N:P (Figure 3) ($p < 0.001$). The soil enzyme C:N ratio notably decreased from 8.30% to 11.35% with increasing temperature from 15 to 35 °C relative to that at 5 °C. Correspondingly, soil enzyme C:P and N:P ratios significantly increased, with increases of 2.91% to 18.12% and 9.69% to 23.24%, respectively. Compared with CK, the soil enzyme C:N decreased significantly with increasing sludge addition ratio, with those of the S5 to S20 treatment groups decreasing by 14.77 to 21.01%, respectively. Concurrently, the soil enzyme C:P and N:P ratios underwent substantial enhancements, increasing from 18.14% to 24.08% and 32.71% to 50.39%, respectively, under S5 to S20 relative to CK.



Figure 3. *Cont.*

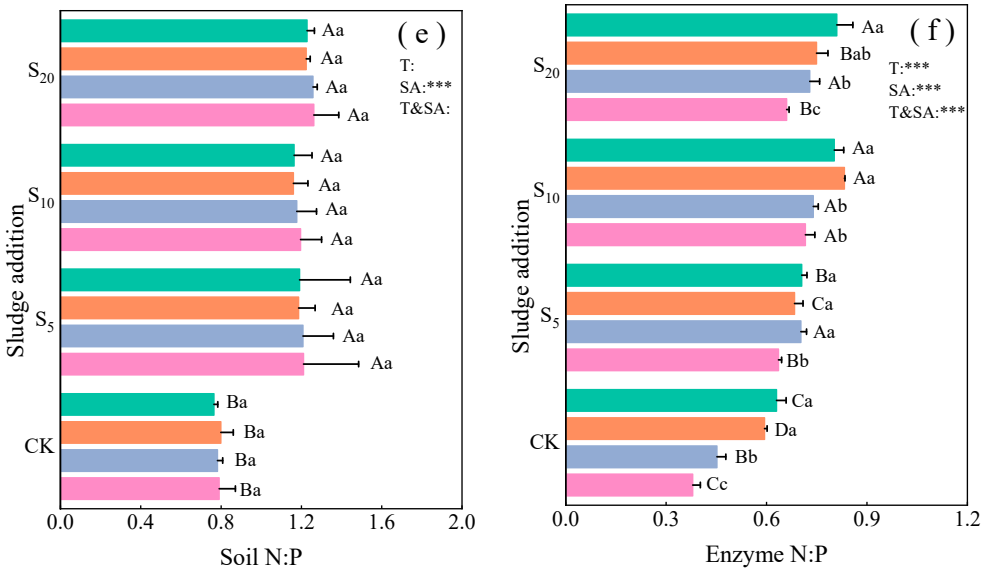


Figure 3. Effect of incubation temperature and sludge addition on the ecological stoichiometry ratios of soil nutrients and soil enzyme activities. (**a**) Soil C:N. (**b**) Soil enzyme C:N. (**c**) Soil C:P. (**d**) Soil enzyme C:P. (**e**) Soil N:P. (**f**) Soil enzyme N:P. Note: Different uppercase letters indicate significant differences among sludge addition treatments at the same incubation temperatures ($p < 0.05$). Different lowercase letters indicate significant differences among different incubation temperatures at the same sludge addition treatment ($p < 0.05$). T: Incubation incubation temperature, SA: Sludge sludge addition, T&SA: Interactive interactive effect of incubation temperature and sludge addition. Statistical significance is indicated as follows: ***, $p < 0.001$.

3.4. SOC and Nitrogen Mineralization Characteristics

The interaction effects of incubation temperature and sludge addition profoundly modulated the cumulative mineralization rates of net SOC and nitrogen (Figure 4) ($p < 0.001$). With increasing incubation temperature, there was a marked increase in the cumulative mineralization rates of both net SOC and nitrogen. Relative to the baseline at 5 °C, the mineralization rates for net carbon and net nitrogen at 15 to 35 °C increased from 171.64% to 429.48% and 208.22% to 542.30%, respectively. Sludge addition proportionally increased the cumulative mineralization rates of net carbon and net nitrogen in the soil. Compared to the CK, the treatment groups with 5% to 20% sludge addition exhibited increased mineralization rates of net carbon (125.85% to 151.10%) and net nitrogen (19.61% to 89.50%). There was a significant linear correlation between the cumulative mineralization rates of net carbon and net nitrogen in the soil (Figure 5c). Among the various incubation temperatures, a significant linear correlation between the cumulative mineralization of net carbon and net nitrogen was observed at 25 °C (Figure 5b). Across varying proportions of sludge, a consistent and significant linear correlation was found between the cumulative mineralization rates of net organic carbon and nitrogen (Figure 5a).

