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Diversity of Functional Soil Microbes in Manures and Its Effect on Organic Mustard Green (*Brassica juncea*) Production

Ari Kurniawati[A]*, Maya Melati[B], Sandra Arifin Aziz[B], Purwono[B]

[A] Study Program of Agrotechnology, Nahdlatul Ulama University of Purwokerto, Jl. Sultan Agung No. 42, Karangklesem, Purwokerto 53144, Central Java Indonesia
[B] Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia

*Corresponding author, e-mail: mayamelati14@gmail.com

Abstract

The application of organic fertilizer, especially manures, for crop production has long-term effects for soil nutrients availability and improvement of soil structure. The improvement of soil properties involves interactions of various types of microorganism in the soil. The research aimed to study the effects of different types of manures on the diversity of functional soil microbes and its effects on organic green mustard production. The experiment was conducted at IPB organic research field, Cikarawang, Darmaga, Bogor, from April to June 2015. The experiment used a randomized complete block design with two factors; the first factor was types of manure, i.e. chicken, cow, and goat manures; the second factor was manure rates, i.e. 0 and 10 ton.ha^{-1}, so there were six treatments in total, replicated three times. The results showed that chicken manure application resulted in a higher mustard green yield and soil-P content than application of goat manure. The addition 10 ton.ha^{-1} of manures increased C-organic, N, K, and C/N ratio in soil significantly, but mustard green production was not affected. The diversity of microbe population of the soil treated with all types of manures was high. The addition 10 ton.ha^{-1} of manures decreased the total number of microbes, but increased the number of cellulose-degrading microbes. The population of cellulose-degrading and phosphate-solubilizing microbes in the soil applied with cow manure was higher than those applied with the other manures.

Keywords: Brassicas, chicken manure, cow manure, crop residues, goat manure, soil organic matter

Introduction

The application of organic fertilizer aims to increase the soil organic matter and to improve microbe's activity in soil because agricultural practices tend to decrease the soil quality and fertility in the long term. Application of organic fertilizer can improve of physical, chemical, and biological soil properties (Sutanto, 2002; Barus, 2012). The organic matter will increase the role of functional microbes in soil through providing carbon source for the microbes. Moreover, the improvement of soil physical structure with manure can provide more optimal environment for microbe's growth. Sahwan et al. (2011) showed that the optimal environment for microbe growth, including humidity, the availability of oxygen, temperature, C/N ratio, and soil pH, increase microbe reproduction exponentially.

Organic fertilizers can be derived from animal manures and agricultural wastes. There are abundant availability of manures, particularly of chicken, cow, and goat manures around the farm, because generally Indonesian farmers grow the animals around the farm. Animal manures, both in solid and liquid form, mixed with food wastes, can increase soil nutrients when applied to the soil (Maruapey, 2011). In addition, crop residues that were returned into the field will supply additional nutrients. Crop residues from rice straw increased bacterial population in paddy soil (Wu et al., 2011).

Agricultural land in Indonesia consists of lowlands and highlands. One of vegetables which is suitable to cultivate in both of lowland and highland is mustard green (*Brassica juncea*). Mustard green requires a lot of nitrogen for their vegetative growth, and organic matter is one of nitrogen sources. The organic matter which has been applied in the agricultural land will slowly be released as organic fertilizer (Sharma dan Singh, 2011) and resulted in improvements of soil functions up to more than 15 years (Diacono and Montemurro, 2010). The process of organic matter decomposition is supported by microbe's activity to maintain sustainability of ecosystem (Zak et al., 1994) and soil productivity (Juan et al., 2015), so the application of manures should be optimized. One of the important role of microbes is to increase the availability of nutrients for plants (Widyati, 2013) which will then improve plant growth. Therefore, this research aimed to study the application of different types of manure on the diversity of functional soil microbes and mustard green production.

Materials and Methods

The experiment was conducted at IPB organic experimental station, Cikarawang, Darmaga which geographically located between 6°30'- 6°45' South Latitude and 106°30'- 106°45' East Longitude, at 250 m above sea level, Bogor, Indonesia, from April to June 2015. This experiment was carried out in the second season following soybean cultivation in the first season. The first season experiment on soybean used three types of manures, namely chicken, goat and cow manures, at 20 ton.ha^{-1} each. The soybean biomass residues from the first season were then applied in the second season prior to mustard green planting.

