

# Soil Microbiome

## in the Upper Northeast of Thailand

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## Introduction

Thailand is agriculture-based country, primarily in the Northeast of Thailand (NE). Major cultivar plants in Thailand are rice (>50%), followed by assorted vegetables and fruits (e.g. tea, corn and banana) (NESDB, 2005).

NE encompasses one-third of the country's land (168,854 km<sup>2</sup>), and contains a majority of population. The NE population nominates in agriculture for living. In contrast to other regions of Thailand, the NE soil is rather inferior for cultivation (Fukai, Sittisuang and Chanphengsay, 1998). The soil is of fine-grained sand texture. The NE geography has limited to water access and the annual rainfall is unpredictable. Examples include a long drought season and then a flood. Irrigation relieves the problem but leaving the soil salinity problem. Besides, many cultivar lands come from deforestation. Together, the crop yields per area are poor, and promote farmers to use industrial agriculture (IA), which further accelerates the soil quality problems (Fedra, Winkelbauer and Pantulu, 1991; NESDB, 2005).

A modern era of industrialization arises a conventional farming, also known as industrial agriculture (IA). IA refers to farming system that utilizes chemical fertilizer, pesticide, herbicide and other synthetically inorganic inputs, as well as genetic modified organism, concentrated animal feeding, and extensive irrigation or tillage. This allows a large sum of food production on less land and with less human labor (Seufert, Ramankutty, and Foley, 2012). However, many activities of this non-naturally high resource and energy intense IA affect environmental health, such as chemical leaching, land degradation and loss in biodiversity resources. Researches comparing between IA and organic agriculture (OA), a contrast method to IA, also reported generally more species diversity and abundance in OA than IA (Bengtsson, Ahnström, and Weibull, 2005). In addition, the chemical inputs, e.g. pesticide, pose harm to human health. Nonetheless, with the instant great yield, IA remains commonly used by Thai farmers, and poor soil quality problems occur increasingly (Fukai, Sittisuang and Chanphengsay, 1998).



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## Organic agriculture and importance of soil microbiome

OA, also known as sustainable farming, prohibits those chemical and artificial inputs, and genetic modified organisms, albeit the use of agricultural machines is still allowed for energy and human labor efficient (Hole et al., 2005; Paull, 2011). For instances, OA utilizes animal and plant product fertilizers, hand weeding and biological pest control, in replace of chemical fertilizer, herbicide and pesticide. OA also uses polyculture (or companion planting), and crop rotation to increase soil diversity. The goal of OA is to maintain the healthiness of nature, including, but not limited to, soil, water, animals and humans. The OA processes rely on ecological biodiversity and metabolic food networks that suit local geography (Rigby and Cáceres, 2001). Office of Agricultural Economics of Thailand reported the OA farms in Thailand comprise of rice (approximately 68%), vegetables (12%), fruits (8%), tea (8%), and herbs and others, and the OA produces can generally have the accepted higher market price than the non-OA produces due to their merit in green agriculture.

Microorganisms serve the major beginning and end (recycling) steps in almost every food network routes. Recently, researches attempt to increase arable yield through the role of prokaryotic and eukaryotic (mainly fungi, nematodes and annelids) microbiome. When farmers grow and harvest crops, some nutrients were removed from the soil. Continuingly, the land suffers nutrient depletion and become non-arable soil. To replenish nutrients in soil, maintenance of suitable microbial diversity balance, including prokaryotic bacteria and archaea, and eukaryotic fungi, protists and small animals i.e. nematodes, serve primary roles in nutrient cycles. Fertile (arable) soil microbiome is an ultimate goal of OA, hopefully to provide sustainably cultivar land with fertile microbial abundance and metabolic food networks. Studies demonstrated agrotechniques affected soil microbial diversity, and suggested long-term

IA change soil microbial structures likely in a way to decrease species abundance and activities compared with OA (Bossio et al., 1998; Letourneau and Goldstein, 2001; Berkelmans et al., 2003). OA soils also showed decreasing plant pathogens through the arm of beneficial soil flora (Griffiths et al., 1994). Whilst individual microbe application showed inconsistent results, the databases of diverse soil microbiome suggestive to core microbiome applications have resolved various agriculture plant and soil dilemma, i.e. salt-tolerant plant and improved soil health (Vandenkoornhuysen et al., 2015; Qin et al., 2016; Schmidt, Bowles and Gaudin, 2016). However, soil microbiome vary across geographic regions and crop types (Bulgarelli et al., 2012; Lakshmanan, 2015; Agler et al., 2016).