3.5. Factors Impacting the Variation of SOC and Nitrogen Mineralization

PCA elucidated pronounced variations in soil chemical properties, enzyme activities, ecological stoichiometric ratios, and cumulative mineralization of SOC and nitrogen across different incubation temperatures and sludge additions (Figure 6). PC1 and PC2 explained 53.28% and 26.63% of the total variance in soil properties, respectively. Notable disparities in soil chemical properties, enzyme activities, ecological stoichiometric ratios, and rates of cumulative carbon and nitrogen mineralization were observed under S5, S10, and S20 across the PC1 and PC2 axes. Additionally, marked variations were apparent in soil chemical properties, enzyme activities, ecological stoichiometric ratios, and the cumulative mineralization of carbon and nitrogen at incubation temperatures of 15, 25, and 35 °C along the PC1 and PC2 axes.

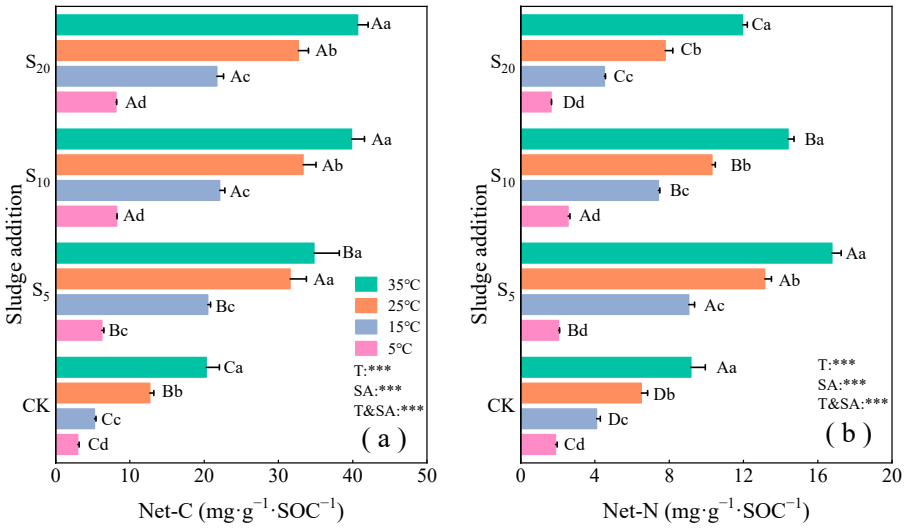


Figure 4. Effect of incubation temperature and sludge addition on the cumulative mineralization of net soil organic carbon and nitrogen. (**a**) Net cumulative soil carbon mineralization. (**b**) Net cumulative mineralization of soil nitrogen. Note: Different uppercase letters indicate significant differences among sludge addition treatments at the same incubation temperatures ($p < 0.05$). Different lowercase letters indicate significant differences among different incubation temperatures at the same sludge addition treatment ($p < 0.05$). T: Incubation incubation temperature, SA: Sludge sludge addition, T&SA: Interactive interactive effect of incubation temperature and sludge addition. Statistical significance is indicated as follows: ***, $p < 0.001$.

