Insights into soil nematode diversity and microbiome of Thai Jasmine rice rhizosphere from different paddy fields in Thailand (#93845)

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Insights into soil nematode diversity and microbiome of Thai Jasmine rice rhizosphere from different paddy fields in Thailand

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Globally, phytonematodes cause significant crop losses. Understanding the functions played by the plant rhizosphere soil microbiome during phytonematodes infection is crucial. This study examined the distribution of phytonematodes in the paddy fields of five provinces in Thailand, as well as determining the keystone microbial taxa in response to environmental factors that could be considered in the development of efficient biocontrol tactics in agriculture. The results demonstrated that *Meloidogyne graminicola* and *Hirschmanniella* spp. were the major and dominant phytonematodes distributed across the paddy fields of Thailand. Soil parameters (total P, Cu, Mg, and Zn) were the important factors affecting the abundance of both nematodes. Illumina next-generation sequencing demonstrated the levels of bacterial diversity among all locations were not significant difference. The *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, *Myxococcota*, *Chloroflexi*, *Verrucomicrobiota*, *Bacteroidota*, *Gemmatimonadota*, and *Desulfobacterota* were the most abundant bacterial phyla presented at all sites. The numbers of classes of the *Acidobacteriae*, *Clostridia*, *Bacilli*, and *Bacteroidia* influenced the proportions of *Hirschmanniella* spp., *Tylenchorhynchus* spp., and free-living nematodes in the sampling dirt, whereas the numbers of classes of the *Polyangia* and *Actinobacteria* affected the amounts of *Pratylenchus* spp. in both roots and soils. Soil organic matter, N, and Mn were main factors that influenced the structure of bacterial community. Correlations among rhizosphere microbiota, soil nematodes, and soil properties will be informative data in considering phytonematodes management in a rice production system.

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Abstract

Globally, phytonematodes cause significant crop losses. Understanding the functions played by the plant rhizosphere soil microbiome during phytonematodes infection is crucial. This study examined the distribution of phytonematodes in the paddy fields of five provinces in Thailand, as well as determining the keystone microbial taxa in response to environmental factors that could be considered in the development of efficient biocontrol tactics in agriculture. The results demonstrated that *Meloidogyne graminicola* and *Hirschmanniella* spp. were the major and dominant phytonematodes distributed across the paddy fields of Thailand. Soil parameters (total P, Cu, Mg, and Zn) were the important factors affecting the abundance of both nematodes. Illumina next-generation sequencing demonstrated the levels of bacterial diversity among all locations were not significant difference. The *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, *Myxococcota*, *Chloroflexi*, *Verrucomicrobiota*, *Bacteroidota*, *Gemmatimonadota*, and *Desulfobacterota* were the most abundant bacterial phyla presented at all sites. The numbers of classes of the *Acidobacteriae*, *Clostridia*, *Bacilli*, and *Bacteroidia* influenced the proportions of *Hirschmanniella* spp., *Tylenchorhynchus* spp., and free-living nematodes in the sampling dirt, whereas the numbers of classes of the *Polyangia* and *Actinobacteria* affected the amounts of *Pratylenchus* spp. in both roots and soils. Soil organic matter, N, and Mn were main factors that influenced the structure of bacterial community.

Correlations among rhizosphere microbiota, soil nematodes, and soil properties will be informative data in considering phytonematodes management in a rice production system.

Introduction

Presently, a major challenge for agro-industrial operations is identifying the way to secure food for world's citizenry, which at its current rate of increase is estimated to reach nearly 10 billion. While global food demand is expected to double by 2050, the availability and quality of natural resources that support agricultural practices continue to diminish, their scarcity and degradation will become more pronounced (*World Bank, 2007*). Rice, as a staple nourishment, holds significant importance of global food for humankind, extensively traded and captivated in over 110 countries globally (*Corrêa et al., 2007*; *Gnamkoulamba et al., 2018*; *Musarrat et al., 2016*). The climate change which has already had adverse global damages on agriculture (*Nimnoi, Pongsilp & Lumyong, 2017*), phytonematodes recorded as the leading agents that cause significant yield loss of rice worldwide (*Bridge, Luc & Plowright, 1990; De Waele & Elsen, 2007*; *Mantelin, Bellafiore & Kyndt, 2017*; *Ruanpanun & Khun-In, 2015*). These pests can adapt well and survive in flooded and irrigated conditions (*Fernandez, Cabasan & De Waele, 2014*; *Win et al., 2013*). Among the nematodes known as pathogens of rice, only 29 species have caused considerable yield losses (*Bridge, Plowright & Peng, 2005*). *Criconema* spp., *Dolichodorus* spp., *Helicotylenchus* spp., *Heterodera* spp., *Hirschmanniella* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Scutellonema* spp., *Tylenchorhynchus* spp., and *Xiphinema* spp. have been predominantly found in the rice rhizosphere and roots (*Gnamkoulamba et al., 2018*; *Musarrat et al., 2016*; *Shahabi et al., 2016*). Damage levels in both direct and indirect ways in rice caused by phytonematodes result in deferred plant growth and maturation, stunting, yellowing, and grain yield loss that subsequently reduce the income (*Onkendi et al., 2014*; *Sharma-Poudyal et al., 2005*; *Win, Kyi & De Waele, 2011*). Phytonematode infestations around the world create an estimated annual yield loss up to USD 150 billion (*Singh, Singh & Singh, 2015*).

To cope with this problem, the relationship among phytonematodes, the microbial community and physiochemical soil characteristics is one promising and important way to provide useful information for the implementation of an appropriate soil management program, to limit harmful nematodes to crops. Previous research demonstrated many physiochemical soil traits, such as pH, texture and elements of soil, and organic matter (OM) are correlated with the existence and density of nematodes, as well as with bacterial diversity and bacterial community levels (*Huang et al., 2020*; *Renčo, Gömöryová & Čerevková , 2020*; *Zhou et al., 2019*). Bacterial communities have been revealed to suppress nematode infection with mechanisms that regulate nematode population densities (*Zhou et al., 2019*). Although the impact of soil-borne nematodes on plant-associated microbial communities has been theorized to play significant roles in plant development and yield (*Markalanda et al., 2022*; *Pieterse, De Jonge & Berendsen, 2016*; *Zhou et al., 2019*), less is understood about the specific relationships between phytonematodes and the microbiome in root and of rice. Recently, in Vietnam, *Masson et al. (2020)* studied the microbiome of infected and uninfected rice roots for *Meloidogyne graminicola* (rice root-knot nematode). They revealed that *M. graminicola* infection results in the huge restructuring of bacterial communities by influencing species richness and taxa abundance.

To our knowledge, no research has looked at the association between the phytonematodes population density and the rice root-associated microbiome from the same rice cultivar under different natural environmental conditions, especially in Thailand. Hence, this study's goals were aimed at bacterial diversities and communities of the rhizosphere soil from Thai Hom Mali rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 (KDML105) from 5 natural paddy fields in Thailand and to elucidate the relationships between phytonematode populations, root-associated bacterial communities, and soil physicochemical factors. The massive data generation and the derived comprehensive versatile knowledge may aid soil management as well as resource conservation in agroecosystem of economic importance.

Materials & Methods

Soil sample collection

During September 2022, surveillance was conducted of the phytonematode distribution in five notable paddy fields of KDML105 in tillering stage in: Chachoengsao, CCS (13°36'46.1"N 101°16'54.3"E); Nakhon Nayok, NYK (14°16'25.4"N 101°08'37.0"E); Prachin Buri, PAR (14°10'01.3"N 101°35'23.1"E); Pathum Thani, PTT (14°09'32.7"N 100°43'55.4"E); and Phra Nakhon Si Ayutthaya, AYY (14°26'43.6"N 100°45'52.5"E). In each field, 3 sampling areas were random to collect soil samples depending on field size. The sampling area measuring 20×20 m was methodically subdivided into a grid pattern of 5×5 m mesh lines to facilitate the collecting of samples. In total, 16 subsamples of rice roots and their rhizosphere soils were collected from 16 points of the grid area and then the soils were meticulously combined to create a composite sample weighing 10 kg, which aimed to represent the overall soil composition throughout the study spot. (*Win, Kyi & De Waele, 2011*). The samples were carefully enclosed in individual plastic pouches, securely sealed, and promptly delivered to the Agricultural Nematology and Microbiology Laboratory for investigation. The soil samples were partitioned into 3 parts; 3 kg for nematode extraction, 5 kg were air-dried for physical and chemical property analysis, and the last 2 kg were kept at 4°C for microbiome preparation.