Therefore, the database and knowledge of soil microbiome in cultivar farms in Thailand is priceless. In Thailand, the agriculture soil microbiome database is still a tip of an iceberg since the next generation sequencing is a recent advance technique. Several essential studies thereby rely on bacterial culture, individual clone sequencing and non-high throughput sequencing (i.e. community profiles separation by automated ribosomal intergenic spacer analysis and terminal restriction fragment analysis) (Chawanakul et al., 2009; Doi and Ranamukhaarachchi, 2009; Sooksa-Nguan et al., 2010; Chaiyasen et al., 2014; Chunjaturas et al., 2014; Siripattanakul-Ratpukdi et al., 2015); hence, the true microbiota remains to fulfill the missing puzzles.

## Study objectives

To support the understanding and sustainable management of the NE soil, we obtained a complete database of soil prokaryotic (bacteria and archaea) and eukaryotic (fungi, protists, plants and animals) microbiome in an upper NE named Sakon Nakhon province using metagenomics combined with 16S and 18S rRNA genes next generation sequencing. The correlations between microbial metabolic networks

and microbiota were also analyzed to retrieve putative links of key metabolic pathways and microbes that may be lost or overgrowth by IA. As the agrotechnical soil microbiome database in Thailand remains limited, the present study represents an initial database of the NE (Sakon Nakhon province), and this article includes a part of our results on prokaryotic and eukaryotic diversity (excluded soil characteristics correlation, and metabolic potential correlation analysis). Sakon Nakhon is one of the 19 provinces in NE, with 0.3 m. ha rice cultivar area in 2005. This considers moderately high compared to other provinces in the NE.

## Methods

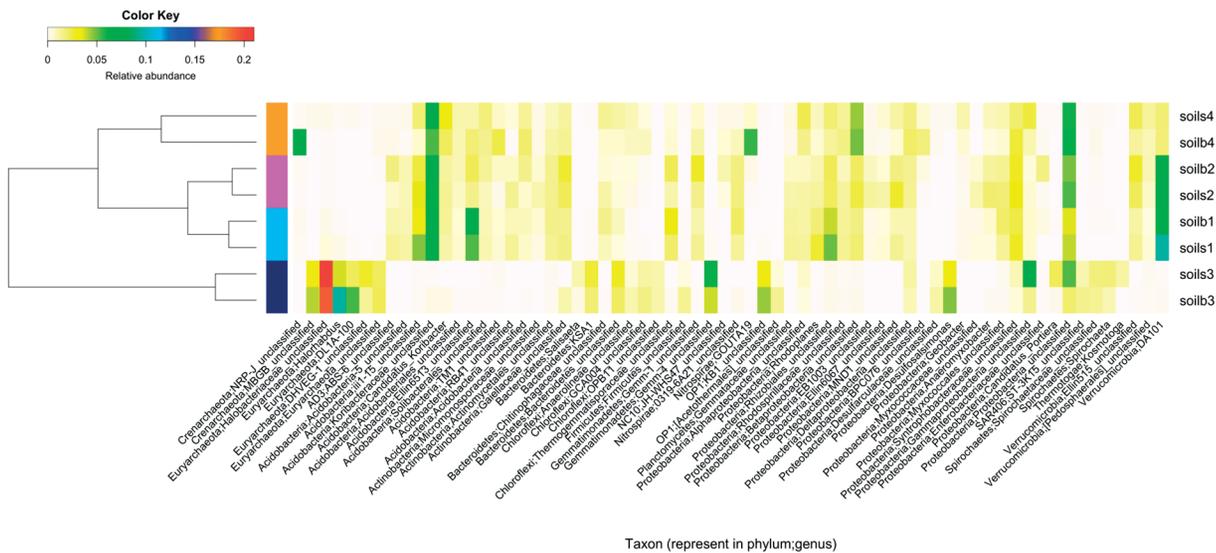
Four different agriculture sites in Sakon Nakhon province were selected to cover the soil agricultural diversity in the NE: (1) OA rice farm with polyculture and crop rotation in Kusuman District (site 1); (2) OA plus IA rice farm (near salt pan) with crop rotation in Wanorn Niwat District (site 2); (3) salt pan in Wanorn Niwat District (site 3); and (4) IA rice farm with crop rotation in Phanna Nikhom District (site 4). Samples were collected on 21-22 June 2014. Of each site, 10 independently soil sampling collections of 2 depths (~1-5 cm represent 'surface' microbiota and ~20-30 cm belowground represent 'root' associated microbiota) of 15 g each 1 m apart were collected in sterile containers. The surface and root microbiota are abbreviated 's' and 'b', respectively. With four total sites, soils1 represents surface at site 1, soilb1 represents root at site 1, soils2 represents surface at site 2, and so on.