The experiment used a randomized complete block design with two factors. The first factor was types of manures, and the second factor was rates of manures, i.e., 0 and 10 ton.ha^{-1}, so there were six combinations of treatments with three replications. Data were analyzed by analysis of variance and means analysis were statistically compared with a Tukey test at α= 0.05 using SAS 9.1.3.

Leaf and soil nutrient content were measured in Laboratory of Soil Science and Land Resources Department, Bogor Agricultural University (IPB) using Walkley and Black method for C-Organic, Kjeldahl for N-total, Bray method for P and K measurement of soil (Eviati dan Sulaeman, 2009). Leaf samples were collected from the harvested mustards; soil samples from five points of each plot with the total weight of 1 kg per sample. Measurement of manure nutrient content (C-organic, N, P, K, Ca, Mg, C/N ratio) was conducted at The Soil Research Institute, of Land Research Center, Bogor. Analyses of functional soil microbes used plate count method (Napitupulu, 2012) conducted at Soil Biotechnology Laboratory, IPB.

Crop residues of 8.63 ton.ha^{-1} from the previous season were chopped into small pieces before application to all plots by top dressing. At this point, the field was left fallow for about two months prior to planting. Mustard "Brisk Green" seeds were sown on nursery media consists of an equal volume of soil, manure, and husk charcoal. While waiting for the mustard seeds to grow, the manures were applied into rows at the rate of 3 kg each plot. Three weeks after sowing, the seedlings were transplanted into 1.5 m x 2 m field plots at 20 cm x 20 cm spacing. Mustard green crops were harvested at four weeks after transplanting.

Results and Discussion

There was no interaction between manure types and rates on mustard green production. However, manure types affected mustard green weight per hectare. Mustard green yields were higher when treated chicken or cow manures (Table 1). The production of mustard green in this study was still below yield potential based on information available on manufactured label of "Brisk Green" seeds which were up to 40 ton.ha^{-1}. However, the level of

Table 1. The production of organic mustard green with different types and rates of manure application

Treatments	Percentage of harvestable crop (%)	Fresh shoot weight per plant (g)	Mustard green yield (ton.ha^{-1})
Manure types			
Chicken manure	71	126.10	19.48 a
Cow manure	84	73.00	19.20 a
Goat manure	70	105.53	15.76 b
Manure rates (ton.ha^{-1})			
10	73	103.40	17.80
0	77	99.69	18.49
Types of manures x rates	ns	ns	ns

Note: Values followed by the same letter within the same column were not significantly different according to Tukey test at α=0.05 level production in this study was still in higher than the average production of mustard green in Indonesia which was 9.9 ton ha^{-1} in 2014 (Kementan, 2015).

Fresh shoot weight per plant increased with the application of 10 ton.ha^{-1} of manure, but mustard green yield decreased to 1.31 ton.ha^{-1} with the increase of manure rates (P>0.05) (Table 1). The decrease in yield was likely due to the low percentage of harvestable crops, possibly because of the excessive nutrients from soybean crop residues from the previous season, and the addition of manures in the current experiment.

Types and rates of manures did not affect the percentage of harvestable crops (Table 1). However, the application of cow manure tended to produce more harvestable crops with higher yields than the application of chicken and goat manure (Table 1). Susanto (2002) showed that the application of high rates of chicken manure can damage vegetable seeds and transplants, possibly due to the allelopathic compounds in the chicken manure which can function as herbicides.

The crops treated with chicken and goat manures had greater fresh weight than those applied with cow manures (P>0.05) (Table 1). This might be related to higher nutrient content in chicken and goat manures than those of cow manures (Table 2). Compared to application of the other manures, application of cow manures resulted in the lowest fresh shoot weight per plant, although the cow manures contain more nitrogen than those of chicken manures (Table 2). Based on these results, cow manure alone did not seem to be able to support mustard growth.

