Effects of Goat Manure-Biochar Compost on Soil Microbial Activities Relating Soil Carbon Sequestration

ผลของปุ๋ยหมักถ่านชีวภาพมูลแพะต่อกิจกรรมจุลินทรีย์ในดินที่สัมพันธ์กับการกักเก็บคาร์บอนในดิน

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บทคัดย่อ: การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการตอบสนองของจุลินทรีย์ในดินที่เกี่ยวข้องกับกิจกรรมของเอนไซม์หมุนเวียนคาร์บอนต่อการใช้ปุ๋ยเคมีหรือวัสดุอินทรีย์ที่สัมพันธ์กับการสะสมคาร์บอนอินทรีย์ในดิน กรรมวิธีทดลองประกอบด้วย 4 กรรมวิธี ดังนี้ 1) ควบคุม (ไม่มีการใส่ปุ๋ยอินทรีย์และปุ๋ยเคมี) 2) ปุ๋ยเคมี (CF) (N-P-K = 15-15-15) 3) มูลแพะ (GM) และ 4) ปุ๋ยหมักถ่านมูลแพะ (GMBC) ผลการศึกษาพบว่า ภายหลัง 3 ชั่วโมงของการใส่วัสดุอินทรีย์ ค่าการหายใจของจุลินทรีย์ต่อมวลชีวภาพจุลินทรีย์คาร์บอน (qCO_2) สูงสุดในกรรมวิธี GM ตามด้วยกรรมวิธี GMBC มีค่าเท่ากับ 0.3617 และ 0.26 mg CO_2-C/g MBC/day ตามลำดับ การปลดปล่อย CO_2-C สะสมมีค่าสูงสุดในกรรมวิธี GM ภายหลัง112 วันหลังการใส่ (2,578 mg CO_2-C/m^2/day) ตามด้วยกรรมวิธี GMBC CF และกรรมวิธีควบคุม มีค่าเท่ากับ 1,481 685 และ 613 mg CO_2-C/m^2/day ตามลำดับ ทั้งนี้ภายหลัง 21 วันหลังการใส่พบกิจกรรมจำเพาะของเอนไซม์บีตา-กลูโคสิเดสเพิ่มขึ้นสูงภายใต้กรรมวิธี GM มีค่าเท่ากับ 878 µg *p*-nitrophenol/g MBC/h ขณะเดียวกันพบว่า กิจกรรมจำเพาะของเอนไซม์ฟีนอลออกซิเดสมีค่าสูงสุดในวันที่ 21 และ77 (0.044 และ 0.036 µmol dicq/g MBC/h) ทั้งนี้พบสหสัมพันธ์เชิงบวกระหว่างกิจกรรมของบีตา-กลูโคสิเดส และการปลดปล่อย CO_2 (r = 0.6648***) รวมถึงมวลชีวภาพจุลินทรีย์คาร์บอน (r = 0.6438***) การศึกษานี้ชี้ให้เห็นว่ารูปของคาร์บอนจากวัสดุอินทรีย์ส่งผลต่อกิจกรรมของเอนไซม์หมุนเวียนคาร์บอนในดินมากกว่าไนโตรเจนที่เป็นองค์ประกอบ ปุ๋ยหมักถ่านมูลแพะเหมาะสมใช้การปรับปรุงดินทรายจากการมีคาร์บอนทั้งรูปที่ง่ายและยากต่อการย่อยสลาย และมีค่า C/N ที่เหมาะสมต่อการนำไปใช้โดยจุลินทรีย์

คำสำคัญ: คาร์บอนอินทรีย์ในดิน เอนไซม์ในดิน ปุ๋ยหมักถ่านมูลแพะ บีตา-กลูโคสิเดส ฟีนอลออกซิเดส

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Abstract: This research aimed to study the responses of soil microorganisms associated with carbon cycling enzyme activity to the application of chemical fertilizers or organic materials concerning soil organic carbon accumulation. The experiment consisted of four treatments: 1) control (unamended soil); 2) chemical fertilizer (N-P-K = 15-15-15) (CF); 3) goat manure (GM); and 4) GM-biochar compost (GMBC). The study found that after 3 hours of organic input incorporation, the soil metabolic quotient (qCO_2) was the highest in the GM treatment, followed by GMBC treatments (0.3617 and 0.26 mg CO2-C/g MBC/day, respectively). The cumulative CO_2-C evolution was the highest in the GM treatment after 112 days of incorporation (2,578 mg CO_2-C/m2/day), followed by the GMBC, CF, and control treatment (1,481, 685, and 613 mg CO_2-C/m2/day, respectively). The specific ß-glucosidase activity increased sharply with the GM treatment (878 µg p-nitrophenol/g MBC/h) after 21 days of organic inputs. Therefore, the specific phenoloxidase activity in the GMBC treatment had the highest enzyme activity of 0.044 and 0.036 µmol dicq/g MBC/h on days 21 and 77 ($P < 0.05$). Absolute ß-glucosidase was positively correlated with CO_2 emissions (r = 0.6648***), while microbial biomass C was also governed by the activity of this enzyme (r = 0.6438***). Our study indicated that the C form of organic inputs had a stronger influence on C-cycling enzyme activities than the influence of nitrogen. The goat manure biochar composts were the best suited for improving soil the organic C content in sandy soil, as it contains both easily and resistant C compounds coupled with an appropriate C/N ratio for microbial use.

