

# Soil Bacterial Diversity and Productivity of Coffee - Shade Tree Agro-ecosystems

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## ABSTRACT

Coffee productions should have environmental values such as providing high soil microbial diversity while producing high yield. To examine that purposes, two experimental plots were conducted at benchmark site of *Conservation and Sustainable Management of Below-Ground Biodiversity* (CSM-BGBD), in Sumberjaya Subdistrict, West Lampung, Indonesia, during 2007-2010. Types of coffee agro-ecosystem to be examined were *Coffea canephora* with shade trees of *Gliricidia sepium*, *Erythrina sububrams*, *Michelia champaca*, and no shade. Two plots were constructed at 5-years-coffee and 15-years-coffee. Diversity of soil bacteria was determined based on DNA finger printing of total soil bacteria using Ribosomal Intergenic Spacer Analysis (RISA) method. The results showed that: (1) For mature coffee (15 years old), shade-grown coffee agro-ecosystems had higher soil bacterial diversity than those of no shade coffee agro-ecosystem, (2) Shaded coffee agro-ecosystems were able to conserve soil bacterial diversity better than no-shade coffee agro-ecosystem. Soil organic C and total litter biomass had positive effect on soil bacterial diversity, (3) Types of agro-ecosystem significantly affected the bean yield of 15 years coffee. Coffee agro-ecosystems shaded by legume trees had higher yield than those of non-legume shade and no shade coffee agro-ecosystem, (4) Shannon-Weaver indices of soil bacterial diversity together with weed biomass and N content of coffee leaf had positive effect on coffee bean yield.

**Keywords:** agro-ecosystem , bacterial diversity, coffee, RISA, shade

## INTRODUCTION

Sustainable agro-ecosystem is becoming important issue on coffee production that could be achieved by soil conservation and biodiversity conservation (Conservation International 2001). Biodiversity as a part of ecological aspect covers both aboveground and belowground biodiversity. There are strong interaction between aboveground and belowground communities (He *et al.* 2008). Plant diversity and soil microbial communities including bacterial diversity are linked. Plant communities govern ecosystems function including structure and function of soil microbe. Such relationship might be driven by plant biomass and productivity (Liu *et al.* 2008; Lamb *et al.* 2011).

Moreover, biodiversity including soil bacteria diversity is highly related to agro-ecosystem functioning (Moonen and Barberi 2008). Biodiversity and agro-ecosystem functioning also have impact on stability of agro-ecosystem that need

to be considered to develop of sustainable agro-ecosystem (Shennan 2008).

Bacterial community is very diverse and predominant in soil. Moreover, soil bacteria plays an important role on ecosystem services such as nutrient cycle and biological N fixation (Rao 2007). Methods to understand the link between soil bacterial diversity and ecosystem functions are needed to be develop to address question of how diversity influences function. Molecular-based methods have been developed to study soil bacterial diversity to overcome problems associated with non-culturable soil bacteria (Kirk *et al.* 2004). Recently, method of extracting total bacterial community DNA from fresh soil is applied to perform DNA fingerprinting (He *et al.* 2012).

Planting shade trees are the main measurement for soil conservation in coffee agro-ecosystems beside constructing terrace that is only effective in the first couple of years in reducing soil loss (Agus 2011; Bernas 2011). Shade trees of legume in particular have an ecological function such as litter fall production, N fixation, reducing soil erosion, utilizing nutrient from deep soil, and improving soil biological processes (Young 1990).

The objective of this study was to assess coffee agro-ecosystems based on shade tree systems that would sustain soil bacterial diversity and produced high yield of coffee bean.

## MATERIALS AND METHODS

### Study Site and Plot

The research was based on survey and experimental plot of coffee agro-ecosystems, was conducted at benchmark of *Conservation and Sustainable Management of Below-Ground Biodiversity* (CSM-BGBD), in Sumberjaya Sub-district, West Lampung, Indonesia, situated at 4°64' – 5°10' S and 104°15' – 104°20' E. Types of coffee agro-ecosystem to be examined were *Coffea canephora* with shade trees of *Gliricidia sepium*, *Erythrina sububrams*, *Michelia champaca*, and without shade tree. Two plots consisted of 5 years old and 15 years old coffee trees were established.

