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Original Research

Impacts of Adding Municipal Sewage Sludge on Soil Enzyme Activity and Stoichiometry in a Chinese Loess Soil

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Abstract

Municipal sewage sludge (MSS) disposal through land application was a cost-effective option for improving soil quality. It is inconsistent about the effects of MSS addition on the variations of soil enzyme activities and stoichiometries, which have been frequently used as indicators of soil quality. A laboratory incubation experiment was conducted for 60 days with six treatments: non-amended soil (CK), soil amended with synthetic fertilizer (N or P addition), and different doses (5%, 10%, and 15%) of MSS. Soil samples were collected after 1, 2, 3, 5, 7, 10, 15, 30, 45, and 60 days of incubation, and C-, N-, and P-acquiring enzyme activities (β-glucosidase [BG], N-acetyl-glucosaminidase [NAG], L-leucine aminopoptidase [LAP], and phosphatase [AP]) were determined and enzyme stoichiometries were calculated. BG, NAG+LAP, and AP activities logarithmically increased with incubation time. The daily variation of BG, NAG+LAP, and AP activities first increased and then decreased with incubation time, with the highest value on the 7th day incubation. Compared with CK, the cumulative enzyme activities increased by 323.8-389.8% in the 15% MSS addition treatment, whereas those under the N and P treatments significantly decreased by 18.0%-51.4% and 31.8%-53.0%, respectively. Enzyme C:N decreased but C:P and N:P increased during incubation with MSS addition. However, synthetic N and P addition increased the enzyme C:N and C:P but decreased enzyme N:P. These results indicated that N and P addition decreased the enzyme activities, and MSS addition significantly increased the secreting of enzymes and would be better for alleviating soil C and N limitations.

Keywords: incubation experiment, soil quality, organic amended, enzyme activity, Calcaric cambisol

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Introduction

Soil erosion is one of the most serious environmental problems on the worldwide [1]. Soil erosion caused soil organic matter (OM) loss from soil surface is one of the principal mechanisms of land degradation [2]. The continuous depletion of OM in many areas globally is of major concern, and it is typically associated with a loss of both soil fertility and the capacity of ecosystem services (e.g. climate regulation, food production, carbon sequestration) [3, 4]. At the same time, the output of municipal sewage sludge (MSS) had rapidly increased with the high speed economic development and urbanization [5]. For example, the MSS production in China increased from 54.27 million ton in 2010 to 74.36 million tons in 2017 [6]. Therefore, the disposal and utilization of MSS is crucial for sustainable development. Since MSS contains high levels of OM and nutrient, so MSS disposal through land application could be used for increasing soil OM and remediation of degraded land, and which might be a cost-effective option for sustainable sludge treatment [7]. However, MSS may contain toxic and harmful substances, such as organic micro-pollutants, pathogens and heavy metals [8, 9], which are the major risks for land application of MSS. So, monitoring the changes of soil quality after MSS addition is useful for finding environmentally, friendly and socially acceptable MSS management.

Soil enzymes are the key mediators for OM decomposition and nutrient mineralization [10]. Compared with soil physical and chemical properties, enzyme activities are more sensitive indicators of changes in land management, especially under short-term conditions [11, 12]. Changes in enzyme activities are associated with the soil biochemical processes, and there is a growing interest in using soil enzyme activities to access and monitor soil quality [13, 14]. Numerous studies have monitored the variations in soil enzyme activities to evaluate the effects of adding MSS on remediating the quality of degraded soil [11, 15, 16]. For example, Wang et al. [17] reported that adding MSS to aeolian sandy soil improved soil urease, sucrase, neutral phosphatase, and catalase activities in relation to the input of organic materials and microorganisms. Siebielec et al. [18] found that the long-term (10 years) application of sewage sludge in agriculture stimulated both microbial activity and the number of microorganisms and promoted the activity of alkaline phosphatase and dehydrogenases. Furthermore, the studies of Kayikcioglu et al. [19] and Hamdi et al. [16] found that sewage sludge increased the soil microbial biomass and soil enzyme activity, particularly when high doses of sludge were applied. However, Paz-Ferreiro et al. [11] found that adding 8% sludge to soil significantly decreased the geometric mean of enzyme activities, indicating that the high dose application of sewage sludge harmed soil microorganisms. Similarly, Xue and Huang [20] reported that sludge had high electrical conductivity and a high heavy metal content, which might cause a decline in microbial biomass and enzyme activity in soil ecosystems. Therefore, the responses of soil enzyme activities to the addition of sewage sludge may be related to duration of application, soil type, and incubation conditions [16, 18, 20]. The real-time monitoring the variation of soil enzyme activities after sewage sludge addition was not available. So, further research is necessary to monitor the changes of enzyme activities with sewage sludge addition and to determine whether its application is beneficial for improving degraded soil.

Soil microorganisms decompose complex OM into simpler products by secreting various enzymes into soils. To maintain nutrient requirements, soil microorganisms produce different enzymes that target specific compounds of C, N, or P [21, 22]. The stoichiometry of soil C-, N-, and P-acquiring enzyme reflects the nutrient limitation of microbial activities and the supply of nutrients in the soil [23]. Studies of soil enzyme stoichiometry were useful for understanding the process of nutrient turnover, circulation and balance in soil systems. Zheng et al. [24] found that soil enzyme stoichiometries were significantly associated with soil chemical properties, and chemical fertilizer addition increased the negative relationships between the soil nutrient ratios and enzyme stoichiometries. Yan et al. [25] reported that nitrogen addition decreased N-acquiring enzyme activities and increased P-acquiring enzyme activities, thus decreased enzyme N:P. MSS contains high levels of OM, N, and P, and its land application would alter the relative allocation of C-, N-, and P-acquiring enzyme activities. A better understanding of the soil enzyme stoichiometries after MSS addition would be conducive to evaluate the available nutrients supply in the soil.

