รูปแบบกรดไขมันฟอสโฟลิปิดและกิจกรรมเซลลูเลสของชุมชนจุลินทรีย์ในดินนาข้าว ภายหลังการเติมปุ๋ยเคมี

นางสาวธัญภัทร พรหมศร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์สิ่งแวดล้อม (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

PHOSPHOLIPID FATTY ACID PATTERNS AND CELLULASE ACTIVITIES OF MICROBIAL COMMUNITIES IN RICE FIELD SOILS FOLLOWING CHEMICAL FERTILIZER AMENDMENT

Miss Thanyaphat Promson

Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Environmental Science

(Interdisciplinary Program)

Graduate School

Chulalongkorn University

Academic Year 2009

Thesis Title	PHOSPHOLIPID FATTY ACID PATTERNS AND CELLULASE ACTIVITIES OF MICROBIAL COMMUNITIES IN RICE FIELD SOILS FOLLOWING CHEMICAL FERTILIZER AMENDMENT
By	Miss Thanyaphat Promson
Field of Study	Environmental Science
Thesis Advisor	Supawin Watcharamul, Ph. D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Graduate School
(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Charnwit Kositanont, Ph.D.)

..... Thesis Advisor
(Supawin Watcharamul, Ph. D.)

..... Examiner
(Associate Professor Alisa Vangnai, Ph.D.)

..... Examiner
(Assistant Professor Onruthai Pinyakong, Ph.D.)

..... External Examiner
(Sirawut Klinbunga, Ph.D.)

ธัญภัทร พรหมศร : รูปแบบกรดไขมันฟอสโฟลิปิดและกิจกรรมเซลลูเลสของชุมชนจุลินทรีย์ในดินนาข้าวภายหลังการเติมปุ๋ยเคมี. (PHOSPHOLIPID FATTY ACID PATTERNS AND CELLULASE ACTIVITIES OF MICROBIAL COMMUNITIES IN RICE FIELD SOILS FOLLOWING CHEMICAL FERTILIZER AMENDMENT) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ดร. ศุภวิน วัชรมูล, 129 หน้า.

การศึกษาชุมชนจุลินทรีย์จากรูปแบบกรดไขมันฟอสโฟลิปิดและกิจกรรมของจุลินทรีย์ดินจากกิจกรรมเซลลูเลสในดินนาข้าวภายหลังการเติมปุ๋ยเคมีสูตร 16-20-0 อัตรา 25 กิโลกรัมต่อไร่ (กลุ่มทดลอง) ตามอัตราที่แนะนำโดยกรมวิชาการเกษตร เปรียบเทียบกับดินนาข้าวที่ไม่เติมปุ๋ยเคมี (กลุ่มควบคุม) ในช่วงเวลา 50 วัน โดยทำการเก็บตัวอย่างในวันที่ 0, 1, 3, 7, 14, 21, 28, 35, 42, และ 49 พบว่ากรดไขมันฟอสโฟลิปิดอิ่มตัว โดยเฉพาะ 16:0 และ 18:0 รวมถึงกรดไขมันฟอสโฟลิปิด i15:0 และ a15:0 ซึ่งเป็นกรดไขมันฟอสโฟลิปิดเฉพาะของแบคทีเรียแกรมบวก เป็นกรดไขมันฟอสโฟลิปิดเด่นตลอดการทดลอง ทั้งในกลุ่มทดลองและกลุ่มควบคุม จากการวิเคราะห์ทางสถิติด้วยการวิเคราะห์องค์ประกอบหลัก (Principle Component Analysis; PCA) พบว่ารูปแบบกรดไขมันฟอสโฟลิปิดในกลุ่มทดลองและกลุ่มควบคุม ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ การศึกษากิจกรรมเซลลูเลสพบว่ากิจกรรมเซลลูเลสในกลุ่มทดลองมีค่าใกล้เคียงกับกลุ่มควบคุมและไม่มีความแตกต่างกันทางสถิติตลอดการทดลอง โดยกิจกรรมเซลลูเลสมีค่าสูงสุดในวันที่ 21 ของการทดลอง (170.21±19.21 และ 194.46±12.44 $\mu g\ GE \cdot g^{-1} dm \cdot 24h^{-1}$ ในกลุ่มทดลองและกลุ่มควบคุมตามลำดับ) นอกจากนี้การวัดกิจกรรมของจุลินทรีย์ดินจากปริมาณคาร์บอนไดออกไซด์ที่เกิดขึ้นจากการหายใจของดินตลอด 50 วันของการทดลองพบว่า ปริมาณคาร์บอนไดออกไซด์ที่เกิดขึ้นในกลุ่มทดลองมีแนวโน้มสูงกว่าในกลุ่มควบคุม โดยปริมาณคาร์บอนไดออกไซด์สะสมตลอด 50 วัน มีค่าเท่ากับ 3,339.79 และ 2,663.84 $\mu g\ CO_2 \cdot g^{-1}$ dm ตามลำดับ การวิเคราะห์สมบัติของดินบางประการ ซึ่งวิเคราะห์ในวันที่ 0, 18, และ 49 ของการทดลอง มีเพียงค่าความเป็นกรดเป็นด่างของดินที่ได้รับผลกระทบจากการใช้ปุ๋ยเคมี โดยมีค่าลดลงอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 ค่าความเป็นกรดเป็นด่างที่ลดลงนี้สอดคล้องกับปริมาณคาร์บอนไดออกไซด์ที่วัดได้ในแต่ละวันที่ทำการทดลองซึ่งมีแนวโน้มลดลงเมื่อเวลาผ่านไป

สาขาวิชา วิทยาศาสตร์สิ่งแวดล้อม ลายมือชื่อนิสิต.....ธัญภัทร พรหมศร.....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....ศุภวิน วัชรมูล

508 71445 20 : MAJOR ENVIRONMENTAL SCIENCE
KEYWORDS : CHEMICAL FERTILIZERS / RICE FIELD SOIL / PHOSPHOLIPID FATTY ACID (PLFA) / CELLULASE ACTIVITY / SOIL RESPIRATION

THANYAPHAT PROMSON : PHOSPHOLIPID FATTY ACID PATTERNS AND CELLULASE ACTIVITIES OF MICROBIAL COMMUNITIES IN RICE FIELD SOILS FOLLOWING CHEMICAL FERTILIZER AMENDMENT.
THESIS ADVISOR : SUPAWIN WATCHARAMUL, Ph.D., 129 pp.

Phospholipid fatty acid (PLFA) patterns and cellulase activities of microbial communities in rice field soils were investigated after chemical fertilizer grade 16-20-0 at 25 kg·rai^{-1} as the recommended dose by Department of Agriculture was amended. The fertilizer amended (FA) soils were tested against the rice soils without chemical fertilizer amendment (control; C) for 50 days of the experiment and the samples were collected on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49. PLFA data showed that saturated fatty acids, especially 16:0 and 18:0, and i15:0 and a15:0 which are signature PLFA of Gram-positive bacteria, were predominant throughout the experiment period in both C and FA treatment sets. Principle component analysis (PCA) data showed that there was also no significant difference between the total PLFA patterns of C and FA sets. The study of cellulase activity indicated that the cellulase activities of FA set were similar to C set and there was also no significant difference, and the highest cellulase activities were occurred on Day 21 in both FA and C sets (170.21±19.21 and 194.46±12.44 μg GE·g^{-1}dm·24h^{-1}, respectively). The soil microbial community activity was also measured by the determination of carbon dioxide (CO_2) evolution from soil respiration over a 50 day-period. The results showed that the CO_2 evolution trend in FA set was higher than that of C set and the 50-day of CO_2 accumulation of FA set was higher than C set (3,339.79 and 2,663.84 μg CO_2·g^{-1} dm, respectively). The soil physico-chemical properties were analyzed on Day 0, 18, and 49 of the experiment. The results showed that soil pH was the only parameter in this study that was affected by chemical fertilizer amendment which decreased significantly at the 95% of confidence level. The decreasing trend of soil pH was correlated to the detected CO_2 amount which declined through the experiment period.

Field of Study : Environmental Science Student's Signature Thanyaphat Promson
Academic Year : 2009 Advisor's Signature Supawin Watcharamul

ACKNOWLEDGEMENTS

It is a pleasure to thank those who made this thesis possible. This thesis could not be done without their help and support.

I would like to express my gratefulness to my thesis advisor, Supawin Watcharamul, Ph.D., for his knowledge, encouragement, support and his value time from the beginning to the final which enable me to develop not only my efficiency of learning, but also my lifestyle attitudes.

It is pleasure to thank the thesis committee, Assistant Professor Charnwit Kositanont, Ph.D., Associate Professor Alisa Vangnai, Ph.D., Assistant Professor Onruthai Pinyakong, Ph.D., and Sirawut Klinbunga, Ph.D., for their value time, comments, and advices.

I wish to acknowledge THE 90th ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Ratchadaphiseksomphot Endowment Fund) and Graduate School, for financial supports. I also appreciate Department of General Science, Faculty of Science, for chemical and laboratory sustenance.

I would like to thank staff of Department of General Science, Faculty of Science for laboratory support. I would like to show my recognition to Mrs. Sunee Pakpraphun for her help with the GC analysis and I am thankful for Ms. Monrawee Juntogul and Mr. Prachon Petch-chong for the collection of soil.

I owe my deepest gratitude to my parents and my relatives for encouragement, understanding and financial support. I am also indebted to my friends, Ms. Aujjima Prianan, Ms. Peerapa Suwannachote, and Mr. Abhichart Krissanaprasit, to their help and inspiration and encouragement. Finally, I would like to appreciate Mr. Torarit Senanimit for his patient, understanding, and the joy he brings to me.

CONTENTS

CONTENTS (CONT.)

CONTENTS (CONT.)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

ABBREVIATION

°C	degree Celsius
μg	microgram
BAME	bacterial acid methyl ester
CO_2	carbon dioxide
FAME	fatty acid methyl ester
g	gram
G-	Gram-negative bacteria
G+	Gram-positive bacteria
kg	kilogram
L	liter
M	molar
ml	milliliter
N	normality
nm	nanometer
PCA	principle component analysis
PLFA	phospholipid fatty acid
ppm	part per million
SatFA	saturated fatty acid

CHAPTER I

INTRODUCTION

1.1 Rationale

Chemical fertilizers are used worldwide in most agro-systems to help increase agricultural product yields and maintain soil quality. Chemical fertilizers are composed, in varying proportions, of three important macro-elements, nitrogen (N), phosphorus (P), and potassium (K), and other micro- and trace-elements such as calcium (Ca), magnesium (Mg), and sulfur (S). Due to their “easy-to-use” properties and their nutrient elements availability for plant to uptake, hence, chemical fertilizers are used broadly.

Thailand is an agricultural country. Millions tons of chemical fertilizers are applied on plantation areas annually whilst rice fields are named one of the places where chemical fertilizers are heavily used in the country (The National Statistical Office; NSO, 2003). Rice is cultivated nationwide, especially in the central region (Phra Nakhon Si Ayutthaya Province and Suphanburi Province, for instance). Paddy soil in the central region is fertile alluvial soil which is clay or clay loam (Kyuma, 2004). In process of rice field preparation, straws (left from harvesting) and weeds are tilled and help increase paddy soil fertility. Although paddy soil is very fertile, continuous plantation and tillage could cause soil degradation and loss of soil fertility. Consequently, chemical fertilizers are applied to maintain soil quality and product yields. However, there are many suggestions that long term or improper use of chemical fertilizers might change soil physico-chemical (Sarathchandra *et al.*, 2001; Demoling, Ola Nilsson, and Bååth, 2008; Zhang *et. al.,* 2007) and biological properties (Nayak, Babu, and Adhya, 2007; Marschner, Kandeler, and Marschner, 2003) which effected to soil living organisms.

Paddy soil agro-ecosystem is very unique and most effected by anthropogenic activities such as irrigation, tillage, and chemical fertilizer application (Attanandana, 1988; Thai Rice Foundation under Royal Patronage, 2006; Rice Department, 2008). Water level in the process of lowland rice cultivation is an

important factor changing soil properties. As a result, a unique agro-ecosystem with high soil microbial diversity is created. When water is filled in the rice field created submerged soil or anaerobic soil. When water is released from the rice field caused aerobic soil. These conditions do affect the soil microbial diversity including aerobes, facultative anaerobes, and anaerobes in paddy soils (Kyuma, 2004). These microbes have important roles as decomposers and associate in soil physical, chemical, biological, and biochemical properties. Some of them are tolerant, but some of them are sensitive to environmental changing or soil pollutions, for example, oxygen level (Bossio and Scow, 1998; Zabaloy *et al.*, 2008), heavy metal contamination (Frostegård, Tunlid and Bååth, 1993; 1996), pH (Aciego Pietri and Brookes, 2008; Bååth and Anderson, 2003), and nutrient level (Allison *et al.*, 2007; Chang, In, and Pil, 2004). At present, it is confirmed that soil microorganisms are the most rapid response to environmental changes which may cause the shift of microbial community structures and their activities that lead to the change of soil properties (Böhme, Langer, and Böhme, 2005; Li *et al.*, 2008; Liao *et al.*, 2002; Lin *et al.*, 2004; Zhang *et al.*, 2006).

Many methods have been used to study soil microbial communities and their shift affected by environmental changes. The well known “traditional technique”, for examples; plate count technique, strain isolation, most probable number (MPN) method, and colony morphology, are culture dependent technique used to study soil microbial communities. However, it cannot reveal entire communities because only 1-5% of soil microbial that observed by microscopy can culture on solid media (Eldor, 2007). Therefore, many researchers have been developing various culture independent methods, such as, 16S rRNA (Purdy *et al.*, 1997), restriction fragment length polymorphism (RFLP) (Hartmann *et al.*, 2005; Tom-Petersen *et al.*, 2003), and denaturing gradient gel electrophoresis (DGGE) (Agnelli *et al.*, 2004; Asakawa and Kimura, 2008; Matsuyama *et al.*, 2007), to elucidate the structure of soil microbial communities and their functions on nutrient cycles.

Phospholipid fatty acid (PLFA) analysis is one of the popular culture independent methods to study soil microbial communities and their changes (White *et al*. 1979; Frostegård and Bååth, 1996; Zelles, 1999). Phospholipids are cell membrane component. Each group of microbes has specific phospholipids fatty acid patterns which can use to identify them as their fingerprints or their signatures (Zelles, 1999; Kaur *et al.*, 2005; Marschner, 2007). For instance, *iso*- and *antei-iso*-

fatty acids are abundance in Gram-positive bacteria, whereas monounsaturated fatty acids, cyclopropane fatty acids, and ß-hydroxy fatty acids are rich in Gram-negative bacteria (Zelles, 1996; Kaur *et al.,* 2005; Marschner, 2007). Since PLFA degrade rapidly after cell death and not found in storage cell, therefore, they can used to detect viable cells (Jjemba, 2004). Furthermore, change in their patterns can predict stress, starving, and change in environment that impact to the microbial communities and their activities (Bai, Gattinger, and Zelles, 2000; Frostegård and Bååth, 1996; Hedrick, Peacock, and White, 2007; Kaur *et al.*, 2005; Yan *et al.*, 2008; Zelles, 1997)

The shift of soil microbial communities from environment change could change their activities. Decomposition is the important activity of soil microbes with a lot of enzymes association. Thus, studies of soil enzymes are keys to understand the effects of environment on soil microbial activities. The major groups of soil enzymes are involve to carbon cycle because most of soil microbial are heterotrophs that need carbon as energy source (Tate, 2000). In paddy soil, most of organic matter is derived from plant materials which are tilled during rice field preparation process. There is up to 60% cellulose in plant component (Killham and Prosser, 2007), hence, cellulase is the most important enzyme in paddy soil.

Cellulase is a group of extracellular enzymes, and the products of their catalytic are oligosaccharide, cellobiose, and glucose (Deng and Tabatabai, 1994; Sharrock, 1988). Thereby, detection of cellulase activity can imply to soil fertility level and microbial activity. Moreover, when plant residue and organic matter are completely decomposed by microbial, the end products are biomass, energy, and carbon dioxide (CO_2). Thus, detection of CO_2 or soil respiration is another way to explore soil fertility and microbial activity (Watcharamul and O'Donnell, 2002).

The effects of chemical fertilizers on soil microbial communities and their activities are studied in many agro-ecosystems (Chang, Chung, and Tsai, 2007; Wei *et al.*, 2008; Toyota and Kuninaga, 2006; Mandal *et al.*, 2007; Saha *et al.*, 2008; Sarathchadra *et al.*, 2001). There are some researches on soil fertilizer amendments in Thailand. Most are focused on the effects of fertilizer amendments to soil physical and chemical properties, nutrients uptake, and product yields but very rarely to soil biological and biochemical properties (Khentha, 2008; Methaluk, 2004; Photong, 2008). Therefore, this thesis could provide more useful information which is necessary for sustainable agro-ecosystem management.

1.2 Objectives of the Thesis

1) To investigate soil microbial communities in paddy soil using phospholipid fatty acid patterns and their cellulase activity with and without chemical fertilizer amendment.

2) To determine the effects of chemical fertilizer amendment on paddy soil physico-chemical properties.

1.3 Scopes of the Thesis

Soil samples were taken from a rice field at Tumbon Ban-rom, Amphur Tha-rua, Phra Nakhon Si Ayutthaya Province, Thailand. Samples were separated into two experiment sets, treatment set (FA) and control set (C), referring to with and without chemical fertilizer amendment, respectively. A chemical fertilizer used throughout this study was 16-20-0 mixed fertilizer at 156.25 kg·ha^{-1} (25 kg N·ha^{-1} and 31.25 kg P_2O_5·ha^{-1}) (25 kg·rai^{-1}; 4 kg N·rai^{-1} and 5 kg P_2O_5·rai^{-1}). This fertilizer ratio is recommended by Department of Agriculture, Ministry of Agricultural and Cooperatives, Thailand (2004), for low land rice culture.

Comparisons and analysis of phospholipid fatty acid patterns and cellulase activities between FA and C sets were determined triplicately on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49. Phospholipid fatty acid analysis was described by Bligh and Dyer (1959) and modified by Watcharamul (2005). Cellulase activity was measured by the methods of von Mersi and Schinner (1996). Soil respiration, according to Öhlinger (1996), was determined by titration daily for fifty days in five replicates. Soil physical and chemical properties were measured on Day 0, 18, and 49. Phospholipid fatty acids were detected by gas chromatography (GC), and data were analyzed statistically by principle component analysis (PCA). Cellulase activities, soil respiration, and soil physico-chemical properties data were statistically analyzed with t-test. The period of experiments is December 2008 – September 2009.

1.4 Anticipated Benefits

The data of this thesis could be used to reveal soil microbial community structure and their activities in Thailand paddy soils. Also, the effects of chemical fertilizer amendment on soil microbial community through the change in phospholipids fatty acid profiles, cellulase activity, and soil respiration. These data are also related to soil physical and chemical properties in paddy soil followed by chemical fertilizer amendment. This information can be used as the fundamental data for soil microbial biodiversity in Thailand and the effects of agricultural activities on soil microbial communities. Moreover, this thesis has a potential to publish on the international journal which could be a reference source for the highest sustainable agricultural management especially on soil resource in order to maintain soil fertility and natural equivalents.

1.5 Components of the Thesis

The thesis comprises of 5 chapters. Chapter 1 is the introduction. Theoretical and literature reviews are presented in Chapter 2 which focused on paddy soil properties, rice cultivation, chemical fertilizer application in paddy soil and environmental problems, soil microbial organisms and their activities, and phospholipid fatty acid as a tool to investigate soil microbial communities. Literature reviews included the effects of chemical fertilizer amendment on soil properties, soil microbial communities and their activities in paddy soil ecosystem and other agro-ecosystems. Methodology and equipments, and methods for statistical analysis are presented in Chapter 3 and the results of this thesis appeared in Chapter 4. Finally, discussion, summary, and suggestions are shown in Chapter 5.

CHAPTER II

LITERATURE REVIEW

2.1 Rice Culture

Rice is a main source of carbohydrate for Thais and has been the most important agricultural exported product. Rice planted area in Thailand is one of the major cereal planted areas of the world. With warm subhumid tropics and fertile land, rice can be grown in every region of Thailand (Maclean *et al*., 2002). In the past, rice cultivation was practiced only in rainy season because water is the most important factor for rice to grow. After the Green Revolution, modified plants, agricultural machines, chemical fertilizers and pesticides, including irrigation development have major roles on intensive agriculture. Rice cultivation can be practiced off the rain season and over the year with these factors (Department of Agriculture, 2002).

There are two patterns of rice culture in Thailand divided by topography. Lowland rice culture is established in every region with high soil water holding capacity, while upland rice culture is set up in upland areas, the lower altitudes of high hill, and drought areas which lands or soil are less capacity to hold the water (Attanandana, 1988).

The central region is an intensively cultivated alluvial area. Alluvial soil is suitable for lowland rice culture because it has high water holding capacity and very fertile. Rice growing season is begun on May to July which is rainy season, so most of rice areas are rainfed rice system. After harvesting on November to December, the second lowland rice culture can be practiced under irrigated condition. The harvesting period of the second crop is on May to June (Maclean *et al.*, 2002). The important rice areas on the central region are Phra Nakhon Si Ayutthaya, Suphanburi, Chainat, and Pathumthani Provinces (NSO, 2003).

2.1.1 Rice Transplanting System

Rice transplanting system is traditional practice on rainfed lowland rice area. Soil preparation is begun in early rainy season. Paddy plough soil layer is tilled to mix rice straws, stubbles, leaves, and some husks that left in rice field after

harvesting that help to increase soil organic matter and maintain soil fertility. Tilling is also oxygenated to plough soil layer and defeat weeds. Paddy soil is left to dry to eliminated weeds and some plant diseases. Water is then stored in paddy field in order to soften soil texture and created soil puddling that easier to plough and allow remained weed to grow (Rice Department, 2008).

Tilled puddle soil is submerged for 1-2 weeks. After that, farmer repeats the second tillage to defeat weeds that grow after the first tillage and minimize soil clump. Finally, paddy soil is harrowed to remove weeds and minimize soil clod. Moreover, harrow is done to flatten soil level for control water level in rice field to make rice seeds grow properly. Water is drained if this field is seeding field or remain submerge to be transplanted field (Figure 2.1). The prepared rice seed is sown on seeding field and then transplanted to transplanted field in 20-25 day after seeding (Figure 2.2). The water level is maintained to support rice growth and eventually drained to stimulate rice to ripe. About 10 days after draining, rice will be proper to harvest (Thai Rice Foundation under Royal Patronage, 2006; Rice Department, 2008). The whole processes need a lot of labors and time consuming.

Figure 2.1 Rice field soil for cultivation after tillage preparation [Photographed by Thanyaphat Promson (January 2nd, 2010)]

Figure 2.2 Farmers are transplanting rice (Food and Agriculture Organization; FAO, 2002 : online)

2.1.2 Paddy-sown Field System

Paddy-sown field system is a lowland rice culture practice and becoming of interests because it consumes less time and fewer labors. The puddling preparation and growing process are similar to rice transplanted system except seeding stage. The prepared rice seed is sown directly to prepared paddy field without transplanting, so rice is grown on that seeding field. This cultivation technique is suitable for rice area with irrigation facility or rainfed area that water is well controlled (Thai Rice Foundation under Royal Patronage, 2006; Rice Department, 2008).

2.2 Paddy Soil Environment

Paddy soil is a unique agro-ecology and disturbed by human actions. Water controlling in each stage of lowland rice cultivation, tillage, fertilizer amendment, and using of pesticide is very influence on changes of paddy soil properties. These conditions affect soil organisms, especially soil microbial communities, which also play major roles in changing soil physico-chemical, and biological properties. Most of lowland rice cultivation areas are clay soil or clay-loam soil texture (Kyuma, 2004). Paddy soil has high surface area because clay particles create more pore space.

These soil pores are habitat for soil organisms and play important roles in water and air movement. Water holding capacity (WHC) of paddy soil is high which related to small pore in clay soil texture. Cation exchange capacity (CEC) of paddy soil is also high because the more percent of clay particle, the more cations are on soil surface. CEC is important for soil microorganisms because it controls nutrient adsorption and releasing, pH control (Hartei, 2005), and also attachment of bacteria through cation bridge on clay particle (Maier and Pepper, 2009). With high surface area, WHC, and CEC make paddy soil is a good habitat for soil microorganisms. Moreover, with high soil fertility level of paddy soil and water control in each stage of cultivation, paddy soil ecology is highly diverse that aerobes, facultative anaerobes, and anaerobes could be ubiquitously found (Kyuma, 2004).

2.2.1 Effect of Rice Cultivation Processes on Paddy Soil Properties

As mentioned earlier, anthropogenic activities on paddy soils could affect the changes in paddy soil properties depending on water level in paddy field. After harvesting, water in paddy field is drained, makes soil drought and covered with rice residues and weed. With highly percent of clay, paddy soil becomes aggregated, compact, and hard (Greenland, 1997). Rice cultivation season is begun after the first rainfall in rainfed agriculture area. For soil preparation, moisten paddy soil is tilled in order to turn the lower soil over to contact the air, dry, mix organic residues into soil, and defeat weeds and some plant diseases (Rice Department, 2008). The important activity of soil microbial in this shortly stage is decomposition of plant residue while soil is on aerobic condition which increases soil fertility. Soil aeration creates yellow or red mottles on blue-grey soil surface due to ferrous oxidation reaction (Singer and Munns, 2006; Kyuma, 2004).

Rain or irrigated water is then filled in paddy field in order to soften soil texture which easier to plough and allow water weeds to grow (Rice Department, 2008). Soil become submerge in this stage and soil air is instead with water creates anaerobic condition. Oxygen level in soil is decreasing rapidly in a few days. This condition favours anaerobes and facultative anaerobes still survive. Anaerobes can use other electron acceptors instead of oxygen, such as nitrate (NO_3), ferric iron (Fe^{3+}), and carbon dioxide (CO_2) (Kyuma, 2004).

The important activity of soil microorganisms in this stage is fermentation of rice residues. Tilled paddy soil is left submerged for 1-2 weeks (Rice

Department, 2008). Meanwhile, methane (CH_4) and loss of nitrogen are occurred via CO_2 and NO_3 reduction (Greenland, 1997). After that, farmer repeats the second tillage to destructed water weeds that have grown after the first tillage and minimize soil clump. Finally, paddy soil is harrowed to remove weeds and minimize soil clod. Moreover, harrow is done to flatten soil level for control water level in rice field to make rice seeds grow properly (Rice Department, 2008).

After soil preparation is done, water is filled in create submerge soil and thus ready for rice plantation. In paddy-sown field system, rice seed is pre-cultivated by soaking in water overnight. Drained rice seed then kept moistened to grow for 2 days before sowing (Attanandana, 1988). Water level has to be maintained at a proper level so that rice can grow properly. As the result, paddy soil is under anaerobic condition.

Reduction occurs when paddy soil is flooded. Electron acceptors are used in sequential reduction process depends on redox potential (mV). While oxygen is not completely consumed, NO_3 is first reduced to nitrite (NO_2) and nitrogen (N_2), and managic (MnO_2) is reduced to manganous (Mn^{2+}). These reduction reactions decrease redox potential which effects to soil chemical properties (e.g., pH). Ferric (Fe^{3+}) reduction does not happen until O_2 and NO_3 is almost completely run out and sulphate (SO_4^{2-}) reduction occur only after O_2 and NO_3 is completely depleted. Reduction of CO_2 to CH_4 is happened in this stage too. Organic matter is still decomposed in fermentation process. Organic acids are intermediate product and finally converted to CO_2 or CH_4 (Kyuma, 2004; Greenland, 1997).

2.3 Fertilizers

Fertilizer is the material either inorganic or organic compound which derived from natural sources or synthesized. Fertilizer is applied for plant to provide needs plant nutrient elements or created change on soil chemical properties in order to promote plant growth, their qualities, and product yield (Plaster, 2009). Fertilizer is classified in many ways. Source by its original material is one.

After the World War II, the green revolution was broadening around the world. The mission to increase agricultural products to support the world's citizen was rising continuously. Modified plants and agricultural machines are used to increase

efficiency and product yields (Department of Agriculture, 2002). Since agricultural areas tend to decrease and transform to resident or industrial areas, fertilizer has a major role for gain product yield in limited area which has intensively culture.

2.3.1 Classification of Fertilizer

1) Organic fertilizer

Organic fertilizer is originated from organic materials, such as plant residues, animal dung, and sewage sludge, which pass several biological or chemical processes before apply to plants. Compost, animal manure, and green manure are represented to organic fertilizer. According to Fertilizer Act B.E. 2518 (A.D. 1975), organic fertilizer is the fertilizer that from organic material which made by moistening, cutting, grinding, composting, or another processes (คณาจารย์ภาควิชาปฐพีวิทยา, 2548)

2) Chemical fertilizer or inorganic fertilizer

Chemical fertilizer is combination of synthesized chemical inorganic compounds. After Fertilizer Act B.E. 2518 (A.D. 1975), it also include straight fertilizer, mixed fertilizer, compound fertilizer, and the combination of organic and chemical fertilizer, but not cover lime, marl plaster, and gypsum (คณาจารย์ภาควิชาปฐพีวิทยา, 2548)

2.3.2 Chemical Fertilizer and Classification

Chemical fertilizers are in variety forms; liquid as pressurized liquids or fluid fertilizer; solid as granules, powder, or prills (Plaster, 2009). There are several ways to classify chemical fertilizer. Categorize by their composition compounds is a basis (Troch and Thompson, 2005).

Single compound fertilizers, also called fertilizer materials or fertilizer carriers (Plaster, 2009), are mostly supply only fertilizer element. Examples are ammonium sulfate, ammonium nitrate, and potassium chloride. A few fertilizers combine two elements in one compound, for instance, diammonium phosphate.

Manufactured fertilizers are marketing mixed fertilizers. They are mixing of chosen N-P-K ratio and add some trace elements that appropriate for each plant and market needs. These mixed fertilizers are ready to use. Some of mixed fertilizers provided complete N-P-K compounds; some are only supply N-P, N-K, or P-K. Bulk-blend fertilizers are another mixed fertilizer. Fertilizer carriers are mixed follow the order ratio just before packed and sent to the grower. Some micronutrients are added before apply by sprayed (Troch and Thompson, 2005).

2.3.3 Fertilizer Carriers

Fertilizer carriers can be used as single compound fertilizers. They can also be used as fertilizer materials for manufactured mixed fertilizers and bulk-blend fertilizers. The nutrients in fertilizer carriers are form as available forms which plant can uptake them immediately when they are in soil solution. The major compositions in chemical fertilizers are nitrogen, phosphorus, and potassium. The same fertilizer grade from other manufactured fertilizers may have provided from different fertilizer carriers (Plaster, 2009).