Isolation and identification of Nematodes

A modified Baermann tray technique was used for extracting nematodes from the sampling soils (*Schindler, 1961*). The rice roots were washed carefully under running water to eliminate all traces of dirt particle, meticulously chopped into small pieces (1–2 cm), and then blended in 0.8% NaOCl for 30 s. For 10 minutes, the mixes were left at room temperature before applying a modified Baermann funnel method (*Hooper, 1986*). Nematode suspensions derived from soil and root samples were obtained following a 48-h incubation period, subsequently subjected to inspection using a stereomicroscope. (Olympus SZ51; Tokyo, Japan). The nematodes without any stylets were identified as free-living nematodes. The identification of phytonematodes was conducted at the genus level through the analysis of morphological traits (*Tarjan, Esser & Chang, 1977*). The body size, stylet length, and vulva position were measured using the CellSens imaging software (V1.6) with an Olympus DP26 camera (Tokyo, Japan). *Meloiodogyne* species were identified using the perineal pattern of adult females (*Hunt and Handoo, 2009*) and species-specific primers (*Htay et al., 2016*).

Soil physical and chemical property analysis

Dried sampling soils were passed through a sieve (2 mm and 10 mesh size). The pH and electrical conductivity (Ec) were examined (*Slavich & Petterson*, 1993). Using a pipette-based technique, the samples' particle-size distribution was evaluated (Gee & Bauder, 1986). The amount of OM in the soil was measured (*Walkley & Black 1934*). The analysis of the available N was conducted (*Guebel, Nudel & Giulietti, 1991*). The quantity of total P was analyzed (*Bray & Kurtz, 1945*). The amount of Ca, K, and Mg were estimated (Del Castilho & Rix, 1993). Elements including, Cu, Fe, Mn, and Zn were also quantified (*Lindsay & Norvell, 1978*).

DNA isolation and next generation sequencing

Total DNA extraction was performed on three duplicates of soil samples from each location by a DNA soil extraction kit of NucleoSpin (Macherey-Nagel, Germany). The amplification of 16S rDNA was performed as follows *Apprill et al. (2015)* and *Parada, Needham & Fuhrman (2016)*. The PCR results were cleaned up by Vivantis gel extraction kit (Vivantis, Malaysia). The amplified DNA libraries were created and determined using Illumina-HiSeq2500 (Illumina, USA). Through parallel amplification and sequencing, negative controls (reactions with sterile water) were performed.

Bioinformatics analyses

The FLASH program was employed to combine raw reads (*Magoč & Salzberg, 2011*). The raw reads were screened by QIIME program for choosing of high-quality sequences (*Caporaso et al., 2010*). The UCHIME program employed for determination and elimination of chimera (*Haas et al., 2011*). The Uparse program was applied to perform the clustering and species annotation of operational taxonomic unit (OTU) (*Edgar, 2013*). The Mothur program was performed to annotate bacterial species (*Quast et al., 2013*; *Schloss et al., 2009*). All OTUs obtained from representative reads were analyzed phylogenetically using the MUSCLE tool (*Edgar, 2004*).

Statistical determination

The beta and alpha diversity parameters as well as sequencing depth were computed with the QIIME program (*Caporaso et al., 2010*). The R program was used to display the analyzed data (*R Core Team, 2013*). For determination and reducing dimensionalities of data, the PCoA and NMDS were used. The QIIME program was used to determine the similarity between samples by UPGMA method (*Caporaso et al., 2010*). The LEfSe analysis was computed by LEfSe program to discover high-dimensional biomarker between samples (*Segata et al., 2011*). The AMOVA and ANOSIM were computed to disclose the significant differences of bacterial communities. The PAST program was employed for canonical correlation analysis (CCA) (*Hammer, Harper & Ryan, 2001*). The ANOVA with Tukey's test was employed to signify the alpha diversity indices, phytonematodes, free-living nematodes, and soil physicochemical parameters. Based on spearman's correlation, the relationships between soil physicochemical factors and phytonematodes, free-living nematodes, and bacterial populations were clarified. The spearman's between-group analysis as well as ANOVA were computed by the SPSS program (IBM, USA).

Results

Diversity of soil nematodes associated with *Oryza sativa* L.

The diversity of phytonematodes and free-living nematodes was determined within the rhizosphere soils and roots of the rice collected from five paddy fields of notable provinces in Thailand (Supplementary Table 1). Among all the rhizosphere soils, site PTT had the highest number of *M. graminicola* (141.66±38.10 nematodes/500 g soil) which was significantly different compared to the other sites, followed by site CCS, PAR, AYY, and NYK, respectively (Table 1). Site PTT also had the highest number of *Hirschmanniella* spp. (22.00±4.58 nematodes/500 g soil), which was significantly different compared to the other sites. *Pratylenchus* spp. were found only at sites PAR (11.00±3.60 nematodes/500 g soil) and NYK (4.66±0.57 nematodes/500 g soil). *Helicotylenchus* spp. were found only at sites PTT and CCS, with site PTT having a significantly higher number compared to site CCS. Sites CCS and NYK were the only ones where *Tylenchorhynchus* spp. were identified; however, their numbers did not differ from each other. The number of free-living nematodes was highest at site AYY (117.66±29.36 nematodes/500 g soil) which was significant difference compared to the other sites.

Besides the phytonematodes and free-living nematodes in the rhizosphere soils, we also examined their presence within roots. The findings demonstrated that site CCS had the most *M. graminicola* (3,280.00±603.98 nematodes/3 g root), which was noticeably different from the other sites, followed by sites AYY, PTT, PAR, and NYK, in that order, respectively (Table 2). The number of *Hirschmanniella* spp. was highest at site AYY and was not significantly different compared to the other sites. *Pratylenchus* spp. were found at sites PAR (9.33±2.51 nematodes/3 g root) and NYK (6.33±3.05 nematodes/3 g root). *Helicotylenchus* spp. and *Tylenchorhynchus* spp. were detected at the PTT and CCS sites, respectively. The PAR site had the greatest density of free-living nematodes. This study demonstrated that the rhizosphere of rice in PTT province was confronted with severe epiphytotic levels of *M. graminicola, Hirschmanniella* spp., and *Helicotylenchus* spp., whereas the greatest concern in regarding the distribution of free-living nematodes in the rhizosphere soil was in AYY (Supplementary Fig. 1).

Soil parameters

The soil physicochemical parameters of each sampling site were characterized. All observed locations, pH and Ec values were in the ranges 4.77–7.81 and 0.37–10.52 ds/m, respectively (Table 3). Site AYY had the highest soil pH, whereas site PAR had the lowest soil pH. The Ec value for site CCS was the highest, while the lowest was at site NYK. Site AYY had the highest amounts of OM (4.41±0.35%) and available N (0.21±0.01%), which were significantly different compared to the other sites, followed by sites PTT, NYK, PAR, and CCS, respectively. The amounts of total P at each sampling site differed significantly with each other. Site CCS had the highest concentration of total K (235.93±2.37 mg/kg) with both being significantly different compared to the other sites. Total Ca (5301.19±99.21 mg/kg) was significantly the highest at site AYY, whereas it was significantly the lowest at site PAR (381.06±27.27 mg/kg). Site PTT had the highest amount of total Mg (674.91±3.19 mg/kg) which was significantly different compared to the other sites. Total Fe (379.33±4.86 mg/kg) was significantly the highest at site PAR. The amounts of total Mn at each sampling site differed significantly from each other. Site AYY had the greatest amount of total Mn (61.78±0.32 mg/kg) followed by sites PTT, PAR, NYK, and CCS. Site PTT had the highest amounts of total Cu (46.12±0.70 mg/kg) and Zn (15.51±0.86 mg/kg), which differed significantly with the other sites. The pH value and amounts of OM, available N, total Ca, and Mn at site AYY were the greatest. While concentrations of total P, Mg, Cu, and Zn in site PTT were highest. Site CCS presented the highest Ec value and amount of total K. Amount of total Fe was significantly highest in site PAR.