The availability of nutrients in the manures correlated with the availability of functional microbes in the manures (Table 2). The number of microbes in the three types of manures ranged from 7.00 x 10^{3} to 3.85 x 10^{7} (Table 3), which correlate to their ability to supply nutrients for the crops. The highest count of Rhizobium was in goat manures, i.e. 1.07 x 10^{5} CFU/g. Goat manures also had the highest nitrogen content (Table 2). Goat manures also had the highest number of microbes, P content, number of cellulose-degrading microbes, and C-organic.

The goat manures were in granule form, so they are more difficult to break down to release the nutrients to the soil (Sutanto, 2002). On the other hand, the total number of microbes in goat manures was high (Table 3), which demonstrated that the microbes of the goat manures were still active, and the nutrients were possibly not ready to be released. The application of goat manures could be correlated with the lowest percentage of harvested crops; possibly because of the low availability of phosphate (P) nutrient in soil (Table 4) although P levels in the manure was the highest (Table 2).

The application of manures affect the soil nutrient levels due to the biological process by microbes in the soil. The microorganisms play important roles in the dynamic of soil nutrient levels through the processes of humification and mineralization (Emmerling et al., 2002). Table 4 showed that the types of manures did not affect soil nutrient levels, except for P P; application of chicken manures increased soil P levels. According to Hartatik and Widowati (2006), chicken manures has a relatively higher P content compared to the other animal manures. In addition, the low number of phosphate-solubilizing microbes in

Table 2. Nutritional values of different types of manures*

Manure types	C-organic	N	P	K	Ca	Mg	C/N ratio
				%			
Chicken manure	10.57	1.43	2.49	1.07	6.63	0.88	7
Cow manure	8.43	1.60	0.35	0.20	1.01	0.25	5
Goat manure	17.65	2.04	2.65	1.42	1.60	1.01	9

*From Eviati dan Sulaeman (2009), Laboratory of Land Research, Bogor

Table 3. Microbes population in manures*

Manure types	∑ Total microbe	∑ Rhizobium	∑ Phosphate-solubilizing microbes	∑ Cellulose-degrading microbes
	(SPK per g)			
Chicken manure	1.71 x 10^{7}	2.90 x 10^{4}	1.00 x 10^{4}	7.00 x 10^{3}
Cow manure	1.37 x 10^{7}	7.60 x 10^{4}	2.00 x 10^{4}	3.80 x 10^{4}
Goat manure	3.85x 10^{7}	1.07 x 10^{5}	2.10 x 10^{4}	6.90 x 10^{4}

* Based on plate count method (Napitupulu, 2012), Soil Biotechnology Laboratory, IPB

chicken manure (Table 3) indicated the high levels of P. According to Richardson and Simpson (2011) the increase of activity of phosphatases might occur in response to P deficiency, and vice versa.

Manure application at 10 tons.ha^{-1} significantly increased the levels of carbon, potassium and C/N ratio (Table 4). According to Adiku et al. (2008) the addition of organic matter to soil will lead to an increase of soil organic carbon, so that the soil microbial activities increased as the result of carbon mineralization.

The application of goat manure reduced C-organic levels and C/N ratio compared to application of the other manures, but the values were not significantly different (Table 4). The increase in C/N ratio was caused by the high number of cellulose-degrading microbes in goat manure (Table 3). The decomposition process decreases with time and make the nutrients available for the crops.

The types and rates of manures interacted in affecting soil available P (Table 5). Goat manure application at 10 ton.ha^{-1} significantly increased the levels of soil available P. However, the application of chicken manure resulted lower available-P level, even though the effects were not significantly different. The decrease the soil available-P might have been caused by immobilization of P in the soil. Uptake of P from soil solution occurs as orthophosphate; addition of organic matter was associated with a significant decline of soluble orthophosphate in the soil (Richardson and Simpson, 2011).

The soil available-P in the control treatment (without manures) was higher (P>0.05) than those with chicken manure application at 10 ton.ha^{-1} (Table 5), possibly due to P residues from the applied chicken manures in the first-season experiment. As mentioned in the method, chicken manure with the rate of 20 ton.ha^{-1} was applied for cultivating soybean in the first-season. Therefore, when chicken manure was applied in the second season, it might result in immobilization of P and reduced the available-P in the soil.