2.3.3.1 Nitrogen Carriers

Most of nitrogen carriers are derivatives of ammonia (NH_3). In many fertilizer factories, NH_3 is received by fixing nitrogen gas from air and attach it to hydrogen from natural gas. This reaction needs heat, pressure, and catalysts. Ammonium can be used as fertilizer carrier or changed to the other forms via the Haber-Bosch process (Figure 2.3). Most of these nitrogen carriers are acidic when they are solutions except for sodium nitrate and calcium nitrate are basic (Plaster, 2009).

2.3.3.2 Phosphorus Carriers

Natural rock phosphate is a main source of phosphorus fertilizers (White, 2006). Phosphate rock deposited in very long time contains various apatite minerals. Clay, calcium carbonate, and silica are separated from phosphate rock. After that, phosphate rock is grinded and ready for use as mineral fertilizer but will be dissolve in soil solution very slowly (Plaster, 2009). Hence, treating with acid will improve P solubility (Singer and Munns, 2006). Triple superphosphate is a derivative of phosphate rock treated with phosphoric acid. Treating with 90% sulfuric acid and ammonia produce ammonium phosphate, while the reaction between 70%

sulfuric acid make superphosphate. The schemes of these processes are illustrated in Figure 2.4.

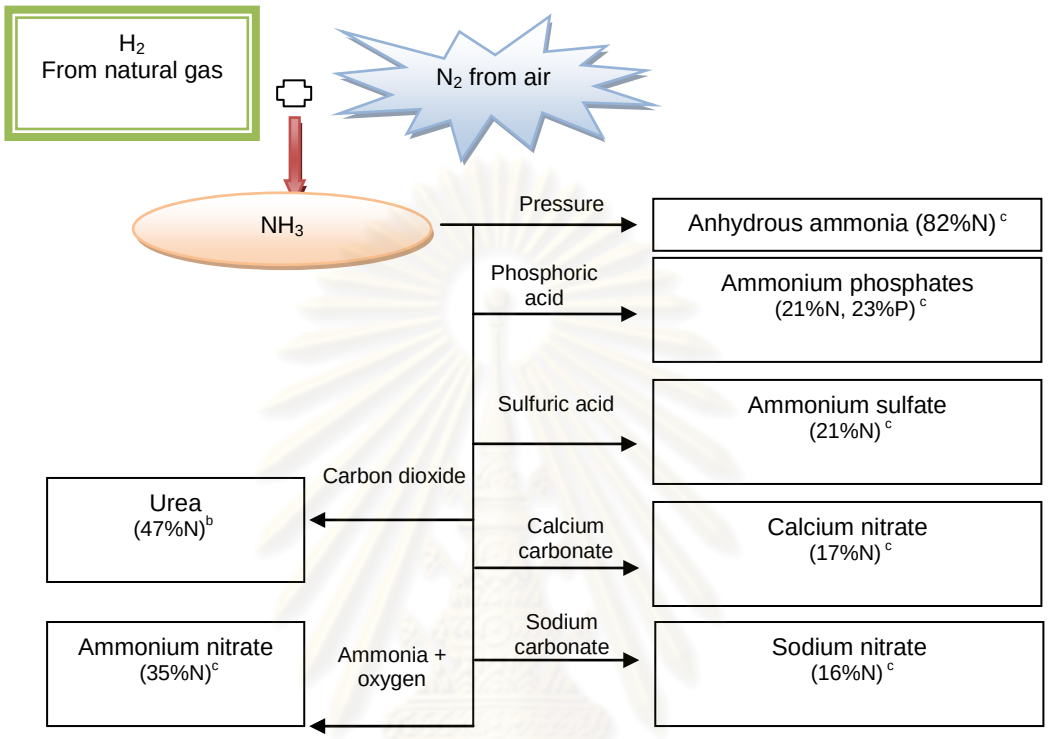


Figure 2.3 Schemes of Haber-Bosch process[a] and %N contents[b,c] (after Plaster, 2009[a]; Singer and Munns, 2006[b]; White, 2006[c])

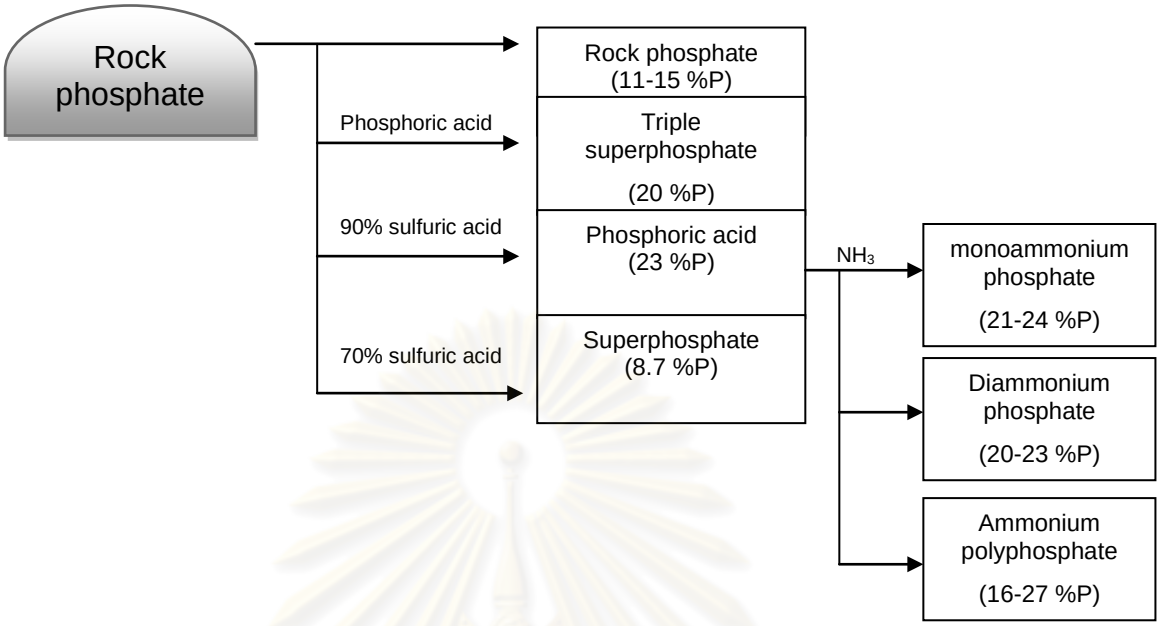


Figure 2.4 Schemes of phosphorus carriers' productions and %P contents (after Plaster, 2009)

2.3.3.3 Potassium Carriers

Potassium chloride (KCl) is the most widely used potassium fertilizers. This salt deposit is provided by mining of deep sedimentary or from salt-lake deposits. Not only KCl occurs in these deposits, but also includes NaCl, $MgSO_4$, and K_2SO_4. Thus, KCl has to be separated by floatation or recrystallization (White, 2006). The term "potash" is common used when referring to potassium fertilizers (Plaster, 2009). There are many forms of potash carriers which contain various potash percent. The lists are shown in Table 2.1.

Table 2.1 Potash carrier forms and %K content (after Plaster, 2009)

Potash carriers	K content (%)
Potassium chloride (KCl)	60
Potassium sulfate (K_2SO_4)	49
Potassium nitrate (KNO_3)	42
Sulfate potash magnesia	22

2.3.4 Fertilizer Grade

On every fertilizer bag should show the fertilizer grade which indicates to primary nutrients contains. There are 3 numbers on the label that is percent contain of nitrogen, phosphorus, and potassium (N-P-K), respectively (as shown in Figure 2.5). The first number is imply to % total nitrogen, the second number is infer to % available P_2O_5 and the last number is indicate to % K_2O (Plaster, 2009). For example, fertilizer with grade 16-20-0 is 16 percent total nitrogen and 20 percent of available P_2O_5 without potash. Some fertilizer companies also give percent of other compounds such as sulfur (S), and calcium (Ca).

2.3.5 Chemical Fertilizer Amendment on Rice Culture

Using chemical fertilizers on rice culture depends on many factors. Rice culture systems (rice transplanting, paddy-sown rice, or upland rice system); source of water (rain or irrigation); soil types (clay, clay-loam, sandy, or sandy-loam); and plant photoperiod sensitivity, are keys to select which grades of chemical fertilizer should be used and when to apply (Department of Agriculture, 2004). To increase productivity and decrease cost of planting, it is important to select grades and rates, and application periods. It must be note on fertilizer package that this fertilizer is used for rice.

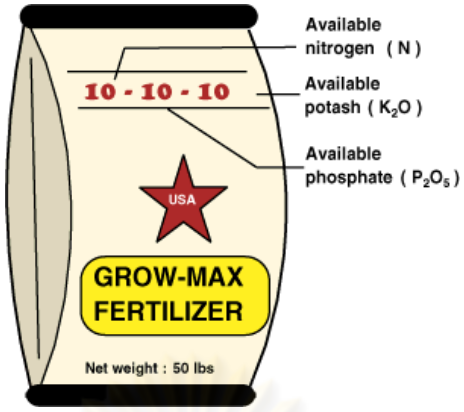


Figure 2.5 The sample of chemical fertilizer bag. The numbers on the bag are represented to N-P-K percent contains. (Agronomic Division, 2010: online]

Many farmers use improper fertilizer grades that not suitable for soil type and sometimes they use chemical fertilizer less than recommended rate because they are quite expensive. These are the reasons why their productivities are low. On the other hand, some farmers use more chemical fertilizer than what they are recommended to insure that their plants will received enough nutrients. Some of them apply chemical fertilizer on improper duration. Over nutrition and using on wrong period may increase weeds and pests in rice field. For these reasons the farmers have to use more pesticides and herbicide which are expand their cost of plantation and lead to environmental problems. The excess N and P may lead to eutrophication and highly N fertilizer use may induce high nitrate concentration in plants or well waters by leaching, lead to health problems (White, 2006).

Department of Agriculture, Ministry of Agricultural and Cooperatives (2004), has suggested farmers on chemical fertilizer applications for sustainable agriculture in order to maintain soil fertility and safe the environment while they can increase their productivities and spend less expense on their plant cost. Soil property analysis is recommended. Therefore, farmers should be selecting the proper grades and rates of chemical fertilizers according to these results. Only chemical fertilizer applications on clay and clay-loam soil are defined in this section, and categorized by rice culture systems and plant photoperiod sensitivity.

2.3.5.1 Rice Transplanting System

According to Department of Agriculture suggestions (2004), chemical fertilizer should be applied 2 times on culture season; the first is on transplanting day or 15 days after rice was transplanted, and the second is on 30 days before flowering stage. On the first time of chemical fertilizer application, 25 $kg \cdot rai^{-1}$ (156.25 $kg \cdot ha^{-1}$) of ammonium phosphate in vary grades (depends on soil type and the other soil properties analysis) such as 16-20-0, 18-22-0, 20-20-0, and 18-46-0, is recommended to applied on photoperiod sensitivity rice field.

For photoperiod insensitivity rice, 187.5 $kg \cdot ha^{-1}$ (30 $kg \cdot rai^{-1}$) of ammonium phosphate is recommended. On the second time, 62.5 $kg \cdot ha^{-1}$ (10 $kg \cdot rai^{-1}$) of urea (46-0-0) is suggested to apply for photoperiod sensitivity rice and 125 $kg \cdot ha^{-1}$ (20 $kg \cdot rai^{-1}$) of urea is suggested for photoperiod insensitivity rice. Instead of urea, 21-0-0 ammonium phosphate may apply at rates 125 $kg \cdot ha^{-1}$ (20 $kg \cdot rai^{-1}$) and 250 $kg \cdot ha^{-1}$ (40 $kg \cdot rai^{-1}$) for photoperiod sensitivity rice and photoperiod insensitivity rice, respectively.

2.3.5.2 Paddy-sown Field System

Double use of chemical fertilizer applications on this culture system is also recommended like the above system. The first time of the application should be set on 20-30 days after sowing and the second time is suggested to set on 30 days before flowering stage (as shown in Figure 2.6(a) and (b)).

For photoperiod sensitivity rice 156.25 $kg \cdot ha^{-1}$ (25 $kg \cdot rai^{-1}$) of vary grades of ammonium phosphate, and 62.5 $kg \cdot ha^{-1}$ (10 $kg \cdot rai^{-1}$) of urea (46-0-0) or 125 $kg \cdot ha^{-1}$ (20 $kg \cdot rai^{-1}$) of 21-0-0 ammonium phosphate, are recommended on the first and the second time of chemical fertilizer using periods, respectively. For photoperiod insensitivity rice, using 187.5 $kg \cdot ha^{-1}$ (30 $kg \cdot rai^{-1}$) of ammonium phosphate on the first application time is suggested. 125 $kg \cdot ha^{-1}$ (20 $kg \cdot rai^{-1}$) of urea or 250 $kg \cdot ha^{-1}$ (40 $kg \cdot rai^{-1}$) (21-0-0) ammonium phosphate should be used on the second fertilizer application duration.

The other grades and rates of mixed fertilizers may be used depending on soil and plant of planting area. Also, the farmers may use fertilizer

carriers to make their own proper ratio of mixed fertilizers for their rice culture base on soil and plant requirements instead of using manufactured fertilizers.

(a)

(b)

Figure 2.6 Chemical fertilizer applications on paddy-sown field system (a) 20-30 days after sowing and (b) 30 days before flowering stage. (Rice Department, 2008: online)

2.4 Soil Microorganisms

Soil is a habitat for various organisms, from vertebrate animals to soil microorganisms that only visible under microscope lens. By size classification, soil organisms are divided to 3 groups (White, 2006). Macro-faunas are vertebrate animals which live in soil temporary or permanent. The samples are burrowing animals such as rabbit, rat, and mole. Micro-faunas are invertebrate animals such as mollusk, arthropods, and earthworm, and microorganisms are invertebrate animals that smaller than 0.2 mm and soil microbes.

Soil microbes are diverse. There are many criteria to categorize them into a group. According to source of energy, they can divide to heterotrophs and autotrophs. Heterotrophs are received their food from organic molecular decomposition, while autotrophs produce their food by using light energy (photoautotrophs) or inorganic molecular oxidation (chemoautotrophs) to convert simple inorganic compound to their food (Standing and Killham, 2007). Soil microbes also classified on their oxygen demand. Aerobes need oxygen as terminal electron reception. Anaerobes, on the other hand, do not need oxygen because it toxic to them. They use inorganic compounds (e.g., NO_3^-, SO_4^{2-}, and Fe^{3+}) as terminal electron reception. Facultative anaerobes are lie between aerobes and anaerobes. Usually, they live in oxygenated environment and use oxygen as their terminal electron acceptor. When oxygen is exhausted or soil is saturated with water, they can adapt inorganic compounds to be their terminal electron acceptor (White, 2006).

In addition, soil microbes can classify by their evolution to prokaryotes and eukaryotes. Prokaryotes are different to eukaryotes because they don't have a unit membrane-bound nucleus and lack of other cell organelles (Killham and Prosser, 2006). Bacteria, Actinomycetes, and Archaea belong to prokaryotes, while fungi, algae, and protozoa are grouped in eukaryotes (White, 2006).

2.4.1 Bacteria

Bacteria are the most abundance of soil microbes. They are estimated about 10^8- 10^9 cells in a gram of soil (Sylvia *et al.,* 2005) and their miscellaneous are more than 20,000 species (White, 2006). They are 0.5-1 μm diameter and 1-2 μm length and found in various shapes (e.g., rod, cocci, or spiral) (Roane *et al.,* 2009).

Many bacteria use flagella for their locomotion. They live in water film around soil particle and division rapidly in suitable environment (Killham and Prosser, 2006).

Bacteria can be separated by their cell wall component arrangement with Gram staining technique. Gram-positive bacteria has thick layer of peptidoglycan which adsorb crystal violet (purple stained) while Gram-negative bacteria has thinner peptidoglycan layer. Instead, their cell wall contain with phospholipids and lipopolysaccharides, therefore they cannot retain crystal violet dye but stained with safranin (red stain) (van Elvas *et al.,* 2007). The drawing of Gram-positive and Gram-negative bacteria is shown below in Figure 2.7.

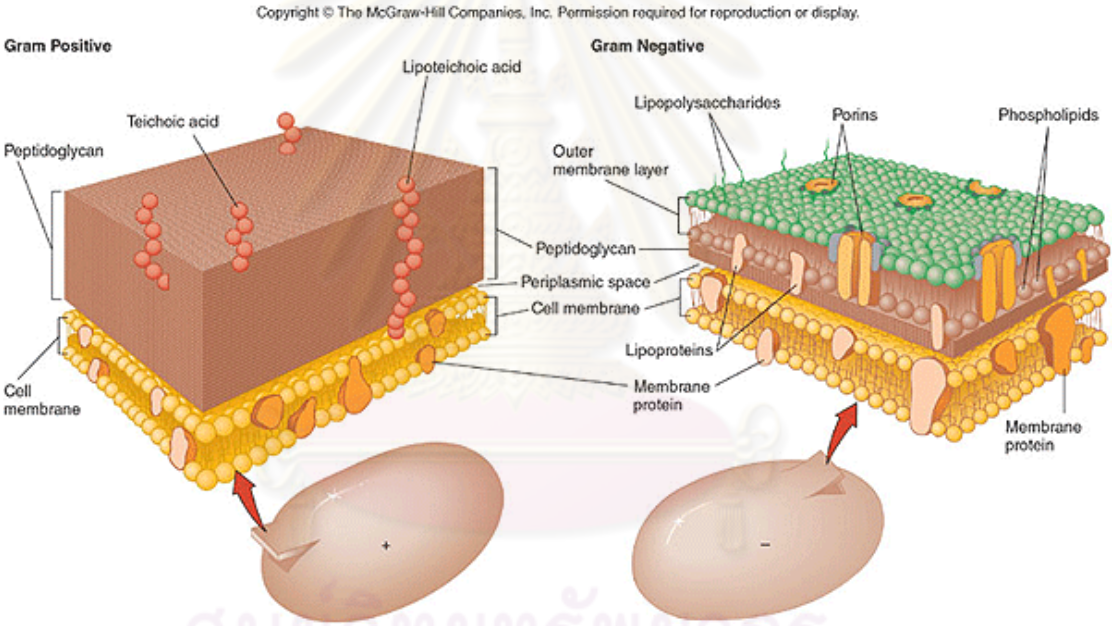


Figure 2.7 Bacteria cell wall components. (Environmental Microbiology, 2009: online)

2.4.2 Actinomycetes

Actinomycetes have both bacteria and fungi features. Their cell wall components are like Gram-positive bacteria, but their filament or branched colony forming and spore producing are resemble to fungi. Although they form filaments like fungi, but their hypae diameter is smaller (Maier and Pepper, 2009). Most of

Actinomycetes are saprophyte. Some of them can fix nitrogen in the air and have symbiosis relation by create nods on plant roots. Some of them, such as *Streptomyces*, are useful for medication by producing natural antibiotics. Some of them are plants and animals pathogens (White, 2006). The numbers of actinomycetes in a gram of soil are 10^7 to 10^8 cells (Sylvia *et al.,* 2005).

2.4.3 Fungi

Fungi are divided to five phyla; Chyridiomycota (*Blastocladiella* sp.), Zygomycota (*Rhizopus nigricans*), Deuteromycota (Fungi Imperfecti), Ascomycota (*Aspergillus* sp., *Penicillium* sp.), and Basidiomycota (*Saccharomyces cerevisiae*, mushrooms) (Jjemba, 2004). Their numbers in soil are about 10^5-10^6 cells in a gram of soil (Sylvia *et al.,* 2005), less than bacteria and Actinomycetes but fungi's filamentous forming make their biomass larger than both of them (Maier and Pepper, 2009). Fungi can appear as a small unicellular (yeast) to a large fruiting body filamentous form (mushroom). Their filaments are used for reaching their food source spread on soil surface make soil to be more aggregate (Singer and Munns, 2006).

Fungi are aerobes, except yeast that have ability to adapt their metabolism to fermentation in anaerobic environment (Maier and Pepper, 2009). All fungi are heterotrophs (Finlay, 2007; Jjemba, 2004). Most of them are saprophytes which are important organic materials decomposers. Many fungi have an important role on complex organic materials degradation such as lignin and cellulose (Singer and Munns, 2006). Some of them have symbiosis relation to plant (mychorrhiza) and algae (lichens) (Jjemba, 2004). Many of fungi species are used in several industries, such as brewery, medical, and food industry.

2.4.4 Algae

Algae are the primary producer in soil. They have chlorophyll or pigments. Using light as their energy source, they can convert CO_2 to simple organic compound by photosynthesis. Hence, algae are most found on wet soil surface (Maier and Pepper, 2009). Algae are divided to prokaryotes, Cyanobacteria (blue-green algae) and eukaryotes, green algae. *Nostoc* and *Anabaena* are representing to the blue-green algae. Their nitrogen fixation ability is very important in N cycle in wet soil (White, 2006). Therefore, when they died, their residues are carbon and nitrogen sources for the other microorganisms. Many algae are free-living cell. Some algae

have symbiotic association with fungi (lichens), mollusks, protozoa, and bryophytes and vascular plants (*Azolla*) (Roane *et al.,* 2009; Jjemba, 2004)

2.4.5 Protozoa

Protozoa are unicellular organisms, living in water film around soil particle. They use water as media for their locomotion. Protozoa move by cilia, flagellae in case of *Euglena*, or means of cytoplasmic streaming in *Amoeba* (White, 2006). Most of them are heterotrophs. Protozoa have an important role on microorganism population control. They live by consuming bacteria, fungi, and algae which release immobilized nutrients to environment (Roane *et al.,* 2009; Coleman and Wall, 2007). Some protozoa have ability to release extracellular enzymes to decompose complex organic materials (e.g., cellulose) (Roane *et al.,* 2009)

2.5 Study of Soil Microbial Communities

There are many ways to study soil microbial communities. The plate count technique is the culture dependent technique to discover structure of the communities. Strain isolation, most probable number (MPN) method, and colony morphology are well known “traditional technique”. These methods are easy, not expensive, and taking a few days of experiment. However, it cannot reveal entire communities because only 1-5% of soil microbial that observed by microscopy can culture on solid media (Eldor, 2007). Moreover, there are many factors that effect to their growth such as temperature and selective media (Trolldenier, 1996). Biolog™ is also culture dependent technique which provided more information about substrate utilization system of soil microbial communities that the researchers interested in (Kandeler, 2007). Though, this method is useful and give the researchers more details about the communities and their functional groups (Zhang *et al.*, 2006), there are still having disadvantages as the plate count technique. Therefore, the culture independent techniques can help us to explore more details and more accurate.

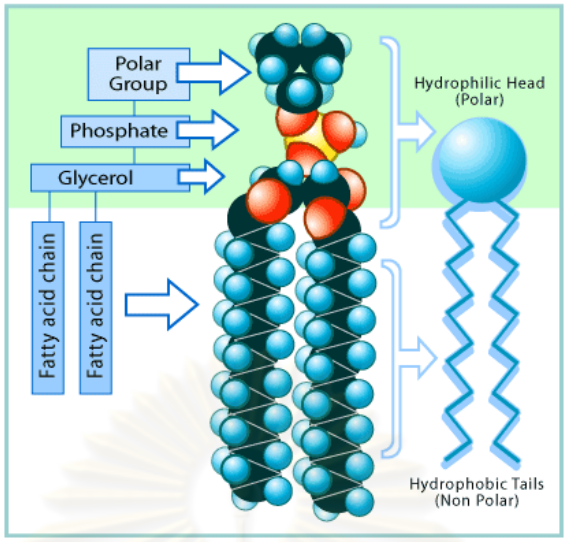
Since the culture independent techniques have been developed, they are very useful tools to study the soil microbial community structures and give us more data. For the examples, using the electrophoresis techniques such as 16S rRNA (Purdy *et al.*, 1997), restriction fragment length polymorphism (RFLP) (Hartmann *et al.*, 2005; Tom-Petersen *et al.*, 2003) or denaturing gradient gel electrophoresis

(Agnelli *et al.*, 2004; Asakawa and Kimura, 2008; Matsuyama *et al.*, 2007) can be very useful tools to explore species of microbes in ecosystem. Furthermore, the researchers can use phospholipids fatty acid (PLFA) to classify the groups of soil microbial communities in the interesting agroecosystem (Bai *et al.*, 2000; Bhattacharyya *et al.*, 2005; Chen *et al.*, 2007; Kaur *et al.*, 2005; Okabe, Toyota, and Kimura, 2000; Zelles *et al.*, 1994)

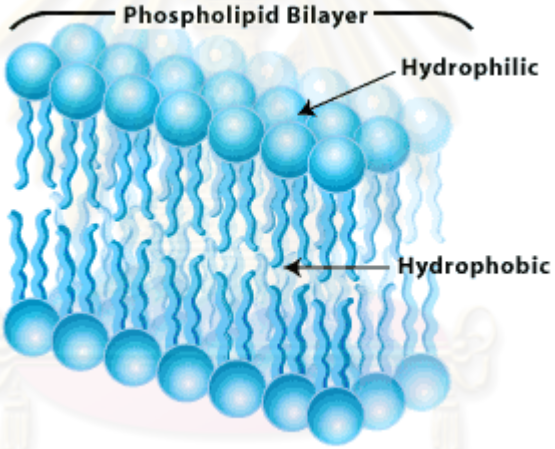
2.5.1 Phospholipid Fatty Acid Analysis

Phospholipids are crucial membranes components of all organisms (Zelles, 1999). The structure of phospholipids, as shown in Figure 2.8(a), consists of the polar head and the non polar tails. The polar head of phospholipids consist of glycerol, phosphate, and polar group, so they are hydrophilic. The non polar tails contain two chains of fatty acids which hydrophobic. As they form lipid bilayers, the hydrophilic heads are the outer surface of cell membrane while hydrophobic tails are inside (Figure 2.8(b)) (Roane *et al.,* 2009; Kaur *et al.*, 2005). Phospholipids are not found in storage product (Zelles, 1999) and are dephosphorylated rapidly to diglycerides (neutral lipid) after cell death (Jjemba, 2004; Kandeler, 2007). Therefore, phospholipids are biomarker for living cells (Marschner, 2007).

Each group of microbial has specific phospholipids fatty acid (PLFA) patterns which can use to identify them as their fingerprint or their signature (Frostegård *et al.,* 1993a; Zelles, 1996). Saturated fatty acids are found in all organisms (Hedrick, Peacock, and White, 2005). *Iso* and *antei-iso* fatty acids are abundance in Gram-positive bacteria, whereas monounsaturated fatty acids, cyclopropane fatty acids, and ß-hydroxy fatty acids are rich in Gram-negative bacteria (Zelles, 1996; Kaur *et al.,* 2005; Marschner, 2007). While several signature fatty acids are bacteria fingerprint, a few fatty acids are indicated to fungi. Polyunsaturated fatty acid (18:2ω6c, and 18:3ω3c) are represented to fungi (Hedrick *et al.,* 2005; Marschner, 2007; Bossio and Scow, 1998). Mid-chain branched, 10me16:0 and 10me18:0 are found in Actinomycetes (Marschner, 2007). The summaries of signature fatty acids are presented in Table 2.2.



(a)



(b)

Figure 2.8 Phospholipid structure (a) and phospholipid bilayers structure on cell membrane (b). (Targeting Cancer Cells Using Lipid-based Drug Delivery Vehicles, 2010: online)

Since PLFA degrade rapidly after cell death and not found in storage cell, therefore, they can used to detect viable cells. Furthermore, changing of their patterns can predict stress, starving, and change in environment that impact to the microbial communities and their activities (Bai *et al.*, 2000; Frostegård and Bååth, 1996; Hedrick *et al.* 2007; Kaur *et al.*, 2005; Yan *et al.*, 2008; Zelles, 1997).

Table 2.2 Fatty acid signatures. Nomenclature is based on the ratio of number of carbon atoms:number of double bonds in the fatty acid, followed by the position of the double bond from the methyl end of the molecule. *Cis*- and *trans*- configurations are indicated by c and t, respectively; prefixes a and i indicate *anteiso*- and *iso*- branching; 10Me indicates a methyl group on the tenth C atom from the carboxyl end of the molecule; cy refers to cyclopropane fatty acids (Frostegård *et al*., 1993a)

Group	Signature Fatty Acids
Bacteria	
Gram-positive	*Iso*- and *antei-iso*- of 15:0, 16:0, and 17:0 10Me16:0, 18:1ω9
Gram-negative	Mono unsaturated fatty acid (ω4, ω7, ω9, and ω11), Cyclopropane fatty acid (cy17:0 and cy19:0), ß-hydroxy fatty acid (2-OH15:0, 3-OH15:0, 2-OH16:1)
Fungi	18:2ω6c, 18:3ω3c, and 18:3ω6c
Actinomycetes	10Me16:0, 10Me18:0
Anaerobic bacteria	Branched monounsaturated fatty acid
Sulphate reducing (*Desulfobacter* sp.)	cy17 : 0 and 10Me16 : 0 without high level of bacteria 10Me 18 : 0
Methanogens	
Type I	16 : 1ω8
Type II	18 : 1ω8

PLFA analysis can extract either from pure culture (Zelles, 1997) or environmental samples such as sediments (White *et al.,* 1979), heavy metal contaminated soils (Frostegård *et al*., 1993b), or paddy soil incorporated with organic materials or chemical fertilizers (Kimura and Asakawa, 2006; Peacock *et al.*, 2001; Zhang *et al.,* 2007; Nakamura *et al.,* 2003). The procedures of phospholipid fatty acid analysis divided to four parts. First, lipids are extracted the samples in chloroform - methanol - buffer solution (Bligh and Dyer, 1959) and then phospholipid fatty acids are separated from neutral lipids and glycolipids using solid phase extraction column. After that, phospholipid fatty acids are converted into fatty acid methyl ester (FAME) and determined with gas chromatograph (White *et al.,* 1979).

2.6 Soil Microbial Activities

Soil heterotrophs are keys of nutrients turn over via decomposition. With several biochemical reactions, they convert complex organic materials to simpler organic compounds and inorganic compounds (Singer and Munns, 2006). These processes called mineralization (van Elvas *et al.,* 2007). Soil microorganisms do not only mineralize organic materials and return nutrients to environment, they also use these released nutrients for their growth. This process calls immobilization. When soil microbes die, the immobilized nutrients are substrates to mineralize by living organisms (White, 2006).