The Loess Plateau of China suffered serious soil erosion due to unreasonable land use and scare plant coverage. The Chinese government implement "Grain for Green" program to control soil erosion and improve ecological environment [26]. While no the loess soil on the Loess Plateau was characterized by low OM and nutrient content, this limited the speed and quality of plant restoration in this fragile ecosystem [27]. In this respect, the current study evaluated the effects of adding different doses sludge on soil enzyme activities and enzyme stoichiometry to gain further insights into how adding sewage sludge effects the restoration of degraded soil ecosystems. A laboratory microcosm incubation experiment was conducted to evaluate their effects on the variations of soil enzyme activities and stoichiometries. Usually, the laboratory experiments incubate a small amount of disturber soil in closed bottles, and the structure of the incubated soil is altered and cannot always reflect what actually occurs in the field. As a complement to field studies, laboratory incubation experiments have the advantages of saving time and effort and allowing real-time direct measurements under controlled conditions without the influence of other factors. The aims of the study were

thus to evaluate the changes in soil enzyme activities and stoichiometries during incubation under the addition of different doses sludge. We hypothesized that the soil enzyme activities would significantly increase with sludge addition due to the high OM content. Owing to the significantly higher of C:P and N:P ratios in the sludge compared with loess soil, we further expected that soil enzyme C:P and N:P ratios would significantly increase with incubation time. It is considered that the results of this research will contribute to the resource utilization of sludge in remediating degraded soil ecosystems.

Materials and Methods

Soil and Sewage Sludge Sampling

Soil samples were collected from the surface zone (0-20 cm) of agricultural land in Suide County, Shaanxi Province, China. The region has a continental monsoon climate, with average annual precipitation and a mean annual temperature of 513 mm and 10.2ºC, respectively. The area is located on the northern Loess Plateau, and the soil type is primarily loessial (Calcaric Cambisols, FAO), which is a calcareous soil with low OM content that results in severe soil and water losses and a very fragile ecosystem. The fresh soil samples were sieved through a 2-mm mesh to remove plant roots and other debris.

Municipal sewage sludge was collected from the Xi'an Wastewater Treatment Plant, which processes domestic wastewater. The collected sewage sludge was air-dried, ground, and sieved through 2-mm mesh prior to conduct the incubation experiments.

Table 1 lists the primary chemical properties of the soil samples and municipal sewage sludge.

Experimental Design and Soil Incubation

To study the effects of the addition of different nutrient types on enzyme activities and stoichiometries within the loess soil, a laboratory microcosm incubation experiment was conducted using six different treatments as follows: soil with no added nutrients (CK), soil with three different doses of sewage sludge added, soil with added synthetic N, and soil with added synthetic P. For the experiments with the addition of three different doses of sewage sludge, dried sludge was artificially mixed with soil at doses of 5%, 10%, and 15% (dry weight), respectively. For soil with the addition of N and P, 0.364g CH_4N_2O (equal to 0.167 g N) and 0.384 g Na_2HPO_4 (equal to 0.079 g P) were dissolved in distilled water and sprayed in the 100g soil, and the amount of N and P addition was equal to the treatment of 10% of sludge addition.

The processed soils were rewetted to 60% of field capacity (FC) and then incubated in 1 L jars at 25ºC for 60 days. The moisture content of each soil sample was maintained at 60% FC throughout the incubation period by adding deionized water once every two days. Soil samples were analyzed after 1, 2, 3, 5, 7, 10, 15, 30, 45, and 60 days of incubation, and four replicates of each treatment were analyzed. There were a total of 240 jars (6 treatments×10 times sampling×4 replicates). The soil samples were stored at –20ºC for less than one week and then used to determine enzyme activity [22].

Enzyme Assays

The potential enzyme activities relating to C, N, and P cycling (β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), L-leucine aminopoptidase (LAP), and phosphatase (AP)) were measured following standard fluorometric microplate techniques according to Saiya-Cork et al. [28]. The corresponding substrates and functions of the four enzymes were shown in Table 2. The microplates were incubated in the dark at 25ºC for 3 h, and fluorescence was measured using a microplate reader (SpectraMax iD5, Molecular Devices) with excitation and emission of 365 nm and 450 nm, respectively. After correction using quench control, enzyme activities were expressed in units of nmol h^{-1} g^{-1} soil. The soil enzyme C:N, C:P, and N:P ratios were calculated using ln(BG):ln(NAG+LAP), ln(BG):ln(AP), and ln(NAG+LAP):ln (AP), respectively.

The daily variation of soil enzyme activities were calculated using the following formula,

$$E_v = \frac{E_{t_2} - E_{t_1}}{t_2 - t_1}$$

Where E_v is the daily variation of enzyme activity, E_{t1} and E_{t2} is the enzyme activity at the incubation time of t_1 and t_2.

To better compare treatment effects, the cumulative enzyme activity and cumulative enzyme stoichiometry

Table 1. The basic chemical properties of loess soil and sewage sludge.

	pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	aP (mg kg^{-1})	C:N	C:P	N:P
Loess soil	8.68	3.01	0.36	0.39	0.36	8.14	0.15	8.36	7.72	0.92
Sewage sludge	6.94	121.32	16.74	7.91	6.32	8.47	0.26	7.25	15.33	2.12

Note: SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; aP, available phosphorus.