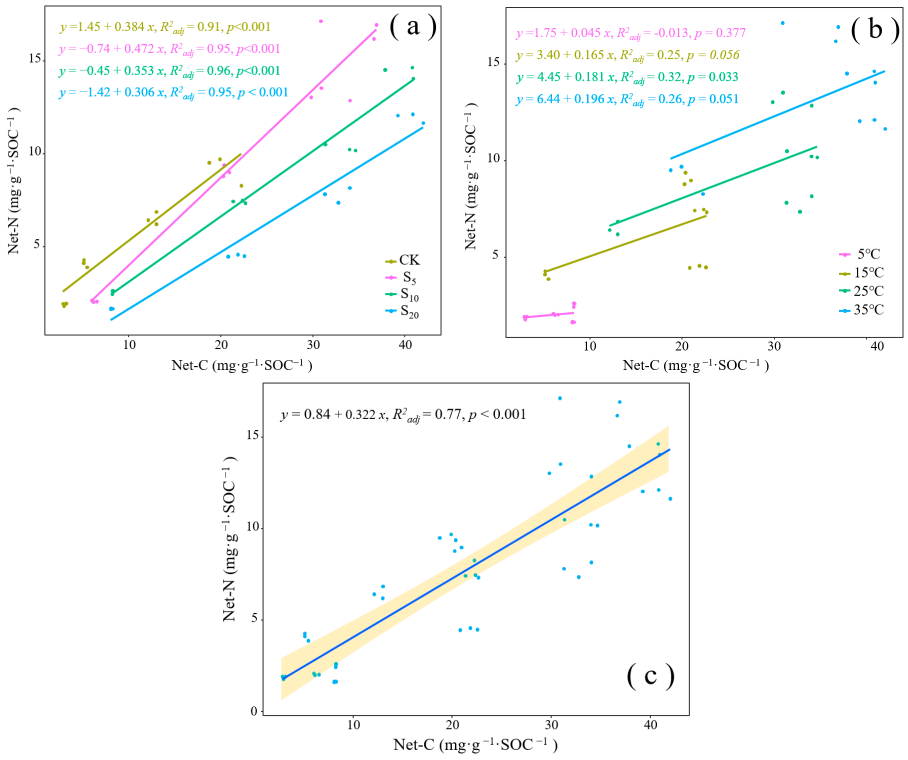


Figure 5. Correlation between cumulative net soil organic carbon mineralization and cumulative net nitrogen mineralization. (**a**) Relationship between the cumulative mineralization of soil net carbon and nitrogen under different sludge additions. (**b**) Relationship between soil net carbon and nitrogen cumulative mineralization at different incubation temperatures. (**c**) Relationship between overall net carbon cumulative mineralization and net nitrogen cumulative mineralization.

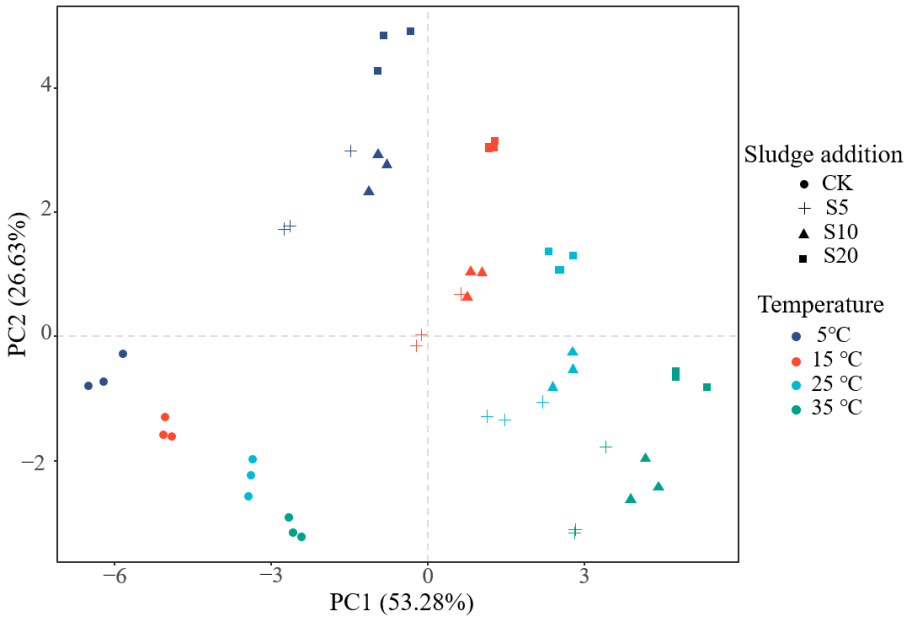


Figure 6. Principal component analysis of soil chemical properties, soil enzyme activities, and their ecological stoichiometry, and net soil organic carbon and nitrogen cumulative mineralization under different incubation temperatures and sludge additions.

Soil nutrient content, enzyme activity, ecological stoichiometry, and cumulative mineralization of net SOC and nitrogen under different incubation temperatures and sludge additions were significantly correlated (Figure 7) ($p < 0.05$, $p < 0.01$, or $p < 0.001$). This coincides with Hypothesis (1). Cumulative net carbon mineralization in the soil exhibited strong positive correlations with incubation temperature, sludge addition, SOC, TN, TP, DOC, NO_3^-, AP, CBH, BG, NAG, ALP, soil N:P, enzyme C:P, and enzyme N:P, while showing significant negative correlations with soil C:N and enzyme C:N. Similarly, cumulative net nitrogen mineralization in the soil displayed marked positive correlations with incubation temperature, DOC, NO_3^-, AP, and soil enzymatic activities, while it was inversely associated with NH_4^+ and soil C:N.

