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Impact of Agricultural Intensification Practices on Bacterial Community in Agro-ecosystems of Southern Sumatra, Indonesia

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ABSTRACT

Soil microbes are important contributors to primary productivity and are very sensitive to disturbance caused by agricultural practices. Nevertheless, the understanding of this circumstance is very limited. The impact of agricultural intensification practices on the microbial community was investigated at 4 different agro-ecosystems. Carbon, nitrogen and phosphorus contents of the soil samples were analyzed. The bacterial community was investigated by using a nucleic acid profiling by means of ribosomal intergenic spacer analysis and the results were analyzed for their similarity by the "Simple matching" coefficient and the dendrograms were obtained by the UPGMA method. The tree-based agricultural system had less carbon, nitrogen and phosphorus content than the crop-based ones. Less intensive agricultural systems had less carbon, nitrogen and phosphorus content compared to that of the intensive ones. Intensive systems had less RISA bands indicating less bacterial diversity. Cluster analysis of the RISA bands indicated that bacterial diversity in crop-based agricultural systems was more vulnerable to intensification practices compared to the one in tree-based systems. © 2012 Friends Science Publishers

Key Words: Agricultural practices; Bacterial community; RISA

INTRODUCTION

Increasing poverty has posed pressure on the land in the humid tropic that is becoming a major drive of global change. It is leading to accelerated habitat modification. Although hardly apparent to the naked eye, soil is one of the most complex habitats. It contains one of the most diverse assemblages of living organisms. Soil microbes are important contributors to the assemblage and play important roles in primary productivity. They provide services such as catalysis of nutrient transformations, (temporary) storage of nutrients, formation and stabilization of soil structure, and control of plant pathogens (Anderson & Domsch, 1989; Oades, 1993; Smith *et al*., 1994; Kennedy & Papendick, 1995), which are important for the existence and functioning of natural ecosystems.

On high-input farms, microbes are generally thought to play a minor role in soil fertility because most nutrients are in the form of in organic fertilizers and are readily available for the plants. While on reduced tillage systems and with low usage of inorganic fertilizers and pesticides, the role of soil microbes in the decomposition and mineralization of complex organic compounds as well as in the reduction of plant pathogens will increase (Schnürer *et al*., 1986; Lebbink *et al*., 1994; McCaig *et al*., 1999). Recently, it has been shown that changes in the microbial community composition of soils can be brought about by either abusive or improved management practices (Baath *et al*., 1995; Frostegard *et al*., 1996; 1997; Zelles *et al*., 1996; Reichardt *et al*., 1997). Variation in microbial population can be correlated with fertilization and drainage (Ajwa *et al*., 1999; Bardgett *et al*., 1999), above ground plant species (Cheng *et al*., 2003; Salas *et al*., 2003) and farming systems (Bloem *et al*., 1994; Bossio *et al*., 1999).

To date, only limited information exists on microbial diversity in agricultural soils (Bell *et al*., 1982; Bloem *et al*., 1994; Zogg *et al*., 1997). As part of a move to make up the result of neglect in this aspect of biodiversity, a project has been initiated to assess impacts of land use change on belowground diversity. Indonesia, together with Brazil, Cote d'Ivorie, India, Kenya, Mexico and Uganda, is participating in the project. The participating countries are expected to sample four types of land use i.e., forest, tree-crop production systems, annual-food crop systems and pasture (Van Noordwijk *et al*., 2004). The district of Sumberjaya at Lampung, Southern Sumatra, Indonesia was selected as one of windows for the project. One of the objectives of the project is to gain knowledge on bacterial diversity in different agricultural soil (Feest & Madelin, 1988; Zogg *et al*., 1997; Bossio *et al*., 1999). Molecular-based methods were applied to soil samples as the use of molecular techniques for the detection and analysis of

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microbes in the environment is increasing steadily (Borneman *et al*., 1996; Gelsomino *et al*., 1999; McCaig *et al*., 1999). Ribosomal intergenic spacer analysis (RISA) has been used to estimate bacterial diversity (Martin-Laurent *et al*., 2001). The present study described the impact of agricultural intensification practices on the bacterial community at four different agro-ecosystems located at Sumberjaya using RISA.

