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Microbial diversity in pesticidal and non-pesticidal paddy soil microbiomes

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Abstract. *Susanti R, Kenarni NR, Jaya AF, Nisa' FF, Mukaromah RL, Widiatningrum T, Martuti NKT, Rahayuningsih M. 2023. Microbial diversity in pesticidal and non-pesticidal paddy soil microbiomes. Biodiversitas 24: 4723-4730.* The use of pesticides can affect the physical and chemical properties of paddy soil. Soil physical and chemical properties can indicate soil quality because they affect soil microbial activity. This study used an exploratory observational method to analyze the diversity and abundance of microbes in the soil microbiome of pesticide paddy fields (PPF) and non-pesticide paddy fields (PF). The PPF samples were taken from Purwosari Village, Mijen Subdistrict, Semarang City, while the PF samples were taken from Tambangan Village, Mijen Subdistrict, Semarang City. Soil samples were taken for chemical and metagenomic analysis based on 16S rRNA gene marker region V3-V4. The results showed that microbial PPF diversity was higher than PF's based on the Shannon and Simpson index. The PPF microbiome is dominated by 3 bacterial phyla, namely Proteobacteria (26.61%), Actinobacteriota (15.13%) and Acidobacteriota (11.44%). Meanwhile, the PF microbiome was dominated by Proteobacteria (28.73%), Acidobacteriota (12.80%), and Chloroflexi (11.41%). The Archaea phylum that dominates PPF are Halobacterota (62.78%), Euryarchaeota (20.82%), and Crenarchaeota (10.89%), while in PF it is dominated by Halobacterota (80.43%), Crenarchaeota (11.03%) and Euryarchaeota (5.04%). The phylum that plays an important role in the biodegradation of organochlorine pesticides is Actinobacteria, which dominates in PPF; low abundance of Nitrospirota in PPF as a bioindicator of organochlorine pesticide contamination.

Keywords: Diversity, metagenomics, non-pesticide paddy field, pesticide paddy field, soil microbes

INTRODUCTION

Lately, a rapid evolution of farming technology has caused many deleterious impacts. The environment and organisms are exposed to enormous amounts and kinds of chemicals, such as caused by fertilizer and pesticides (Sharma and Singhvi 2017). Massive use of pesticides is due to its appointment as the most efficient and effective in farm defense against pests as part of pest management (Piwowar 2021). It is reinforced by a trend among farmers to exploit pesticides as much as possible because of the distress of crop failure (Mishra et al. 2021). These induce bioaccumulation of hazardous chemicals within food chains and stipulate an imbalanced ecosystem. Furthermore, the residues persist in the environment, such as in the plant organs, soil, air, and even groundwater, and negatively impact environments and organisms (Ali et al. 2021). Some disrupt the environment and the organisms within (Kumar et al. 2023).

Similar types of ecosystem problems are also found in some main paddy fields. Excessive doses and or mixing of several types of pesticides cause a negative effect in which the contamination of paddy soil is getting higher (Mberulata et al. 2022). This, in turn, affects the soil nutrient balance and acidity (pH) (Qaswar et al. 2020). Nutrient imbalances affect soil fertility, microbial species diversity, and soil abundance (Eo and Park 2016).

On the opposite of this damaged ecosystem is an environmental balancer, paddy farming in the form of organic control. The farmer implements plant-based pesticides at this paddy field to prevent and overcome plant disease attacks (Haritha et al. 2021). Plant-based pesticides can provide the nutrients needed by plants so that soil fertility is maintained. In addition, using plant-based pesticides can produce agricultural products free of pesticide residues. In organic paddy fields, organic fertilizers such as manure, compost, green manure, crop residues (straw, stover, corn cobs), livestock waste, industrial waste, and biological fertilizers are also used (Giri and Pokhrel 2021).

The presence of microorganisms in the soil indicates soil fertility because microbes have an important role in the decomposition and mineralization of nutrients and nitrogen fixation by rhizobia (Zhu et al. 2020). Several indicators, such as the availability of organic matter, temperature, water availability, and soil ecological conditions, can be observed by analyzing the diversity of species and the abundance of microbes in the soil (Blesh and Ying 2020). The higher the microbial population in the soil, the higher the biochemical activity and soil quality index (Zhu et al. 2020).

