Final Research Report

Impact of Pesticide Applications on Soil Microorganisms and Properties at Surin Province

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Abstract

This study aims to investigate (a) which of the soil variables (chemical, physical and biological properties) can be used as indicators reflecting differences in soil quality among organically, young organically, semi-chemically and conventionally managed rice fields, and (b) effects of the agricultural management practices of paddy field on soil variables. Dynamics of soil properties also were compared between before planting and after post-harvesting. Distribution of soil microorganism populations (bacteria, fungi, actinomycetes, and cellulolytic microbes) among agricultural management practices was statistically analyzed by Principal Component Analysis (PCA). The results clearly showed that mainly soil moisture content, bulk density, available phosphorus, soil pH, nitrogen content, organic matter, exchangeable Ca^{++}, Mg^{++}, Na^{+}, and K^{+} were considered to be soil quality indicators. In addition, organic and young organic fields are related to soil bacterial and cellulolytic microbes while semi-chemical, and chemical soils are related to soil fungi and actinomycetes, respectively. Microbial populations varied among agricultural management practices. The order of soil microorganism levels was found from highest to lowest as follows: organic field > young organic field > chemical field > semi-chemical field in both cases of before planting and after harvesting soils. In addition, the proportion of soil population varied among different soil sites. Organic and young organic areas contained the soil cellulolytic microbes greater than bacterial population, actinomycete population, fungal population, respectively. For semi-chemical and chemical areas, the proportion of soil microorganisms showed highest number of soil actinomycete followed bacteria, cellulolytic microbes, and soil fungi, respectively. DGGE profiles showed that the structure of microbial communities were different among soil sites. The patterns in PCR-DGGE profile were clustered into two groups: one group comprising the organic and young organic sites and second one from semi-chemical and chemical soils. These results suggest that the microbial diversity in the chemical fields is completely different from organic fields.

Keywords: Agricultural management practice, organic field, chemical field, soil microbial population, bacteria, fungi, actinomycetes, cellulolytic microbes, DGGE, PCA

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CHAPTER 1

INTRODUCTION

1.1. Research question

Thai Hom Mali rice is a major agricultural export products of Thailand, which is accounted statically around 50%, (40,341.9 baht in 2006, and 63,520.8 million baht in 2010) and it is growing dramatically (Office of Agricultural Economics, 2011). The increase in export values in Thai Hom Mali rice resulted in the expanding of rice cultivation and land use. Although the huge amount upto 49.76% of the total land use are paddy fields but grain yield was rather low rate. There was only 343 kgs per rai, which is lowest in Asia (average yield in Asia was 625 kgs per rai i.e. China 1,013 kgs per rai, Japan 1,027 kgs per rai, Korea 1,087 kgs per rai, Indonesia 730 kgs per rai, Vietnam 602 kgs per rai, etc.) and also under the standardization of the world (average grain yield of the world was 612 kgs per rai) (Department of Agricultural Extension, 2011). Eighty percentage of Thai Hom Mali rice production is from Northeast regions as Burirum, Srisaket, Ubonratchatanee, especially from Surin province. Thai Hom Mali rice from Surin province is well known and high quality due to a variety of topography and suitable climate. Surin province shows a varieties of rice cultivation patterns such as direct dried-seeding, wet-seeding and indirect-seeding and under organic, young organic, semi-chemical and chemical management practices. In addition, rice cultivation is grown in wet and dry seasons.

In many areas, massive amounts of rice residue after post harvest were normally eliminated by burning in open-field, because rice straw is considered as disease infestation, unstable nutrients and slow rate in degradation. Effects of burning process of rice straw in the fields are concern seriously all over the world due to emission of many seriously toxic pollutants such as carbon dioxide, carbon monoxide, methane, nitrous oxide, and sulphur dioxide, polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins (PCDs) (Gadde, Bonnet, Menke, & Garivait, 2009; Kausar, Sariah, Mohd Saud, Zahangir Alam, & Razi Ismail, 2010). These toxic compounds represent a threat to public health and pose an environmental pollution problem. In addition, many studies have been shown that agricultural management practices strongly influenced soil property changes in chemical, physical, and biological variables, especially, the abundance and diversity of soil fauna (Briar, et al., 2011), and soil microbial communities (Braun, Böckelmann, Grohmann, & Szewzyk, 2010; Meriles, et al., 2009; Wang, et al., 2010; Whitelaw-Weckert, Rahman, Hutton, & Coombes, 2007). The effects of continuous plant cover on soil microbial counts have been shown by Whitelaw-Weckert, et al (2007). They found that hot water extractable soil C in the vine row was increased by 73% after 3 years of planting and most of the soil bacterial counts. The numbers of bacteria were decreased from the fast growing cellulolytic bacteria, slow growing cellulolytic bacteria, copiotrophic bacteria, fast growing low nutrient bacteria, oligotrophic bacteria, and copiotrophic pseudomonads along the herbicide applications. The fraction of total soil soluble organic C was positively correlated with fungal counts and with cellulolytic, pseudomonad, copiotrophic and oligotrophic bacterial counts. Bulluck, Brosius, Evanylo, and Ristaino (2002) found that organic and synthetic fertility amendments significantly influenced soil microbial, physical and chemical properties on organic and conventional farms. Especially, beneficial soil fungi in the genus *Trichoderma*, the numbers of *Trichoderma* sp. were higher in soils from fields with a history of organic than conventional production. The densities were increased over time in fields with a conventional history, but were remained higher over time in soils from organic compared to conventional fields. Soils with alternative amendments had also higher propagule densities of *Trichoderma* sp. than soils amended with synthetic fertilizers in vegetable farms in Virginia and Maryland (Bulluck, et al., 2002). Alternative fertility amendments enhanced beneficial soil microorganisms and they could reduce pathogen populations. In addition, alternative fertility amendments increased soil organic matter, total carbon, and cation exchange capacity (CEC), and lowered bulk density thus improving soil quality, while synthetic fertility decreased soil organic matter, total carbon, and cation exchange capacity (CEC), and heighten bulk density. The cropping patterns such as mono-cropping pattern to mixed cropping pattern resulted soil microbial communities and soil properties. Vargas Gil, et al. (2011) reported that microbial community structure based on PLFA analysis was the lowest PLFA content in soils treated by reduced tillage and soybean monoculture, whereas the highest one was found in the reduced tillage and corn-soybean treatment. A similar trend was also found for the zero tillage samples with higher values in the corn-soybean treatment samples than in the soybean monoculture samples. In paddy soils, Das & Debnath (2006) reported that the effect of four systemic herbicides viz., butachlor [N-(butoxymethyl)-2-chloro-2′,6′-diethyl-acetanilide], fluchloralin [N-(2-chloroethyl)-(2,6-dinitro-N-propyl-4-trifluoromethyl) aniline], oxadiazon [5-terbutyl-3-(2,4-dichloro-5-isopro poxyphenyl)-1,3,4-oxadiazol-2-one] and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene] at their recommended field rates (2.0, 1.5, 0.4 and 0.12 kg ha^{-1}, respectively). These herbicides significantly affected on growth and activities of non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms. These herbicides are frequently used by the farmers in their rice fields to eradicate the unwanted weeds for better crop growth, which are classified as chloroacetanilide, 2,6-dinitroaniline, oxadiazoline, diphenylether, respectively. They also found that these herbicides related to availability of nitrogen and phosphorus in the rhizosphere soils as well as yield of the rice crop. Soil microorganisms are the main bioorganisms responsible for long-term sustainability of soil ecosystems since they control the breakdown of organic matter and nutrients through decomposition, mineralization, and immobilization processes. Thus, soil microorganisms can be used as bioindicators of soil quality changes (Winding, Hund-Rinke, & Rutgers, 2005; Zhang, et al., 2010). In Surin province, the recent policy concerned with more environmentally sensitive farming practices has led to a widespread interest in organic farming, but there still rarely was an accredited organic commercial farm. Farmers still have pesticide, synthetic fertilizer application, and rice straw burning and with a variety of rice cultivation patterns by direct dry seeding, wet seeding and indirect seeding. In this study, the effects of organic, conventional and chemical management practices on soil health and to determine soil quality indicators was investigated based on physical, chemical, and biological parameters among various agricultural systems.

1.2 Objectives

1. To investigate the distribution of microorganism populations in various agricultural soil managements at Surin rice fields; organic, young organic, semi-chemical, and chemical organic management practices; and
2. To evaluate soil physical and chemical properties of Surin rice fields by agricultural management practices

1.3 Expected results

This investigation on the impact of pesticide applications on soil microorganisms and properties at Surin province will provide the distribution patterns of soil microorganisms, soil physical and chemical properties, among various agricultural management practices for improving soil quality and soil indicators.

CHAPTER 2

LITERATURE REVIEWS

2.1 Significance of soil quality

Soil is one of the most important bases of the lives for humans and other microorganisms, essentially, for human land use. Soil as a resource is not renewable which often leading to conflicts between concurrent types of land use. Soil quality is best defined as the capacity of a soil to promote the growth of plants and to protect watersheds by regulating the infiltration and partitioning of precipitation, and to prevent water and air pollution by buffering potential pollutants such as agricultural chemicals, organic wastes, and industrial chemicals (Committee on Long-Range Soil and Water Conservation, 1993). Franzluebbers (2002) defined that soil quality as a concept, based on the premise that management can deteriorate, stabilize, or improve soil ecosystem functions. The quality of a soil is also determined by a combination of physical, chemical, and biological properties such as soil texture, water-holding capacity, porosity, organic matter content and depth. Since these attributes differ among soils in their quality. Some soils, because of their texture or depth, for example, are inherently more productive because they can store and make available larger amounts of water and nutrients to plants. Similarly, for their organic matter content, some soils are able to immobilize or degrade larger amounts of potential pollutants. Soil management can either improve or degrade soil quality. The soil parameters such as erosion, compaction, salinization, sodification, acidification, and pollution with toxic chemicals can degrade soil quality. On the other hand, increasing soil protection by crop residues and plants as adding organic matter to the soil through crop rotations, manures, or crop residues, and careful management of fertilizers, pesticides, tillage equipment, and other elements of the farming system can improve soil quality. In Thailand, soil quality problems caused by agricultural practices are increased national attention and perceived by society as a major environmental problem comparable to other national environmental problems as such as air quality, water quality and the release of toxic pollutants from industrial sources. Soil quality provides the foundation for environmental quality and sustainable land use.

