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Soil microbial activities in Alfisol with different green manure application

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Abstract The result showed the highest soil respiration in green manure from African sesbania application was 0.5478 mgCO_2/g soil. The lowest microbial biomass nitrogen (MBN) found in green manure from cowpea application was 29.68 mgCO_2/g soil. MBN was found at the highest value in non-application of green manure (control) at 2.20 ng/g soil after 30 days application. Urease activity was the highest in green manure from African sesbania at 12.58 µg NH_4-N/g dwt. However, activities of cellulase, protease and acid-phosphatase were not statistically differed between treatments. Enzymatic activities increased with the highest activities found at 20^{th} and 30^{th} days after application. The green manure from Soybean promoted the highest microbial biomass while green manure from jack bean promoted the highest soil respiration. The enzymes were changed, except for urease. Decomposition rate related to plant quality to control activity in soil.

Keywords: Green manure, Enzymatic activity, Microbial activity

Introduction

Repeated monoculture decreases the abundance and diversity of soil biology (Hunt and Wall, 2002), and lack of soil fertility is a major problem around the world. Soil organic matter (SOM) is an indicator of soil quality (Lal *et al*., 1995) a major pool for carbon (C), nitrogen (N), phosphorus (P) and sulfur (S). Cycling and availability of these elements are constantly changed by microbial activity and mineralization (Feichtinger *et al*., 2004). Increase in organic matter improves physical properties of soil, conserves water and increases available nutrients. These improvements ultimately lead to greater biomass and crop yield (Bauer and Black, 1994; Onemli, 2004). Green manure is an alternative way to increase soil fertility and reduce disease accumulation. Legumes are used as a cover crop or green manure because of their high biomass production, high nutrient content and deep root system (Balota and Chaves, 2010). In Thailand, are available as green manure such as sunn hemp,

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mung bean and sesbania. After plowing at the blooming stage, the residual is left to decompose by the action of extracellular enzymes from soil microorganisms. Soil enzymes play an important role in maintaining the ecosystem and can be used as an indicator of soil fertility. Major enzymes related to soil nutrients and organic matter included urease, protease, phosphatase and cellulase. Urease rapidly hydrolyzes urea to ammonium and carbon dioxide (Harre *et al*., 1971), while protease accelerates the hydrolysis of proteins to polypeptides and oligopeptides and then to amino acids (Handa *et al*., 2000). Both urease and protease are associated with the nitrogen cycle in soil (Moreno *et al*., 2003). Phosphatase stimulates the hydrolysis of ester bonds between phosphate and carbon in organic compounds which increases phosphorus availability (Turner and Haygarth, 2005). Cellulase breaks down cellulose molecules, as the main component of plant cells, to glucose (Reese *et al*., 1950). Soil enzyme activity can be used as an indicator of certain biochemical processes (Balota and Chaves, 2010). Use of green manure focuses on nutrient release to the soil during or after degradation; however, induction of enzyme activity and microorganisms in soil by green manure is another benefit from green manure application. Cultivation of diverse plant species increases microbial activity and soil fertility in different (Balota and Chaves, 2010).

This study focused on the activities of soil microorganisms and enzymes as urease, protease, phosphatase and cellulase during green manure decomposition under aerobic condition.

Materials and Methods

Experimental design

The experiment was set as completely randomized design (CRD) with 8 treatments and 3 replications, using different green manure application as follows: T1: no green manure application (control), T2: sunn hemp (*Crotalaria juncea*), T3: jack bean (*Canavalia ensiformis* L.), T4: cowpea (*Vigna sinensis*), T5: mung bean (*Vigna radiata*), T6: soybean (*Glycine max*), T7: African sesbania (*Sesbania rostrata*) and T8: earleaf acacia (*Acacia auriculiformis*). The experiment was conducted in 12" plastic pots with 8 kg of soil and each type of green manure which was 5.98 kg N/rai (0.18 gN/pot) for each treatment, except for the control (non-green manure application). The green manure was analyzed nitrogen content before application, that found nitrogen content as follow: sunn hemp had 2.84 %N, jack bean had 1.91 %N, cowpea had 2.90 %N, mung bean had 2.59 %N, soybean had 3.74 %N, African sesbania had 3.52 %N and earleaf acacia had 2.66 %N. The green manure was dried and crushed

before weighed according to its nitrogen content and applied in the treatments. The soil moisture was kept constantly at field capacity throughout the time of study. Soil samples were collected at 0, 10, 20 and 30 days for biological analysis.