The novel technology of direct DNA sequencing of soil microbial communities allows researchers to gain a more in-depth and diverse view of each soil microorganism's

taxonomic potential and function. Metagenomic analysis is the most suitable method of sequencing and analyzing DNA from various ecosystems, including microbial ecosystems (Prayogo et al. 2020). This method can be run quickly and effectively, so it has become a routine method to characterize the functional potential of microbial communities. However, environmental conditions are not always uniform, so only the most abundant microorganisms can be detected, or in other words, the total community and population heterogeneity can hamper the efficiency of the assembly. Therefore, to reduce the inadequacy, all metagenomic analyses should begin with DNA quality control, which aims to minimize bias by removing nucleotide sequences, low-quality base pairs, and sequence contaminants that come from the outside environment of the sample (Taş et al. 2021).

Metagenomic analysis among the paddy fields of both managements in Indonesia has never been done, even though it is an important effort to obtain markers of microbiota stability in each paddy field soil ecosystem and to expose the environment safety. Therefore, this research aimed to maintain metagenomic analysis to detect species diversity and abundance of microbials in pesticide and non-pesticide paddy fields using the Next Generation Sequencing (NGS) techniques.

MATERIALS AND METHODS

This exploratory observational research reveals the species diversity and abundance of microbes in pesticide and non-pesticide paddy field soil. This research was conducted at the Biology Laboratory of Universitas Negeri Semarang, Central Java, Indonesia. Soil chemical analysis with soil macro-micro element test parameters was conducted at BPTP Yogyakarta. Organochlorine pesticide residue test was conducted at the Agricultural Environmental Research Center (Balingtan) Central Java. Metagenomic analysis of soil samples was conducted at PT Genetika Science Indonesia. Determination of the research sample location was carried out by purposive sampling with certain criteria. Pesticides paddy field soil (PPF) samples were taken from Purwosari Village, Mijen District, Semarang City, Central Java, Indonesia (S 07°04'39.2" E110°20'22.2"). At the same time, non-pesticide paddy field soil (PF) samples were taken from Tambangan Village, Mijen Subdistrict, Semarang City, Central Java, Indonesia (S 07°04'49.1" E110°18'35.7"). The distance between PPF and PF is approximately 4.4 km (Figure 1). Soil sampling was carried out during the dry season, when the rice plants entered the ripening phase.

Sampling and sample preparation

Soil samples of pesticide and non-pesticide paddy fields were taken at 5 points each in one plot. Each sample point was taken as much as 500 g of soil with a depth of 10 cm from the soil surface. Soil samples that have been taken are put into a plastic zip-lock and then closed tightly. The zip-lock plastic containing the pesticide and non-pesticide paddy soil samples was then stored in a cool box to be taken to the laboratory.

The soil from each sample point was then put into a large tub and combined into one (polled) at the Biology Laboratory of Universitas Negeri Semarang. The pesticide and non-pesticide paddy-field soil samples were divided into three parts for different test analyses: soil macro-micro chemical content test, organochlorine pesticide residue test, and metagenomic analysis.