A random forest model was employed to quantitatively determine the key predictors influencing the cumulative mineralization of net SOC and nitrogen (Figure 8). Incubation temperature, sludge addition, DOC, NO_3^-, CBH, BG, NAG, ALP, and enzyme C:P ratio were identified as critical predictors of soil net carbon mineralization, while incubation temperature, sludge addition, TP, NO_3^-, AP, CBH, BG, NAG, ALP, and enzyme C:P ratio were crucial predictors of soil net nitrogen mineralization. A SEM was applied to elucidate the direct, indirect, and total effect contributions of the pivotal predictors to the cumulative mineralization of net SOC and nitrogen (Figure 9). Sludge addition, NO_3^-, NAG, ALP, and enzyme C:P ratio exerted direct positive influences on net SOC mineralization, whereas DOC and BG had direct negative impacts. Conversely, sludge addition and incubation temperature indirectly modulated net SOC mineralization via alterations in soil chemical properties and enzymatic activities. Temperature, NO_3^-, AP, and ALP had a direct positive effect on net cumulative soil N mineralization. The total effects of incubation temperature on soil net cumulative carbon mineralization (0.666) and net cumulative nitrogen mineralization (0.587) were greater than those of sludge addition (0.311 and 0.229) (Tables 3 and 4). Furthermore, NO_3^- was the main factor affecting soil net cumulative carbon and nitrogen mineralization.

Table 3. Direct, indirect, and total effects of sludge addition, incubation temperature, soil chemical properties, and soil enzyme activities on net cumulative soil carbon mineralization.

	Sludge Addition	Incubation Temperature	DOC	NO_3^-	CBH	BG	NAG	ALP	EnzymeC:P
Direct	0.249	-	−0.105	0.516	-	−0.422	0.358	0.270	0.407
Indirect	0.062	0.666	−0.036	0.252	−0.077	0.377	-	−0.204	-
Total	0.311	0.666	−0.141	0.768	−0.077	−0.045	0.358	0.066	0.407

Table 4. Direct, indirect, and total effects of sludge addition, incubation temperature, soil chemical properties, and soil enzyme activities on cumulative mineralization of net soil nitrogen.

	Sludge Addition	Incubation Temperature	NO_3^-	AP	CBH	BG	ALP	EnzymeC:P
Direct	-	0.196	0.970	0.019	-	-	0.106	0.319
Indirect	0.229	0.683	-	−0.006	−0.059	0.302	−0.158	-
Total	0.229	0.879	0.970	0.013	−0.059	0.302	−0.052	0.319