Keywords: Soil organic carbon, soil enzymes, goat manure-biochar compost, ß-glucosidase, phenoloxidase

Introduction

Soil microorganism activity is the key factor determining soil fertility and carbon (C) sequestration in soil. Therefore, it is crucial to investigate how the metabolic activity of soil microorganisms responds to the differentiation of C sources (e.g., easily and resistant C compounds), particularly in highly weathered sandy soil where original nitrogen (N) concentrations are low. The activity of soil C-cycling enzymes has received increasing attention as an indicator of soil fertility, particularly as influenced by the C availability of organic inputs (Kamolmanit *et al*., 2013). Although it has been reported that the application of contrasting quality organic inputs can influence soil microbial activities concerning enzyme activities in soil, the pattern of changes in specific enzyme activities is still unclear. Generally, the activities of easily decomposable C compound-degrading enzymes are positively related to C and also N concentrations in organic inputs or soils (Mazzon *et al*., 2018). Studies have shown that adding N has a potent influence on the inhibition of phenoloxidase activity, but the opposite is true for the labile C compounds (Carreiro *et al*., 2000). However, the positive effects of N on phenoloxidase activity resulted in the depletion of soil organic matter (SOM), leading to the decline of indigenous soil organic carbon (SOC) (Kuzyakov, 2010; Sinsabaugh, 2010). Since C-cycling enzyme activity plays a crucial role in residue decomposition, we chose both labile- and resistant-C compound degrading enzymes as a comparative factor, as it is a significant component of soil function, particularly in highly weathered soil.

Recycling animal manure into low-cost organic fertilizers has a beneficial effect on the growth and productivity of various crops and promotes the restoration of the ecological and economic functions of the soil. Another approach to improve some properties of goat manure is to create biochar. The resulting manure-biochar created yields with a high percentage of fixed C (Kamolmanit *et al.,* 2018). Many herbivore manures have been investigated as materials for biochar production (Situmeang *et al*., 2019; Tsai *et al*., 2012). However, compared to goat manure, other types of manure such as poultry and pig manure contain high levels of plant macronutrients and high C/N ratios, so farmers prefer to incorporate them directly into the soil or make compost. Fresh goat manure reportedly has a fixed carbon content of 129 g/kg, which can be used as feedstock for the production of biochar (Kamolmanite and Lawongsa. 2021). In 2022, the Department of Livestock Development in Thailand published data on goat husbandry, which revealed over 1,500,000 goat heads (Department of Livestock Development, 2022). According to Osuhor *et al*. (2002), the mean manure production per head per day was 0.38 kg. It's possible to use goat manure for biochar production since it can yield around 570,000 per head per day Animal manure biochar can also increase C stabilizers and plant nutrients more than fresh manure (Kanouo *et al*., 2018; Kiran *et al*., 2017; Sarfaraz *et al*., 2020; Zwetsloot *et al*., 2016). Biochar is a C-rich material with a more stable structure than natural materials and leads to soil stability compared with other organic C materials when applied to the soil approach for soil C sequestration (Kuzyakov *et al*., 2009; Skjemstad *et al*., 2002; Zimmerman *et al*., 2011). However, insight into the decomposition process during biochar product decomposition and how C availability exerts an influence on the activities of C-cycling enzymes are lacking. There is limited information on how the use of goat manure biochar composts as organic fertilizer is involved in the microbial decomposition process related to the pattern of soil C storage. Thus, in this study, we hypothesized that C sequestration in form of microbial biomass was controlled by C availability and concentrations of N compounds in organic inputs, particularly in goat manure-biochar compost. This study aimed to investigate the responses of soil microorganisms to fertilizer or organic inputs through the expression of microbial activities, particularly the relationship between C-cycling enzyme activities and microbiological properties involved in C dynamics and its accumulation.

Materials and Methods

Soil and organic materials

The soil was taken from a field experiment at the Agricultural Training and Research Center, Nakhon Ratchasima Rajabhat University, Thailand. The soil texture was sandy loam comprising sand, silt, and clay particles at 73.4, 13.6, and 13 %, respectively. At 0-15 cm depth, the initial topsoil characteristics were soil density 1.58 g/cm^3, pH 5.3, soil organic C 2.2 g/kg, total N 0.18 g/kg with a soil C/N ratio of 12.22, available P 10.3 mg/kg, and exchangeable K 20.79 mg/kg. Sunflowers (*Helianthus annuus* L.) have dominated the plants grown at the site each year since late October 2015. This year will be the 7th sunflower planting in which chemical fertilizer was applied, as well as the first year with organic input incorporation. The organic input treatments of the experiment for the first year was applied in late October, 2021.