2.2 Soil quality indicators

Soil quality indicators (chemical, physical or biological property of soil) are sensitive to disturbance and represents performance of ecosystem function in the soil of interest. Indicators are dynamic soil properties. Scientists use soil quality indicators to evaluate how well soil functions since soil function indirectly. Measuring soil quality is an exercise to identify soil properties that are responsive to soil managements, inturn correlated with environmental outcomes that are capable of being precisely measured within certain technical and economic constraints. Soil quality indicators may be qualitative as fast drainage or quantitative as infiltration rate (2.5 in/hr). Doran & Parkin (1996) reported that ideal indicators for soil quality should correlate well with ecosystem processes, integrate soil physical, chemical, and biological properties and processes being accessible to many users, being sensitive to management and climate, being components of existing databases, and being interpretable.

There are three main categories of soil indicators: chemical, physical and biological. Typical soil tests only look at chemical indicators. Soil quality attempts to integrate all three types of indicators. The categories do not neatly align with the various soil functions thus integration is necessary. The table below shows the relationship between indicator types and soil function.

Table 2-1. Relationship between indicator types and soil function (Doran & Parkin, 1996)

Indicator category	Related soil function
Chemical	Nutrient Cycling, Water Relations, Buffering
Physical	Physical Stability and Support, Water Relations, Habitat
Biological	Biodiversity, Nutrient Cycling, Filtering

Organic matter, or more specifically carbon content and transcends all three indicator categories, have the most widely recognized influence on soil quality. Organic matter is related to all soil functions which affects the other indicators such as aggregate stability (physical), nutrient retention and availability (chemical), and nutrient cycling (biological).

Some indicators are descriptive and can be used in the field as part of a health card. Others must be measured using laboratory analyses. Examples of the three broad categories of chemical, physical and biological are provided below.

2.2.1 Chemical indicators give information about the equilibrium between soil solution (soil water and nutrients) and exchange sites (clay particles, organic matter); plant health; the nutritional requirements of plant and soil animal communities; and levels of soil contaminants and their availability for uptake by animals and plants. Indicators include measures of electrical conductivity, soil nitrate, soil reaction (pH).

2.2.2 Physical indicators provide information about soil hydrologic characteristics such as water entry and retention, that influences availability to plants. Some indicators are related to nutrient availability by their influence on rooting volume and aeration status. Indicators include measures of aggregate stability, available water capacity, bulk density, infiltration, slaking, soil crusts, soil structure and macropores.

2.2.3 Biological indicators show the organisms that form the soil food web that are responsible for decomposition of organic matter and nutrient cycling. Information about the numbers of organisms, both individuals and species, that perform similar jobs or niches, can indicate a soil's ability to function or bounce back after disturbance (resistance and resilience). Indicators include measures of earthworms, particulate organic matter, potentially mineralizable nitrogen, respiration, soil enzymes, total organic carbon.

Integration of multiple soil parameters (Fig. 2-1) representing soil biology, nutrient recycling and supply, and suitability of physical conditions provide a logical representation of overall soil quality for crop production used in many researches (Bhardwaj, Jasrotia, Hamilton, & Robertson, 2011).

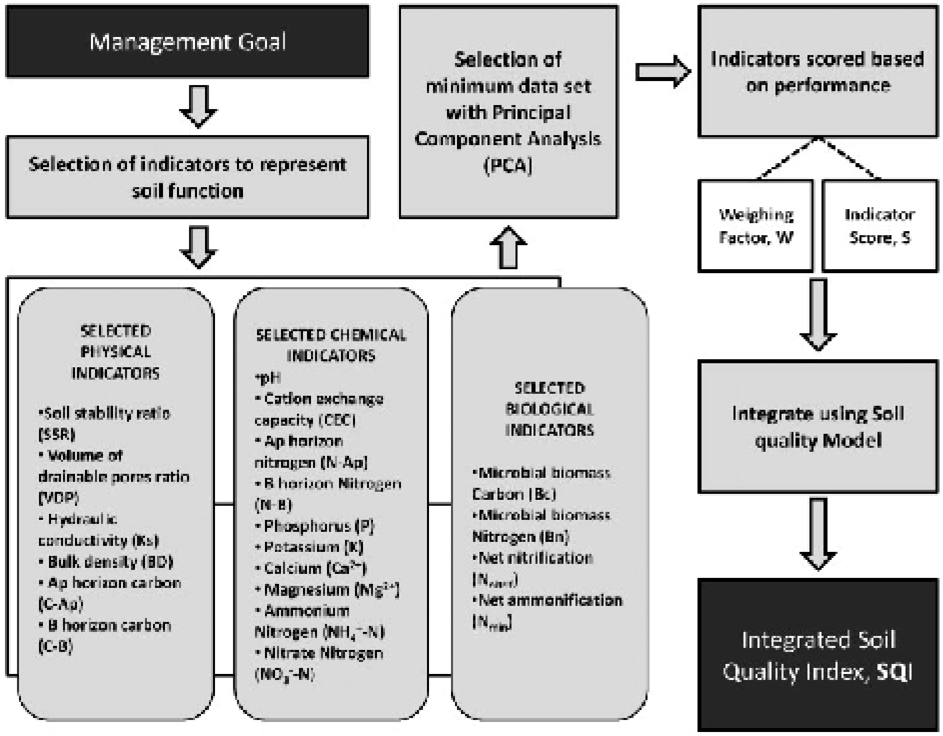

Fig. 2-1. Conceptual framework for soil quality assessment. (Bhardwaj, et al., 2011).

2.3 Chemical and physical indicators of soil quality

Soil organic matter is commonly recognized as one of the key chemical parameter of soil quality (Franzluebbers, 2002; Mazzoncini, et al., 2010). They found that the C_{org} mean value was significantly higher in organic than in conventional plots. High stratification ratios of soil C and N pools could be good indicators of dynamic soil quality, independent of soil type and climatic regime, because ratios >2 would be uncommon under degraded conditions. Miralles et.al (2009) studied the variations of soil physical and chemical characteristics along agricultural land use. The results have been shown that most characteristics were significantly correlated with total organic C (mean equal to 28.5 ± 4.6 g kg^{-1}), which demonstrates the central role of the organic matter in the functioning of the whole ecosystem. Marinari et.al (2006) found that chemical properties were chosen as indicators of soil quality and were measured at soil depth intervals of 5–20 and 20–35 cm, after 7 years of organic certified and conventional management methods. The field under organic management showed significantly better soil nutritional condition with increasing level of total nitrogen, nitrate and available phosphorus. No consistent increase in total organic carbon was observed. Results of the study suggest that, over the period of 7 year, organic management method strongly affects soil quality indicators.

2.4 Biological indicators of soil quality

Many researches have been reported that biological indicators varied agricultural management systems as crop rotation, management practices etc. Microbiological property was chosen as an indicator of soil quality and was measured at soil depth intervals of 5–20 and 20–35 cm, after 7 years of organic certified and conventional management methods. The field under organic management showed significantly better soil nutritional and microbiological conditions; with an increased level of microbial biomass content, and enzymatic activities (acid phosphatase, protease and dehydrogenase) (Marinari, et al., 2006). Benintende, Benintende, Sterren, & De Battista (2008) studied on the conservationist management applied by the use of rice pasture rotation. They found that this rotation showed the highest values of variables related to the size of the microbial communities (MBC, MBN), to nitrogen availability (PMN-AI) and biochemical variables (FDA, urease). In contrast, rice monoculture showed high values of C to N ratio in the microbial biomass, resulting in differences in microbial community composition due to the low quality of the stubble. Rice soybean rotation system behaved similar to the monoculture, but the rotation in which maize was included tended to maintain higher values of the variables associated to soil quality. The differences found in microbial analysis indicate that microbiological variables (MBC, MBN), nitrogen availability index (PNM-AI), and biochemical variables (FDA) were sensitive variables to measure soil rotations effects. These variables might be used as good soil quality indicators once their critical values have been determined for different conditions. In addition, agro-ecosystem management can affect soil quality in the long-term by modifying soil physical, chemical and biological characteristics at a rate that is largely dependent on climate conditions and farming practice (Mazzoncini, et al., 2010)

CHAPTER 3

MATERIALS AND MATHODS

3.1 Study area and sampling location

This study was conducted in Surin province, the Northeast of Thailand ($14^{\circ} 53^{\prime}$ N; $103^{\circ} 30^{\prime}$ E), which higher than above sea level of 150 m. The study site has a mean annual rainfall of 1,542.80 mm and a mean annual temperature of 27.5°C, with a minimum of 23.7°C in January and a maximum of 30.4°C in April. Surin province also shows a variety of soil management practices; chemically, semi-chemically, conventionally and organically managed soils, which were started to operate since only one crop season up to five crop seasons and over. Thus, the soil samples were classified into 4 management practices, according to:

1. fields operated under “chemical management” (involved the application of inorganic fertilizers and synthetic pesticides)
2. fields operated by converting from chemical to organic management as “semi-chemical management” (one to two years into transitional change from chemical to organic practices and including fields in which synthetic pesticides or fertilizers are combined with organic farming techniques)
3. fields operated as “young organic management” (at three years to five years under organic management)
4. fields managed organically as “organic management” (over five years under the organic practices).

3.2 Soil sampling

Samples were collected twice, in May and November, before rice cultivation period and post harvest season. At each soil sample, the soil surface layer (0-15 cm) and subsoil (15-30 cm) were taken and transported to the laboratory on ice, and also stored at -20 °C in dark plastic bags. For chemical and physical analyses, the collected soil samples were air dried and then ground and sieved through a 0.5 cm mesh.

3.3 Physical and chemical analyses

Soil bulk density was determined by using the core method. Soil texture (mass of sand–silt-clay sized particles) was determined by using a hydrometer. Freshly sieved samples were weighed onto dried aluminum trays, heated at 105°C for 48 hr, and reweighed to determine the moisture lost. A 10 g portion of each sample was mixed with water at a ratio of 1:5 and 1:10 and then soil pH was determined after 1 hr by using pH-meter with a glass combination electrode. The available phosphorus (Pi) was extracted by Bray II and measured by spectrophotometry. Organic carbon (C_{org}) was followed by Walkley and Black method (Bonifacio, et al., 2008; Li, et al., 2010).

3.4 Culture media and conditions

Bacteria were counted at 25°C for 5 days on nutrient agar at pH 6.8 ± 0.1. Actinomycetes were cultivated at 25°C for 7 days on glycerol-yeast extract medium at pH 7.0 ± 0.1. Streptomycin and cycloheximide were added to inhibit the growth of bacteria and fungi at a final concentration of 10 μg/ml. Fungi were grown at 25°C for 5 days on Rose Bengal medium at pH 6.8 ± 0.1. Cellulolytic microbes were assayed at 25°C after 7 days incubation on Mandels–Reese medium with carboxymethylcellulose (CMC, Sigma) as the sole carbon source and sprayed with Congo red to show a clear zone around the colonies (Cho, Tsai, Ravindran, Selvam, & Yang, 2008; Tsai, Selvam, & Yang, 2007). All the experiments were carried out in triplicates.