Commonly, soil microorganisms decompose plant debris, animal excrete, and death organisms and turn these complex components such as plant cell wall to simpler organic compounds (e.g., glucose, amino acids). Several extracellular enzymes secreted from soil microbes involve in these mineralization processes. In agriculture areas or forest, Carbohydrate of plant debris is most abundant carbon source for soil organisms. Sugar and starch are rapidly decomposed whereas cellulose, hemicelluloses, and lignin, which are plant cell wall components, are more resistant to degraded because their complex structures. Compare to these three plant cell wall components, cellulose is most abundant and easier to degrade (Standing and Killham, 2007).

2.6.1 Cellulase Activity

Cellulose is the polymer of glucose unit with ß-1, 4 linkages (Figure 2.9) (Chernin and Chet, 2003). It is a component of plant cell wall. It may contain up rise to 60 percent of fiber in plant tissue (Killham and Prosser, 2007). The proportion of cellulose and the other cell wall components of wood and rice straw are shown in Table 2.3.

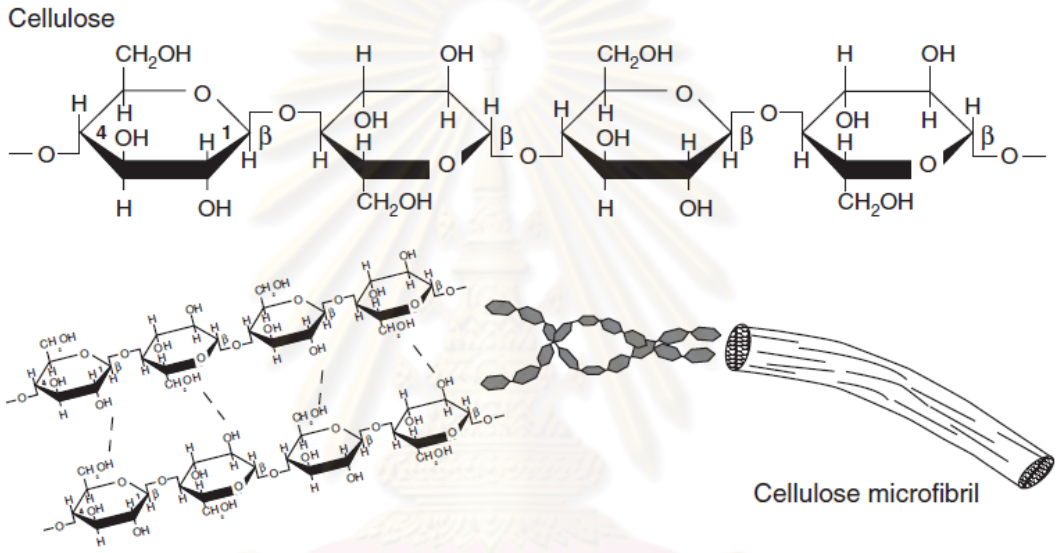


Figure 2.9 Cellulose composition in plant cell wall (Horwath, 2007).

Table 2.3 Constituents of cellulose, hemicellulose, and lignin in wood and rice straw. [Data were modified from Yao *et al.* (2008)]

Constituent (%)	Wood		Rice straw			
	Softwood	Hardwood	Husk	Whole straw	Leaf	Stem
Cellulose	40-45	45-50	35-45	41-57	37-41	24-46
Hemicellulose	25-30	21-36	19-25	33	22-25	24-28
Lignin	26-34	22-30	20	8-19	7-8	4-6

Soil microbial cellulose decomposition processes involve hydrolytic enzymes, cellulase. Cellulase is a group of extracellular enzymes consisting of (1) endo-ß-1,4-glucanase (EC 3.2.1.4) which randomly breaks cellulose chain to smaller polysaccharide or cellobiose, (2) exo-ß-1,4-glucanase (EC 3.2.1.91) splits glucose from the end of cellulose chain or polysaccharide chain, and (3) ß-glucosidase (EC 3.2.1.21) separates glucose from cellobiose or cellodextrins (Deng and Tabatabai, 1994; Sharrock, 1988). The scheme of cellulose degradation is illustrated in Figure 2.10. The completely degradation of cellulose is given glucose as energy source for heterotrophs in soil.

Since cellulose is the most abundant and important carbohydrate source for soil microbial, hence, cellulase activity is a key of carbon turn over process. There are many assays to determine cellulase activity. Manning (1981) determined cellulase activity derived from *Trichoderma viride* by observing carboxymethyl cellulose (CMC) viscosity. Crude cotton, filter paper, and cellophane membrane were also used as substrate in many literatures (for example; Wang *et al.,* 2004; Romero *et al.,* 1999; Semenov *et al.,* 1996). Dyed carbohydrate was used as substrate and the dye release with the release of reducing sugars was colourimetrically analysis in the study of Šnajdr *et al.* (2008).

Determination of released reducing sugar was used in many researches (Allison *et al.,* 2007; Nayak *et al.,* 2007; Chang *et al.,* 2007). Basically, soil sample is incubated with soluble cellulose derivative, usually is CMC, in buffer solution. After incubation, reducing sugars are released, react with solution for colour development using 3,5-Dinitrosalicylic acid (DNS) reagent (Wood and Bhat, 1988) or Somogyi-Nelson regents (Deng and Tabatabai, 1994), and then determined colourimetrically.

Schinner and von Mersi (1990) developed the Prussian blue method to measure reducing sugars. This method is more sensitive than Somogyi-Nelson method (Deng and Tabatabai, 1995). Since natural cellulose is insoluble, soluble CMC, the derivative of cellulose (the structure was shown in Figure 2.11), is used as substrate to measure cellulase activity. Soil sample is incubated with 0.7% w/w CMC in acetate buffer pH 5.5 at 50°C for 24 hours. Released reducing sugars then reduce potassium hexacyanoferrate (III) in an alkaline solution. Reduced potassium hexacyanoferrate (II) later reacted with ferric ammonium sulfate in an acidic solution to form ferric hexacyanoferrate (II) (Prussian blue), which was measured by

spectrophotometer at 690 nm. The diagram of Prussian blue reaction was shown in Figure 2.12.

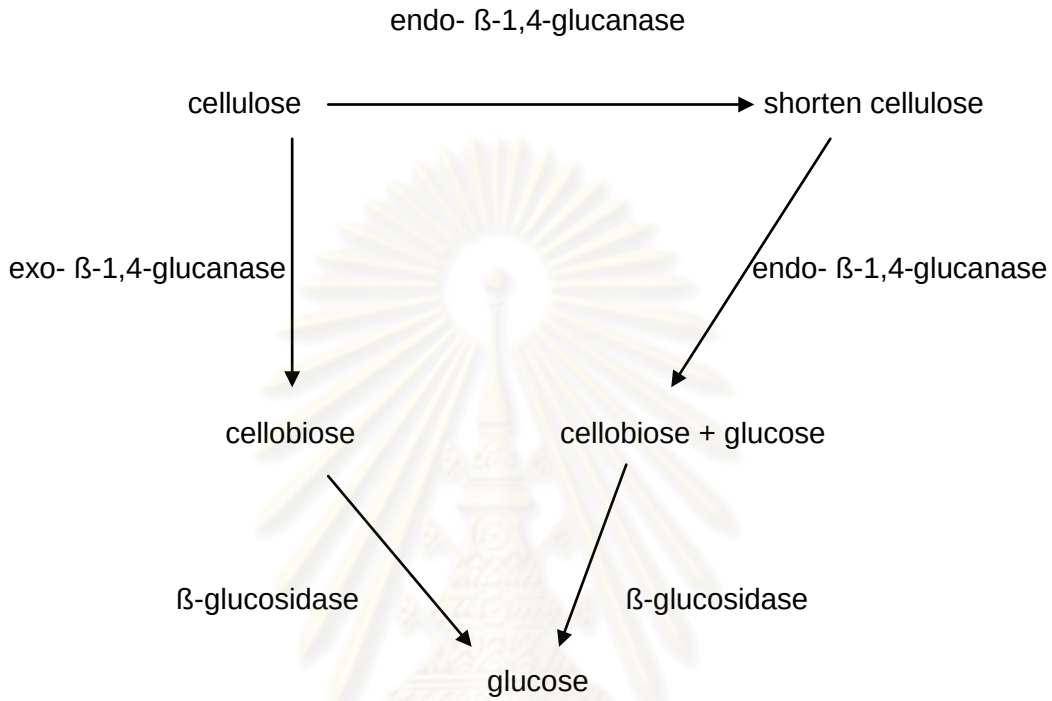


Figure 2.10 Scheme of cellulose degradation (modified from Sharrock, 1988)

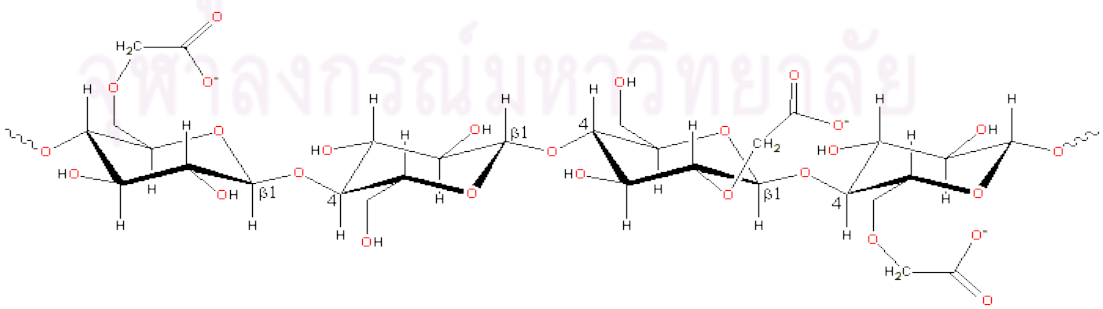


Figure 2.11 Carboxymethyl cellulose (CMC) (Chaplin, 2009: online)

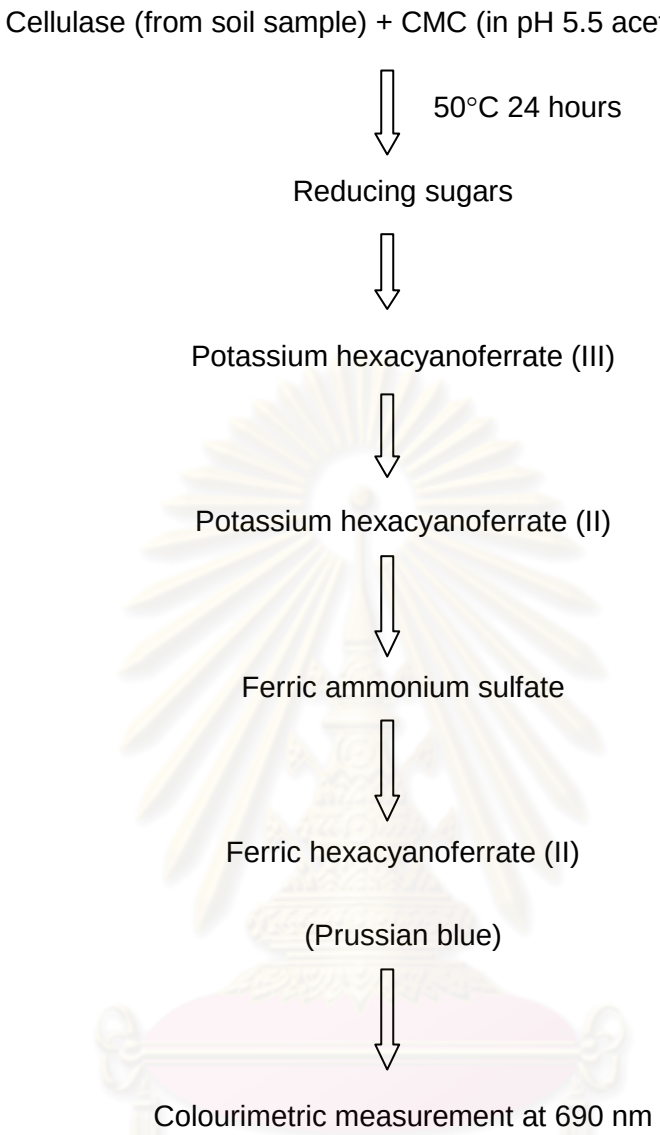


Figure 2.12 The diagram of Prussian blue reaction

2.7 Soil Respiration

As mentioned earlier, soil microbes play a vital role on carbon mineralization. Degradation of organic matter under aerobic condition release CO_2, water and energy. This process called respiration. Degradation of organic matter also happen under low oxygen concentration or anaerobic condition, called fermentation, also release CO_2, acid or alcohol, and energy (Singer and Munns, 2006). Carbon dioxide also release to soil environment via plant root respiration and soil microorganisms

biochemical by-product (such as TCA cycle). All carbon dioxide release by organism activities is soil respiration (Hopkins, 2008; Luo and Zhou, 2006). Therefore, measurement of soil respiration is implied to soil microorganism activities (Öhlinger, 1996).

Determination of CO_2 evolution from soil respiration could be performed in opened chamber or closed chamber (Luo and Zhou, 2006; Hopkins, 2008). In closed chamber approaches, CO_2 accumulation in the headspace could be detected by using an infrared gas analysis or gas chromatograph (Beck, 1996; Jensen *et al.,* 1996; Hashimoto, 2002) or absorb occur CO_2 in alkaline solution (Öhlinger, 1996; Hopkins, 2008; Hopkins *et al.,* 2008).

The alkaline trapping method is an easy method. It is inexpensive and does not need complicated equipment (Hopkins, 2008). Soil sample is incubated with alkaline solution (e.g., NaOH) in a close container. Carbon dioxide release by soil microbial metabolisms and respiration is trapped in alkaline solution and form sodium carbonate (Na_2CO_3).

$$CO_2 + 2NaOH \longrightarrow Na_2CO_3 + H_2O$$

Carbon dioxide is absorbed as Na_2CO_3 then precipitated by add barium chloride ($BaCl_2$) which turn to barium carbonate ($BaCO_3$) and sodium chloride (NaCl). The total CO_2 produced is calculate by back titrate the excess NaOH with HCl using phenolphthalein as an indicator (Öhlinger, 1996). This procedure could be used to determine basal soil respiration or substrate-induced respiration (SIR) (Beck, Öhlinger, and Baumgarten, 1996).

$$Na_2CO_3 + BaCl_2 \longrightarrow BaCO_3 + NaCl$$

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

2.8 Effects of Environmental Factor on Soil Microbial Community and Their Activities.

Soil microorganisms live in soil pores, many of them attach themselves to soil particles, and many of them live in water film surround soil particles. Hence, soil microbial community and their activities are affected by surrounding soil environment.

Soil physical and chemical properties are important factors that regulate community pattern and activity rate.

2.8.1 Nutrient

Nutrient is the most important factor for all organisms. Soil microorganisms could be classified by their energy source (prototroph, chemoheterotroph, or chemoautotroph), carbon source (CO_2 or organic compound), and electron acceptor (Standing and Killham, 2007). For heterotrophic microorganisms, they mineralize general organic matter to reach their carbon source and nutrient elements which are the keys of their growth and activities. C/N ratio is an important key of mineralization and immobilization. If C/N ratio is > 20, immobilization is more occur, where as mineralization is good if C/N ratio is < 20 (White, 2006). In agriculture area, fertilizers are applied to increase productivity. Not only plant takes advantage from these nutrients supplements, but soil microbes are also take benefit from the available elements.

2.8.2 Soil Aeration and Soil Moisture

Soil aeration controls soil microbial communities and their activities. Aerobic microorganisms definitely need oxygen and use O_2 as terminal electron acceptor. Facultative anaerobes can grow in both oxic and anoxic soil, while obligated anaerobes grow only in absence of oxygen (Hartei, 2005). Bacteria and actinomycetes are found in both oxygenated soil and anoxic soil, while fungi, except for yeast, are obligate needs oxygen for their living and activities (Maier and Pepper, 2009).

Soil moisture is important to soil microorganisms. Available nutrients are in soil solution. Water surround soil particle is habitat for many soil microorganisms and help motile microorganisms for their movement. Bacteria and soil algae are not tolerant in low available water, while fungi and actinomycetes are more tolerant (Davet, 2004).

Soil aeration has relation to soil moisture. Both air and water fill in soil pores, therefore, oxygen decrease when soil moisture increase. Oxygen level controls oxidation and reduction process in soil. Respiration by aerobes turns to fermentation by fermenting bacteria which change polysaccharide to alcohol and organic acid and finally convert to CO_2 and methane (CH_4) by methanotrophs.

Reductions of inorganic compounds occur in anoxic soil. When oxygen is run out, NO_3^- is the first inorganic compound that facultative anaerobes and anaerobes use as their terminal electron reception. After NO_3^- is depleted, Mn (IV), Fe^{3+}, SO_4^{2-}, and CO_2 are respectively used by redox potential as the terminal electron acceptor. Nitrate reducer, iron reducer, and sulfate reducer have vital roles on these processes (Singer and Munns, 2006; Kyuma, 2004).

In paddy soil, water is the major factor that regulates soil microbial community and their activities via oxygen level in soil. When water is drained off the rice field, soil is oxygenated. Aerobes and facultative anaerobes are predominant microorganisms in oxic soil. Their main activity is decomposition and the end product of this process and their respiration is CO_2. When the soil is waterlogged, oxygen is depleted rapidly by aerobes consumption and oxidation reaction. Without oxygen, the predominant microorganisms are anaerobes and facultative anaerobes.

Studies by several researchers on paddy soil microbial communities and decomposition activity during cultivation periods were set. Bossio and Scow (1998) indicated that aerobic bacteria and fungi were reduced under flooded condition while Gram-positive bacteria were increased and actinomycetes had no effect of flooding. Nakamura *et al.* (2003) studied microbial response on the decomposition of rice straw using PLFA pattern under upland and flooded condition. The studies showed that Gram-negative bacteria and fungi were predominant under upland condition while Gram-positive bacteria and anaerobic Gram-negative bacteria were predominant under flooded condition. Kimura *et al.* (2001) reported Gram-positive bacteria as major decomposers of rice straw incorporated into a paddy soil microcosm under submerged conditions from PLFA composition.

2.8.3 Soil pH

Most of soil microorganisms and plants prefer pH 6-7 because the availability of most soil nutrients is best in this pH range (Hartei, 2005). The chart of nutrients availability and related soil pH is shown in Figure 2.13. However, soil microorganisms can survive in a broad range of optimum soil pH. Bacteria and actinomycetes are predominant in neutral soil pH (Jjemba, 2004; Davet, 2004), whereas fungi have capability to live in more wide range (pH 3.5-8.5) (Davet, 2004). Soil pH is another criterion to classified soil microorganisms. Acidophilic microorganisms such as *Acetobacter*, prefer low pH (pH 1-4), while neutrophilic

microorganisms are more like pH 5-8, whereas alkaliphilic microorganisms (*Bacillus sp.*, for example) are favorite to pH 9-11 (Standing and Killham, 2007).

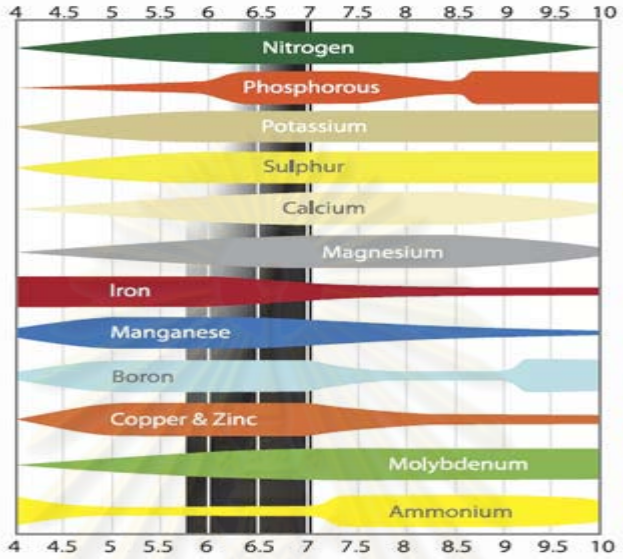


Figure 2.13 Nutrients availability in varies soil pH (Stealth Growth, LLC, 2010: online)

Not only soil pH is factor that control soil microbial community, but also regulate their activities in soil. Soil pH regulates chemical reactions and a multiplicity of enzymes in microorganisms (Luo and Zhou, 2006). Increasing of Gram-negative and Gram-positive bacteria when soil pH was increased after wood ash and lime amendment in forest soil was investigated (Frostegård *et al.*, 1993a). Basal soil respiration and SIR are tend to increase when soil pH is increasing (Aciego Pietri and Brookes, 2008; Demoling *et al.*, 2008; Enwall *et al.,* 2007). The reports of Bååth and Anderson (2003) and Rousk, Brookes, and Bååth (2010) were similar and confirmed positive in correlation of soil pH to SIR-biomass C, total PLFA and soil respiration.

2.8.4 Soil Temperature

Soil microorganism communities and activities are also regulated by soil temperature. Classification of soil microorganisms according to temperature preference divide to three subgroup; phychrophile grow in low temperature range in -5 – 20 °C, the optimum growth temperature for mesophile is 37°C and their prefer

temperature range in 15 – 45°C, and thermophile survive in high temperature environment from 40- 95°C (Standing and Killham, 2007).

CHAPTER III

METHODOLOGY

3.1 Sample Site Information

The rice field which is the sample site on this thesis belongs to Mr. Prachon Petch-chong, located on number 49 Moo 8 Tumbon Ban-rom, Amphur Tha-rua, Phra Nakhon Si Ayutthaya Province (14°33'54.59"N 100°42'3.61"E) (Figure 3.1(a) and (b)). This sample site has been cultured lowland rice more than 50 years. There are two times a year wet-seeded rice plantations. Chemical fertilizer has been applied on this site. First, 16-20-0 formula at 156.25 kg·ha^{-1} (25 kg·rai^{-1}) is applied on 20-30 days after seeding and then 46-0-0 formula at 93.75 kg·ha^{-1} (15 kg·rai^{-1}) is applied 20 days later or on rice tillering period. These chemical fertilizers amendment rates are recommended by Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. There is no organic fertilizer or compost manure applied on the sample site and no other crop cultures after rice harvesting period.

3.2 Soil Sampling and Preparation

Soil samples were collected before rice harvesting period. Water was drained out of the rice field but soil was still moist (Figure 3.2). The surface soil (0-15 cm) was simple random sampling with a shovel (Land Development Department, 2008). The samples were mixed then packed in polypropylene bags for transported to laboratory on the sampling day. Soil samples were then mixed again and plant residues were removed as much as possible. The samples were pre-incubated for 7 days at 30°C with moist sheet covered on in order to maintained soil moisture. A little amount of soil was taken to analyzed water holding capacity (WHC) described by Öhlinger (1996) and soil dry mass at 105°C overnight on Day 0 and Day 7 of pre-incubation period.

(a)

(b)

Figure 3.1 Sample site from aerial photograph (a) (Google Earth, 2008) and actual picture on sampling date (b).

Figure 3.2 Soil sample

After 7 days of pre-incubation, samples were weighted and divided into 2 parts equally, control (C) and fertilizer amendment (FA) sets. The soil sample of FA set was absolutely mixed with mixed chemical fertilizer 16-20-0 grade at 156.25 $kg \cdot ha^{-1}$ (25 $kg \cdot rai^{-1}$) in solution (see **APPENDIX A** for solution preparation). Mixed chemical fertilizer solution was prepared in sterilized distilled water and filtered through sterilized Millipore. The sample of control set was mixed with sterilized distilled water at the same amount of the mixed chemical fertilizer solution to controlled soil moisture content at 55% (w/w).

All glasswares were washed with non-phosphate detergent, rinsed with tap water twice and rinsed with distilled water before oven dried at 105°C overnight. Glasswares used on soil biological property experiment were acid washed, rinsed with distilled water, and autoclaved at 121°C for 20 minutes.

3.3 Soil Physico-chemical Properties Analysis

In this thesis, not only the effect of chemical fertilizer amendment on paddy soil biological and biochemical properties were interested, but also the influence of chemical fertilizer amendment on soil physical and chemical properties. The soil preparation was as same as previously described. The microcosms were set by weighing control soil and FA soil into each sterilized glass beakers. The initial weight

was recorded and weighted everyday in order to maintain soil moisture with sterilized distilled water. The beakers were covered with aluminum foil with small holes puncture for air circulation then incubated in the dark at 30°C. Soil samples were collected on Day 0, 18, and 49. The analyzed parameters performed after Soil Analysis Division (2001) were soil pH, organic carbon (OC) and organic matter (OM), and available phosphorus. Total nitrogen and cation exchange capacity (CEC) were analyzed as described by Ariyakanon (2007). Soil texture analysis was measured as described by Tan (2005). The equipment and solution preparation are shown in **APPENDIX B**.

3.4 Soil Microbial Community Patterns and Cellulase Activity.

3.4.1 Microcosm

To explore the effects of chemical fertilizer amendment on soil microbial communities through the change of phospholipid fatty acid patterns and cellulase activities, the 250 ml Erlenmeyer flasks were set as the microcosms. The 60 Erlenmeyer flasks (30 flaks for control set and 30 flaks for FA set) were sterilized for 20 minutes at 121°C with an autoclave. Sixty grams of the soil sample was weighted in each flask for determined phospholipid fatty acid and cellulase activity. All flaks were closed with sterilized cotton as shown in Figure 3.3 and incubated in the dark at 30°C and weighed every day in case there was some weight loss from evaporation in the microcosm.

Figure 3.3 Microcosm set in sterilized flasks for the experiments.

Sterilized distilled water was then dropped to maintained soil moisture content. The experiments were set on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49. Three replicates of microcosms of control set and FA set were randomly selected on the experiment day. Twenty grams of soil sample in each microcosm were determined for cellulase activities immediately. The rest of soil samples were stored at -20°C to determine phospholipid fatty acid on later day.

3.4.2 Cellulase Activities

This method was described by von Mersi and Schinner (1996). In this experiment carboxymethyl sodium salt (CMC) (Fluka 21 900) was used as substrate to determined cellulase activity. Ten grams of sample soil was incubated with 15 ml substrate solution (0.7% w/v CMC in 2 M acetate buffer, pH 5.5) and 15 ml 2 M acetate buffer (pH 5.5) for 24 hours at 50°C. Reducing sugars released during incubation period reduced potassium hexacyanoferrate (III) in an alkaline solution. Reduced potassium hexacyanoferrate (II) later reacted with ferric ammonium sulfate in an acidic solution to form ferric hexacyanoferrate (II) (Prussian blue), which was measured colourimetrically. Reagents and preparation are shown in **APPENDIX B**.

Two sets of 10 g of fresh soil in each microcosm were weighed then added to 150 ml beaker. Fifteen milliliters of substrate solution and 15 ml of acetate

buffer pH 5.5 solutions were added into soil beaker as the treatment beaker. In another beaker only 15 ml of acetate buffer pH 5.5 was added as the control. Soil and the solutions were mixed, covered the beaker with aluminum foil before incubated at 50°C for 24 hours. Fifteen milliliters of substrate solution was added into the control beaker and mixed briefly after 24 hours incubation period. Soil mixture was then filtered through Whatman® No. 42 filter paper to collect the filtrate. 0.5 ml of the filtrate was transferred to a new 50 ml Erlenmeyer flask which contained 19.5 ml of distilled water.

The flask was then shaken briefly to mix the solution. A milliliter of diluted filtrate was transferred to a test tube before added 1 ml of reagent A solution and 1 ml of reagent B solution. The tube was closed and mixed with a vortex shaker, followed by boiled in boil water for 15 minutes. After cooled down in water bath at room temperature, 5 ml of reagent C was added and mixed with a vortex shaker. The test tube was stood for 60 minutes for colour development. The Prussian blue solution was then transferred to a cuvette in order to measure the absorbance at 690 nanometer (nm) wavelengths within 30 minutes and compared with glucose standard curve.

To prepare the glucose standard curve, 0 (reagent blank), 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of working standard were pipette to each test tube and adjusted to 1 ml with distilled water and treat like soil mixture filtrate. Prepare glucose standard curve with glucose concentration on the X axis against the absorbance at 690 nm on the Y axis. The colour development of glucose standard is shown in Figure 3.4. Conversion the absorbance at 690 nm of sample to µg glucose equivalents (GE) was shown in **APPENDIX B**.

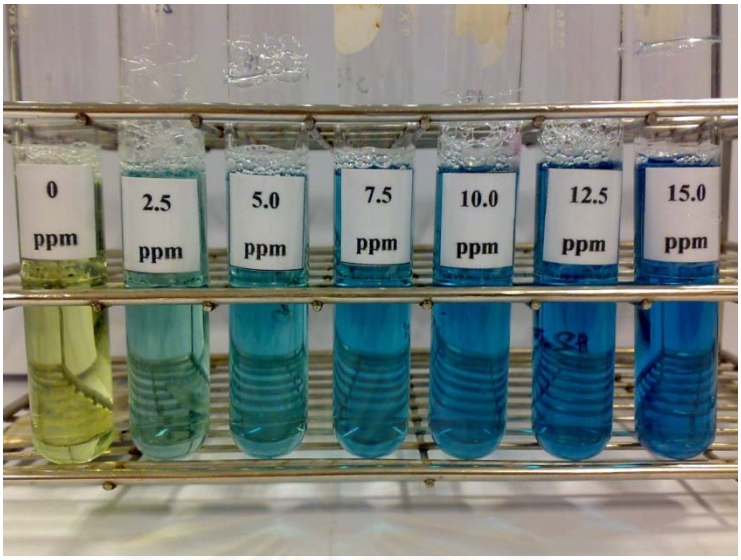


Figure 3.4 The colour development of glucose standard after the Prussian blue reaction.

3.4.3 Phospholipid Fatty Acid Analysis

In this thesis, PLFAs were used to observe the effects of chemical fertilizer amendment on soil microbial communities of paddy soil. The change of PLFA patterns mean there are changes on soil microbial communities. In this experiment part, PLFA patterns extracted from chemical fertilizer amendment paddy soil on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49 were compared against PLFA patterns extracted from control. This method was developed by Bligh and Dyer (1959) and modified by Watcharamul (2005). The equipments and reagents preparation were shown in **APPENDIX B**.

Lipid extraction

Two grams of fresh soil was weighted into a Duran bottle followed by adding 16 ml of chloroform : methanol : citrate buffer solution (1:2:0.8 v/v/v) and shaken for 2 hours. Another 10.5 ml chloroform and 10.5 ml sterilized distilled water were added, and then shook the bottle overnight. Next, the upper phase was discarded before filter the lower phase through the Whatman® No.42 filter paper which previously rinsed with chloroform : methanol (2:1 v/v) solution. Collected filtrate was filled in a test tube with screw cap and dried under nitrogen stream at 37°C.