MATERIALS AND METHODS

Site and samples: Soil samples from 8 sites of 4 different agro-ecosystems of Conservation and Sustainable Management of Below Ground Biodiversity Project located at Sumberjaya, Lampung Barat, Lampung, Indonesia (104°26'-104°27' E & 05°01'-05°02' S), were generously given by Dewi *et al*. (2006). Sample A and B were taken from monoculture coffee (*Coffea canephora*) farms. Sample C and D were taken from cowpea (*Vigna unguiculata*) field and eggplant (*Solanum melongena*) field, respectively. Sample E and F were taken from coffee tree-based agro forestry, where coffee tree were grown in the existence of other trees (>15 % & > 5 tree species). Sample G and H were taken from cassava (*Manihot esculenta*) fields.

Soil carbon, nitrogen and phosphorus analyses: Triplicate air-dried <2-mm particle size samples were analyzed according to standard methods. Organic carbon content was analyzed by the Walkley–Black Procedure (Nelson & Sommers, 1982), total nitrogen content was determined by the Kjeldahl method (Bremner & Mulvaney, 1982), while total phosphorus was examined by the ammonium paramolybdate-ascorbic acid colorimetric procedure after extraction using dilute acid fluoride (Olsen & Sommers, 1982).

DNA isolation: For the isolation of DNA, the protocol described by Gabor *et al*. (2002) was used. Air-dried soil (0.5 g) was ground and 800 µL lysis buffer (100 mM Tris-HCl pH 8.0, 100 mM Na-EDTA pH 8.0, 1.5 M NaCl & 1% CTAB) containing 2.0 mg lysozyme were added. The mixture was incubated at 37°C for 30 min, 200 µL 10% SDS were then added and incubation was continued at 68°C for 2 h with mild mixing every 20 min. Supernatant obtained after centrifugation at 10,000 × *g* for 10 min was transferred to a new tube. Extraction steps were repeated using 500 µL lysis buffer and 500 µL 20% SDS. Strong mixing was accomplished by vortexing for 10 sec and incubation was performed at 68°C for 10 min. Supernatant obtained after centrifugation of the mixture was added to the previously obtained supernatant, and chloroform was then added at the same volume of the combined supernatant. After extraction and centrifugation, the upper layer was transferred to a new tube, isopropanol was then added at 0.6 volume of the transferred upper layer, and the suspension was then incubated overnight at 4°C. DNA obtained after centrifugation at 10,000 × *g* for 5 min was then resuspended in TE buffer (100 mM Tris HCl pH 8.0 & 1 mM Na-EDTA pH 8.0). A same volume of phenol-chloroform-isoamylalcohol mixture (25:24:1) was then added, mixed gently, stand at room temperature for 10 min, and then centrifuged at 10,000 × *g* for 10 min. The supernatant was transferred to a new tube, added with 0.1 volumes of 3 M Na acetate and 2 volume of cold ethanol, and incubated overnight at -20°C. DNA pellet obtained after centrifugation at 10,000 × *g* for 5 min was then washed using 70% cold ethanol. After drying up, the DNA was resuspended in TE buffer.

