ASIAN JOURNAL OF AGRICULTURE
Volume 8, Number 2, December 2024
Pages: 124-133

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g080206

Detection of available phosphorus and diversity of culturable phosphate-solubilizing bacteria after organic farming conversion

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Manuscript received: 20 June 2024. Revision accepted: 12 September 2024.

Abstract. *Waithaisong K. 2024. Detection of available phosphorus and diversity of culturable phosphate-solubilizing bacteria after organic farming conversion. Asian J Agric 8: 124-133.* Organic Agriculture (OA) has increased in Thailand since 2000. Most studies on OA in Thailand focus on socio-economic sectors. Soil properties and microorganisms in OA soils have been little explored. This study aimed to investigate the soil nutrient status nitrogen (N), phosphorus (P), and biodiversity of Phosphate-Solubilizing Bacteria (PSB) in OA soils. Topsoil samples were collected from two organic paddy soils (OS1 and OS2). The soils were analyzed for particle distribution, cation exchange capacity, exchangeable elements, Organic Matter (OM), electrical conductivity, pH, N content, and P content. Soil bacteria were isolated on LB agar medium and screened for PSB on NBRIP agar. The sequences of the 16S rRNA gene were used to identify PSB. The results showed that in all the sites, soils were silty clay, and high accumulation of OM, high N availability, and ammonium was the main form of N. Phosphorus was present in low concentration indicating the limitation of P may reduce the nitrification process, while organic P was mostly found in soil. Analysis of microbial P revealed that P was mainly found in microbial cells of OS2. PSB identification showed that the main PSB genera found in OA soils were *Enterobacter*, *Pantoea*, *Bacillus*, and *Pseudomonas*. All results indicated that OS1 and OS2 soils had low P which affected N cycling. The reduction of P may result from the conversion of rice soil to organic agricultural practices. Further, PSB should be characterized and applied with rock phosphate in OA soils to maintain rice productivity.

Keywords: Organic farming, paddy soil, phosphate-solubilizing bacteria, phosphorus limitation, soil properties

INTRODUCTION

Organic Agriculture (OA) has increased in Thailand since 2000 due to the export of Thai organic products and the modification of the national strategy of economic and social development (Kongsom and Panyakul 2016; Sitthisuntikul et al. 2018). In 2018, Thailand presented around 300,000 rai of organic agricultural land (1 rai = 0.16 ha) as reported by Suwanmaneepong et al. (2020) and the exportation of rice presented 11.13 million tons (Seerasarn et al. 2020). The Thai government has launched a 4-year program to convert 160,000 ha of land for organic rice production with the certification to supply the demand of the Chinese market (Hérique and Faysse 2021). Thai farmers were encouraged to take part in the National Program for the Promotion of Organic Rice Production (PPORP) to promote organic rice production and obtain organic certification. The certification and the number of organic farmers progressed slowly due to the lack of human resources to support the transition of OA and promoted the sustainability of the sector (Hérique and Faysse 2021). Moreover, organic rice farmers need more support from the Thai government for water and nutrient management in soils (Sitthisuntikul et al. 2018).

The research about the impact of OA on Thai farmers has increased. Several studies reported the impacts of socio-economic of OA. Among them, it was reported that small farmers in the North of Thailand produced organic products such as rice and vegetables for family consumption (Sitthisuntikul et al. 2018). The authors suggested the transformation of small production to commercial production to help household expenses by supporting their OA practices knowledge. The case studies of organic agricultural producers in Chachoengsao Province, the East of Thailand reported the need for innovative technology for OA production, promoting the new strategy for organic agricultural members and enhancing the quality of OA products for consumers and for reaching the criteria of organic certification. In the same area in Chachoengsao Province, the comparative study of the cost and return structure of organic and conventional rice farming systems showed that organic farming had a high benefit for farmers than conventional farming by increasing of rice price for selling with the difference value of 4.32 THB/kg (Suwanmaneepong et al. 2020). Moreover, in the Central Plains of Thailand, in Nakhon Pathom Province, the organic rice farmers transformed their Community Enterprise as an agro-tourism place for local visitors and supply the knowledge of organic agricultural practices as well as selling organic rice products (Poorahong et al. 2022).

Besides nutrient management in OA, it was also reported that farmers applied green manure, plant residues and rice straw (Suwanmaneepong et al. 2020). Some farmers added organic fertilizer after converting the rice fields to OA. In addition, some farmers produce compost by themselves and use it in their farms (Sitthisuntikul et al. 2018; Hérique and Faysse 2021; Poorahong et al. 2022).

Organic rice cultivation promoted carbon sequestration in the soils compared to conventional rice cultivation by increasing Soil Organic Carbon (SOC) (Arunrat et al. 2022).

In microbial population analysis, the study of 15 organic rice soils showed that the grain yield of rice was related to soil quality indicators, such as microbial biomass, total phosphorus, total nitrogen (N), SOC, and soil Organic Matter (OM) (Thuithaisong et al. 2011). The analysis of Phosphate-Solubilizing Bacteria (PSB) from one hundred and fifty rice fields at various locations in Northern Thailand, including some organic rice fields, revealed that *Acinetobacter* strain CR 1.8 was the best PSB among 216 isolates to solubilize tricalcium phosphate and produced acid phosphatase. The strain *Klebsiella* SN 1.1 was the best PSB in terms of indole acetic acid production (Chaiharn and Lumyong 2011).

Organic agriculture is an agricultural system that uses a natural process to produce plant biomass and reduce inputs, such as synthetic fertilizers, pesticides, and herbicides (Meemken and Qaim 2018). In terms of nutrient availability, the systems used animal manures, biofertilizers, and green manures instead of synthetic fertilizers (Gomiero et al. 2011). It is often found that the systems present the limitation of nutrients such as N and P (Bedoussac et al. 2015; Meemken and Qaim 2018). In England, it was reported that organic farming reduced available P (labile Pi) after 15 years of land conversion (Gosling and Shepherd 2005). OA also promoted organic phosphorus (labile Po) (Xavier et al. 2009) and microbial biomass (Crystal-Ornelas et al. 2021). Moreover, OA promoted Cation-Exchange Capacity (CEC), and potassium availability (Bulluck et al. 2002), and promoted the accumulation of OM in the soils (Reganold and Wachter 2016). In terms of microbial biodiversity, OA increased soil biodiversity (Tuck et al. 2014) and promoted some phyla of soil bacteria, such as Acidobacteria, Firmicutes, and TM7 (Bonanomi et al. 2016) and increased carbon storage in soil (Gomiero et al. 2011).