Solid phase extraction

Dried lipid filtrate was redissolved with 0.5 ml chloroform, after that it was transferred into an activated silicic column (0.5 g silicic acid 200-400 mesh, Sigma). The apparatus was shown in Figure 3.5. Ten milliliters of chloroform was added, neutral lipid was contained in this fraction. This fraction could discard or save for further analysis. Next, the column was rinsed with 10 ml acetone, this fraction contains glycolipids which could also discard or save for further analysis. Finally, 10 ml methanol was pulled into the column and phospholipid was separated. This fraction was collected in a test tube with screw cap before drying under nitrogen stream at 37°C.

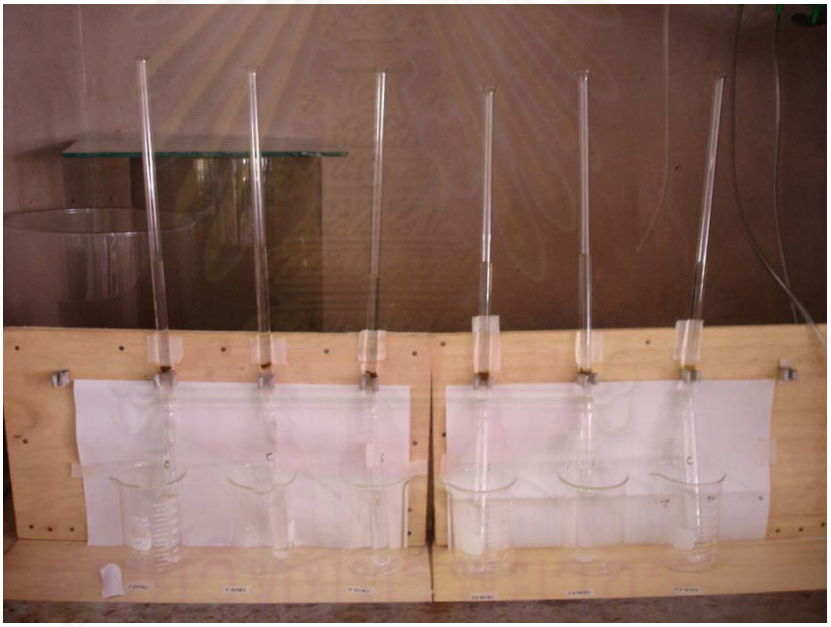


Figure 3.5 Lipid extraction columns

Mild alkaline methanolysis

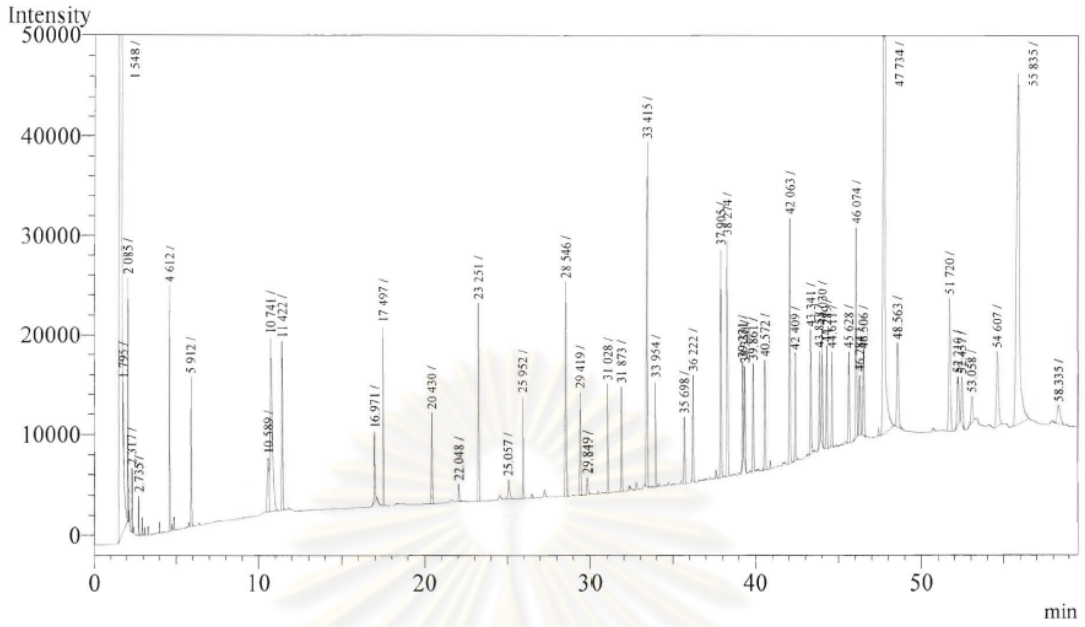
The phospholipid fraction was resuspended with 1 ml methanol and 1 ml 30% KOH (w/v) and incubated at 75°C overnight. After cooling, pH of the suspension was adjusted to pH 1 with 10% HCl:H_2O (v/v) before added 1 ml diethyl ether. The suspension was mixed and removed the upper phase to a new test tube

with screw cap, and this step was repeated twice. Combined upper phase was dried under nitrogen stream at 37°C followed by adding 1 ml dichloromethane, 1 ml phase transfer catalysis solution (0.1 M tetrabutylammonium hydrogen sulphate in 0.2 M NaOH), and 25 μl iodomethane. The test tube was then shaken for 30 minutes at room temperature. The lower phase was collected after shaken solution was left for phase separation. This fraction was dried under nitrogen stream at 37°C This final fraction was fatty acid methyl esters (FAMEs) and was preserved in 0.5 hexane and store at -20°C for further GC detection.

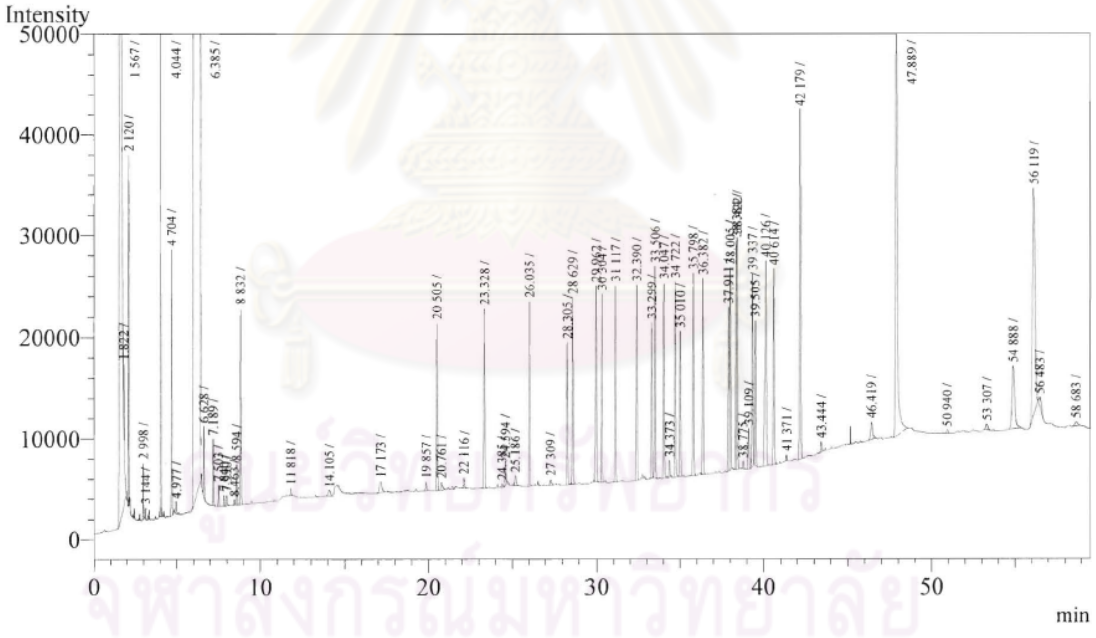
Gas chromatograph detection

FAMEs solution was dried under nitrogen stream at 37°C and redissolved in 200 μl hexane before analyzed by gas chromatography (Shimazu, Model GC-2010) on a 30 m BP20 capillary column. Helium was used as the carrier gas and peaks were detected by flame ionization. Peaks were identified reference to the Supelco® 37 component FAME mix and bacterial acid methyl esters (BAMEs) mix (Figure 3.6(a) and (b), respectively). Nomenclature is based on the ratio of number of carbon atoms:number of double bonds in the fatty acid, followed by the position of the double bond from the methyl end of the molecule. *Cis*- and *trans*- configurations are indicated by c and t, respectively; prefixes a and i indicate *anteiso*- and *iso*-branching; cy refers to cyclopropane fatty acids (Frostegård *et al*., 1993a)

The fatty acids 12:0, 13:0, 14:0, 15:0. 16:0, 17:0, and 18:0 were chosen to represent saturated fatty acid (SatFA). i15:0, a15:0, 15:1, i16:0, i17:0 and 17:1 were represented Gram-positive (G+) and 16:1, cy17:0, 18:1ω9 and 20:1ω9 were represented Gram-negative (G-). 18:2ω6c, 18:3ω6, and 18:3ω3 were indicated to fungi. 20:2, 20:3ω6, 20:3ω3, 20:4ω6, 20:5ω3, and 22:6ω3 were implied to protozoa and microeukaryotes (Fierer *et al*., 2003; Waldrop *et al*., 2000; Marschner, 2007; Bossio and Scow, 1998; Zelles, 1997; Hedrick *et al*., 2005; and Frostegård *et al.*, 1993a)



(a)



(b)

Figure 3.6 Standard reference peaks with retention time labels on the Supelco® 37 component FAME mix (a), and bacterial acid methyl esters (BAMEs) mix (b).

3.5 Soil Respiration

Soil respiration is created by soil microorganisms. CO_2 is a product of organic decomposition; therefore, measuring of CO_2 or soil respiration could be implied to the activities of soil microbes. This experiment part was followed by Öhlinger (1996). The concept of this method is CO_2 from soil respiration is trapped in alkaline solution which is 0.5 N sodium hydroxide (NaOH) in this experiment. The remaining of NaOH is then titrated with 0.1M hydrochloric (HCl) with a few drops of phenolphthalein as an indicator to the end point and back calculated to CO_2 from soil respiration. The equipments and reagents preparation were detailed in **APPENDIX B**.

3.5.1 Microcosm

To determined soil respiration, twelve of 50 ml sterilized glass beakers were set as microcosms. Twenty-five grams (fresh weight) of control soil was put into 5 sterilized glass beakers and 25 g (fresh weight) of FA soil was put into another 5 sterilized glass beakers. Two replicates of blank set were set up with 50 ml sterilized glass beakers which left without soil. Each soil beaker was transferred into a chamber which had a 20 ml of 0.05N NaOH inside. The lid was closed firmly and sealed with parafilm M (American National Can, Greenwich, CT 06836, USA) as illustrated in Figure 3.7. All chambers were incubated in the dark at 30°C.

Detection of CO_2 evolution was performed with titration. A beaker of 20 ml of 0.05 N NaOH was taken out of the chamber and following by add 2 ml of 0.5 M $BaCl_2$ into the beaker. Another few drops of phenolphthalein were dropped into the NaOH beaker and shaken briefly before titrated with 0.1 M HCl. The volume of 0.1 M HCl at the end point was noted when the solution in the beaker turned from pink to colourless as S value. These methods were repeated to the blank and noted the volume of 0.1 M HCl at the end point as C value. Soil respiration was express as mg $CO_2 \cdot g^{-1}dm \cdot 24h^{-1}$ (see the calculation in **APPENDIX B**).

Soil respiration was determined daily for 50 days. A fresh 0.05 N NaOH beaker was replaced in each chamber every day. Soil moisture was controlled by weighted ever day. In case there was some weight loss from evaporation in a soil beaker, sterilized distilled water was dropped to maintain soil weight.

Figure 3.7 A closed chamber for CO_2 trapped contains a sample soil microcosm and a beaker of 0.05 N NaOH.

3.6 Statistical Analysis

All data were arranged in Microsoft Excel 2007 format. The soil physical and chemical properties results were compared by t-test and one-way ANOVA (with a 95% confidence level) in SPSS version 17 (SPSS Inc., USA). Simple and multiple correlations between soil parameters were also analyzed using SPSS version 17 to establish possible statistical relationship.

Cellulase activity was expressed as mean of $\mu g\ GE \cdot g^{-1}dm \cdot 24h^{-1} \pm$ standard error (SE). Soil respirations were presented as CO_2 accumulation expressed as mg $CO_2 \cdot g^{-1}dm$ and were compared by t-test and one-way ANOVA. The significant of mean difference was set at the 0.05 level.

For PLFA analysis, the individual chosen signature fatty acids were express as %peak area. Log10 of %peak area were subject to principle component analysis (PCA) (SPSS version 17) to elucidate the major variation and co-variation patterns.

CHAPTER IV

RESULTS

4.1 Soil Physico-chemical Properties Analysis

Soil samples were taken on 30th November, 2008. It was pre-harvest period; therefore, water was drained out of the rice field. Ambient temperature was 22.8°C and soil temperature was 21°C. Soil pH was about pH 5 determined by soil pH tester and universal pH indicator paper. Soil samples were moist. Top soil surface was brown and there were orange mottles. 0-15 cm soil was taken as a sample. Soil sample colour was gray with some orange mottles. Soil texture of sample was analyzed by hydrometer method as described by Tan (2005). Soil sample has 28.66% clay, 42.43% silt, and 28.92% sand which is clay loam texture when read soil texture triangle. Change in some soil physical and chemical properties after chemical fertilizer amendment (FA) on paddy soil were detected against unamended soil (control; C) on Day 0, 18, and 49 of experiment day.

Soil pH was determined in 3 solutions, water, 1 N KCl, and 0.01 M $CaCl_2$. The trend of soil pH was significantly decreased in C and FA sets ($p < .05$). On Day 0, FA soil pH was significant higher than C when detected with H_2O and 0.01 M $CaCl_2$ while it was equal when detected with 1 N KCl. On Day 18 soil pH was decrease, soil pH of C and FA were even in 1 N KCl, and 0.01 M $CaCl_2$ solution, when FA soil pH was lower than C. On Day 49, FA soil pH was significant lower than C in every solution. The data was shown in Table 4.1.

Organic carbon and organic matter were slightly decreased after 49 days of experiment in C and FA. There was no significant difference between two experiment groups. While available phosphorus of C was less than FA and the trend was decreasing constantly, available phosphorus of FA was decline on Day 18 then rise on Day 49. Total nitrogen of C and FA were no significant difference on entire of experiment period. CEC of C and FA were constant on Day 0 and 18. On Day 49 of the experiment, CEC of C was decline while FA was rise. There was significant

difference between C and FA only on Day 0 at the .05 level (p = .044). The data was shown in Table 4.2.

4.2 Cellulase Activity

Cellulase activity was determined using carboxymethyl cellulose as substrate and measured reducing sugar occurrence (expressed as μg glucose equivalent) with spectrophotometer (von Mersi and Schinner, 1996) The experiments were performed on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49. Compared to Day 0, cellulase activity of control group increased on Day 1 and 3, and then slightly decreased on Day 7 and 14. The highest cellulase activity occurred on Day 21 (194.46±12.44 μg $GE \cdot g^{-1}$ $dm \cdot 24h^{-1}$). After that the activity declined continuously from Day 28 to Day 42, then ascended again on Day 49. Cellulase activity of FA, also compared to Day 0, increased on Day 1. The activity was steady until Day 7 and slightly increased on Day 14. Like the cellulase activity of C, the highest cellulase activity occurred on Day 21 and slightly drops on Day 28 (170.21±19.21 and 168.71±10.44 μg $GE \cdot g^{-1} dm \cdot 24h^{-1}$, respectively). The activity then decreased on Day 35 and 42 before increased again on Day 49. Cellulase activity of FA tend to higher than C. However, there was no significant difference between C and FA. The data was shown in Table 4.3 and Figure 4.1.

Table 4.1 Soil pH of control (C) and fertilizer amendment (FA) sets. All data are expressed as mean (n = 3) ± standard error (S.E.). The star (*) means there is significant difference between groups at the 0.05 level.

Day	(soil : H_2O (1:2.5))		soil : 1N KCl (1:2.5)		soil :0.01M $CaCl_2$ (1:2.5)	
	C	FA	C	FA	C	FA
0	5.25±0.02	5.38±0.01*	4.12±0.00	4.12±0.00	4.54±0.00	4.60±0.00*
18	5.18±0.00	4.96±0.01*	4.07±0.00	4.07±0.00	4.48±0.00	4.48±0.00
49	4.96±0.01	4.78±0.00*	4.04±0.00	3.99±0.01*	4.39±0.00	4.33±0.00*

Table 4.2 Some soil physico-chemical properties of control (C) and fertilizer amendment (FA) sets. All data are expressed as mean (n = 3) ± standard error (S.E.) except total nitrogen data that n = 5. The star (*) means there is significant difference between groups at the 0.05 level.

Day	% Organic matter		% Organic carbon		Available P (Bray II) (ppm)		C/N		Total Nitrogen (%N)[b]		CEC (cmol/kg)	
	C	FA	C	FA	C	FA	C	FA	C	FA	C	FA
0	2.26±0.02	2.33±0.05	1.31±0.01	1.35±0.03	13.48±0.27	14.31±0.18	9.02	10.54	0.145±0.012	0.128±0.002	16.253±2.042	10.214±0.384*
18	2.29±0.03	2.29±0.01	1.33±0.02	1.33±0.01	12.11±0.47	12.29±0.34	8.10	11.22	0.164±0.027	0.119±0.003	16.854±6.035	10.185±0.179
49	2.19±0.02	2.03±0.18	1.27±0.01	1.18±0.11	11.92±0.64	14.41±0.10	8.53	8.59	0.149±0.009	0.137±0.003	12.218±0.228	12.099±0.048

Table 4.3 Cellulase activity express as mean±SE μg GE·g^{-1} dm·24h^{-1} (n=3)

Day	Control	Fertilizer amendment
0	88.15±7.58	99.00±15.07
1	101.96±13.11	116.17±19.23
3	120.76±8.26	118.57±7.27
7	114.18±2.61	118.17±12.54
14	107.02±7.08	126.00±10.91
21	194.46±12.44	170.21±19.21
28	147.19±6.23	168.71±10.44
35	128.27±20.54	113.94±7.80
42	116.17±13.96	133.06±22.92
49	159.54±9.08	153.13±9.66

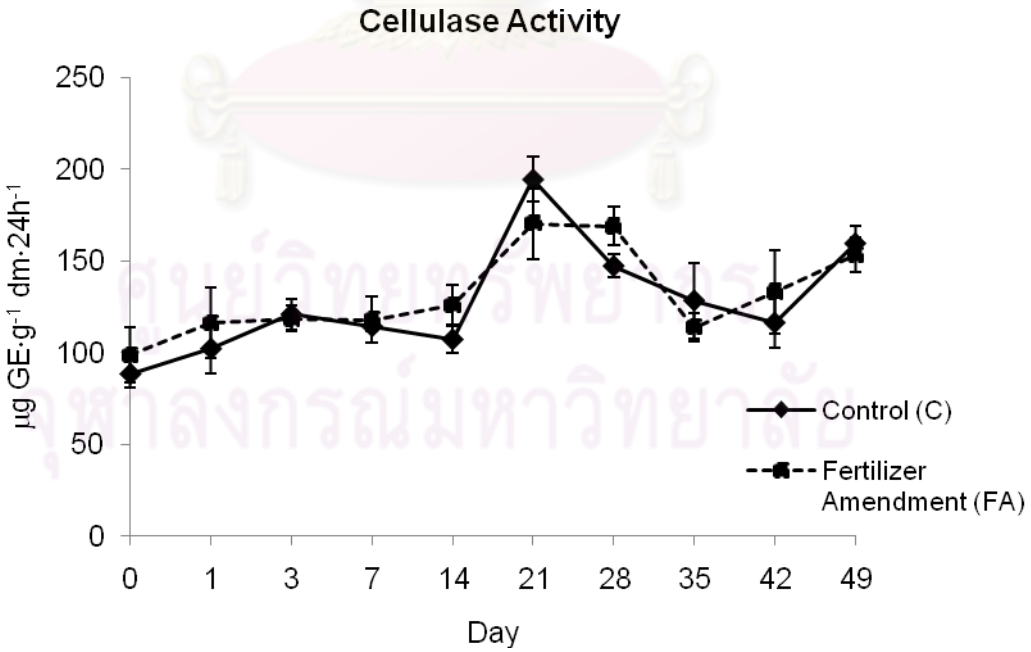
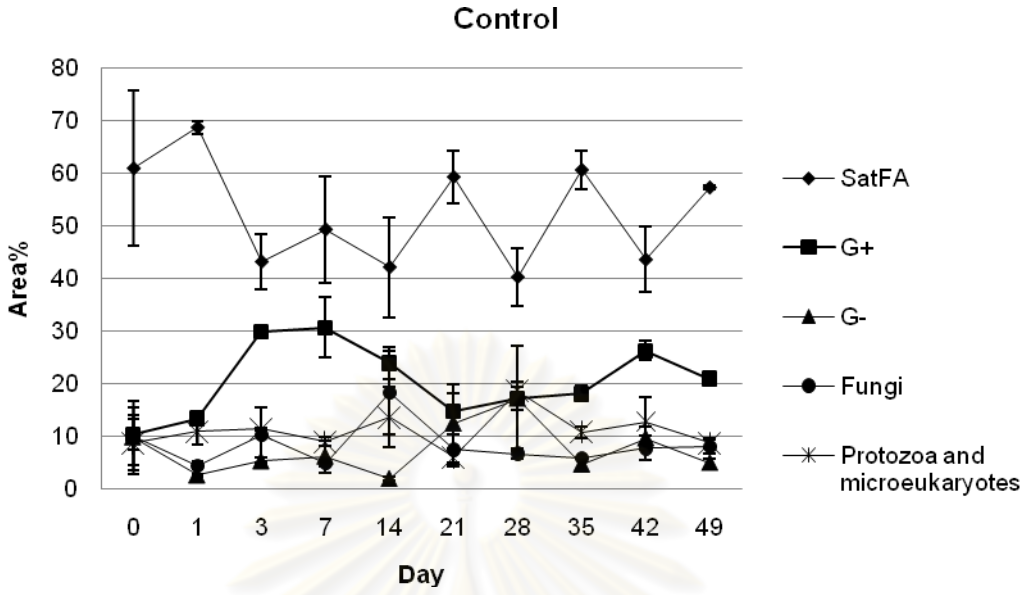


Figure 4.1 Cellulase activity of control (C) and fertilizer amendment (FA) paddy soil (mean±SE ; n=3).

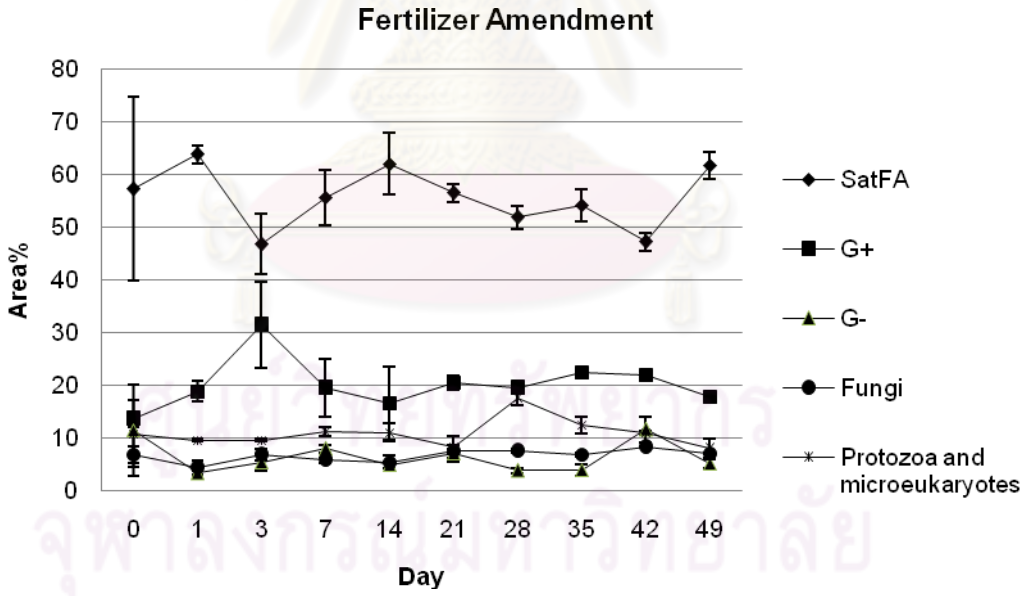
4.3 Phospholipid Fatty Acid Analysis

To study soil microbial community structure in paddy soil and the effect of chemical fertilizer amendment, PLFA was used in this Thesis. After fatty acid methyl esters (FAMEs) were analyzed on GC, signature fatty acids were identified by comparing retention time with Supelco® 37 component FAME mix and bacterial acid methyl esters (BAMEs) mix. The peak area of selected fatty acids were calculated to area% Saturated fatty acids (SatFA) were represented by 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, and 18:0. Since SatFA were found in every organism, they are not represented signature fatty acids (Zelles, 1997). Fatty acids i15:0, a15:0, 15:1, i16:0, i17:0, and 17:1 were Gram-positive bacteria's (G+) signature fatty acids. Fatty acids 16:1, cy17:0, 18:1ω9, and 20:1ω9 were chosen to represent Gram-negative bacteria (G-), and 18:2ω6c, 18:3ω6, and 18:3ω3 were presented to fungi. Protozoa and microeukaryotes were represented by, 20:2, 20:3ω6, 20:3ω3, 20:4ω6, 20:5ω3, and 22:6ω3.

Comparison of FA's total PLFA to C's, the highest area% belonged to SatFA in both groups everyday as shown in Figure 4.2(a) and (b). Their area% was range from 40.3±5.4% - 68.7±1.1% and 46.8±5.8% - 63.9±1.8% in C and FA, respectively, as showed in Figure 4.3. Their patterns were interval through the experiment period. There was no significant different between C's and FA's total SatFA.



(a)



(b)

Figure 4.2 Total area% PLFA of SatFA and groups of microorganisms (a) control set and (b) fertilizer amendment set. Data were presented by mean (n =3) and error bar indicate standard error (SE).

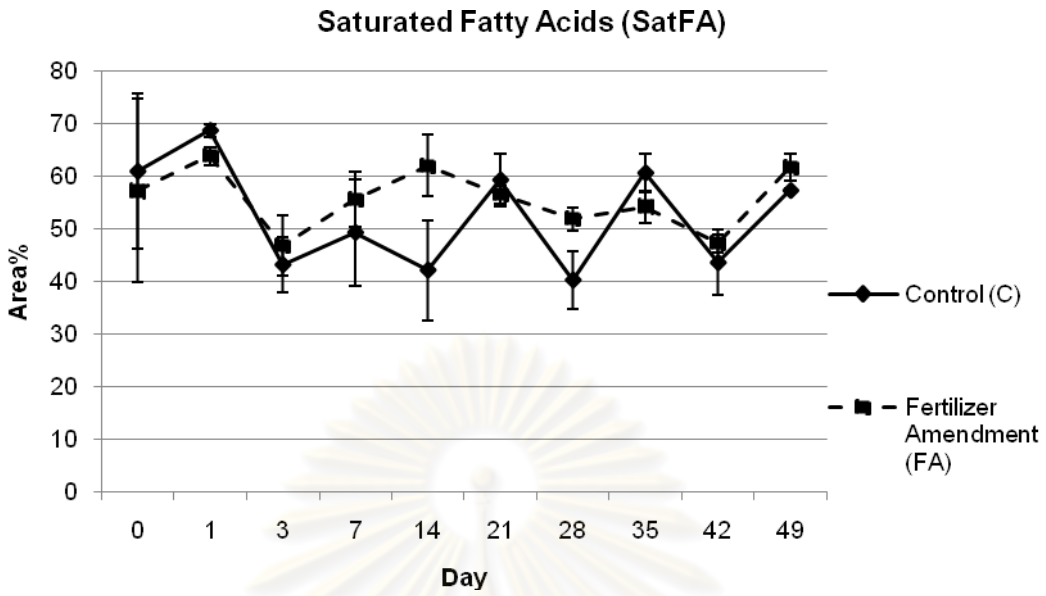


Figure 4.3 The area% of saturated fatty acids (SatFA) of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3) and error bar indicate standard error (SE).

With highly area% compared to the other group of microorganisms, Gram-positive bacteria was a dominant group of soil microbial community in both C and FA (see Figure 4.2). Area% of G+ was varied in C but more steady in FA (Figure 4.4). The C's area% of G+ increased from Day 0 to Day 3 and the highest area% occurred on Day 7 (30.6±5.7%). The area% decline continuously on Day 14 and 21 then slightly increased on Day 28 to Day 42 before descended on Day 49. Similar to C, the FA's area% of G+ increased on first 3 days. The highest area% was presented on Day 3 (31.4±8.1%) before decreasing sharply on Day 7 and then steady until Day 49 (see Figure 4.4). There were significant differences between G+ total area% on Day 35 and 49. On Day 35, FA's area% was significant higher than C's at the .05 level (22.5±0.4% and 18.1±1.4% in orderly, p = .042). On Day 49, C's area% was significant higher than FA's at the .05 level (21.0±0.5% and 17.9±0.7% severally, p = .022).

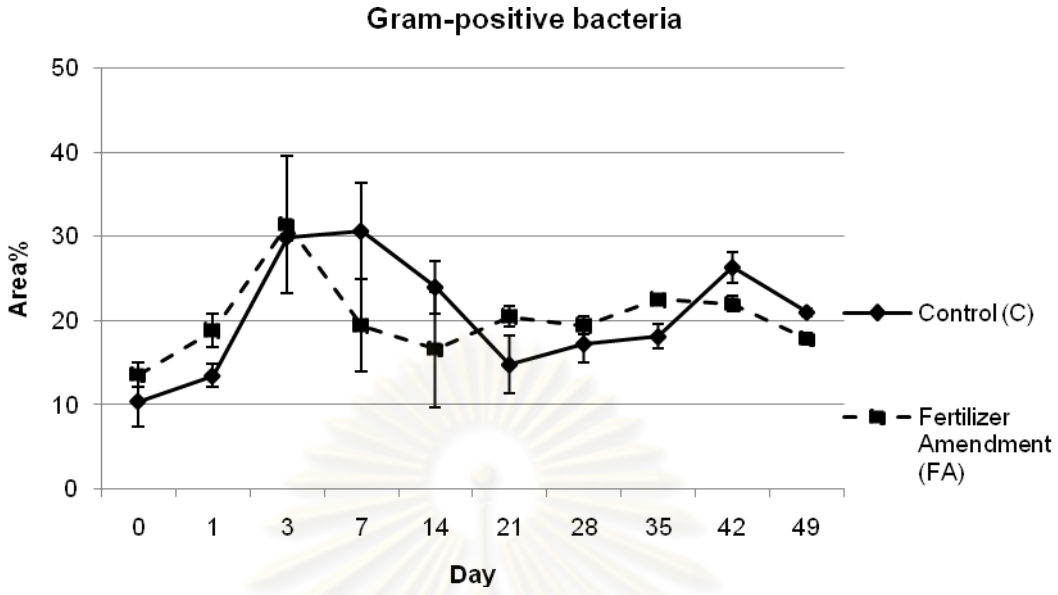


Figure 4.4 The area% of Gram-positive bacteria's total PLFA of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3) and error bar indicate standard error (SE).

