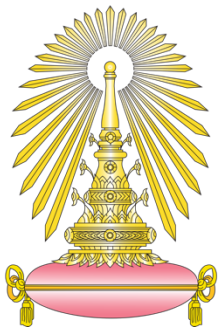

Senior Project

โครงการการเรียนการสอนเพื่อเสริมประสบการณ์

Project Title	**The relationships between microbial communities and environmental factors in agricultural soils in Saraburi**
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Department of Environmental Science

Faculty of Science, Chulalongkorn University

The relationships between microbial communities and environmental factors in agricultural soils in Saraburi

การหาความสัมพันธ์ระหว่างสังคมจุลินทรีย์และปัจจัยสิ่งแวดล้อมในดินเกษตรกรรมจังหวัดสระบุรี

Pimonpan Pakdee

A Senior Project Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Science Program in Environmental Science, Faculty of Science, Chulalongkorn university, Academic Year 2020

Project Title	**The relationships between microbial communities and environmental factors in agricultural soils in Saraburi**
Name	**Ms. Pimonpan Pakdee Student ID 603 33360 23**
Project Advisor	**Assistant Professor Pasicha Chaikaew, Ph.D.**
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Department	**Environmental Science**
Academic Year	**2020**

Accepted by the Department of Environmental Science, Faculty of Science, Chulalongkorn University in Partial Fulfilment of the Requirements for the Bachelor's Degree

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บทคัดย่อ

จุลินทรีย์ดินมีความสามารถในการควบคุมกิจกรรมในดินทั้งด้านโครงสร้างและวัฏจักรชีวเคมี ในขณะเดียวกันโครงสร้างและส่วนประกอบของดินที่ถูกรบกวนจากการใช้งานทางการเกษตรสามารถส่งผลกระทบถึงสังคมจุลินทรีย์ได้ งานวิจัยนี้มีวัตถุประสงค์เพื่อ 1) หาความหลากหลายของจุลินทรีย์ในดินเกษตร 2) หาความสัมพันธ์ระหว่างสังคมจุลินทรีย์และปัจจัยด้านสิ่งแวดล้อม ได้แก่ ค่าความเป็นกรดด่าง ค่าการนำไฟฟ้า อินทรียวัตถุ ปริมาณไนโตรเจน และฟอสฟอรัสที่เป็นประโยชน์ในดินเกษตรจังหวัดสระบุรี โดยเก็บตัวอย่างดิน 30 ตัวอย่าง ที่ความลึก 15 ซม. จากดินเกษตรใน 30 ตำบลของจังหวัดสระบุรี เพื่อหาความหลากหลายของจุลินทรีย์ในดินเกษตรกรรม จากดัชนีความหลากหลาย 3 ดัชนี ได้แก่ ดัชนีความหลากหลายแชนนอน (Shannon, H') ดัชนีความหลากหลายซิมป์สัน (Simpson's) และดัชนีความสม่ำเสมอ (Evenness) ผลลัพธ์ที่ได้คือ 1.77, 0.74, 0.19 ตามลำดับ ผลจากการวหาความสัมพันธ์ระหว่างความหลากหลายของจุลินทรีย์และคุณสมบัติของดินด้วย Principal component analysis (PCA) พบว่าดัชนีความหลากหลาย (Shannon, **H'**) มีความสัมพันธ์เชิงบวกกับฟอสฟอรัสที่เป็นประโยชน์ และมีความสัมพันธ์เชิงลบกับค่าความเป็นกรดด่าง ค่าการนำไฟฟ้า และอินทรียวัตถุในดิน นอกจากนี้ยังได้มีการใช้ Canonical correspondence analysis (CCA) เพื่อหาความสัมพันธ์ระหว่างสังคมจุลินทรีย์กับคุณสมบัติของดิน โดยไฟลัมที่พบมากที่สุด 5 ไฟลัม ได้แก่ *Firmicutes* (26.9%), *Proteobacteria* (16.9%), *Bacteroidetes* (16.8%), *Acidobacteria* (11.5%) และ *Actinobacteria* (8.3%) โดยที่ไฟลัม *Firmicutes* มีความสัมพันธ์เชิงบวกกับค่าความเป็นกรดด่าง อินทรียวัตถุ และค่าการนำไฟฟ้า ไฟลัมนี้ได้แสดงความสัมพันธ์เชิงลบกับปริมาณไนโตรเจนและฟอสฟอรัสที่เป็นประโยชน์ ไฟลัม *Proteobacteria* มีความสัมพันธ์เชิงบวกกับธาตุอาหารทั้งสองประเภท ไฟลัม *Bacteroidetes* มีความสัมพันธ์เชิงบวกกับค่าการนำไฟฟ้า และอินทรียวัตถุ ไฟลัม *Acidobacteria* มีความสัมพันธ์เชิงบวกกับฟอสฟอรัสที่มีอยู่ และมีความสัมพันธ์เชิงลบกับอินทรียวัตถุ และไฟลัม *Actinobacteria* แสดงความสัมพันธ์เชิงลบกับค่าความเป็นกรดด่างของดิน ผลการวิจัยนี้ชี้ให้เห็นว่าคุณสมบัติของดินมีอิทธิพลต่อสังคมจุลินทรีย์ ผลลัพธ์ที่ได้จากการศึกษานี้ช่วยให้มีความรู้ความเข้าใจที่มากขึ้นเกี่ยวกับอิทธิพลของชุมชนจุลินทรีย์และปัจจัยด้านสิ่งแวดล้อมในดิน และยังเป็นข้อมูลที่มีประโยชน์ สามารถช่วยให้เกษตรกรเลือกใช้ปุ๋ยในการปรับปรุงคุณภาพดินเพื่อส่งผลต่อความหลากหลายของสังคมจุลินทรีย์ในดินได้อย่างมีประสิทธิภาพมากขึ้น

คำสำคัญ: ดินเกษตรกรรม ความหลากหลายของจุลินทรีย์ สังคมจุลินทรีย์ คุณสมบัติของดิน ธาตุอาหาร

Project Title	**The relationships between microbial communities and environmental factors in agricultural soils in Saraburi**
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Abstract

The diversity of soil microbial communities are the main factors in the biogeochemical cycles due to their ability to control underground activities, meanwhile the disturbed soils from agricultural practices often perturb microbial communities. This study aims to 1) investigate the microbial diversity in agricultural soils. 2) determine the relationships between the relative abundance of microbes and soil environmental factors such as pH, organic matter (OM), electrical conductivity (EC), nitrogen (N) and available phosphorus (P) in the agricultural soil of Saraburi. Thirty soil samples at 15 cm depth were collected from agricultural soil in thirty sub-districts of Saraburi Province in August 2020. Three diversity indices indicated microbial diversity as follows: 1.77 for Shannon diversity index (H'), 0.74 for Simpson's diversity index, and 0.19 for Evenness index. Principal component analysis (PCA) was conducted to observed the relationship between Shannon's diversity index of microbial communities and soil properties. Shannon's diversity index correlated with available P in a positive direction and negative correlation with pH, EC and OM. Canonical correspondence analysis (CCA) determined the relationship between soil microbial communities and soil properties. The phyla *Firmicutes* (26.9%), *Proteobacteria* (16.9%), *Bacteroidetes* (16.8%), *Acidobacteria* (11.5%), and *Actinobacteria* (8.3%) were abundant in all soil samples. *Firmicutes* was a positive correlation with soil pH, OM, and EC, this phylum also shows the negative correlation between N and available P. *Proteobacteria* were positive correlation with both nutrients. *Bacteroidetes* was positive significant correlation with EC and OM. *Acidobacteria* indicating that a strong positive correlation with available P and negative correlation with OM. *Actinobacteria* was negatively correlated with soil pH. The findings indicated that soil properties influence on the microbial community. The distribution of microbial communities and relationships with environmental factors could help farmers for balancing fertilizer application of soil improvement.