Metagenomic extraction for prokaryotes and eukaryotes were performed on freshly collected soil samples using Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA), and determined quality and concentration via agarose gel electrophoresis and nanodrop spectrophotometry. The library constructions were performed using universal 16S rRNA gene (for prokaryotes) and 18S rRNA gene

(eukaryotes) primers with appended 5' Illumina adapter and 3' Golay barcode, following Caparaso et al. (2012). Each amplicon library was size-purified using GenepHlow™ Gel Extraction Kit (Geneaid Biotech Ltd., New Taipei City, Taiwan) and quantified with Picogreen (Invitrogen, Eugene, Oregon, USA), and a minimum of independent triplicates were performed and equally pooled to minimize stochastic PCR bias. Sequencing was performed by MiSeq300 platform (Illumina, San Diego, CA, USA) at Chulalongkorn Medical Research Center, along with appropriate sequencing primers and index sequence (Caporaso et al., 2012). For bioinformatic analyses, Mothur's standard operating procedures (Schloss et al., 2009) were followed unless noted. Pearson product-moment correlation coefficient was used to evaluate an association between community structures and soil characteristics. For representative species of the community structures, Spearman's correlation was performed.

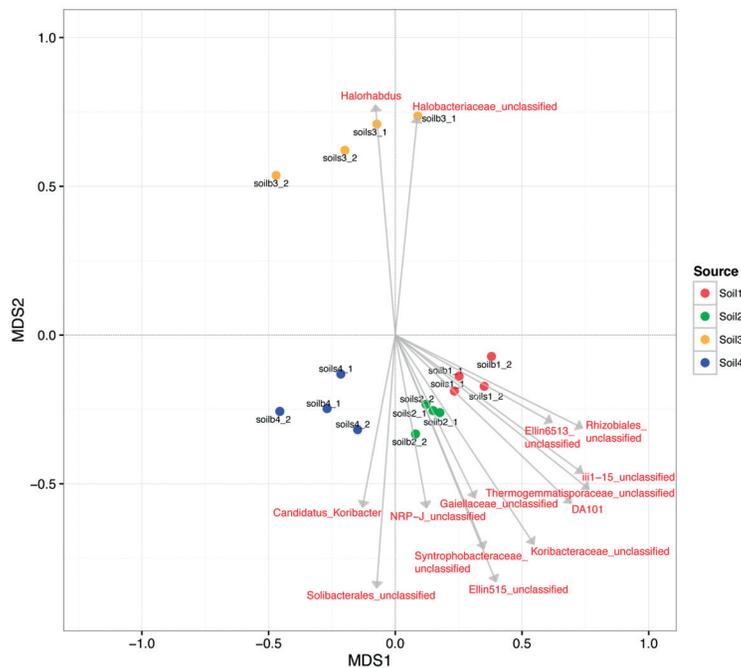
## Diversity of bacteria and archaea populations