Table 2. Soil enzymes and their corresponding substrates and functions.

Enzyme	Abbreviation	Substrate	Function
β-1,4-glucosidase	BG	4-MUB-β-D-glucoside	Releases glucose from cellulose
N-acetyl-glucosaminidase nidase	NAG	4-MUB-N-acetyl-β-glucosaminide	Releases N-acetyl glucosamine from oligosaccharides
L-leucine aminopeptidase	LAP	L-leucine-7-amido-4-methylcoumarin	Catalyzes the hydrolysis of leucine and other amini acid residues
Phosphatase	AP	4-MUB-phosphate	Anhydrides of phosphoric acid to release phosphate groups

over the incubation period were calculated using the following formula,

$$E_C = \sum_{i=1}^{n} E_i T_i$$

where E_C is the cumulative enzyme activity or cumulative enzyme stoichiometry, n is the number of incubation days, E_i is the mean enzyme activity of two successive measurements, and T_i is the time between the two measurements [29, 30].

Statistical Analyses

A one-way analysis of variance (ANOVA) was conducted to determine the effects of different treatments or incubation time on soil enzyme activity and enzyme stoichiometries, and the effects of different treatments on cumulative enzyme activities and stoichiometries were determined by one-way ANOVA. Post-hoc means were determined using Duncan post hoc tests using SPSS 16.0, where the significance level of the post hoc test was set at 5%. Changes in soil enzyme activities and stoichiometries according to incubation time were characterized by logarithmic functions.

Results

Effects of Soil Nutrient Addition on Enzyme Activities

BG, NAG+LAP, and AP enzyme activities significantly increased with incubation time (Fig. 1), and such changes could be characterized by a logarithmic function (Table 3). Enzyme activities increased rapidly during the first 30 days of incubation, but the rate of increase then decreased in the following 30 days of incubation. For example, for the CK treatment, the BG, NAG+LAP, and AP enzyme activities increased by 56.81, 244.67 and 161.94 nmol h^{-1} g^{-1} in the first 30 days incubation, while these enzyme activities only further increased by 5.85, 10.27, and 8.44 nmol h^{-1} g^{-1} in the later 30 days of incubation (Table 4). The other treatments showed similar patterns to that of the CK treatment during the 60 day incubation period.

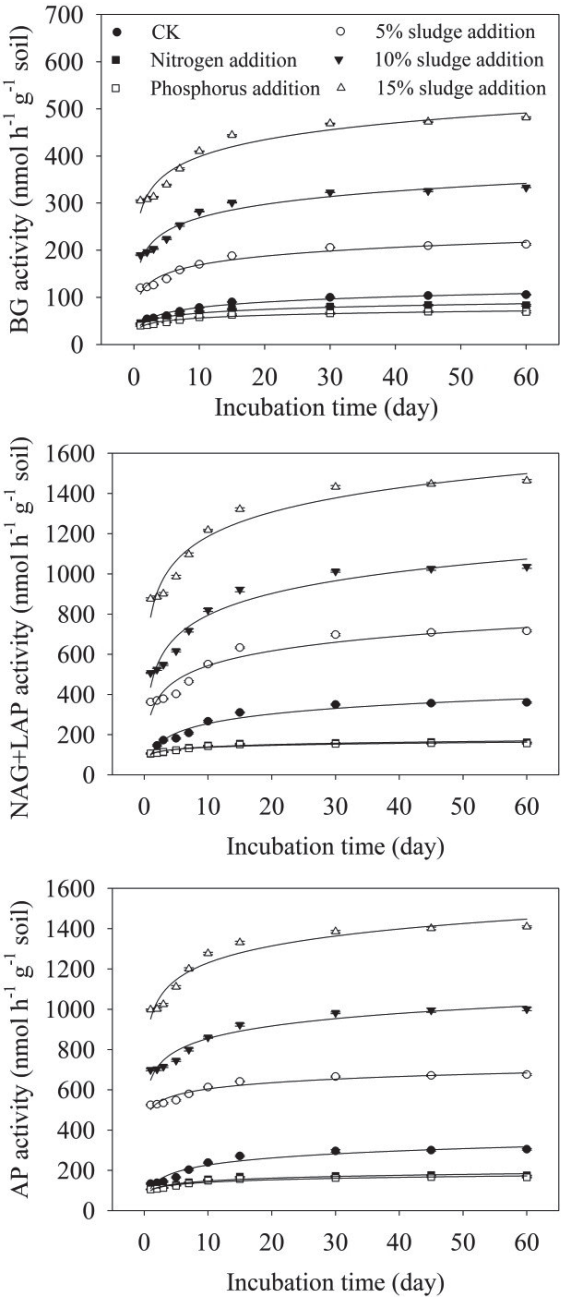

Fig. 1. Effects of sewage sludge and synthetic fertilizer addition on soil enzyme activities over 60-day incubation. BG, β-glucosidase; NAG+LAP, N-acetyl-glucosaminidase and L-leucine aminopoptidase; AP, phosphatase; CK, no fertilizer. Data are presented as mean±standard deviation (n = 4).

Table 3. Simulating equations of soil enzyme activities with incubation time.