Keywords: Agricultural soil, Microbial diversity, Microbial community, Soil properties, Nutrients

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CHAPTER 1
INTRODUCTION

The difference between landscapes and plant management is the cause of the diversity of terrestrial organisms from plants, animals to microbes. The distribution of soil microbial are the main factors in the biogeochemical cycles due to their ability to control underground activities. In agricultural areas, often disturbed by agricultural applications such as tilling and applying pesticides, herbicides and other chemical fertilizers. These practices can result to the change of microbial community and diversity and consequently degrade soil health. Some useful microbial are not be able to adapt themselves to the new environment, however, some communities prefer to live in the changing situations.

Beneficial microbial in soil play an important role in agriculture as it is related to soil environmental factors. Microorganisms degrade various organic materials into nutrients causing nutrients recycling and reuse. The transformation from organic to inorganic substances increases the usefulness of plant nutrients. Moreover, soil microorganisms increased the production of plants, made up the soil coagulate, and improve soil stability. Each source of soil have different types and amount of microorganisms, It depends on soil quality such as moisture, temperature, pH, and soil nutrients. The transformation of organic matter from macromolecules to subunits and release of nutrients circulating into the soil, increasing the number of nutrients in the soil. The macronutrients and micronutrients that are productive to plants are amino acids, ammonium phosphate, and potassium, etc. (Jacoby et al., 2017). By using different types of enzyme activity that microorganisms produce. Many studies have examined N and P transformations because of their importance for plant nutrition and, thus, agricultural production.

Nowadays, there are many other beneficial microorganisms that can be developed and used in soil improvement to increase agricultural production and reduce production costs. The role of microorganisms in soil is important should not be overlooked as a country of agriculture manufacture. Thai agriculture plays an important role in food products especially in Saraburi, where rice fields are grown and exported a lot of rice products, it is necessary to maintain soil resources to suit various types of plants. Increase the potential of Thai agriculture to be able to produce food every year and make sustainable use of soil resources. And in general soils do not have enough organic matter as a food source for microorganisms. Therefore, organic matter should be added to increase the number and activity of microorganisms.

Most publications often report the distribution of microbes at the microscale or at a few given sites and claimed the results as spatial variation. However, their impact on the agricultural landscape is not limited to miniscule. The primary query when considering microbes and ecology is in "how microbial activity is manifested at larger spatial scales and how that activity controls nutrient cycling, decomposition, primary productivity, and other microbially medicated ecosystem functions at scales that are relevant to humans" (Franklin and Mills, 2007). The abundance of microbes, community, diversity, and linkage to nutrients are rarely considered in the agricultural landscape. Thus, this study aims to enhance a better knowledge of the dominant population of microorganisms in agricultural soil in Saraburi Province. This work also presents a detailed analysis of the microbial community and the relationships between the abundance of microbial and environmental factors (pH, electrical conductivity (EC), Organic matter (OM), nitrogen (N) and available phosphorus (P) across a large scale.

1.1 Objectives

1. To investigate the microbial diversity in agricultural soils.
2. To determine the relationships between the relative abundance of microbes and soil environmental factors (pH, EC, OM, N, and P).

1.2 Outcomes of research

1. This research provides useful information to farmers about the condition of soils for plant growth.
2. This research helps to select beneficial microbial community composition to decompose organic matter, including the release of nutrients from plants or inorganic substances, because microorganisms can facilitate nutrient solubilization to plants and soil.

CHAPTER 2
LITERATURE REVIEW

2.1 Soil microbial diversity

Microorganisms are generally divided into five major taxonomic kingdoms: algae, bacteria, fungi, protists and viruses (Giri et al., 2005). Often, microbes can be found as single cells or as microcolonies embedded in a matrix of polysaccharides (Giri et al., 2005). The diversity of microorganisms has a much longer evolutionary history than plants or animals and thus had more time to evolve into diverse forms. The microbial diversity of soil and the interactions between different trophic levels were elucidated in a simple ecosystem model in which primary producers (plants) and decomposers (microbes) were linked through the cycling of a limiting nutrient factor for the primary producers. Microbial diversity in soil ecosystems exceeds, by far, that of eukaryotic organisms. One gram of soil may harbor up to 10 billion microorganisms of possibly thousands of different species. As less than 1% of the microorganisms observed under the microscope are cultivated and characterized, soil ecosystems are, to a large extent, uncharted. Microbial diversity describes complexity and variability at different levels of biological organization.

2.2 Soil properties

2.2.1 Soil pH

Soil pH or soil reaction is an indication of the acidity or alkalinity of soil and is measured in pH units. Soil pH is defined as the negative logarithm of the hydrogen ion concentration. The pH scale goes from 0 to 14 with pH 7 as the neutral point. As the amount of hydrogen ions in the soil increases the soil pH decreases thus becoming more acidic. From pH 7 to 0 the soil is increasingly more acidic and from pH 7 to 14 the soil is increasingly more alkaline or basic. The effect of soil pH is great on the solubility of minerals or nutrients. Fourteen of the seventeen essential plant nutrients are obtained from the soil. Before a nutrient can be used by plants it must be dissolved in the soil solution. Most minerals and nutrients are more soluble or available in acid soils than in neutral or slightly alkaline soils. Phosphorus is never readily soluble in the soil but is most available in soil with a pH range centered around 6.5. The soil pH can also influence plant growth by its effect on activity of beneficial microorganisms. In the natural environment, the pH of the soil has a huge influence on soil biogeochemical processes. Therefore, soil pH is described as the master soil variable that influences soil biological, chemical, and physical properties and processes that affect plant growth (Neina, 2019).

2.2.2 Soil group

The soil series designation of the soil taxonomy serves as one of the fundamental units of the taxonomy. It is the most detailed designation and comprises the most essential information needed for the most common issues in managing soils. In the past soil mappers have taken local names as the preliminary designation of a soil series. The process of forming a soil series can begin with an initial experience with a specific soil, perhaps in terms of using the soil in support of a particular type of agriculture. Soil resources surveys and research division has divided the Thailand soil group into 62 groups, with Saraburi Province having 21 soil groups. The study area has the following main soil group:

1) Soil group 1

This group of soils has a clay texture. The topsoil cracks in small grooves in summer, most of which are black or gray. The soil may have some yellow dots covering the topsoil. The lower floor is often mixed with cement. The soil is naturally fertile.

2) Soil group 4

The soil group with soil texture is clay. The topsoil is brown to gray or brown. The lower soil is brown to gray or brown or gray to olive green. The soil has a dark brown dotted. There are iron and manganese deposits in the lower soil. This group of soils can be found in flat or lowlands. The drainage is quite bad and the soil is moderately fertile.

3) Soil group 16

The soil texture is loamy soil. The soil is light brown or grayish-brown with dark brown, yellow, or red dots. Iron and manganese may be found in the subsoil. This group of soils can be found in the flat areas along the low river yard. It is very deep, poorly drained, and low in fertility. The soil series in this group are Hin Kong soil.

4) Soil group 28

This group of soils has a very clay texture. In the lower soil, there may be a layer of marl. This series of soils is black, dark gray, or brown. The soil has reddish-brown dots but is found in small quantities. In the topsoil, there is a flat or relatively smooth area. The soil is well-drained and is moderately fertile. The soil series in this group are the Dong Lan soil and the Lopburi soil.