The experiment used a randomized completely block design (RCBD) with three replications. Coffee trees were spaced at 2 × 2 m and shade trees were spaced at 4 × 4 m. Manual weeding was done 4 times a year while pruning was 2 times a year. No pesticide was applied. In experimental plot 1, shrub was cleared and Robusta coffee seedlings were planted with four shade treatments as mentioned above. Size of experiment plot 1 was 0.48 ha consisted of 12 unit plots of 400 m<sup>2</sup>. Fertilizer dose of 75-25-50 NPK was applied. Plot 2 was a mature Robusta coffee field of 15 years old with the same types of shade trees. Size of experimental plot 2 was 2.4 ha consisted of 12 unit plots of 0.2 ha. Fertilizer of NPK was applied 150-50-100.

### Productivity, Soil and Plant Analysis

Productivity consisted of coffee yield and litter biomass production. Coffee productivity was calculated based on the yield of fresh bean per plot (Hagggar *et al.* 2011). Biomass production (dry weight) was determined based on the production of litter fall, pruning of coffee and shade trees, and weed biomass. Litter fall was sampled using 3 litter traps of 1X2 m wide per plot. Weed biomass was harvested every 3 months before weeding, based on quadrant of 1X1m wide (Evizal *et al.* 2009a). At each plots, soils sample were sampled composite at 0-20 cm depth to analyze organic C content (Walkley and Black), total N (Kjeldahl), and diversity of total soil bacteria (RISA). To analyze N content, leaves were sampled from the third and the fourth pairs of leaves from branches located in

the middle up the sample trees (Snoeck and Lambot 2004).

### Bacterial Analysis

Molecular analysis was conducted to measure soil bacterial diversity. DNA finger printing of total soil bacteria was done by extracting soil samples (Gabor *et al.* 2002) using FastDNA Kit for Soil (Biomedical, USA). To extract DNA of bacteria, it took 0.5 g soil samples and then followed the manufacturer's protocol. Homogenization was done using Fast Prep 24 MP (Biomedical, USA) at the speed of 6.0 m sec<sup>-1</sup> for 40 sec. Prior to PCR, the DNA was further purified by using GeneClean Kit (Biomedical, USA).

To analyze soil bacterial diversity, method of Ribosomal Intergenic Spacer Analysis (RISA) was done by using forward primer 1400F (5' > TGYACACACCGCCCGT <3' and reverse primer 23sR (5' > GGGTTBCCCCATTCRG <3' (Borneman and Triplett 1997). The intergenic spacer region on bacteria DNA was amplified in 20 ìl PCR mixtures of 10 ìl Mega-Mix-Blue (Microzone, UK), 6 ìl sterile distilled water, 1 ìl each of 1406F and 23sR primer (25 pmol ìl<sup>-1</sup>), 2 ìl DNA (25 ng ìl<sup>-1</sup>). DNA was amplified in a GeneAmp PCR System 9700 (Biosystems, USA) as follows: denaturation of DNA at 95°C for 5 min; 30 cycles of denaturation (95°C for 1 min), annealing (53°C for 45 sec), and elongation (72°C for 1 min 30 sec) with final elongation time of 7 min at 72°C.

The PCR products were analyzed in 8% Polyacrilamide Gel Electrophoreses (PAGE). To make the gel, it took 5.3 ml of 30% acrilamide, 12.5 ml destilate water, 2 ml TBE 10X, 180 ml 10% APS and 20 ìl TEMED. Then 9 ìl PCR products were loaded on polyacrilamide gels in 1X TAE buffer. Electrophoreses were run with a constant voltage of 100 V for 6 hours.

### Data Analysis

Intensity of PAGE bands as representation of Operational Taxonomic Unit (OTU) (Cetecioglu *et al.* 2009) were quantified by Adobe Photoshop software. Using software of Biodiversity Analysis Package, Shannon-Weaver diversity indices (H') were calculated based on formula (Ge *et al.* 2008):  $H' = - \sum \pi_i (\ln \pi_i)$  where  $\pi_i = n_i/N$ ,  $n_i$  = intensity of OTU,  $N$  = total intensity of OTU.