The goat manure (GM) biochar production was pyrolyzed by modern kiln sizes [3.5 m (width) x 5 m (length) x 2 m (height)]. In a preliminary study, the pyrolysis temperature of the kilns tested ranged from 350 - 450 °C. We prepared compost from rice straw, goat manure, and GM biochar at an initial C/N ratio of 30 : 1. Some properties of the rice straw used in the composting process were: pH (materials: water = 1 : 10) 5.85, organic C 520 (g/kg), total N 4.2 (g/kg) with a C/N ratio of 123.8. The mixed ratio was calculated based on the percentage of total organic carbon (TOC), total nitrogen (TN), and the moisture content of the material. The rice straw, goat manure, and GM biochar amount were 150, 650, and 200 kg (wet weight), respectively, by mixing 1,000 kg of composting material with a microbial activator. Mix the organic material homogeneously and take a sample to calculate the amount of water to be added up to 60 % of the dry weight. Turn the compost pile an average of twice a week. Adjust the moisture control when the temperature is at maturity for 2 weeks. Continue composting until 49 days. Both goat manure and goat manure biochar compost were added directly to the plot without sifting, but had to sort out branches, stones, and other contaminants.

Field experiment set-up and maintenance

The experiment was carried out along with the sunflower planting. The experiment had four treatments organized in a randomized complete block design (RCBD) with three replications, consisting of 1) control (unamended soil), 2) chemical fertilizer (CF), 3) goat manure (GM), and 4) GM-biochar compost (GMBC). In this study, seedlings of sunflower were transplanted into plots with a plant spacing of 45 cm and a row spacing of 70 cm, then rows were divided for treatment and fertilizer was applied along the planting row. For the fertilized plots, chemical fertilizer (N - P - K) (15 - 15 - 15) was applied to the soil at the rate of 312.5 kg/ha by dividing the chemical fertilizer twice, the first time before transplanting and the second time when the sunflower has an average of 5 - 7 leaves. Organic inputs were used in treatments 3 and 4 at a rate of 3,125 kg/ha, 20 days after transplanting, with only single sprinkle along the planting row. All materials were evenly distributed on the soil surface in a 0.16 ha plot and subsequently ploughed into the soil to a 15 cm depth. Soil samples were randomly collected between rows within each treatment at 0 (3 h), 7, 21, 49, 63, 77 and 112 days after incorporation of all inputs.

Soil and plant analyses

The soil pH (1 : 2.5, soil: water) was determined using a pH meter. Organic C was oxidized using dichromate ($K_2Cr_2O_7$), followed by titration with ferrous sulfate ($FeSO_4$) (Black, 1965). Total N was determined using the Kjeldahl method (Alef and Nannipieri, 1995). Mineral N (NH_4^+-N and NO_3^--N) was measured by extracting the fresh soil samples and calorimetrically determined via a flow injection analyzer according to the Tecator Application Notes ASN 65-31/84 and ASN 65-32/84 (Emteryd, 1989). Microbial biomass C was measured through the chloroform fumigation-extraction method (Amato and Ladd, 1988). The soil was extracted with 0.5 *M* K_2SO_4 and then oxidized with $K_2Cr_2O_7$. The C content in the microbial biomass significantly differed in the fumigated and unfumigated soil samples, using a *k*EC factor of 0.33 (Sparling and West, 1988). A brief description and the calculation of CO_2-C

evolution were conducted as follows: Carbon dioxide was determined using the alkaline trapped method in which a glass bottle containing 1 *N* NaOH was placed in a polyethylene glycol container (ø = 20 cm). Trapped CO_2 was determined by titration with 0.5 *M* HCl (Rowell, 1994). The evolution rate of CO_2-C was expressed in mg/kg soil/day or mg/m^2/day. The activity of ß-glucosidase was measured, as described by Alef and Nannipieri (1995) using 25 m*M* *p*-nitrophenyl-ß-d-glucopyranoside as a substrate. The specific activity was expressed as µg p-nitrophenol/g MBC/h. The activity of phenoloxidase was measured, as described by Hendel *et al*. (2005); using 5 m*M* L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate. The specific activity was expressed as µmol 2,3-dihydroindole-2-carboxylate (dicq)/g MBC/h. Total P and total K were digested by nitric-perchloric acids. Phosphorus concentration was developed with a vanadomolybdate solution and measured at a wavelength of 420 nm with spectrophotometer (Olsen and Sommers, 1982). Potassium concentration was analyzed by an atomic absorption spectrophotometer. Biochar analyses for proximate valuation were carried out according to ASTMD 1762-84 (ASTM International, 2007) in which moisture analysis was determined in the samples at 105 °C until constant weight. Volatile matter (VM) was achieved by calcining each treatment at 950 °C for 6 min. Ash content was measured by burning the sample at 750 °C for 6 hours, during which the carbon was constant (fixed C).