Soil that were treated with goat manures had the highest potassium level (Table 4), resulting in high levels of potassium in plant tissues (Table 6). Silahooy (2008) reported that the uptake of potassium in groundnut gradually increased following the increases of KCl fertilizer application, which in line with the results in this study that increasing rates of manures increased the potassium levels in the mustard green tissues (Table 6). Different from K content, the levels of N and P in the leaf tissue were not significantly different among the types and rates of manure application.

Table 4. Soil nutrient content

Treatments	C (%)	N (%)	P (ppm)	K (me/100 g)	C:N ratio
Manure types					
Chicken manure	2.73	0.16	55.18 a	0.54	17.20
Cow manure	2.70	0.16	27.13 b	0.51	17.04
Goat manure	2.68	0.17	20.83 b	1.01	16.25
Manure rates (ton.ha^{-1})					
10	3.59 a	0.16	37.88	1.04 a	22.06 a
0	1.81 b	0.16	30.88	0.33 b	11.59 b
Manure types x rates	ns	ns	**	ns	ns

Note: Values followed by the same letter within the same column were not significantly different according to Tukey test at α=0.05 level

Table 5. The effect of interaction between manure types and rates to soil available P 1) 2)

Treatments	Chicken manure	Cow manure	Goat manure
Manure rates (ton.ha^{-1})		(ppm)	
10	45.95	38.63	29.06 A
0	64.40 a	15.63 b	12.60 bB

Note: 1. Values followed by the same letters within the same rows were not significantly different according to Tukey test at α=0.05
2. Values followed by the same capital letters within the same column were not significantly different according to Tukey test at α=0.05

The levels of N, P and K in the leaf of *Brassica oleracea* were higher than the sufficiency levels of N, P, and K in the leaf tissues according to IFA (1992) of 3.3, 0.5, and 3.1%, respectively. The results showed that the mustard greed had reached sufficient nutrient levels from the application of manures. Although cow manure had a lower phosphate and potassium content compared to other animal manures (Table 2), the number of phosphate-solubilizing microbes in cow manure was quite high (Table 3), which could potentially increase available P in the soil.

Comparison of soil nutrient levels before and after mustard green cultivation indicates the efficiency of manure application. The differences in the nutrient levels might be caused by biological processes or microbes activity in the soil which might be different in different types of soil and the crops grown. The total counts of microbes and Rhizobium after mustard green cultivation has declined compared to before planting (Figure 1), possibly due to the unavailability of plant root exudates as one of energy source for the microbes (Richardson and Simpson, 2011). We could conclude that the effects of soil microbes on crop growth were direct, because there were differences in the number of soil microbes with and without the crops. In addition, plant species affected the presence of specific types of microbes, such as Rhizobium, will likely be available in large quantities when legume crops were grown.

The diversity of functional microbes in the soil previously grown with mustard can be related to different composition of organic fertilizer which affects the availability of substrate for microbes (Juan et al., 2015). The decline in the count of total microbes had occurred after addition of manures (Figure 1) due to the presence of microbe competition between soil and manure. Furthermore, from the three functional soil microbe types, the population of cellulose-degrading microbes was the highest. The application of manure will increase the population of degrading-cellulose microbes (Figure 1) due to the increase availability of carbon in the soil. According to Zhong et al. (2010) metabolic activities of soil microbes are triggered by long-term fertilization that caused by the accumulation

Table 6. Mustard green leaf nutrient content

Treatments	N	P	K
		(%)	
Manure types			
Chicken manure	3.94	0.53	3.60 a
Cow manure	3.46	0.54	3.09 b
Goat manure	3.74	0.52	3.65 a
Manure rates (ton.ha^{-1})			
10	3.59	0.54	3.64 a
0	3.84	0.53	3.25 b
Types of manure x rates	ns	ns	ns

Note: Values followed by the same letters within the same column were not significantly different according to Tukey test at α<0.05