Despite the increase in organic rice cultivation, few studies examined soil nutrient status after the conversion of land and microbial population, especially microorganisms involved in the phosphorus (P) cycling in organic rice fields in Thailand such as PSB. PSB has been reported to increase labile Pi through the solubilization and mineralization processes which encapsulates the ability of bacteria to produce organic acid and phosphatase enzyme and can use as biofertilizer in organic farming (Alori et al. 2017). The property of P solubilization and mineralization was naturally induced by labile Pi and labile carbon in soils (Huang et al. 2021). It has been reported that PSB belong to the genera; *Pseudomonas* spp., *Agrobacterium* spp., *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kushneria*, *Paenibacillus*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Serratia*, *Bradyrhizobium*, *Salmonella*, *Sinomonas*, and *Thiobacillus* (Alori et al. 2017). This study aimed to investigate the soil nutrient status (N, P) and biodiversity of PSB for further use as a biofertilizer in OA soils.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected from an organic paddy field at Ban Chanote, Klong Yong-Lantakfa Community Enterprise, located in Lantakfa, Nakhon Chaisri, Nakhon Pathom, Thailand (Figure 1). The area belongs to the Central plain region of Thailand. The soil in the undisturbed native land was classified according to the USDA soil taxonomy as Alfisols. The typical characteristics were dark-brown clay loam with low drainage, moderate organic matter content, and slight acidity (pH 5.0–6.5). The area was used as a traditional rice field in 1961 and converted to an organic rice field in 2011. Poultry and swine manure were added to the soils before rice planting at an unknown rate (Hunnark et al. 2017). Six composite samples of soil were collected from organic paddy soil1 (OS1), and same number of soil samples were collected from organic paddy soil2 (OS2) presented in Figure 1 by using the protocol as described by Waithaisong et al. (2022a). The soil samples were randomly collected from 10 Rai (16,000 m^2). Five hundred grams of soil samples were collected at a depth of 0-17 cm using a shovel and stored in plastic bags at 4°C placed in a cooling tank for PSB isolation and soil analysis.

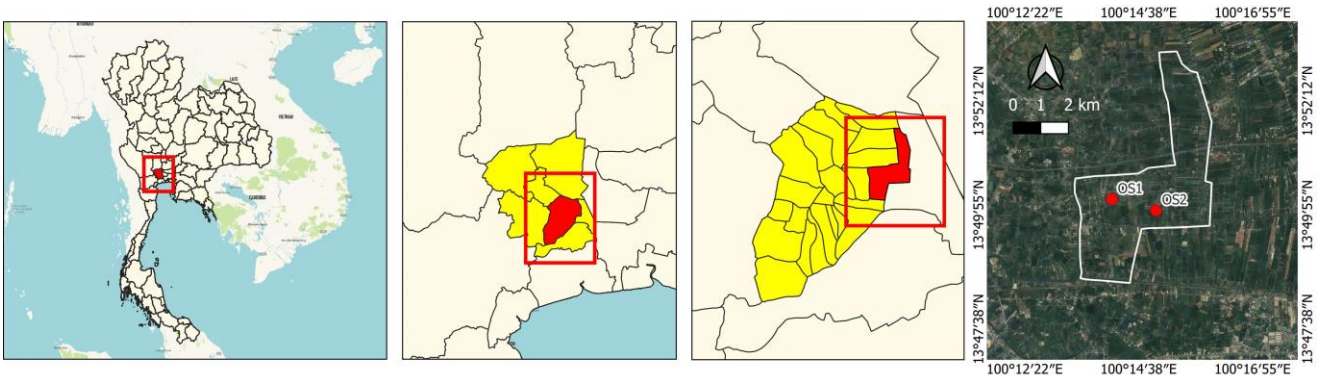

Figure 1. Study area in Nakhon Pathom Province, Thailand. Two red spots represent Organic Paddy Soil1 (OS1) and Organic Paddy Soil2 (OS2)

Soil analysis

The soils were sent to the soil plant and agricultural material testing and research units of Kasetsart University, Bangkok, Thailand, for analysis of soil particle distribution, CEC, exchangeable elements, OM, electrical conductivity (Ece), potential of hydrogen (pH), and N availability. In brief, the particle distribution of soil was analyzed by pipette method (Sheldrick and Wang 1993). The soil CEC and exchangeable elements were determined using the method of 1N ammonium acetate, pH 7 (FAO 2022). The exchangeable elements were then analyzed with atomic spectroscopy. The soil samples were digested using a mixture of $H_2SO^{4-}Na_2SO^{4-}Se$ for analysis of soil OM. The soil Ece was measured in saturated soil paste extract by using an EC meter. The soil pH (in water) was measured using a pH meter. The available of ammonium and nitrate measured by the KCl extraction-distillation method (Wheatley et al. 1989).

The availability of P was estimated by Olsen method (Olsen et al. 1954). The soil extraction was performed with the intact fresh soils to evaluate labile Pi and labile Po. The soil samples were mixed with 0.5M $NaHCO_3$, pH 8.5 (1/10, w/v) and then shaken end-over-end for 30 min at room temperature. The samples were centrifuged 6000 rpm for 15 min. The supernatant was divided into two fractions to measure the concentration of (i) labile Pi after humic acid precipitation by using 6N HCl (1/300, v/v) and (ii) total P after acid digestion with 12N HCl (1/1, v/v) (Ali et al. 2009). The labile Po was calculated from the subtraction of total P and labile Pi. The availability of P in soil microorganisms was evaluated in autoclaved soils (Waithaisong et al. 2022a). For this, fresh soil samples were autoclaved twice (110°C for 60 min) at 24h intervals then performed the Olsen extraction as previously described. The labile Pi of soil extract was labeled as microbial Pi and labile Po was labeled as microbial Po.

Isolation of PSB

Total bacteria were isolated from fresh soil samples on Luria-Bertani (LB) agar medium (10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl, 15 L^{-1} agar). By selecting the different colony morphology on LB agar, PSB was screened on National Botanical Research Institute's Phosphate growth medium (NBRIP) agar medium (10 g L^{-1} D-glucose, 5 g L^{-1} Ca_3 $(PO_4)_2$, 5 g L^{-1} $MgCl_2$ $6H_2O$, 0.25 g L^{-1} $MgSO_4$ $7H_2O$, 0.2 g L^{-1} KCl, 0.1 g L^{-1} $(NH_4)_2SO_4$, 15 g L^{-1} agar) (Jorquera et al. 2008). For each site, two hundred and eighty-eight colony were screened. The isolates that formed clear zones on NBRIP agar were considered as PSB. They were purified by streak plate method and stored at –80°C in 30% glycerol for identification.

16S rRNA gene amplification, sequencing, and phylogenetic analysis

The purified PSB were grown on LB broth and performed DNA extraction by using a Thermo Scientific GeneGET genomic DNA purification kit. The purified PSB were identified by amplifying and sequencing the 16S rRNA gene with the universal bacterial primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'GGTTACCTTGTTACGACTT-3'). Polymerase Chain Reaction (PCR) was performed by using Thermo Scientific DreamTaq PCR Master Mix (2X). The PCR mixture consisted of a final volume of 50 µl; 0.25 µM of each primer, 2 mM MgCl2, 1X DreamTaq Master Mix buffer containing Taq DNA polymerase, and 1 µg DNA template. The following cycle conditions were: initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 3 minutes (35 cycles) and final extension at 72°C for 10 minutes (Waithaisong et al. 2022b). PCR products were checked on 1% agarose gel electrophoresis at 100 voltages for 40 minutes and then purified by using Invitrogen PCR purification Kit. The PCR products were then sequenced. The nucleotide sequences of 16S rRNA genes were corrected using the HVDR online program (Bell and Kramvis 2013). The corrected sequences were compared with reference sequences in GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify PSB genera. The phylogenetic trees were constructed with MEGA11 program. The trees were reconstructed using the Neighbor-joining method with the Kimura two-parameter method and uniform rates. Bootstrap values were determined from 1000 replications.