Statistical analysis

One factor analysis of variance (ANOVA) along with least significant differences (LSD) was used to analyze the treatment effects on the soil and microbial parameters ($P < 0.05$) (Statistix 8.0). Pearson's correlation analysis was conducted to evaluate the relationships between enzyme activities and the other parameters.

Results

For biochar production, the pyrolysis temperature of the incinerator was observed to change with a slight increase in temperature in the late production lot. Goat manure biochar produced approximately 36.2 % less volatile matter than the fresh one, but increased ash and fixed C by 47.2 and 2.32 %, respectively. About 215 kg of GM-biochar (GMB) with a turnover percentage of 33 % was obtained from a total of 650 kg of dried goat manure. The pH of GM-biochar compost indicated that it was slightly basic (8.17). This manure-derived biochar was also high in P and K compared with the fresh manure, notably, the N contents decreased (Table 1). After 112 days after transplanting, the highest pH was found by the GMBC treatment at 6.91, followed by the GM and control treatment at 6.67 and 6.60, respectively. The chemical fertilizer treatment resulted in the lowest soil pH at 6.21. The addition of organic inputs resulted in the highest SOC content in the GM treatment (3.04 g/kg), followed by the GMBC treatment (2.88 g/kg), while the chemical fertilizer treatment had the lowest SOC content (1.65 g/kg) compared to the organic input-treated treatments ($P < 0.05$). Although the soil treated with chemical fertilizer had lower SOC than the control soil on day 112 of the investigation, no statistically significant difference was observed. (Table 2).

Table 1. Organic material properties used in the experiment

Parameter	Goat manure (GM)	GM-biochar (GMB)	GM-biochar compost (GMBC)
pH (materials: water = 1: 10)	7.79	8.68	8.17
Organic C (g/kg)	316.5	372.4	195
Total N (g/kg)	13.6	9.8	8.5
C/N ratio	23.30	38	22.94
Total P (g/kg)	1.8	2.5	2.1
Total K (g/kg)	26	103.6	54
Volatile matter (%)	79.12	50.48	nd
Ash (%)	20.03	47.2	nd
Fixed C (%)	0.85	2.32	nd

nd: not determined

Table 2. Soil chemical properties (<1 mm) after 112 days of organic inputs incorporation

Parameter	Treatment								$\|P\|$	SED
	Control		CF		GM		GMBC			
pH (soil : water = 1: 2.5)	6.59	ab	6.21	b	6.67	a	6.91	a	*	0.19
NH_4^+-N (mg/kg)	0.71	b	36.12	a	0.87	b	2.8	b	***	1.83
NO_3^--N (mg/kg)	1.58	c	20.35	a	6.25	b	0.05	c	***	1.77
SOC (g/kg)	1.89	bc	1.65	c	3.04	a	2.88	ab	*	0.52
total N (g/kg)	0.14	b	0.24	a	0.19	ab	0.18	b	*	0.03
Soil C/N ratio	13.56	a	6.92	b	15.93	a	16.2	a	*	3.01

*, *** Standard error of the difference (SED) at $P < 0.05$, and $P < 0.001$, respectively

Means for the same character within the row with the same letter are not significantly different by LSD at 0.05

After 3 h of organic inputs, the highest and second highest metabolic quotient (qCO_2) of soil was found in the treatments GM and GMBC (0.3617 and 0.26 mg CO_2-C/g MBC/day, respectively) ($P < 0.001$). However, all treatments had no statistical difference after day 77 of the organic inputs incorporation. It was found that the qCO_2 of the CF treatment was lower than that of the GM treatment during days 0 - 63 (Figure 1), while the control treatment had a microbial biomass carbon of about 49 - 94 mg C/kg, which was the lowest value (data not shown). The cumulative CO_2-C evolution was highest in the GM treatment within 112 days after incorporation (2,578 mg CO_2-C/m^2/day), followed by the GMBC, CF, and control treatment (1,481, 685, and 613 mg CO_2-C/m_2/day, respectively). The study found that the GM treatment had the highest cumulative CO_2 emissions over all periods, with the GMBC treatment scoring second ($P < 0.001$), whereas the CF treatment did not disrupt the CO_2 emissions differently from that of the control treatment (Figure 2). Activities of ß-glucosidase and phenoloxidase showed different

short-term responses to the chemical fertilizer and organic inputs. The absolute ß-glucosidase was positively correlated with CO_2 emissions (r = 0.6648***). Microbial biomass C was also governed by the activity of this enzyme (r = 0.6438***). Therefore, the absolute activity of phenoloxidase was positively correlated with soil pH (r = 0.5334***) (Table 3).