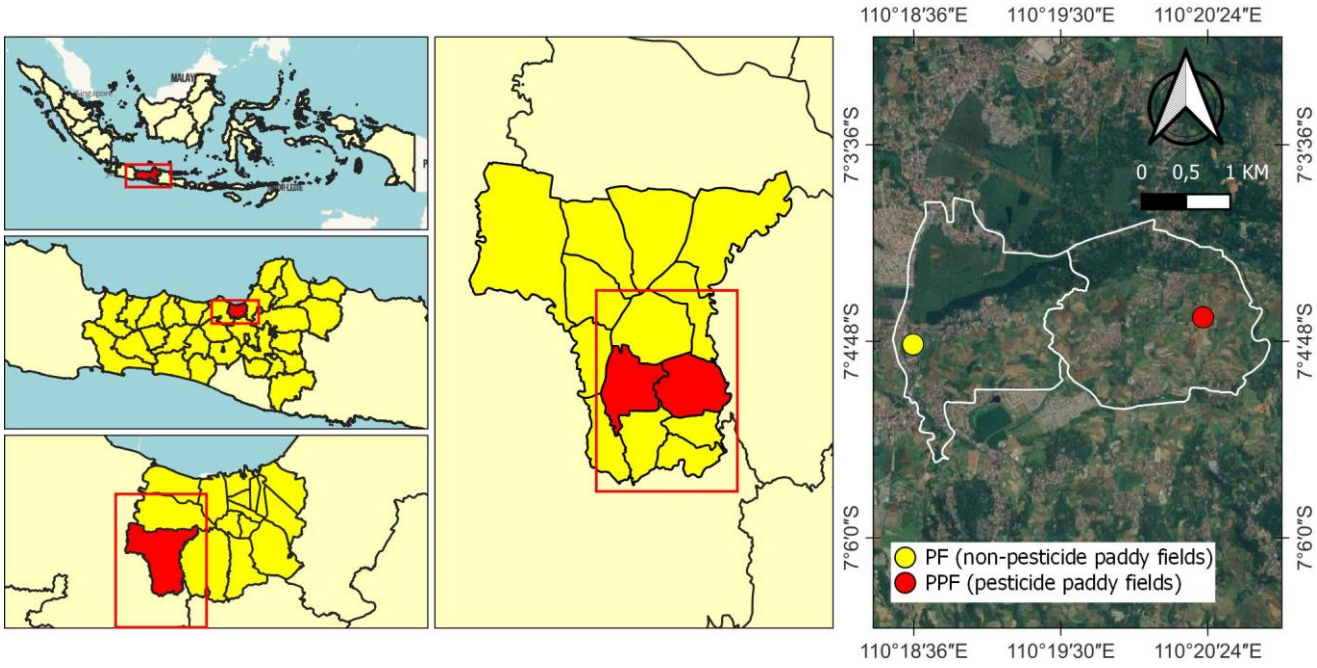

Figure 1. Location map of the research sample points: Pesticides paddy field soil (PPF) in Purwosari Village and non-pesticide paddy field (PF) in Tambangan Village; both location in Mijen Subdistrict, Semarang City, Central Java, Indonesia

Analysis of soil physical and chemical characteristics (Soon and Hendershot 2008)

Therefore, when sampling, soil samples of pesticide and non-pesticide paddy fields taken from the location are analyzed for soil physics in temperature, pH, moisture, color, and soil texture. The soil chemical analysis by soil macro-micro element test includes C-organic, N-total Kjeldahl method, K-available 25% HCl method, P_2O_5, Ca-dd, Mg-dd, Na-dd, Fe, and S parameters with standard methods. The organochlorine pesticide residue test was conducted using the gas chromatography method.

DNA isolation

Microbial DNA was extracted from soil samples using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, California, US) according to the manufacturer's protocol. The extracted DNA was stored in a -20°C freezer before being used for further analysis. Pesticide and non-pesticide paddy-field soil samples were isolated from bacterial DNA using 16S rRNA gene amplification in the V3-V4 region. The amplicon was then sequenced using Next-Generation Sequencing (NGS): metagenomic method (Holm et al. 2019).

Next Generation Sequencing (NGS)

DNA samples were amplified with 16S rRNA gene markers V3-V4 region (Yarza et al. 2014). The amplification program with PCR (Polymerase Chain Reaction) was 94°C denaturation for 3 minutes, followed by 27 cycles (denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds) and final extension at 72°C for 10 minutes. The primers used were 338F (5′-GGACTACHVGGGTWTC TAAT-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), which bind to the barcode, an eight-base sequence specific to each sample. PCR reactions were performed with a total volume of 20 mL containing 0.5 µL (5 U/µL) Easy Taq DNA polymerase, 2 µL 10× Easy Taq buffer, 2 µL 0.25 mmol/L dNTPs, 0.2 µmol/L primers, 10 ng template DNA and up to 20 µL ddH_2O (Holm et al. 2019).

Data analysis

Soil physical and chemical data were analyzed descriptively by relating the diversity and abundance of microbes in each soil microbiome to various soil physical and chemical parameters. The soil microbial metagenomic analysis was performed using QIIME2 (Ver. 2019). The paired-end files were demultiplexed using the demux plugin. Then, quality control was performed on each sample using the DADA2 plugin (Callahan et al. 2019). Furthermore, the diversity index value was generated using 4 diversity indices: Shannon, Simpson, Chao1, and Observed OTUs. Furthermore, taxonomy was compiled based on the Greengenes 13_8 99% OUT database. Then, the heatmap was prepared using the Heatmap plugin, and the taxa barplot was prepared using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Physical and chemical characteristics of soil in PPF and PF

Physical data of pH value in pesticide and non-pesticide soils are almost identical, including in neutral pH conditions. Temperature and humidity of pesticide and non-pesticide soils have the same value. Pesticide paddy field soil has a dense, fine clay texture, while non-pesticide paddy field has a liquid fine clay texture. The soil color of the non-pesticide paddy field is darker brown than the pesticide paddy field (Table 1).