Statistical analysis

The statistical analysis was conducted with R software (version 2.11.1, The R Foundation for Statistical Computing). In the statistical analysis, the paired comparison was conducted by using Student's t-Test. The Analysis of Variance (ANOVA) was used when more than three groups were compared with Duncan's multiple range test for post hoc test. The principal component analysis was used to examine the interaction of soil properties.

RESULTS AND DISCUSSION

Characteristics of soils

The two organic paddy soils did not different for the soil type. The soils were silty clay. In terms of particle distribution, soils were significantly different in silt and clay contents. The amount of silt (44.14%) in OS1 was lower than the amount (50.45%) of silt in OS2. However, the amount of clay in OS1 was higher (53.66%) than in OS2 (48.14%). The analysis of exchangeable elements exhibited the same quantity of Ca and of Mg in the two sites. Contrary, the quantity of K and CEC was significantly different. The quantity of these soil properties was lower in OS1 than in OS2. The quantity of K was 0.66 cmol kg^{-1} and 0.76 cmol kg^{-1}, respectively. The CEC content was 28.17 cmol kg^{-1} and 30.79 cmol kg^{-1} in OS1 and OS2, respectively. In addition, 4.10% and 5.18% OM was recorded from the two study sites which were significantly different. The Ece and pH in water did not different in the two sites (Table 1).

Nitrogen content

The quantity of available N (NH_4^+-N, NO_3^--N) was 125.53 mg kg^{-1} and 84.08 mg kg^{-1} in OS1 and OS2,

respectively. Comparing the quantity of N species inside the site, soil accumulated more ammonium than nitrate (Table 2). In different organic paddy sites, the amount of ammonium was indifferent in two soils (Figure 2.A). In contrast, the quantity of nitrate was significantly different. It was higher in OS1 than OS2 with the value of 29.61 mg kg^{-1} and 7.11 mg kg^{-1}, respectively (Figure 2.B).

Phosphorus content

In general, Po was accumulated in two organic paddy soils. Comparing inside the sites, the quantity of labile Pi and labile Po (bicarbonate-extractable P in intact soils) showed significantly different in all sites. However, the values were wider in OS2 compared to OS1. The quantity of P was 11.15 mg kg^{-1} for labile Pi and 14.98 mg kg^{-1} for labile Po in OS1. In OS2, the quantity of P was 3.41 mg kg^{-1} for labile Pi and 11.58 mg kg^{-1} for labile Po. In autoclaved soils, the quantity of microbial Pi and microbial Po (bicarbonate-extractable P in autoclaved soils) showed the same trend as labile P. The quantity of microbial P was 7.56 mg kg^{-1} for microbial Pi and 21.64 mg kg^{-1} for microbial Po in OS1. In OS2, microbial P was 4.29 mg kg^{-1} for microbial Pi and 19.11 mg kg-1 for microbial Po (Table 3).

In different organic paddy sites, the quantity of labile Pi was significantly different. It was higher in OS1 than in OS2. The quantity of microbial Pi showed the same trend as that of labile Pi. It was also found higher in OS1 than in OS2 (Figure 3.A). The quantity of labile Po and microbial Po did not different in OS1 and OS2 (Figure 3.B).

Considering the effect of autoclaving, in OS1 site, the quantity of microbial Pi was decreased in autoclaved soils compared to the quantity of labile Pi in intact soil. In OS2 site, the quantity of microbial Pi was not different in autoclaved soils compared to the quantity of labile Pi in intact soil (Figure 3.A). In addition, the quantity of microbial Po was slightly increased but not significantly different in autoclaved soils compared to the quantity of labile Po in intact soil for two study sites (Figure 3.B).

Table 1. Characteristics of organic paddy soils (OS1) and (OS2)

Characteristics of soils	OS1	OS2	p-value
Soil types (USDA)	Silty clay	Silty clay	
Particle distribution (%)			
Sand	2.20±0.62	1.41±0.58	NS
Silt	44.14±1.33	50.45±2.60	<0.01
Clay	53.66±1.88	48.14±2.48	<0.01
Exchangeable elements (cmol kg^{-1})			
K	0.66±0.06	0.76±0.07	0.03
Ca	15.51±0.67	16.35±0.54	NS
Mg	1.59±0.05	1.68±0.11	NS
CEC	28.17±1.33	30.79±0.74	<0.01
Other soil properties			
Organic matter (%)	4.10±0.46	5.18±0.37	<0.01
Electrical conductivity	4.31±0.30	4.77±0.47	NS
pH	5.76±0.19	5.86±0.15	NS

Note: Values are mean ± SD (n=6 replication). The p-values indicate significant differences according to the Student's t-Test (P≤0.05). NS: not significant

Table 2. Comparison of nitrogen forms in OS1 and OS2

Nitrogen forms (mg kg^{-1})	OS1	OS2	Total
NH_4^+-N	95.92±14.50	76.97±42.46	125.53±17.36
NO_3^--N	29.61±15.02	7.11±0.00	84.08±42.46
p-value	<0.01	0.01	NS

Note: Values are mean ± SD (n=6 replication). The p-values indicate significant differences according to the Student's t-Test (P≤0.05). NS: not significant

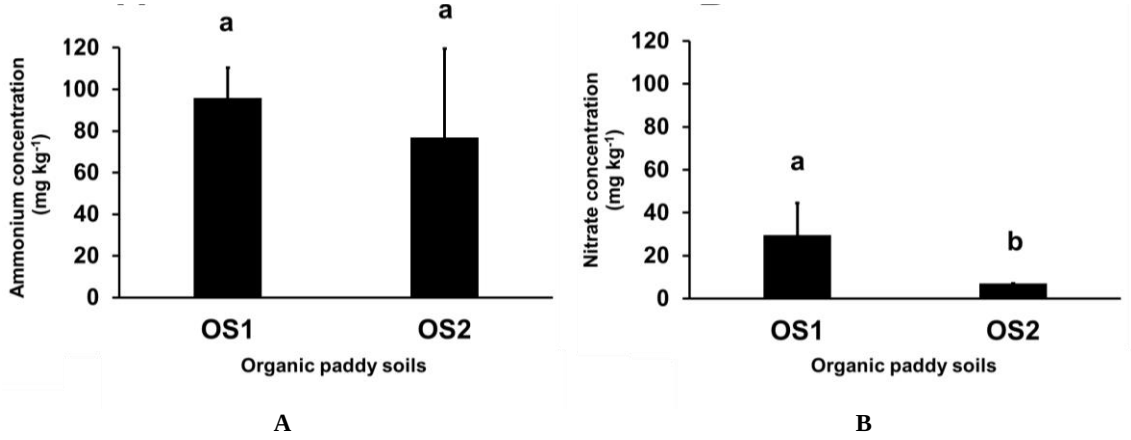

Figure 2. The concentration of nitrogen forms (mg kg^{-1}) of OS1 and OS2. A. Ammonium concentration (NH_4^+-N), B. Nitrate concentration (NO_3^--N). Values are mean ± SD (n=6 replication). Distinct letters indicate significant differences according to ANOVA (P≤0.05)