3.5 DNA extraction

Genomic DNA of the soil samples was extracted following a modified protocol of Zhou et.al (1996). Soil samples of 10-15 g were mixed with 13.5 ml of DNA extraction buffer (100 m MTris-HCl [pH 8.0], 100 mM sodium EDTA [pH 8.0], 100 mM sodiumphosphate [pH 8.0], 1.5 MNaCl, 1% CTAB) and 100 ml of proteinase K (10mg/ml) in Oakridge tubes by horizontal shaking at 225 rpm for 30 min at 37 ºC. After the shaking treatment, 1.5 ml of 20% SDS was added, and the samples were incubated in a 65 ºC water bath for 2h with gentle end-over-end inversions every 15 to 20 min. The supernatants were collected after centrifugation at 7000 rpm for 20 min at room temperature and transferred into 50 ml centrifuge tubes. Supernatants were combined and mixed well with an equal volume of chloroform/ isoamylalcohol (24:1,vol/vol). The queous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 14,000 rpm for 30 min at room temperature, washed with cold 70% ethanol, and resuspended with 300 µl of sterile deionized water.

3.6 PCR amplification

Total bacterial 16S rDNA was amplified using the universal DNA primer set 338F (5′-C GCCCGC CGCGCG CGGC GGGCG GGGC- GGG GGCA CGGGG GGA CTCCTA CGGGA GGCA-3′) and 518R (5′-ATTA CCGC GGC- TG CTGG-3′) (Siddique, Zhang, Okeke, & Frankenberger, 2006). The PCR was performed with the following reaction conditions (Table 3-1).

Table 3-1. PCR primers and PCR condition

Nested PCR	PCR conditions						
	No. of cycles	Denaturation		Annealing		Elongation	
		°C	min	°C	min	°C	min
Primer: 338-GC-F/518r	30	95	0.50	60	0.30	72	0.50

3.7 Denaturing gradient gel electrophoresis

DGGE analyses were conducted following the protocol of Siddique et al. (2006). Electophoresis was performed on 7.5% polyacrylamide gels using with gradients of 40–70% denaturants and a running time of 12 h at 100 V were selected. Gels were immerged in 1x TAE buffer (Trizma base 40mM, EDTA 1 mM, sodium acetate 20 mM, pH 7.5) at at 60°C in a DGGE 2000 system (CBS Scientific Company, Del Mar, CA). After electrophoresis, gels were stained with SYBR green and visualized by UV transillumination. The gel image was captured using a CCD camera and Biocapt software (Vilber Lourmat). Image analysis was performed using Bio-1D++ software version 11.06 (Vilber-Lourmat, Marne-La-Valle, France), which allows fragment detection and quantification. Statistical differences between groups determined from the dendrogram by unweighted-pair group method with arithmetic means (UPGMA).

3.8 Statistical analysis

Data analyses were performed using PC-ORD for Windows, 5th version from MjM software. Discriminant analysis of agricultural management practices was analyzed based on the principal component analysis (PCA). In addition, One-way analysis of variance (ANOVA) was also used to detect significant differences in soil variables from different management practices.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Soil Physical and Chemical Properties

The studied soils were generally were sandy clay loam. Most of soil texture of paddy fields in Surin province was composted of sand particle between 30-60 % (Table 4-1 to 4-2). They were classified into sandy clay loam, sandy clay loam, loam, and sandy loam under organic, young organic, semi-chemical and chemical management practices, respectively.

Table 4-1. Soil texture class under various agricultural management practices

Agricultural Management Practices	Soil depth (cm.)	Soil texture class
Organic	0-15	Sandy clay loam
	15-30	Sandy clay loam
Young organic	0-15	Sandy clay loam
	15-30	Sandy clay loam
Semi-chemical	0-15	Loam
	15-30	Loam
Chemical	0-15	Sandy loam
	15-30	Loam

Some physical characteristics of soil under organic, young organic, semi-chemical, and chemical management practices before planting (before rice cultivation) were shown in Table 2. Soil moisture content under organic and young organic areas was higher than semi-chemical and chemical areas. Bulk density of soils varied among agricultural management practices. Soil moisture content before planting was 13.62%, 13.19%, 11.37%, and 7.66%, in organic, young organic, semi-chemical, and chemical soils, respectively. Soil bulk density was 1.38 g/cm^3, 1.45 g/cm^3, 1.56 g/cm^3, and 1.67 g/cm^3 in organic, young organic, semi-chemical, and chemical soils, respectively (Table 4-2).

Table 4-2. Soil physical properties at various agricultural management practices before planting; before rice cultivation period (mean values ± standard deviation)

Variables	Depth (cm)	Organic	Young Organic	Semi-chemical	Chemical
		Agricultural Management Practices			
Sand (%)	0-5	60.61 ± 6.76	47.83 ± 9.11	34.60 ± 8.13	54.64 ± 3.00
	10-15	56.86 ± 7.75	47.19 ± 7.12	36.46 ± 7.32	52.37 ± 5.73
Average		**58.73 ± 7.25[a]**	**47.51 ± 8.11[b]**	**35.53 ± 7.72[c]**	**53.51 ± 4.37[d]**
Silt (%)	0-5	18.77 ± 7.38	23.88 ± 9.77	47.89 ± 9.10	29.02 ± 2.23
	10-15	22.09 ± 7.64	23.92 ± 5.66	45.05 ± 8.57	32.12 ± 5.66
Average		**20.43 ± 7.51[a]**	**23.90 ± 7.71[a]**	**46.47 ± 8.84[b]**	**30.57 ± 3.95[c]**
Clay (%)	0-5	20.62 ± 2.71	28.29 ± 8.18	17.51 ± 2.43	16.34 ± 1.65
	10-15	21.05 ± 2.23	28.89 ± 7.57	18.50 ± 2.77	15.50 ± 1.70
Average		**20.84 ± 2.47[a]**	**28.59 ± 7.88[b]**	**18.00 ± 2.60[ac]**	**15.92 ± 1.67[c]**
MC (%)	0-5	14.70 ± 12.80	13.37 ± 4.95	11.66 ± 6.09	8.33 ± 3.20
	10-15	12.54 ± 11.82	13.02 ± 5.63	11.08 ± 5.26	6.98 ± 2.79
Average		**13.62 ± 12.31[a]**	**13.19 ± 5.29[a]**	**11.37 ± 5.67[b]**	**7.66 ± 2.99[bc]**
Db (g/cm^3)	0-5	1.35 ± 0.11	1.45 ± 0.07	1.54 ± 0.06	1.62 ± 0.07
	10-15	1.42 ± 0.10	1.45 ± 0.12	1.59 ± 0.15	1.71 ± 0.14
Average		**1.38 ± 0.10[a]**	**1.45 ± 0.09[a]**	**1.56 ± 0.10[b]**	**1.67 ± 0.11[c]**

Mc: soil moisture content, Db: bulk density. Different letters correspond to statistically significant differences ($P < 0.05$).

For after post harvesting, soil moisture content was 17.91%, 16.02%, 14.70%, and 7.19%, in organic, young organic, semi-chemical, and chemical soils, respectively. Soil bulk density was 1.30 g/cm^3, 1.43 g/cm^3, 1.62 g/cm^3, and 1.90 g/cm^3 in organic, young organic, semi-chemical, and chemical soils, respectively (Table 4-3). The results clearly showed that semi-chemical and chemical were greater in soil bulk density values than in organic and young organic fields at all soil layers and both before and after harvesting. The highest percentage of soil moisture content was found only in organic fields both before planting and after harvesting. In addition, organic and young organic management practices increased soil moisture content over 31% while semi-chemical and chemical soil decreased soil moisture content. Organic and young organic management practices also decreased with oil bulk density, whereas semi-chemical and chemical increased soil bulk density was over 3-13%. The results on physical properties confirmed that organic and young organic practices could improve soil quality under rice fields.

Table 4-3. Soil physical properties at various agricultural management practices after rice cultivation period; post harvesting (mean values ± standard deviation)

Variables	Depth (cm)	Organic	Young Organic	Semi-chemical	Chemical
		Agricultural Management Practices			
Sand (%)	0-5	58.72 ± 4.59	42.72 ± 11.91	36.22 ± 13.69	60.08 ± 11.35
	10-15	59.04 ± 6.05	39.89 ± 8.92	32.83 ± 3.96	53.46 ± 13.10
Average		**58.88 ± 5.32[a]**	**41.31 ± 10.42[b]**	**34.52 ± 8.82[c]**	**56.77 ± 12.22[d]**
Silt (%)	0-5	16.89 ± 7.08	30.67 ± 10.39	37.56 ± 11.70	19.33 ± 12.08
	10-15	17.78 ± 7.10	31.78 ± 9.77	41.56 ± 7.13	24.44 ± 12.44
Average		**17.33 ± 7.09[a]**	**31.22 ± 10.08[b]**	**39.56 ± 9.41[c]**	**21.89 ± 12.26[a]**
Clay (%)	0-5	24.39 ± 4.83	26.61 ± 5.64	26.22 ± 5.51	20.59 ± 3.47
	10-15	23.18 ± 4.71	28.33 ± 5.30	25.62 ± 5.59	22.10 ± 7.39
Average		**23.79 ± 4.77[a]**	**27.47 ± 5.47[b]**	**25.92 ± 5.55[b]**	**21.34 ± 5.43[c]**
MC (%)	0-5	19.63 ± 3.77	17.34 ± 6.68	15.31 ± 4.20	7.06 ± 2.71
	10-15	16.18 ± 2.61	14.70 ± 6.58	14.09 ± 4.11	7.33 ± 3.11
Average		**17.91 ± 3.19[a]**	**16.02 ± 6.63[ab]**	**14.70 ± 4.15[b]**	**7.19 ± 2.91[c]**
Db (g/cm^3)	0-5	1.18 ± 0.04	1.47 ± 0.20	1.61 ± 0.12	1.90 ± 0.05
	10-15	1.43 ± 0.11	1.40 ± 0.13	1.62 ± 0.07	1.90 ± 0.08
Average		**1.30 ± 0.08[a]**	**1.43 ± 0.16[b]**	**1.62 ± 0.10[c]**	**1.90 ± 0.06[d]**

Mc: soil moisture content, Db: bulk density. Different letters correspond to statistically significant differences ($P < 0.05$).