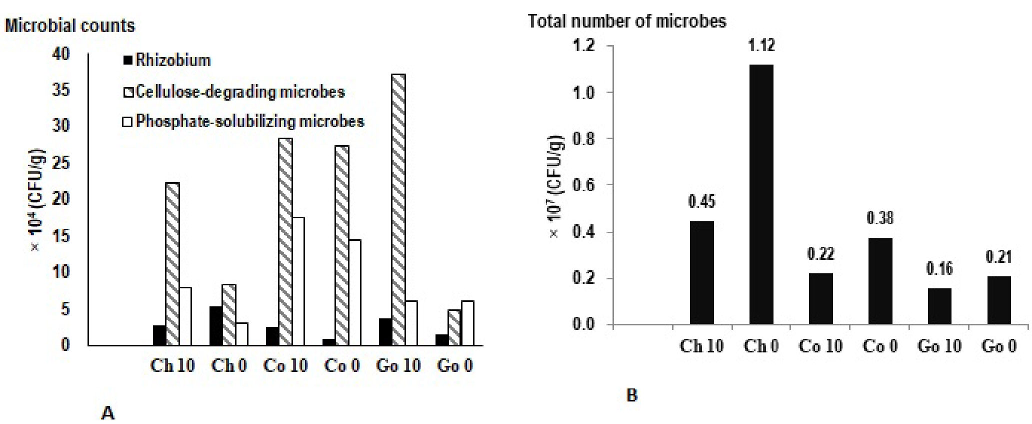

Figure 1. Counts of Rhizobium, cellulose-degrading and phosphate-solubilizing microbes (A) and total number of microbes (B) in the soil treated with 10 ton.ha^{-1} of chicken manure (Ch10), without chicken manure (Ch0), with 10 ton.ha^{-1} of goat manure (Go 10), and without goat manure (Go 0).

of carbohydrates and amino acids derived from the release of plants root exudates to the soil.

The increasing rates of different manures showed that the population of cellulose-degrading microbes was the highest in goat manure. Goat manures at 10 $t.ha^{-1}$ resulted in an in immediate increase of cellulose-degrading microbial count compared to the control. Application of cow manures, on the other hand, had longer term effects on cellulose-degrading microbial count; soil that were treated with cow manures in the first season still had high cellulose-degrading microbial count in the second season. According to Yang et al. (2014), the differences in carbon and nitrogen sources influenced the number and activity of cellulose-degrading microbes because of their cellulose production. The low C/N ratio in different types of manures (Table 2) promoted the decomposition process and stimulated the activities of the specific microbes, resulting in the availability of nutrients for plants (Juan et al., 2015), as shown in nutrient uptake of the leaves (Table 6).

The population of phosphate-solubilizing microbes in the soil applied with cow manure tended to be higher than the in soils with other manures (Figure 1). This microbe was also found in the cow manure and it was more abundant than those in chicken manure (Table 3). On the contrary, the population of phosphate-solubilizing microbes in the soil applied with goat manure was lower than the in soil with cow manure although the number of phosphate solubilizing microbes in goat manure was the highest (Table 3). Kim et al. (1998) showed that the population of phosphate-solubilizing microbes depends on soil physical and chemical properties, organic matter content, and P levels in the soil. Juan et al. (2015) stated that the differences in organic matter composition and substrate availability are likely to be the main reasons for the differences in microbial community. It is evident from this study that soil microorganisms are responsible for the degradation of organic matter, and releasing nutrients from organic matter to stimulate crop growth. In addition, soil microorganisms are also important for maintaining soil structure for a sustainable organic farming.

Conclusion

The application of chicken manures resulted in highest yield of mustard green, but it was not significantly different from the yield with cow manure application. The soil available-P was the highest with chicken manure application. The increasing rates of animal manures increased C-organic, nitrogen, potassium, and C/N ratio in the soil, but had no effects on mustard yields. The soil microbe diversity increased following mustard cultivation. The increasing rates of manures decreased the total microbe counts after mustard cultivation, but increased the cellulose-degrading microbes. The population of cellulose-degrading and phosphate-solubilizing microbes in cow manure were higher than those of the other manures.

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