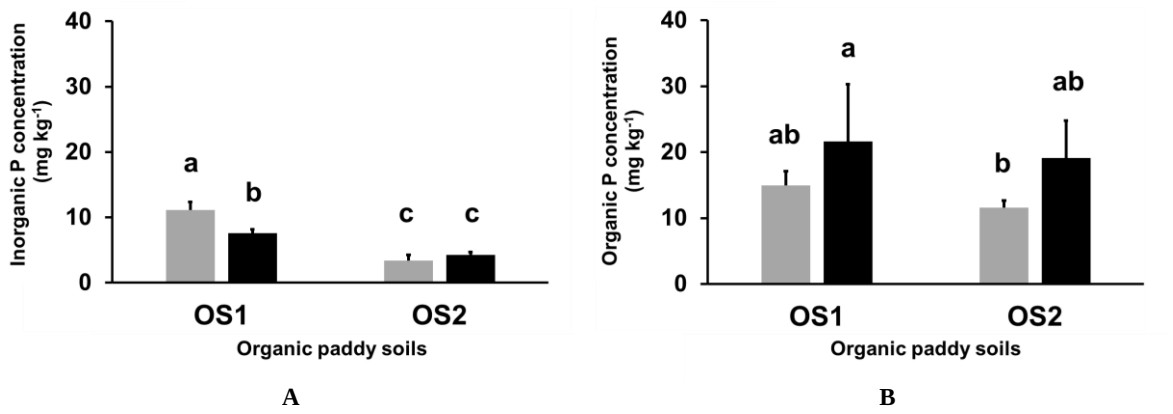

Figure 3. The concentration of bicarbonate extractable P forms (mg kg-1) of OS1 and OS2. A. Inorganic P extracted from intact soil (grey, labile Pi) and autoclaved soil (black, microbial Pi), B. Organic P extracted from intact soil (grey, labile Po) and autoclaved soil (black, microbial Po). Values are mean ± SD (n=6 replication). Distinct letters indicate significant differences according to ANOVA (P≤0.05)

Table 3. Comparison of Pi and Po concentration of intact and autoclaved soils of OS1 and OS2

Phosphorus forms (mg kg^{-1})	OS1	OS2
Intact soil		
Pi	11.15±1.18	3.41±0.85
Po	14.98±2.13	11.58±1.05
p-value	0.01	<0.01
Autoclaved soil		
Pi	7.56±0.62	4.29±0.39
Po	21.64±8.70	19.11±5.66
p-value	0.02	<0.01

Values are mean ± SD (n=6 replication). The p-values indicate significant differences according to the Student's t-Test (P≤0.05)

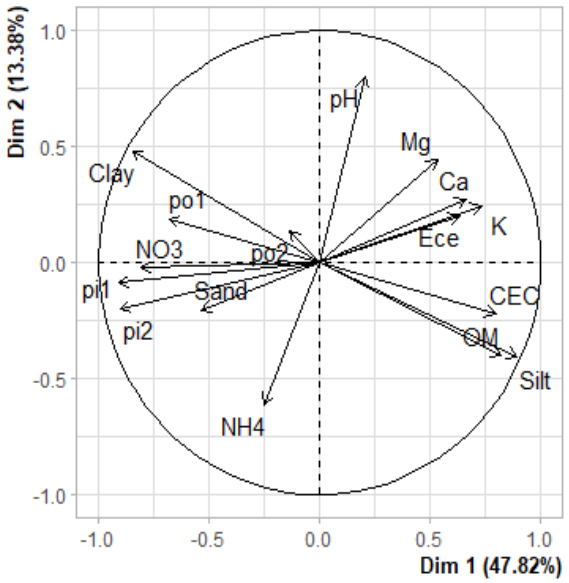

Figure 4. Principal component analysis of soil properties of two organic paddy soils. Pi1: Labile Pi, pi2: Microbial Pi, po1: Labile P0, po2: Microbial Po, Ece: Electrical conductivity

Table 4. Identities of the PSB strains isolated from OS1 site

Strains	Identification	% Gene identity
OSN8	*Enterobacter* sp.	99.90
OSN9	*Enterobacter* sp.	99.65
OSN10	*Enterobacter* sp.	99.62
OSN15	*Enterobacter* sp.	100.00
OSN21	*Enterobacter* sp.	98.84
OSN24	*Enterobacter* sp.	100.00
OSN27	*Enterobacter* sp.	99.62
OSN28	*Enterobacter* sp.	99.42
OSN29	*Enterobacter* sp.	99.12
OSN35	*Enterobacter* sp.	99.34
OSN40	*Enterobacter* sp.	99.90
OSN45	*Enterobacter* sp.	99.42
OSN1	*Pantoea* sp.	98.33
OSN3	*Pantoea* sp.	99.72
OSN4	*Pantoea* sp.	99.42
OSN47	*Pantoea* sp.	99.90
OSN83	*Pantoea* sp.	99.19
OSN87	*Pantoea* sp.	99.80
OSN89	*Pantoea* sp.	99.71
OSN19	*Bacillus* sp.	99.80
OSN22	*Bacillus* sp.	99.69
0SN38	*Bacillus* sp.	99.79
OSN60	*Bacillus* sp.	99.13
OSN66	*Bacillus* sp.	99.82
OSN82	*Bacillus* sp.	99.15
OSN91	*Bacillus* sp.	98.46
OSN5	*Pseudomonas* sp.	99.90
OSN11	*Pseudomonas* sp.	99.07
OSN12	*Pseudomonas* sp.	99.89
OSN16	*Pseudomonas* sp.	99.90
OSN71	*Pseudomonas* sp.	98.91
OSN18	*Acinetobacter* sp.	99.91
OSN54	*Burkholderia* sp.	99.38