The effects of the agricultural management practices on soil chemical properties were shown in Table 4-5. The amount of extractable Ca^{++}, Mg^{++}, K^+, and Na^+ of soil samples before planting were high with the range of 1.37-4.50, 1.28-1.87, 1.46-1.54, and 1.08-1.09 me/100 g soil, respectively. The highest amount of Ca, Mg, and K was found in organic soils, while the lowest amount was shown in chemical soil. The amounts of EC and CEC were slightly low with the range of 90.56-111.91 μS/cm, and 3.02-6.33 me/100 g soil. Available P content in the soils was moderately high (15.38-16.22 ppm) except for the soil from semi-chemical field. Nitrogen and organic matter ranged between 0.25-0.67 %, and 1.68-2.57 %. The highest OM and N found in organic field. ANOVA showed that the concentrations of Ca++, Na+, total N, soil pH, CEC, and organic matter differed significantly among sampling areas, while differences in the Mg++, K+, EC, and available P concentration were not significant ($P>0.05$). From comparisons among organic, young organic, semi-chemical, and chemical fields, we found that chemical fields exhibited the lowest amounts of Ca++, Na+, pH, CEC, and organic matter. The values of chemical variables in organic areas (> 5 years) were similar to those recorded in the young organic areas (3-5 years). Between two soil layers, the surface soil (1-15 cm.) and sub soil (15-30 cm.), higher organic matter was recorded in surface soil.

For after harvesting period, most of tested chemical soil variables differed significantly among organic, young organic, semi-chemical, and chemical fields, shown in Table 5. The extractable Ca, Mg, K, and Na of soil samples were in the range of 1.67-9.30, 1.35-3.08, 1.08-1.17, and 0.79-1.28 me/100 g soil respectively. The highest amount of Ca, Mg, K, and Na was found in organic soils. The amounts of EC and CEC were in the range of 21.38-40.26 μS/cm, and 3.76-4.61 me/100 g soil, respectively. Available P content was between 6.32-15.03 ppm. Nitrogen and organic matter ranged between 0.25-0.80 %, and 1.36-3.09 %. ANOVA showed that the concentrations of Ca++, Mg++, Na+, total nitrogen, soil pH, EC, CEC, organic mater, and available phosphorus differed significantly among four soil management practices. The values of chemical variables in semi-chemical areas were clearly similar to those recorded in the chemical areas, whereas organic areas were closely similar to young organic areas. Especially, the total nitrogen, and organic matter variables were significantly differed between organic areas and chemical areas.

Table 4-4. Soil chemical properties at various agricultural management practices before planting; before rice cultivation period (mean values ± standard deviation)

Variables	Depth (cm)	Organic	Young Organic	Semi-Chemical	Chemical
		Agricultural Management Practices			
Ca^{++} (me/100 g soil)	0-5	4.70 ± 4.31	2.59 ± 2.87	2.57 ± 2.50	1.47 ± 1.27
	10-15	4.31 ± 3.80	2.14 ± 2.33	2.33 ± 2.39	1.27 ± 1.14
Average		**4.50 ± 4.05^{a}**	**2.37 ± 2.60^{b}**	**2.45 ± 2.45^{b}**	**1.37 ± 1.21^{c}**
Mg^{++}(me/100 g soil)	0-5	1.84 ± 1.13	1.67 ± 0.82	1.24 ± 0.97	1.48 ± 1.11
	10-15	1.89 ± 1.35	1.54 ± 0.69	1.31 ± 1.02	1.41 ± 1.17
Average		**1.87 ± 1.24**	**1.61 ± 0.75**	**1.28 ± 1.00**	**1.44 ± 1.14**
K^{+} (me/100 g soil)	0-5	1.64 ± 0.05	1.61± 0.05	1.61 ± 0.03	1.60 ± 0.03
	10-15	1.44 ± 0.10	1.35 ± 0.13	1.31 ± 0.14	1.32 ± 0.11
Average		**1.54 ± 0.07**	**1.48 ± 0.09**	**1.46 ± 0.08**	**1.46 ± 0.07**
Na^{+}(me/100 g soil)	0-5	1.32 ± 0.40	2.15 ± 0.94	1.49 ± 0.75	1.31 ± 0.35
	10-15	0.86 ± 0.06	0.84 ± 0.07	0.87 ± 0.09	0.86 ± 0.05
Average		**1.09 ± 0.23^{a}**	**1.49 ± 0.50^{a}**	**1.18 ± 0.42^{a}**	**1.08 ± 0.20^{b}**
N (%)	0-5	0.70 ± 0.15	0.34 ± 0.05	0.25 ± 0.06	0.24 ± 0.04
	10-15	0.64 ± 0.19	0.34 ± 0.05	0.26 ± 0.03	0.25 ± 0.07
Average		**0.67 ± 0.17^{a}**	**0.34 ± 0.05^{b}**	**0.25 ± 0.05^{c}**	**0.25 ± 0.06^{c}**
OM (%)	0-5	2.59 ± 0.84	2.60 ± 0.36	1.88 ± 0.44	2.03 ± 0.22
	10-15	2.55 ± 0.82	2.37 ± 0.40	1.48 ± 0.45	1.91 ± 0.24
Average		**2.57 ± 0.83^{a}**	**2.49 ± 0.38^{a}**	**1.68 ± 0.44^{b}**	**1.97 ± 0.23^{b}**
EC (µS/cm)	0-5	101.52 ± 43.39	122.27 ± 57.60	87.78 ± 23.37	98.71 ± 23.51
	10-15	79.61 ± 25.86	95.58 ± 33.19	136.04 ± 29.56	123.15 ± 33.27
Average		**90.56 ± 34.63**	**108.93 ± 45.40**	**111.91 ± 26.47**	**110.93 ± 28.39**
CEC (me/100 g soil)	0-5	6.31 ± 1.88	6.73 ± 1.67	4.67 ± 0.86	2.97 ± 0.29
	10-15	6.34 ± 1.87	6.58 ± 1.70	4.20 ± 0.26	3.08 ± 0.32
Average		**6.33 ± 1.87^{a}**	**6.65 ± 1.69^{a}**	**4.43 ± 0.56^{b}**	**3.02 ± 0.31^{b}**
Pi (ppm)	0-5	17.75 ± 11.79	11.32 ± 4.46	27.25 ± 21.97	17.35 ± 9.77
	10-15	14.69 ± 10.09	19.43 ± 26.20	18.40 ± 18.48	13.47 ± 8.81
Average		**16.22 ± 10.94**	**15.38 ± 15.33**	**22.82 ± 20.22**	**15.41 ± 9.29**

Table 4-4. (Cont.)

Variables	Depth (cm)	Agricultural Management Practices			
		Organic	Young Organic	Semi-Chemical	Chemical
pH 1:10	0-5	5.40 ± 0.43	5.38 ± 0.36	17.35 ± 9.77	4.94 ± 0.95
	10-15	5.41 ± 0.37	5.26 ± 0.23	13.47 ± 8.81	5.23 ± 0.17
Average		**5.40 ± 0.40[a]**	**5.32 ± 0.29[a]**	**15.41 ± 9.29[a]**	**5.08 ± 0.56[b]**

OM: organic matter, EC: electric conductivity, CEC: cation exchangeable capacity, and Pi: available phosphorus. Different letters correspond to statistically significant differences ($P < 0.05$) among different sites.

Most of soil chemical variables clearly indicated that amount of chemical compositions greater in organic field than young organic field, semi-chemical field, and chemical field, respectively. In addition, organic matter and nitrogen contents clearly differed in the chemically and organically managed areas. For organic and young organic management, soil organic matter was increased around 16-18%, whereas chemical management practice significantly decreased in organic matter content of 31%. The nitrogen content was decreased over 45% in chemical soil at the top soil layer. Soil from various practices was slightly acidity (pH 5.08-5.40). Only chemical management practices showed strong acidity (pH 4.94). The content of organic matter in upper (0-5 cm.) was higher than subsoil (15-30 cm.) in all agricultural practice systems.

Table 4-5. Soil chemical properties at various agricultural management practices after rice cultivation period; post harvesting (mean values ± standard deviation)

Variables	Depth (cm)	Agricultural Management Practices			
		Organic	Young Organic	Semi-Chemical	Chemical
Ca^{++} (me/100 g soil)	0-5	9.36 ± 1.10	7.42 ± 2.41	2.37 ± 1.05	1.58 ± 0.90
	10-15	9.24 ± 1.25	7.08 ± 2.31	2.87 ± 1.33	1.75 ± 0.85

Table 4-5. (Cont.)

Variables	Depth (cm)	Agricultural Management Practices: Organic	Young Organic	Semi-Chemical	Chemical
Average		**9.30 ± 1.17[a]**	**7.25 ± 2.36[b]**	**2.62 ± 1.19[c]**	**1.67 ± 0.88[c]**
Mg^{++}(me/100 g soil)	0-5	3.44 ± 0.68	2.20 ± 0.41	1.40 ± 0.25	1.46 ± 0.41
	10-15	2.71 ± 0.73	1.90 ± 0.56	1.34 ± 0.33	1.23 ± 0.29
Average		**3.08 ± 0.70[a]**	**2.05 ± 0.48[b]**	**1.37 ± 0.29[c]**	**1.35 ± 0.35[c]**
K^{+} (me/100 g soil)	0-5	1.14 ± 0.21	1.11 ± 0.35	1.25 ± 0.50	1.13 ± 0.58
	10-15	1.07 ± 0.11	1.05 ± 0.26	0.93 ± 0.49	1.20 ± 0.44
Average		**1.10 ± 0.16**	**1.08 ± 0.30**	**1.09 ± 0.50**	**1.17 ± 0.51**
Na^{+}(me/100 g soil)	0-5	1.37 ± 0.50	1.21 ± 0.47	0.79 ± 0.13	0.86 ± 0.15
	10-15	1.19 ± 0.41	1.01 ± 0.17	0.78 ± 0.12	0.86 ± 0.13
Average		**1.28 ± 0.45[a]**	**1.11 ± 0.32[a]**	**0.79 ± 0.12[b]**	**0.86 ± 0.14[b]**
N (%)	0-5	0.85 ± 0.02	0.65 ± 0.07	0.35 ± 0.25	0.37 ± 0.25
	10-15	0.76 ± 0.05	0.51 ± 0.06	0.20 ± 0.07	0.14 ± 0.13
Average		**0.80 ± 0.04[a]**	**0.58 ± 0.07[b]**	**0.28 ± 0.16[c]**	**0.25 ± 0.19[c]**
OM (%)	0-5	3.37 ± 1.00	3.04 ± 0.59	1.88 ± 0.41	1.51 ± 0.40
	10-15	2.80 ± 0.62	2.74 ± 0.56	1.58 ± 0.36	1.21 ± 0.24
Average		**3.09 ± 0.81[a]**	**2.89 ± 0.57[a]**	**1.73 ± 0.39[b]**	**1.36 ± 0.32[b]**
EC (μS/cm)	0-5	32.69 ± 30.67	38.26 ± 23.94	44.42 ± 15.43	22.80 ± 5.15
	10-15	23.79 ± 15.67	31.87 ± 17.35	36.10 ± 11.43	19.96 ± 4.12
Average		**28.24 ± 23.17[a]**	**35.07 ± 20.64[ab]**	**40.26 ± 13.43[b]**	**21.38 ± 4.64[c]**
CEC (me/100 g soil)	0-5	4.65 ± 0.74	4.32 ± 0.66	4.29 ± 0.89	3.90 ± 0.37
	10-15	4.57 ± 0.88	4.39 ± 0.63	4.28 ± 1.04	3.63 ± 0.39
Average		**4.61 ± 0.81[a]**	**4.36 ± 0.65[a]**	**4.29 ± 0.96[a]**	**3.76 ± 0.38[b]**
Pi (ppm)	0-5	17.53 ± 10.74	15.80 ± 8.98	10.89 ± 7.10	7.18 ± 4.16
	10-15	12.53 ± 7.90	10.08 ± 5.32	9.15 ± 5.48	5.45 ± 4.01
Average		**15.03 ± 9.32[a]**	**12.94 ± 7.15[ab]**	**10.02 ± 6.29[bc]**	**6.32 ± 4.09[c]**