Microbial activity

Soil respiration was measured using a titration method. Twenty grams of moist soil was placed in 500 ml Erlenmeyer flasks along with a vial of 5 ml of 1*N* NaOH. Alkali traps were titrated after 48 h of incubation. Unreacted alkali in the NaOH traps was back-titrated with 0.5*N* HCl to determine CO_2-C. Soil respiration was calculated as follow:

$$\text{Soil respiration} = (B - V) \times 0.5 \times 22$$

Where B = ml of HCl used for titrated blank and V = ml of HCl used for titrated the sample.

Microbial biomass carbon (MBC) was measured by chloroform fumigation and extraction methods (Vance *et al*., 1987). Twenty grams of soil was incubated with chloroform under dark condition for 48 h. The soil after incubation was extracted with 0.5*M* K_2SO_4 (1:4 w/v) followed by dichromate oxidation (Kalembasa and Jenkinson, 1973; Vance *et al*. 1987). Four milliliters of filtrate was titrated with 0.01*N* $(NH_4)_2Fe(SO_4)_2.H_2O$. The unfumigated was used as a control. MBC was calculated as:

$$MBC = E_C/k_{EC}$$

Where E_C = (organic C extracted from fumigated soils) - (organic C extracted from unfumigated soil) and k_{EC} = 0.38 (Vance *et al*., 1987).

Microbial biomass nitrogen (MBN) was measured by chloroform fumigation and extraction methods (Vance *et al*., 1987). Twenty grams of soil was incubated with chloroform under dark condition for 48 h. The soil after incubation was extracted with 0.5*M* K_2SO_4 (1:4 w/v) followed by Kjeldahl method (Brookes *et al*., 1968). Ten milliliters of filtrate was distilled with 20 ml of 10*N* NaOH. Ammonium was trapped with 20 ml of 2% H_3BO_3 and then titrated with 0.005*N* HCl (Brookes *et al*., 1968). The unfumigated was used as a control. MBN was calculated as:

$$MBN = E_N/k_{EN}$$

Where E_N = (total N from fumigated soils) - (total N extracted from unfumigated soil) and k_{EN} = 0.45 (Brookes *et al*., 1968).

Soil enzymes

For protease, one gram of moist soil was mixed with 5 ml of Tris-buffer and 5 ml of sodium caseinate solution. After incubation for 2 h at 50 ℃, 5 ml of

trichloroacetic acid solution was added and centrifuged at 10,000-12,000 rpm for 10 min. Then, 7.5 ml of alkaline reagent was added to 5 ml of clear supernatant and incubated at room temperature for 1 h. Absorption of the sample was measured at 700 nm (Ladd and Butler, 1972). Tyrosine was used as a standard.

For urease, 2.5 ml of urea solution and 20 ml of borate buffer was added to 5 g of moist soil and then incubated at 37 ℃. After 2 h, 30 ml of KCl solution was added to the sample, then shaking at 120 rpm for 30 min. The solution was filtrated through Whatman no.1 filter paper and 1 ml of filtrate was transferred to a new tube before adding 9 ml of distilled water, 5 ml of Na salicylate/NaOH solution, and 2 ml of sodium dichlorocyanide solution. Absorption of the sample was measured at 690 nm (Kandelar and Gerbe, 1972). Ammonium was used as a standard solution.

For cellulase, 15 ml of acetate buffer and 15 ml of carboxymethyl cellulose sodium salt solution was added to 5 g of moist soil. The sample was filtrated and 1 ml was transferred to a new tube before adding 1 ml of reagent A (0.15*M* $NaCO_3$ + 0.0138*M* KCN) and 1 ml of 0.003*M* $K_3Fe(CN)_6$. The sample was then boiled at 100 ℃ for 15 min. After cooling at 20 ^oC for 5 min, 5 ml of reagent C (0.003*M* $NH_4Fe(SO_4)_2 \cdot 12H_2O$ + 0.003*M* $NaC_{12}H_{25}SO_4$ + 0.04*M* H_2SO_4) was added, with incubation for 1 h at 20 ℃. Absorption of the sample was measured at 690 nm (Schinner and Von, 1972). Glucose was used as a standard.