5) Soil group 62

This group of soils consists of mountainous areas. Soil contains both deep and shallow soils. The soil texture and fertility differ depending on the type of rock originating in that area. Most of them are covered with various types of forests. This group of soils should not be used

for agricultural purposes. This is because many problems are affecting the ecosystem. This group of soil sets in the provincial map is called "The slope of the complex"

2.3 Macronutrients

These nutrients include nitrogen (N), phosphorus (P), and potassium (K) and are commonly found to be deficient in poor soils. Mismanagement of these nutrients is not only bad for crop production but can also be harmful to the environment. Excessive use of fertilizers, manures and sludges, composts, etc. can lead to groundwater contamination issues.

2.3.1 Soil nitrogen

Nitrogen availability is a key factor regulating the biological productivity of many ecosystems (Capone, 2000). Soil microorganisms have long been recognized as important agents affecting N pools through various transformations. Total Nitrogen is an essential nutrient for plants and animals. However, an excess amount of nitrogen in a waterway may lead to low levels of dissolved oxygen and negatively alter various plant life and organisms. Sources of nitrogen include wastewater treatment plants, runoff from fertilized lawns and croplands, failing septic systems, runoff from animal manure and storage areas, and industrial discharges that contain corrosion inhibitors.

2.3.2 Soil phosphorus

Phosphorus (P), next to nitrogen, is often the most limiting nutrient for crop and forage production. Phosphorus' primary role in a plant is to store and transfer energy produced by photosynthesis for use in growth and reproductive processes. Soil phosphorus cycles in a variety forms in the soil. Adequate phosphorus levels promote root growth and winter hardiness, stimulate tillering, and hasten maturity. Phosphate soil test levels are an excellent indicator of P-cycling in soils and are an index of the likelihood of crop response to phosphorus fertilizer.

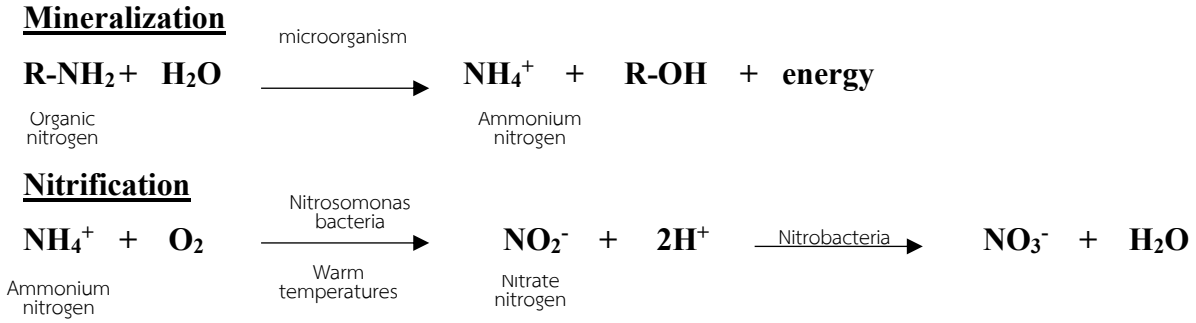
2.4 Soil organic matter

Organic matter is any living or dead animal and plant material. It includes living plant roots and animals, plant and animal remains at various stages of decomposition, and microorganisms and their excretions. The main sources of organic matter are plant litter (plant roots, stubble, leaves, mulch) and animal manures. Earthworms and microorganisms decompose these materials. The process of decomposition releases nutrients which can be taken up by plant roots. The end product of decomposition is humus, a black crumbly material resistant to further decomposition. A complex chemical substance, humus stores plant nutrients, holds moisture

and improves soil structure (Berns & Knicker, 2014). Organic matter is the key to sustain the agricultural productivity.

2.5 Mineralization

Mineralization is a biological process in which organic substances are converted to inorganic substances by soil microorganisms whereas decomposition could be due to physical, chemical or biological processes (Gregorich et al., 2001). Organic matter, organic fertilizers, and some slow-release fertilizers are broken down or transformed by soil microorganisms to supply plants with available ammonium and nitrate. Mineralization is a three-step process involving aminization, ammonification, and nitrification. Aminization and ammonification are stages of the mineralization process in which proteins, amines, and amino acids (usually from organic matter or humus) are converted to ammonium, a source of nitrogen utilized by plants. Mineralization and nitrification are described in the following equation:



Following mineralization, ammonium nitrogen (NH_4^+) is absorbed by plants or undergoes further transformation to become nitrate (NO_3^-). Mineralization is a process through which organic phosphorus in soil is converted into inorganic phosphorus with the help of soil microbes. Immobilization, on the other hand, is the reverse of mineralization. During immobilization, inorganic phosphorus forms are converted back to organic forms and are absorbed into the living cells of soil microbes. Immobilization typically occurs when crop residues are incorporated in the soil. As crop residues decompose, more phosphorus becomes available in the soil solution through mineralization. Because mineralization and immobilization processes are biological processes, they are highly influenced by soil moisture, temperature, pH, organic carbon to organic phosphorus ratio of crop residues, microbial population, etc (Prasad & Chakraborty., 2019).

2.6 Diversity indices

A diversity index is a mathematical measure of phylum diversity in a microbial community. Diversity indices provide more information about community composition than

simply richness (i.e., the number of operational taxonomy unit (OTU) present). Diversity indices provide important information about rarity and commonness of OTU in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure.

2.6.1 Shannon diversity index (*H'*)

The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled. In the Shannon index, p_i is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations, and *s* is the number of species (Daly et al., 2018).

$$Shannon\ index\ (H) = -\sum_{i=1}^{s} p_i \ln p_i \qquad \text{Eq. 1}$$

2.6.2 Simpson's diversity index

The Simpson index is a dominance index because it gives more weight to common or dominant OTUs. In this case, a few rare species with only a few representatives will not affect the diversity. In the Simpson index, n is the total number of organisms of a particular species, N is the total number of organisms of all species, Σ is still the sum of the calculations, With this index, 0 and 1 represents infinite diversity and no diversity, respectively. That is, the bigger the value of D, the lower the diversity. (Daly et al., 2018).

$$Simpson\ index\ (D) = \frac{\sum n(n-1)}{N(N-1)} \qquad \text{Eq. 2}$$

2.6.3 Evenness index

Species evenness refers to how close in numbers each species in an environmental. Mathematically it is defined as a diversity index, a measure of biodiversity which quantifies how equal the community is numerically. The evenness of a community can be represented by Pielou's evenness index (Pielou 1966):

$$J' = \frac{H'}{H'_{max}} \qquad \text{Eq. 3}$$

Where *H'* is the number derived from the Shannon diversity index and H'_{max} is the maximum diversity possible

2.7 Principal component analysis (PCA)

Principal component analysis (PCA) is a multivariate technique that analyzes a data table in which information is described by several inter-correlated quantitative dependent variables. PCA involves a mathematical procedure that transforms several correlated variables into many uncorrelated variables called principal components. The first principal

component accounts for as much of the variability in the data as possible (Abdi & Williams, 2010).

2.8 Canonical correspondence analysis analysis (CCA)

Canonical correspondence analysis (CCA) is a multivariate statistical method that analyzes the relationship between two sets of variables, in which each set contains at least two variables. It is the most general type of the general linear model, with multiple regression, multiple analysis of variance, analysis of variance, and discriminant function analysis all being special cases of CCA (Blumentritt, 2012).

2.9 Related research

Soil is a structured and heterogeneous system with complex trophic interactions that provide an enormous diversity of microbial populations estimated that about 10,000 different microbial species are present in 1 g of boreal forest soil (Zhalnina et al., 2014). Agricultural soils are commonly fertilized with nitrogen, and this could be another possible factor regulating the diversity of microorganism.

Zhalnina et al., (2014) analyzed the microbial communities in The Park Grass Experiment (PGE) with different pH gradients (pH 3.6-7) and nitrogen-based fertilizer inputs using 16S ribosomal RNA (rRNA) gene Illumina sequencing. The results showed that *Clostridium, Bacteroides, Bradyrhizobium, Mycobacterium, Ruminococcus, Paenibacillus*, and *Rhodoplanes* were the most abundant genera found at the PGE. The main soil parameter that determined microbial composition, diversity, and biomass in the PGE soil was pH. Plant responded to the nitrogen treatments with an increase in productivity and a decrease in the species richness.