Ribosomal intergenic spacer analysis (RISA): The intergenic spacer region between the small subunit and largesub unit of rRNA genes existing in the DNA extracted from soil samples was amplified using puReTaq™ Ready-To-Go™ PCR Beads of Amersham Biosciences Ltd. (Buckinghamshire, UK) in in 25-µL PCR mixtures at the following final concentrations or total amounts: 25 ng of soil DNA, 25 µM (each) primer, 2.5 U of puReTaq DNA polymerase, 10 mM Tris HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP and stabilizers, including bovine serum albumin. The PCR primers were 1406F (TGYACACACCGCCCGT) (universal rRNA small subunit) and 23SR (GGGTTBCCCCATTCRG) (bacterial 23S rRNA large subunit). All reagents were combined and heated at 94°C for 2 min. Thirty cycles of PCR were performed with a My cycler (Biorad) at 94°C for 15 s, 50°C for 15 s, and 72°C for 45 s, followed by additional 2 min elongation at 72°C for the last cycle. The PCR products were then separated in 6% polyacrylamide gel electrophoresis after addition of loading buffer (15% ficoll, 0.25% bromophenol blue, 0.25% xylene cyanol FF) at 0.2 volumes. The electrophoresis was performed at 100 V for 4 h. Gel was silver stained following the protocol described by Sambrook *et al*. (1989). The gel was soaked into 10% glacial acetic acid for 40 min, followed by 3 times rinse by soaking into aquabidest for 5 min each. The gel was then soaked into a solution containing 0.1% $AgNO_3$ and 0.56% formaldehyde for 45 min, followed by quick washing in double distilled water. The gel was then destained using a mixture of 3% Na_2CO_3, 0.56% formaldehyde, and 2 mg mL^{-1} $Na_2S_2O_3$ until bands of DNA visualized. The destaining process was terminated by adding 10% glacial acetic acid into the destaining solution.

Community percent similarity based on RISA: The RISA banding patterns were analyzed manually. The relative mobility of each band was calculated using the 100-bp molecular size marker as the reference. A similarity matrix of bands presence (1) and absence (0) was calculated by the "Simple matching" coefficient and the dendrograms were obtained by the UPGMA method from NTSYS-pc package (Rohlf, 1998). TREEVIEW (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) software package (Page, 1996) was used to visualize the dendrogram.

RESULTS AND DISCUSSION

The impact of agricultural intensification practice son the microbial community was investigated at four different agro-ecosystems located at Sumberjaya, Lampung Barat, Lampung, Indonesia. The soil was dominated by clay fraction in all depth. The farmland was dominated by coffee plantation and annual crops fields.

Considering that monoculture coffee plantation could not grow adequately without addition of fertilizer, samples A and B were considered to represent tree-based intensive agricultures (TBI). Intensive application of fertilizers is also needed in growing horticultural crops and vegetables. Therefore, samples C and D may represent annual crop-based intensive agricultures (ABI). On the other hand, local practices give inadequate fertilizers in growing cassava and coffee grown in agroforestries. Therefore, samples E and F were classified as tree-based less intensive agricultures (TBLI), while samples G and H were classified as annual crop-based less intensive agricultures (ABLI).

Carbon, nitrogen and phosphorus contents of the soil samples: Analysis of organic carbon, total nitrogen, and total phosphorus of the soil samples were used to verify the above classification. Indices of [C][N][P] were calculated from the results of the analysis (Table I). Sample F has a high [C][N][P] index. It may indicate the addition of fertilizers. When plotted into a graph (Fig. 1), the [C][N][P] indices of intensive agricultures (samples A, B, C & D) were higher than those of less intensive agriculture (E, G, & H). Therefore, [C][N][P] indices can be used to approach the agricultural intensification practices quantitatively. Fig. 1 also showed that sample F is more appropriate to be classified into tree-based intensive agriculture.

Community differences based on RISA: Demonstration of the relevance and the sensitivity of the RISA approach for microbial diversity analysis has been previously reported (Ranjard *et al*., 2000). The RISA patterns are shown in Fig. 2. The patterns comprised 7 to 24 bands, including one or more major bands in each sample. Each band may represent a single member of microbial community. More dominant bands can be observed in intensive agriculture than in less intensive agriculture. It may be caused by soil environmental changes formed by the agricultural practices, which furnish favorable conditions to certain microorganisms.