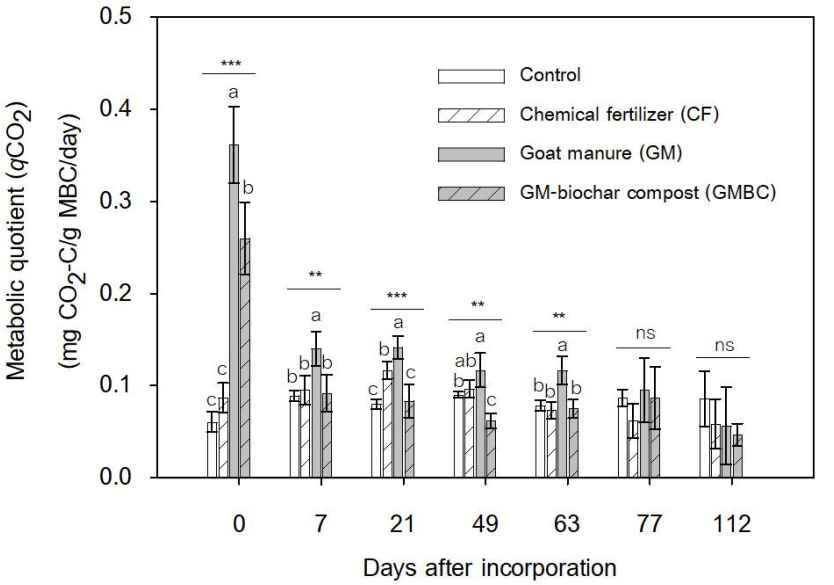


Figure 1. Metabolic quotient (qCO_2) in soil after chemical fertilizer and organic inputs

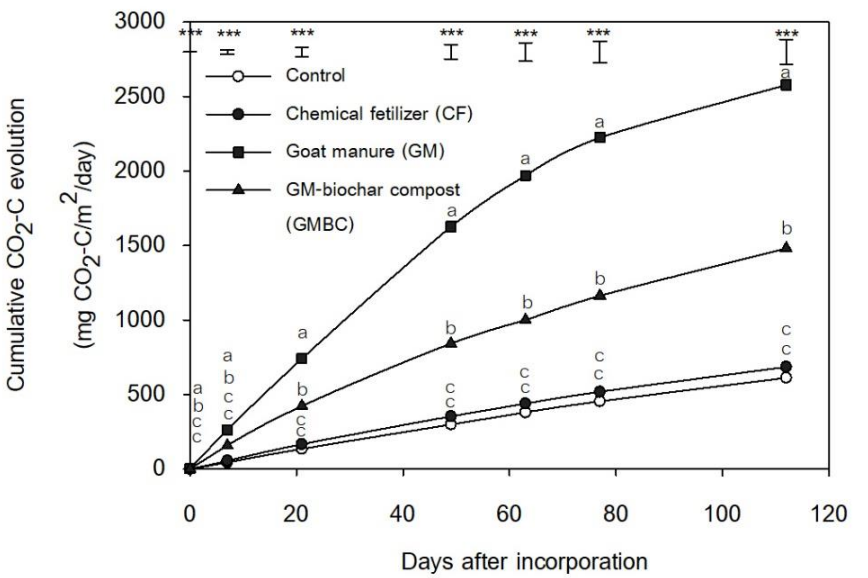


Figure 2. Cumulative CO_2-C emissions as affected by chemical fertilizer and organic inputs

Table 3. Pearson correlation coefficients (*r*) between enzyme activities and microbial parameters after 112 days of organic material incorporation

Parameters	ß-glucosidase activity	Phenoloxidase activity
Microbial biomass C	**0.6438** ***	**0.5676** ***
CO_2 emissions	**0.6648** ***	0.3687
NH_4^+-N	0.1412	-0.2110
NO_3^--N	0.1250	-0.3308
pH	0.2618	**0.5334** ***

The specific ß-glucosidase activity increased sharply in the GM treatment during the first 21 days of organic inputs (0.143 - 0.878 µg *p*-nitrophenol/g MBC/h). Meanwhile, the specific activity of this enzyme in the GMBC treatment was lower than that in the soil treated with the fresh one, except for days 77 and 112 (Figure 3a). The specific phenoloxidase activity in the GMBC treatment had the highest enzyme activity of 0.044 and 0.036 µmol dicq/g MBC/h on days 21 and 77 ($P < 0.05$) (Figure 3b). The GM treatment indicated an increase in the activity of this enzyme after 21 days of organic inputs. The specific phenoloxidase activity of the soil amended with fresh goat manure demonstrated a significantly higher activity, but only on day 49 (0.042 µmol dicq/g MBC/h).