Among groups, the bacteria of Proteobacteria phylum was dominated (avg. 31.43%), followed by Acidobacteria, Verrucomicrobia, Actinobacteria and Chloroflexi. For archaea, phyla Crenarchaeota, Euryarchaeota and Parvarchaeota were found. According to statistic computation, independent replicate sequencing results were merged (data not shown). Surface- and root-associated microbiota showed relatively close community structures (Yue & Clayton theta similarity coefficient, p-value 0.780; Jaccard similarity coefficient, p-value 0.824), while of different agriculture sites showed disparate structures. While the soil microbiota between (1) and (4) should be of relative due to the land characteristics (data not shown: geography, temperature, pH and texture identity of soil) and crop plantation, the soil microbiota of (1) and (2) were most related. Examples of dominated species in (1) are several members in



**Figure 1** Percent composition of prokaryotic genera, along with the dendrogram clustering on the left (branch in different color denotes different soil groups). Of each group, relative percent abundance was shown bases on a color chart on the top left. To fit a limited space, genus with less than 1% relative abundance was omitted.

Source: Somboonna (2015)



**Figure 2** NMDS showing prokaryotic communities relatedness, and representative genera calculated by Spearman’s correlation

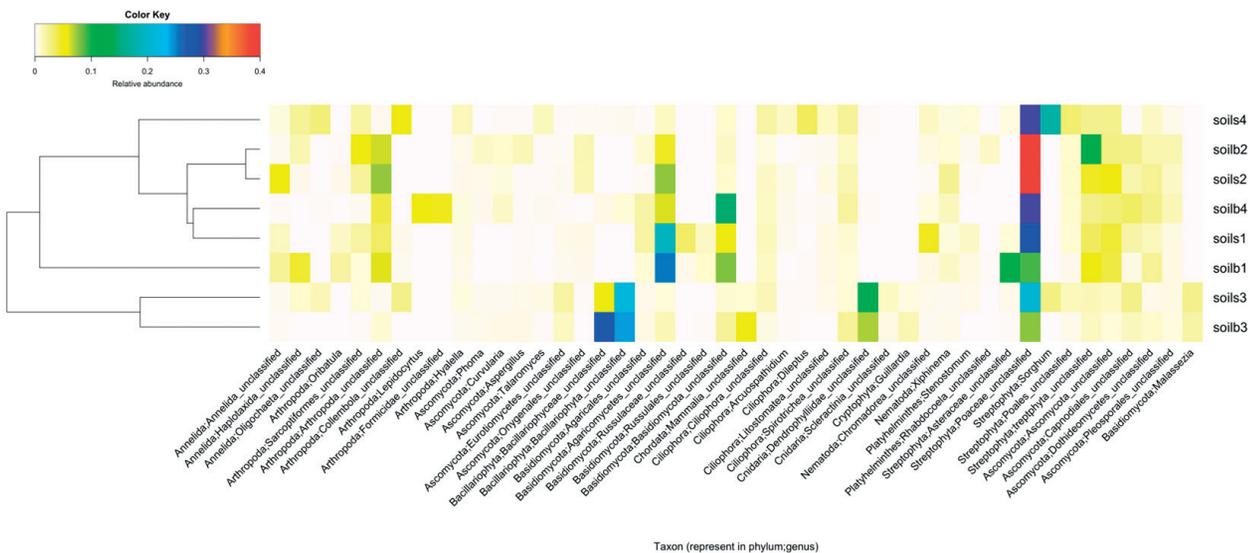
Source: Somboonna (2015)

phylum Acidobacteria (e.g. iii1-15 and Ellin6513), unclassified in family Thermogemmatissporaceae (phylum Chloroflexi), Gemm-1 in phylum Gemmatimonadetes, 0319-6A21 in phylum Nitrospirae, Rhodoplanes and unclassified genus in order Rhizobiales (phylum Proteobacteria), unclassified in order Myxococcales (phylum