When the numbers of bands were plotted against the [C][N][P] indices (Fig. 3), it showed that intensive agricultural practices (both crop-based as well as tree-based) have less RISA bands indicating the existence of a fewer kind of microorganisms in such systems compared to that of less-intensive agriculture. Agricultural activities, such as tillage, weeding, terracing, sub soiling, deep ploughing, manure, compost and fertilizer applications, liming, draining, irrigation, and biocides applications on cultivated crops, may affect soil chemical, physical or biological properties (Bridges & De Bakker, 1997).

Table I: Carbon, nitrogen and phosphorus contents and [C][N][P] index of soil samples use in this study

Sample	Carbon (%)	Nitrogen (%)	Phosphorus (ppm)	[C][N][P] index
A	3.20	0.28	119.21	106.81
B	2.86	0.27	221.80	171.27
C	4.39	0.42	97.93	180.56
D	2.28	0.28	179.41	114.54
E	2.36	0.23	118.26	64.19
F	2.58	0.32	241.41	199.31
G	2.45	0.29	140.39	99.75
H	2.78	0.30	97.13	81.01

Fig. 1: Indices of [C][N][P] across a land use intensity. TBI: Tree-based intensive; CBI: crop-based intensive; TBLI: tree-based less-intensive; and CBLI: crop-based less-intensive

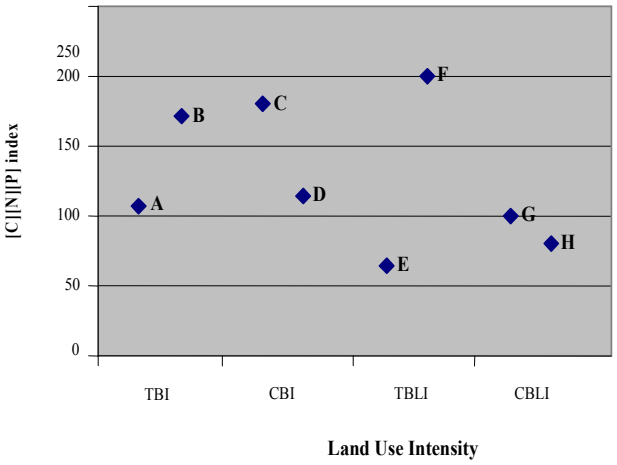


Fig. 2: RIS analysis of different land use.M, 100-bp ladder DNA molecular marker.TBI: Tree-based intensive; CBI: crop-based intensive; TBLI: tree-based less-intensive; and CBLI: crop-based less-intensive

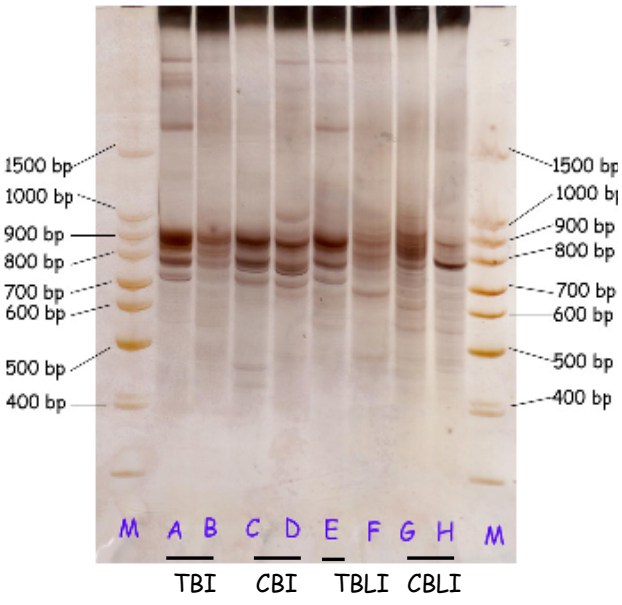


Fig. 3: Correlation between the number of RIS bands and the [C][N][P] indices. A and B: Tree-based intensive; C and D: crop-based intensive; E: tree-based less-intensive; G and H: crop-based less-intensive