Analysis of variance and Duncan Multiple Range Test was carried out to test effect of type of agro-ecosystems on soil bacterial diversity, soil organic C, total N, weed biomass, N content of coffee leaf, and coffee bean yield by using SAS

software. Regression analysis and t-test were performed using SPSS software. Variables of soil organic C, total N, weed biomass, and total litter biomass were regressed with soil bacterial diversity. Variables of soil bacterial diversity, weed biomass, N leaf content, and total litter biomass were regressed with coffee bean yield. To compare variables between 5-years-coffee and 15-years-coffee agro-ecosystem, t-test was carried out.

**RESULTS AND DISCUSSION**

**Soil Bacterial Diversity**

Types of coffee agro-ecosystem influenced soil bacterial diversity which is shown by the profile of DNA bands (Figure 1). Table 1 showed diversity indices of total bacteria DNA extracted from soil samples from different agro-ecosystems based on Shannon-Weaver indices. Types of agro-ecosystem did not affect soil bacterial diversity of 5-years-coffee. On the contrary, types of agro-ecosystem significantly affected soil bacterial diversity of 15-years-coffee. Shaded coffee agro-ecosystems had higher soil bacterial diversity than those of no shade coffee agro-ecosystem. Result of both Duncan and contrast test showed that bacteria diversity from soil of legume (*Gliricidia* and *Erythrina*) shade coffee agro-ecosystems were not significantly different with those of non-legume (*Michellia*) coffee agro-ecosystem.

**Variables Effect on Soil Bacterial Diversity**

As soil decomposer, soil bacterial community is related to soil organic C, soil N, and litter input.

Status of soil organic C in 5-years-coffee agro-ecosystems was high (3.16%) while in 15-years-coffee was moderate (2.45%) according to Balai Penelitian Tanah (2005). Status of soil N was low both in 5-years and 15-years-coffee agro-ecosystems. Based on t-test, weed biomass of 5-years-coffee (9.0 Mg) was higher than those of 15-years-coffee agro-ecosystems (4.9 Mg) but total litter biomass (weed, litter fall, and pruning litter) was relatively similar (11.5 Mg for 5-years-coffee and 11.4 for 15-years-coffee agro-ecosystems). Based on Duncan’s test, types of coffee agro-ecosystems did not have effect on soil organic C, total N, and total litter both in 5-years and 15-years-coffee (Table 2). Souza *et al.* (2012) reported that soil organic C and total N in shaded (agroforest) coffee systems did not differ significantly from full-sun coffee after 13 years of experiment.

Regression analysis result showed that soil organic C and total litter biomass (litter fall, coffee and shade tree pruning residues, and weed biomass)

Table 1. Diversity indices of soil bacteria from coffee agro-ecosystems.

Coffee agro-ecosystems	Shannon-Weaver indices diversity (H')	
	5-years-coffee	15-years-coffee
No shade	2.60 a	2.33 b
<i>Gliricidia</i> shade	2.74 a	2.76 a
<i>Erythrina</i> shade	2.73 a	2.86 a
<i>Michelia</i> shade	2.76 a	2.90 a

Note: Means followed by the same letter in a column are not significantly different using Duncan’s test at  $\alpha = 5\%$ .