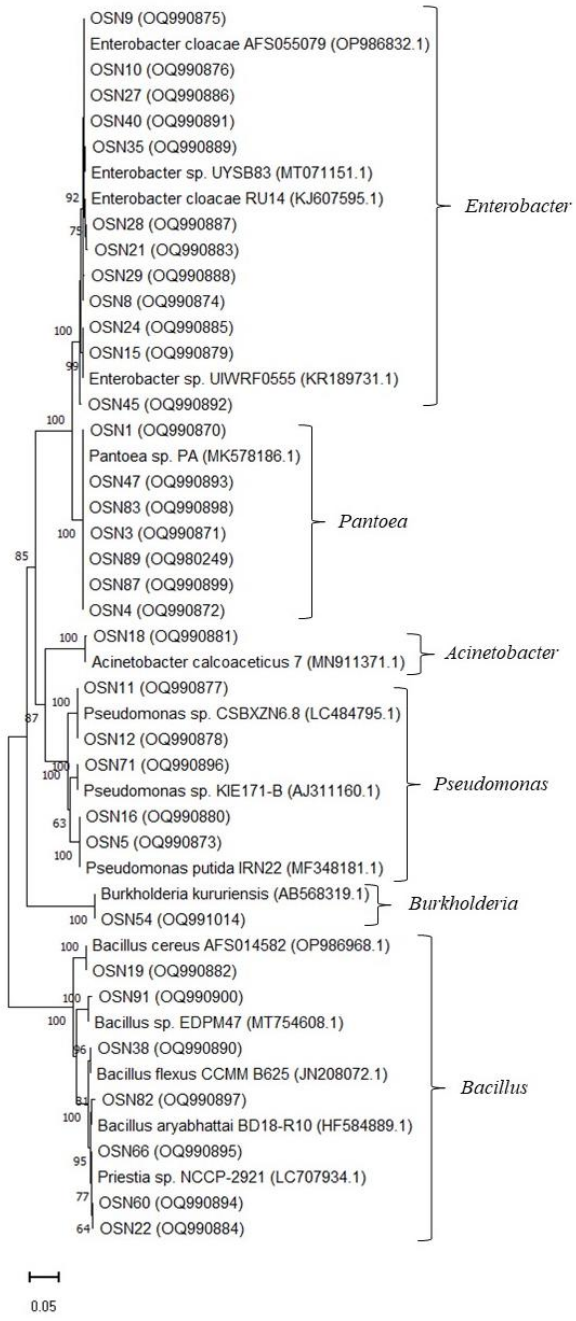

Figure 5. Phylogenetic tree of phosphate-solubilizing bacteria isolated from organic paddy soil 1 (OS1) based on 16S rRNA gene. The tree was reconstructed using the Neighbor-joining method with the Kimura two-parameter method and uniform rates. Bootstrap values were determined from 1000 replications. Bar, 0.05 substitutions per site

PCA analysis of soil properties

In PCA analysis, 47.82% of the vectors representing soil properties were separated into two groups along the horizontal axis. This percentage explained more relationship between the soil properties than the vertical axis that represented the vectors of soil properties only 13.38%. The relationship of the soil properties on the horizontal axis revealed that the quantity of phosphorus (labile P, labile Po, and microbial P), nitrate, clay particles, and sand were found to increase in the OS1 site, whereas only OM, CEC, silt particle, Ece, and exchangeable elements were found increased at the OS2 site (Figure 4).

Diversity of culturable phosphate- solubilizing bacteria

In the OS1 site, molecular characterization of PSB strains, based on 16S rRNA sequences, is presented in Figure 5 and Table 4. The percentage of similarity of query sequences and subject sequences in the NCBI database were more than 98% for all samples. Thirty-three strains from 288 colonies were capable of producing a halo zone on NBRIP agar supplemented with $Ca_3(PO_4)_2$. Six genera were identified: *Enterobacter*, *Pantoea*, *Bacillus*, *Pseudomonas*, *Burkhodelria*, and *Acinetobacter*. Among them, twelve strains (OSN8, OSN 9, OSN10, OSN15, OSN21, OSN24, OSN27, OSN28, OSN29, OSN35, OSN40, OSN45) were identified as *Enterobacter*. Seven strains (OSN1, OSN3, OSN4, OSN47, OSN83, OSN87, OSN89) were identified as *Pantoea*. Seven strains (OSN19, OSN22, OSN38, OSN60, OSN66, OSN82, OSN91) were identified as *Bacillus*. Five strains (OSN5, OSN11, OSN12, OSN16, OSN71) were identified as *Pseudomonas*. One strain (OSN 18) was identified as *Burkhodelria* and one other strain (OSN 54) was identified as *Acinetobacter*.

In OS2, the percentage of similarity of query sequences and subject sequences in the NCBI database were more than 98% for all samples. Nineteen strains from 288 colonies were capable of producing a halo zone on NBRIP agar supplemented with $Ca_3(PO_4)_2$. Six genera were identified: *Enterobacter*, *Pantoea*, *Bacillus*, *Pseudomonas*, *Shigella*, and *Aeromonas*. There were ten strains (OSP52, OSP57, OSP58, OSP59, OSP62, OSP78, OSP81, OSP85, OSP90, OSP93) belonged to the genus *Bacillus*. Three strains (OSP7, OSP41, OSP53) were identified as *Pseudomonas*. Two trains (OSP48, OSP79) and (OSP76, OSP86) were identified as *Enterobacter* and *Pantoea*, respectively. One strain (OSP68) was identified as *Shigella* and one other strain (OSP51) was identified as *Aeromonas* (Figure 6 and Table 5).

Table 5. Identities of the PSB strains isolated from OS2 site

Strains	Identification	% Gene identity
OSP52	*Bacillus* sp.	99.50
OSP57	*Bacillus* sp.	99.59
OSP58	*Bacillus* sp.	99.53
OSP59	*Bacillus* sp.	99.89
OSP62	*Bacillus* sp.	99.90
OSP78	*Bacillus* sp.	99.91
OSP81	*Bacillus* sp.	99.79
OSP85	*Bacillus* sp.	99.11
OSP90	*Bacillus* sp.	98.92
OSP93	*Bacillus* sp.	98.55
OSP7	*Pseudomonas* sp.	100.00
OSP41	*Pseudomonas* sp.	99.89
OSP53	*Pseudomonas* sp.	98.86
OSP48	*Enterobacter* sp.	99.91
OSP79	*Enterobacter* sp.	99.13
OSP76	*Pantoea* sp.	99.41
OSP86	*Pantoea* sp.	99.25
OSP68	*Shigella* sp.	99.90
OSP51	*Aeromonas* sp.	99.57

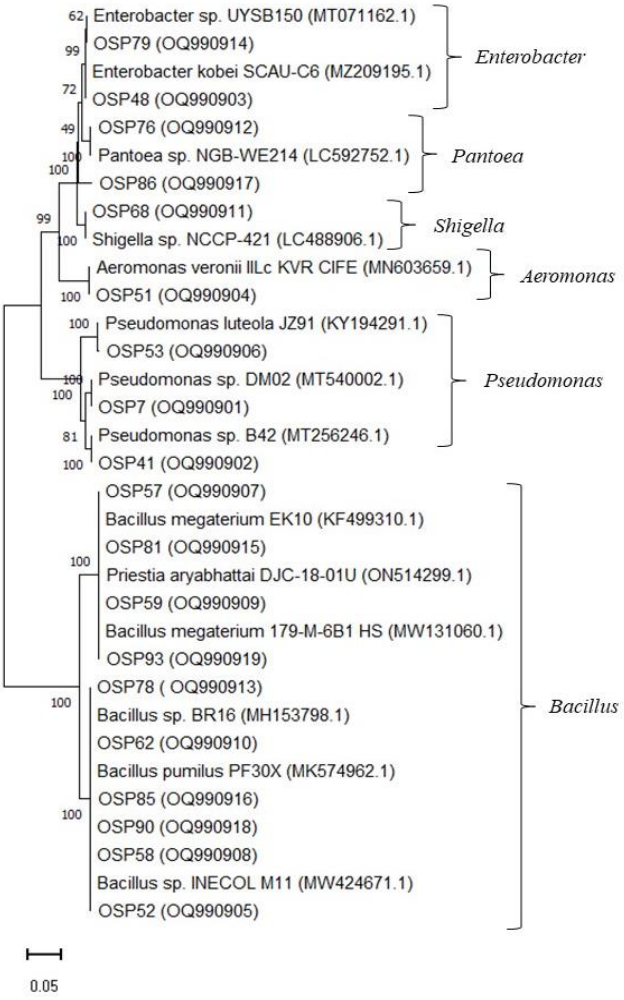

Figure 6. Phylogenetic tree of phosphate-solubilizing bacteria isolated from organic paddy soil 2 (OS2) based on 16S rRNA gene. The tree was reconstructed using the Neighbor-joining method with the Kimura two-parameter method and uniform rates. Bootstrap values were determined from 1000 replications. Bar, 0.05 substitutions per site