Table 4-5. (Cont.)

Variables	Depth (cm)	Agricultural Management Practices			
		Organic	Young Organic	Semi-Chemical	Chemical
pH 1:10	0-5	5.76 ± 0.44	5.48 ± 0.26	5.26 ± 0.25	5.37 ± 0.28
	10-15	5.92 ± 0.53	5.56 ± 0.30	5.57 ± 0.48	5.51 ± 0.23
Average		**5.84 ± 0.49^{a}**	**5.52 ± 0.28^{b}**	**5.41 ± 0.36^{b}**	**5.44 ± 0.26^{b}**

OM: organic matter, EC: electric conductivity, CEC: cation exchangeable capacity, and Pi: available phosphorus. Different letters correspond to statistically significant differences ($P < 0.05$) among different sites.

The principal Component Analysis (PCA) demonstrated that agricultural management practices affected soil chemical and physical properties. The studied soils distributed in three main clusters: 1A (organic and young organic soils), 1B (semi-chemical soils), and 2A (chemical soils), shown in Fig. 4-1. Organic and young organic soil samples were classified towards the left end of the first axis, while semi-chemical and chemical soil samples were positioned towards the right end of the second axis indicating that differentiation between organic, young organic soils and semi-chemical, chemical soils. Soil samples were clearly distributed along agricultural management practices. Organic soils were closely related to young organic soils, while semi-chemical soils were related to chemical soils. Soil moisture content, bulk density, available phosphorus, soil pH, nitrogen content, organic matter, exchangeable Ca++, Mg++, Na+, and K+ were considered to be soil quality indicators (Fig. 4-1), because they were sensitive to agricultural management systems. Soil obtained from organic and young organic fields were related to soil moisture content, available Ca++, Mg++, Na+, organic matter, total nitrogen, available phosphorus, and soil pH while semi-chemical, and chemical soils related only available K+, and bulk density.

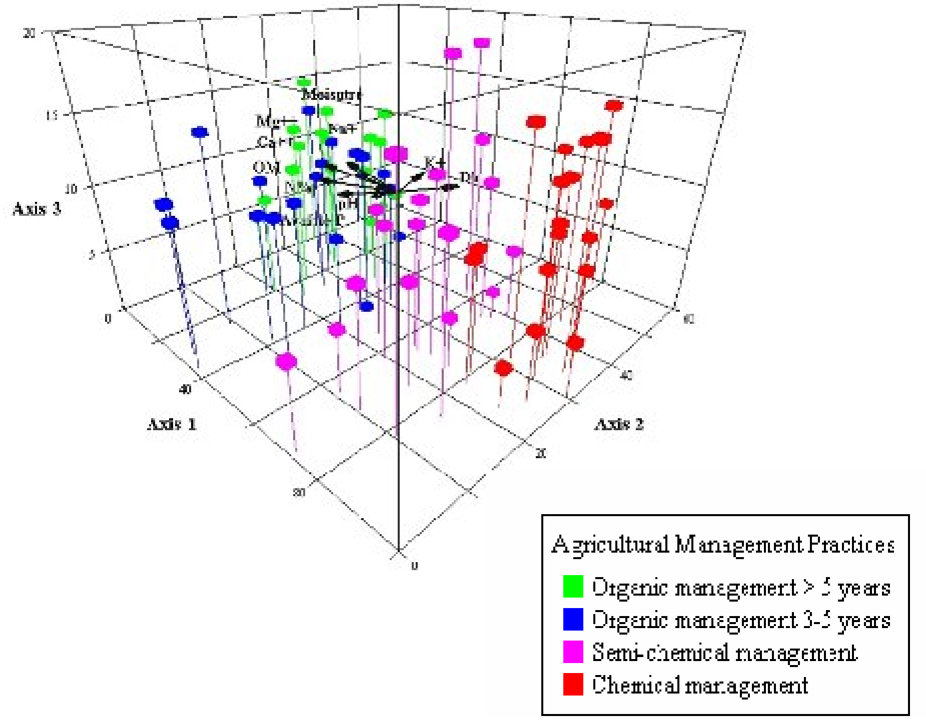

Figure 4-1. Discrimination analysis of samples in relation to soil chemical and physical variables by PCA

4.2 Soil Microbial Populations

4.2.1 Bacterial population

Microbial populations of four soil tested sites (organic, young organic, semi-chemical and chemical fields) were presented in Table 4-6. The bacterial populations of Surin rice fields were in the range of 2.56 – 7.69 x 10^4 CFU/g before planting, 15.15 - 33.61 x 10^5 CFU/g after harvesting. Organic areas had the highest bacterial population (7.69 x 10^4, and 7.63 x 10^5 CFU/g in soil before planting and after harvesting), followed by young organic fields (3.95 x 10^4, and 6.03 x 10^5 CFU/g in soil before planting and after harvesting), semi-chemical fields (3.12 x 10^4, and 3.27 x 10^5 CFU/g in soil before planting and after harvesting), and chemical areas (2.56 x 10^4, and 2.84 x 10^5 CFU/g in soil before planting and after harvesting), respectively. Most topsoil layers, excepted in chemical areas, was observed to contain higher bacterial populations than those of subsoil, because of high organic matter content and high total nitrogen. Statistical analysis of bacterial population showed significantly difference among organic area, young organic and semi-chemical areas, and chemical area for soil obtained from soil before planting, while soil after harvesting significant differences ($P<0.05$) the bacteria population was found between organic and young organic areas, and between semi-chemical and chemical areas shown in Table 4-6.

4.2.2 Fungal population

The highest fungal population was observed in organic field while the lowest fungal population was observed in chemical field. The fungal populations were 5.82, 1.58, 1.62, and 1.34 x 10^3 CFU/g in soil before planting. After harvesting, the fungal populations in soil were 8.93, 6.23, 3.44, and 2.44 x 10^4 CFU/g. The fungal population was found statistically significant in organic fields and differed from soil in other fields for before planting rice cultivation soils, whereas after harvesting soils organic and young organic fields significantly was found to differ from semi-chemical and chemical fields.

4.2.3 Actinomycete population

The population numbers of actinomycete were in the range of 4.72-5.74, 6.04-6.06, 6.16-7.58, and 6.26-11.27 x 10^5 CFU/g for organic, young organic, semi-chemical, and chemical fields, respectively. The highest population was found in only chemical area. The population number of actinomycete showed high in chemical > semi-chemical > young organic > organic areas in both soils; before and after harvesting soils.

4.2.4 Cellulolytic microbes

Cellulolytic microbes of Surin rice fields were 8.88, 6.27, 0.87, and 0.64 x 10^4 CFU/g for organic, young organic, semi-chemical, and chemical fields, respectively. The highest population of cellulolytic microbes were found in organic soil, whereas, the lowest cellulolytic population was recorded in chemical area. For after harvesting soils, cellulolytic populations were similar to soils collected form before planting soils. Most topsoil layers had higher bacterial populations than those of subsoil. Statistical analysis of cellulolytic population showed that significant differences found among organic, young organic, and semi-chemical and chemical areas, while after harvesting significant differences found among organic, young organic, and semi-chemical and chemical areas.

Table 4-6. Population numbers of soil bacteria, fungi, actinomycetes and cellulolytic bacteria at various agricultural management practices before planting; before rice cultivation period (mean values ± standard deviation)

Agricultural Management Practices	Soil depth (cm.)	Microbial populations (CFU/g dry soil)			
		Bacteria (X 10^4)	Fungi (X 10^3)	Actinomycetes (X 10^5)	Cellulolytic microbes (X 10^4)
Organic	0-15 cm.	8.44 ± 3.27	7.05 ± 3.61	5.62 ± 2.39	9.74 ± 2.49
	15-30 cm.	6.94 ± 2.62	4.59 ± 2.38	5.86 ± 2.81	8.02 ± 1.76
Average number		**7.69^a ± 2.94**	**5.82^a ± 3.00**	**5.74^a ± 2.60**	**8.88^a ± 2.12**
Young organic	0-15 cm.	4.19 ± 1.53	1.95 ± 1.34	6.11 ± 1.80	7.71 ± 1.68
	15-30 cm.	3.70 ± 1.44	1.22 ± 0.60	5.97 ± 2.63	4.82 ± 2.11
Average number		**3.95^b ± 1.48**	**1.58^b ± 0.97**	**6.04^a ± 2.21**	**6.27^b ± 1.90**

Different letters correspond to statistically significant differences (P < 0.05) among different sites.

Table 4-6. (Cont.)