For phosphatase, 4 ml of modified universal buffer and 1 ml of 15*mM* p-nitrophenol phosphate solution was added to 1 g of moist soil and incubated for 1 h at 37 ℃. Then, 0.25 ml of toluene and 4 ml of 0.5*M* NaOH were added to the sample and incubated at room temperature for 30 min before filtration using Whatman no.2 filter paper. Absorption of the sample was measured at 400 nm (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977). Nitrophenol was used as a standard.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA). Treatment means were compared with Duncan's multiple range test (DMRT) at 95% confidence level ($p \leq 0.05$).

Results

Microbial activity

At the end of 30 days experimental period, amount of CO_2 released was statistically different ($p<0.05$) when applying green manure to the soil.

Application of jack bean and African sesbania gave higher soil respiration than sunn hemp, cowpea, mung bean, soybean, earleaf acacia and control (Table 1.). Soil respiration increased during the study period (Figure 1A). Respiration rate rapidly increased during the first 10 days and then remained constant, except for mung bean which decreased and African sesbania which increased after 10 days. Microbial biomass increased significantly ($p<0.05$) when applying green manure to the soil, except for cowpea. Fluctuation of MBC was similar in every treatment. MBC was highest after 10 days of application and then reduced, except for African sesbania which gave lowest MBC after 10 days of application and then increased (Figure 1 B). By contrast, microbial biomass nitrogen (MBN) decreased significantly under green manure application. MBN values of all treatments followed the same trend as highest after 20 days of application and then reducing (Figure 1 C).

Enzymatic activity

Enzymatic activity during the study period showed statistical difference only for the urease enzyme (Table 2.). Application of African sesbania increased urease activity but application of other green manures was not statistically different from control ($p<0.05$). Activities of protease, acid-phosphatase and cellulase did not differ between the tested treatments. Enzymatic activity of urease in earleaf acacia and African sesbania increased after 20 days of application, contrasting with other green manure applications that reduced (Figure 2A). Urease activity relates to plant cellulose that protects cell degradation from microbes. Activity of protease, phosphatase and cellulase were not difference after 30 days of study period (Figure 2B-D).

Table 1. Microbial activity after 30 days of green manure application

Treatment	Soil respiration ($mgCO_2/g$ soil)	MBC (mg/g soil)	MBN (ng/g soil)
T1[1/]	0.2514 c[2/]	36.49 bc	2.20 a
T2	0.3861 abc	79.28 ab	1.89 e
T3	0.4760 ab	72.01 ab	1.91 de
T4	0.2156 c	29.68 c	2.03 bc
T5	0.3502 bc	86.42 a	1.89 e
T6	0.2695 c	94.77 a	2.09 b
T7	0.5478 a	82.37 ab	1.91 de
T8	0.2964 c	88.15 a	1.98 cd
F-test	*[3/]	*	*
CV (%)	27.60	27.99	10.81

[1/] T1 = control, T2 = sunn hemp, T3 = jack bean, T4 = cowpea, T5 = mung bean, T6 = soybean, T7 = African sesbania, T8 = earleaf acacia

[2/] different letters in each column are significantly different according to DMRT