The availability of essential soil nutrients could influence the activities, biomass, and compositions of soil microbial communities (He et al., 2008). Nutrient availability greatly regulates soil microbial processes and functions in tropical forests. However, few studies have explored the impacts of nitrogen addition (100 kg P ha^{-1} $year^{-1}$), phosphorus addition (100 kg N ha^{-1} $year^{-1}$), and nitrogen with phosphorus interaction on soil microbial biomass and microbial community composition in tropical forests. They established a field nutrient manipulation experiment in a secondary tropical forest of South China. Soil physicochemical properties and microbial community composition were measured. Analysis of phospholipid fatty acids (PLFAs) was used to determine soil microbial biomass and composition, and both were related to environmental factors by the redundancy analysis (RDA) and principal response curves (PRC). They demonstrated that nitrogen addition usually did not affect microbial

biomass, which was increased by phosphorus addition over 3 years of fertilization. Nitrogen addition decreased soil bacterial biomass but did not affect soil fungal biomass after 3 years of fertilization. After phosphorus addition, soil fungal biomass increased faster than soil bacterial biomass, indicating a more sensitive response of soil fungi to phosphorus addition than bacteria. Phosphorus addition increased fungi/bacteria ratio (F/B) ratios after 3 years of fertilization. Both nitrogen and phosphorus additions had different effects on soil microbial community in this tropical forest and, thus, probably altered ecosystem functioning (Li et al., 2015).

Jacobsen & Hjelmsø, (2014) comparing the effects of ten very different pesticides on soil microbial functional diversity and enzyme activity, the experiments were carried out over a period of 12 months, likely allowing for huge differences of the pesticide bioavailability either due to different sorption or degradation in the soil. The effect of pesticides on microbial diversity is mainly affected by the type of pesticide used. In general, the strongest effects are seen from the soil fumigants. Analysis of the phospholipid fatty acid (PLFA) profiles in soils fumigated by the active ingredient methyl isothiocyanate showed an increase in the gram positive bacteria and a decrease in the gram negative bacteria and fungi. A recent study using a similar fumigant and 454 16s amplicon sequencing showed a marked increase in the relative abundance of *Bacillus* and *Burkholderia* species (Hjelmsoe et al., 2013). Other pesticide types not targeting soil bacteria have also been shown to affect the soil bacteria diversity: for example, for herbicides, the reduction of growth-promoting bacteria in rhizosphere by glyphosate and the increase of gram negative bacteria following treatment with napromide. Effects have also been seen for insecticides and fungicides. Often though, the results are difficult to compare because of differences in experimental setups, dose concentrations and methods.

Upton et al., (2019) investigated both seasonal (within year) and annual (across sampling years) changes of discrete microbial communities in soil aggregate fractions, large macroaggregrates (LM) and microaggregates (MICRO) in three different bioenergy management systems. They hypothesized that 1) seasonal changes due to plant phenology and aggregate turnover will be most pronounced within the MICRO aggregate soil microbial community; 2) inter-annual variability will lead to changes in microbial diversity across aggregate sizes and the magnitude of change will be mediated by management regime. They found that LM and MICRO aggregates have unique microbial communities within soil. MICRO aggregate microbial communities are more diverse and change more dynamically across the sampling season, peaking in diversity at peak plant growth and maximum biomass.

The number of family indicative of specific MICRO aggregate habitats increases over the growing season for both bacteria (from 3 to 51) and fungi (from 8 to 14). The LM aggregates harbored less diverse, yet more stable, communities within a growing season. By contrast, between years the LM aggregates were the most responsive to inter-annual variability. Targeted analysis of the MICRO aggregates can contribute to deeper understanding of potential diversity and functioning of soil microbial communities for ecosystem maintenance as well as the response to climatic events and environmental change.

CHAPTER 3
METHODOLOGY

3.1 Study site

The study area was agricultural area in Saraburi Province, located in the central region of Thailand. Saraburi is on the east side of the Chao Phraya River valley. The eastern part of the province is covered by high plains and plateaus, while the western part is mostly low flat plains. Saraburi has a tropical savanna climate. The climate is arid with little rain in winter, relatively high temperatures in summer, cool in winter, and rain from May to October, about 70–90 day. The average annual temperature is 28-29 degrees Celsius. The study area covered all 30 sub-districts of Saraburi Province. Most of them are rice fields. The study area consists of 5 groups of soil group such as soil group 1, soil group 4, soil group 16, soil group 28 and soil group 62. The locations within Saraburi province in Thailand and soil sampling points are indicated in Fig 1 and Fig 2.

Fig. 1. Saraburi Province

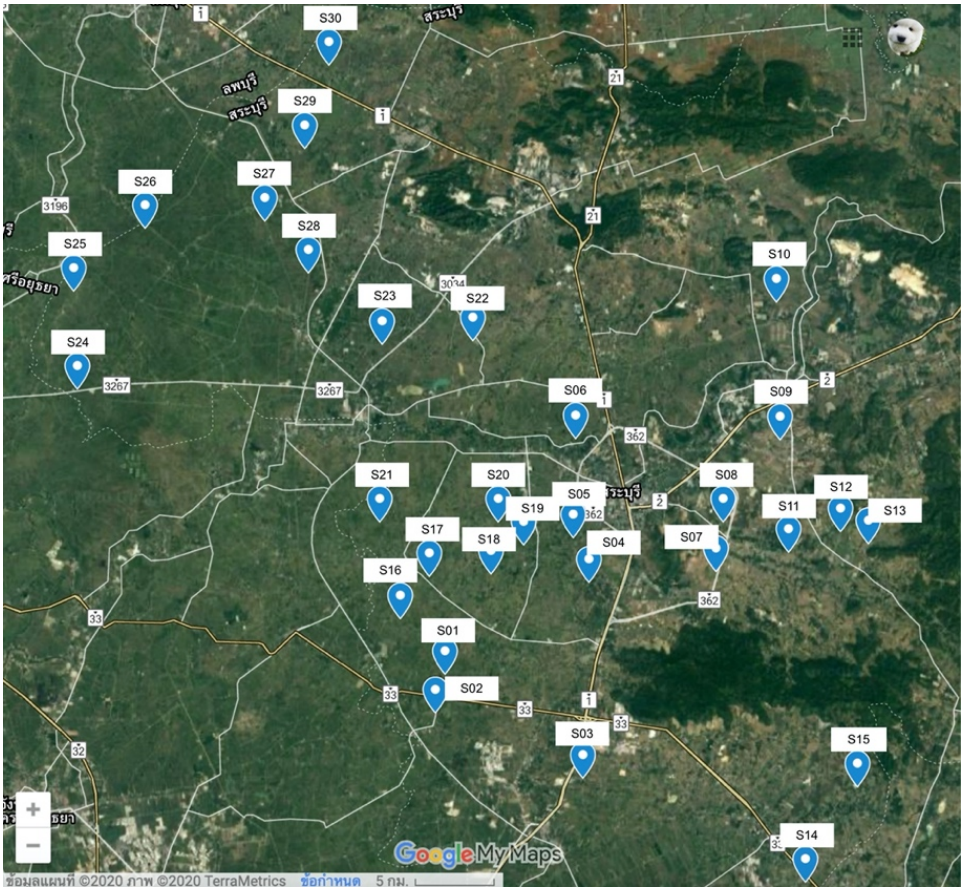


Fig. 2. Thirty soil sampling locations across sub-districts within Saraburi Province.