Agricultural Management Practices	Soil depth (cm.)	Microbial populations (CFU/g dry soil)			
		Bacteria ($\times 10^4$)	Fungi ($\times 10^3$)	Actinomycetes ($\times 10^5$)	Cellulolytic microbes ($\times 10^4$)
Semi-chemical	0-15 cm.	3.52 ± 1.71	1.70 ± 1.23	6.36 ± 2.86	0.97 ± 0.51
	15-30 cm.	2.72 ± 1.79	1.54 ± 1.19	5.95 ± 1.52	0.76 ± 0.44
Average number		**3.12^{b} ± 1.75**	**1.62^{b} ± 1.21**	**6.16^{a} ± 2.19**	**0.87^{bc} ± 0.47**
Chemical	0-15 cm.	2.50 ± 0.89	1.03 ± 0.31	6.15 ± 1.77	0.56 ± 0.27
	15-30 cm.	2.62 ± 1.22	1.65 ± 0.79	6.37 ± 1.32	0.71 ± 0.28
Average number		**2.56^{bc} ± 1.06**	**1.34^{b} ± 0.55**	**6.26^{b} ± 1.55**	**0.64^{bc} ± 0.28**

Different letters correspond to statistically significant differences ($P < 0.05$) among different sites.

Table 4-7. Population numbers of soil bacteria, fungi, actinomycetes and cellulolytic bacteria at various agricultural management practices after rice cultivation period; post harvesting (mean values ± standard deviation)

Agricultural Management Practices	Soil depth (cm.)	Microbial populations (CFU/g dry soil)			
		Bacteria ($\times 10^5$)	Fungi ($\times 10^4$)	Actinomycetes ($\times 10^5$)	Cellulolytic microbes ($\times 10^5$)
Organic	0-15 cm.	8.57 ± 2.42	9.49 ± 1.76	6.05 ± 2.76	11.06 ± 2.43
	15-30 cm.	6.70 ± 1.34	8.36 ± 1.43	3.39 ± 1.93	9.41 ± 2.68
Average number		**7.63^{a} ± 2.34**	**8.93^{a} ± 2.08**	**4.72^{a} ± 2.81**	**10.24^{a} ± 2.82**
Young organic	0-15 cm.	6.39 ± 1.23	6.61 ± 1.73	7.59 ± 2.03	7.62 ± 2.87
	15-30 cm.	5.67 ± 2.29	5.85 ± 1.15	4.53 ± 2.81	6.34 ± 2.94
Average number		**6.03^{a} ± 1.84**	**6.23^{b} ± 1.78**	**6.06^{ab} ± 2.00**	**6.98^{b} ± 3.04**
Semi-chemical	0-15 cm.	3.40 ± 1.82	3.44 ± 1.78	8.58 ± 2.35	0.81 ± 0.61
	15-30 cm.	3.14 ± 1.37	3.43 ± 2.89	6.59 ± 3.03	0.70 ± 0.40
Average number		**3.27^{b} ± 1.84**	**3.44^{c} ± 1.72**	**7.58^{b} ± 2.22**	**0.76^{c} ± 0.70**

Different letters correspond to statistically significant differences ($P < 0.05$) among different sites.

Table 4-7. (Cont.)

Agricultural Management Practices	Soil depth (cm.)	Microbial populations (CFU/g dry soil)			
		Bacteria ($X 10^5$)	Fungi ($X 10^4$)	Actinomycetes ($X 10^5$)	Cellulolytic microbes ($X 10^5$)
Chemical	0-15 cm.	3.02 ± 1.49	2.60 ± 3.10	12.93 ± 2.95	0.64 ± 3.04
	15-30 cm.	2.66 ± 1.23	2.29 ± 2.71	9.60 ± 2.04	0.58 ± 0.04
Average number		**2.84^b ± 2.40**	**2.44^c ± 2.14**	**11.27^c ± 3.08**	**0.61^c ± 0.44**

Different letters correspond to statistically significant differences ($P < 0.05$) among different sites.

The order of soil microorganism numbers ranging from highest to lowest was as following; organic field > young organic field > chemical field > semi-chemical field in both soils; before planting and after harvesting soils (Table 4-8 to 4-9). In addition, the proportion of soil population varied among soil sites. The organic soils had the highest microbial population among soil tested sites. The organic field contained soil cellulolytic microbes (38.79%), greater than bacterial population (33.61%), actinomycete population (25.06%), and fungal population (2.54%) , respectively. For young organic area, the proportion of soil microorganisms contained the soil cellulolytic microbes (40.79%) > actinomycete population (32.50%) > bacterial population (25.68%) > fungal population (1.03%) respectively. In semi-chemical and chemical areas, the proportion number of soil microorganisms showed that they were similar in the order of actinomycete population > bacterial population > cellulolytic > fungal population respectively. The proportions of soil microorganisms were 60.58, and 80.30% for actinomycetes, 29.65, and 15.15% for bacteria, 8.22, and 3.76% for cellulolytic microbes, 1.54, and 0.79% for soil fungi under semi-chemical and chemical field respectively.

After post harvesting, the proportion of soil microorganisms in organic soil areas were similar to young organic areas and similarly between semi-chemical and chemical areas. Organic and young organic areas contained the soil cellulolytic microbes > bacterial population > actinomycete population > fungal population respectively. For semi-chemical and chemical areas, the proportion of soil microorganisms showed soil actinomycete, greater than bacteria, cellulolytic microbes, and soil fungi, respectively. The proportions of organic field were 43.91, 32.56, 19.70, and 3.84% in the soil cellulolytic microbes, bacteria, actinomycete, and fungi, respectively. For young organic area, the proportions of soil microorganisms were 35.61, 30.93, 30.27, and 3.19% in the soil cellulolytic microbes, bacteria, actinomycete, and fungi, respectively. In semi-chemical and chemical areas, the proportion numbers of soil microorganisms showed 63.22 and 75.09% for actinomycetes, 27.53 and 19.13% for bacteria, 6.35 and 4.13% for cellulolytic microbes, 2.90 and 1.65% for soil fungi under semi-chemical and chemical fields, respectively (Table 4-9).

Table 4-8. Proportions of soil microorganisms at various agricultural management practices before planting; before rice cultivation (mean values ± standard deviation)

Agricultural Practices	Soil depth (cm.)	Proportion (%)				Total number (X 10^5)
		Bacteria	**Fungi**	**Actinomycetes**	**Cellulolytic bacteria**	
Organic	0-15 cm.	34.46	2.88	22.92	39.75	2.45
	15-30 cm.	32.63	2.16	27.52	37.69	2.13
Average number		**33.61**	**2.54**	**25.06**	**38.79**	**2.29**
Young organic	0-15 cm.	23.88	1.11	31.05	43.96	1.75
	15-30 cm.	28.09	0.92	34.42	36.57	1.32
Average number		**25.68**	**1.03**	**32.50**	**40.79**	**1.54**
Semi-chemical	0-15 cm.	31.18	1.50	58.72	8.60	1.13
	15-30 cm.	27.89	1.58	62.74	7.79	0.98
Average number		**29.65**	**1.54**	**60.58**	**8.22**	**1.05**
Chemical	0-15 cm.	15.05	0.62	80.94	3.38	1.66
	15-30 cm.	15.25	0.96	79.67	4.12	1.72
Average number		**15.15**	**0.79**	**80.30**	**3.76**	**1.69**

Table 4-9. Proportions of soil microorganisms at various agricultural management practices after rice cultivation period; post harvesting (mean values ± standard deviation)

Agricultural Practices	Soil depth (cm.)	Proportion (%)				Total number (X 10^6)
		Bacteria	Fungi	Actinomycetes	Cellulolytic bacteria	
Organic	0-15 cm.	32.18	3.57	22.71	41.54	2.66
	15-30 cm.	32.93	4.11	16.68	46.27	2.03
Average number		**32.56**	**3.84**	**19.70**	**43.91**	**2.35**
Young organic	0-15 cm.	28.72	2.97	34.10	34.21	2.23
	15-30 cm.	33.13	3.42	26.44	37.01	1.71
Average number		**30.93**	**3.19**	**30.27**	**35.61**	**1.97**
Semi-chemical	0-15 cm.	25.92	2.62	65.30	6.16	1.31
	15-30 cm.	29.13	3.18	61.15	6.54	1.08
Average number		**27.53**	**2.90**	**63.22**	**6.35**	**1.20**
Chemical	0-15 cm.	17.90	1.54	76.77	3.79	1.68
	15-30 cm.	20.36	1.75	73.42	4.47	1.31
Average number		**19.13**	**1.65**	**75.09**	**4.13**	**1.50**

Principal component analysis (PCA) was able to discriminate among organic, young organic, semi-chemical, and chemical areas. Actenomycete and fungi were correlated with chemical and semi-chemical fields, while cellulolytic and bacteria were positively correlated with organic and young organic areas. Soils under organic and young organic management practices were classified towards the right end of the first axis, while semi-chemical and chemical soil samples were positioned towards the left end of the first axis indicating that more differences in between organic, young organic soils and semi-chemical, chemical soils. Soil samples were clearly distributed along agricultural management practices and soil microorganism groups (Fig. 4-2).

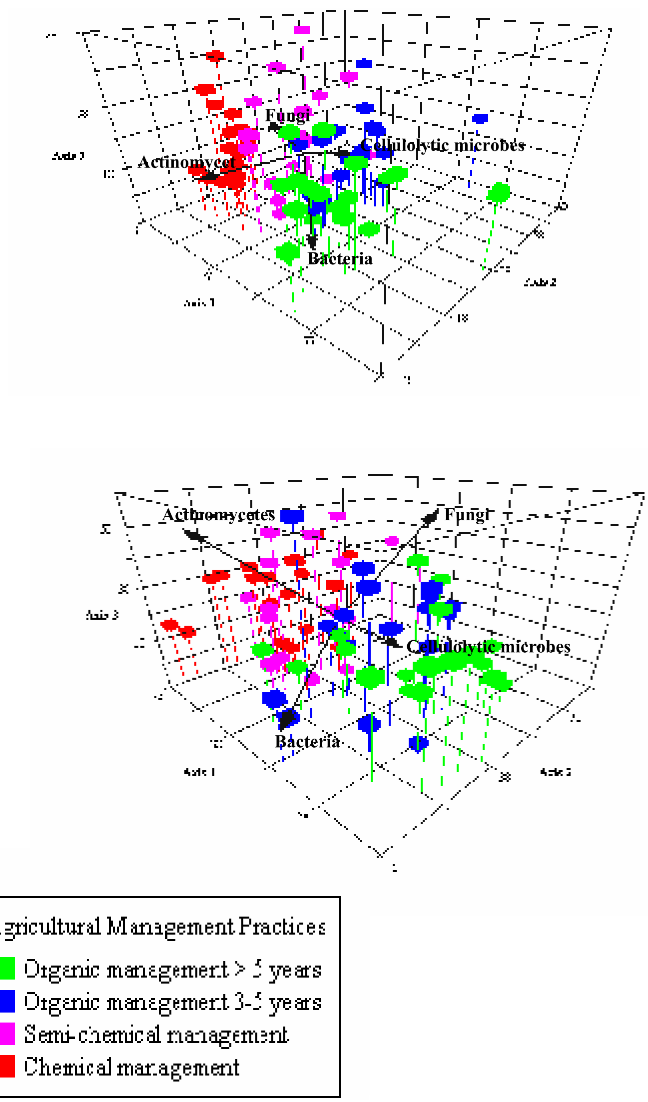

Figure 4-2. Ordination biplot of principal component analysis (PCA) for various soil microbial populations: a) before planting and; b) after post harvesting.