Discussion

Organic inputs properties

The temperature used for the biochar production was measured between 350 - 450 °C, which may affect the uniformity of the charcoal produced. In this study, we found that GM-biochar compost (GMBC) with an initial practical C/N ratio of 31.9 (data not shown) and an added microbial activator required a shorter time to reach maturity (around day 42) than previously reported by Kamolmanit *et al.* (2018), as shown by ambient temperature coupled with a final C/N ratio close to 20 : 1 (22.94). A previous lab-scale study by Kamolmanit *et al.* (2018); used hardwood biochar, which has a higher structural resistance to degradation than manure biochar. Therefore, our results showed that using manure biochar as an ingredient in compost instead of wood biochar has several advantages, such as increasing the nutrients and reducing the decomposition time. Although the C/N ratio between GM and GMBC was quite close, GMBC was found to increase plant nutrients, which could be due to biochar has a higher percentage of fixed carbon (2.32 %) than fresh manure (0.85 %). Meanwhile, GMBC had 37.5 % less total N than fresh goat manure (Table 1). This is because N concentrations found in the biochar were reported to be negatively associated with pyrolysis temperature (Ippolito *et al.*, 2020). Generally, biochar production at temperatures below 350 °C is often found in pyrolysis by traditional methods using a traditional kiln (Butnan *et al.*, 2016). Therefore, biochar produced attemperatures below 350 °C will result in higher soil cation exchange capacity (CEC), and create a porous surface area, thereby improving the soil for cultivation (Jiang *et al.*, 2021).

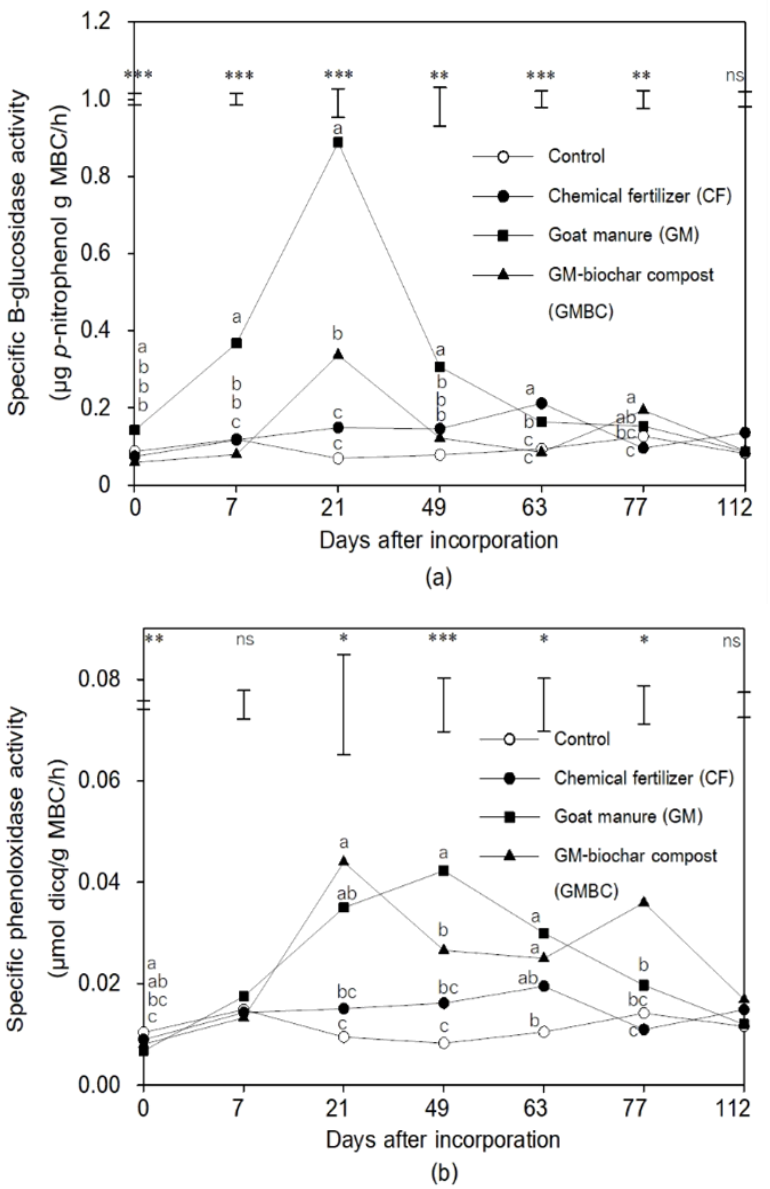


Figure 3. Specific activities of ß-glucosidase (a) and phenoloxidase (b) in the soil