Sequence analysis, bacterial diversity, and richness indices

The bacterial diversity and richness of all sampling sites were determined. In total, 1,955,800 raw sequences were acquired from fifteen DNA samples (3 replicates/field). Tag merge and sequence quality control were performed to retrieve a total of 1,936,693 qualified tags (99.20% of the raw reads). In total, 1,263,601 taxon tags were obtained after removing the potential chimera tags. The tags with ≥97% resemblance were assigned into the same OTU. In total, 16,338 OTUs were obtained from all sampling sites with 98.79±0.00% of Good's coverage. Fig. 1A displays the total tags, taxon tags, unclassified tags, unique tags, and OTU numbers for each replicate. A Venn diagram (Fig. 1B) was used to present the numbers of unique, common, and overlapping OTUs between sampling sites. This diagram showed 1,659 OTUs presented across all sampling sites. The greatest number of unique OTUs was found at site CCS (2,560), followed by sites PAR, AYY, PTT, and NYK, in that order. Additional analysis investigated the number of observed species, diversity as indicated by the Shannon-Weaver and Simpson indices, while richness was indicated using Chao1 and ACE for each sampling site (Table 4). The results demonstrated that site CCS presented the greatest number of observed species (5,260.00±291.82), followed by sites PAR, PTT, NYK, and AYY, respectively. The higher values for the Shannon-Weaver indice implied greater bacterial diversity at site CCS, followed by sites PTT, AYY, PAR, and NYK, respectively, though the values did not differ with all sites significantly. Furthermore, the Chao1 and ACE values indicating bacterial richness illustrated that site CCS had the highest amount of bacterial richness, followed by sites PAR, PTT, and NYK, respectively, whereas site AYY had the lowest amount of bacterial richness. We found that site CCS had the highest numbers of unique OTUs and observed species, and the greatest diversity and richness of bacteria, whereas site NYK showed the lowest number of unique OTUs and the least bacterial diversity. The least number of detected bacterial species and richness were found at site AYY.

NGS and bacterial communities

In all sampling sites, the phylum *Acidobacteriota* was most abundant (10.18–36.26%), followed by *Proteobacteria* (12.80-30.24%), *Firmicutes* (5.25-10.40%), *Actinobacteriota* (3.08-9.11%), *Chloroflexi* (4.27-9.01%), *Myxococcota* (2.12-8.84%), *Verrucomicrobiota* (3.36-7.33%), *Bacteroidota* (1.64-5.63%), *Gemmatimonadota* (1.43-5.33%), and *Desulfobacterota* (0.66-4.23%). Based on the biomarker analysis, the LDA score illustrated statistically unique communities at each sampling site. As depicted in Supplementary Fig. 2A, there were differences in the bacterial community composition at each sampling site. The relative abundance of the phyla *Chloroflexi*, *Gemmatimonadota*, and *Bacteroidota* exhibited a statistically significant increased at site PTT. The phyla *Actinobacteriota* and *Cyanobacteria* were more abundant at site PAR, whereas the *Verrucomicrobiota* were significantly abundant at site NYK and similarly, the *Nitrospirota* at site CCS and both the *Myxococcota* and *Desulfobacterota* were the significantly predominant phyla at site AYY. However, there were also statistically distinguishable variations in the phyla among samples. For example, the variability of the phylum *Edwardsbacteria* was shown to be statistically significant across the sites PTT, AYY, and PAR. Similarly, the phylum *Latescibacterota* exhibited considerable variability across the sites CSS, NKY, and PTT. The phylum *Nitrospirota* was variable among all sites significantly (Supplementary Fig. 2B). Moreover, evaluating the top-10 predominant bacterial classes distributed in the soil at each sampling site (Table 5), site NYK had the highest numbers of *Acidobacteriae* and *Verrucomicrobiae*, which differed significantly with the other sites. The numbers of alpha-proteobacteria and *Anaerolineae* were highest at sites CCS and PTT, respectively. The amounts of *Bacilli* and *Bacteroidia* were highest at site PTT. Site AYY had the highest number of gamma-proteobacteria. Site PAR had the highest numbers of *Clostridia* and *Actinobacteria*. Sites AYY and PTT had the highest numbers of *Polyangia*, which differed significantly with other sites.

Heat map analysis was employed to determine more clearly the distribution at the genus level which contributed to the structure of community at each sampling site (Fig. 2). At site NYK, the more predominant genera were the *Bryobacter*, ADurb.Bin063-1 (*Verrucomicrobia* bacterium), *Candidatus_Soilbacter*, *Candidatus_Koribacter*, and *Candidatus_Udaeobacter*. The *Bifidobacterium*, WPS-2 (*Eremiobacterota*), WD2101 soil group (*Planctomycetes*), *Acidibacter*, *Pseudomonas*, *Burkholderia*, *Caballeronia*, *Paraburkholderia*, *Pantoea*, *Faecalibacterium*, and *Bacteroides* were the predominant genera at site PAR. The most abundant genera at site PTT were the *Gemmatimoonas*, *Sphingomonas*, and *Latescibacterota*. The *Thioalkalispira-*

Sulfurivermis, *Sporacetigenium*, Ellin6067 (*beta-proteobacteria*), *Anaeromyxobacter*, *Mycoplasma*, MND1, and *Thiobacillus* were the common genera at site AYY. The A21b (uncultured bacterium), Subgroup_13 (*Acidobacteria*), Subgroup_2 (*Acidobacteria*), RCP2-54 (uncultured bacterium), and Bacteriap25 (uncultured bacterium) were more abundant than other genus at site CCS.

The AMOVA results showed major variations in the structure of community among the sites (Fs=14.22; P<0.001). In addition, the inter- and inner-site variations in the bacterial community composition were measured using ANOSIM. The results showed that the inter-site variations in the bacterial community composition were greater than for inner-site variations (R=1). The PCoA and NMDS analyses provided convincing evidence of variations in the bacterial community composition across the different sampling sites (Fig. 3A and B). The bacterial community structures of sites PTT and AYY were closer to each other. Furthermore, the beta diversity heat map representing an explicit comparison of bacterial communities based on their composition confirmed that the bacterial community composition of site PTT was most closely related to AYY at 0.183 (Fig. 4), whereas the bacterial community composition of site AYY was the most dissimilar to site NYK (0.341) followed by site PAR (0.338). These results were supported by the UPGMA dendrogram (Fig. 5) that showed the relationships for the relative abundance of each sampling site at the phylum level. The created dendrogram consisted of 2 main different clusters. The first cluster formed of sites PTT, AYY, and CCS, with sites PTT and AYY closer to each other but linked together with site CCS. The second cluster was composed of site NYK together with site PAR.

Effect of environmental constituents on soil bacterial community and nematodes distribution

Regarding the influences of the soil physicochemical parameters on the soil bacterial communities, the analysis presented the soil pH was the most positively correlated with the members of the *Polyangia* and most negatively correlated with the members of the Acidobacteriae (Supplementary Table 2). The Ec and total K content were the most positively correlated with members of the *Anaerolineae* and the most negatively correlated with members of the *Verrucomicrobiae*. The OM was the most positively correlated with members of the *Polyangia* as well as the bacterial community and the most negatively correlated with members of the alpha-*proteobacteria*. The available N was the most positively correlated with the bacterial community and most negatively correlated with members of the alpha-*proteobacteria*. Total P and Mn were the most positively correlated with members of the *Bacteroidia* and the most negatively correlated with members of the *Acidobacteriae*. Total Ca was the most positively correlated with members of *Polyangia* and the most negatively correlated with members of the alpha-proteobacteria and *Acidobacteriae*. Total Mg was the most positively correlated with members of the *Anaerolineae* and the most negatively correlated with members of the *Verrucomicrobiae*. Total Fe was the most negatively correlated with members of the

Anaerolineae. Total Cu and Zn were the most positively correlated with members of the *Anaerolineae* and the most negatively correlated with members of the *Acidobacteriae*.

In addition, after evaluating the effect of the soil physicochemical parameters on phytonematodes and free-living nematodes in the rhizosphere soils, soil pH was the most positively correlated with free-living nematodes and the most negatively correlated with *Pratylenchus* spp. (Supplementary Table 2). The Ec was the most positively correlated with *Helicotylenchus* spp. and the most negatively correlated with *Pratylenchus* spp. The OM and available N were the most negatively correlated with *Tylenchorhynchus* spp. The total P was the most positively correlated with *Hirschmanniella* spp. and the most negatively correlated with *Tylenchorhynchus* spp. The total K and Mg were the most positively correlated with *Helicotylenchus* spp. and the most negatively correlated with *Pratylenchus* spp. In contrast, the total Fe was the most positively correlated with *Pratylenchus* spp. and the most negatively correlated with *Helicotylenchus* spp., whereas total Ca was negatively correlated with *Pratylenchus* spp. Total Mn was the most positively correlated with free-living nematodes and the most negatively correlated with *Tylenchorhynchus* spp. Total Cu and Zn were the most positively correlated with *M. graminicola* and the most negatively correlated with *Pratylenchus* spp. The correlations between soil nematodes and the microbiome were investigated (Supplementary Table 3). The results showed that *Hirschmanniella* spp. were the most positively correlated with members of the *Bacteroidia*. *Pratylenchus* spp. were the most positively correlated with members of the *Actinobacteria* and the most negatively correlated with members of the *Polyangia*. *Tylenchorhynchus* spp. were the most negatively correlated with members of the *Clostridia* and *Bacilli*. Free-living nematodes in soils were the most negatively correlated with members of the *Acidobacteriae*.