3.2 Soil sampling collection

Thirty soil samples at 15 cm depth were collected from agricultural soil in thirty sub-districts of Saraburi Province in August 2020. Field soil samples were frozen and sent to Omics Sciences & Bioinformatics center for further analysis. Samples are minimally processed in the field and must be sealed and kept in sterile conditions to prevent cross-contamination from other microbes while in storage or transit. Once shipped to the analytical facility, samples are further prepared for laboratory analysis and DNA is extracted from the homogenized soil subsamples (Tate, 2020). Soil samples for DNA sequencing were analyzed by Illumina sequencing of 16S rRNA genes.

For other parameters, soil samples were dry and sieved 0.5 mm and 2.0 mm to analyze nitrogen and available phosphorus.

3.3 Soil DNA extraction, Bioinformatics sequence analysis

The analysis of microorganisms in soil samples were analyzed at the Omics Sciences & Bioinformatics Center. DNA were extracted from the 30 samples. The extracted DNA from the soil samples were amplified and conducted for 16S microbial sequencing to determine diversity and abundance of microorganism in the agricultural soil. Primers targeting the regions

(V3-V4) of the 16S rRNA gene were used for polymerase chain reaction because sequences in that regions are conservative and provide the greatest microbial identification to genus level.

3.4 Analysis of soil pH

The soil pH were measured by pH meter in soil to water ratios 1:1. Calibrate the pH meter over the appropriate range using the standard buffers. Soil 20 g of sieved, air-dried, soil into 100 ml. beaker. Add 20 mL distilled water to the sample. Stir for 15 seconds and let stand for 30 minutes. Place electrodes in the slurry, swirl carefully, and read the pH immediately (Eckert & Sims, 2009).

3.5 Analysis of electrical conductivity

Prepare a 1:5 soil:water suspension by weighing 4 g air-dry soil into a 100 ml beaker. Add 20 mL water. shake for 15 seconds and let stand for 30 minutes. Calibrate the conductivity meter according to the manufacturer's instructions using the KCl reference solution to obtain the cell constant. Measure the electrical conductivity of soil samples. Record the value indicated on the conductivity meter. Rinse the cell with water between samples (Soil Survey Standard Test Method Electrical Conductivity).

3.6 Analysis of nitrogen

1. Place the sample amounts 1 g into the digestion tube, Ideally, the particle size should be < 0.5 mm. Add 20 ml conc. sulfuric acid and swirl until the acid is thoroughly mixed with the sample. Allow the mixture to stand for cooling. Add 7 g of the catalyst (K_2SO_4: $CuSO_45H_2O$: Se portion of mixed 100:10:1). Bring the digestion tube and mixture into the digestion unit and into a heating block. Heat the mixture (350 – 380 °C) until white fumes can be seen. Continue the heating for about 180 minutes. The digestion is finished when the sample had totally transparent with a slightly blue or green color due to the Cu from the catalyst. The sample is allowed to cool to room temperature and cautiously approx.

2. Sample already digested with sulfuric acid. Place the digested samples in digestion tubes to the distilling unit. The sample is distilled until 60 mL of distillate water in the tube. 80.0 mL of 32% NaOH and 65.0 mL of 2% boric acid were automatically added by distillation unit program.

3. Titrate with H_2SO_4 0.1 N until the solution has a slightly pinkish color. With the volume and concentration of H_2SO_4 used, we can calculate the total nitrogen content.

4. Calculation of total nitrogen

$$\% \text{ TKN in soil } = \frac{(\text{ml standard acid} - \text{ml blank}) \text{ x Normality of } H_2SO_4}{\text{Weight of soil sample(g)}} \text{ x } 1.4 \qquad \text{Eq. 4}$$

3.7 Analysis of available phosphorus

The soil samples were collected and air-dried for 7 days. These samples were sieved using a sieve mesh size of 2.0 mm. About 5 g of the sieved soil sample was accurately weighed and transferred to a 250 ml volumetric flask and 200 mg of Darco G60 activated charcoal, 20 ml of HCl and H_2SO_4 (ratio of 3 to 1) was added and shaken in a mechanical shaker for 10 minutes. This was then filtered using filter paper Whatman No. 42. About 5 ml of the filtered digested sample was taken, 10 ml of ammonium molybdate, 4 ml of mixed reagent between antimony – ascorbic acid was added. The blue color observed was measured spectrophotometrically using the wavelength of absorbance around 840 nm. The absorption spectral data of the Potassium dihydrogenphosphate was used as a standard against the reagent blank (water). Pipette standard KH_2PO_4 0, 0.5, 1.0, 1.5, 2.0 and 5.0 ml. Then, repeat the above procedures without a soil sample. After that, make the standard curve define the x-axis as phosphorus volume and y-axis as measured absorbance. Absorbance data of the sample soil was compared to determine the phosphorus content in the soil.

3.8 Index and statistical analyses

Statistical analyses to determine the microbial diversity in agricultural soils were conducted using diversity indices include Shannon diversity index (*H'*), Simpson's diversity index, and Evenness index. The QIIME2 software was used to facilitate exploratory analysis and visualization of microbial taxonomy. Principal component analysis (PCA) was used to determine the relationship between microbial diversity and soil environmental factors. Canonical correspondence analysis (CCA) was used to identify the relationship between soil microbial community and soil environmental factors by PAST software.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Soil properties

The environmental factors of soil at different sampling points are shown in Table 1. Soil environment factors included pH, nutrients (N, available P), electrical conductivity (EC) and soil organic matter. Descriptive statistic values, such as maximum, minimum, mean and standard deviation (SD), from a total of 30 soil samples are shown in Table 1.

Table 1 The descriptive statistics of soil environmental factors

Explained statistics	pH	EC (dS/m)	OM (%)	N (%)	Available P (mgP/kg)
Max	7.80	0.408	4.62	0.121	0.100
Min	4.92	0.023	1.17	0.004	0.004
Mean	-	0.092	2.66	0.038	0.030
SD	-	0.076	0.89	0.037	0.022

4.1.1 Soil pH

The soil pH levels were measured by pH meter in soil to water ratios 1:1 range of the agricultural soil in Saraburi was 4.92 - 7.80. In general, soil pH range between 6 to 7.5 is suitable for availability phosphorus, while pH values below 5.5 and between 7.5 and 8.5 limit availability phosphorus (USDA., 1994). Soil pH influences the microbial community composition, the diversity and the biomass of the microorganisms. Soil pH was the most likely effect on microbial community structure in the regulation of the availability of nutrients in the soil (Zhalnina et al., 2014).

4.1.2 Electrical conductivity

Electrical conductivities of the soil samples were measured in saturated extracts of soil to water ratios 1:5 ranged from 0.023 to 0.408 dS/m. The electrical conductivity of soil solution extracts was the method for determining soil salinity. Salinity has harmful effects on soil microbial communities, resulting in a decrease of soil respiration, microbial biomass, and related decomposition and mineralization processes (Wichern et al., 2006).

4.1.3 Soil organic matter

The average quantity of organic matter in agricultural soil was 2.66%, ranged from 1.17 to 4.62 %. Soil organic matter generally originates from plant and animal residues, which are

decomposed and stabilized via physical, chemical, or microbial pathways. Soil microbes decompose plant litter and carrion by excreting extracellular enzymes. The plant received organic matter is decomposed by soil microbes, transformed into microbial biomass and becomes stabilized as soil organic matter in the form of microbial necromass after death. The circulation of soil organic matter is limited by sorption/stabilization which slows the OM decomposition process, that was the reason why the content of soil organic matter had low quantity (Hu et al., 2020).