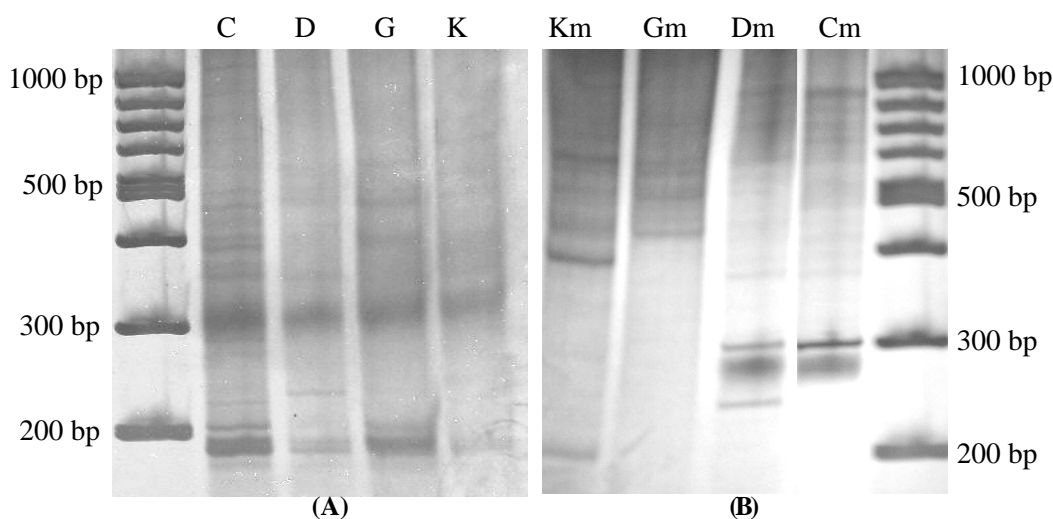


Figure 1. Profile soil bacterial diversity based on DNA bands (A = 15-years-coffee with C, D, G, K = *Michelia*, *Erythrina*, *Gliricidia*, no shade tree, B = 5-years-coffee with Km, Gm, Dm, Cm = no shade, *Gliricidia*, *Erythrina*, *Michelia* shade tree).

Table 2. Soil organic C and total N, weed and total litter biomass of coffee agro-ecosystems.

Agro-ecosystems	Organic C (%)	Total N (%)	Weed biomass (Mg ha <sup>-1</sup> y <sup>-1</sup> )	Total litter biomass (Mg ha <sup>-1</sup> y <sup>-1</sup> )
<b>5-years-coffee</b>				
No shade	3.10 a	0.24 a	9.64 a	11.76 a
<i>Gliricidia</i> shade	3.10 a	0.24 a	8.81 b	11.20 a
<i>Erythrina</i> shade	3.15 a	0.26 a	9.01 ab	11.77 a
<i>Michelia</i> shade	3.30 a	0.24 a	8.55 b	11.43 a
<b>15-years-coffee</b>				
No shade	2.40 a	0.24 a	6.93 a	10.38 a
<i>Gliricidia</i> shade	2.43 a	0.24 a	4.44 b	10.92 a
<i>Erythrina</i> shade	2.51 a	0.25 a	4.33 b	12.25 a
<i>Michelia</i> shade	2.46 a	0.22 a	3.79 b	12.10 a
t calculated (significancy)*	8.43 (0.00)	0.41 (0.69)	7.35 (0.00)	1.40 (0.89)

Note: Means followed by the same letter in a column are not significantly different using Duncan's test at  $\alpha = 5\%$ . \* Comparing 5-years-coffee with 15-years-coffee.

had positive effect on soil bacterial diversity (Table 3). It indicated that soil organic C and litter input are important for bacteria growth including its diversity. Evizal *et al.* (2009b) reported that different types of coffee agro-ecosystem produced different composition of litter. Bohlen (2006) reported that changes in plant communities would be followed by changes of litter composition and by changes of soil microbe diversity.

Figure 2 showed regression between total litter biomass and soil bacterial diversity. Litter produced by coffee agro-ecosystems reached 10-13 Mg per ha per year. Soil bacterial diversity increased with the increasing of litter productivity. Continues litter supply from agro-ecosystems stands are important for conserving soil bacterial diversity. Young-Mathews *et al.* (2010) reported that type of agro-ecosystem affected soil bacterial diversity due to different biomass productivity.