Discussion

The soils in the present study were collected from the Central Plain region of Thailand. It was reported that, in this area, agricultural practices are mainly paddy fields (Jindaluang et al. 2013). In this study, the amount of sand, silt and clay were 1.41-2.20%, 44.14-50.45%, and 48.14-53.66%, respectively. These results were the same range as the previous report of the Central Plain area by Jindaluang et al. (2013). They reported that the amount of sand ranged from 3.60% to 49.00%, silt ranged from 8.30% to 46.10%, and the clay ranged from 31.60% to 88.00%. In addition, in OS1 and OS2 sites, the CEC values ranged from 28.17-30.79 cmol kg^{-1}. The exchangeable cations in soils were 0.66-0.76 cmol kg^{-1} for K, 15.51-16.35 cmol kg^{-1} for Ca, and 1.59-1.68 cmol kg^{-1} for Mg. The values of CEC and exchangeable cations were close to the report of Intorpetch et al. (2014), who collected soil samples from the Central Plain area. He reported that the CEC value was 16.20-42.50 cmol kg^{-1}, and the exchangeable cations were 0.14-0.98 cmol kg^{-1} for K, 5.80-20.20 cmol kg^{-1} for Ca, and 2.40-10.20 cmol kg^{-1} for Mg. The quantity of OM was 4.10-5.18% and the pH was 5.76-5.86 in the present study soils. Intorpetch et al. (2014) reported that OM was 0.61-5.94% and the pH values ranged from 3.7-6.8.

Comparing two study sites, the CEC values and some cations such as K increased in OS2 site. In addition, the PCA analysis showed that OS2 soil was characterized by the accumulation of Ca and Mg. Results exhibited that OS2 site accumulated more OM than OS1 site, while clay content was lower than OS1 site. These results showed that the quantity of OM had a greater impact on soil cation retention than the amount of clay. The same result was also reported in Swiss top soils. It was reported that the effective Cation Exchange Capacity (CEC eff.) or the available soil surfaces that are available for cation fixation, was related strongly to the quantity of OM in Swiss forest top soils (<30 cm depth). The concentration of OM contributed between 35 and 50% to the total CEC eff., indicating that OM plays a significant role in cation retention in topsoil reducing the loss of cation by erosion and leaching processes (Solly et al. 2020).

The quantity of total N was 84.08-125.53 mg kg^{-1} in the present study soils. These values are considered to be moderated as reported by Carson and Phillips (2024) suggesting that the application of poultry and swine manures is N sufficient to grow rice in these OA soils. Analysis of N species in the soils had shown that all two sites accumulated more ammonium than nitrate. The result can be attributed to several factors. The first one is the low concentration of labile Pi in the OA soils. It was 11.15 mg kg^{-1} for OS1 and 3.41 mg kg^{-1} for OS2. In OA soils, it is often found that there is a limitation of nutrients such as N and P (Bedoussac et al. 2015; Meemken and Qaim 2018). It is possible that P was a limiting factor for the growing of nitrifiers, that was in turn inhibited the nitrification process which converts ammonium (NH_4^+-N) to nitrate (NO_3^--N), resulting in the accumulation of ammoniums in the soils. In P-limiting soil, the activities of nitrifiers were reduced (O'Neill et al. 2022) and it was generally observed the reduction of nitrate concentration in the soil (Wang et al. 2022). Moreover, the application of poultry and swine manures, as organic fertilizers in the paddy soils, can take part in the accumulation of ammonium. It was reported that poultry and swine manures contain a lot of urea that will convert to ammonium through the enzymatic action of urease (López-Bellido et al. 2014). In addition, the quantity of nitrate in OS2 site was lower than OS1 site but the concentration of ammonium ion was indifferent. The limitation of nitrifiers activities was higher on OS2 than OS1 because this site was lowest in term of labile Pi, that explain why the quantity of nitrate was low in this site.

In intact soils, the bicarbonate-extractable Pi was 11.15 mg kg^{-1} for OS1 site and 3.41 mg kg^{-1} for OS2 site. The same results were also found in the Central Plain region of Thailand. It was reported that labile Pi in the area ranged from 2.8 mg kg^{-1} to 23.00 mg kg^{-1} (Intorpetch et al. 2014). The bicarbonate-extractable Po was 14.98 mg kg^{-1} for OS1 site and 11.58 mg kg^{-1} for OS2 site. These values were significantly different compared to labile Pi indicating that

the soils accumulated phosphorus in organic fraction more than inorganic fraction. This study showed similar results as reported by Xavier et al (2009) that the conversion of land to organic agriculture promoted the accumulation of labile Po. The low concentration of labile Pi was also observed in other studies after the conversion of agricultural practices to organic farming (Bedoussac et al. 2015; Meemken and Qaim 2018). However, it can not be certain that the reduction of labile Pi is due to the conversion of the paddy soils to organic agricultural practices because the data on soil nutrient status was not monitored before the conversion. Moreover, the soils in this study were silty clay which presented a high concentration of clay. The amount of labile Pi in the area was low due to high clay content. Clay minerals play a crucial role in labile Pi adsorption (Xiong et al. 2022).

The technique of autoclaved soils used to extract P contained in microbial cells (Waithaisong et al. 2022a). The results revealed that microbial Pi released from the soil after autoclaving was reduced compared to labile Pi using intact soil in OS1 site. In OS2 site, the quantity of microbial Pi and labile Pi was indifferent. It was reported that if the adsorption of P did not occur or the effect was minor, the quantity of inorganic P and organic P after autoclaving was increased due to the release of P from microbial cells in sandy soils (Waithaisong et al. 2022a). Normally, the autoclaving method uses a moist heat environment to kill the microorganisms contained in the soil and release Pi and Po from microbial cells. The autoclaving technique gives equivalent results to the chloroform fumigation method, that is used to estimate microbial P in soils (Sinegani and Hosseinpur 2010). However, this method cannot be used for silty clay soil in these sites. It was reported that autoclaving soil reduced soil aggregates leading to an increase in clay fractions and change a chemical structure of OM (Berns et al. 2008). This may increase the soil adsorption capacity of P. The quantity of P in the organic fraction was indifferent after autoclaving compared to intact soils. This also due to the effect of P adsorption after autoclaving. However, the results of autoclaved soils showed that P in the study sites was mainly in organic fraction, especially in OS2 soil. These results were similar to the results obtained from intact soils. It was reported that in P-limiting soil, the P fraction was mainly held in microbial cells (Waithaisong et al. 2022a). The P-limiting soils promoted P immobilization by soil microorganisms that were revealed by a high P incorporation into microbial cells and used as the composition of biomolecules (Pistocchi et al. 2018). The conversion of land to organic farming increased microbial biomass (Crystal-Ornelas et al. 2021). This may be attributed to the accumulation of P in organic fraction that observed in this study.

PCA analysis of all soil properties revealed that, at the OS1 site, the concentration of P fractions (labile Pi, labile Po, microbial P) and nitrate increased with the concentration of clay content. It seems that clay may play an important role in anions retention such as N and P in this site. It was reported that clay can adsorb P in the form of orthophosphate ions and N in the form of nitrate on the surface, and inside the mineral contributing to nutrient retention (Xiong et al. 2022). At OS2 site, OM concentration increased with CEC and cation value, indicating the role of OM which plays a major role in the retention of cations, such as K, Ca and Mg. This situation was also observed in forest soils in Switzerland, where OM in topsoil was the main soil component that adsorbed cation and reduced the loss of cation by erosion and leaching processes (Solly et al. 2020).