Variation of total PLFA's area% of Gram-negative bacteria in C and FA occurred through the experiment period. There was similar pattern and amount of G-'s area% in C and FA from Day 0 to Day 14. The mean of FA's G- area% on Day 14 was significant higher than C's at the .05 level (p = .015). The area% increased sharply on Day 21 and continued increased steeply on Day 28 in C set and then descended on Day 35 while it quite steady in FA set from Day 21 to 35. After that, the similar pattern and very closed amount of G-'s area% in C and FA was occurred again on Day 35 to Day 49. The data of G-'s area% was presented in Figure 4.5. Total bacteria area% data were the combination of G+ and G- area%. Both C and FA had an interval pattern of total bacteria area% as shown in Figure 4.6. There were significant differences between C and FA data set on Day 1 and 49 at the .05 level (p = .024 and .019, orderly).

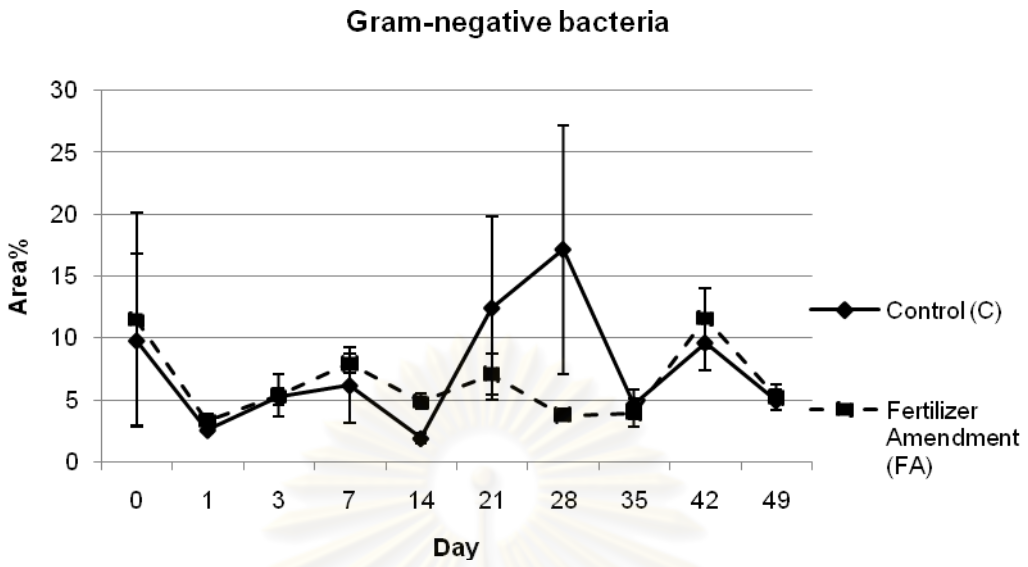


Figure 4.5 The area% of Gram-negative bacteria's total PLFA of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3) and error bar indicate standard error (SE).

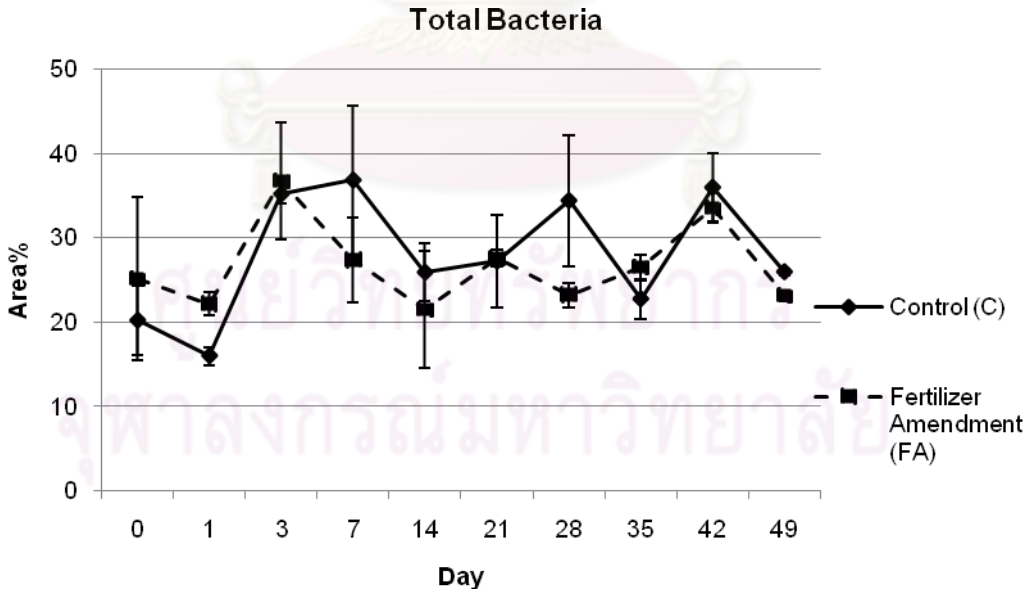


Figure 4.6 The area% of total bacteria of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3), and error bar indicate standard error (SE).

The area% pattern of C's fungi was varies since Day 0 to Day 21.The highest area% (18.3±7.8%) was occurred on Day 14 then descended sharply on Day 21. While there was an interval on control set's fungi area% pattern, fungi area% in FA set was low and stable from Day 0 to Day 49 (Figure 4.7).

The similar total PLFA area% of protozoa and microeukaryotes patterns was presented in C and FA (Figure 4.8). There was significant difference between both C and FA only on Day 3 (p = 0.004). The highest area% in FA was occurred spire on Day 28, and the highest area% in C was appeared steeply on the same day (17.4±1.3% and 18.6±1.8%, severally).

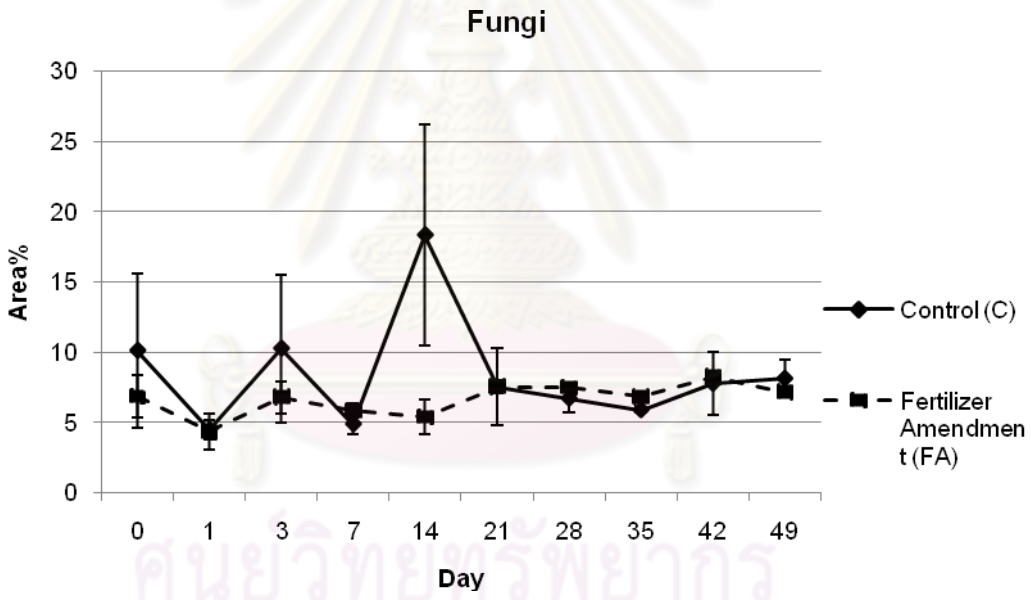


Figure 4.7 The area% of fungi of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3) and error bar indicate standard error (SE).

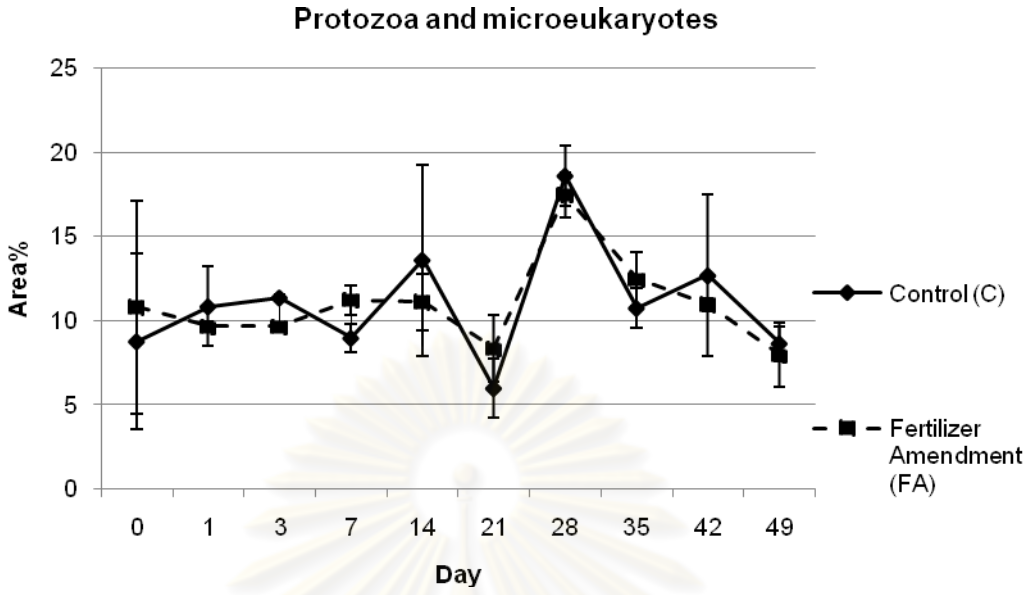


Figure 4.8 The area% of protozoa and microeukaryotes of control (C) and fertilizer amendment (FA) sets. Data were presented by mean (n =3) and error bar indicate standard error (SE).

Saturated fatty acids and signature fatty acids pattern of C and FA on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49 were presented as bar charts in Figure 4.9(a) – (j) . Fatty acids 16:0 and 18:0 were the most abundant SatFA in C and FA. Bacteria were the dominant microorganisms and Gram-positive bacteria were the most abundant in control and chemical fertilizer amendment paddy soil. Fatty acids i15:0 and a15:0 were major signature fatty acids in both C and FA. The pattern of these fatty acids and i16:0 in C set were more varied than FA set which all of signature fatty acids of Gram-positive bacteria were more stable. Among these signature fatty acid, 15:1 and 17:1 had the lowest area% in both C and FA over the experiment period. The area% of i16:0 also flat in FA while the area% of i17:0 was close to a15:0 except on Day 3.

As shown in Figure 4.4, most of total area% of G+ in FA appeared to slightly greater than C. On Day 1, a15:0 in FA was significant higher than C at the .05 level (p = .028). Fatty acid a15:0 in FA was also significant higher than C at the .05 level on Day 14, and at the .01 level on Day 35 (p = .018 and .002, respectively). Significant difference at the .05 level of another Gram-positive's signature fatty acids were also found on Day 21 and Day 35. Fatty acid i15:0 in FA on Day 21 was higher

than C (p = .021) and FA's i17:0 was found significant higher than C's on Day 35 (p = .030).

Monounsaturated fatty acids 18:1ω9 and 20:1ω9 were stood out in C while all of Gram-negative's signature fatty acids of FA had similar trends. Some significant differences of area% were occurred between C and FA. On Day 14, FA set's 16:1 and cy17:0 were significant higher than C set at the .01 level (p = .009 and .01, severally). 20:1ω9 of FA was significant higher than C set at the .01 level on Day 35 with p value = .004.

There was no significant difference between fungi's signature fatty acids of C and FA. However, there were variations of 18:3ω6 area% in C set from Day 0 to Day 14. The steeple was occurred on Day 14 before declined dramatically on Day 21 then stable until Day 49. 18:2ω6c, 18:3ω6, and 18:3ω3 in FA were steady over the experiment period. There were more alternations of signature fatty acids pattern of protozoa and microeukaryotes in C than FA. Fatty acid 20:2 was dominant fatty acid in both sets. The area% of 20:3ω6, 20:3ω3, 20:4ω6, 20:5ω3, 22:6ω3 were stable in FA throughout the experiment period while 20:5ω3 and 22:6ω3 area% pattern in C were shift on Day 14 to Day 35. Although there were variations of individual PLFA data on sampling day, however, there was no significant difference between experiment period mean of SatFA, G+, G-, fungi, and protozoa and microeukaryotes as illustrated as bar charts in Figure 4.10.

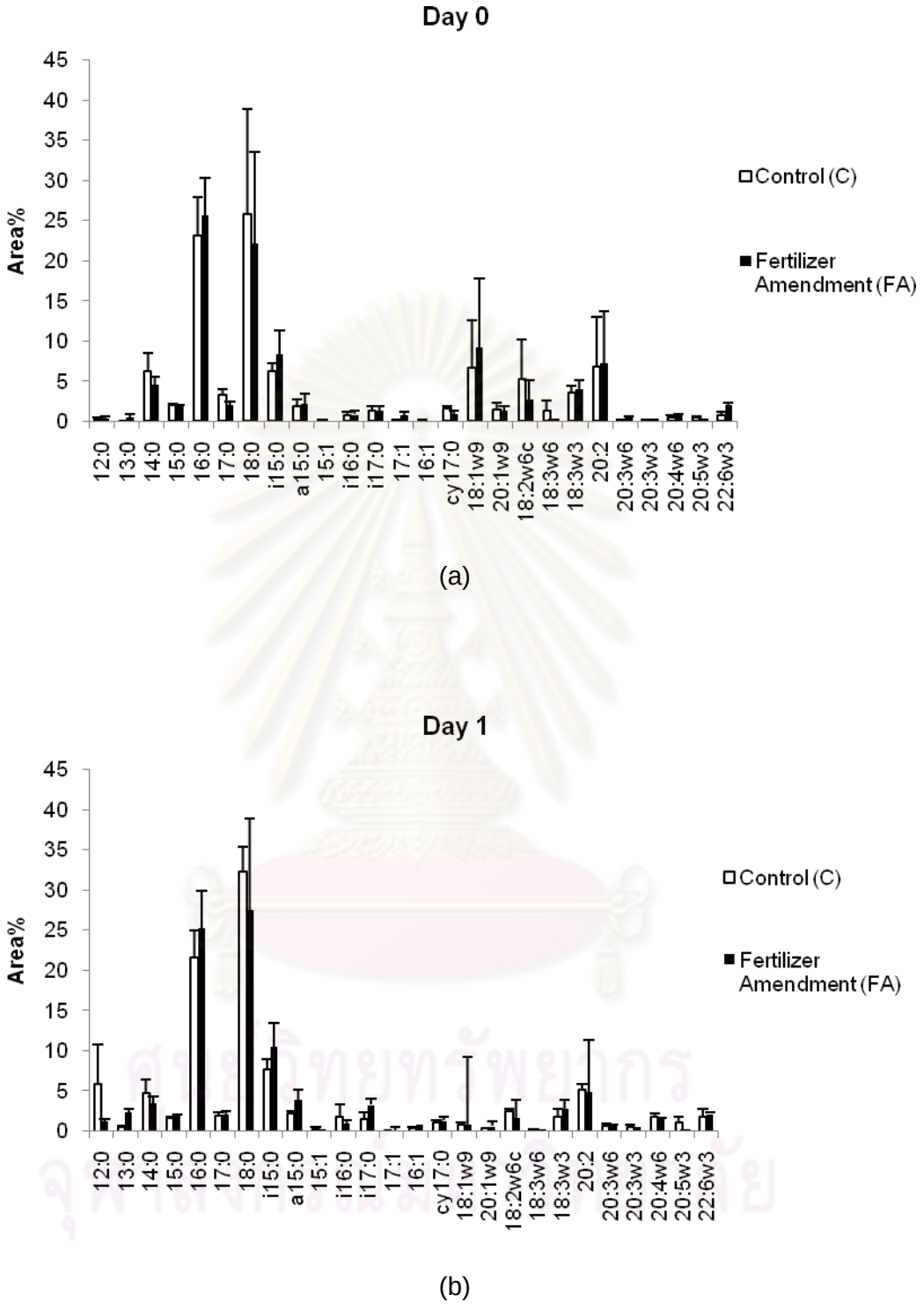
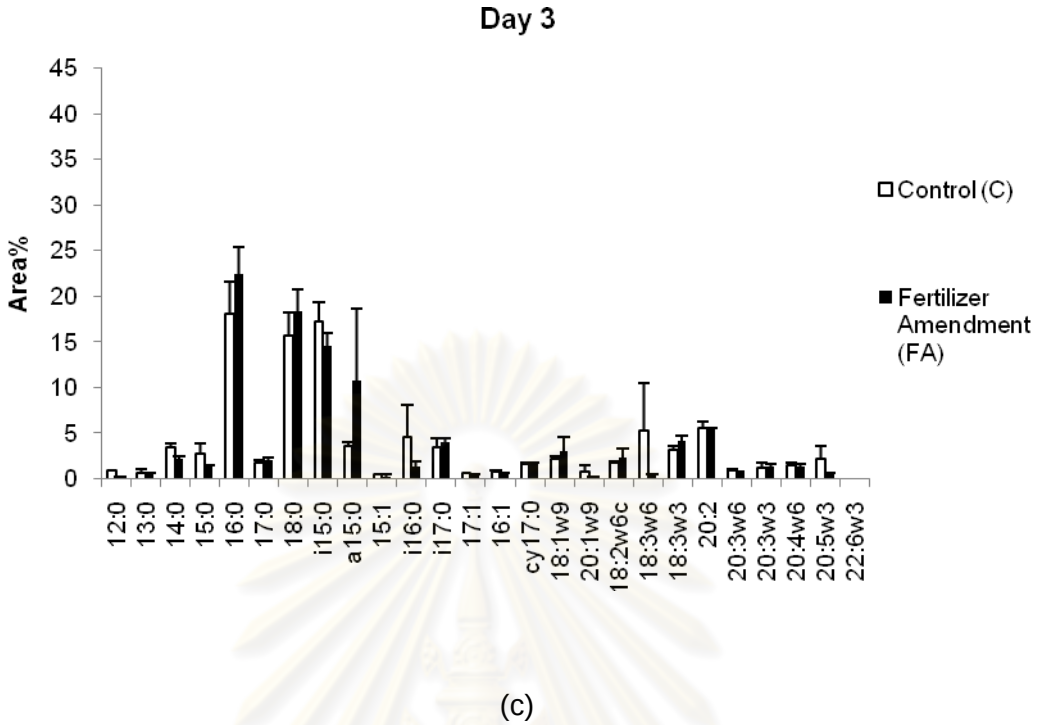
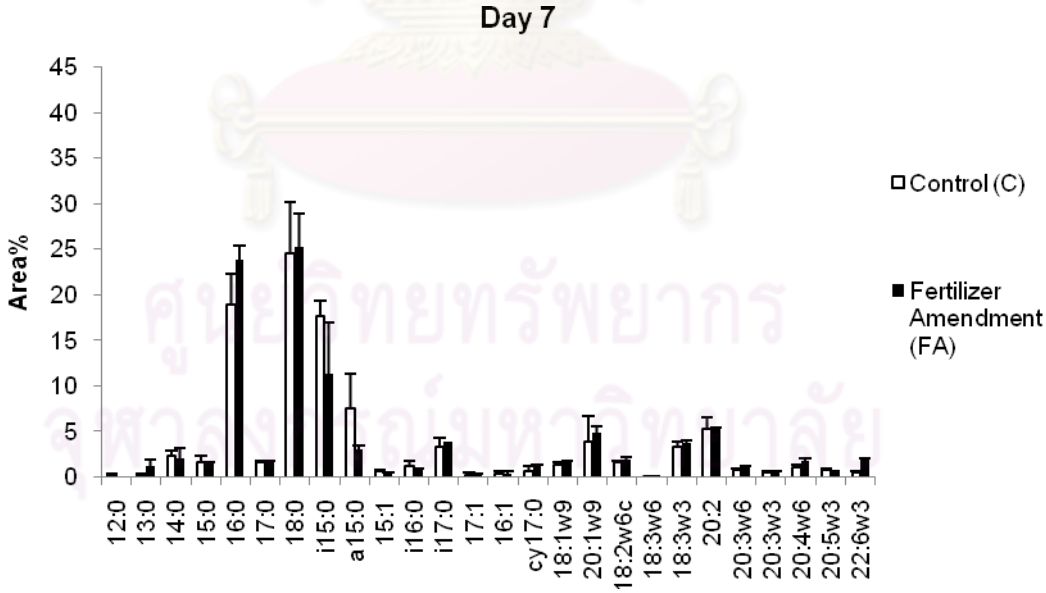


Figure 4.9 Saturated fatty acids and signature fatty acids patterns of control (C) and fertilizer amendment (FA) sets on Day 0, 1, 3, 7, 14, 21, 28, 35, 42, and 49 (a-j). Data were presented by mean (n =3) and error bar indicates standard error (SE).

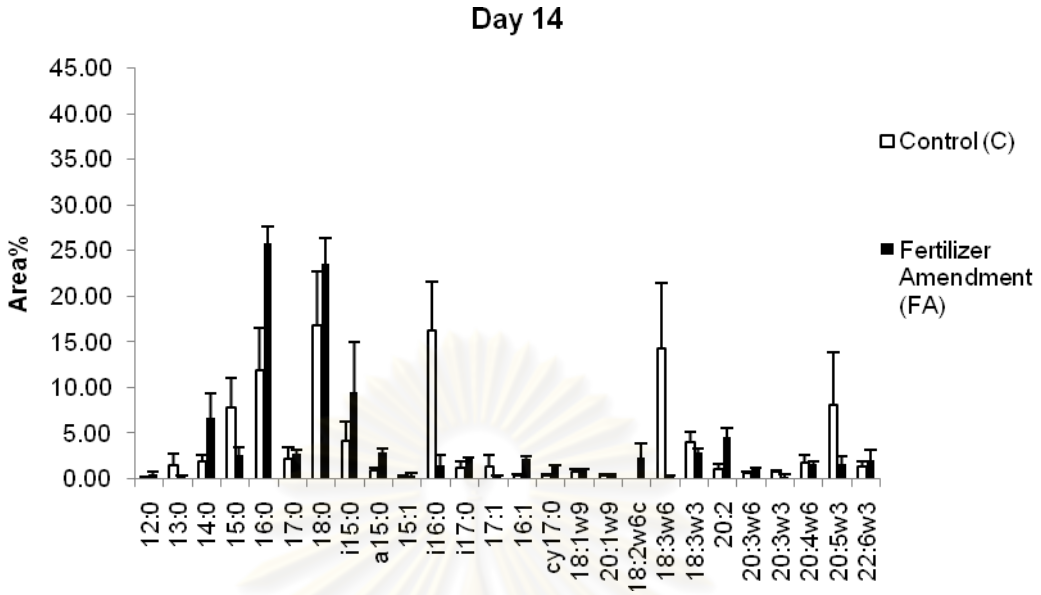


(c)

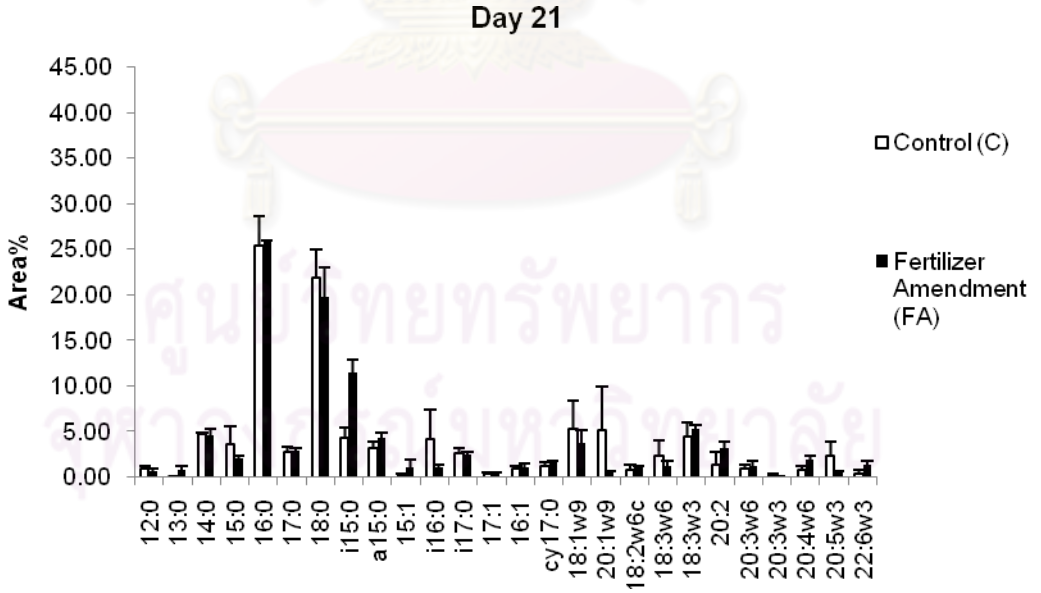


(d)

Figure 4.9 (Continue)

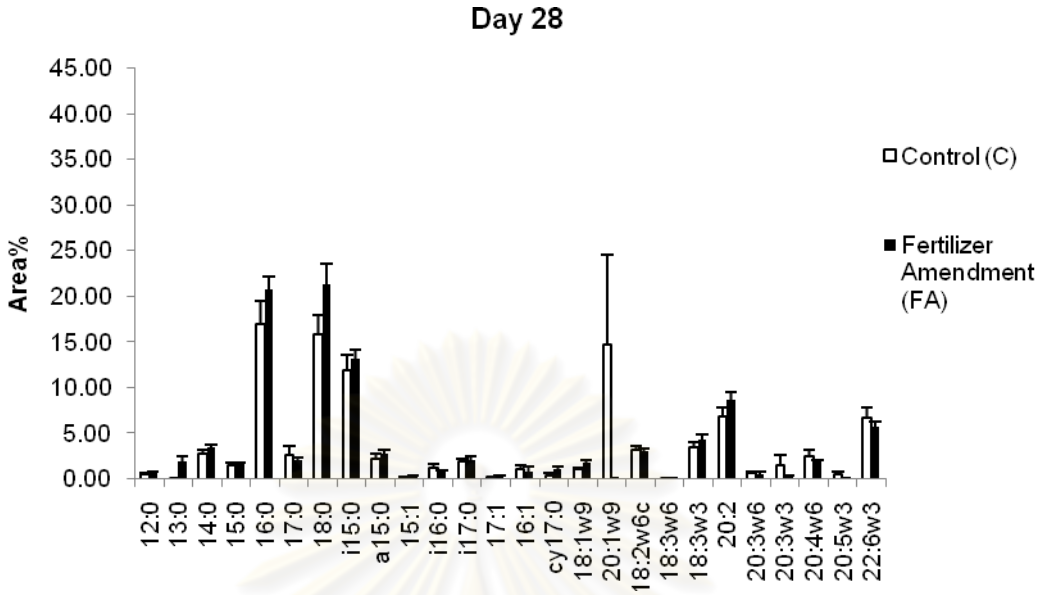


(e)

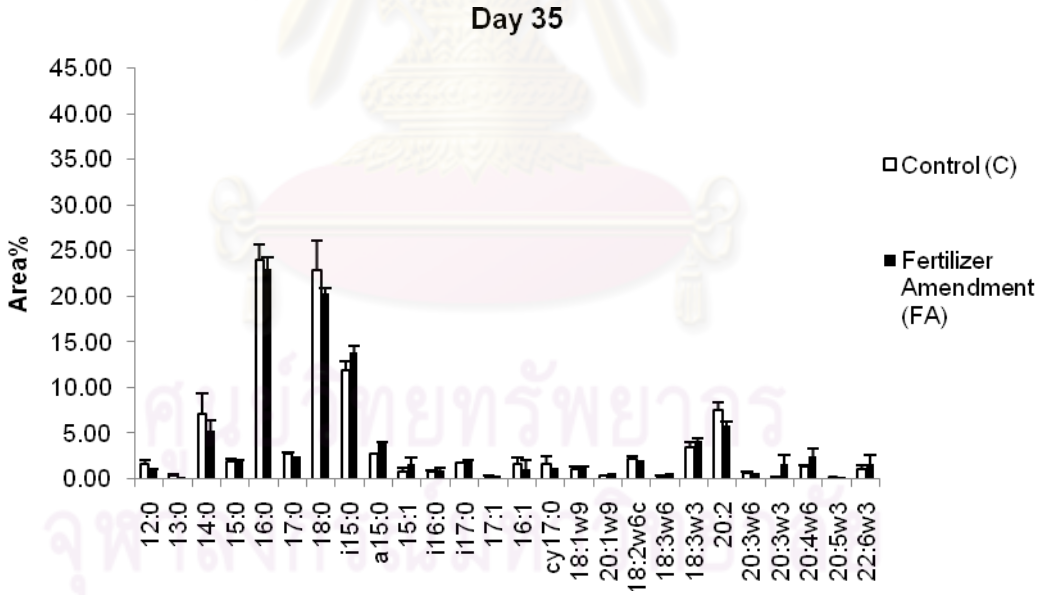


(f)

Figure 4.9 (Continue)