Soil enzymes	Treatment	Equation	*R*	*F*	*P*
BG	CK	y = 40.29 + 16.54ln(x)	0.99	362.82	<0.001
	5% sludge addition	y = 106.86 + 26.86ln(x)	0.98	170.96	<0.001
	10% sludge addition	y = 174.37 + 40.95ln(x)	0.98	156.62	<0.001
	15% sludge addition	y = 279.32 + 51.77ln(x)	0.97	123.55	<0.001
	Nitrogen addition	y = 41.01 + 11.10ln(x)	0.98	161.57	<0.001
	Phosphorus addition	y = 36.57 + 8.45ln(x)	0.98	170.92	<0.001
NAG+LAP	CK	y = 96.71 + 68.71ln(x)	0.98	222.31	<0.001
	5% sludge addition	y = 298.20 + 106.19ln(x)	0.96	96.04	<0.001
	10% sludge addition	y = 435.61 + 155.87ln(x)	0.97	141.69	<0.001
	15% sludge addition	y = 784.74 + 174.43ln(x)	0.97	131.65	<0.001
	Nitrogen addition	y = 100.74 + 16.56ln(x)	0.97	116.94	<0.001
	Phosphorus addition	y = 103.32 + 14.42ln(x)	0.97	122.54	<0.001
AP	CK	y = 109.73 + 50.61ln(x)	0.97	117.76	<0.001
	5% sludge addition	y = 501.62 + 44.47ln(x)	0.97	118.20	<0.001
	10% sludge addition	y = 647.84 + 89.86ln(x)	0.97	126.37	<0.001
	15% sludge addition	y = 951.27 + 121.23ln(x)	0.97	127.52	<0.001
	Nitrogen addition	y = 99.83 + 20.48ln(x)	0.97	113.19	<0.001
	Phosphorus addition	y = 100.04 + 17.66ln(x)	0.97	122.41	<0.001

Note: BG, β-glucosidase; NAG+LAP, N-acetyl-glucosaminidase and L-leucine aminopoptidase; AP, phosphatase; CK, no fertilizer.

Table 4. The changes of soil BG, NAG+LAP, and AP enzyme activities in the first 30 days and later 30 days incubation.

Enzyme type	Time Period	CK	5% addition	10% addition	15% addition	N addition	P addition
BG	1-30d	56.81±1.45d	85.50±2.11c	132.44±1.26b	163.73±1.30a	35.01±1.73e	25.99±2.25f
	30-60d	5.85±0.99b	6.98±1.61b	10.80±0.42a	12.53±2.63a	2.99±1.46c	3.00±1.37c
NAG+LAP	1-30d	244.67±4.19d	334.84±8.21c	506.25±7.64b	556.73±5.82a	53.25±3.94e	46.81±1.84e
	30-60d	10.27±6.54cd	18.89±6.89bc	22.90±8.23ab	31.31±7.37a	3.15±3.26d	2.08±4.08d
AP	1-30d	161.94±8.22c	140.38±3.73d	283.42±4.92b	387.55±8.04a	66.00±3.00e	57.00±2.95f
	30-60d	8.44±5.36bc	9.95±7.55bc	18.63±11.78ab	24.03±6.57a	3.01±1.82c	3.00±2.27c

Note: BG, β-glucosidase; NAG+LAP, N-acetyl-glucosaminidase and L-leucine aminopoptidase; AP, phosphatase; CK, no fertilizer. Data are presented as mean±standard deviation (n = 4). Different lowercase letters indicate significant difference between different treatment (P<0.05).

The daily variation of BG, NAG+LAP, and AP enzyme activities significantly increased in the first 7 days, and then significantly decreased with incubation time (Fig. 2). Compared with CK, the daily variation of BG, NAG+LAP and AP enzyme activities increased with sludge addition amount, while, N and P addition decreased daily variation of enzyme activities. Compared with CK, the daily variation of BG, NAG+LAP and AP activity on the 7th day incubation increased 277.7%, 308.4%, and 134.3%, respectively, while the daily variation of BG, NAG+LAP and AP activity decreased by 11.1%, 51.0%, and 65.8%, respectively, with N addition, and by 44.4%, 66.0%, and 68.4%, respectively, with the P addition.

The addition of sludge and synthetic fertilizer significantly affected the cumulative enzyme activities of BG, NAG+LAP, and AP (Fig. 3). Sludge addition significantly increased cumulative enzyme activity. Compared with the CK treatment, sludge addition significantly increased the cumulative enzyme activities of BG, NAG+LAP, and AP by 378.2%, 323.8%, and 389.8%, respectively, with 15% sludge addition treatment (Fig. 3); the increments increased with the dose of sludge added. N and P addition significantly