Soil properties and SOC accumulation

The significant rise in pH in the soil treated with GMBC (6.91) and GM (6.67), compared to CF soil (6.21) (Table 2), may be due to the reduction capacity of some volatile matter (VM) compounds; including catechol, gallic acid, and tannic acid (Hue *et al*., 2001). Both ash concentrations and the reduction potential of VM, which occurs when Mn^{4+} or Mn^{3+} is reduced to Mn^{2+} may have contributed to the pH increase brought on by the addition of biochar (Butnan *et al*., 2016). Compared to the GMBC soil, therefore, the lower pH found in the soil after applying fresh goat manure may have resulted from easily decomposable organic C being degraded to organic acid by the acid-forming bacteria observed in the fresh manure. The pH drop in the GM treatment

may also be caused by the mineralization of organic acids that occurred through the decomposition process in the soil, as well as the large quantities of CO_2 released within this treatment. The GMBC is different from any other organic material, as it consists of easily decomposable C, which can be immediately used as an energy source for microorganisms. The compost that contains GM-biochar (GMB) also has high resistance C compounds that increase the accumulation of organic matter in the soil, and result in a yield with a high microbial biomass carbon.

SOC analysis on day 112 indicated that C concentrations in the soil increased through by adding organic inputs in both fresh manure and compost forms (Table 2). Notably, adding inorganic fertilizer to the soil did not increase the C content of the soil. As seen in Table 2, fresh goat manure contained a high concentration of total organic C (316.5 g/kg), increasing the SOC content up to 1.65 g/kg when added to the soil. This is further substantiated by observing the lowest SOC accumulation under long-term fertilizer applications in the field since 2015, demonstrating the complete decomposition of easily decomposable C substrates. Schulz and Glaser (2012) found that, in the first cycle of oat (*Avena sativa*) cultivation, crop growth was best in soil treated with compost, followed by biochar-compost, biochar-chemical fertilizer, and chemical fertilizer. In the second cycle, they found that the soil treated with a biochar-chemical fertilizer resulted in the highest increases in SOC, CEC, and soil pH. The above studies have shown that compost contains the significant nutrients needed for plant growth, compared to that of pure biochar. As a result, the crops in the compost-treated soil produced higher yields than those of other organic materials in the first cycle. The second crop, considered as long-term production, demonstrated that biochar compost promotes an increase in SOM accumulation and the release of plant nutrients.

Microbiological parameters and microbial activities

A high metabolic quotient (qCO_2) will indicate the ratio of CO_2 to the carbon used to make microbial cells. Higher values suggest that microorganisms' potential for C use in organic materials is low (Figure 1). Similar to qCO_2, high values of qCO_2 in the GM treatment reflect the greater reductions in SOC than those in microbial activities when the soil was amended with labile C compounds. Consequently, GMBC-containing biochar proved to be a better source of C substrate for soil microorganisms. There was significant cumulative CO_2-C evolution in soil amended with goat manure compared to the more resistant materials e.g., GMBC at all stages of decomposition, indicating that the labile C compound was the main factor regulating microbial activities. This was found in positive correlations between CO_2-C emissions and absolute ß-glucosidase activity ($r = 0.6648^{***}$). The activity of this enzyme has been reported to be related to the C dynamics in soil and can indicate the degradation of labile C in soil (Kang *et al*., 1998). Our findings confirm the ecological importance of the chemical properties of organic inputs in highly weathered sandy soil expressed in three months of data. Our study also indicates that organic inputs with high fixed C content, like GMBC, were most effective among treatments in accumulating soil microbial biomass C by reducing microbial CO_2-C emissions.

The activities of extracellular enzymes provide insights into the biochemical controls of soil C storage because this enzyme catalyzes rate-limiting steps in organic matter degradation and

the correlations between the rates of plant litter decomposition and microbial production (Sinsabaugh, 2010; Sinsabaugh *et al*., 2005; Waldrop and Harden, 2008). In soil studies, enzyme activity is generally presented in absolute terms but does not reflect the microbial part of its production (Acosta-Martínez *et al*., 2003). Consequently, some studies have shown the importance of enzyme assessment activities related to soil microbial biomass or soil organic carbon, also referred to as a specific enzyme activity (Medeiros *et al*., 2015). A rapid increase in specific enzyme activities in soil treated with organic inputs indicated that physiologically capable C exists in biomass. This is because these specific activities involving living microbial communities (Waldrop *et al*., 2000) were more metabolic (Lagomarsino *et al*., 2011) compared to soil suffering from land use changes that were previously fed with chemical fertilizers over a long period.