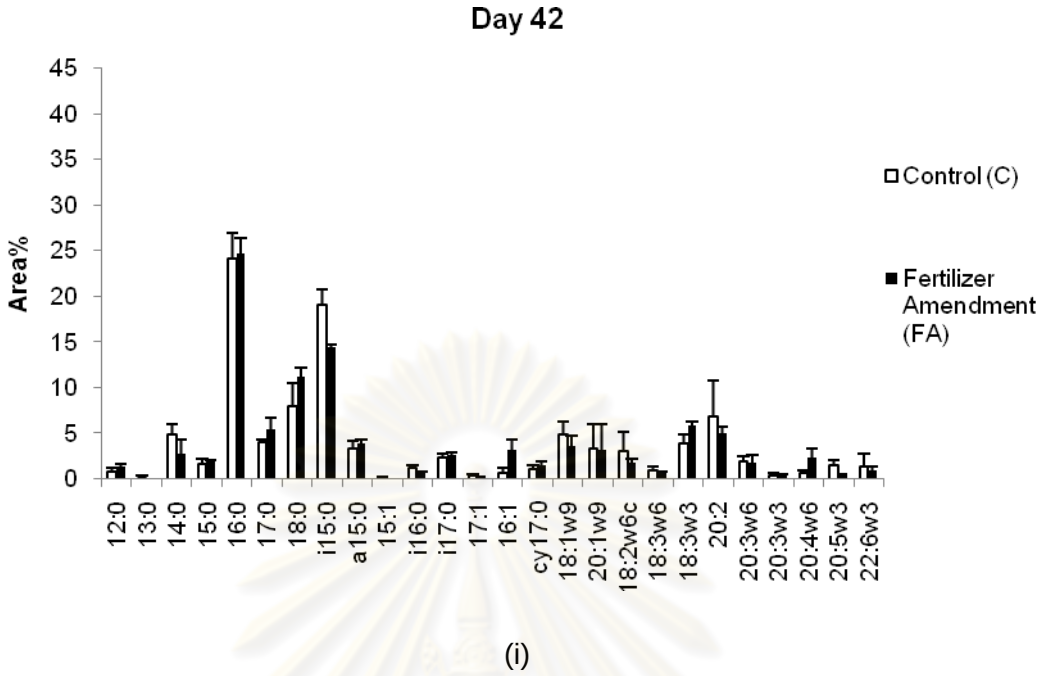


(g)

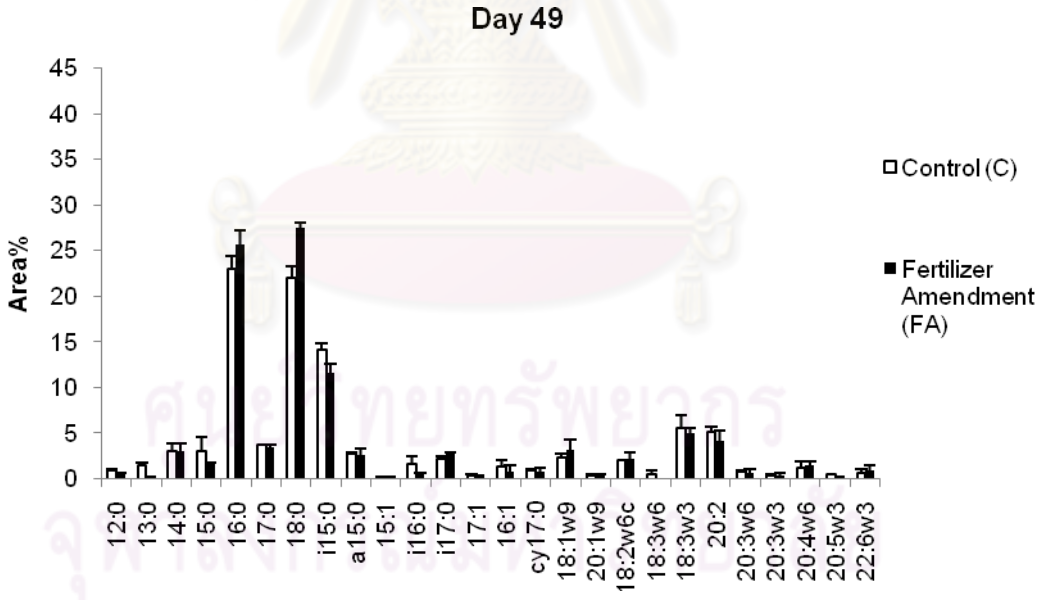


(h)

Figure 4.9 (Continue)



(i)



(j)

Figure 4.9 (Continue)

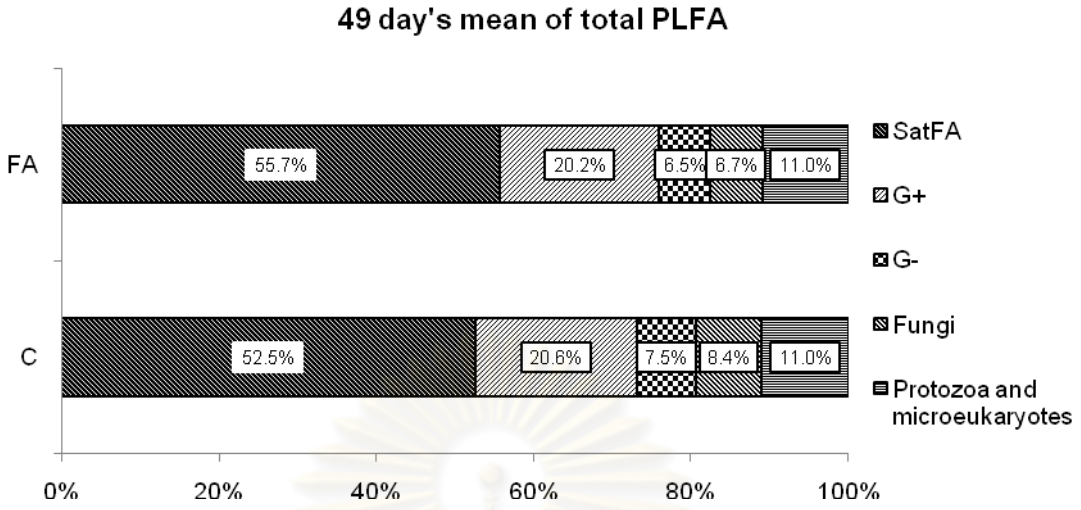


Figure 4.10 Mean of total PLFA of control (C) and fertilizer amendment (FA) sets of 49-day experiment.

Mean area% of total selected PLFAs were transformed to log10 area% then was analyzed with principle component analysis. 66.388% of total PLFA variance could be explained by the first two principal components (PC), 44.423% and 21.960%, respectively as shown in Figure 4.11. Most of variables on the same day were in the same PC. FAD28, CD35, FAD1, CD49, CD1, FAD0, FAD35, FAD7, FAD49, FAD21, FAD14, CD7, and CD28 were on PC1, and FAD42, CD42, CD21, and CD0 were on PC2. Variables were the same PC indicated there are high correlations between them. CD3, CD14, and FAD3 were separated from those variables above and their positions were near intersection (0,0) indicated there was no relation between them and the other variables on difference components.

Pearson's correlation (r) between C and FA were shown in Table 4.4. Positive correlation was high between C and FA every day except on Day 14 that the correlation was low. These indicated that there were on the same component and no difference between the experiment sets.

Log10 mean of area% saturated fatty acids and signature fatty acids were also analyzed by PCA. Only 29.920% of variance could be explained on first two principle component. PC1 and PC2 could be described severally 15.161% and 14.760% of variance. Factor loading of individual selected fatty acids were illustrated

in Figure 4.12. No systematic differences in PLFA were found in either set of samples. 14:0, 16:0, cy17:0 of C set were found on the right hand side of the plot. i15:0, a15:0 and i17:0 of C set were found on quadrant 1 on PC2 while i17:0 of FA set was found quadrant 2 on PC1. Most of SATFA in both sets were discovered on quadrant 3 and 4.

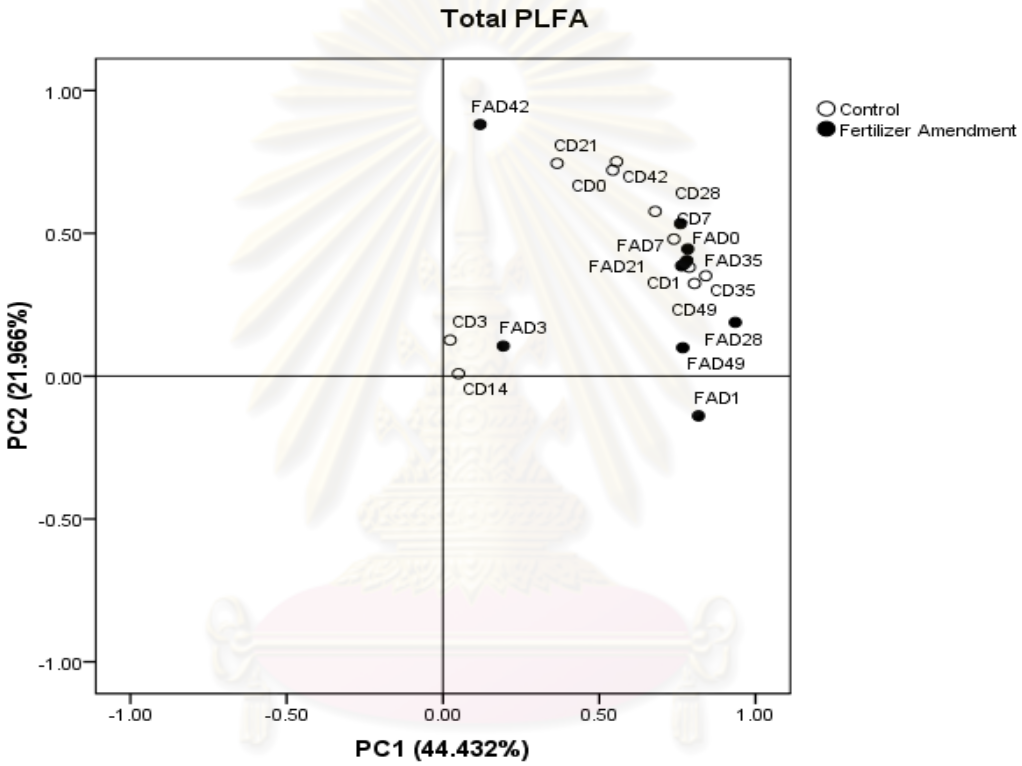


Figure 4.11 Principle component analysis of total PLFA profiles (log10 of mean area%) of paddy soils with and without chemical fertilizer amendment. Hollow symbols indicate control soil (C) and solid symbols indicated fertilizer amended soil (FA). Alphabet D follow by numbers indicate day of microcosm sampling.

Table 4.4 Pearson's correlation (r) between variables.

Correlation Matrix																				
	CD0	CD1	CD3	CD7	CD14	CD21	CD28	CD35	CD42	CD49	FAD0	FAD1	FAD3	FAD7	FAD14	FAD21	FAD28	FAD35	FAD42	FAD49
CD0	1.000	0.719	0.284	0.747	0.017	0.831	0.664	0.770	0.887	0.768	0.865	0.275	0.346	0.738	0.788	0.804	0.694	0.766	0.616	0.351
CD1	0.719	1.000	0.182	0.736	0.050	0.620	0.723	0.849	0.734	0.815	0.738	0.504	0.266	0.685	0.862	0.744	0.833	0.796	0.406	0.616
CD3	0.284	0.182	1.000	0.346	0.075	0.431	-0.020	0.243	0.263	0.365	0.147	-0.026	0.967	0.178	0.199	0.271	0.025	0.231	0.143	0.041
CD7	0.747	0.736	0.346	1.000	0.103	0.699	0.833	0.759	0.798	0.747	0.784	0.545	0.473	0.908	0.832	0.756	0.711	0.806	0.499	0.704
CD14	0.017	0.050	0.075	0.103	1.000	0.317	-0.028	0.048	0.079	0.170	0.049	-0.138	0.035	0.127	0.132	0.250	0.047	0.104	0.022	-0.091
CD21	0.831	0.620	0.431	0.699	0.317	1.000	0.580	0.637	0.842	0.713	0.690	0.121	0.433	0.650	0.737	0.735	0.474	0.578	0.623	0.279
CD28	0.664	0.723	-0.020	0.833	-0.028	0.580	1.000	0.646	0.771	0.624	0.720	0.587	0.089	0.793	0.769	0.546	0.658	0.713	0.601	0.729
CD35	0.770	0.849	0.243	0.759	0.048	0.637	0.646	1.000	0.741	0.870	0.795	0.600	0.352	0.715	0.904	0.920	0.893	0.878	0.455	0.586
CD42	0.887	0.734	0.263	0.798	0.079	0.842	0.771	0.741	1.000	0.801	0.837	0.295	0.331	0.777	0.821	0.764	0.683	0.700	0.658	0.460
CD49	0.768	0.815	0.365	0.747	0.170	0.713	0.624	0.870	0.801	1.000	0.833	0.492	0.471	0.770	0.854	0.878	0.893	0.789	0.336	0.526
FAD0	0.865	0.738	0.147	0.784	0.049	0.690	0.720	0.795	0.837	0.833	1.000	0.568	0.260	0.814	0.759	0.815	0.836	0.737	0.408	0.597
FAD1	0.275	0.504	-0.026	0.545	-0.138	0.121	0.587	0.600	0.295	0.492	0.568	1.000	0.131	0.493	0.431	0.439	0.629	0.581	0.113	0.759
FAD3	0.346	0.266	0.967	0.473	0.035	0.433	0.089	0.352	0.331	0.471	0.260	0.131	1.000	0.312	0.312	0.381	0.181	0.354	0.172	0.213
FAD7	0.738	0.685	0.178	0.908	0.127	0.650	0.793	0.715	0.777	0.770	0.814	0.493	0.312	1.000	0.803	0.771	0.777	0.738	0.314	0.592
FAD14	0.788	0.862	0.199	0.832	0.132	0.737	0.769	0.904	0.821	0.854	0.759	0.431	0.312	0.803	1.000	0.859	0.842	0.841	0.567	0.621
FAD21	0.804	0.744	0.271	0.756	0.250	0.735	0.546	0.920	0.764	0.878	0.815	0.439	0.381	0.771	0.859	1.000	0.858	0.846	0.421	0.438
FAD28	0.694	0.833	0.025	0.711	0.047	0.474	0.658	0.893	0.683	0.893	0.836	0.629	0.181	0.777	0.842	0.858	1.000	0.834	0.252	0.615
FAD35	0.766	0.796	0.231	0.806	0.104	0.578	0.713	0.878	0.700	0.789	0.737	0.581	0.354	0.738	0.841	0.846	0.834	1.000	0.533	0.509
FAD42	0.616	0.406	0.143	0.499	0.022	0.623	0.601	0.455	0.658	0.336	0.408	0.113	0.172	0.314	0.567	0.421	0.252	0.533	1.000	0.282
FAD49	0.351	0.616	0.041	0.704	-0.091	0.279	0.729	0.586	0.460	0.526	0.597	0.759	0.213	0.592	0.621	0.438	0.615	0.509	0.282	1.000

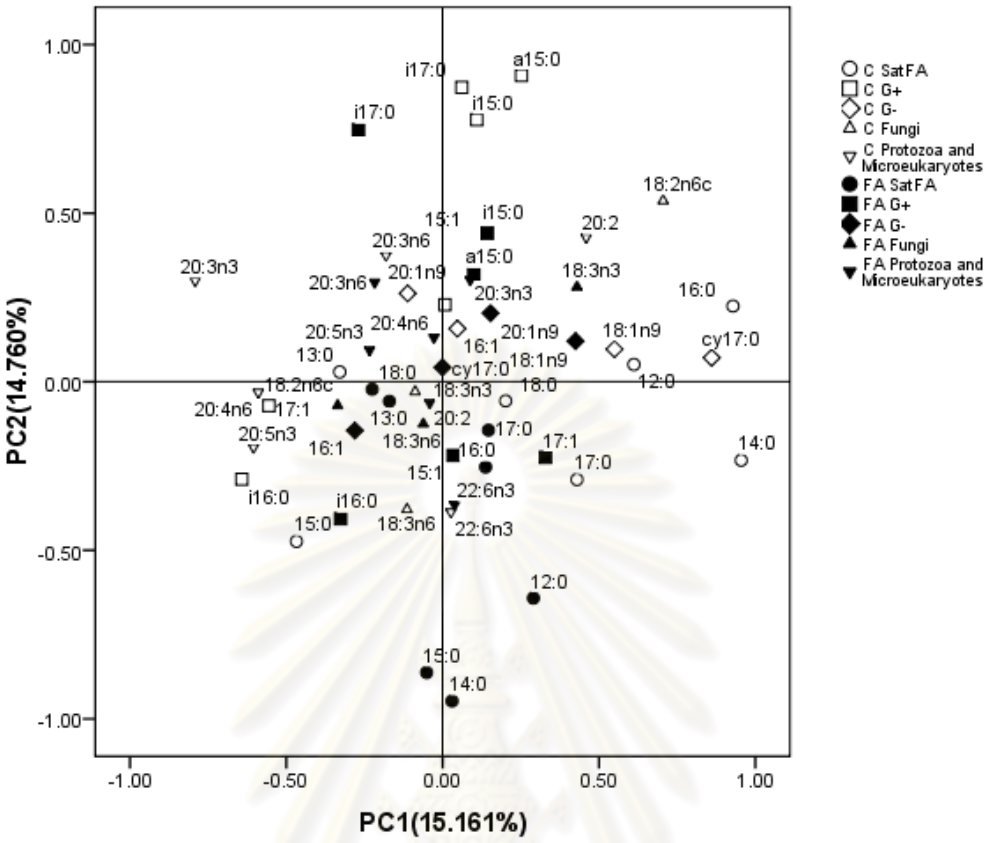


Figure 4.12 Principle component analysis of selected PLFA profiles (log10 of mean area%) of paddy soils with and without chemical fertilizer amendment. Hollow symbols indicate control soil (C) and solid symbols amended soil (FA). The symbol ○ indicate saturated fatty acid, □ indicate PLFA of Gram-positive bacteria, ◇ imply to PLFA of Gram-negative bacteria, ▽ imply to PLFA of fungi, and △ indicate to protozoa and microeukaryotes.

4.4 Soil Respiration

Detection of CO_2 from soil respiration is one way to determine soil microbial activity. In this experiment, soil respiration was detected daily. CO_2 was trapped in alkali solution, and then titrated with mild acid. CO_2 amount was expressed as mean±SE of μg $CO_2 \cdot g^{-1}$ dm·$24h^{-1}$ (Figure 4.13). Variation of CO_2 evolution occurred every day. The highest CO_2 of FA and C was presented on Day 1 (142.07 μg $CO_2 \cdot g^{-1}$ dm·$24h^{-1}$) and Day 9 (107.61 μg $CO_2 \cdot g^{-1}$ dm·$24h^{-1}$), respectively. There was only one significant difference between C and FA on Day 21 at the .01 level (p = .019). Most of

FA's soil respiration was higher than C and the trends of CO_2 evolution were declined on both C and FA sets. The decline trend of soil respiration was related to soil pH as shown in Figure 4.14. CO_2 accumulation of 50 day of experiment as shown in Figure 4.15 was 2663.84 and 3339.79 μg $CO_2 \cdot g^{-1}$ dm in C and FA, in orderly.

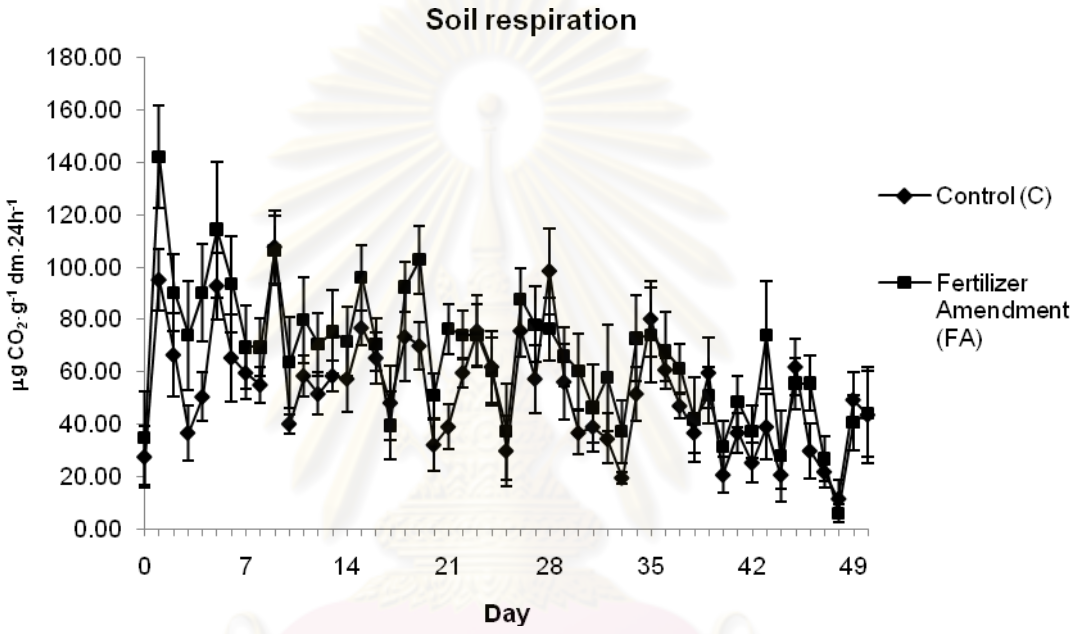
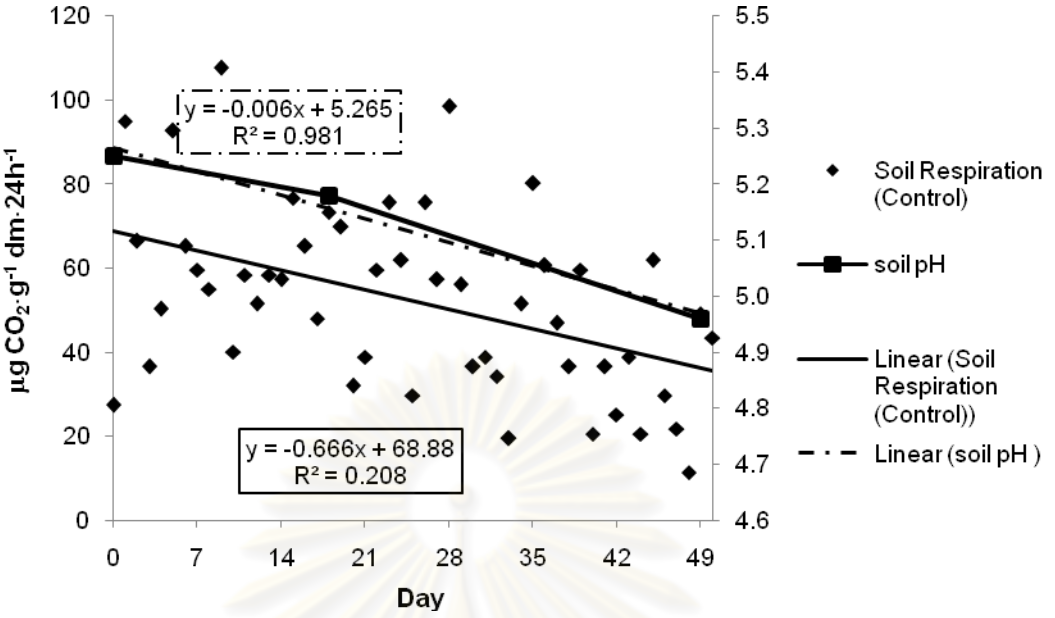
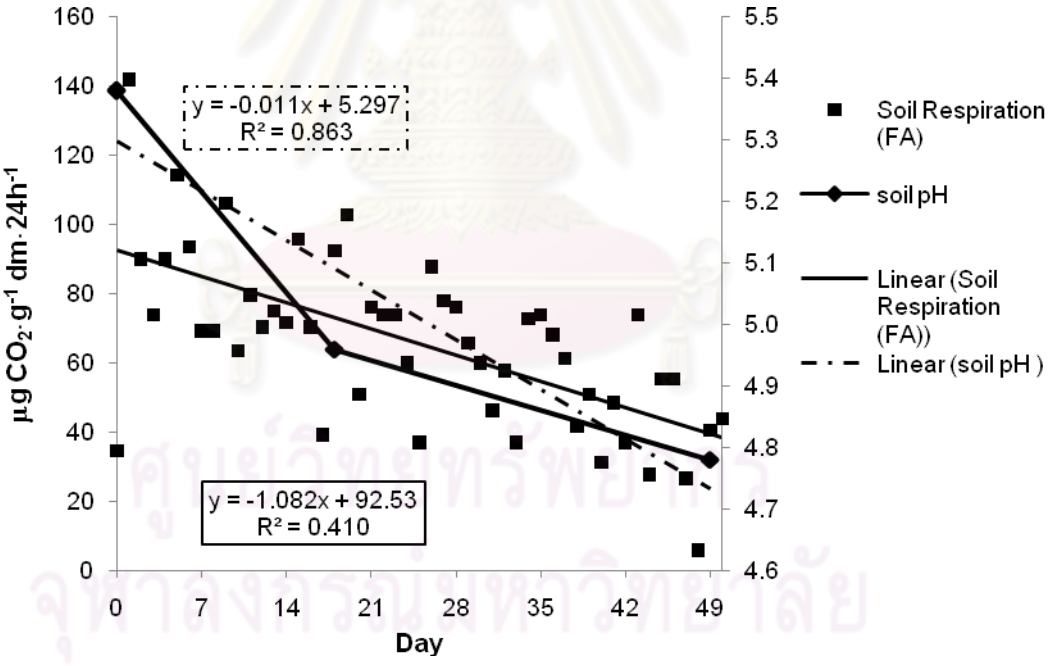


Figure 4.13 Mean of daily soil respiration of control (C) and fertilizer amendment (FA) sets (n = 5). The bar indicates standard error (SE).



(a)



(b)

Figure 4.14 The relationship trend between soil respiration and soil pH (1:2.5 soil to water ratio) in control (a) and fertilizer amendment paddy soil (b).

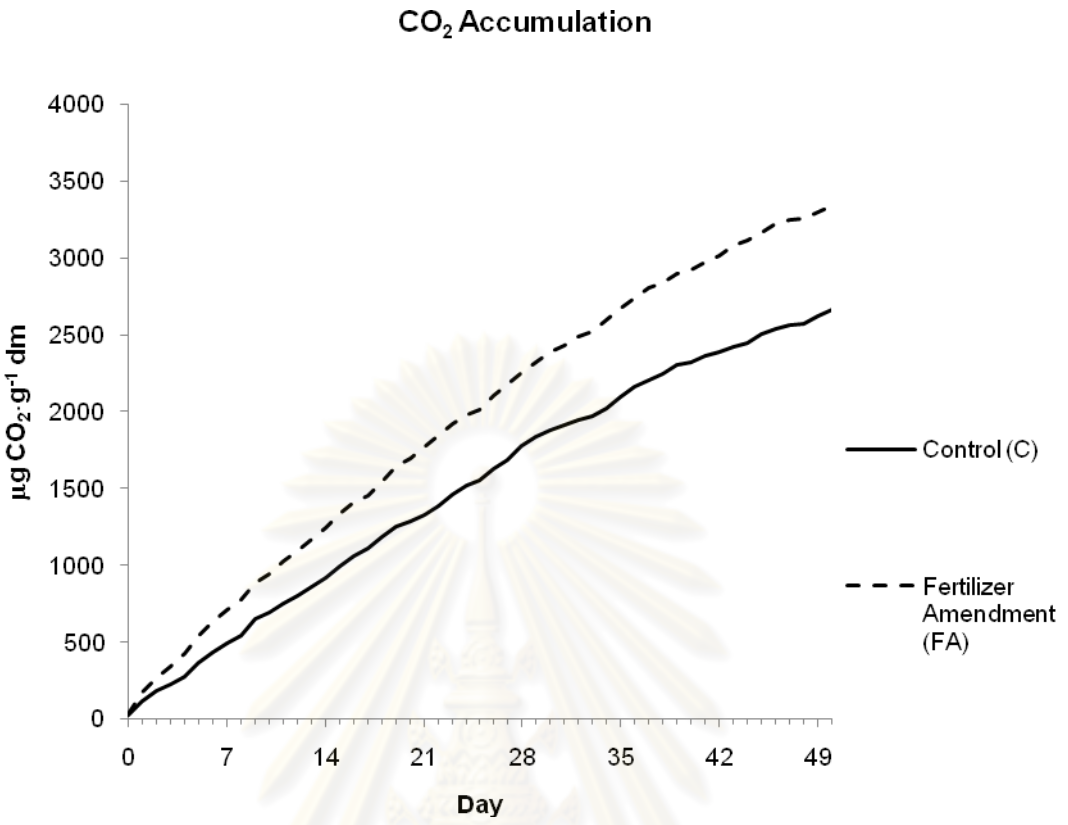


Figure 4.15 CO2 accumulations of control (C) and fertilizer amendment (FA) sets express as μg $CO_2 \cdot g^{-1}$ dm.

CHAPTER V

DISCUSSION AND CONCLUSION

5.1 Discussion

The effects of chemical fertilizer amendment in paddy soil were shown in this thesis. Several parameters were measured against control set (C) (unamended paddy soil) after the recommended rate of 156.25 $kg \cdot ha^{-1}$ (25 $kg \cdot rai^{-1}$) mixed chemical fertilizer 16-20-0 (N-P-K) was amended into paddy soil (FA). The results showed that chemical fertilizer amendment affected to soil physical and chemical properties. Soil pH decreased in both C and FA and FA soil pH was lower than C when pH determination was performed in water and 0.01 M $CaCl_2$ (1:2.5 soil to solvent ratio). However, when soil pH was measured in 1 N KCl which used to detect active and potential acidity (Tan, 2005), there was no difference between C and FA until Day 49, which the lower pH occurred in FA.

The lower soil pH occurred due to the mixed chemical fertilizer used in this study contained $(NH_4)_2SO_4$ as nitrogen carrier and sulphur (S) as trace element. Therefore, this mixed chemical fertilizer solution was acidic which lower soil pH (Krishnamurthy *et al.*, 2009). Moreover, decreasing of soil pH might due to increasing of organic acids and CO_2 (as carbonic acid in soil colloid) which were byproducts of decomposition and soil microbial metabolisms.

Total nitrogen of FA set was lower than C set. The lower total nitrogen in chemical fertilizer amended soil due to rapidly change of NH_4-N forming after N fertilizer amendment was easier to access by NH_4 utilizing bacteria (Sarathchandra *et al.*, 2001). No significant difference of cation exchange capacity (CEC) occurred between FA and C. The lower CEC was found in FA set due to NH_4^+ from chemical fertilizer solution replaced the exchangeable cation on soil particle (Krishnamurthy *et al.*, 2009*)*. Hence, when CEC was measured by ammonium replacement method, CEC of FA set was lower than C set.