In addition, the discrimination of samples in relation to chemical and biological soil variables is presented in Figure 4-3. Soils were classified along soil physical, chemical properties, and microorganisms. Organic and young organic fields could improve soil quality. The soil microorganisms were used as soil bioindicators.

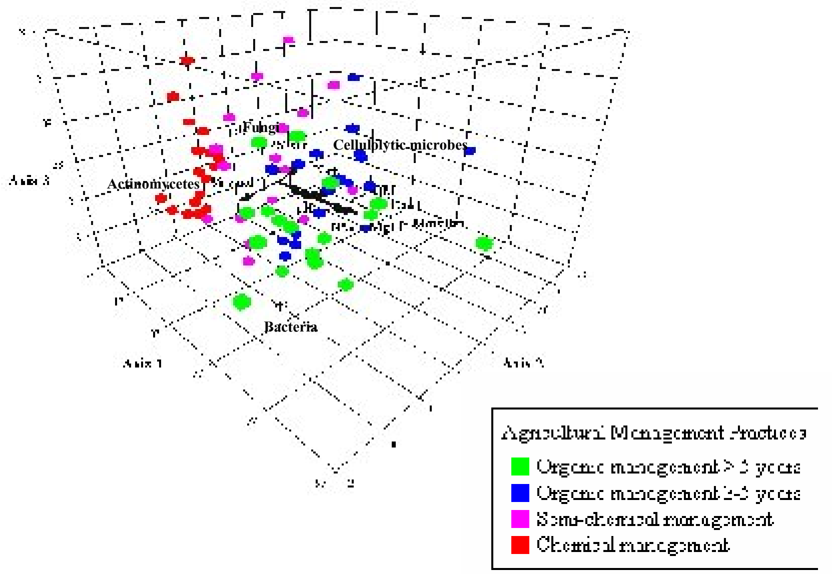

Figure 4-3. Discrimination of soil samples according to chemical, physical and biological variables.

4.3 PCR-DGGE diversity profiles of different soil management practices

DGGE profiles showed that the structure of microbial communities were different among soil sites. The unweighted pair group method with arithmetic means (UPGMA) cluster analysis was used to separate data based upon band intensity. The similar dendrogram (Fig. 4-4 and Fig. 4-5) was clustered into two groups: one group comprising the organic and young organic sites and other one from semi-chemical and chemical soils.

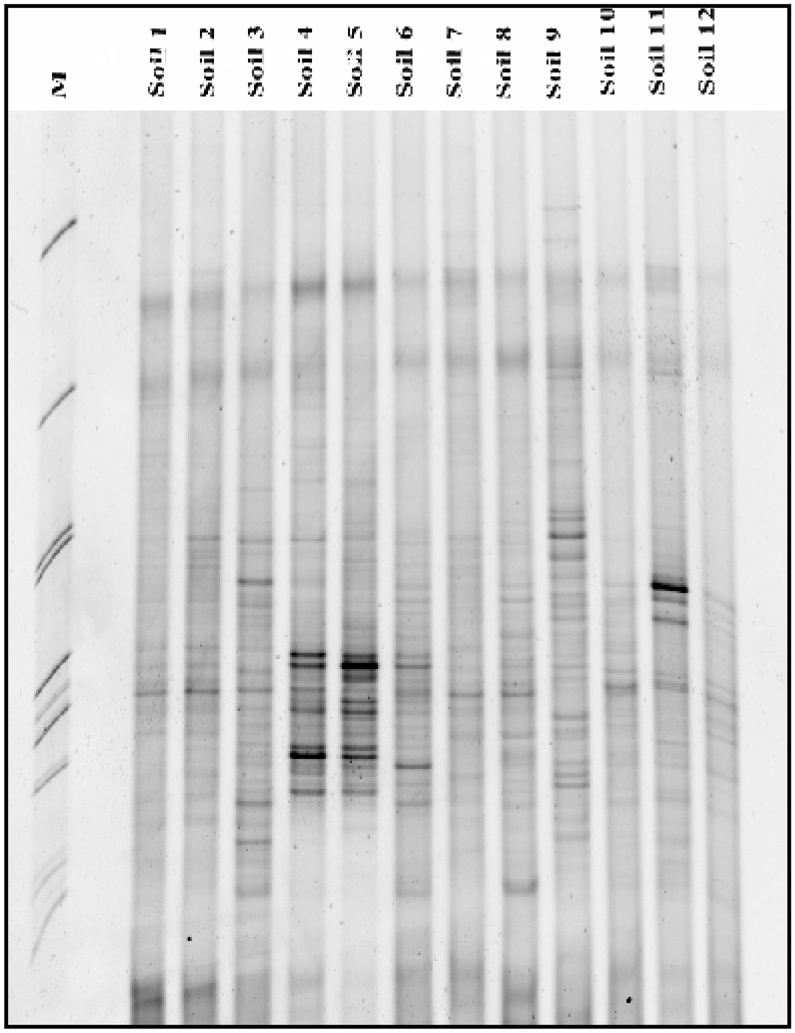

Figure 4-4. Bacterial diversity as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) profiles of soil samples from the organic, young organic, semi-chemical and chemical fields (1-3: organic > 5 years, 4-6: young organic, 7-9: Semi-chemical, 10-12: Chemical fields)

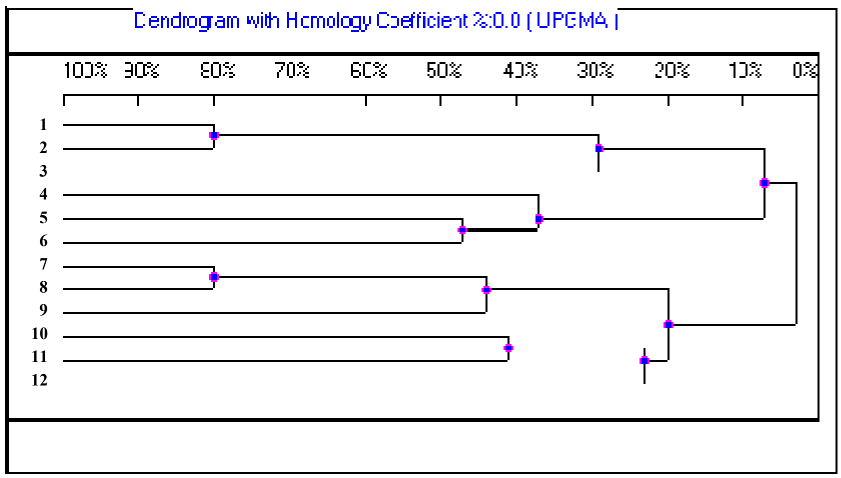

Figure 4-5. Dendrogram indicating the relationships among soil samples according to polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) bacterial diversity profiles of soil samples from various fields (1-3: organic > 5 years, 4-6: young organic, 7-9: Semi-chemical, 10-12: Chemical fields)

5. Conclusion

The study on impact of pesticide applications on soil microorganisms and soil properties at Surin province clearly showed soil physical, chemical, and biological properties varied among management systems. The highest percentage of soil moisture content (17.91%) was found only in organic fields in both soils; before planting and after harvesting. Organic and young organic management practices increased soil moisture content over 31%, while semi-chemical and chemical soil decreased soil moisture content. In addition, organic and young organic management practices also decreased soil bulk density, whereas semi-chemical and chemical increased soil bulk density over 3-13%. For chemical variables, the highest amount of Ca, Mg, and K was found in organic soils, while the lowest amount was shown in chemical soil. ANOVA showed that chemical variables in organic areas (> 5 years) were similar to those recorded in the young organic areas (3-5 years). Especially, the total nitrogen, and organic matter variables were significantly different between organic areas and chemical areas. The results on physical and chemical properties confirmed that organic and young organic practices could improve soil quality in the rice fields.

Microbial populations play a key role in organic matter decomposition and nutrient transformation in organic soils. Microbial populations varied among agricultural management systems. Organic and young organic fields are related to soil bacterial and cellulolytic microbes, while semi-chemical, and chemical soils are related soil fungi and actinomcetes, respectively. The order of soil microorganism levels from highest to lowest was as follows: organic field > young organic field > chemical field > semi-chemical field could be found in before planting and after harvesting soils. The proportion of soil population varied among soil sites. Organic and young organic areas contained the soil cellulolytic microbe number > bacterial population > actinomycete population > fungal population, respectively. For semi-chemical and chemical areas, the proportion of soil microorganisms showed soil actinomycete > bacteria > cellulolytic microbes > soil fungi, respectively. DGGE profiles showed that the structure of microbial communities were different among soil sites. The patterns in PCR-DGGE profile are clustered into two groups: one group comprising the organic and young organic sites and other one from semi-chemical and chemical soils. These results also revealed that soil moisture content, bulk density, available phosphorus, soil pH, nitrogen content, organic matter, exchangeable Ca^{++}, Mg^{++}, Na^{+}, and K^{+} were considered to be soil quality indicators for rice paddy fields.