The results of soil chemical analysis showed that most of the macro-micro element content was higher in non-pesticide paddy field (PF) soil, namely C-organic, total N, K-available, P_2O_5, Ca-dd, Mg-dd, and Na-dd. The content of microelements Fe and S in the soil of pesticide paddy fields (PPF) is higher than the non-pesticide paddy fields (PF) (Table 2).

Soil texture in PPF looks denser than in PF based on soil physics data (Table 1). This condition may be caused by excessive use of chemical pesticides in PPF, which ultimately disturbs biodiversity in the soil. Prashar and Shah (2016) stated that soil organisms, such as earthworms, insects, and other macrofauna, play an important role in the decomposition process of organic matter in the soil (straw, stover, and animal manure). Soil chemistry data showed that the C-organic content in PPF was lower than that in PF, suggesting that the activity of soil organisms in PPF decreased due to exposure to chemical pesticides (Table 2).

Table 1. Soil physical data of pesticide paddy field (PPF) and non-pesticide paddy field (PF)

	PPF	**PF**
Temperature (°C)	37	37
pH	7.54	7.56
Humidity (%)	42	42
Color	Brown	Grayish brown
Texture	Dense clay	Liquid clay
Vegetation	Paddy	Paddy

Table 2. Soil chemistry data of pesticide paddy field (PPF) and non-pesticide paddy field (PF)

Chemicals	**PPF**	**PF**
C-organic (%)	2.19	2.72
N-total (%)	0.13	0.33
K- available (ppm)	82	117
P_2O_5 (ppm)	82	107
Ca-dd (cmol/Kg)	4.48	7.96
Mg-dd (cmol/Kg)	2.44	3.60
Na-dd (cmol/Kg)	0.26	0.39
Fe (ppm)	2.86	2.60
S (%)	0.37	0.24

As an example of such organisms, Earthworms feed on straw and other organic matter, process them in the digestive tract, and excrete the residue containing nutrients and organic carbon into the soil. The result of earthworms' organic matter processing is worm humus, which is rich in stable organic carbon and remains in the soil for longer periods (Desie et al. 2020). Disruption of these soil organism populations due to exposure to chemical pesticides can affect soil aggregation and cause it to become denser, as is the case with PPF.

The organochlorine pesticide residue test results showed that the pesticide paddy field (PPF) and non-pesticide paddy field (PF) soil had values below the LoQ (Limit of Quantification) too small to be detected (Table 3).

Microbial diversity and abundance in PPF and PF

The total microbiota sequences analyzed in this study were 189,421 OTUs (Operational Taxonomic Unit). The number of OTUs in non-pesticide paddy field soil (97,848) was more than in pesticide paddy field soil (91,573). Chao1 index value in non-pesticide paddy field soil (4,100,860) is smaller than in pesticide paddy field soil (4,306,099), as well as ACE value in non-pesticide paddy field soil (4,242,065) is smaller than pesticide paddy field soil (4,435,911). This data suggests that some bacteria are more abundant in pesticide paddy fields than non-pesticide paddy fields. Analysis of the microbiome diversity of paddy field soils showed that the Shannon index diversity value was higher in pesticide paddy fields (9.924) than in non-pesticide paddy fields (9.800). While the Simpson index diversity between pesticide and non-pesticide paddy fields has the same value. Based on the diversity index, the diversity and abundance of microbes in the soil of pesticide paddy fields (PPF) are higher than in non-pesticide paddy fields (PF) (Table 4).

In this study, bacterial diversity in non-pesticide paddy field soil was smaller than in pesticide paddy field soil (Table 4). Different control factors or ecological processes in land cultivation influence the distribution of microbial phylum diversity in PPF and PF. Certain types of bacteria are more abundant in pesticide paddy soil because they can metabolize or survive in extreme soil conditions due to the use of pesticides and chemical fertilizers (Lovecka et al. 2015). All abundance index parameters showed that pesticide-treated paddy field soils had higher bacterial abundance than non-pesticide paddy fields.