4.1.4 Nitrogen

The contents of total nitrogen ranged from 0.004 - 0.121%, the average of total nitrogen in soil was 0.038%, indicating that the contents of total nitrogen in the studied site were low in quantity. Generally, soil organic matter (OM) is the source of total nitrogen, and the correlation coefficient between these two can usually reach over 60 %. Total nitrogen is largely contained in the topsoil, which is the important zone of root development and biological activity. These microbial activities help in decomposing organic matter to form humus by mineralization and release plant nutrients in inorganic form, indicating that total nitrogen is mainly stored in the topsoil (Wang et al., 2021).

4.1.5 Available phosphorus

The available phosphorus content in Saraburi ranged from 0.004 to 0.100 mg/kg with an average value of 0.030±0.002 mg/kg. Phosphorus in the form of available phosphorus is low in soil because of phosphorus fixation as insoluble. The conversion of insoluble phosphorus form into soluble form requires a process of phosphate solubilizing bacteria (Alia et al., 2013).

4.2 Analysis of microbial community structure

4.2.1 The relative abundance of microbial community

Most soils collected from the agricultural area were weakly acidic. The relative abundance of the microbial community at the phylum in agricultural soils is presented in Fig. 3. 407,940 sequence reads were obtained from 16S rRNA microbial gene sequencing to determine the diversity and abundance of microorganism, 99.5% of all sequences were classified as bacteria and 0.5% as archaea. The species composition of the top ten species at the phylum, mainly including *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes*, *Verrucomicrobia* and *Fusobacteria*. The different microbial communities in each area are influenced by many factors such as soil properties, land use, geography and vegetation (Kuramae et al., 2012).

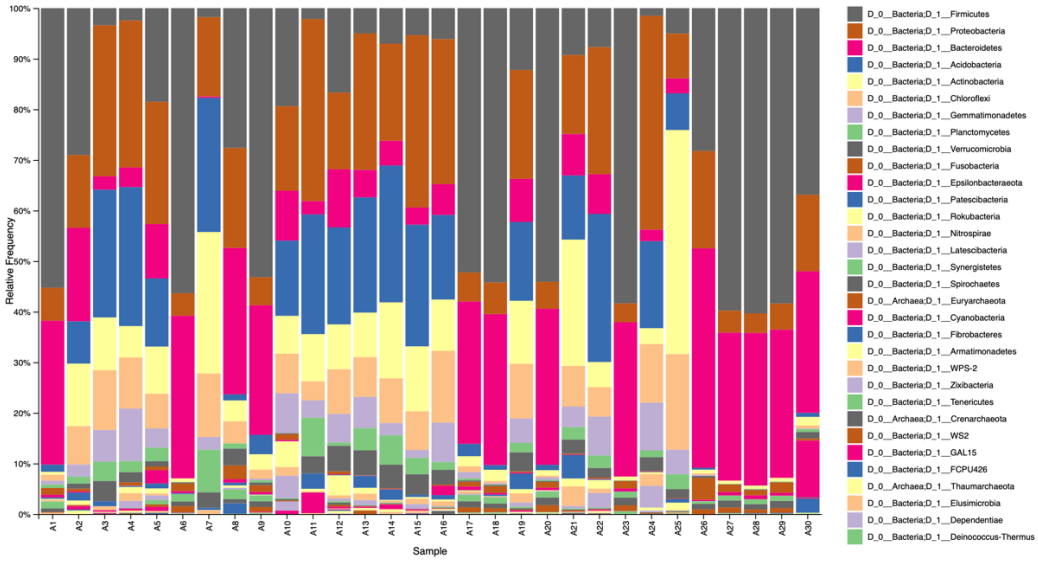


Fig. 3. The relative abundance of microbial community at the phylum level in samples.

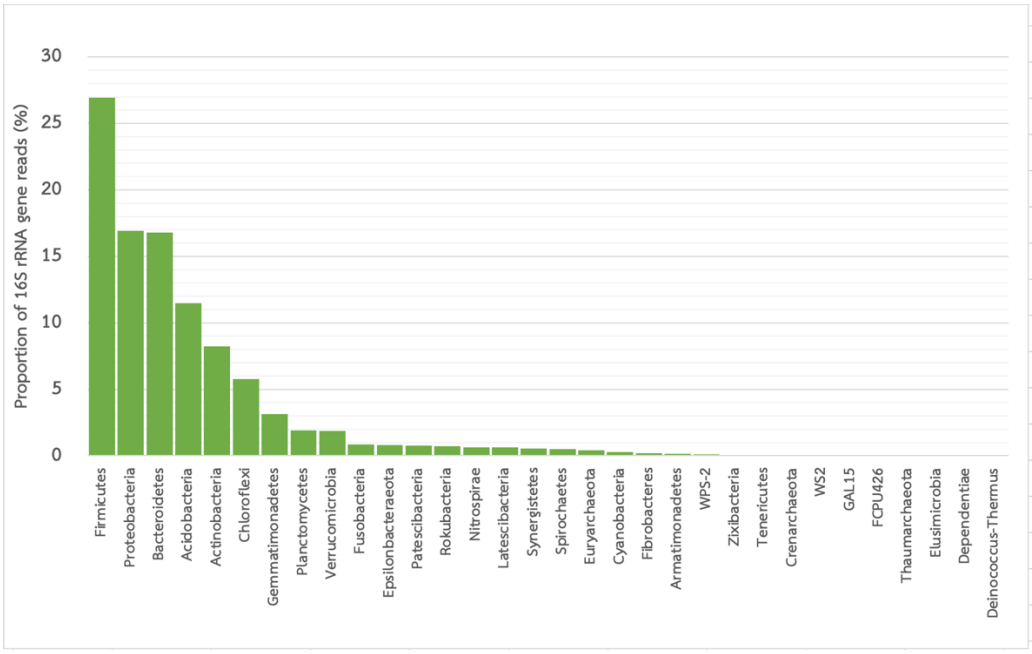


Fig. 4. Microbial community composition of Saraburi. The relative abundance is represented as a proportion of 16S rRNA gene reads of the total number of read

Table 2 The taxonomic and functional aspects of microbial communities in agricultural soils in Saraburi.

Phylum	Family Name	% Abundance Family	Sources	Role	References
Bacteroidetes	*Prevotellaceae*	0.21 – 23.81 %	• Human intestinal • Rumen and hind gut of cattle and sheep • Soils, oceans and freshwater	They can degradation of high molecular weight organic matter such as proteins and carbohydrates.	(Thomas et al., 2011)
Firmicutes	*Ruminococcaceae*	0.14 – 17.70%	• Mammalian gut • soils	They role as degradation of complex plant material and degrade a wide variety of recalcitrant substrates such as cellulose.	(Biddle et al., 2013)
Firmicutes	*Lachnospiraceae*	0.17 – 16.10 %	• Mammalian gut • soils	They are more highly specialized for the degradation of complex plant material. In gut environments, the ability to degrade cellulose of plant material enables to decompose of substrates that are indigestible by the host.	(Biddle et al., 2013)

Phylum	Name	% Abundance	Sources	Role	References
Gemmatimonadetes	*Gemmatimonadaceae*	0.18 – 7.86 %	• soils and sediments	One of the important organisms that are relevant to phosphorus removal from wastewater.	(Albers & Siebers, 2014)
Firmicutes	*Clostridiaceae*	0.06 – 4.98 %	• large bowel • soils	Clostridiaceae are important in nutrient digestibility.	(Bermingham et al., 2017)
Bacteroidetes	*Bacteroidaceae*	0.04 – 13.25 %	• Human intestinal • Rumen and hind gut of cattle and sheep • Soils, oceans and freshwater	Bacteroidaceae are specialized in the degradation of complex organic matter in the biosphere, especially in the form of polysaccharides and proteins.	(Thomas et al., 2011)
Proteobacteria	*Enterobacteriaceae*	0.12 – 17.78 %	• Soils and manure • Intestinaltract of animals	They play an important role in the dissemination of antibiotic resistance genes.	(Pourcher et al., 2014)

4.2.2 Shannon diversity index

Table 3 shows the descriptive statistics of the diversity index of agricultural areas in Saraburi Province by using the Shannon-Wiener diversity Index (H'). The Shannon index varies from 1.07 to 2.31 (mean 1.77, SD 0.424). The top two areas with the highest diversity values were S10 and S19. The soil with the lowest diversity value was S28. Typical values are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4 (Ifo et al., 2016). The Shannon index increases as both the richness and the evenness of the community increase. Therefore, this research has found that the Shannon diversity of microorganisms is slightly low.