The influences of the soil physicochemical parameters on phytonematodes and free-living nematodes within roots were evaluated. The results presented that soil pH and Ec were the most positively correlated with *M. graminicola* and the most negatively correlated with *Pratylenchus* spp. The OM and total Mn were the most negatively correlated with *Tylenchorhynchus* spp., whereas total P was the most positively correlated with *Helicotylenchus* spp. The total contents of K, Ca, and Mg were the most positively correlated with *M. graminicola* and the most negatively correlated with *Pratylenchus* spp. Fe concentration was the most positively correlated with *Pratylenchus* spp. and the most negatively correlated with *Tylenchorhynchus* spp. The total Cu and Zn were the most positively correlated with *Helicotylenchus* spp. and the most negatively correlated with *Pratylenchus* spp. The results of the correlations between nematodes within roots and the microbiome are provided in Supplementary Table 3. *M. graminicola* was the most negatively correlated with the members of the Actinobacteria. *Pratylenchus* spp. were the most positively correlated with members of the Actinobacteria and the most negatively correlated with members of the *Polyangia*. In addition, the CCS analysis showed that soil pH, Ec, total Ca, K, Mg, and Fe were factors that affected the bacterial community composition and diversity of phytonematodes and free-living nematodes in the rhizosphere and roots of rice (Supplementary Fig. 3).

Discussion

Rice is a globally significant agricultural crop cultivated on a massive scale (*Gnamkoulamba et al., 2018*). Phytonematodes are one of the barriers to improved rice production. Even though over 4,100 species of phytonematodes have been reported, including endo- and ecto-parasites (*Decraemer & Hunt, 2006*) a mere 29 species have been identified as having a direct correlation with yield reductions in rice production (*Bridge, Plowright & Peng, 2005*). The occurrence of nematode attacks has the potential to facilitate the infection process of other pathogens (*De Waele & Elsen, 2007*). Our results demonstrated that the most prevalent genera of phytonematodes associated with rice agriculture in various agroecological zones of Thailand included *M. graminicola*, *Hirschmanniella* spp., *Pratylenchus* spp., *Helicotylenchus* spp., and *Tylenchorhynchus* spp. Notably, *M. graminicola* and *Hirschmanniella* spp. had their highest density in soil collected from site PTT. In addition, they were both predominant within roots collected from sites CCS and AYY, while the amounts of free-living nematodes in the soil and roots were highest at sites AYY and PAR. The widespread presence of nematodes in rice fields in Luzon, the Philippines, was investigated by *Pascual et al. (2014)*. They reported that *Helicotylenchus*, *Hirschmanniella*, *Meloidogyne*, *Criconema*, *Xiphinema*, *Pratylenchus*, and *Tylenchorhynchus* were the more prevalent and abundant genera. The genera *Meloidogyne*, *Suctellonema*, *Heterodera*, *Hirschmanniella*, *Pratylenchus*, and *Helicotylenchus* contained the major phytonematodes mainly distributed in paddy fields in Togo (*Coyne et al., 2000*). *Gnamkoulamba et al. (2018)* recorded the genera *Helicotylenchus*, *Hirschmanniella*, *Meloidogyne*, and *Suctellonema* were in both soil and root samples of rice in different agroecosystems in Togo. According to our findings, *M. graminicola* and *Hirschmanniella* spp. were reported as the dominant group of nematodes more frequently found within the soils and roots of rice (*Bridge, Plowright & Peng, 2005*; *Eche et al., 2013*), with *M. graminicola* has been documented as being highly adapted to flooded environments, leading to better survival in soil environments (*Bridge, Plowright & Peng, 2005*). *Musarrat et al. (2016)* found these nematodes in a rice-growing area in Pakistan. The present study detected free-living nematodes from rice roots. This was not surprising, as nematode-fungal pathogen disease complexes have been reported in general with nematode infection. Some species of free-living nematodes are fungivores, which have feeding disposition on fungi, including fugal plant pathogen, so they can invade plant roots to obtain food (*Zhang et al., 2020*). Different factors affect the distribution of nematodes, such as the production system, rice variety, intercropping with other crops, and rainfall (*Gnamkoulamba et al., 2018*). However, the high levels of nematode density and diversity observed in the present study suggested that the rice cultivation in Thailand is being confronted with the severe endo- and epi-phytotic nematodes. These results could be ascribed to the escalating intensification of rice cultivation in Thailand.

The activity of soil-dwelling organisms is influenced by physicochemical soil qualities, whereas the establishment of a nematode population in the soil is influenced by a range of abiotic and biotic factors (*Al-Ghamdi, 2021*). *De Oliveira Cardoso et al. (2012)* have reported the physicochemical properties of soil had an impact on the density and structural diversity of nematode communities. Our findings demonstrated that soil at site PTT that had the highest numbers of *M. graminicola*, *Hirschmanniella* spp., and *Helicotylenchus* spp., contained the significantly highest concentrations of total P, Mg, Cu, and Zn. The rice roots at site AYY had the highest number of *Hirschmanniella* spp. Also had the significantly highest levels of soil OM, available N, total Ca, Mn, and pH. Rice roots at site CCS that had the significantly highest number of *M. graminicola* also had the highest soil Ec value and total K content. These results suggested that the establishment of infection by *M. graminicola* could be significantly associated with soil parameters including Ec and the levels of total K, P, Mg, Cu, and Zn. Many soil factors (including the quantity of OM, levels of available N, total Ca, and Mn, and pH) could support infection by *Helicotylenchus* spp. These findings appeared to be consistent with other reports that demonstrated the contents of soil elements, such as OM, P, Ca, Mg, and K, supported the establishment of phytonematodes in the soil (*Al-Ghamdi, 2021*; *Dias-Arieira et al., 2021*; *Leiva et al., 2020*). Furthermore, our results showed that a higher soil pH and level of OM supported higher populations of phytonematodes and free-living nematodes. This finding was consistent with *Casto et al. (1990)* and *Al-Ghamdi (2021)* who reported that pH and OM played important roles in the proportion of soil nematodes. The distribution of soil nematodes was negatively correlated with the pH; soil acidity affected nematode populations, such as *M. incognita* and *Radopholus similis* that were presented at reduced levels in acidic soils (*Davide, 1980*; *Gade & Hiware, 2017*). The soil OM content positively supported the high proportions of free-living nematodes by promoting bacteria and fungi growth that were essential foods for the nematodes (*Cadet & Spaull*, 2003).