### Coffee Productivity and Soil Bacterial Diversity

N leaf analysis is important to identify deficiencies that determine coffee growth and production (Snoeck and Lambot 2004). This

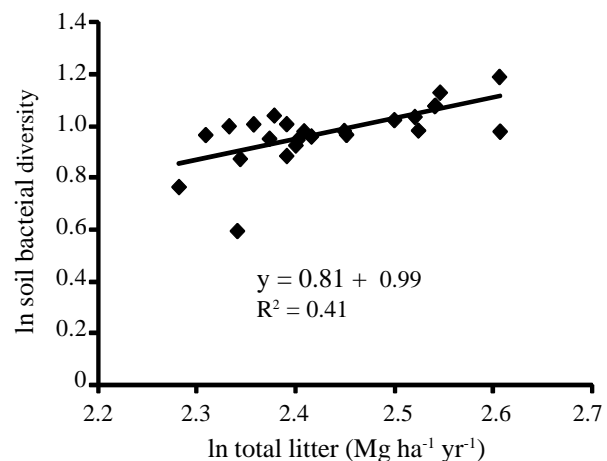


Figure 2. Regression between soil bacterial diversity and litter biomass.

research finding showed that 15-years-coffee trees with legume trees agro-ecosystems were likely to have higher leaf N content than those of no shade coffee and non-legume shade coffee agro-ecosystem. N leaf content of 5-years-coffee trees were not significantly different (Table 4). Coleman and Whitman (2005) stated that there were linkages between ecosystem processes and plant diversity.

Table 3. Regression of soil bacterial diversity of coffee agro-ecosystems.

Variable	Coefficient	t calculated	Significancy
Constant	0.63	1.04	0.31
Soil organic C (%)	0.26*	1.84	0.08
Soil total N (%)	-2.05	-1.05	0.31
Weed biomass (Mg ha <sup>-1</sup> year <sup>-1</sup> )	-0.04	-1.55	0.14
Total litter biomass (Mg ha <sup>-1</sup> year <sup>-1</sup> )	0.18**	4.28	0.00
F calculated (significancy)		5.49 (0.004)	
R <sup>2</sup>		0.54	

Note: \* Significant at  $\alpha 5\%$  and \*\* at  $\alpha 0.1\%$ .

Table 4. N content of coffee leaf.

Coffee agro-ecosystems	Leaf N (%)	Status	Leaf N (%)	Status
	5-years-coffee		15-years-coffee	
No shade	2.34 a	Suboptimum	1.86 c	Deficient
<i>Gliricidia</i> shade	2.73 a	Optimum	3.12 a	Luxurious
<i>Erythrina</i> shade	2.73 a	Optimum	2.59 ab	Optimum
<i>Michelia</i> shade	2.51 a	Optimum	2.07 bc	Suboptimum
Mean	2.58	Optimum	2.41	Optimum
t calculated			1.13	
(significancy)*			(0.28)	

Note: Means followed by the same letter in a column are not significantly different using Duncan's test at  $\alpha = 5\%$ . Status of coffee N content according to Snoeck and Lambot (2004): deficient (< 2%), optimum (2.5-3%), luxurious (> 3.5%). \* Comparing 5-years-coffee with 15-years-coffee.

Table 5. Coffee bean yield of different agro-ecosystems.

Coffee agro-ecosystems	Coffee bean yield (kg ha <sup>-1</sup> )	
	5-years-coffee	15-years-coffee
No shade	1321.5 a	641.0 c
<i>Gliricidia</i> shade	1534.3 a	822.4 b
<i>Erythrina</i> shade	1584.1 a	961.5 a
<i>Michelia</i> shade	1405.3 a	512.1 c
t calculated (significancy)*	9.11 (0.00)	

Note: Means followed by the same letter in a column are not significantly different using Duncan's test at  $\alpha = 5\%$ . \* Comparing 5-years-coffee with 15-years-coffee.

The functional traits of plants such as nitrogen-fixing symbionts, are generally strong drivers of ecosystem processes. Snoeck *et al.* (2000) reported an evidence of the transfer of nitrogen which was fixed by legume trees to coffee trees.