Table 3 The descriptive statistics of microbial diversity

Explained statistics	Shannon diversity index (H')	Simpson's diversity index	Evenness
Max	2.31	0.88	0.50
Min	1.07	0.54	0.14
Mean	1.77	0.74	0.30
SD	0.426	0.116	0.105

4.2.3 Simpson's diversity index

In addition to using the Shannon diversity index, this research has also using Simpson's diversity index. The biodiversity of microorganisms is calculated from the number of phylum present if the area has a large number of phylum and each has a high population, results in high diversity index. Simpson's diversity index varied with range between 0 and 1 (Barcelona Field Studies Center, 2012). The Simpson's diversity index shown in Saraburi agricultural soils ranged from 0.54 to 0.88. The lowest and highest diversity was found at S28 and S12, respectively.

4.2.4 Evenness index

The evenness diversity index provides the distribution of each species in agricultural soil in Saraburi. From Table 3, The evenness diversity index was the range from 0.11 to 0.24. The results showed that the evenness index of the S5, S10, S12, S19 and S21 were higher than other samples. The average evenness index of microbial in agricultural soil in Saraburi was 0.19, indicating that this study area has a low distribution of each species.

Principal component analysis (PCA) was conducted to observe the relationship between microbial diversity (Shannon index) and soil environmental factors. The PCA of Shannon index with soil environmental factors showed that PC1 and PC2 explained 67.29% and 23.02% of the variation, respectively (Fig. 5). Shannon diversity index and available P showed a positive correlation. Soil pH, OM, EC and N had negative correlation on microbial diversity. PCA showed that pH and OM were the main factors that influenced microbial diversity.

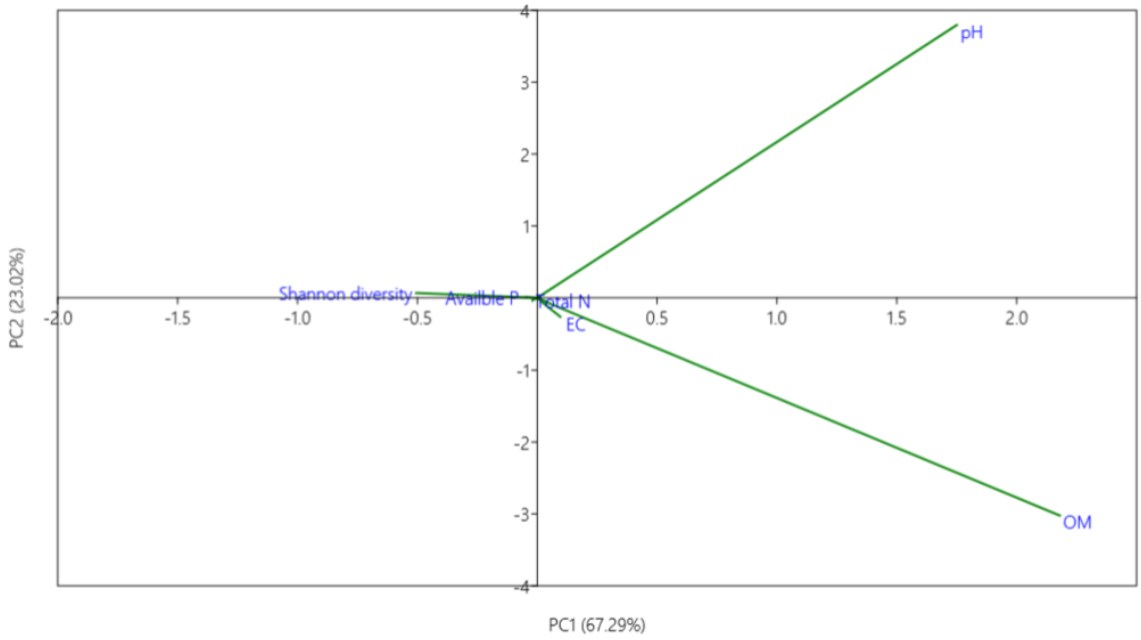


Fig. 5. Principal component analysis (PCA) to observed the correlation between Shannon diversity index of microbial communities at phylum level and soil properties.

Wang et al., (2020) suggested that soil pH has a significant influence on microbial diversity in cropland from China's Yangtze River Basin, when diversity was measured by Shannon diversity index microbial diversity was higher in acidic soil.

He et al., (2013) reported that microbial diversity was significantly increased by low N treatment, they observed that the higher N fertilization was not suitable for the diversity of soil microbial community.

Wang et al., (2018) reported that Shannon index values were lower in nitrogen addition. Shannon index was positively correlated with acidic soil, but was negatively correlated with total nitrogen, phosphorus addition did not significant affect soil microbial communities.

Whereas, Fierer & Jackson, (2006) indicating that soil bacterial diversity as estimated by Shannon index, soil pH was the main predictor of Shannon index with the lowest of diversity in acid soils.

Thus, results from this study suggest that maintaining beneficial microbial communities in soils could be altered by control pH levels, nitrogen, phosphorus, and organic matters at a suitable range to promote the soil healthy with high microbial diversity.

Table 4 Shannon's diversity on other sites.

Other studies	Shannon's diversity index	Referennces
Saline soil	4 – 5.5	(Li et al., 2021)
Nitrogen addition	5.32	(Wang et al., 2018)
Phosphorus addition	5.77	(Wang et al., 2018)
Alpine meadow	10.07	(Canfora et al., 2014)(B. Kang et al., 2021)
Desert grassland	9.73	(Kang et al., 2021)
Saline soil in wetland	3.22 – 5.95	(Vera-Gargallo et al., 2019)
Silty grassland soil	3.8 – 4.8	(Baumann et al., 2013)
Conservation agriculture	1.5 – 3.5	(Habig & Swanepoel, 2015)
Loam silt (agricultural soil)	3.08	(Øvreås & Torsvik, 1998)
Organic soil (agricultural soil)	3.84	(Øvreås & Torsvik, 1998)

Based on literature, pH level is the environmental parameter that affect soil properties such as nutrient availabilities and organic matters, thus consequently promote the abundance of soil microbes and microbial diversity. Many studies reported that the main driver of soil microbial diversity was soil pH, since the diversity and richness of soil bacterial communities differed by ecosystem type, land management and type of vegetation, these variables affect varied soil pH. In this study, the level of microbial diversity index in agricultural soils in Saraburi was slightly low in acidic soil. Microbial have different tolerance ranges of soil pH, and bacteria generally tolerate a narrower pH range. This explanation was supported by Kang et al., (2021), who found soil pH range was from 5.15 to 8.71, and bacterial classes such as *Actinobacteria* and *Thermodesulfovibrionia* were significantly positively and negatively correlated with soil pH, respectively. Another explanation is that soil pH affects the altered community structure through environmental factors (such as nutrient availability, organic C, and soil water condition), which often changes together with changes in soil pH. One possible explanation is N-limited due to application of nitrogen in the soil can lead to changes in niche dimensions, these changes can alter bacterial diversity. Long-term N-fertilizer caused increases in nitrogen content might lead to decreases in soil pH and further increases content of available

nutrients, thus stimulating bacterial growth (Kang et al., 2021). From table 4, many studies showed the differences of Shannon diversity index with each of the sites. Habig & Swanepoel, (2015), reported that the majority of the results obtained, it is obvious that the application of conservation agriculture resulted in increased soil microbial diversity and activity in the different cropping systems more under no-till than under conventional tillage, which the value of Shannon index of this study resembled with our study.