PSB is the group of soil microorganisms that solubilize rock phosphate and/or mineralization of organic phosphorus. Their ability can increase labile Pi for plant growth in organic farming as microbial inoculants (Alori et al. 2017). In the OS1 and OS2 sites, molecular characterization of PSB strains revealed that PSB belonged to six genera: *Enterobacter*, *Pantoea*, *Bacillus*, *Pseudomonas*, *Burkholderia*, and *Acinetobacter*. The PSBs isolated in this study were similar to other studies that isolated PSBs from soils and rhizosphere soils. They belonged to the genera: *Pseudomonas*, *Agrobacterium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Kushneria*, *Paenibacillus*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Serratia*, *Bradyrhizobium*, *Salmonella*, *Sinomonas*, and *Thiobacillus* (Alori et al. 2017). PSB was isolated from the rhizosphere of perennial ryegrass, white clover, wheat, oat, and yellow lupin. Isolated PSB's belonged to the genera *Pseudomonas*, *Enterobacter*, and *Pantoea* (Jorquera et al. 2008). In addition, results showed that the genus *Acinetobacter* was isolated only from the OS1 site. This bacteria was reported to be PSB and isolated from bulk soil (Wan et al. 2020). *Shigella* and *Aeromonas* were found only in OS2. These bacteria were found as PSB that can solubilize $Ca_3(PO_4)_2$, and isolated from vermicompost (Raimi et al. 2022). PSBs, isolated in this study, have the potential to be used as microbial inoculants in rice organic agricultural production after their characterization.

In this study, the analysis of soil properties, collected from two organic paddy soils, that were converted to organic agriculture more than 7 years ago detected the reduction of nutrient availability for P but not N in soils. All study sites were P-limited. The P limitation influences the quantity of N species accumulated from the soils as the accumulation of ammoniums in all study sites. Phosphorus was present mostly in organic fractions. The analysis of microbial P indicated that the OS2 site accumulated P in microbial cells which was unavailable for rice growth. Soil properties indicated that these organic paddy soils decrease labile Pi and affect N cycling, resulting in a decrease in rice yield in the future. The application of manure was insufficient to provide labile Pi for rice growth. In addition, the isolation of PSBs showed that PSBs belonged mainly to the genera; *Enterobacter*, *Pantoea*, *Bacillus*, and *Pseudomonas*. Further, the PSB strains must be characterized for their phosphate solubilization to be applied as biofertilizers. The data of this study may be valuable to the farmers and suggestions should be made to increase the organic fertilization rate or use the combination of rock phosphate and microbial inoculants such as PSB to increase labile Pi for rice growth.

ACKNOWLEDGEMENTS

This work was supported by the Coordinating Center for Thai Government Science and Technology Scholarship Students (CSTS), the National Science and Technology Development Agency (NSTDA), and the grant SRIF-JRG-2565-04 from the Faculty of Science, Silpakorn University, Thailand. I would like to thank Ms. Nantha Prasarnwong, who gave the information about the study site and permission for soil collection. I also thank the researcher assistant, Ms. Korapan Sawetsuwannakun, and many students of the Microbiology Department at Silpakorn University, Thailand who helped with the experiments. I also thank Asst. Prof. Supharerk O-in, who helped in drawing the sampling locations.

REFERENCES

Ali MA, Louche J, Legname E, Duchemin M, Plassard C. 2009. *Pinus pinaster* seedlings and their fungal symbionts show high plasticity in phosphorus acquisition in acidic soils. Tree Physiol 29: 1587-1597. DOI: 10.1093/treephys/tpp088.

Alori ET, Glick BR, Babalola OO. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol 8: 971. DOI: 10.3389/fmicb.2017.00971.

Arunrat N, Sereenonchai S, Chaowiwat W, Wang C, Hatano R. 2022. Carbon, nitrogen and water footprints of organic rice and conventional rice production over 4 years of cultivation; a case study in the lower north of Thailand. Agronomy 12: 380. DOI: 10.3390/agronomy12020380.

Bedoussac L, Journet EP, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, Jensen ES, Prieur L, Justes E. 2015. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agron Sustain Dev 35: 911-935. DOI: 10.1007/s13593-014-0277-7.

Bell TG, Kramvis A. 2013. Fragment merger: An online tool to merge overlapping long sequence fragments. Viruses 5: 824-833. DOI: 10.3390/v5030824.

Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur J Soil Sci 59: 540-550. DOI: 10.1111/j.1365-2389.2008.01016.x.

Bonanomi G, De Filippis F, Cesarano G, La Storia A, Ercolini D, Scala F. 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biol Biochem 103: 327- 336. DOI:10.1016/j.soilbio.2016.09.005.

Bulluck III LR, Brosius M, Evanylo GK, Ristaino JB. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Appl Soil Ecol 19: 147–160. DOI:10.1016/S0929-1393(01)00187-1.

Carson J, Phillips L. 2024. Soil Nitrogen Supply. https://soilquality.org.au/factsheets/soil-nitrogen-supply

Chaiharn M, Lumyong S. 2011. Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. Curr Microbiol 62: 173-181. DOI: 10.1007/s00284-010-9674-6.

Crystal-Ornelas R, Thapa R, Tully KL. 2021. Soil organic carbon is affected by organic amendments, conservation tillage, and cover cropping in organic farming systems: A meta-analysis. Agric Ecosyst Environ 312: 107356. DOI:10.1016/j.agee.2021.107356.

FAO. 2022. Standard Operating Procedure for Cation Exchange Capacity and Exchangeable Bases 1N Ammonium Acetate pH 7.0 Method. https://www.fao.org/3/cc1200en/cc1200en.pdf

Gomiero T, Pimentel D, Paoletti MG. 2011. Environmental impact of different agricultural management practices: conventional vs. organic agriculture. Crit Rev Plant Sci 30: 95-124. DOI: 10.1080/07352689.2011.554355.

Gosling P, Shepherd M. 2005. Long-term changes in soil fertility in organic arable farming systems in England, with particular reference to phosphorus and potassium. Agric Ecosyst Environ 105: 425-432. DOI: 10.1016/j.agee.2004.03.007.

Hérique O, Faysse N. 2021. A large-scale public program to promote organic rice farming in Thailand: Building solid foundations to enable farmers to engage? Org Agric 11: 27-40. DOI: 10.1007/s13165-020-00320-4.

Huang Y, Dai Z, Lin J, Li D, Ye H, Dahlgren RA, Xu J. 2021. Labile carbon facilitated phosphorus solubilization as regulated by bacterial and fungal communities in *Zea mays*. Soil Biol Biochem 163: 108465. DOI: 10.1016/j.soilbio.2021.108465.

Hunnark C, Plainbangyang S, Atsvadachachanyut W. 2017. Strengthening community development through the knowledge management process in organic farming in Nakhon Pathom Province. ASJPSU 28: 158-166. [Thai]

Intorpetch B, Wisawapipat W, Arunlertaree C, Teartisup P. 2014. Soil physicochemical status and nutrient management for paddy soils in the lower central plain of Thailand after the flood disaster in 2011. Environ Nat Resour J 12: 57-67.