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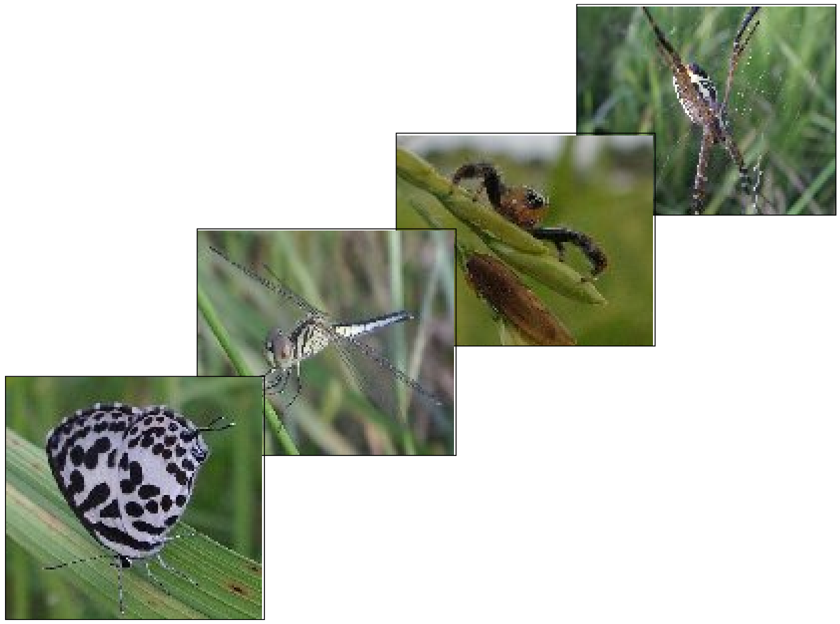
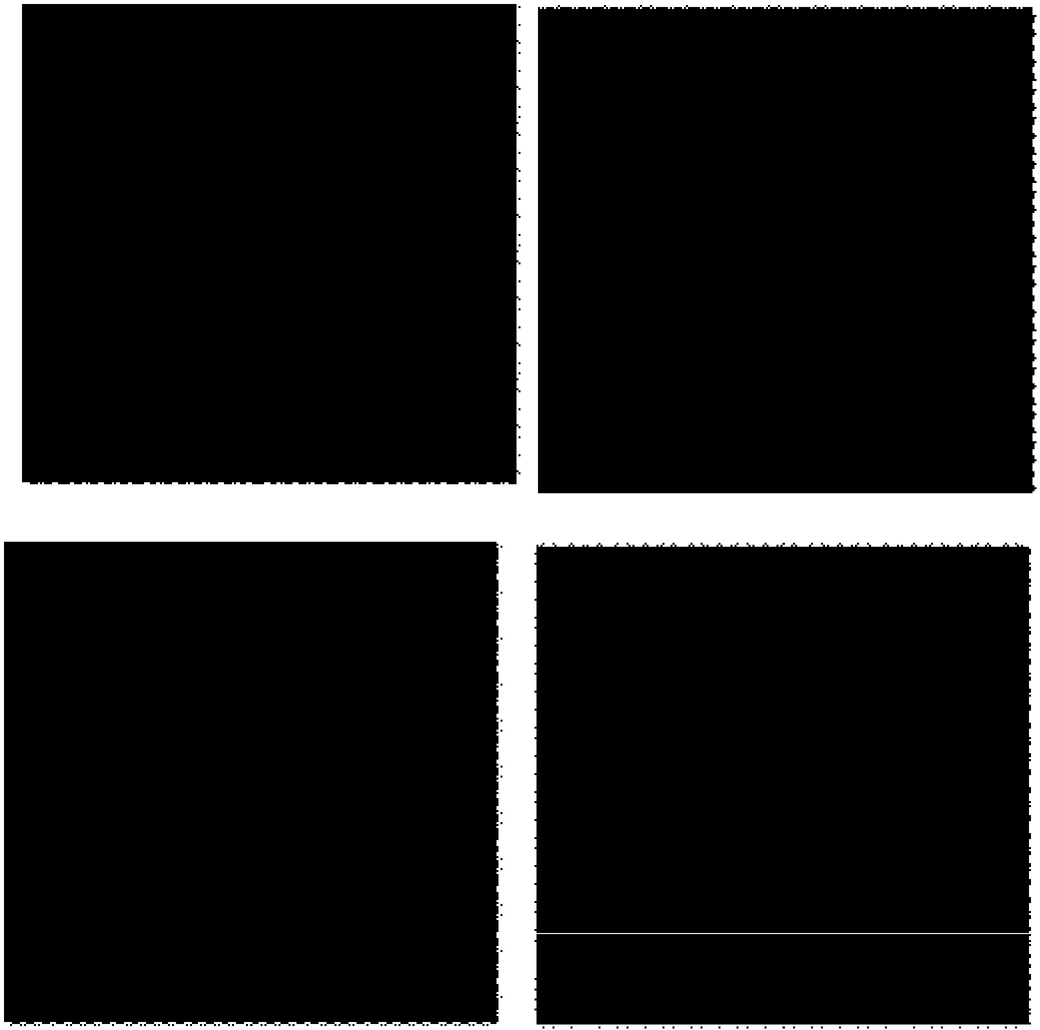
Appendix



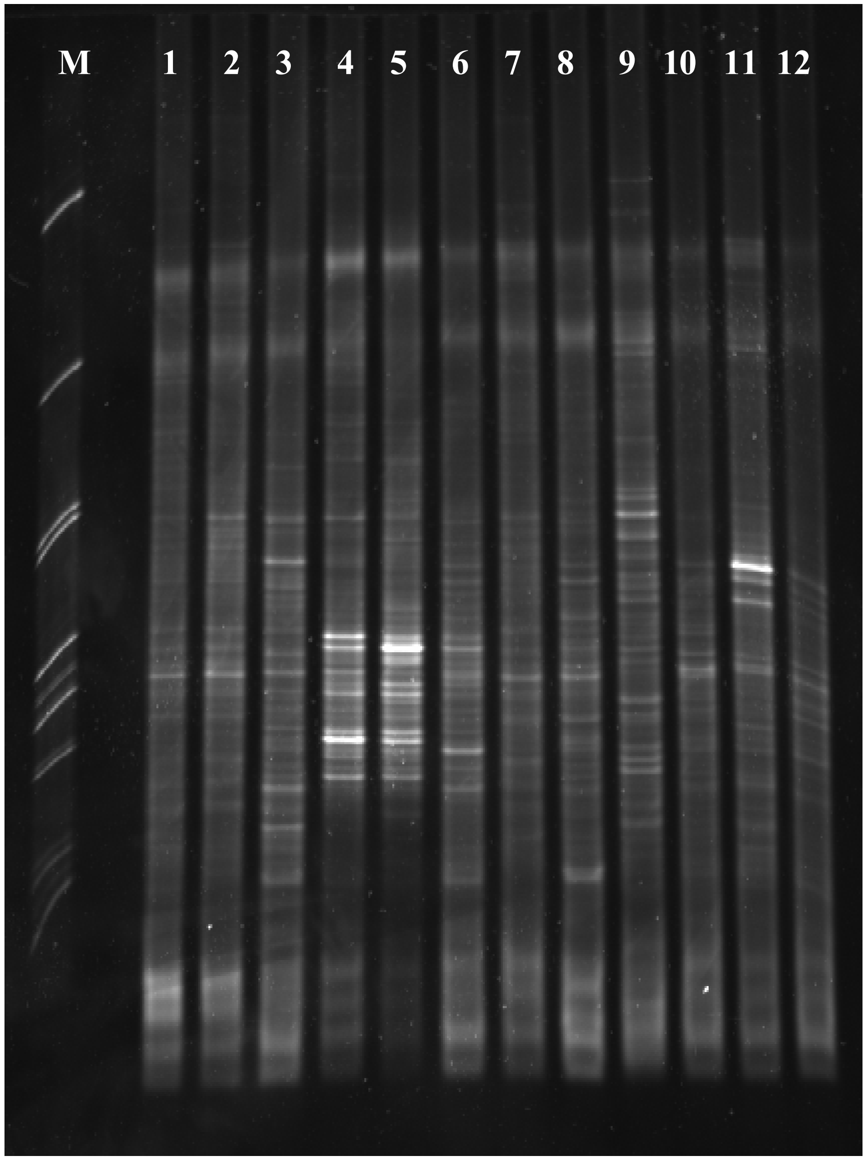
Living things found in organic fields at Surin province

Massive amount of the post-harvest rice residue are eliminated by open-field burning under chemical management system in Surin province.

Agricultural management practices under organic and chemical fields



Microbial population (Organic field; left, Chemical field; right



PCR-DGGE profiles under UV transillumination

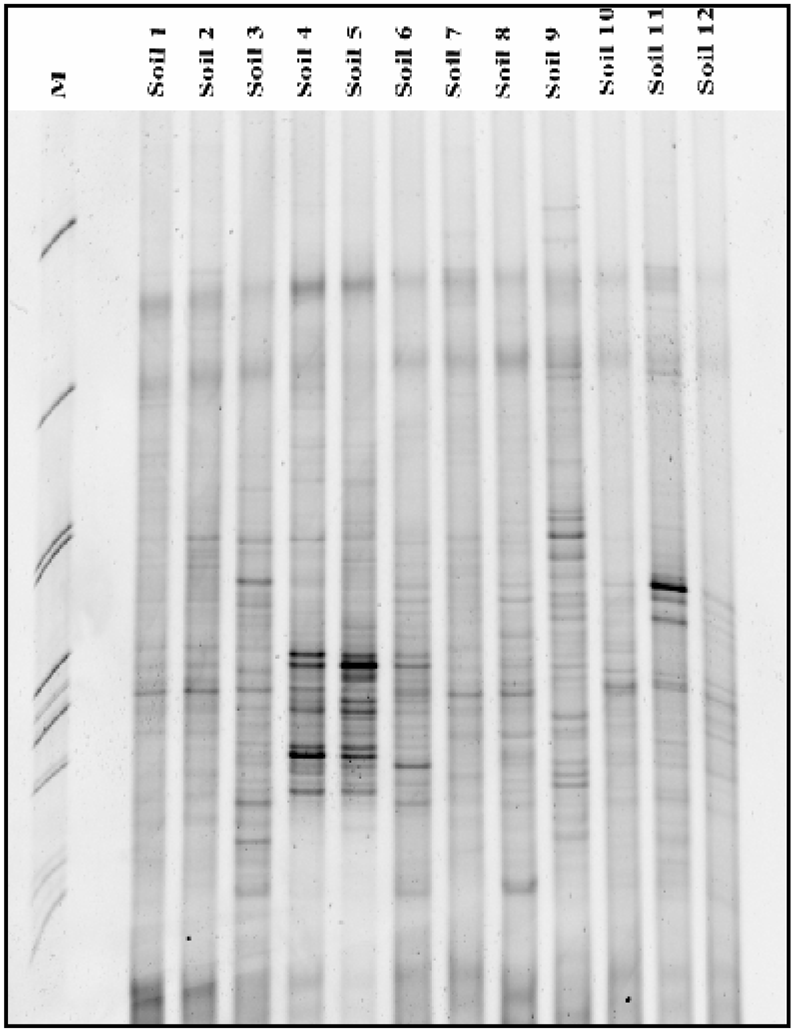


Image analysis was performed using Bio-1D++ software version 11.06 (Vilber-Lourmat, Marne-La-Valle, France