Understanding the role that the plant rhizosphere soil microbiome plays during PPN infection is considerable and should be investigated. Previous research documented the significance of microbial communities present in soil in control of phytonematodes (*Silva et al., 2022*; *Zhou et al., 2019*). Thus, the current study conducted an extensive evaluation of bacterial diversity and community composition in soils infested with nematodes, with a focus on the influence of environmental conditions. The findings showed there were not any significant differences in bacterial diversity across the sampled locations. Nevertheless, site CCS exhibited the greatest number of detected species and bacterial richness. Our results may indicate an effect of nematode density and diversity on the observed species and bacterial richness. Nematodes have been reported to graze on bacteria, which may affect the bacterial community by accelerating bacterial turnover (*Cheng et al., 2016*; *Djigal et al., 2004*). *Silva et al. (2022)* concluded that bacterial richness in a community was reduced as a response to the numbers of nematodes in infested soil. Furthermore, the present study found that the phyla *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, *Myxococcota*, *Chloroflexi*, *Verrucomicrobiota*, *Bacteroidota*, *Gemmatimonadota*, and *Desulfobacterota* were the top-10 regarding bacterial abundance in all the sampled soils. This data was according to the results from other studies. For example, *Silva et al. (2022)* reported that *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, and *Gemmatimonadota* were the most common bacterial groups in soil samples in Brazil. *Masson et al. (2020)* found that the *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, *Verrucomicrobia*, *Nitrospirae*, and *Fibrobacteres* were the predominant phyla of highly *M. graminicola*-infested fields in Vietnam. The bacterial phyla *Acidobacteriota*, *Proteobacteria*, *Actinobacteriota*, and *Gemmatimonadota* were the highest abundant in phytonematode-suppressive soils (*Harkes et al., 2020*). Members of the *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, and *Actinobacteriota* exhibited a broad metabolic diversity and possess the ability to colonize various ecosystems. These bacterial groups possess a multitude of genes associated with stress resistance, carbon degradation, phosphate solubilization, and antibiotic production. These genetic traits contribute to their adaptive capabilities and enable their successful survival in soil, thereby establishing them as dominant microbial groups (*Pongsilp & Nimnoi, 2022*). In addition, our results demonstrated that the soil pH, OM, Ec, and total Ca, K, Mg, and Fe were factors affecting bacterial diversity. Our result is compatible with prior research that has noted the influence of many soil variables, including pH, OM, N, K, Mg, and Zn concentrations, as well as nutrient availability and hydrocarbon bioavailability, on the variability of bacterial communities and their diverse composition (*Achife, Bala & Oyeleke, 2021*; *Nimnoi &Pongsilp, 2022*; *Pongsilp & Nimnoi, 2020*). By changing the amount of nutrients available in the environment, the relationship between soil minerals and bacteria can influence biogeochemical cycling *(Pongsilp & Nimnoi, 2020*; *Vu et al., 2022*). Correlations between the bacterial microbiome and phytonematodes were found, which were greater than in the roots. The numbers of *Acidobacteriae*, *Clostridia*, *Bacilli*, *Bacteroidia*, *Polyangia*, and *Actinobacteria* influenced the proportions of *Hirschmanniella* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., and free-living nematode in the soil samples, whereas only the numbers of *Polyangia* and *Actinobacteria* affected the numbers of *M. graminicola* and *Pratylenchus* spp. within the roots. *Castillo, Vivanco & Manter (2017)* reported correlations between dominant bacteria and nematode populations. The alpha-*proteobacteria*, *Rhodoplanes*, *Phenylobacterium*, and *Kaistobacter* have been found to be correlated with the *Meloidogyne*, while the *Bacteroidia* and gamma-*proteobacteria* have been reported to be correlated with the *Pratylenchus*. Members of the *Bacilli*, *Polyangia*, *Actinobacteria*, and *Acidobacteriae* are important contributors to ecosystems, since they are particularly abundant and ubiquitous in nature, such as in the soil, roots, water, and sediment (*Eichorst, Breznak & Schmidt, 2007*; *Nimnoi, Pongsilp & Lumyong, 2011*; *Nimnoi & Pongsilp, 2022*). Furthermore, they have been noted for their effects on the control of biogeochemical cycles, the degradation of biopolymers, the release of exopolysaccharides, and the encouragement of plant development (*Kalam et al., 2020*; *Matsumoto et al., 2021*; *Puri, Padda & Chanway, 2018*; *2020*). Additionally, they can synthesize diverse natural compounds that produce biomedically and industrially useful chemicals, such as antifungals, antibiotics, and antinematodal agents, which can be applied in regulating and affecting diverse microorganisms in ecosystems (*Atta, 2009*; *Crits-Christoph et al., 2018*; *Hadjithomas et al., 2015*; *Mahajan, 2012*; *Nigris et al., 2018*; *Parsley et al., 2011*; *Reichenbach, 2001*).

Notably, biocontrol plant diseases and plant growth-promoting bacteria, such as genera *Bryobacter*, *Acidibacter*, *Pseudomonas*, *Burkholderia*, *Caballeronia*, *Paraburkholderia*, and

Sphingomonas, were the predominant bacterial groups identified in the sampling soils in the present investigation. These genera have been found in soils where there has been significant suppression of soilborne disease through biocontrol, including phytonematodes, as well as plant growth promotion. *Pseudomonas* spp. are chitinolytic and hydrogen cyanide-producing bacteria that can be applied for the biocontrol of nematodes (*Ha et al., 2014*; *Kang, Anderson & Kim, 2018*; *Lee et al., 2011*). *Burkholderia*, *Caballeronia*, *Paraburkholderia*, and *Sphingomonas* have been stated to function biological nitrogen fixation, increase nutrient uptake, and confer diseases resistance against a bacterial pathogen (*Matsumoto et al., 2021*; *Puri, Padda & Chanway, 2018; 2020*). The establishment of *Acidibacter* in many plant species has been reported in association with soil iron and nutrient cycles, and soil pollution treatments (*Huang et al., 2020*; *Jiao et al., 2018*; *Liu et al., 2016*). The genus *Bryobacter* has been noted as beneficial microorganism for leguminous plants by playing roles in the degradation of minerals, promotion of plant growth, nitrogen fixation, and the suppression of plant disease (*Li et al., 2023*; *Luis et al., 2018*; *Xiao et al., 2017*). Nematodes and the microbial community have been reported for their activities in response to environmental impacts and eco system conversions (*Briar, Grewal & Somasekhar, 2007*; *Renčo, Gömöryová & Čerevková, 2020*). The present results have provided comprehensive data that could be beneficial for designing an appropriate cultivation method to control rice diseases caused by phytonematodes and to preserve soil quality for sustainable management.

Conclusions

The differences in soil properties, and numbers of nematodes and bacterial communities in the soils sampled reveal the clear impact of biotic and abiotic soil characteristics on ecosystem variables. We demonstrated that *M. graminicola, Hirschmanniella, Pratylenchus, Helicotylenchus*, and *Tylenchorhynchus* were the dominant phytonematodes distributed in soil across rice fields in Thailand. The high-throughput sequencing analysis clarified that *Acidobacteriota*, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, *Myxococcota*, *Chloroflexi*, *Verrucomicrobiota*, *Bacteroidota*, *Gemmatimonadota*, and *Desulfobacterota* were the predominant bacterial phyla that had established niches in the sampled soils. The numbers of *Acidobacteriae*, *Clostridia*, *Bacilli*, and *Bacteroidia* influenced the proportions of *Hirschmanniella* spp., *Tylenchorhynchus* spp., and free-living nematodes in the soil samples, whereas the numbers of *Polyangia* and *Actinobacteria* affected the numbers of *Pratylenchus* spp. in both the roots and soils. The components of total P, K, Mg, Ca, Cu, and Zn, as well as the pH, Ec, and OM of the soil influenced the composition of the bacterial and nematode communities. Our findings provided insights into correlations among rhizosphere microbiota, nematode, and soil properties, contributing to the potential development of suitable management programs to reduce phytonematodes in rice production systems.

Acknowledgements

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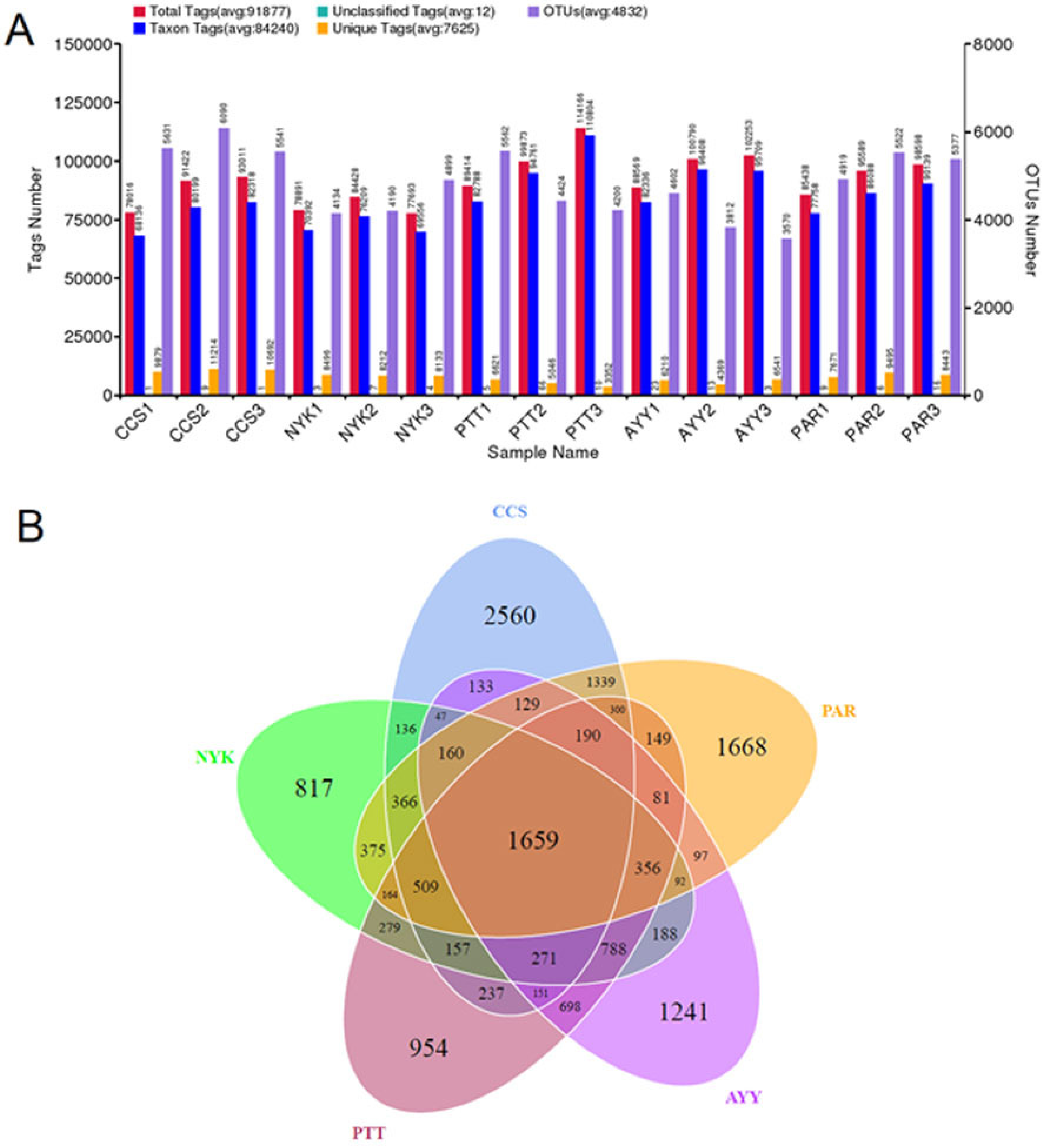
Figure 1

Tags and OTU numbers of each sampling location (A) and Venn diagram presenting the numbers of unique, common, and overlapping OTUs between each sampling site (B).