Jindaluang W, Kheoruenromne I, Suddhiprakarn A, Singh BP, Singh B. 2013. Influence of soil texture and mineralogy on organic matter content and composition in physically separated fractions soils of Thailand. Geoderma 195-196 : 207-219. DOI: 10.1016/j.geoderma.2012.12.003.

Jorquera MA, Hernández MT, Rengel Z, Marschner P, De La Luz Mora M. 2008. Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol Fertil Soils 44: 1025–1034. DOI: 10.1007/s00374-008-0288-0.

Kongsom C, Panyakul V. 2016. Production and market of certified organic products in Thailand. Intl J Econ Manag Eng 10: 2723-2727. DOI: 10.5281/zenodo.1125969.

López-Bellido L, Muñoz-Romero V, Fernández-García P, López-Bellido R.J. 2014. Ammonium accumulation in soil: The long-term effects of tillage, rotation and N rate in a Mediterranean Vertisol. Soil Use Manag 30: 471-479. DOI: 10.1111/sum.12147.

Meemken EM, Qaim M. 2018. Organic agriculture, food security, and the environment. Ann Rev Resour Econ 10: 39-63. DOI: 10.1146/annurev-resource-100517-023252.

O'Neill RM, Duff AM, Brennan FP, Gebremichael AW, Girkin NT, Lanigan GJ, Krol DJ, Wall DP, Renou-Wilson F, Müller C, Richards KG, Deveautour C. 2022. Linking long-term soil phosphorus management to microbial communities involved in nitrogen reactions. Biol Fertil Soils 58: 389-402. DOI:10.1007/s00374-022-01627-y.

Olsen SR, Cole C, Watanabe FS, Dean LA. 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. U.S. Dept. of Agriculture, Washington, D.C.

Pistocchi C, Mészáros É, Tamburini F, Frossard E, Bünemann EK. 2018. Biological processes dominate phosphorus dynamics under low phosphorus availability in organic horizons of temperate forest soils. Soil Biol Biochem 126: 64-75. DOI: 10.1016/j.soilbio.2018.08.013.

Poorahong A, Chetanont S, Satsananan C, Setsri P. 2022. Marketing development of agro-tourism and tourism supply chain of Klong yong-Lan tak fa community dee house group, a case study of the community mill, Ban chanote community enterprise, Klong yong-Lan tak fa community Lan tak fa Sub-district, Nakhonchaisri District, Nakhon Pathom Province based on community innovation resources. Sripatum Chonburi J 19: 146-158. [Thai]

Raimi AR, Atanda AC, Ezeokoli OT, Jooste PJ, Madoroba E, Adeleke RA. 2022. Diversity and predicted functional roles of cultivable bacteria in vermicompost: bioprospecting for potential inoculum. Arch Microbiol 204: 261. DOI:10.1007/s00203-022-02864-3.

Reganold JP, Wachter JM. 2016. Organic agriculture in the twenty-first century. Nat Plants 2: 15221. DOI: 10.1038/NPLANTS.2015.221.

Seerasarn N, Miller SA, Wanaset A. 2020. Transitioning to organic rice farming in Thailand: Drivers and factors. Asian J Agric Rural Dev 10: 740-748. DOI: 10.18488/journal.ajard.2020.103.740.748.

Sheldrick BH, Wang C. 1993. Particle size distribution. In: Carter MR, Gregorich EG (eds). Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, FL. DOI: 10.1201/9781420005271-66.

Sinegani AAS, Hosseinpur A. 2010. Evaluation of effect of different sterilization methods on soil biomass phosphorus extracted with $NaHCO_3$. Plant Soil Environ 56: 156-162. DOI:10.17221/86/2009-pse.

Sitthisuntikul K, Yossuck P, Limnirankul B. 2018. How does organic agriculture contribute to food security of small land holders?: A case

study in the North of Thailand. Cogent Food Agric 4: 1429698. DOI: 10.1080/23311932.2018.1429698.

Solly EF, Weber V, Zimmermann S, Walthert L, Hagedorn F, Schmidt MWI. 2020. A critical evaluation of the relationship between the effective cation exchange capacity and soil organic carbon content in Swiss Forest Soils. Front For Glob Chang 3: 98. DOI: 10.3389/ffgc.2020.00098.

Suwanmaneepong S, Kerdsriserm C, Lepcha N, Cavite HJ, Llones CA. 2020. Cost and return analysis of organic and conventional rice production in Chachoengsao Province, Thailand. Org Agric 10: 369-378. DOI: 10.1007/s13165-020-00280-9.

Thuithaisong C, Parkpian P, Shipin OV, Shrestha RP, Naklang K, Delaune RD, Jugsujinda A. 2011. Soil-quality indicators for predicting sustainable organic rice production. Commun Soil Sci Plant Anal 42: 548-568. DOI: 10.1080/00103624.2011.546930.

Tuck SL, Winqvist C, Mota F, Ahnström J, Turnbull LA, Bengtsson J. 2014. Land-use intensity and the effects of organic farming on biodiversity: A hierarchical meta-analysis. J Appl Ecol 51: 746-755. DOI: 10.1111/1365-2664.12219.

Waithaisong K, Kliangpradith K, Punnasiri P, Thaingthaum P. 2022b. Insoluble phosphate solubilization and acid phosphatase activity of bacteria isolated from organic paddy soils. Naresaun Phayao J 15: 20-29.

Waithaisong K, Robin A, L'Huillery V, Abadie J, Sauvage FX, Chemardin P, Mareschal L, Bouillet JP, Laclau JP, Gonçalves JLM, Plassard C. 2022a. Organic phosphorus immobilization in microbial biomass controls how N_2-fixing trees affect phosphorus bioavailability in two tropical soils. Environ Adv 8: 100247. DOI: 10.1016/j.envadv.2022.100247.

Wan W, Qin Y, Wu H, Zuo W, He H, Tan J, Wang Y, He D. 2020. Isolation and characterization of phosphorus solubilizing bacteria with multiple phosphorus sources utilizing capability and their potential for lead immobilization in soil. Front Microbiol 11: 753. DOI: 10.3389/fmicb.2020.00752.

Wang R, Bicharanloo B, Hou E, Jiang Y, Dijkstra FA. 2022. Phosphorus supply increases nitrogen transformation rates and retention in soil: A global meta-analysis. Earth's Future 10: e2021EF002479. DOI: 10.1029/2021EF002479.

Wheatley RE, MacDonald R, Smith AM. 1989. Extraction of nitrogen from soils. Biol Fertil Soils 8 : 189-190. DOI: 10.1007/BF00257765.

Xavier FA da S, de Oliveira TS, Andrade FV, de Sá Mendonça E. 2009. Phosphorus fractionation in a sandy soil under organic agriculture in Northeastern Brazil. Geoderma 151: 417-423. DOI: 10.1016/j.geoderma.2009.05.007.

Xiong J, Liu Z, Yan Y, Xu J, Liu D, Tan W, Feng X. 2022. Role of clay minerals in controlling phosphorus availability in a subtropical Alfisol. Geoderma 409: 115592. DOI: 10.1016/j.geoderma.2021.115592.