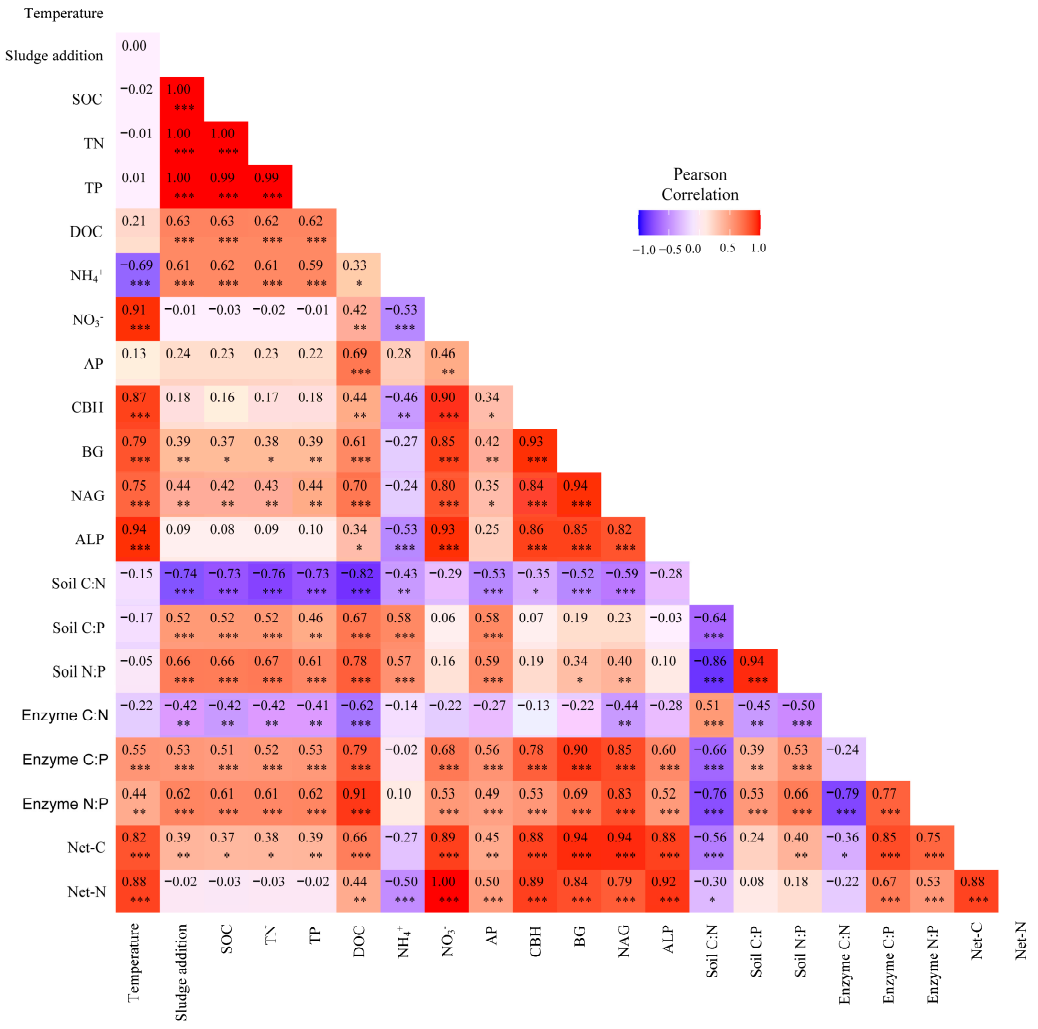


Figure 7. Correlation analysis of soil chemical properties, soil enzymes, and their ecological stoichiometry, and cumulative net soil organic carbon and nitrogen mineralization. significance is indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

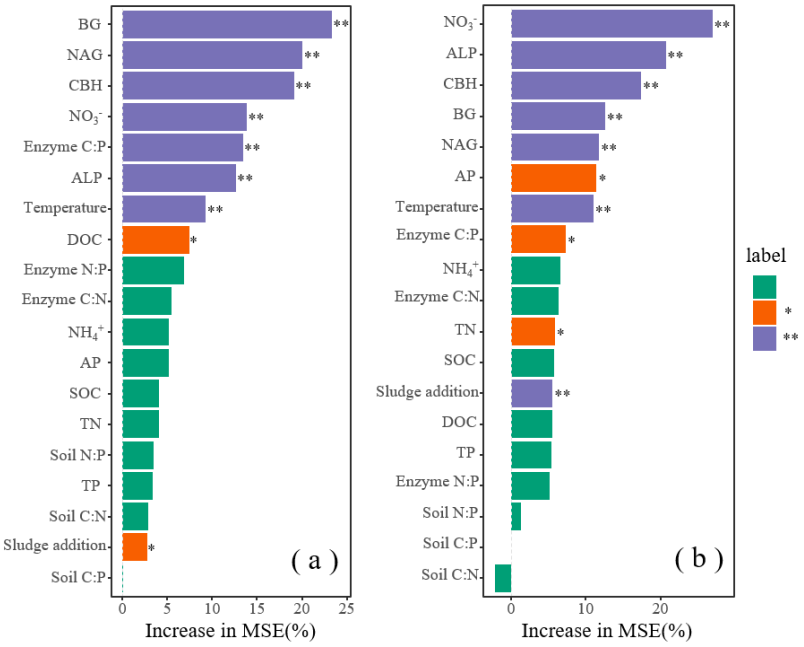


Figure 8. Random forest model elucidating the influence of soil chemical properties, enzyme activities, and their ecological stoichiometry on net soil organic carbon and nitrogen mineralization across different incubation temperatures and sludge additions. (**a**) Significance of predictor variables for net cumulative soil organic carbon mineralization. (**b**) Significance of predictor variables for cumulative net nitrogen mineralization. Level of significance denoted as *, $p < 0.05$ and **, $p < 0.01$.