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.


A
Total Tags(avg:91877)
Taxon Tags(avg:84240)
Unclassified Tags(avg:12)
Unique Tags(avg:7625)
OTUs(avg:4832)
Tags Number
OTUs Number
150000
125000
100000
75000
50000
25000
0
8000
6000
4000
2000
0
78016
68136
9979
5631
91422
80199
11214
6090
93011
82318
10692
5541
78891
70392
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4134
84428
76209
8212
4190
77693
69556
8133
4899
89414
82788
6621
5562
99873
94761
66
5046
4424
114166
110994
18
3352
4200
88569
82336
23
6218
4602
100790
96408
13
4369
3812
102253
95709
6541
3579
85438
77758
7871
4919
95589
86088
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5522
98598
90139
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8443
5377
CCS1
CCS2
CCS3
NYK1
NYK2
NYK3
PTT1
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PTT3
AYY1
AYY2
AYY3
PAR1
PAR2
PAR3
Sample Name
B
CCS
PAR
NYK
AYY
PTT
2560
1668
817
1241
954
1659
133
1339
129
300
47
136
190
149
160
366
81
375
97
356
509
92
164
279
188
157
271
788
237
151
698

Figure 2

Heat map analysis of distribution of genus in each sampling location.

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

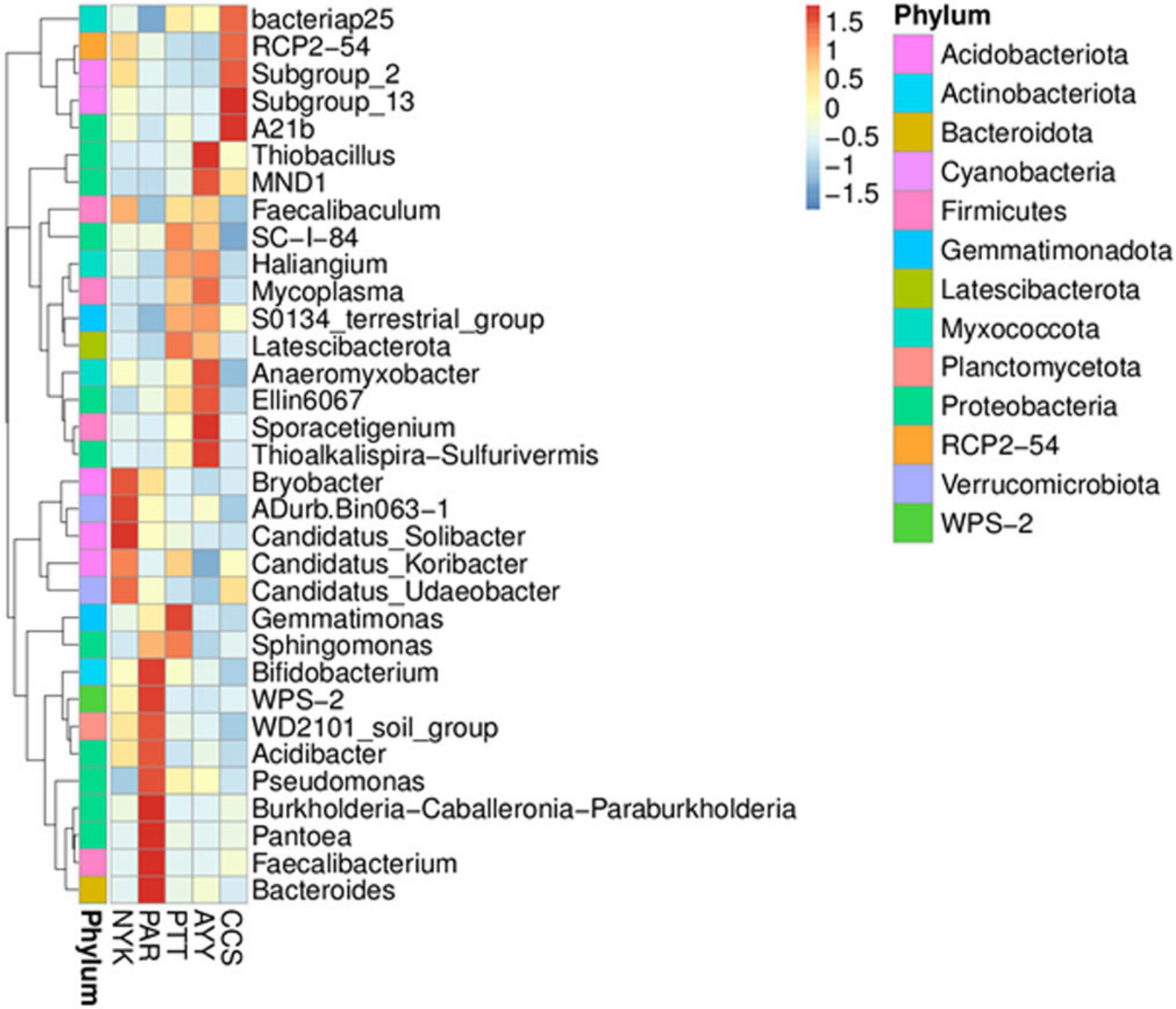

Figure 3

PCoA (A) and NMDS (B) analyses bacterial composition similarity among sampling sites.