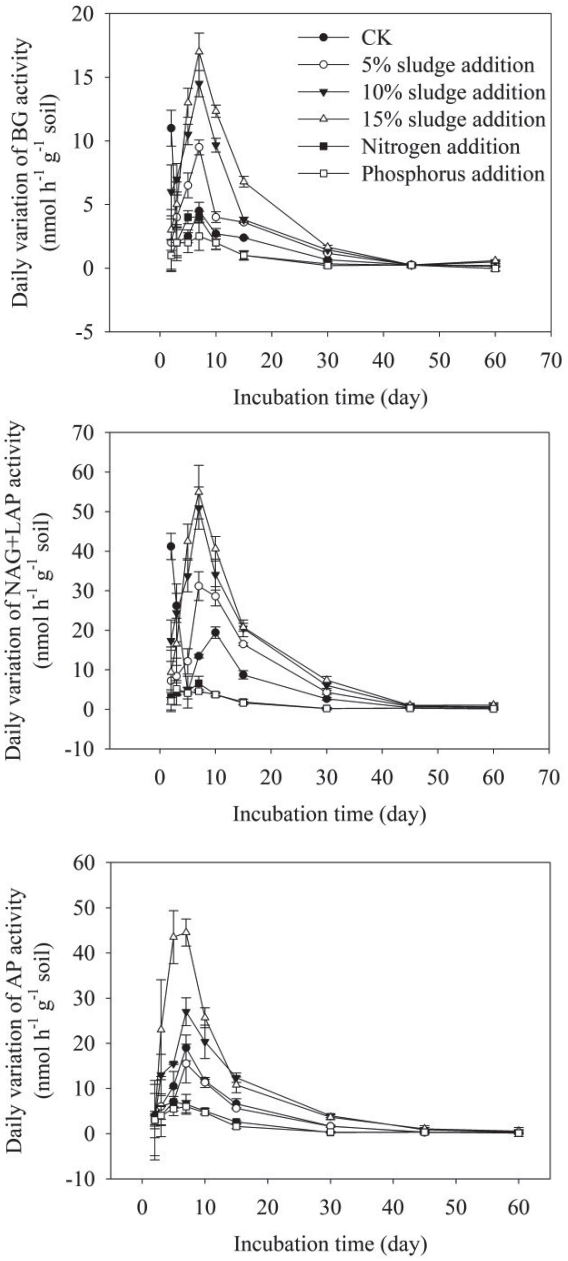

Fig. 2. Effects of sewage sludge and synthetic fertilizer addition on daily variation of soil enzyme activities. BG, β-glucosidase; NAG+LAP, N-acetyl-glucosaminidase and L-leucine aminopoptidase; AP, phosphatase; CK, no fertilizer. Data are presented as mean±standard deviation (n = 4).

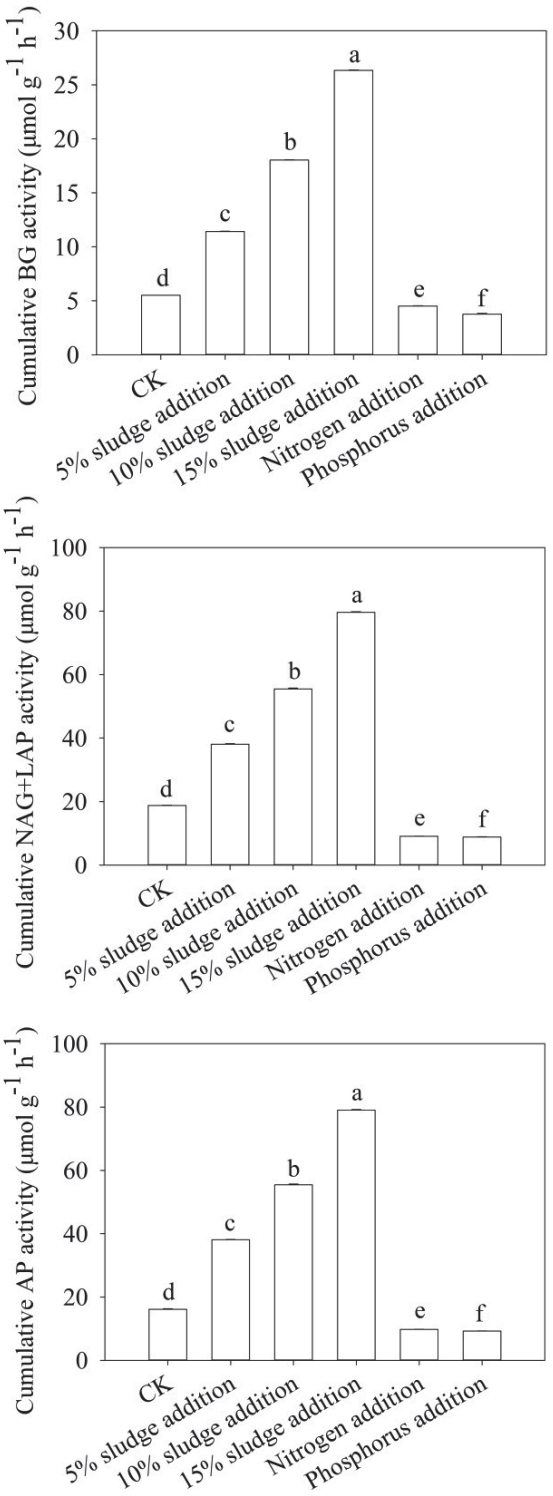

Fig. 3. Effects of sewage sludge and synthetic fertilizer addition on cumulative enzyme activities over 60-day incubation. BG, β-glucosidase; NAG+LAP, N-acetyl-glucosaminidase and L-leucine aminopoptidase; AP, phosphatase; CK, no fertilizer. Letters above the mean±standard deviation (n = 4) indicate significant differences among different treatments at $P<0.05$ using Duncan post hoc tests.

decreased cumulative enzyme activity. Compared with CK, the cumulative enzyme activities of BG, NAG+LAP, and AP decreased by 18.0%, 51.4%, and 39.4%, respectively, with the N addition, and by 31.8%, 53.0%, and 42.7%, respectively, with the P addition.