In conclusion, the characteristics of agricultural soils in Saraburi were acidic and low microbial diversity. A long-term chemical fertilization to accelerate the nutrient turn-over in agricultural soils possibly altered soil pH. Soil pH alteration can result in changes in niche dimensions of microbes. Therefore, the low diversity of soil microorganisms may be due to altered soil properties, and different land management had various influences on microbial diversity.

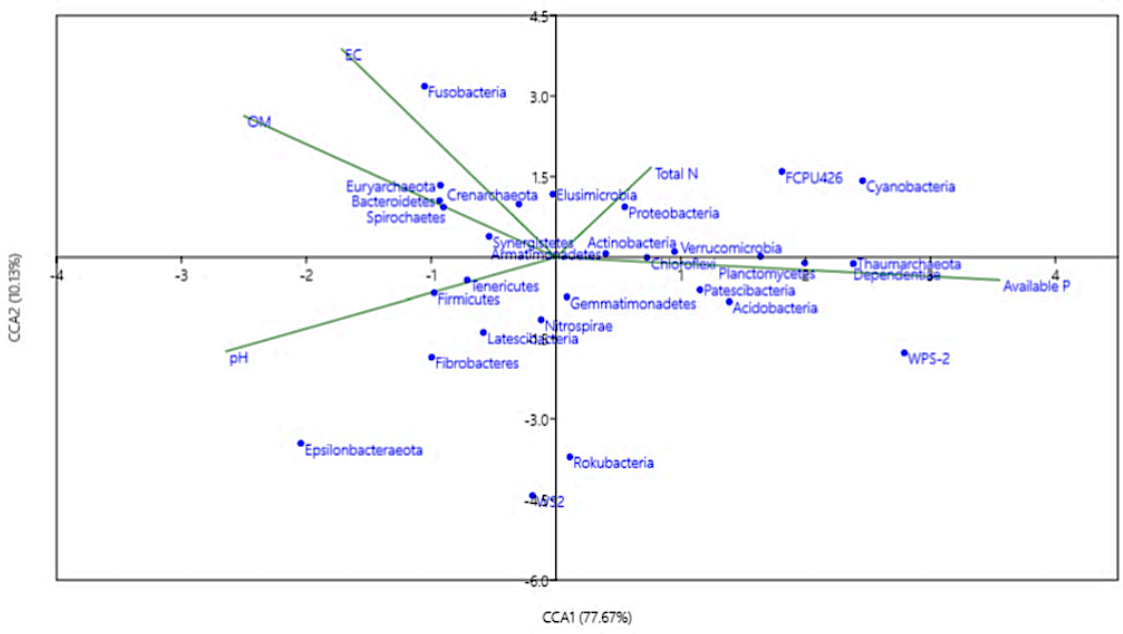


Fig. 6. Canonical correspondence analysis (CCA) illustrating the influences of soil properties on the microbial community.

4.3 Relationship analysis between soil environmental factors and microbial community

To determine the relationship between microbial communities in agricultural soil and soil properties, this study used the Canonical correspondence analysis (CCA). CCA results showed that bacteria and archaeal community structure were influenced by soil environmental factors including pH, EC, OM, N and available P. All read in soil samples were classified into 32 phyla, and their relative abundances were shown in Fig. 4.

The phyla *Firmicutes* (26.9%), *Proteobacteria* (16.9%), *Bacteroidetes* (16.8%), *Acidobacteria* (11.5%), and *Actinobacteria* (8.3%) were dominant in all soil samples. Fig. 6, which reveals that these soil variables explained 87.8% of the variation in the bacterial community by the two axes of CCA, with the first axis explaining 77.67% and the second 10.13%.

Firmicutes were the most abundant phyla, which *Firmicutes* was a positively related with soil pH, OM, and EC, this phylum also showed the negative relationship between N and available P. In general, *Proteobacteria*, *Bacteroidetes, Actinobacteria* and *Firmicutes* are copiotrophic bacteria, while *Acidobacteria* is an oligotrophic bacterium (Yang et al., 2020). Copiotrophic bacteria grow fast and rapidly favored by selection where the resource is abundant. In contrast, oligotrophic bacteria grow slowly but more efficiently and are highly adaptive in low nutrient environments (Koch, 2001). The largest family of *Firmicutes* was *Ruminococcaceae* (Table 2), which is well known to be involved in the degradation of complex materials, the second largest was *Clostridiaceae*, which can degrade cellulose and nutrient digestibility. They part a common role as active plant degraders. From the above information, *Firmicutes* is copiotrophic bacteria that resulted in the increase in abundance under high organic matter, which is consistent with our study.

The second most abundant phyla were *Proteobacteria*. This phylum revealed a positive link with both nutrients. The availability of phosphorus to plants also depends on the amounts of different phosphorus fractions present in soils. *Proteobacteria* was the main soil phosphorus-solubilizing bacteria, which played an important role in the mineralization (Zhang et al., 2021).

Bacteroidetes was positive correlation with EC and OM. Fierer et al., (2007) found that *Bacteroidetes* was a copiotrophic characteristic, changing their abundances in a predictable manner to changes in soil carbon availability. The abundance and distribution pattern of *Bacteroidetes* in marine environments involved in the degradation of high molecular weight compounds such as cellulose, agar, and chitin in both dissolved and particulate fractions of marine organic matter. Members of *Bacteroidetes* have been described as their role in the mineralization of organic matter in the ocean (Dorador et al., 2009). Li et al., (2021), showed that salinity is the main factor controlling the abundance and diversity of microbial and affects the distribution of the microbial community. Naturally, saline soil is also a changing environmental parameter where rain and water movements can change the distribution of salts, these reasons indicating that soil organic matter and salinity had a positive relationship with the relative abundances of *Bateroidetes* (Fig. 6.)

The abundance of *Acidobateria* indicates that a strong positive correlation with available P and negative correlation with OM. *Actinobacteria* have negative correlation with soil pH. *Acidobacteria* are oligotrophic bacteria that are extremely weak to nutrient availability. Cheng et al., (2020) reported *Proteobacteria* and *Bacteroidetes*, which are primary consumers, that could be enriched in high carbon availability conditions, and *Acidobacteria* and *Actinobacteria* had little change under different phosphorus fertilizer level, that is consistent with our study.

CHAPTER 5
CONCLUSIONS

In this study, using 16S microbial sequencing analysis, we obtained the relative abundance of microbial communities in agricultural soil in Saraburi for conducted to find the microbial diversity, revealed low level of microbial diversity in Saraburi. The phyla *Firmicutes* (26.9%), *Proteobacteria* (16.9%), *Bacteroidetes* (16.8%), *Acidobacteria* (11.5%), and *Actinobacteria* (8.3%) were dominant in all soil samples. The research results, indicated that soil properties promoted the abundances of microbial community, and soil pH was the main factor affecting to microbial communities and soil properties such as available nutrients and organic matter. Long-term fertilization result in altered soil pH, that can lead to changes in niche dimensions of microbial communities. The difference in microbial communities and diversity in agricultural soils in Saraburi may be influenced by different soil groups in each sampling area. One possible explanation is differences of each soil group such as structure, composition, texture and mineral content in the soil, these things may affect the soil microbial communities structure and diversity. The findings from this study contributes to a better understanding of the influences of microbial communities and soil environmental factors which could help farmers for balancing fertilizer application to achieve the most beneficial in improving soil microbial diversity and may facilitate higher product of the plant.

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