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

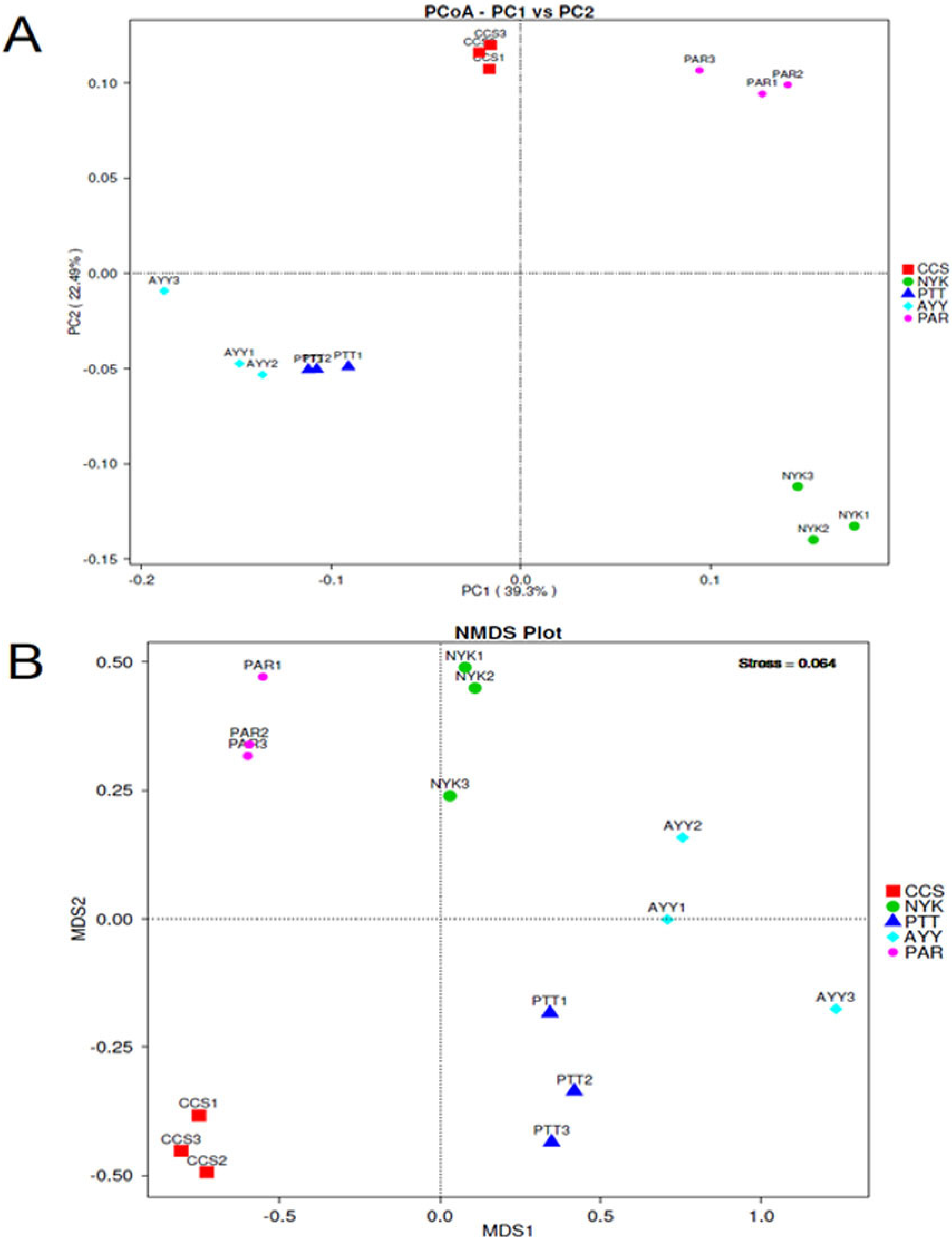
A
PCoA - PC1 vs PC2
PC1 (39.3%)
PC2 (22.49%)
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.2
-0.1
0.0
0.1
CCS3
CCS2
CCS1
PAR3
PAR2
PAR1
AYY3
AYY1
AYY2
PTT3
PTT2
PTT1
NYK3
NYK1
NYK2
CCS
NYK
PTT
AYY
PAR
B
NMDS Plot
Stress = 0.064
MDS1
MDS2
0.50
0.25
0.00
-0.25
-0.50
-0.5
0.0
0.5
1.0
PAR1
PAR2
PAR3
NYK1
NYK2
NYK3
AYY2
AYY1
AYY3
PTT1
PTT2
PTT3
CCS1
CCS3
CCS2
CCS
NYK
PTT
AYY
PAR

Figure 4

Beta diversity heat map of the dissimilarity coefficient between each sample.

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

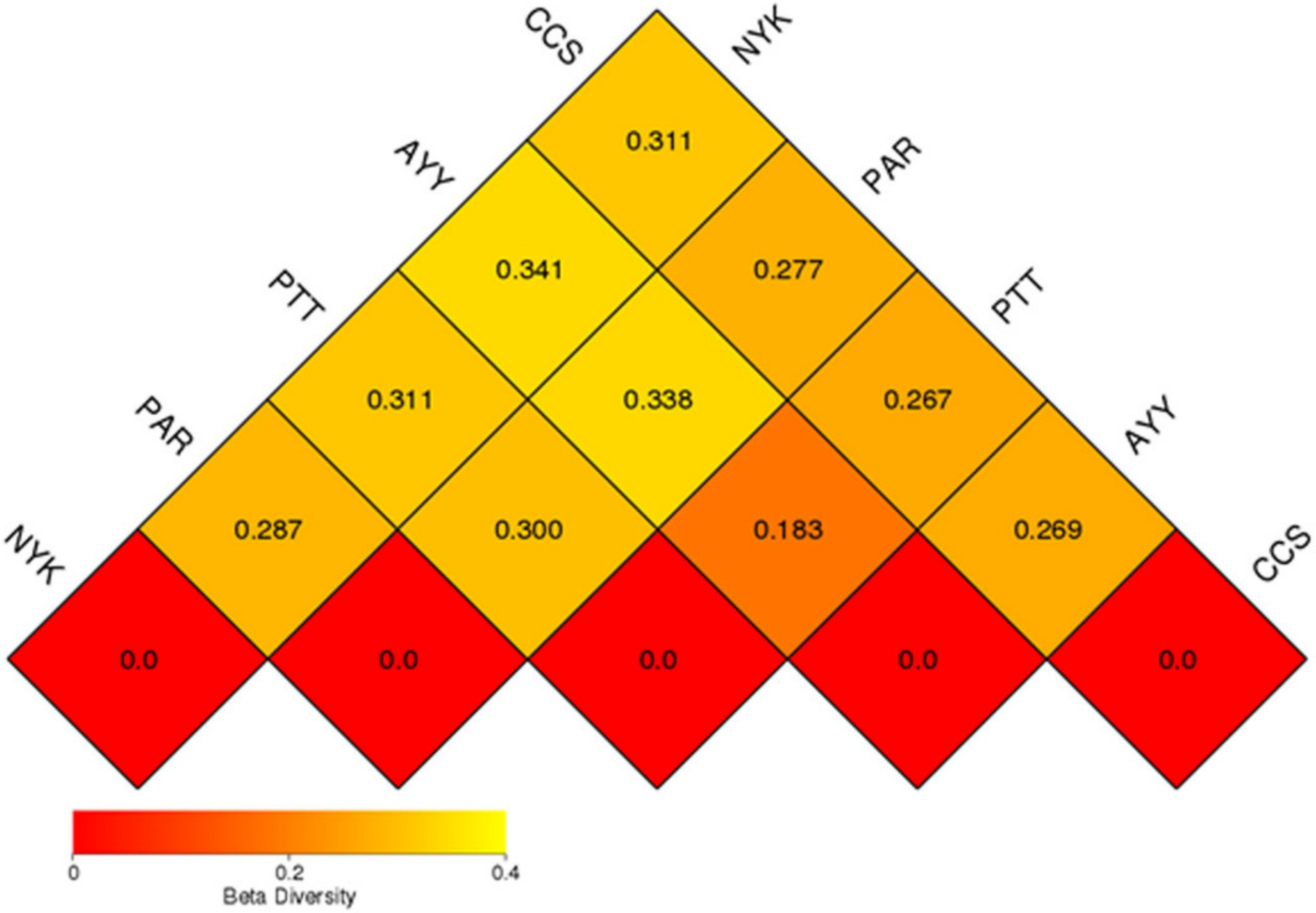

Figure 5

The UPGMA dendrogram of relative abundance at phylum level from each sampling site.

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

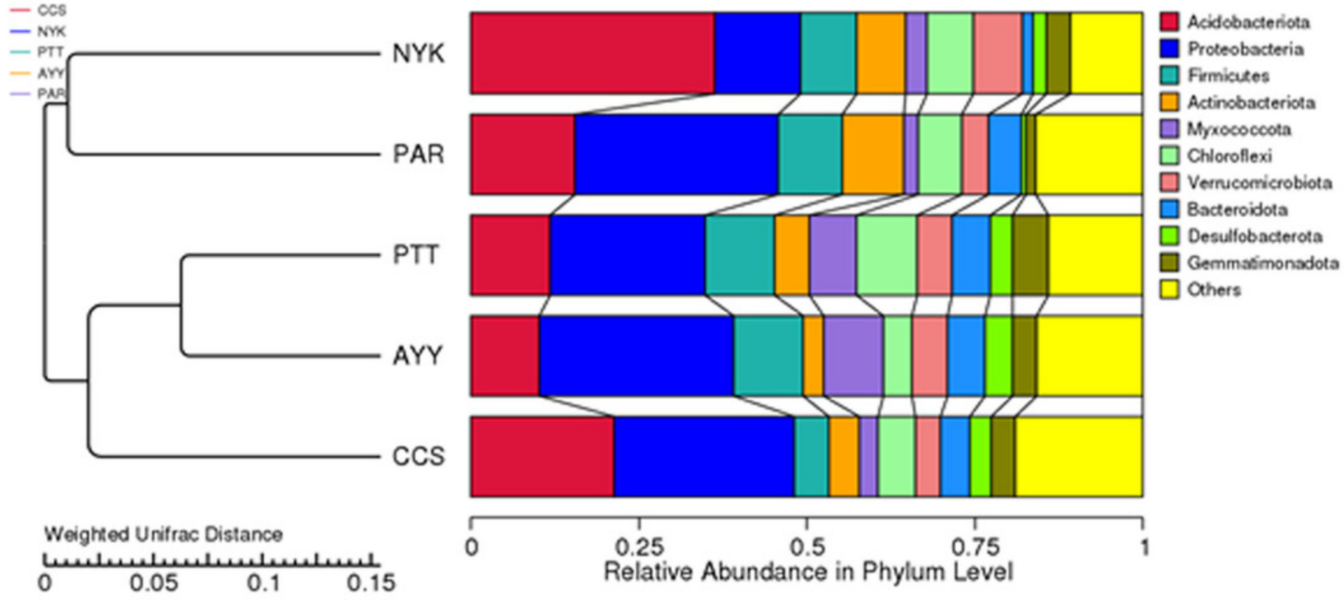

Table 1(on next page)

Soil nematodes from rhizosphere collected from rice fields.