Proteobacteria), and DA101 in class Spartobacteria (phylum Verrucomicrobia) (Figure 1). This despite the fact that site 2 is a neighbor to site 3, a salt pan, and hence share soil characteristics to site 3. The separate cluster of site 4 potentially highlights OA apart from IA factors, give more extensive chemical inputs in site 4 (synthetic fertilizer, pesticide and

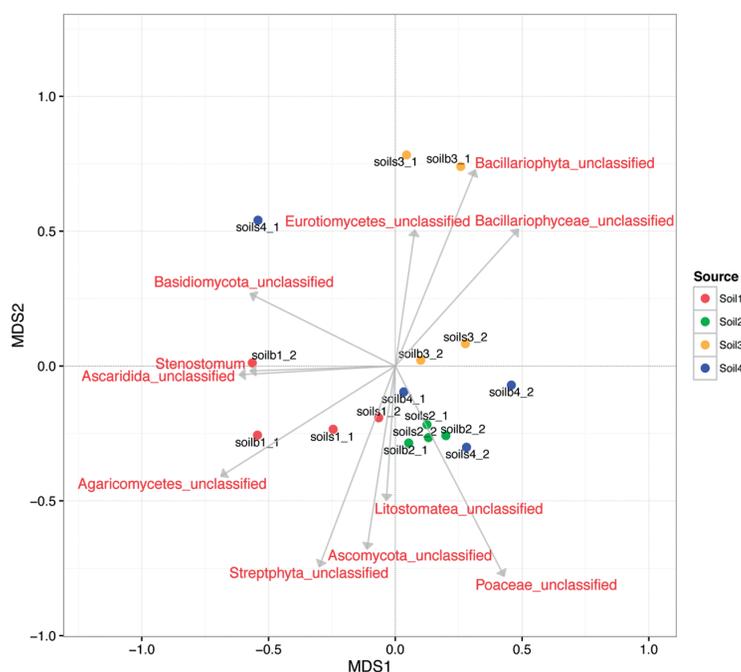
herbicide) than in site 2, which only experienced synthetic fertilizer. On the other hand, the uniqueness of site 3 highlighted the specific structure of the salt farm characteristics and hence soil microbial structure, as demonstrated by the more common archaea (e.g. family Halobacteriaceae and Halorhabdus in

phylum Euryarchaeota), bacterial members of phyla Bacteroidetes, Firmicutes and Spirochaetes. Soil of site 3 also denoted none to minute presence of Acidobacteria, Actinobacteria, Nitrospirae, Proteobacteria (the common phylum for soils of site 1 and 2, and fewer in 4), and Verrucomicrobia (Figure 1).



**Figure 3** Percent composition of eukaryotic genera, along with the dendrogram clustering on the left. Of each group, relative percent abundance was shown based on a color chart on the top left. To fit a limited space, genus with less than 1% relative abundance was omitted.

Source: Somboonna (2015)



**Figure 4** NMDS showing eukaryotic communities relatedness, and representative genera calculated by Spearman's correlation

Source: Somboonna (2015)

Prokaryotic community structures were plotted onto a non-metric multidimensional scale (NMDS) that showed IA may affect the soil characteristic in a way to separate microbiota site 4 from the others (data not shown). Figure 2 demonstrated many bacterial flora (e.g. Ellin6513, Rhizobiales, iii1-15 and DA101) that were statistically suggested to drive the OA soil of site 1 characteristics, diverged from site 44, given the vector length indicates the strength of the association and the vector direction indicates the direction of the effect. For the salt pan soil microbiota, family Halobacteriaceae (including genus *Halorhabdus*) in phylum Euryarchaeota were representative. These microbiota results allow prediction of microbial metabolic networks, and analysis for key metabolic pathways and associated microbes to OA (data not shown). This opens a venue for IA minimal core microbiome remediation, attempt to convert soil to once an OA soil microbial resources.

## Diversity of microbial eukaryote populations

Figure 3 demonstrated detail genera composition of microbial eukaryotes among sites, with independent replicate sequencing results (not shown) were merged. The community structures of soils of site 2 were clustered, embraced by soils1 and soilb4, and soils4, and then soilb1, in orderly. This pinpoints a partial community relationship of site 4 to 1 and 2, alternatively the closer between site 4 and 1 for the soil eukaryotic compared with prokaryotic microbiome.