Types of agro-ecosystems did not affect coffee bean yield of 5-years-coffee. It was the second harvest with high yield of coffee bean which was 1.32 Mg ha<sup>-1</sup> for no shade coffee and 1.56 Mg ha<sup>-1</sup> for shaded coffee. On the contrary, types of agro-ecosystems significantly affected the bean yield of 15-years-coffee. Coffee agro-ecosystems shaded by legume trees had higher yield than those of non-legume shade and no shade coffee agro-ecosystem. Between legume trees, coffee agro-ecosystem shaded by *Erythrina* had higher yield than those of coffee agro-ecosystem shaded by *Gliricidia* (Table 5). Evizal *et al.* (2009a) reported that legume shade trees in coffee agro-ecosystems provided ecosystem services such as N fixation by legume nodulating bacteria (LNB) and leaf fall dynamic that important to improve coffee productivity.

Table 6 shows regression analysis of coffee productivity with some variables including N content of coffee leaf and soil bacterial diversity. Shannon-Weaver indices of soil bacterial diversity together with weed biomass and N content of coffee

leaf had positive effect on coffee productivity. Soil bacterial diversity including decomposer and LNB plays an important role on ecosystems functioning such as C cycle and N cycle that have positive effect on land productivity (Barrios 2007).

Weeds play an ambiguous role on coffee agro-ecosystems productivity. No doubt, weeds compete with coffee tree on water, nutrient, and space that can decrease coffee bean yield. If weed is controlled properly, coffee yield might not decrease. Yet weeds produce root exudates and biomass that increase soil bacteria activities and soil organic C. This research finding showed that weed biomass, leaf N content, and soil bacterial diversity were important indicator of coffee productivity.

Using t-test it resulted that for no shade coffee agro-ecosystem, 15-years-coffee had lower soil bacterial diversity than those of 5-years-coffee. However, for shade coffee agro-ecosystems (shaded by legume or non-legume trees), soil bacterial diversity of 15-years-coffee were not significantly different with those of 5-years-coffee agro-ecosystems (Table 7). This finding indicated that shaded coffee agro-ecosystems conserved soil bacteria diversity better than no shade coffee agro-ecosystem.

Tabel 6. Regression of coffee yield and soil bacterial diversity.

Variabes	Coefficient	t calculated	Significancy
Constant	6.34	1.19	0.25
Soil bacterial diversity	1.03*	2.16	0.04
Weed biomass (kg/ha)	0.75**	7.22	0.00
N leaf content (%)	0.96**	4.15	0.00
Total litter biomass (kg/ha/year)	-0.84	-1.41	0.17
F calculated (significancy)		19.48 (0.00)	
R <sup>2</sup>		0.80	

Note: \* Significant at á 5% and \*\* at á 0.1%.

Table 7. Soil bacterial diversity of 15-years-coffee compared to 5-years-coffee.

Coffee agro-ecosystems	t calculated	Significancy
No shade	-2.80	0.05*
<i>Gliricidia</i> shade	-0.97	0.39
<i>Erythrina</i> shade	0.83	0.45
<i>Michelia</i> shade	0.62	0.57

Note: \* Significant at á 5%.

Shade trees of coffee agro-ecosystems have an important role on biodiversity conservation (Conservation International 2001). Moreover, shade trees are key factor of sustainable coffee plantation (Vaast *et al.* 2005). This research results showed that coffee agro-ecosystems shaded by legume trees had higher yield and conserved soil bacteria diversity. It also indicated that legume shaded coffee could conserved belowground biodiversity without decreasing coffee yield compare to nonlegume shaded coffee, as suggested by Borkhataria *et al.* (2012).

## CONCLUSIONS

In the soil of 15-years-coffee agro-ecosystems, shaded coffee had higher soil bacterial diversity than those of no-shade coffee. Shaded coffee agro-ecosystems were able to conserve soil bacterial diversity better than no-shade coffee agro-ecosystem. Soil organic C and total litter biomass (litter fall, coffee and shade tree pruning, and weed biomass) had positive effect on soil bacterial diversity.

Types of agro-ecosystems significantly affected the bean yield of 15-years-coffee. Coffee agro-ecosystems shaded by legume trees had higher yield than those of non-legume shade and no shade coffee agro-ecosystem. Shannon-Weaver indices of soil bacterial diversity together with weed biomass

and N content of coffee leaf had positive effect on coffee bean yield.

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