* All values are represented as (mean±SD) based on triplicate samples **Values with the same letters in the column are not significantly different (*P*>0.05) according to Tukey’s test CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 1

Soil nematodes from rhizosphere collected from rice fields.

Sampling site	No. of plant-parasitic nematode/500 g soil*					No. of free-living nematode/ 500 g soil*
	M. graminicola	*Hirschmanniella* spp.	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Tylenchorhynchus* spp.	
CCS	39.33±15.04a**	7.33±7.50a	0.00	9.66±9.86a	5.00±1.73a	39.66±34.15a
NYK	18.00±5.00a	4.33±2.30a	4.66±0.57a	0.00	4.66±2.08a	26.33±3.05a
PTT	141.66±38.10b	22.00±4.58b	0.00	46.33±16.25b	0.00	51.33±20.55a
AYY	34.00±9.84a	9.00±3.60a	0.00	0.00	0.00	117.66±29.36b
PAR	36.00±10.00a	9.33±4.16ab	11.00±3.60b	0.00	0.00	48.00±9.00a

* All values are represented as (mean±SD) based on triplicate samples

**Values with the same letters in the column are not significantly different (*P*>0.05) according to Tukey’s test

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 2(on next page)

Soil nematodes in roots collected from rice fields.

* All values are represented as (mean±SD) based on triplicate samples **Values with the same letters in the column are not significantly different (*P*>0.05) according to Tukey's test CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 2

Soil nematodes in roots collected from rice fields.

Sampling site	No. of plant-parasitic nematode/3g root*					No. of free- living nematode /3 g root*
	M. graminicola	*Hirschmanniella* spp.	*Pratylenchus* spp.	*Helicotylenchus* spp.	*Tylenchorhynchus* spp.	
CCS	3280.00±603.98c**	6.00±4.35a	0.00	0.00	10.00±8.18a	0.00
NYK	38.66±8.14a	13.00±4.58a	6.33±3.05a	0.00	0.00	3.33±1.52a
PTT	307.66±76.10a	10.00±8.18a	0.00	6.66±3.78a	0.00	4.00±2.00a
AYY	1846.00±406.73b	14.33±3.51a	0.00	0.00	0.00	0.00
PAR	116.00±24.43a	11.66±3.51a	9.33±2.51a	0.00	0.00	16.66±5.68b

* All values are represented as (mean±SD) based on triplicate samples

**Values with the same letters in the column are not significantly different (P>0.05) according to Tukey's test

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

7

Table 3(on next page)

Soil physicochemical properties from each sampling site.

*All values are represented as (mean±SD) based on triplicate samples **Values with the same letters in the row are not significantly different (*P*>0.05) according to Tukey's test CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 3

Soil physicochemical properties from each sampling site.

Parameter	Sampling site				
	CCS	NYK	PTT	AYY	PAR
pH	5.94	5.21	6.53	7.18	4.77
Electrical conductivity (ds m^{-1})	10.52	0.37	0.84	0.79	0.42
Sand (%)	59.20	47.17	6.00	11.10	35.61
Silt (%)	32.19	40.49	34.18	34.04	52.9
Clay (%)	8.61	12.34	59.82	54.87	11.48
Soil texture	Sandy Loam	Loam	Clay	Clay	Silt Loam
Organic matter (%)*	0.51±0.01a**	1.81±0.26c	2.44±0.49d	4.41±0.35e	1.18±0.25b
Available N (%)*	0.02±0.00a	0.09±0.00c	0.11±0.00d	0.21±0.01e	0.07±0.00b
Total P (mg kg^{-1})*	18.57±0.23b	8.49±0.43a	48.48±0.41e	26.23±0.27c	29.64±0.15d
Total K (mg kg^{-1})*	235.93±2.37e	11.98±0.69b	228.05±2.35d	185.51±0.34c	15.23±0.11a
Total Ca (mg kg^{-1})*	3044.04±64.14c	518.55±33.28b	3138.17±31.85c	5301.19±99.21d	381.06±27.27a
Total Mg (mg kg^{-1})*	633.41±28.11d	7.11±0.12a	674.91±3.19e	397.76±3.35c	29.38±0.60b
Total Fe (mg kg^{-1})*	116.76±0.30a	230.37±27.88c	177.65±0.57b	238.90±4.18c	379.33±4.86d
Total Mn (mg kg^{-1})*	2.29±0.07a	8.25±0.33b	59.99±1.18d	61.78±0.32e	16.08±0.38c
Total Cu (mg kg^{-1})*	1.69±0.01b	0.45±0.01a	46.12±0.70d	7.26±0.08c	0.93±0.04ab
Total Zn (mg kg^{-1})*	0.81±0.02a	0.70±0.00a	15.51±0.86c	2.07±0.05b	0.72±0.02a

*All values are represented as (mean±SD) based on triplicate samples

**Values with the same letters in the row are not significantly different (*P*>0.05) according to Tukey's test

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 4(on next page)

Indices of bacterial richness and diversity of soil from each location.

*All values are represented as (mean±SD) based on triplicate samples **Values with the different letters in the column are significantly different (*P*≤0.05) according to Tukey’s test CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 4

Indices of bacterial richness and diversity of soil from each location.

Sampling site	Observed species*	Diversity indices *		Richness indices*	
		Shanon-Weaver	Simpson	Chao1	ACE
CCS	5260.00±291.82b**	10.20±0.32a	0.99±0.00a	5613.21±414.84b	5715.23±408.28c
NYK	4023.66±411.68ba	9.68±0.24a	0.99±0.00a	4378.77±484.18ab	4424.89±466.56ab
PTT	4205.66±774.84ab	9.99±0.54a	0.99±0.00a	4886.85±569.25ab	5024.60±510.50abc
AYY	3644.00±472.95a	9.87±0.31a	0.99±0.00a	3963.25±516.43a	4094.70±493.20a
PAR	4752.66±260.34ab	9.82±0.08a	0.99±0.00a	5318.71±313.10b	5438.49±355.40bc

*All values are represented as (mean±SD) based on triplicate samples

**Values with the different letters in the column are significantly different ($P \leq 0.05$) according to Tukey's test

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 5(on next page)

Top 10 most abundance of bacterial classes presents in each observed location.

*All values are represented as (mean±SD) based on triplicate samples **Values with the different letters in the column are significantly different (*P*≤0.05) according to Tukey’s test CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

Table 5

Top 10 most abundance of bacterial classes presents in each observed location.

Sampling site	Class*									
	Acidobacteriae	gamma-*proteobacteria*	alpha-*proteobacteria*	*Clostridia*	*Verrucomicrobiae*	*Bacilli*	*Bacteroidia*	*Anaerolineae*	*Polyangia*	*Actinobacteria*
CCS	17.35±1.42c	18.68±2.84b	8.11±0.42c	3.25±0.64a	2.57±0.24a	1.49±0.16a	3.39±0.71b	2.39±0.57a	1.43±0.15a	1.49±0.38a
NYK	33.24±4.00d	6.64±0.39a	6.15±0.03ab	4.71±1.22a	7.19±1.22c	3.35±0.19a	1.47±0.98a	1.31±0.91a	1.41±0.10a	2.96±0.12ab
PTT	5.82±0.42a	15.99±3.58ab	7.09±1.11bc	5.41±0.48a	4.74±0.24b	4.74±0.81a	5.30±0.71c	5.64±0.49b	4.53±0.58b	2.82±0.96ab
AYY	4.76±0.87a	24.30±6.56b	4.50±0.40a	5.66±3.11a	4.83±1.12b	4.63±2.68a	4.64±0.22bc	2.74±0.94a	4.53±0.85b	1.44±0.73a
PAR	11.85±0.88b	19.03±4.05b	11.19±0.84d	5.70±1.24a	3.80±0.45ab	3.50±0.51a	4.03±0.50bc	1.47±0.12a	0.95±.011a	4.14±0.90b

*All values are represented as (mean±SD) based on triplicate samples

**Values with the different letters in the column are significantly different (*P*≤0.05) according to Tukey's test

CCS, sampling from Chachoengsao province; NYK, sampling from Nakhon Nayok province; PTT, sampling from Pathum Thani province; AYY, sampling from Phra Nakhon Si Ayutthaya province; PAR, sampling from Prachin Buri province.

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