The dissimilarity indices by the Jaccard (jclass) and Yue & Clayton theta (thetaYC) showed a minor closer of site 1 to 2 than 4 (jclass: soilb1 and soilb2 = 0.510, soils1 and soils2 = 0.503, soilb1 and soilb4 = 0.552, soils1 and soils4 = 0.548; thetaYC: soilb1 and soilb2 = 0.735, soils1 and soils2 = 0.218, soilb1 and soilb4 = 0.639, soils1 and soils4 = 0.448), consistent with the community structure plots in NMDS in Figure 4. Note the dissimilarity index ranges

from 0.000 to 1 and the closer to 0.000 infers the closer community similarity. Possibly, the eukaryotic profiles include organisms of the greater size than bacteria, hence more resistant to chemical inputs in site 4. This reflects the complex of nature microbiota. Signature microbes to site 1 was animal Ascaridida (phylum Nematoda) and fungi Agaricomycetes (phylum Basidiomycota) (Figure 4), and moderate abundance of plant Poaceae (phylum Streptophyta) (Figure 3). Similar to prokaryotes, the eukaryotic community structures of site 3 remain distinct, with relatively low frequency of annelids (earthworms), arthropods and fungi Basidiomycota, but abundance in Bacillariophyta (phylum) plants (Figure 3). Annelids, nematodes and fungi (e.g. Basidiomycota and Ascomycota) that were more common in crop farms (soil of site 1, 2 and 4) (Figure 3) function significant regulators for soil organic nutrient dynamics, including tillage, fertilization, and soil texture (Fonte, Winsome, and Six, 2009). Soil organic matters is crucial to metabolic food networks in soil ecosystem (Lal, 2004; Fonte, Winsome, and Six, 2009).

## Conclusion

Today, people and government are aware of sustainable environments as well as microbial resources, the fundamentals to the diverse metabolic food networks. The market and agrotechnique for organic crops arise. This study represents the first to uncover the agrotechnique-associated microbiota, both prokaryotes and eukaryotes, in the NE of Thailand. The data are useful to academic and public interests, support elucidation of the core microbiome to convert soil health to crop yield in an environmental safe manner.