[3/] * = significant at $p\leq0.05$, ns = not significant

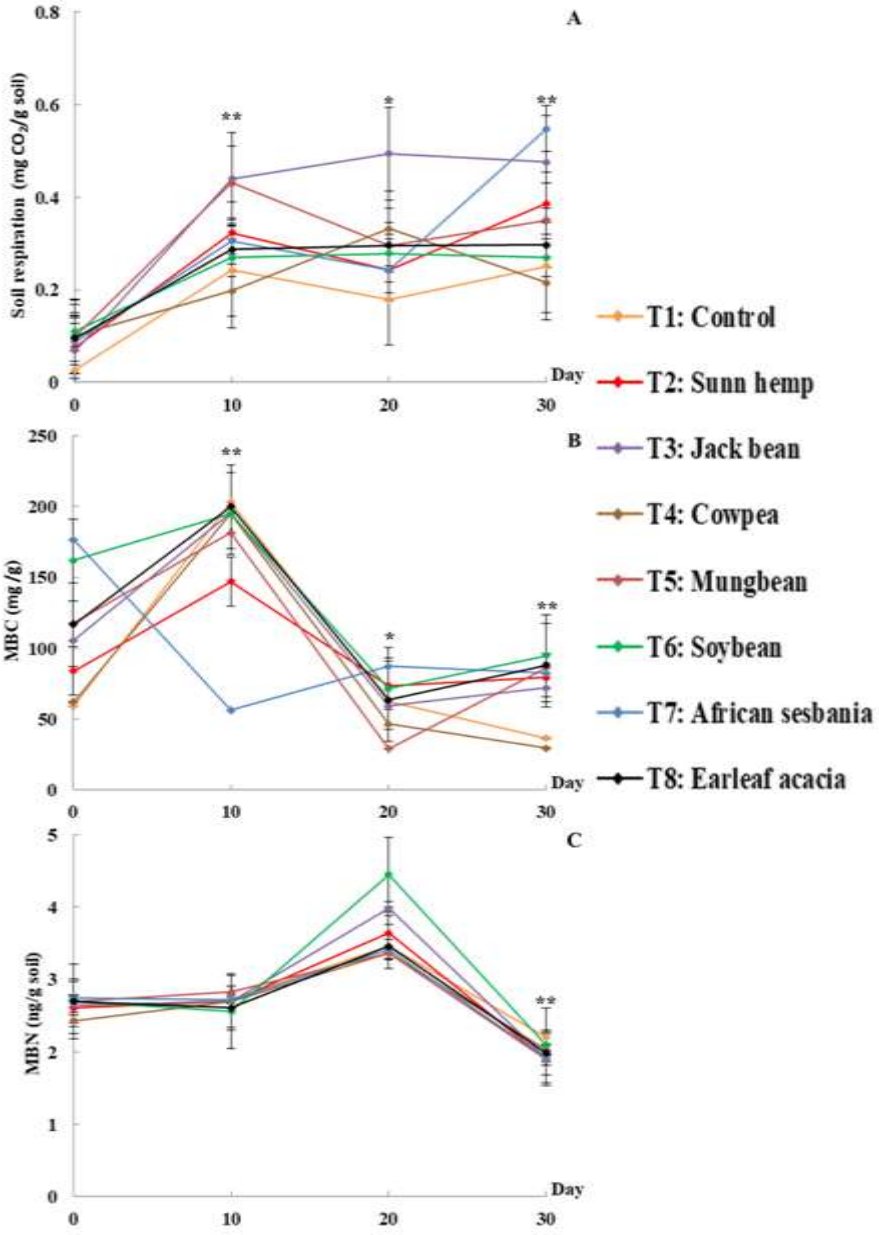

Figure 1. Soil microbial activity during the study period: A: soil respiration, B: MBC, C: MBN

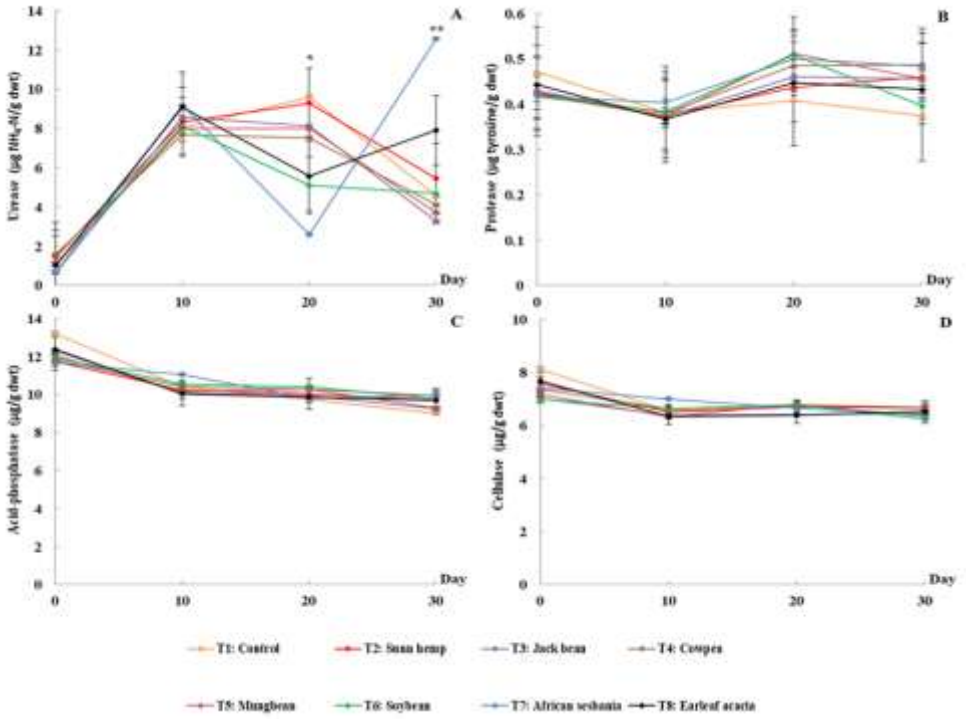

Figure 2. Soil enzyme activity during the study period: A: urease, B: protease, C: acid-phosphatase, D: cellulase