Effects of Soil Nutrient Addition on Enzyme Stoichiometries

Incubation time had a significant influence on the enzyme C:N, C:P, and N:P ratios (Fig. 4), and the changes in enzyme C:N, C:P, and N:P ratios with incubation time could be characterized by a logarithmic function (Table 5). The enzyme C:N ratios decreased with incubation time in the CK and sludge addition

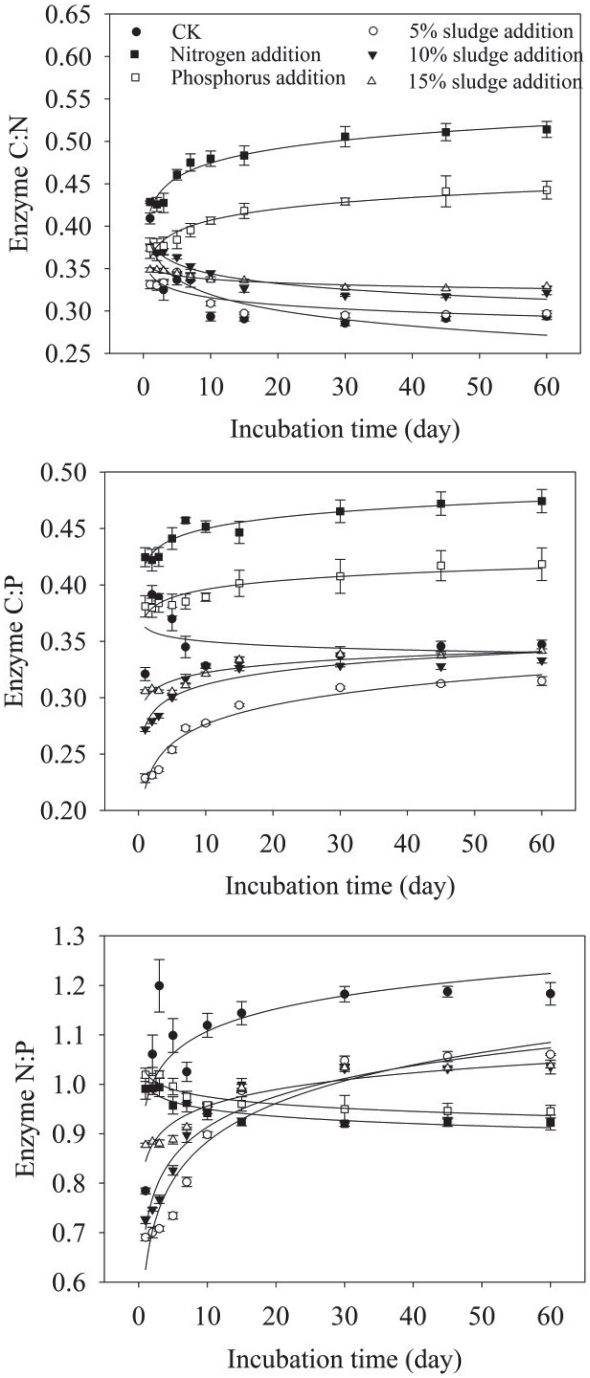

Fig. 4. Effects of sewage sludge and synthetic fertilizer addition on soil enzyme stoichiometries over 60-day incubation. Enzyme C:N, ln(β-glucosidase):ln(N-acetyl-glucosaminidase + L-leucine aminopoptidase); Enzyme C:P, ln(β-glucosidase):ln(phosphatase); Enzyme N:P, ln(N-acetyl-glucosaminidase + L-leucine aminopoptidase): ln(phosphatase); CK, no fertilizer. Data are presented as mean±standard deviation (n = 4).