The OTUs observed 4,055 specific bacterial species in pesticide paddy field soil (PPF) and 3,875 specific bacterial species in non-pesticide paddy field (PF) soil. At the same time, 2,957 species were shared bacteria were observed in both pesticide and non-pesticide paddy fields. This shows that the number of bacterial species observed in pesticide paddy field soil is more than in non-pesticide paddy field soil (Figure 2).

Of all the OTUs observed, the bacterial species observed in the soil of pesticide paddy fields were higher than in non-pesticide paddy fields (Figure 2). The bacterial diversity of pesticide paddy field soil showed greater variability according to the diversity index. The higher bacterial diversity in pesticide paddy field soils is caused by soil conditions with extreme fluctuations due to the use of pesticides and chemical fertilizers.

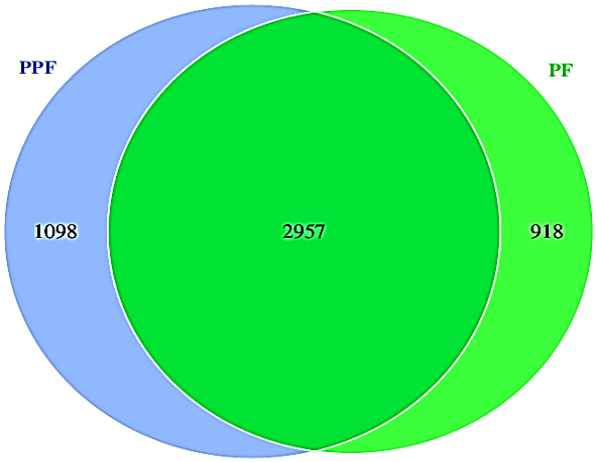

Figure 2. Ven diagram showing the presence of shared species (center) between PPF and PF

Table 4. Diversity index results

Sequencing results and diversity index	PPF	PF
Operational Taxonomic Units (OTUs)	91,573	97,848
Observed-species	4,055	3,875
Index Shannon	9.924	9.800
Index Simpson	0.996	0.996
Index Chao1	4,306,099	4,100,860
ACE (Abundance-base Coverage Estimator)	4,435,911	4,242,065
Good's coverage	0.990	0.991

Table 3. Organochlorine pesticide residue test results

Sample code	Organochlorine residues						
	Aldrin	Lindane	Endrin	Endosulfan	Dieldrin	Heptaklor	DDT
	Ppm						
PF	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
PPF	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
LOD	0.0012	0.0040	0.0009	0.0079	0.0010	0.0086	0.0078
LOQ	0.0200	0.0300	0.0100	0.0300	0.0300	0.0300	0.0500

Note: LoD: Limit of Detection, LoQ: Limit of Quantification

This condition resulted in certain types of bacteria that act as degraders and are more resistant to agrochemicals appearing in the microbiome of pesticide paddy soil (Lovecka et al. 2015). The higher diversity of microbial phyla in PPF is thought to be caused by the dominance of anaerobic bacteria. Excessive use of chemical pesticides can pollute the soil and disrupt the balance of the soil ecosystem, creating anoxic or poor dissolved oxygen environment in PPF. Anoxic conditions that cause very limited oxygen availability tend to be dominated by anaerobic bacteria phylum (Wihardjaka 2015). While non-pesticide paddy fields have more stable land conditions, bacterial diversity is dominated by bacterial species that are generally present in the microbiome of paddy fields and function in maintaining ecosystem balance (Bonanomi et al. 2016). Overall, no anomalies were found, meaning that the diversity index values did not deviate from one or more other values. All diversity index parameters show that the pesticide paddy field soil has higher bacterial diversity than the non-pesticide treated paddy field.

The bacteria domain dominated both pesticide and non-pesticide paddy fields, with 55 phyla in each microbiome. Analysis of the phylum composition of the Bacteria domain of pesticide paddy field soil (PPF) was successively dominated by the 3 highest phyla, namely Proteobacteria (26.61%), Actinobacteriota (15.13%) and Acidobacteriota (11.44%). At the same time, non-pesticide paddy field soil (PF) was successively dominated by the 3 highest phyla, namely Proteobacteria (28.73%), Acidobacteriota (12.80%) and Chloroflexi (11.41%). In both pesticide and non-pesticide paddy field soils, the 10 highest phyla from the Bacteria domain were recorded at >1% (Figure 3).