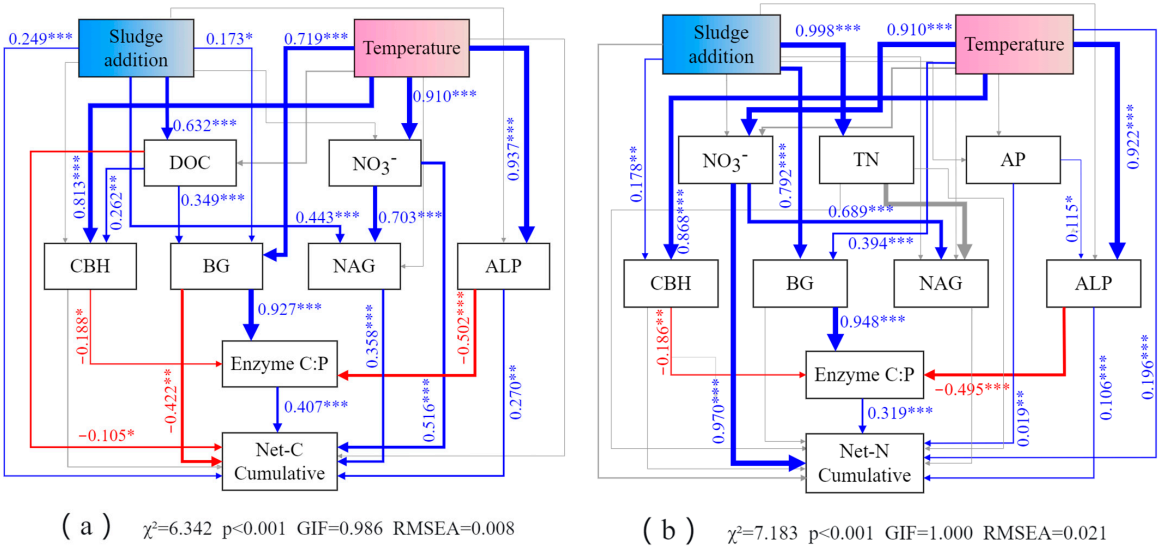


Figure 9. Structural equation modeling delineating the direct and indirect impacts of incubation temperature and sludge addition on soil chemical properties, enzymatic activities, and the cumulative mineralization of soil organic carbon and nitrogen. (**a**) Net cumulative soil carbon mineralization. (**b**) Net cumulative mineralization of soil nitrogen. Within the model, sludge addition is represented by a blue rectangle, and incubation temperature by a red rectangle. Positive and negative influences are encoded by blue and red lines, respectively. The breadth of the arrows quantifies the magnitude of significant standardized path coefficients, with non-significant pathways depicted in gray. TN: total nitrogen, AP: effective phosphorus, DOC: soluble organic carbon, NO_3^-: nitrate nitrogen, CBH: cellobiohydrolase, BG: β-1,4-Glucosidase, ALP: alkaline phosphatase. Statistical significance is indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

4. Discussion

4.1. Soil Nutrients and Enzyme Activity

Soil is the foundational source of nutrients for plant growth, and the status of soil nutrients is pivotal for effective soil remediation [34]. The application of sludge in soil remediation significantly improves soil structure and increases soil nutrient contents [22]. We observed a respective decline and marked increase in soil NH_4^+ and NO_3^- contents with increasing temperature, which is consistent with the findings of Hu et al. [35] regarding the transformation dynamics of TN in soils. This result evidenced that the nitrification process is stronger than the ammonification process in the mineralization of soil TN. Soil DOC and AP concentrations increased significantly with increasing temperature, corroborating the findings of Song et al. [36], who posited that elevated temperatures intensify the breakdown of total soil nutrients, thereby boosting nutrient availability. Lehtinen et al. [37] and Jakubus et al. [38] demonstrated that the use of sewage sludge compost substantially enhanced the concentrations of SOC, TN, phosphorus, and K, establishing sludge as a sustainable fertilizer that concurrently retards the nutrient decomposition process in soils. These results align with our observations that the SOC, TN, and TP concentrations progressively increased with increasing gradients of sludge addition. Furthermore, our data demonstrated that key soil efficacy indicators, including DOC, NH_4^+, NO_3^-, and AP, initially surged and subsequently plateaued with increasing sludge addition rates, which is consistent with the findings of De Lucia et al. [39] and Elsalam et al. [40]. This suggests that the incorporation of composted sludge can rapidly augment the availability of soil nutrients, thereby mitigating the environmental impacts associated with organic waste management.

Soil enzymes are pivotal in facilitating the cycling of carbon and nutrients in terrestrial ecosystems [41]. These enzymes are integral to biogeochemical cycling and the transformation of carbon, nitrogen, and phosphorus in soil ecosystems. Temperature modulates the synthesis of soil enzymes by regulating microbial activity, thereby exerting a profound effect on the mineralization of soil organic matter [42,43]. This study found that the activities of the enzymes CBH, BG, NAG, and ALP increased with increasing temperature, corroborating the observations of Meng et al. [44] on soil enzyme responses to global warming, suggesting that elevated temperatures boost enzyme activity [45]. Skońska et al. [46] demonstrated through both laboratory and field studies that sludge application markedly enhanced soil carbon and nitrogen levels and enzymatic activities. Additionally, our findings revealed that higher sludge addition ratios significantly increased the activities of CBH, BG, NAG, and ALP enzymes, consistent with the insights of Pokharel et al. [47] into the impact of biochar on 12 soil enzyme activities. These results indicated that sludge incorporation substantially augmented soil enzymatic functions. Additionally, they confirmed that incubation temperature, sludge addition, and their combined effects critically influence soil enzyme dynamics.