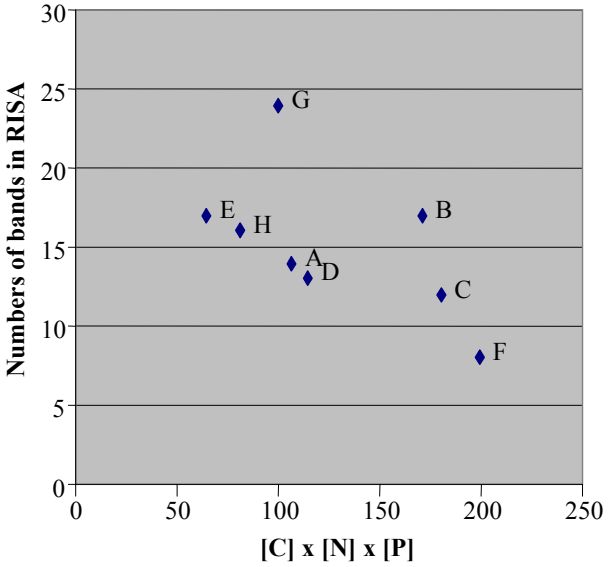
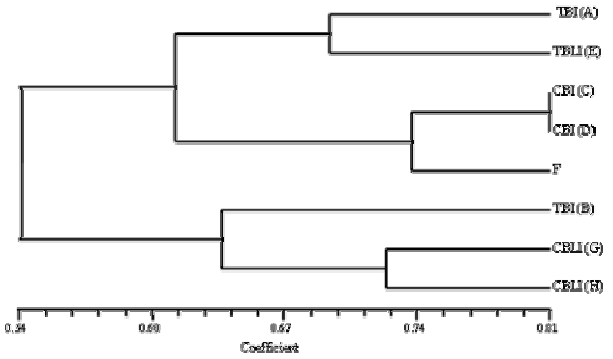


Fig. 4: UPGMA dendrogram clustering analysis based on similarities of the communities. TBI: Tree-based intensive; CBI: crop-based intensive; TBLI: tree-based less-intensive; and CBLI: crop-based less-intensive



Previous studies have shown that transition from high-input to low-input agriculture has caused an increase in pH, total nitrogen phosphorus, potassium, as well as organic matter contents in soil (Scow *et al.*, 1994). Agricultural inputs have also caused deterioration of soil biota (Rana *et al*., 2010). Soil environmental changes formed by the agricultural practices may give favorable conditions to certain microorganisms and give unfavorable condition to other microorganisms. The decline of microbial metabolic diversity in intensive agricultures has also been observed by Mills and Adl (2006). Fig. 3 also showed that sample F is more appropriate to be classified as a sample from intensive agriculture.

Cluster analysis between RISA patterns was conducted (Fig. 4). The results showed that there are distinctive clusters among samples from crop-based agricultures. Samples from crop-based intensive and crop-based less intensive-agriculture had 81 and 72% similarities, respectively. No cluster was observed between tree-based agricultures. Crop based agriculture does not allow the existence of plants other than the crop itself, which may be considered as weeds. In tree based agriculture, some plants other than the intended tree, such as cover crops, were allowed to exist. This condition resulted in the homogeneity of environment in crop based agriculture. Moreover, practices in crop-based agricultures, which are more intensive then the tree-based ones, give a high effect that made greatest community discontinuity between crop-based intensive and crop-based less intensive agricultures. Differences between crops on the fields, where samples were taken for crop-based intensive and crop-based less intensive agricultures in this study may magnify the discontinuity.

CONCLUSION

It can be concluded that tree based agricultural systems have more conserving ability for the belowground biodiversity. The more detailed effort is needed, such as analyses of functional group diversity from these sites that will give more detail information related to the effect of agricultural practices on functional communities in soils.

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