Table 2. Enzymatic activity in soil after 30 days of green manure application

Treatment	Urease (μg NH_4-N/g dwt[2/])	Protease (μg tyrosine/g dwt)	Phosphatase (μg/g dwt)	Cellulase (μg/g dwt)
T1[1/]	4.56 b[3/]	0.3752	9.02	6.63
T2	5.45 b	0.4604	9.68	6.69
T3	3.26 b	0.4558	9.25	6.47
T4	4.10 b	0.4875	9.95	6.68
T5	3.70 b	0.4557	9.29	6.34
T6	4.69 b	0.3964	9.76	6.24
T7	12.58 a	0.4841	9.97	6.39
T8	7.91 ab	0.4326	9.70	6.53
F-test	*[4/]	ns	ns	ns
CV (%)	27.60	15.22	4.61	4.22

[1/] T1 = control, T2 = sunn hemp, T3 = jack bean, T4 = cowpea, T5 = mung bean, T6 = soybean, T7 = African sesbania, T8 = earleaf acacia
[2/] g dwt = gram dry weight of soil
[3/] different letters in each column are significantly different according to DMRT
[4/] * = significant at $p \leq 0.05$, ns = not significant

Discussion

Increase of soil respiration under African sesbania application at the end of the experiment might be due to slower plant decomposition rate than other green manures. Sesbania contains high cellulose and lignin contents. Lignin, pentosan and cellulose contents of *Sesbania bispinosa* varied from 21-23%, 16-18% and 38-43%, respectively depending on growing location (Sarkar *et al*., 2017). Moreover, the C:N ratio control plant degradation rate. Plants with higher nitrogen content actively decompose faster than plants with wider C:N ratios. Cellolose and lignin contents also affect biomass degradation (Tripolskaja *et al*., 2014). Higher soil respiration under jack bean and cowpea applications related to higher plant nutrient content available for microbial activity. Use of *Trifolium pretense* L. as green manure increased soil microbial biomass and soil enzymes (dehydrogenase, urease, phosphatase and arylsulfatase) more significantly than use of *Brassica napus* L. or *Trifolium pretense* mixed with *Brassica napus*. Higher soil activity related to higher nutrient and plant yield depending on different chemicals in the applied green manure (Tejada *et al*., 2008).

Microbial biomass is the living component of soil organic matter (Rice *et al*., 1996). Turnover time of microbial biomass at less than one year shows a rapid response to changes in organic matter. Microbial biomass carbon has high potential for microbial activity (Rice *et al*., 1996). Three years study of applied green manure found that MBC content ranged from 1.94% to 93.07% and MBN from 2.3% to 145.07%, while enzymatic activity of urease, acid-phosphatase and catalase increased from 1.45-56.52%, 2.34-33.17% and 3.33-85.71%, respectively (Ye *et al*., 2014). Different soil environments affect plant species in diverse ways relating to quantity and quality of the plant (Balota and Chaves, 2010). Plant properties impact on soil microorganisms, nutrient cycling and soil organic matter differently (Balota and Chaves, 2010). Our study results ahowed soybean gave the highest MBC.

The urease enzyme catalyzes the hydrolysis of urea to CO_2 and NH_3. This enzyme in soil stimulating the conversion of organic nitrogen and allow release of nitrogen from organic matter for microbial growth. The relationship between MBC and microbial enzyme activity per unit area of soil microorganisms indicates the population and activity of enzymes that are capable of decomposing green manure (Yanyu *et al*., 2019). Application of African sesbania gave highest urease activity and soil respiration after 30 days. Lower urease activity and soil repiration during the early study period with later increase might relate to the rate of degradation; however, no difference between MBN and protease, phosphatase and cellulase activity was recorded. The study

duration might be too short to observe these changes. Moreover, application of green manure at the same rate based on nitrogen content gave similar amounts of nutrition for activation of microbial activity.

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