4.2. Soil Nutrient and Enzyme Stoichiometry and Cumulative Carbon and Nitrogen Mineralization

Soil ecological stoichiometry provides critical insights into microbial activities and metabolic processes and serves as a diagnostic tool for evaluating resource limitations in soil nutrients [48,49]. In the present study, we observed that the soil enzyme C:P and N:P ratios exhibited marked increases with increasing temperatures, whereas the C:N ratio was significantly reduced. This aligns with the results of Bárta et al. [50], who analyzed stoichiometric ratios of enzymes involved in the cycling of carbon, nitrogen, and phosphorus in forest soils, revealing that increasing temperatures preferentially enhanced enzyme activities associated with carbon and nitrogen cycles over those linked to phosphorus cycles, with nitrogen-related enzyme activities surpassing those of carbon-related enzyme activities. Furthermore, our study established a robust correlation between soil and soil enzyme ecological stoichiometry ratios, corroborating the empirical evidence presented by Zhang et al. [51] and Yang et al. [52] regarding the stoichiometric ratios of soil carbon, nitrogen, and phosphorus. In addition, our findings demonstrated that the addition of sludge

led to significant increases in soil C:P, N:P, enzyme C:P, and enzyme N:P ratios, which is consistent with the results of Geng et al. [11] and Chang et al. [53]. This suggests that sludge application notably augments SOC and TN levels, as well as enzymatic activities pertinent to the carbon and nitrogen cycles relative to those of phosphorus. Binh and Shima [54] reported that enhanced sludge application substantially increased soil nitrogen levels and decreased the soil C:N ratio, effectively moderating the net nitrogen mineralization rate and thereby regulating soil nitrogen mineralization processes. These results are consistent with our findings, which demonstrated notable reductions in the soil C:N and soil enzyme C:N ratios with increasing sludge addition, together with a significant decline in soil net nitrogen mineralization when sludge proportions exceeded 5.0%.

SOC and nitrogen mineralization primarily result from enzymatic reactions mediated by soil microorganisms, with elevated temperatures known to accelerate certain enzymatic activities. Consequently, numerous studies have demonstrated a positive correlation between the cumulative mineralization of SOC and nitrogen and ambient temperature [55,56]. Engqvist [57] revealed that increasing temperatures enhanced soil microbial biomass and enzyme activity, thereby accelerating the mineralization of organic carbon in the soil and subsequent CO_2 production. They found that the microbial decomposition of organic matter predominantly governs the mineralization processes of SOC and nitrogen. Moreover, microbial respiration relies on extracellular enzymes to catalyze and hydrolyze organic matter, thereby acquiring the necessary carbon and nitrogen for metabolic functions. Weedon et al. [58] demonstrated that higher temperatures facilitate rapid soil nutrient decomposition with a robust microbial metabolism that secretes an abundance of soil enzymes, thus accelerating the mineralization of SOC and nitrogen. Wei et al. [59] demonstrated that augmenting soil with exogenous carbon increases the active carbon pool within the SOC. Enzyme kinetics theory suggests that soils deficient in organic carbon exhibit elevated activation energies. Consequently, in Wei et al. [59], with an increasing proportion of incorporated exogenous carbon, the soil mineralization rate decreased. In the present study, composted sludge treatments ranging from 10.0% to 20.0% failed to yield substantial net cumulative mineralization across various thermal conditions. As the application rate increased, a decrease in cumulative mineralization was observed, congruent with an enhancement in the capacity of the soil for carbon sequestration. Dais et al. [60] elucidated that biochar application beneficially affects SOC content, stabilizes the soil carbon pool, and bolsters soil carbon sequestration. They observed that biochar supplementation diminished the relative potential of soil mineralization and exerted a suppressive influence on the mineralization potential of SOC upon the addition of exogenous carbon. Furthermore, they ascertained that throughout the mineralization process, the cumulative mineralization of carbon and nitrogen in soil organic matter increased with increasing temperature. The principal rationale is that lower temperatures constrain the activity of soil microbes, thereby curtailing the production of active enzymes and impeding the progression of mineralization. Conversely, elevated temperatures enhance the activity of soil microbes, promoting the synthesis of enzymes involved in the mineralization of SOC and nitrogen. Increasing temperatures predominantly amplify the activity of soil microbes and enzymes, thereby extensively influencing the mineralization of SOC and nitrogen.