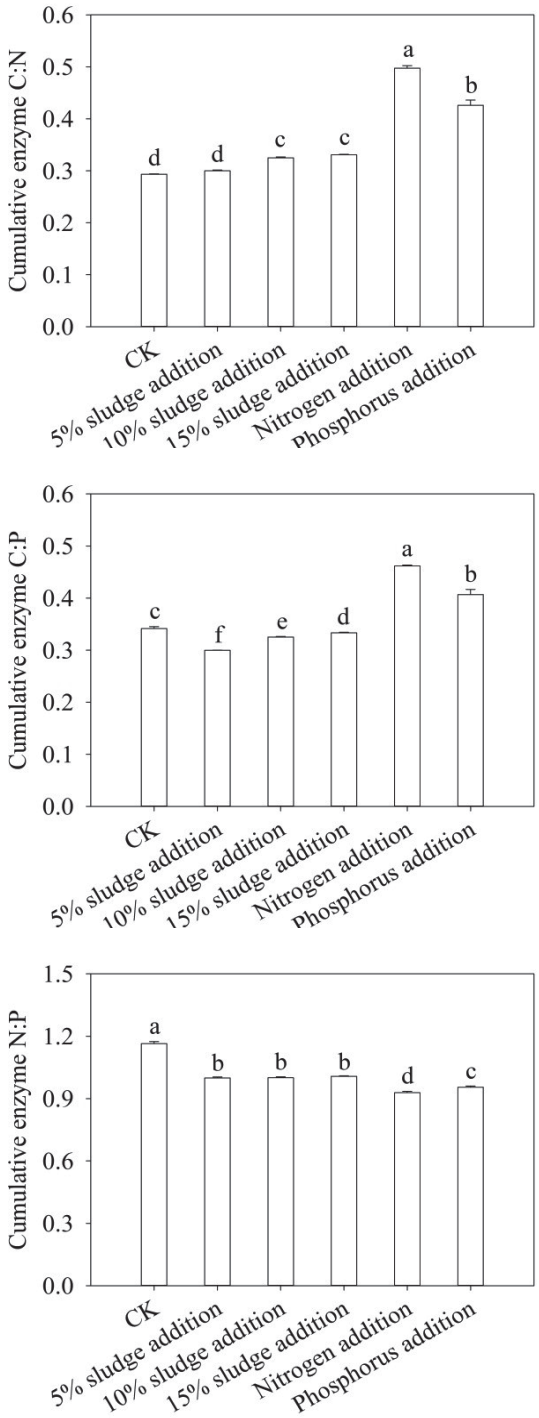

Fig. 5. Effects of sewage sludge and synthetic fertilizer addition on cumulative enzyme stoichiometries over 60-day incubation. Enzyme C:N, ln(β-glucosidase):ln(N-acetylglucosaminidase + L-leucine aminopoptidase); Enzyme C:P, ln(β-glucosidase):ln(phosphatase); Enzyme N:P, ln(N-acetyl-glucosaminidase + L-leucine aminopoptidase): ln(phosphatase); CK, no fertilizer. Letters above the mean±standard deviation (n = 4) indicate significant differences among different treatments at $P<0.05$ using Duncan post hoc tests.

treatments, but increased with incubation time in the N and P addition treatments. In the CK treatment, the enzyme C:P ratios decreased with incubation time but increased with incubation time in the three different sludge addition dose treatments and in the N and P addition treatments. In the CK and sludge addition treatments, enzyme N:P ratios increased with incubation time but decreased in the N and P addition treatments.

Table 5. Simulation equation of soil enzyme stoichiometry with incubation time.

Enzyme stoichiometry	Treatment	Equation	*R*	*F*	*P*
Enzyme C:N	CK	y = 0.38 – 0.03ln(x)	0.89	29.22	<0.001
	5% sludge addition	y = 0.34 – 0.01ln(x)	0.80	14.45	<0.01
	10% sludge addition	y = 0.38 – 0.02ln(x)	0.96	92.68	<0.001
	15% sludge addition	y = 0.35 – 0.01ln(x)	0.97	116.19	<0.001
	Nitrogen addition	y = 0.42 + 0.02ln(x)	0.97	121.49	<0.001
	Phosphorus addition	y = 0.36 + 0.02ln(x)	0.97	149.61	<0.001
Enzyme C:P	CK	y = 0.36 – 0.01ln(x)	0.30	0.79	0.40
	5% sludge addition	y = 0.22 + 0.02ln(x)	0.98	201.03	<0.001
	10% sludge addition	y = 0.27 + 0.02ln(x)	0.93	50.90	<0.001
	15% sludge addition	y = 0.30 + 0.01ln(x)	0.93	48.00	<0.001
	Nitrogen addition	y = 0.42 + 0.01ln(x)	0.94	65.07	<0.001
	Phosphorus addition	y = 0.37 + 0.01ln(x)	0.93	54.55	<0.001
Enzyme N:P	CK	y = 0.96 + 0.07ln(x)	0.71	8.16	<0.05
	5% sludge addition	y = 0.63 + 0.11ln(x)	0.96	89.84	<0.001
	10% sludge addition	y = 0.71 + 0.09ln(x)	0.97	113.42	<0.001
	15% sludge addition	y = 0.84 + 0.05ln(x)	0.95	74.66	<0.001
	Nitrogen addition	y = 1.00 – 0.02ln(x)	0.93	54.12	<0.001
	Phosphorus addition	y = 1.02 – 0.02ln(x)	0.95	66.79	<0.001

Note: Enzyme C:N, ln(β-glucosidase):ln(N-acetyl-glucosaminidase + L-leucine aminopoptidase); Enzyme C:P, ln(β-glucosidase):ln(phosphatase); Enzyme N:P, ln(N-acetyl-glucosaminidase + L-leucine aminopoptidase): ln(phosphatase); CK, no fertilizer

The addition of sludge and synthetic fertilizer significantly altered the enzyme C:N, C:P, and N:P ratios (Fig. 5). Compared with CK, the cumulative enzyme C:N ratios increased by 13.8% after 15% sludge addition, and the enzyme C:P and N:P ratios decreased by 2.9% and 12.9%, respectively (Fig. 5). Compared with CK, the addition of synthetic N and P significantly increased the cumulative enzyme C:N and C:P ratios by 72.4% and 35.3%, 48.3% and 20.6%, respectively, but decreased enzyme N:P ratios by 19.8% and 18.1%, respectively.

Discussion

Soil enzymes produced by microorganisms are agents for the decomposition of soil OM. Variations in the composition and structure of soil microbial communities are closely associated with soil nutrient availability and the physical conditions of the soil, such as moisture content, texture, and temperature [31, 32]. In the studies of Zhou et al. [33] and You et al. [34], optimum soil moisture and temperature conditions during the incubation period remarkably increased soil microbial biomass and activity, which also increased enzyme activities in the soil. In the present study, soil enzyme activities in the six treatments increased rapidly in the first 30 days of incubation and then the increment obviously decreased, which may indicate that the increased soil available nutrient content inhibited the secreting of enzymes by soil microorganisms [24]. Xue and Huang [20] and Mingorance et al. [35] also reported that higher doses of added sludge significantly decreased the microbial biomass and respiration of soil, which resulted in decreased enzyme expression.