Using of chemical fertilizer is not only affected to soil physical and chemical properties, but also have impacted to soil microbial communities and their activities. In this experiment, PLFA was used to investigate the change of soil microbial

communities after chemical fertilizer was amended. Saturated fatty acids (SatFA) were dominant in C and FA set, especially 16:0 and 18:0 which are found generally in every organism (Hedrick *et al.,* 2005). PLFA data was shown that Gram-positive bacteria, indicated by *iso*- and *antei-iso*- of 15:0, 16:0, and 17:0, and 15:1 and 17:1 (Zelles, 1997), were predominant in this paddy soil sample in both C and FA. The variation of total area% of fungi in C, which indicated by 18:2ω6c, 18:3ω6c and 18:3ω3c (Frostegård and Bååth, 1996; Hedrick *et al.*, 2005; Marschner, 2007), was occurred on Day 0 to Day 14 and steady from Day 21 to Day 49 while total area% of fungi in FA was more steady. Log10 of total selected PLFAs area% was analyzed by PCA shown that there was no difference between C and FA.

The effects of chemical fertilizer amendment on soil microbial communities and their biomass was controversy in many reports. The different results might cause by the variation of land management and agricultural system (Stweenwerth *et al.,* 2002; Li, Wu, and Chen, 2007) and kinds of chemical fertilizers and proportion (Marschner, *et al.,* 2003; Li *et al*., 2008). Zhang, Wang, and Yao (2007) found the change of soil microbial community patterns after soil was amended with different chemical fertilizers in paddy soil. They reported that Gram-positive bacteria were stimulated by fertilizers applications and the low fungi PLFA (18:2ω6c) was found in every treatment while actinomycetes had different response to vary chemical fertilizer. Peacock *et al.* (2001) found no significant difference of soil microbial biomass between unfertilized soil and N fertilizer amended soil. They also reported the decreasing of Gram-negative bacteria after N fertilizer was applied. Sarathchadra *et al.* (2001) found that N and P fertilizer applications had no effect on total bacteria but not fungi. Demoling *et al.* (2008) studies demonstrated the negative effects of chemical fertilizers on total PLFA and fungi (PLFA biomarker; 18:2ω6c, and ergosterol) in coniferous forest soils.

Cellulase activity was determined to investigated soil microbial activities. There was no significant difference between C and FA. The highest cellulase activity occurred on Day 21 in both C and FA. PCA data showed non-systematic pattern between signatures PLFA explained the coordination between soil microorganisms on soil microbial activities in this sample. However, PLFA data indicated that bacteria, especially Gram-positive bacteria which were predominant microorganisms, could be a major cellulose decomposer in aerobic paddy soil. This result was contrast to studies of Nakamura *et al.* (2003) of microbial response on the decomposition of rice straw using PLFA pattern under upland and flooded condition.

The studies showed that Gram-negative bacteria and fungi were predominant under upland condition while Gram-positive bacteria and anaerobic Gram-negative bacteria were predominant under flooded condition. This result was also different from Kimura *et al.* (2001) reported that Gram-positive bacteria were the major decomposers of rice straw incorporated into a paddy soil microcosm under submerged conditions while Gram-negative and fungi were predominant in drained soil.

Compare the effects of chemical fertilizer amendment on soil enzyme activities to the other agriculture soil, the similar results were reported by Nayak *et al.* (2007) which their experiments were also determined the effects of fertilizers in paddy soil. They found that cellulase activity and the other enzyme activities were rise after chemical fertilizer applications. Increasing activities also found in chemical fertilizer and compost amended paddy soil due to increasing of nutrients and carbon sources to soil microbes supported biomass building and were primary substrates in decomposition processes. Saha *et al.* (2008) found the similar results that cellulase activities after NP and NK applications in a rainfed soybean–wheat system were increase but no significant difference compared with control whereas protease and urease activities were significantly descended. The studies of Sarathchadra *et al.* (2001) found that N and P fertilizer application had no effect on cellulolytic microbes while the function diversity was decreased when large amount of N fertilizer (urea) was applied but no effect when P fertilizer was amended. The studies of Mandal *et al.* (2007) revealed that soil enzyme activities (dehydrogenase, acid phosphatase and alkaline phosphatase) were raised after chemical fertilizers were applied. Wei *et al.* (2008) and Toyota and Kuninaga (2006) were studied the impacts of fertilizers on substrate utilization in varies crops soil and discovered that the substrate utilization activities of soil microbial in chemical fertilizers amended soils were increased compared with unamended soil. In contrast, declined CM-cellulase, protease, and urease activities after chemical fertilizers in many crops soils were found by Chang *et al.* (2007).

Soil respiration was one of parameters that used to determined soil microbial activity. In this study, there was only significant difference between C and FA on Day 21 which was the same day that the highest cellulase activity occurred. CO_2 accumulation of FA set had trend to higher than C. Daily soil respiration trended to decrease correlated to soil pH. This result confirmed to Demoling *et al.* (2008) report that soil respiration and SIR were declined in acid coniferous forest soil after chemical fertilizers were applied. In the same experiment and the other works that were mentioned earlier, they also found the effect of chemical fertilizers on soil pH

which significantly declined when higher dosages were used. Enwall *et al.* (2007) found that the basal respiration in chemical fertilizers amended soil was rise. In the same study, the substrate-induced respiration (SIR) was decreased in low soil pH indicated the lower efficiency of heterotrophic microorganisms to convert organic carbon into microbial biomass in chemical fertilizer trail. The effects of soil pH on soil respiration and soil bacteria and fungi were studied by Bååth and Anderson (2003) and Rousk *et al.* (2010). They found positive correlation between soil pH to soil respiration, SIR, and bacteria and fungi biomass using PLFA measurement.

5.2 Conclusion

Chemical fertilizers are useful in order to increase agriculture productivity by providing available nutrient elements for plant. But not only plant could take this benefit, soil microbes are also uptake these nutrients and turn them to their energy sources which could affect to their activities at the same time. Moreover, chemical fertilizers could change soil physico-chemical properties which could affect to soil microbes directly or in directly. In this study, chemical fertilizer amendment did affect to soil physico-chemical properties, especially soil pH. The result showed that soil was become more acid after chemical fertilizer was applied.

PLFA data revealed that Gram-positive bacteria were predominant microorganisms in this paddy soil sample. Low rate chemical fertilizer amendment did not affect to soil microbial community. Cellulase activity and CO_2 accumulation were higher in chemical fertilizer amended soil compare to unamended soil, though there was no significant difference occurred. It could be concluded that using chemical fertilizer at the recommended dosage did not affect to organic carbon turnover efficiency of soil microbial communities in paddy soil. However, the effects of soil pH on the other soil enzyme activities should be further investigated.

This study was shown only some effects of low rate chemical fertilizer amendment on soil microbial communities and their activities, and some soil physico-chemical properties in paddy soil. The effect of high rate chemical fertilizers application and the kind of fertilizers on soil microbial communities and their activities in different rice culture periods should be further investigated.

REFERENCES

ภาษาไทย

คณาจารย์ภาควิชาปฐพีวิทยา. 2548. ปฐพีวิทยาเบื้องต้น. ภาควิชาปฐพีวิทยา คณะเกษตร มหาวิทยาลัยเกษตรศาสตร์. กรุงเทพมหานคร: สำนักพิมพ์มหาวิทยาลัยเกษตรศาสตร์.

ภาษาอังกฤษ

Aciego Pietri, J. C., and Brookes, P. C. 2008. Relationships between soil pH and microbial properties in a UK arable soil. Soil Biology and Biochemistry 40(7): 1856-1861.

Agnelli, A., Ascher, J., Corti, G., Ceccherini, M. T., Nannipieri, P., and Pietramellara, G. 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biology and Biochemistry 36(5): 859-868.

Agronomic Division, North Carolina Department of Agriculture & Consumer Services. 2010. A Homeowner's Guide to Fertilizer [online]. Available from: http://www.agr.state.nc.us/cyber/kidswrld/plant/Fertbag.GIF [17 March 2010]

Allison, V. J., Condron, L. M., Peltzer, D. A., Richardson, S. J., and Turner, B. L. 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. Soil Biology and Biochemistry 39(7): 1770-1781.

Ariyakanon, N. 2007. Environmental soil science lab. Bangkok: Department of General Science, Faculty of Science, Chulalongkorn University.

Asakawa, S., and Kimura, M. 2008. Comparison of bacterial community structures at main habitats in paddy field ecosystem based on DGGE analysis. Soil Biology and Biochemistry 40(6): 1322-1329.

Attanandana, T. 1988. Paddy soil science. Bangkok: Kasetsart University Press.

Bååth, E., and Anderson, T. H. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology and Biochemistry 35(7): 955-963.

Bai, Q., Gattinger, A., and Zelles, L. 2000. Characterization of microbial consortia in paddy rice soil by phospholipid analysis. Microbial Ecology 39(4): 273-281.

Beck, T. 1996. Soil Respiration by Infrared Gas Analysis. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in soil biology. Germany: Springer.

Beck, T., Öhlinger, R., and Baumgarten, A. 1996. Substrate-Induced Respiration. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in Soil Biology. Germany: Springer.

Bhattacharyya, P., Chakrabarti, K., and Chakraborty, A. 2005. Microbial biomass and enzyme activities in submerged rice soil amended with municipal solid waste compost and decomposed cow manure. Chemosphere 60(3): 310-318.

Bligh, E. G., and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

Böhme, L., Langer, U., and Böhme, F. 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agriculture, Ecosystems & Environment 109(1-2): 141-152.

Bossio, D. A., and Scow, K. M. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35(3): 265-278.

Chang, E. H., Chung, R. S., and Tsai, Y. H. 2007. Effect of different application rates of organic fertlizer on soil enzyme activity and microbial population. Soil Science and Plant Nutrition 53: 132-140.

Chang, H. L., In, B. L., and Pil, J. K. 2004. Effects of long-term fertilization on organic phosphorus fraction in paddy soil. Soil Science and Plant Nutrition 50(4): 485-491.

Chaplin, M. 2009. Carboxymethylcellulose (CMC) [Online]. Available from: http://www1.lsbu.ac.uk/water/hycmc.html [27 December, 2009]

Chen, A. L., Wang, K. R., Xie, X. L., and Liu, Y. X. 2007. Responses of microbial biomass P to the changes of organic C and P in paddy soils under different fertilization systems. Chinese Journal of Applied Ecology 18(12): 2733-2738.

Chernin, L., and Chet, I. 2002. Microbial enzymes in the biocontrol of plant pathogens and pests. In Burns, R. G., and Dick, R. P. (eds.), Enzymes in the environment : Activity, ecology, and applications. the United States of America: Marcel Dekker.

Coleman, D. C., and Wall, D. H. 2007. Fauna: The engine for microbial activity and transport. In Eldor A., P. (ed.), Soil microbiology, ecology, and biochemistry 3rd ed. the United States of America: Elsevier

Davet, P. 2004. Microbial ecology of the soil and plant growth. India: Science Publishers.

Demoling, F., Ola Nilsson, L., and Bååth, E. 2008. Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. Soil Biology and Biochemistry 40(2): 370-379.

Deng, S. P., and Tabatabai, M. A. 1994. Cellulase activity of soils. Soil Biology & Biochemistry 26(10): 1347-1354.

Deng, S. P., and Tabatabai, M. A. 1995. Cellulase activity of soils: Effect of trace elements. Soil Biology and Biochemistry 27(7): 977-979.

Department of Agriculture, Ministry of Agricultural and Cooperatives. 2002. วิวัฒนาการผลิตข้าวไทย. กรุงเทพมหานคร: นิวธรรมดาการพิมพ์.

Department of Agriculture, Ministry of Agricultural and Cooperatives. 2004. คำแนะนำการใช้ปุ๋ยเคมีในนาข้าวตามค่าวิเคราะห์ดิน [Online]. Rice Department. Available from: http://www.brrd.in.th/rkb/data_004/rice_xx2-04_manage_006.html [27th June 2008]

Eldor, A. P. 2007. Soil microbiology, ecology and biochemistry in perspective. In Eldor A., P. (ed.), Soil microbiology, ecology, and biochemistry 3rd ed. the United States of America: Elsevier

Environmental Microbiology [Online]. 2009. Available from: http://water.me.vccs.edu/courses/ENV108/clipart/cellwall.gif [25 December, 2009].

Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenström, J., and Hallin, S. 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biology & Biochemistry 39 (1): 106–115.

Fierer, N., Schimel, J. P., and Holden, P. A. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35(1): 167-176.

Finlay, R. D. 2007. The fungi in soil. In van Elsas, J. D., Jansson, J. K., and Trevors, J. T. (eds.), Modern soil microbiology 2nd ed., pp. 107-146. the United States of America: CRC Press.

Food and Agriculture Organization. 2002. The State of Food and Agriculture 2002 [online]. Food and Agriculture Organization. Available from: http://www.fao.org/DOCREP/004/Y6000E/y6000e44.jpg [17 May 2010]

Frostegård, Å., and Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22: 59-65.

Frostegård, Å., Bååth, E., and Tunlid, A. 1993a. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology and Biochemistry 25(6): 723-730.

Frostegård, Å., Tunlid, A., and Bååth, E. 1993b. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59(11): 3605-3617.

Frostegård, Å., Tunlid, A., and Bååth, E. 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biology and Biochemistry 28(1): 55-63.

Greenland, D. J. 1997. The sustainability of rice farming. the United Kingdom: CAB International.

Hartei, P. G. 2005. The soil habitat. In Sylvia, D. M., Fuhrmann, J. J., Hartel, P. G., and Zuberer, D. A. (eds.), Principles and applications of soil microbiology 2nd ed., pp. 26-52. New Jersey: Pearson Prentice Hall.

Hartmann, M., Frey, B., Kölliker, R., and Widmer, F. 2005. Semi-automated genetic analyses of soil microbial communities: comparison of T-RFLP and RISA based on descriptive and discriminative statistical approaches. Journal of Microbiological Methods 61(3): 349-360.

Hashimoto, S. 2002. A simple technique to analyze a small volume of soil CO_2 gas using an infrared gas analyzer. Soil Biology and Biochemistry 34(2): 273-275.

Hedrick, D. B., Peacock, A. D., and White, D. C. 2005. Interpretation of fatty acid profiles of soil microorganisms. In Margesin, R., and Schinner, F. (eds.), Soil biology Vol. 5, pp. 251-259. Berlin Heidelberg: Springer-Verlag.

Hedrick, D. B., Peacock, A. R., and White, D. C. 2007. Lipid analyses for viable microbial biomass, community composition, metabolic status, and *in situ* metabolism. In Hurst, C. J., Crawford, R. L., Mills, A. L., Garland, J. L., Stetzenbach, L. D., and Lipson, D. A. (eds.), Manual of environmental microbiology 3rd ed., pp. 112-121. Washington, D.C.: ASM press.

Hopkins, D. W. 2008. Carbon mineralization. In Carter, M. R., and Gregorich, E. G. (eds.), Soil sampling and methods of analysis 2nd ed., pp. 589-598. the United States of America: CRC Press.

Hopkins, D. W., Sparrow, A. D., Shillam, L. L., English, L. C., Dennis, P. G., Novis, P., Elberling, B., Gregorich, E. G., and Greenfield, L. G. 2008. Enzymatic activities and microbial communities in an Antarctic dry valley soil: Responses to C and N supplementation. Soil Biology and Biochemistry 40(9): 2130-2136.

Horwath, W. 2007. Carbon cycling and formation of soil organic matter. In Eldor A., P. (ed.), Soil microbiology, ecology, and biochemistry 3rd ed. the United States of America: Elsevier

Introduction and historical perspective. 2005. In Sylvia, D. M., Fuhrmann, J. J., Hartel, P. G., and Zuberer, D. A. (eds.), Principles and applications of soil microbiology 2nd ed., pp. 8-9. New Jersey: Pearson Prentice Hall.

Jensen, L. S., Mueller, T., Tate, K. R., Ross, D. J., Magid, J., and Nielsen, N. E. 1996. Soil surface CO2 flux as an index of soil respiration in situ: A comparison of two chamber methods. Soil Biology and Biochemistry 28(10-11): 1297-1306.

Jjemba, P. K. 2004. Environmental microbiology : Principles and applications. India: Science Publishers.

Kandeler, E. 2007. Physiological and biochemical methods for studying soil biota and their function. In Eldor A., P. (ed.), Soil microbiology, ecology, and biochemistry 3rd ed. the United States of America: Elsevier

Kaur, A., Chaudhary, A., Choudhary, R., and Kaushik, R. 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Current Science 89(7): 1103-1112.

Khentha, K. 2008. Effect of organic fertilizers on some soil prooperties and Yield of Chinese Teas. Master's Thesis, Department of Soil Science, Kasetsart University, Bangkok.

Killham, K., and Prosser, J. I. 2007. The Prokaryotes. In Eldor A., P. (ed.), Soil Microbiology, Ecology, and Biochemistry 3rd ed. the United States of America: Elsevier Inc.

Kimura, M., and Asakawa, S. 2006. Comparison of community structures of microbiota at main habitats in rice field ecosystems based on phospholipid fatty acid analysis. Biology and Fertility of Soils 43(1): 20-29.

Kimura, M., Miyaki, M., Fujinaka, K. I., and Maie, N. 2001. Microbiota responsible for the decomposition of rice straw in a submerged paddy soil estimated from

phospholipid fatty acid composition. Soil Science and Plant Nutrition 47(3): 569-578.

Krishnamurthy, V. N., Gowariker, S., Dhanokar, M., and Paranjape, K. (eds.). 2009. Ammonium sulphate. The fertilizer encyclopedia p. 52. the United States of America: John Wiley & Sons.

Kyuma, K. 2004. Paddy soil science. Melbourne: Kyoto University Press.

Land Development Department, Ministry of Agricultural and Cooperatives. 2008. การเก็บตัวอย่างดิน [Online]. Land Development Department. Available from: http://osd101.ldd.go.th/services/Soil/Soil%20Manual.doc [30th July, 2008].

Li, J., Zhao, B.-q., Li, X.-y., Jiang, R.-b., and Bing, S. H. 2008. Effects of Long-Term Combined Application of Organic and Mineral Fertilizers on Microbial Biomass, Soil Enzyme Activities and Soil Fertility. Agricultural Sciences in China 7(3): 336-343.

Li, Z. p., Wu, X. c., and Chen, B. y. 2007. Changes in Transformation of Soil Organic C and Functional Diversity of Soil Microbial Community Under Different Land Uses. Agricultural Sciences in China 6(10): 1235-1245.

Liao, M., Xie, X., Subhani, A., and Klose, S. 2002. Combined effect of nutrient and pest managements on substrate utilization pattern of soil microbial population in hybrid rice cropping system. Pedosphere 12(3): 219-228.

Lin, X. G., Yin, R., Zhang, H. Y., Huang, J. F., Chen, R. R., and Cao, Z. H. 2004. Changes of soil microbiological properties caused by land use changing from rice-wheat rotation to vegetable cultivation. Environmental Geochemistry and Health 26(2): 119-128.

Luo, Y., and Zhou, X. 2006. Soil respiration and the environment. Lodon: Academic Press.

Maclean, J. L., Dawe, D. C., Hardy, B., and Hettel, G. P. (eds.). 2002. Rice almanac : Source book for the most important economic activity on earth 3rd ed. p.^pp. Pages. China: CABI Publish.

Maier, R. M., and Pepper, I. L. 2009. Earth environments. In Maier, R. M., Pepper, I. L., and Gerba, C. P. (eds.), Environmental microbiology 2nd ed., pp. 57-82. China: Academic Press.

Mandal, A., Patra, A. K., Singh, D., Swarup, A., and Masto, R. E. 2007. Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology 98: 3585–3592.

Manning, K. 1981. Improved viscometric assay for cellulase. Journal of Biochemical and Biophysical Methods 5(4): 189-202.

Marschner, P. 2007. Soil microbial community structure and function assessed by FAME, PLFA and DGGE – Advantages and limitations. In Varma, A., and Oelmuller, R. (eds.), Soil biology: Advanced techniques in soil microbiology. Vol. 11, pp. 181-200. Berlin: Springer-Verleg.

Marschner, P., Kandeler, E., and Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry 35(3): 453-461.

Matsuyama, T., Nakajima, Y., Matsuya, K., Ikenaga, M., Asakawa, S., and Kimura, M. 2007. Bacterial community in plant residues in a Japanese paddy field estimated by RFLP and DGGE analyses. Soil Biology and Biochemistry 39(2): 463-472.

Methaluk, P. 2004. The effects of organic and chemical fertilizers on yield of chinese kale (*Brassica oleracea* L.) cv. RS1 and some soil properties. Master's Thesis, Department of Soil Science., Kasetsart University, Bangkok.

Nakamura, A., Tun, C. C., Asakawa, S., and Kimura, M. 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: estimation by phospholipid fatty acid analysis. Biology and Fertility of Soils 38: 288-295.

National Statistical Office. 2003. Number of Holdings With Crop by Using Fertilizer, Type of Fertilizers and Size of Total Area of Holding : 2003 Agricultural Census Whole Kingdom [Online]. National Statistical Office,

Ministry of Information and Communication Technology. Source: http://web.nso.go.th/en/census/agricult/cen_agri03.htm [15 December 2009]

Nayak, D. R., Babu, Y. J., and Adhya, T. K. 2007. Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biology and Biochemistry 39(8): 1897-1906.

Öhlinger, R. 1996. Soil respiration by titration. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in soil biology pp. 95-98. Germany: Springer.

Okabe, A., Toyota, K., and Kimura, M. 2000. Seasonal variations of phospholipid fatty acid composition in the floodwater of a Japanese paddy field under a long-term fertilizer trial. Soil Science and Plant Nutrition 46(1): 177-188.

Peacock, A. D., Mullen, M. D., Ringelberg, D. B., Tyler, D. D., Hedrick, D. B., Gale, P. M., and White, D. C. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biology and Biochemistry 33(7-8): 1011-1019.

Photong, P. 2008. The comparison of composts efficiency in improvement of properties and plant nutrient adsorption of kamphaeng sean soil series. Master's Thesis, Department of Soil Science., Kasetsart University, Bangkok.

Plaster, E. J. 2009. Fertilizers Soil science & management 5th ed. New York: Delmar Cengage Learning.

Purdy, K. J., Nedwell, D. B., Embley, T. M., and Takii, S. 1997. Use of 16S rRNA-targeted oligonucleotide probes to investigate the occurrence and selection of sulfate-reducing bacteria in response to nutrient addition to sediment slurry microcosms from a Japanese estuary. FEMS Microbiology Ecology 24(3): 221-234.

Rice Department, Ministry of Agricultural and Cooperatives. 2008. Rice knowledge bank [Online]. Rice Department. Available form: http://www.brrd.in.th/rkb/index.html [27th June, 2008]

Rice Department, Ministry of Agricultural and Cooperatives. 2008. ข้าวนาชลประทาน : นาหว่านน้ำตม [Online]. Rice Department. Available form: http://www.brrd.in.th/rkb/data_004/rice_xx2-04_manage_001-6.html [27th June, 2008]

Roane, T. B., Reynold, K. A., Maier, R. M., and Pepper, I. L. 2009. Microorganisms. In Maier, R. M., Pepper, I. L., and Gerba, C. P. (eds.), Environmental microbiology 2nd ed., pp. 9-28. China: Academic Press.

Romero, M. D., Aguado, J., González, L., and Ladero, M. 1999. Cellulase production by Neurospora crassa on wheat straw. Enzyme and Microbial Technology 25(3-5): 244-250.

Rousk, J., Brookes, P. C., and Bååth, E. 2010. The microbial PLFA composition as affected by pH in an arable soil. Soil Biology and Biochemistry 42(3): 516-520.

Saha, S., Prakash, V., Kundu, S., Kumar, N., and Mina, B. L. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. European Journal of Soil Biology 44(3): 309-315.

Sarathchandra, S. U., Ghani, A., Yeates, G. W., Burch, G., and Cox, N. R. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. Soil Biology and Biochemistry 33: 953-964.

Schinner, F., and von Mersi, W. 1990. Xylanase-, CM-cellulase- and invertase activity in soil: An improved method. Soil Biology and Biochemistry 22(4): 511-515.

Semenov, A. M., Batomunkueva, B. P., Nizovtseva, D. V., and Panikov, N. S. 1996. Method of determination of cellulase activity in soils and in microbial cultures, and its calibration. Journal of Microbiological Methods 24(3): 259-267.

Sharrock, K. R. 1988. Cellulase assay methods: a review. Journal of Biochemical and Biophysical Methods 17(2): 81-105.

Singer, M. J., and Munns, D. N. 2006. Soils : An introduction. 6th ed. the United States of America: Pearson Prentice Hall.

Šnajdr, J., Valášková, V., Merhautová, V., Cajthaml, T., and Baldrian, P. 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry 40(9): 2068-2075.

Soil Analysis Division, Ministry of Agriculture and Cooperatives. 2001. Soil chemical analysis. n.p., n.d.

Soilsensor.com. 2008. Soil Type and Identification [Online]. Available from: http://www.soilsensor.com/images/soiltriangle_large.jpg [25 December, 2009]

Standing, D., and Killham, K. 2007. The soil environment. In van Elsas, J. D., Jansson, J. K., and Trevors, J. T. (eds.), Modern soil microbiology 2nd ed. the United States of America: CRC Press.

Stealth Growth, LLC. 2009. Stealth Grow Room Setup Guidelines [online]. Available from: http://www.jackscreative.com/_stealthdev/site/images/nutrient-ph-range.jpg [17 March, 2010]

Steenwerth, K. L., Jackson, L. E., Calderón, F. J., Stromberg, M. R., and Scow, K. M. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology & Biochemistry 34(11): 1599-1611.

Tan, K. H. 2005. Soil Sampling, Preparation, and Analysis. 2nd ed. the United States of America: CRC Press.

Targeting Cancer Cells Using Lipid-based Drug Delivery Vehicles [Online]. Available from: http://www.bioteach.ubc.ca/Bio-industry/Inex/ [17 March, 2010].

Tate, R. L. 2000. Soil microbiology. 2nd ed. the United States of America: John Wiley & sons.

Thai Rice Foundation under Royal Patronage. 2006. การทำนา [Online]. Thai Rice Foundation under Royal Patronage. Available from: http://www.thairice.org/html/aboutrice/about_rice3.htm [10th December, 2009]

Tom-Petersen, A., Leser, T. D., Marsh, T. L., and Nybroe, O. 2003. Effects of copper amendment on the bacterial community in agricultural soil analyzed by the T-RFLP technique. FEMS Microbiology Ecology 46(1): 53-62.

Toyota, K., and Kuninaga, S. 2006. Comparison of soil microbial community between soils amended with or without farmyard manure. Applied Soil Ecology 33(1): 39-48.

Troch, F. R., and Thompson, L. M. 2005. Soils and soil fertility. 6th ed. India: Blackwell Publish.

Trolldenier, G. 1996. Plate count technique. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in soil biology pp. 20-26. Germany: Springer-Verleg.

van Elsas, J. D., Torsvik, V., Hartmann, A., Øvreås, L., and Jansson, J. K. 2007. The bacteria and archaea in soil. In van Elsas, J. D., Jansson, J. K., and Trevors, J. T. (eds.), Modern soil microbiology 2nd ed., pp. 83-105. the United States of America: CRC Press.

von Mersi, W., and Schinner, F. 1996. CM-cellulase activity. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in soil biology pp. 190-193. Germany: Springer.

Waldrop, M. P., Balser, T. C., and Firestone, M. K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry 32(13): 1837-1846.

Wang, L.-S., Zhang, Y.-Z., Yang, H., and Gao, P.-J. 2004. Quantitative estimate of the effect of cellulase components during degradation of cotton fibers. Carbohydrate Research 339(4): 819-824.

Watcharamul, S. 2005. Microbial communities of Thai rice field soils during rice straw decomposition. Department of Agricultural and Environmental Science, University of Newcastle upon Tyne.

Watcharamul, S., and O'Donnell, A.G. 2002. Cellulase diversity in Thai paddy soils. Proceedings of the 17th World Congress of Soil Science. Bangkok, Thailand.

Wei, D., Yang, Q., Zhang, J.-Z., Wang, S., Chen, X.-L., Zhang, X.-L., and Li, W.-Q. 2008. Bacterial community structure and diversity in a black soil as affected by long-term fertilization. Pedosphere 18(5): 582-592.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbie, R. J. 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40(1): 51-62.

White, R. E. 2006. Principles and practice of soil science : The soil as a natural resource. 4th ed. the United Kingdom: Blackwell Publishing.

Wood, T. M., and Bhat, K. M. 1988. Methods for measuring cellulase activities. In Willis, A. W., and Scott, T. K. (eds.), Methods in enzymology Vol. 160, pp. 87-112: Academic Press.

Yan, H., Zhong, W. H., Li, Z. P., and Cai, Z. C. 2008. Effects of long-term fertilization on phospholipid fatty acids and enzyme activities in paddy red soil. Chinese Journal of Applied Ecology 19(1): 71-75.

Yao, F., Wu, Q., Lei, Y., and Xu, Y. 2008. Rice straw fiber-reinforced high-density polyethylene composite: Effect of fiber type and loading. Industrial Crops and Products 28(1): 63-72.

Zabaloy, M. C., Lehman, R. M., Frey, S. D., and Garland, J. L. 2008. Optimization of an oxygen-based approach for community-level physiological profiling of soils. Soil Biology and Biochemistry 40(12): 2960-2969.

Zelles, L. 1996. Fatty acid patterns of microbial phospholipids and lipopolysaccharides. In Schinner, F., Öhlinger, R., Kandeler, E., and Margesin, R. (eds.), Methods in soil biology pp. 80-92. Germany: Springer.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial community. Chemosphere 35: 275-294.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29 111–129.

Zelles, L., Bai, Q. Y., Ma, R. X., Rackwitz, R., Winter, K., and Beese, F. 1994. Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and poly-hydroxybutyrate in agriculturally-managed soils. Soil Biology and Biochemistry 26(4): 439-446.

Zhang, P., Zheng, J., Pan, G., Zhang, X., Li, L., and Rolf, T. 2007. Changes in microbial community structure and function within particle size fractions of a paddy soil under different long-term fertilization treatments from the Tai Lake region, China. Colloids and Surfaces B: Biointerfaces 58(2): 264-270.

Zhang, Q., Wang, G., and Yao, H. 2007. Phospholipid fatty acid patterns of microbial communities in paddy soil under different fertilizer treatments. Journal of Environmental Sciences 19(1): 55-59.

Zhang, Y. F., Zhong, W. H., Li, Z. P., and Cai, Z. C. 2006. Effects of long-term different fertilization on soil enzyme activity and microbial community functional diversity in paddy soil derived from quaternary red clay. Journal of Ecology and Rural Environment 22(4): 39-44.