4.3. Factors Impacting the Variation of Soil Carbon and Nitrogen Mineralization

Net carbon and nitrogen mineralization in the soil have synergistic effects [61]. The present study established a significant linear correlation between the cumulative mineralization of net organic carbon and net nitrogen in the soil. This corroborates the findings of Wu et al. [62], who reported that fertilization modulates the mineralization of SOC and nitrogen reserves, which coincides with our Hypothesis (3). The mineralization rates of both carbon and nitrogen in the soil increase significantly with increasing temperature [63,64]. In the present study, across various incubation temperatures, the cumulative net carbon and nitrogen mineralization in the soil treated with sludge significantly exceeded that of CK. This suggests that elevated temperatures profoundly influence the availability and net

mineralization of carbon and nitrogen in the soil. The cumulative mineralization of organic carbon and nitrogen in the soil is positively correlated with SOC concentration [65,66]. Sludge addition can enhance carbon sequestration and nutrient availability in the soil, although this effect is contingent on the proportion of sludge used. Our results revealed a significant linear relationship between the cumulative mineralization of net carbon and net nitrogen in the soil and the proportion of sludge used, although the cumulative mineralization levels were not statistically significant after applying 10.0% sludge. However, as the application rate increased, a downward trend in the cumulative mineralization of net nitrogen was observed, concurrent with an enhancement in the soil nutrient retention capacity. A random forest model indicated that temperature, NO_3^-, BG, NAG, CBH, and ALP enzymes were critical determinants of net carbon and nitrogen mineralization in the soil. Correspondingly, various studies have identified temperature and soil enzyme activity as pivotal indicators that facilitate the net mineralization of carbon and nitrogen [67]. Using the SEM, we determined that the overall impacts of incubation temperature on net cumulative soil carbon and nitrogen mineralization surpassed those of sludge addition. This is consistent with the findings of Marzi et al. [61] on the influence of various sources of organic matter on soil carbon and nitrogen mineralization, while also supporting Hypothesis (2). Furthermore, this study identified NO_3^- as the primary factor affecting the cumulative mineralization of net soil carbon and nitrogen [68]. These results demonstrated that sludge addition and temperature influenced the net cumulative mineralization of carbon and nitrogen both directly and indirectly, mediated by soil chemical properties and enzymatic activity. Overall, this laboratory-based study evaluated the effects of temperature and sludge addition on the net mineralization of SOC and nitrogen and elucidated the principal interactions among soil chemical properties, enzyme activity, and ecological stoichiometry throughout the mineralization process.

In summary, we found that soil net carbon mineralization tended to be stable at a 5.0% to 10.0% sludge addition, whereas soil net nitrogen mineralization peaked. This sludge addition rate appears to be the most conducive to the remediation of degraded soils, as the soil carbon sequestration capacity is stable, while the soil mineralized inorganic nitrogen produces the maximum effective nitrogen that can be utilized by plants. Nevertheless, extensive research has confirmed the highly dynamic nature of soil carbon and nitrogen mineralization processes [69,70], showing that these are not only affected by the external environment but are also closely related to the internal nutrient changes in the soil. Therefore, additional field studies are required to assess the characteristics of SOC and nitrogen mineralization in response to temperature changes and the addition of sludge more accurately.

5. Conclusions

Our investigation uncovered substantial variations in soil chemical properties, enzyme activities, ecological stoichiometry, and cumulative net carbon and nitrogen mineralization under different temperatures and sludge addition rates. Elevated temperatures promoted soil DOC, NO_3^-, and AP contents, together with soil CBH, BG, NAG, and ALP enzyme activities, thereby enhancing soil net carbon and nitrogen cumulative mineralization while significantly decreasing soil SOC and NH_4^+ contents and soil C:N. For its part, sludge addition increased the SOC, TN, TP, and NH_4^+ contents, soil CBH, BG, NAG, and ALP enzyme activities, soil enzyme C:P and enzyme N:P, and the net carbon and nitrogen cumulative mineralization capacity of the soil. The total effect of incubation temperature on soil C:N mineralization was greater than that of sludge addition, but NO_3^- was the main factor affecting soil C:N mineralization. Based on these results, we suggest that a sludge application rate of 5.0% to 10.0% can significantly improve the soil nutrient content. Simultaneously, the soil carbon sequestration capacity remains stable, while the effective nitrogen produced by soil inorganic nitrogen mineralization available to plants is maximized, which is the most conducive to the restoration of degraded soils. However, further studies are needed to understand the transformation mechanisms of

incubation temperatures and soil NO_3^- in the soil carbon mineralization-fixation cycle more comprehensively.

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