Sewage sludge is typically rich in OM and contains high levels of N and P [7], which cannot be directly absorbed by plant roots or by the soil microbial community. However, the addition of sludge to soil increases the amount of enzymes secreted by microorganisms when they degrade OM [36]. Therefore, sludge meets the demands of microorganisms for available nutrients in degraded soils, specifically in soils with a low OM content [15]. Siebielec et al. [18] reported a significant increase in soil enzyme activity after sewage sludge was added to soil, and Gryta et al. [37] demonstrated that organic waste amendment supports soil microbial diversity and leads to enhanced functional potentials, which are expressed as enzyme activity. In addition to the positive effect of sewage sludge on the enzyme activity of soils, the results of this study showed that enzymatic activity increased with the dose of sludge added, those results supported our first hypothesis, and these results were consistent with those of Mingorance et al. [35] and Sciubba et al. [38], who reported that soil enzyme activities increased in line with sludge application rates. However, an

enzyme can remain catalytic in the medium, so when its substrate is added in excess in laboratory, it would result in high enzyme activity; while its substrate is not necessarily present in the medium in reality. So, further researches should be conducted in the field conditions to better capture the changes of soil enzyme activities with sludge addition treatments.

Many studies have demonstrated that soil enzyme activity is a highly sensitive and reliable indicator of soil biological activity that can be used to evaluate soil fertility levels [11, 12, 14]. The results of this study showed that the addition of sludge increased cumulative soil enzyme activities, but the additions of synthetic N and P addition decreased them. Similarly, Zhang et al. [39] found that manure fertilizer application increased hydrolytic enzyme activities, while inorganic fertilizers decreased these enzyme activities. Synthetic N and P are available nutrients for plant and microbial community growth, increases in available soil nutrients inhibit enzyme secretion by the soil microbial community [24]. For example, Zhou et al. [40] reported that inorganic N and P additions decreased the NAG activity by 8.0% and 13.6%, respectively and decreased the AP activity by 13.6% and 17.6%, respectively. Dong et al. [41] found that urea addition decreased the activity of LAP by 99.0% in extremely degraded grassland of northern China. Fang et al. [42] also reported that the addition of inorganic P significantly decreased NAG and AP activity by 50.4% and 45.2% in a subtropical forest. These findings are consistent with resource allocation theory, which states that soil microorganisms produce enzymes to degrade OM to obtain resources that are most in demand, and they decrease enzyme production when simple resources are available [43, 44]. In this study, the addition of sludge significantly promoted an increase in soil enzyme activities, which demonstrates that it is suitable for remediating degraded soil and improving soil fertility.

Soil microbial communities prefer to invest their resources in synthesizing enzymes that facilitate the acquisition of the most limiting nutrients [45]; therefore, the enzymes produced by microorganisms generally align with carbon and other nutrients supplied to the soil. Soil enzyme stoichiometry, where the relative abundance of C, N, and P-acquiring enzyme activity is used to characterize the status of C, N, and P, highlights the limitations of the microbial community within soil [23, 25]. In our study, the soil enzyme C:N ratios decreased but C:P and N:P ratios increased during incubation under the sludge addition treatments, which supported our secondary hypothesis. Those results demonstrated that the addition of sludge has a particular effect on increasing the N-acquiring enzymes, and its application could thus provide an available N nutrient supply to soil. In contrast, the addition of synthetic N and P increased the soil enzyme C:N and C:P ratios but decreased enzyme N:P ratios, and this result is primarily related to the decrease in cumulative N and P-acquiring enzymes, which indicates that the addition of synthetic fertilizer has a direct effect on decreasing N and P limitations for the soil microbial community. However, Chen et al. [46] found that the addition of inorganic N and P had no significant effect on soil enzymes involved in C, N, and P cycling or their stoichiometric ratios in Chinese forest and grassland ecosystems. The difference between results thus highlights the fact that uncertainties remain in our understanding of the responses of soil enzyme stoichiometric ratios to nutrient addition. Investigations of soil properties and the type, duration, and amount of nutrient added have all been proposed to explain the effects of the addition of soil nutrients on soil enzyme activities and stoichiometries [19, 47]. Such investigations are also required using different types, amounts, and durations of sludge to determine the associated effects on soil enzyme activities and stoichiometries, and a comprehensive evaluation of their relationships with soil biochemical properties should be made to determine the remediation effects of adding sludge to degraded soil.

It should be noted that the results of the present research was obtained based on laboratory incubation experiment, which might not reflect what actually occurs in the field conditions. Therefore, further researches should be conducted in the field to further evaluate the effects of municipal sewage sludge addition on soil enzyme activities and to quantify their correlation to the laboratory incubation experiments.

Conclusions

In this study, the effects of adding synthetic fertilizer and sludge on soil enzyme activities were evaluated using a 60-day laboratory incubation experiment. Soil enzyme activities logarithmically increased with incubation time in both the synthetic fertilizer and sludge addition treatments. Compared with non-amended soil, the cumulative enzyme activities after 60-day incubation significantly improved with increasing doses of sludge but decreased under the synthetic fertilizer addition treatments. Soil enzyme stoichiometry showed a logarithmic relationship with incubation time. Enzyme C:N decreased but C:P and N:P increased during incubation with sludge addition. Those results demonstrated that sludge addition would beneficial for soil nutrient supply than synthetic fertilizer addition due to the increased soil enzyme activities related to C, N, and P cycling, and sludge addition would especially benefit for alleviating soil C and N nutrient limitation.

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Conflict of Interest

The authors declare no conflict of interest.

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