Soil treated with fresh goat manure showed higher specific ß-glucosidase activities than those of the biochar compost indicating that the critical role of C availability from the substrate, e.g., cellulose, in manure stimulates the activity of this enzyme. The high cumulative CO_2 emissions indicated that fresh manure was used for biomass, and microbes utilized the carbon, and released a significant amount of CO_2 into the atmosphere (Figure 2). Support for this assumption was provided in the soil treated with goat manure, in which ß-glucosidase activity indicated a positive correlation between MBC and CO_2-C emissions. Interestingly, although GMBC had the second highest readily available C (195 g/kg) compared to the fresh manure (316.5 g/kg), it did not translate into the stimulation of specific ß-glucosidase activities, except on day 21 (Figure 3a). Activity stimulation occurred on day 21, which arose from the availability of the previously protected cellulose in the biochar. One hypothesis referred to the pyrolysis temperature, the most important factor affecting the characteristics of the biochar used in this study. Another possible mechanism; there may also be internal holes in this low-temperature pyrolysis of biochar (< 350 °C) containing incomplete combustion cellulose (Kuo *et al*., 2008; Smith *et al*., 2017). This implies that the activity of ß-glucosidase is influenced by the availability of carbon, as well as the defenses against cellulose degradation. This was observed in the unique patterns of microbial activities between the two types of enzymes; phenoloxidase and ß-glucosidase (Figures 3a, b). The specific activity of phenoloxidase in the GMBC treatment increased rapidly, as the biochar in the compost had undergone microbial degradation from the previous decomposition process. It was observed that after 63 days there was an increase in phenoloxidase activity, which could be attributed to the enzymes in the biochar pores or on the surface that were previously protected. Verheijen *et al.* (2009) reported that cellulose decomposes in the temperature range of 250 - 350 °C, while lignin decomposes in the temperature range of 280 - 500 °C. This may also be caused by the incomplete combustion of biochar that resulted in degradation by microorganisms. Similar to ß-glucosidase, the sharp increase in specific phenoloxidase activity was observed after day 7 (Figure 3b), indicating the adjustment of soil microorganisms to the complex C substrates of the inputs (Hadas *et al*., 2004; Rubin, 2008). We expected that not only lignin but also fixed C would become another important factor/parameter regulating phenoloxidase activity. Furthermore, we did not observe the suppressive effects of N inputs on specific phenoloxidase activities, as shown by the increased specific phenoloxidase activities in both biochar and control soil. However, the effects

of N on both C-cycling enzyme activities were not observed in this study.

We may deduce phenoloxidase activity which has a more significant effect on C stimulation than the N source. This assumption was supported by Keeler *et al*. (2009) who studied the non-effectual effects of organic N inputs on resistant C compound-degrading enzymes in forest and grassland soil. Interestingly, our findings do not support the ongoing discussion of the inhibitory effects of N on the activity of resistant C compound-degrading enzymes, as previously reported by Berg and Matzner (1997). It has been reported that the concentration of N has negative effects on enzyme activities (Liu *et al*., 2021). Our study revealed no significant negative effects of N on phenoloxidase activity, as seen in Table 3. Lastly, the global cycling of C through biochar production showed that more than 50 % of the existing C was converted to stable form (Venkatesh *et al*., 2013). For this reason, amended biochar compost has the potential to increase soil C better than fresh manure.

Conclusion

Goat manure has the potential for biochar production, resulting in a biochar with an increased pH, a stable form of carbon, and soil macronutrients, including an increase in phosphorus and potassium. However, the use of fresh goat manure to produce biochar resulted in a significant reduction in total nitrogen, so appropriate pyrolysis processes should be considered. Biochar compost materials that contain carbon-resistant organic materials allow microbes to retain carbon in their cells instead of releasing it into the atmosphere as carbon dioxide, as indicated by C-cycling enzyme activities and lower cumulative CO_2 emissions. The substrate within certain phenoloxidase enzymes had a stronger effect on the usefulness of carbon than on the influence of N during microbial degradation. It is necessary to conduct a more in-depth study on the impact of nitrogen form, NH_4^+ or NO_3^-, on the activity of soil microorganisms. This factor is crucial and requires further examination. Like labile carbon compound degrading enzymes, phenoloxidase was not affected by the nitrogen inputs in the soil samples. Our study investigated the significant role of biochemical interactions between different compounds, as shown in the case of labile carbon and the resistant-organic inputs regulating microbial decomposition, manifested in ß-glucosidase and phenoloxidase activities. To achieve a sustainable practice of soil SOC accumulation, the low carbon inputs in the control soil and the soil treated with chemical fertilizer led to a low SOC environment, which lowers the stimulation of microbial enzyme activities. Although the enzymes studied were specific to the quality of the substrate, the soil environment induced microorganisms to increase enzyme production. While the activity of enzymes in soil indicated the metabolic activity of soil microorganisms, they can also predict the level of soil organic carbon accumulation. Lastly, we showed that goat manure-biochar compost was the most suited to improving soil organic carbon content in highly weathered, sandy soil; it contains both easily decomposable- and resistant carbon compounds paralleled with a suitable C/N ratio for microbial consumption. The long-term addition of a biochar compost mixture can be expected to achieve an increase in soil organic carbon, as microbial biomass carbon parallels plant nutrients.

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