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## References

- Agler, M., Ruhe, J., Kroll, S., Morhenn, C., Kim, S. -T., Weigel, D., et al. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. **PLoS Biology** 14(1): e1002352. doi: 10.1371/journal.pbio.1002352
- Bengtsson, J., Ahnström, J., and Weibull, A. -C. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. **Journal of Applied Ecology** 42: 261–269.
- Berkelmans, R., Ferris, H., Tenuta, M., and van Bruggen, A. H. C. 2003. Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. **Applied Soil Ecology** 23: 223-235.
- Bossio, D. A., Scow, K. M., Gunapala, N., and Graham, K. J. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. **Microbial Ecology** 36: 1-12.
- Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., et al. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. **Nature** 488: 91-95.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **International Society for Microbial Ecology** 6: 1621-1624.
- Chaiyasen, A., Young, J. P. W., Teaumroong, N., Gavinlertvatana, P., and Lumyong, S. 2014. Characterization of arbuscular mycorrhizal fungus communities of *Aquilaria crassna* and *Tectona grandis* roots and soils in Thailand plantations. **PLoS ONE** 9(11): e112591. doi:10.1371/journal.pone.0112591
- Chawanakul, S., Chaiprasert, P., Towprayoon, S., and Tanticharoen, M. 2009. Contributions of available substrates and activities of trophic microbial community to methanogenesis in vegetative and reproductive rice rhizospheric soil. **Journal of Environmental Biology** 30: 119-127.
- Chunjaturas, W., Ferguson, J. A., Rattanapichai, W., Sadowsky, M. J., and Sajjaphan, K. 2014. Shift of bacterial community structure in two Thai soil series affected by silver nanoparticles using ARISA. **World Journal of Microbiology and Biotechnology** 30: 2119-2124.
- Doi, R., and Ranamukhaarachchi, S. L. 2009. Correlations between soil microbial and physicochemical variations in a rice paddy: implications for assessing soil health. **Journal of Bioscience** 34: 969-976.
- Fedra, K., Winkelbauer, L., and Pantulu, V. R. 1991. **Expert systems for environmental screening. An application in the lower Mekong basin.** Laxenburg, Austria: International Institute for Applied Systems Analysis.
- Fonte, S. J., Winsome, T., and Six, J. 2009. Earthworm populations in relation to soil organic matter dynamics and management in California tomato cropping systems. **Applied Soil Ecology** 41: 206-214.
- Fukai, S., Sittisuang, P., and Chanphengsay, M. 1998. Increasing production of rainfed lowland rice in drought prone environments - a case study in Thailand and Laos. **Plant Production Science** 1: 75-82.
- Griffiths, B. S., Ritz, K., and Wheatley, R. E. 1994. Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system. **Soil Use and Management** 10: 20-24.
- Hole, D. G., Perkins, A. J., Wilson, J. D., Alexander, I. H., Grice, P. V., and Evans, A. D. 2005. Does organic farming benefit biodiversity? **Biological Conservation** 122: 133-130.
- Lakshmanan, V. 2015. Root microbiome assemblage is modulated by plant host factors. **Advance Botany Research** 75: 57-79.
- Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. **Science** 304: 1623-1627.
- Letourneau, D. K., and Goldstein, B. 2001. Pest damage and arthropod community structure in organic vs. conventional tomato production in California. **Journal of Applied Ecology** 38: 557-570.
- National Economic and Social Development Board (NESDB). 2005. **Macro economic outlook: gross regional product and national income.** [Online]. Available form: <http://www.nesdb.go.th>
- Office of Agricultural Economics (OAE). 2011. **The Situation of Organic Agriculture.**[Online]. Available form: [http://www.oae.go.th/ewtadmin/ewt/oae\\_baer/download/article/article\\_20111013102515.pdf](http://www.oae.go.th/ewtadmin/ewt/oae_baer/download/article/article_20111013102515.pdf)
- Paull, J. 2011. Attending the First Organic Agriculture Course: Rudolf Steiner's Agriculture Course at Koberwitz, 1924. **European Journal of Social Sciences** 21: 64-70.
- Qin, Y., Druzhinina, I. S., Pan, X., and Yuan, Z. 2016. Microbially mediated plant salt tolerance and microbiome-based solutions for saline agriculture. **Biotechnology Advances** 34: 1245-1259.
- Rigby, D., and Cáceres, D. 2001. Organic farming and the sustainability of agricultural systems. **Agricultural Systems** 68: 21-40.
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. **Applied Environmental Microbiology** 75: 7537-7541.
- Schmidt, J. E., Bowles, T. M., and Gaudin, A. C. M. 2016. Using ancient traits to convert soil health into crop yield: impact of selection on maize root and rhizosphere function. **Frontiers in Plant Science** 7: 373. doi: 10.3389/fpls.2016.00373
- Seufert, V., Ramankutty, N., and Foley, J. A. 2012. Comparing the yields of organic and conventional agriculture. **Nature** 485: 229-234.
- Siripattanakul-Ratpukdi, S., Vangnai, A. S., Sangthean, P., and Singkibut, S. 2015. Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. **Environmental Science and Pollution Research** 22: 320-328.
- Somboonna, N. 2015. **Microbiome representing fertile and healthy soils.** Ratchadaphiseksomphot Endowment Fund 2013 of Chulalongkorn University (CU-56-463-FW).
- Sooksa-Nguan, T., Gympantasiri, P., Boonkerd, N., Thies, J. E., and Teaumroong, N. 2010. Changes in bacterial community composition in the system of rice intensification (SRI) in Chiang Mai, Thailand. **Microbes and Environments** 25: 224-227.
- Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. 2015. The importance of the microbiome of the plant holobiont. **New Phytology** 206: 1196-1206.