The bacteria domain dominated both the soil of pesticide and non-pesticide paddy fields. This follows previous reports showing that the bacteria domain has the highest relative abundance in soil ecosystems (Castañeda and Barbosa 2017). The 3 highest phylum dominance of Bacteria from PPF (Proteobacteria, Actinobacteriota, and Acidobacteriota) and PF (Proteobacteria, Acidobacteriota, and Chloroflexi) are shown in Figure 3. The dominance of the phylum in PF follows previous research, which states that the 3 phyla are specific phyla that dominate paddy field soils in general (Ali et al. 2022).

The heatmap of bacterial density shows that some phyla from the Bacteria domain identified in the pesticide paddy field soil have a darker color than the non-pesticide paddy field. This indicates that certain bacteria are more abundant in the pesticide paddy field soil. The proportion of the contribution of Bacteria domain phylum in pesticide paddy field soil that recorded >10% was Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi, and Firmicutes. At the same time, the phylum Proteobacteria, Acidobacteriota, Chloroflexi, Firmicutes, and Actinobacteriota contributed >10% in non-pesticide paddy fields (Figure 4).

Proteobacteria dominated the microbiome of paddy field soil, both in pesticide fields (26.61%) and non-pesticide fields (28.73%). Following the results of research by Chou et al. (2017), there are more Proteobacteria found in organic soil than in conventional soil. The abundance of Proteobacteria is influenced by the high concentration of C-organic in the soil, which can be obtained from fertilization and adding plant residues (biomass), especially straw (Dai et al. 2018). According to the chemical data table (Table 2), non-pesticide paddy field soil has higher C-organic. The abundance of the Proteobacteria phylum is also related to soil tillage that uses organic fertilizers from animal manure and weathering plants (straw, stover, corn cobs) so that the concentration of C-organic is high and spurs the development of nitrogen-fixing and phosphate-solubilizing bacterial populations, most of which come from the Proteobacteria phylum. Proteobacteria are important in soil organic matter transformation and carbon cycling (Li et al. 2017). The abundance of Proteobacteria in PPF affected by organochlorine pesticides indicates the ability of this group of bacteria to resist the toxicity of organochlorine pesticides and utilize these pesticide residues as nutrients for growth. One family of the Proteobacteria phylum, Enterobacteriaceae, has diverse potential to utilize or metabolize pesticides directly for growth (Wang et al. 2020).