APPENDICES

APPENDIX A

Fertilizer Solution Preparation

Fertilizer Solution Preparation

The area of 1 rai = 1600 m^2 = 16000000 cm^2

The volume of soil at 15 cm dept in 1 rai = $1.6 \times 10^7 cm^2 \times 15$ cm

= $2.40 \times 10^8 cm^3$

Soil density = 1.3 g/cm^3

Therefore, the weight of 1 rai soil = $1.3\ g/cm^3 \times 2.40 \times 10^8 cm^3$

= 3.12×10^8 g or 3.12×10^5 kg

Recommended dosage of 16-20-0 mixed chemical fertilizer is 25 kg·rai or 25×10^3 g.

soil 3.12×10^5 kg take 25×10^3 g of chemical fertilizer

soil 1 kg take 25×10^3 g $\times 1$ kg / 3.12×10^5 kg

soil 1 kg take 8.01 g of chemical fertilizer

APPENDIX B

Soil Properties Analysis

B.1 Water Holding Capacity ; WHC (modified from Öhlinger, 1996)

Methodology

1. Weigh 50 g of 2 mm sieved soil in the cylinder. Put the cylinder into a 1000 ml beaker, and then pour the water into the beaker as shown in Figure B.1. The water level should be as high as the soil column in the cylinder.

2. Leave the cylinder in the beaker for an hour. After that, place the cylinder on a funnel to let the excess water to drain for at least 3 hours. Cover the cylinder with a watch glass.

3. After draining, weigh 30 g of saturated soil on an aluminum foil that already weigh and tare. Dry the sample at 105°C overnight and then cool it down in a desiccator before weigh in.

4. The maximum water holding capacity (WHC), expressed as g water ·100 g^{-1}dm, is calculated follow by this equation

$$\frac{(\text{saturated soil} - \text{dried soil}) \cdot 100}{\text{dried soil}} = \%\text{WHC}$$

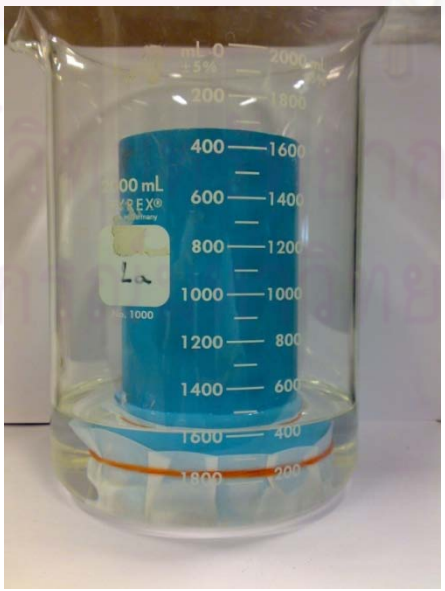


Figure B.1 Water holding capacity apparatus.

B.2 Dry Matter and Water Content (modified from Öhlinger, 1996)

Methodology

1. Weigh 10-30 g fresh soil on pre-weighted aluminum foil, record the weight.

2. Dry sample at 105°C for at least 3 hours.

3. Cool the sample in a desiccator, and weigh again.

4. Calculate soil dry matter and water content follow below equations.

weight after drying – aluminum foil weight = soil dry matter

$$\frac{\text{soil dry matter} \cdot 100}{\text{initial soil weight}} = \%\text{dry matter(dm)}$$

$$100 - \%dm = \%\text{water content (wc)}$$

B.3 Soil Texture (modified from Tan, 2005)

Special Equipments

Standard hydrometer, ASTM No. 1. 152H, with Bouyoucos scale in g L^{-1}

Reagents and preparation

1. Amyl alcohol

2. 4% Calgon® solution

Dissolve 40 g sodium metaphosphate [$(NaPO_3)_x \cdot Na_2O$,(x~13)] with 250 ml distilled water. Adjust to 1000 ml in volumetric flask.

Methodology

1. Weigh 50 g of oven dried, sieved soil and transfer into a blender cup. Fill the blender cup with 400 ml of distilled water, and then add 10 ml of 4% Calgon® solution.

2. Attach the cup to a stirring machine and blend for 15 minutes.

3. Transfer the soil suspension into a cylinder. Wash the remaining soil residue with water from a water bottle. Make up the volume to 1130 ml with distilled water.

4. Mix the soil suspension thoroughly with a plunger. Record the time when stirring stopped. Measure the suspension temperature with a thermometer. Drop a few drops of amyl alcohol if bubbles are occur.

5. Lower the hydrometer carefully into the suspension and take readings after 40 seconds (R40s). Remove the hydrometer, rinse it, and wipe it dry.

6. Mix the soil suspension thoroughly with a plunger again. Reinsert the hydrometer carefully and take another reading after 2 hours (R2h) and measure the suspension temperature with a thermometer.

Calculation of readings

The hydrometer readings have to be adjusted due to the calibration temperature of the hydrometer is 68°F. Corrected reading is calculated followed by this equation.

Hydrometer reading + 0.2 (°F suspension temperature-68)

The corrected readings are used to calculate %sand, %silt, and %clay followed by these equations below.

%sand + %silt + %clay = 100%

%(silt+clay) = (R40s / soil dry weight) × 100 (a)

%sand = 100 - (a)

%clay = (R2h / soil dry weight) × 100 (b)

%silt = (a)-(b)

Read these results on soil texture triangle (Figure B.2) to see what the soil class name of the sample is.

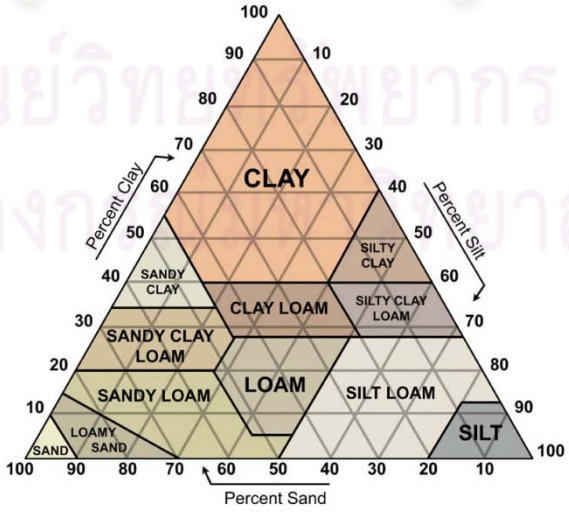


Figure B.2 Soil texture triangle. (Soilsensor.com, 2008 : online)

B.4 Soil pH (Soil Analysis Division, 2001)

Special Equipments

pH meter (Mettler Toledo, Model SevenEasy™ pH)

Reagents and preparation

1. 0.01 M calcium chloride ($CaCl_2$) 100 ml

Dissolve 0.1470 g $CaCl_2$ with a little amount of distilled water. Adjust to 100 ml in 100 ml volumetric flask.

2. 1.0 M potassium chloride (KCl) 100 ml

Dissolve 7.456 g KCl with a little amount of distilled water. Adjust to 100 ml in 100 ml volumetric flask.

3. Buffer solution pH 4 and pH 7

Methodology

1. Weigh 10 g of 2 mm air dried, sieved soil into 50 ml beaker.

2. Add 25 ml distilled water (or 0.01 M $CaCl_2$ or 1.0 M KCl) to sample beaker

3. Mix soil sample and solvent with stirring rod. Allow it to settle for 30 minutes (Figure B.3).

4. Calibrate pH meter with buffer solution pH 4 and pH 7 before taking a measure of the sample pH.

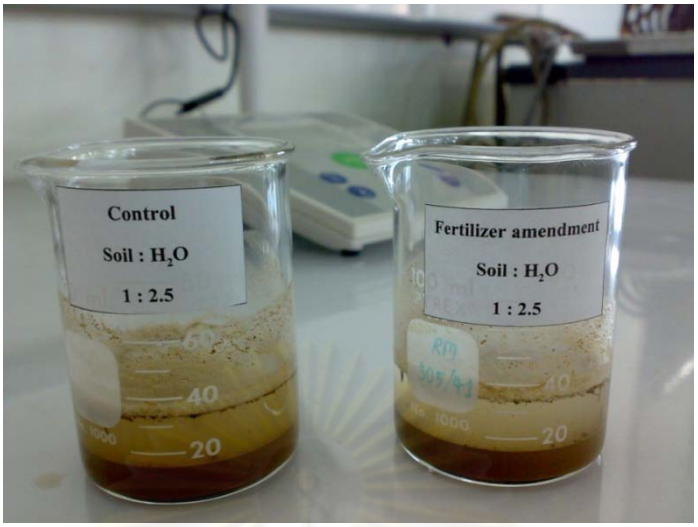


Figure B.3 Soil suspension ready for pH measurement.

B.5 Cation Exchange Capacity ; CEC (Ariyakanon, 2007)

Special Equipments

Büchi distillation unit (model Büchi 339)

Reagents preparation

1. 1 N ammonium acetate (NH_4OAc) pH 7

Dissolve 77.08 g NH_4OAc in distilled water, and then adjust to 1000 ml in volumetric flask.

2. 95% Ethanol

Pour 950 ml absolute ethanol into 1000 ml volumetric flask. Make up the volume to 1000 ml with distilled water.

3. 0.1 N HCl

Add 8.6 ml 37% HCl into 1000 ml volumetric flask contained 800 ml distilled water. Slowly pour distilled water to adjust solution volume to 1000 ml.

4. 32% NaOH

Dissolve 960 g NaOH pellet in 2.5 L distilled water. Stir until pellets thoroughly dissolve. Cool down the solution in water bath before adjust volume to 3 L with distilled water.

5. 2% Boric acid (H_3BO_3)

Dissolve 60 g H_3BO_3 and adjust volume to 3 L with distilled water.

6. 0.1 N H_2SO_4

Add 2.8 ml H_2SO_4 to 800 ml distilled water in 1000 ml volumetric flask. Adjust to 1000ml.

Methodology

1. Weigh 10 g 0.5 mm sieved air dried soil into 500 ml Erlenmeyer flask. Add 50 ml NH_4OAc pH 7 into the flask. Shake it for 30 minutes with shaker.

2. Pour soil suspension on filter paper which placed on Buchner funnel connected to suction flask and vacuum pump. Wash soil with 50 ml NH_4OAc. Turn the vacuum on to drain solution from soil, repeat 3 times. Discard solution in suction flask.

3. Wash soil on filter paper with 50 ml 95% ethanol, repeat 3 times. Discard solution in suction flask.

4. Wash soil on filter paper with 50 ml 0.1 N HCl, repeat 4 times. Collect solution in suction flask and measure solution volume with a cylinder. Divide 1/10 of total volume and pour it into a distillation glass tube. Blank is set up without soil sample and done as the sample.

5. Prepare the distillation unit by filling 32% NaOH, 2% H_3BO_3, and distilled water into its tank and open the condenser. Insert a bare distilled glass tube into the distillation unit. Calibrate pH probe, run pre-heating, priming the distillation unit follow by manuscript. Remove the bare tube and replace with the blank tube.

6. Set the machine to fill 60 ml distilled water, 80 ml 32% NaOH, and 65 ml H_3BO_3 for titration. Run blank test. Replace the blank tube with sample tube, input samples weight as 10% of initial weight, then run the machine.

7. Calculate CEC from this equation

$$\text{CEC (me/100g)} = \frac{(T - B) \times \text{normality of } H_2SO_4 \times 100}{\text{10\% of initial soil weight (g)}}$$

When T = volume of 0.1 N H_2SO_4 titrated with sample (ml)

B = volume of 0.1 N H_2SO_4 titrated with blank (ml)

B.6 Organic Matter; OM (Walkley and Black method)(Soil Analysis Division, 2001)

Reagents and preparation

1. 1 N Potassium dichromate

Heat 98 g of potassium dichromate ($K_2Cr_2O_7$) at 105^oC for 2 hours. Dissolve in distilled water and adjust volume to 2 L.

2. 0.5 N Ferrous ammonium sulphate

Dissolve 400 g of ferrous ammonium sulphate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ in distilled water. Add 50 ml conc. H_2SO_4 before adjust volume to 2 L with distilled water.

3. 0.025 M O – Phenanthroline indicator

Dissolve 0.7 g of ferrous sulphate ($FeSO_4 \cdot 7H_2O$) and 1.48 g of O-phenanthroline in distilled water and adjust to 100 ml.

4. Conc. H_2SO_4

Methodology

1. Weigh 1 g of 2 mm sieved air dried soil into a 250 ml Erlenmeyer flask.

2. Pipette 10 ml of 1 N $K_2Cr_2O_7$ and 15 ml of conc. H_2SO_4. Stir the flask gentle for 1 – 2 minutes then stand it in the fume hood for 30 minutes.

3. Add 50 ml distilled water and leave it cool.

4. Drop 7 – 9 drops of O – Phenanthroline indicator Titrate with 0.5 N Ferrous ammonium sulphate to determined remain potassium dichromate from reaction. The colour of solution at the end point is turn from green to red – brown (Figure B.4 and Figure B.5). Note the volume of potassium dicromate and ferrous ammonium sulphate.

5. Blank is done the same as the sample but without soil.

6. Calculate % Organic carbon and % organic matter from these below equations.

$$\%\text{Organic carbon} = \frac{(B-T)\,N}{B} \times \frac{100}{7} \times \frac{3}{10^3} \times \frac{100}{x} \times 10$$

$$\%\ \text{Organic matter} = \%\ \text{Organic carbon} \times 1.724$$

$$\text{Or}\ \ \%\ \text{Organic matter} = \frac{(B-T)\,N}{B} \times \frac{100}{77} \times \frac{100}{58} \times \frac{3}{10^3} \times \frac{100}{X} \times 10$$

When N = Concentration of $K_2Cr_2O_7$ (Normal)

B = Volume of Ferrous ammonium sulphate titrate with blank (ml)

T = Volume of Ferrous ammonium sulphate titrate with Sample (ml)

X = Soil weight (g)

Figure B.4 The red-brown solution at the end point.

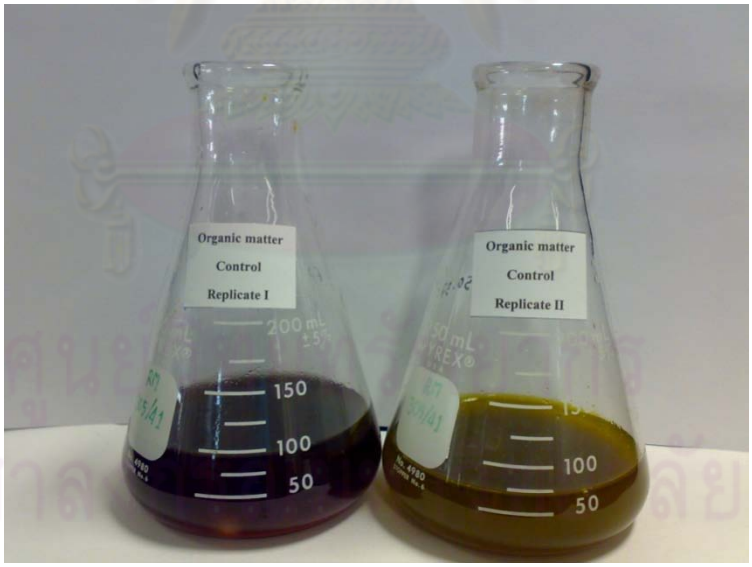


Figure B.5 The green solution on right hand turns to red-brown at the end point on left hand.

B.7 Total Nitrogen Analysis (Ariyakanon, 2007)

Special Equipments

1. Büchi digestion unit (model K-435)

2. Büchi distillation unit (model Büchi 339)

3. Büchi scrubber (model B-414)

Reagent preparation

1. Conc. H_2SO_4

2. 32% NaOH (w/v)

Dissolve 960 g NaOH pellet in 2.5 L distilled water. Stir until pellets thoroughly dissolve. Cool down the solution in water bath before adjust volume to 3 L with distilled water.

3. 2% Boric acid (H_3BO_3)

Dissolve 60 g H_3BO_3 and adjust volume to 3 L with distilled water.

4. 0.1 N H_2SO_4

Add 2.8 ml H_2SO_4 to 800 ml distilled water in 1000 ml volumetric flask. Adjust to 1000 ml.

5. Catalyst

Mix 100 g potassium sulphate (K_2SO_4), 10 g copper sulphate ($CuSO_4 \cdot 5H_2O$), and 1 g selenium (Se) together. Prepare before use.

Methodology

1. Weigh 0.5 g of 0.5 mm sieved, air dried soil into distilled glass tube, then add 7 g catalyst into the tube. The blank tube is added only catalyst.

2. Add 20 ml Conc. H_2SO_4 before inserts the tubes into the digestion unit. Scrubber unit must be on when the digestion unit programme is running.

3. Run the digestion programme until the sample solution colour turn to clear blue-green as shown in Figure B.6. Stop the programme, cool down the tubes in fume hood (scrubber unit must be on).

4. Prepare the distillation unit by filling 32% NaOH, 2% H_3BO_3, and distilled water into its tank and open the condenser. Insert a bare distilled glass tube into the distillation unit. Calibrate pH probe, run pre-heating, priming the distillation unit follow by manuscript. Remove the bare tube and replace with the blank tube.

5. Set the machine to fill 60 ml distilled water, 80 ml 32% NaOH, and 65 ml H_3BO_3 for titration. Run blank test. Replace the blank tube with sample tube, put in all samples weight, then run the machine. The machine is automatic calculate. Total nitrogen percent is calculated follow by this equation.

$$\%\text{total nitrogen} = \frac{(S - B) \times \text{normality of } H_2SO_4 \times 1.4}{\text{Soil weight (g)}}$$

When S = volume of 0.1 N H_2SO_4 titrated with sample (ml)

B = volume of 0.1 N H_2SO_4 titrated with blank (ml)

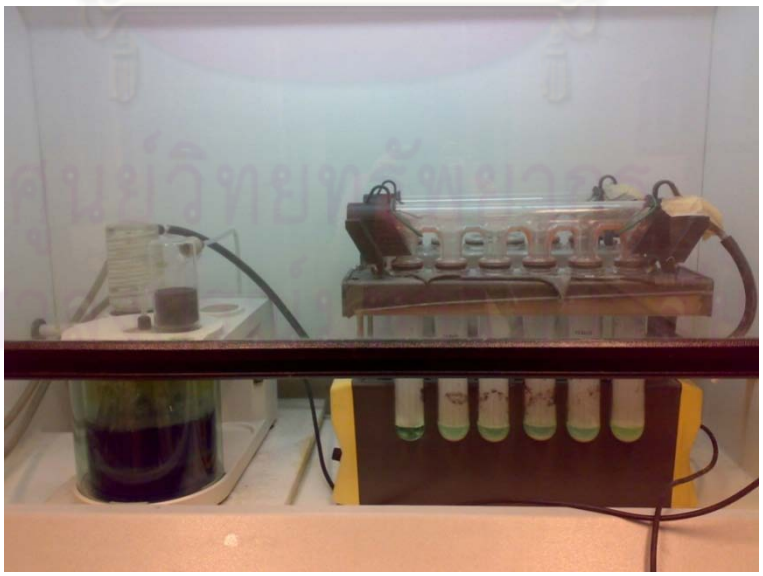


Figure B.6 The sample solution colour turn to clear blue-green after the digestion was done.

B.8 Available Phosphorus (Bray II method) (Soil Analysis Division, 2001)

Special Equipment

Spectrophotometer (GeneSys 20 Thermo spectronic)

Reagents preparation

1. Bray II extract solution (0.03 N NH_4F, 0.1 N HCl)

Dissolve ammonium fluoride (NH_4F) 1.1100 g with in 800 ml of distilled water. Add 8.6 ml of conc. HCl follow by make volume to 1 L with distilled water. Adjust to pH 1.5 – 1.6.

2. Stock solution (Reagent A : Sulfuric – molybdate – tartrate solution)

1) Dissolve 50 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$)] with 200 ml of distilled water. Stir until the solution is thoroughly mixed.

2) Dissolve 1.213 g of antimony potassium tartrate ($KSbO\cdot C_4H_4O_6$) with 50 ml distilled water in new beaker. This solution can be warmed under 60^{o}c incase it is not thoroughly dissolve.

3) Mix two solutions together. Add 700 ml of conc. H_2SO_4 slowly. Stand the solution beaker in water bath to cool down the solution.

4) Adjust volume to 1 L in volumetric flask with distilled water. Transfer this solution into polyethylene bottle of brown Pyrex bottle. Store it in a dark and cool place. This solution expired in 6 months.

3. Working solution; Reagent B

Dissolve 0.088 g of ascorbic acid with 80 ml of distilled water. Add 2 ml of reagent A, mix and adjust the volume to 100 ml in volumetric flask. Leave it to cool down about 2 hours before use. This solution must prepare freshly.

4. Standard phosphorus solution (50 ppm P)

Dissolve phosphorus dihydrogen phosphate (KH_2PO_4) which dried at 40^{o}C for 2 hours 0.2195 g with distilled water. Add a few drops of conc. H_2SO_4 before adjust the volume to 1 L in volumetric flask with distilled water.

5. Standard solution set

Use the solution from number 4 to make dilution of standard solution set with 0, 2, 4, 6, 8, 10 and 15 ppm P Concentrations which diluted with Bray II extract solution.

Methodology

1. Weigh 1.0000 g air dried soil into 50 ml Erlenmeyer flask.

2. Add 10 ml of Bray II extract solution. Shake for 1 minute and filtrate the solution with Whatman® No. 42 filter paper. Collect the filtrate.

3. Pipette the filtrate and mix with working solution with 1 : 16 ratio in test tube. Stand it for 30 minutes for colour development as shown in Figure B.7.

4. The concentration is measured at 882 nm with spectrophotometer against a reagent blank and standard solution set which are done same as the filtrate.

5. Calculation of available P is follow by this equation.

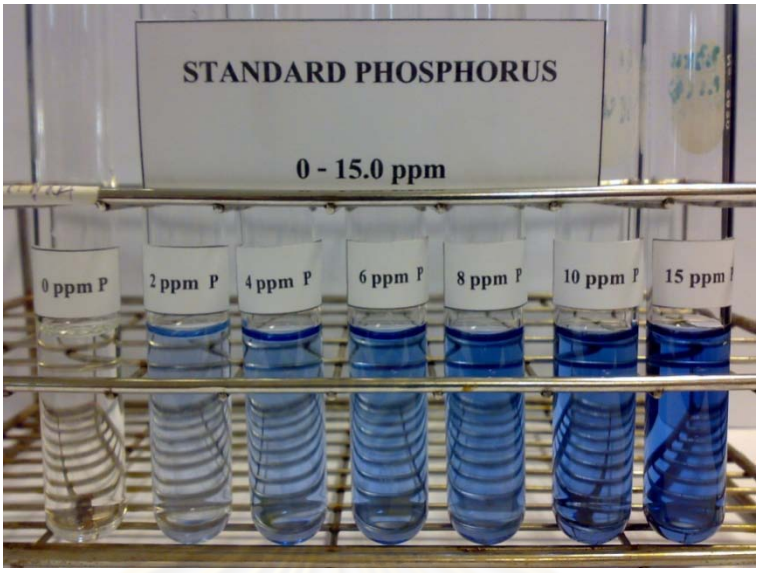
$$\text{Available P} = \frac{B \times DF\ (\text{sample}) \times X}{A \times DF\ (\text{standard})} \quad \text{ppm}$$

When A = soil weight (g)

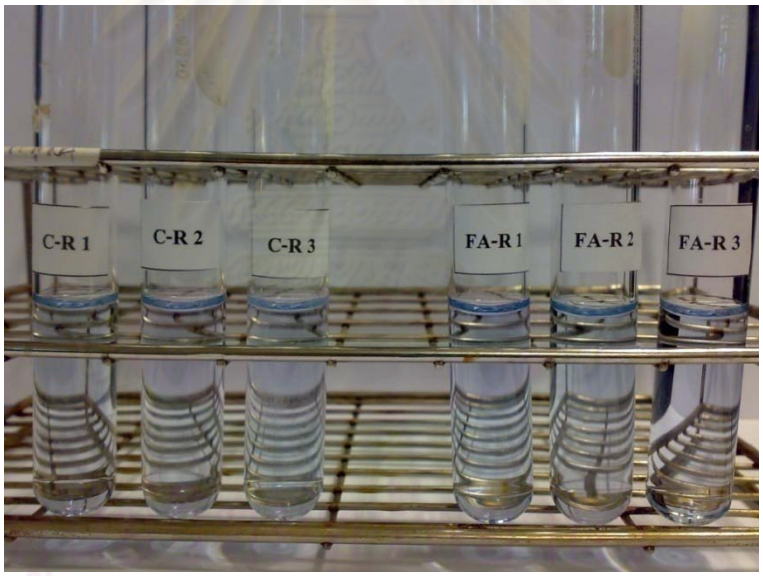
B = volume of extract solution (ml)

X = the concentration of sample compare to standard set

DF = dilution factor



(a)



(b)

Figure B.7 The colour of the standard solutions (a) and the sample solutions (b) change to blue after 30 minutes of the colour development and ready to analyze with spectrophotometer at 882 nm.

B.9 Cellulase Activity (von Mersi and Schinner, 1996)

Reagents and preparation

1. 2 M Acetate buffer pH 5.5

1) Dissolve 164.06 g anhydrous sodium acetate in distilled water. Adjust to 1000 ml in volumetric flask.

2) Add 500 ml distilled water into 1000 ml volumetric flask. Then add 60 ml glacial acetic acid. Adjust to 1000 ml with distilled water

3) Mix 1000 ml 2 M sodium acetate solution and 190 ml diluted acetic solution. Adjust pH to 5.5 with diluted acetic acid solution.

2. Substrate solution 0.7% w/v

1) Weigh 7 g carboxy methyl sodium salt. Dissolve with 800 ml acetate buffer.

2) Heat this solution at 45^{o}C and stir it for 2 hours or until the solution is thoroughly mix.

3) Adjust the volume to 1000 ml in volumetric flask with acetate buffer.

3. Reagent A

Dissolve 16 g anhydrous sodium carbonate and 0.9 g potassium cyanide in distilled water. Mix and adjust the volume to 1000 ml in 1000 ml volumetric flask.

4. Reagent B

Dissolve 0.5 g potassium hexacyanoferrate (III) in distilled water and adjust to 1000 ml. This reagent must be kept in a brown bottle.

5. Reagent C.

1) Dissolve 1.5 g ferric ammonium sulfate and 1 g Sodium dodecyl sulfate in 900 ml distilled water

2) Add 4.2 ml conc. H_2SO_4 then stir this solution with heating magnetic stirrer at 50°C to dissolve absolutely.

Calculation

Conversed the absorbance at 690 nm of sample to µg glucose equivalents (GE) followed by this equation.

$$\frac{(S-C)\times 30\times 40\times 100}{10\times \%dm} = \mu gGE\cdot g^{-1}dm\cdot 24h^{-1}$$

When C is control (µg GE)

S is mean value of samples (µg GE)

30 is volume of incubation mixture (ml)

40 is factor of dilution of the filtrate

10 is initial soil weight

$100\cdot\%dm^{-1}$ is factor for soil dry matter

B.10 Phospholipid Fatty Acid Analysis (PLFA) [Bligh and Dyer (1959), modified by Watcharamul (2005)]

Special Equipments

1. Gas chromatograph (Shimazu, Model GC-2010)

2. 30 m BP20 capillary column

Reagents and preparation

1. 10% HCl:H_2O (v/v)

Add 10 ml of 37% HCl into 100 ml volumetric flask which has 50 ml of distilled water. Adjust the volume to 100 ml with distilled water.

2. 30% KOH (w/v)

Dissolve 30 g KOH in 50 ml of distilled water. Adjust the volume to 100 ml with distilled water.

3. 0.15 M Citrate buffer pH4

1) 0.15 M trisodiumcitrate dihydrate

Dissolve 44.1 g of trisodiumcitrate dehydrate in sterilized distilled water and adjust the volume to 1000 ml with 1000 ml volumetric flask.

2) 0.15 M citric acid

Dissolve 31.5 g of citric acid · H_2O in sterilized distilled water and adjust the volume to 1000 ml with 1000 ml volumetric flask

3) Add 59 ml of 0.15 M citric acid and 49 ml of 0.15 M trisodiumcitrate dihydrate in a 100 volumetric flask. Mix the solvent thoroughly.

3. Phase transfer catalysis solution (0.1 M tetrabutylammonium hydrogen sulphate in 0.2 M NaOH)

Dissolve 0.8 g NaOH and 3.39 g tetrabutylammonium hydrogen sulphate with sterilized distilled water and make up the volume to 100 ml with a volumetric flask.

4. Silicic column

Weigh 0.5 g silicic acid into a 50 ml beaker and activate by heating at 100°C for an hour. Make silicic acid slurry with 3 ml chloroform before add it to a column. Allow excess chloroform to flow before use.

B.11 Soil Respiration (Öhlinger, 1996)

Reagents and preparation

1. 0.1 M HCl

Pour 800 ml of distilled water into 1000 ml volumetric flask. Add 8.6 ml of 37% Hydrochloric acid. Adjust to 1000 ml with distilled water. Store this solution in brown Pyrex bottle.

2. 0.05 M NaOH

Dissolve 2 g of sodium hydroxide (NaOH) in distilled water and adjust to 1000 ml in volumetric flask.

3. 0.5 M $BaCl_2$

Dissolve 10.4 g of barium chloride ($BaCl_2$) in distilled water and adjust to 100 ml.

4. Phenolphthalein

Dissolve 0.5 g of phenolphthalein thoroughly in 100 ml 50% Ethanol (v/v)

Calculation

Calculation of mg $CO_2 \cdot g^{-1}dm \cdot 24h^{-1}$ was followed by with this equation;

$$\frac{(C - S) \cdot 2.2 \cdot 100}{SW \cdot \%dm} = mgCO_2 \cdot g^{-1}dm \cdot 24h^{-1}$$

When C is volume of 0.1 M HCl at the end point of blank (ml)

S is volume of 0.1 M HCl at the end point of sample (ml)

2.2 is conversion factor (1 ml of 0.1 M HCl relates to 2.2 mg CO_2)

SW is soil weight (g)

$100 \cdot \%^{-1}dm$ is factor of soil dry weight

Biography

Miss Thanyaphat Promson was born on February 18th, 1985 in Bangkok. She holds her Bachelor of Science (Biotechnology) from King Mongkut's Institute of Technology Ladkrabang in 2006. In 2007, she furthered her graduate study at Environmental Science Programme (Interdisciplinary Programme), Graduate School, Chulalongkorn University. In 2008, her senior project titled “Genetic abnormality of sperm in smoker” was published in Thai Journal of Genetics volume 1. Her thesis was presented and published in the 1st CMU Graduate Research Conference at Graduate School, Chiang Mai University on November 27th, 2009. She graduated her Master of Science in Environmental Science in 2009.