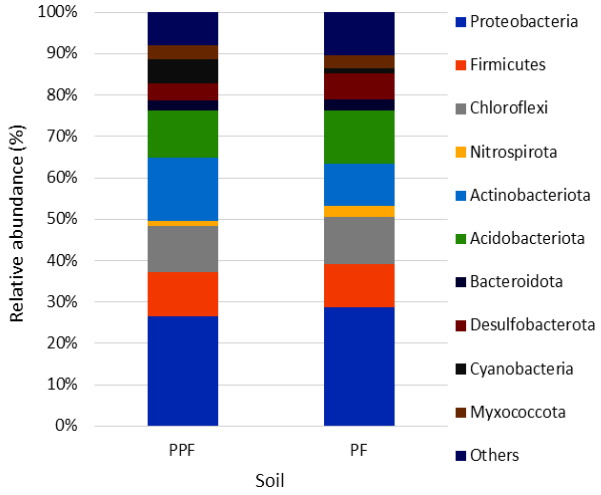

Figure 3. Relative abundance (%) of the top 10 phyla bacteria of PPF and PF

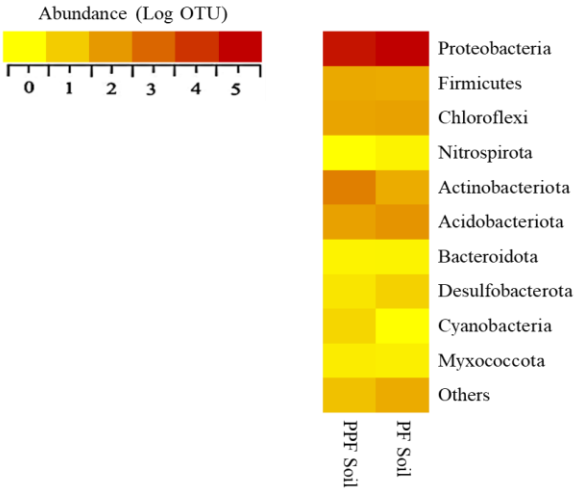

Figure 4. Heatmap of phylum abundance of bacteria in PPF and PF

The Acidobacteriota phylum in non-pesticide paddy fields was higher (12.80%) than in pesticide paddy fields (11.44%). Acidobacteria are usually considered oligotrophs and phylum groups sensitive to soil management. They are usually more abundant in natural ecosystems than in conventional agricultural land because they can metabolize stubborn organic substrates (Pershina et al. 2015). The research of Dai et al. (2018) showed that indirectly increasing C-organic through planting and directly added nutrients greatly determines the abundance of Acidobacteria. This also follows the data in Table 2 that non-pesticide fields have higher C-organic than pesticide fields. Hence, the abundance of acidobacteria is higher in non-pesticide fields. The difference in Acidobacteriota abundance in pesticide and non-pesticide paddy fields is not too high because Acidobacteria are the main flora in agricultural soils, which are reported to be sensitive to organic nutrient inputs and tolerate many pollutants (Cederlund et al. 2014).

The phylum Actinobacteriota showed a different abundance, which was very abundant in the soil of pesticide paddy fields (15.13%) compared to non-pesticide paddy fields (10.27%). This is in line with the research of Bonanomi et al. (2016) that the composition of bacterial phylum Acidobacteria and Firmicutes is higher, while Actinobacteria is lower in organic farming soils compared to conventional farming. The Actinobacteriota phylum degrades agrochemicals or agricultural chemicals, including herbicides, fungicides, and insecticides. The abundance of the phylum Actinobacteriota is associated with selective pressure as it depends on the chemicals used in agricultural tillage (Bonanomi et al. 2016).

The Nitrospirota phylum in the non-pesticide paddy field soil (2.67%) was higher than the pesticide paddy field (1.29%). The low abundance of Nitrospirota phylum in pesticide paddy field soil indicates the possible sensitivity of this phylum to the toxicity of organochlorine residues in polluted ecosystems. The low fertility of PPF affected by pesticide pollution can be seen in the data of soil physical and chemical characteristics influenced by the low abundance and extinction of some microorganisms. The phylum Nitrospirota is a nitrifier in terrestrial and marine ecosystems with the innate ability to convert nitrite to nitrate (Demanou et al. 2019). The relatively low abundance of Nitrospirota in pesticide-contaminated soil compared to non-pesticide-contaminated paddy fields may explain the reduced nitrate content of PPF contaminated with organochlorine pesticides. Some species of the Proteobacteria phylum play an important role in nitrogen fixation and are thus responsible for fixing nitrogen that contributes to low available nitrate in organochlorine pesticide-impacted PPF because other contributors to soil nitrate such as the Nitrospirota phylum have low abundance (Egbe et al. 2021).

The Archaea domain also dominates, with 8 phyla each from pesticide and non-pesticide paddy fields. Analysis of the phylum composition of Archaea of pesticide paddy field soil (PPF) successively dominated by the 3 highest phyla, namely Halobacterota (62.78%), Euryarchaeota (20.82%) and Crenarchaeota (10.89%). While in non-pesticide paddy field soil (PF), the 3 highest phyla are Halobacterota (80.43%), Crenarchaeota (11.03%), and Euryarchaeota (5.04%). In pesticide and non-pesticide paddy field soil, 3 phyla from the Archaea domain were recorded <1%, namely Micrarchaeota, Aenigmarchaeota, and Altiarchaeota (Figure 5).

Several studies confirm that Archaea are important for nitrogen cycling in various terrestrial environments (Gao et al. 2018), which is also evidenced by the abundance of the archaea domain in this study, which is the second highest after the bacteria domain. The highest 3 phylum dominance of Archaea in PPF are Halobacterota, Euryarchaeota, and Crenarchaeota, and in PF are Halobacterota, Crenarchaeota, and Euryarchaeota (Figure 5).

Heatmap of Archaea abundance level shows that some phyla identified in pesticide paddy field soil are darker than non-pesticide paddy fields. This indicates that bacteria from certain phyla are more abundant in pesticide-ridden paddy fields and less in non-pesticide-ridden fields. The highest proportion of the contribution of 3 phyla from the Archaea domain in pesticide paddy field soil, which recorded >10%, were Halobacterota, Euryarchaeota, and Crenarchaeota. While in non-pesticide paddy fields, only Halobacterota and Crenarchaeota phylum contributed >10% (Figure 6).

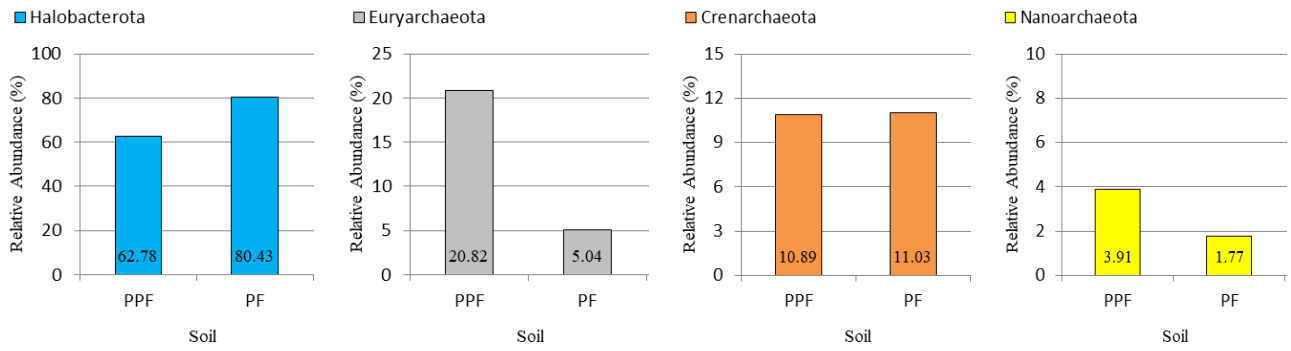

Figure 5. Relative abundance (%) of the 4 highest phyla of PPF and PF

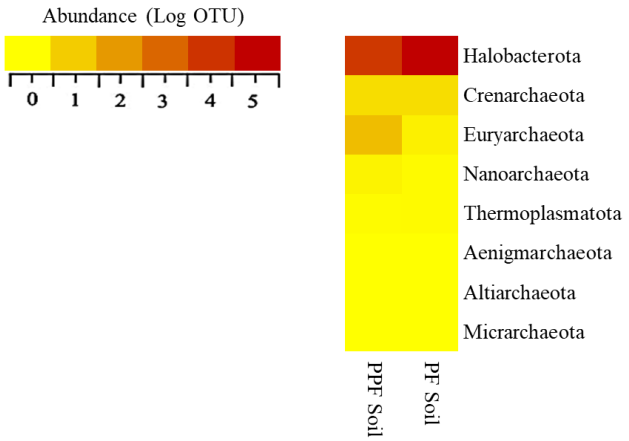

Figure 6. Heatmap of abundance level of archaea domain phylum in PPF and PF

The Halobacterota phylum dominated the microbiome of paddy field soil, both in pesticide paddy field soil (62.78%) and non-pesticide paddy field soil (80.43%). The higher abundance of Halobacterota phylum in non-pesticide paddy field soil is due to using organic fertilizer from animal manure and weathering of plants (straw, stover, corn cobs). Hence, the concentration of C-organic is high. This follows the results of research by Wu et al. (2022) that the application of manure can increase the abundance of Halobacterota phylum in paddy field soil. Halobacterota is one of the common methanogens in soil and is very important for soil methane emission because some organisms in this phylum contain the essential mcrA gene (Brauer et al. 2020).

The Euryarchaeota phylum was more abundant in pesticide paddy soil (20.82%) than in non-pesticide paddy soil (5.04%). Methanogenic Euryarchaeota generally spread in highly reduced and anoxic environments such as wetlands, paddy fields, and marine sediments (Hu et al. 2013). This indicates that the pesticide paddy field soil has reached anoxic conditions or poor dissolved oxygen due to inundation. The high abundance of Euryarchaeota phylum in pesticide paddy fields is also caused by the high concentration of Sulfur (S) and Iron (Fe) in pesticide paddy fields (Table 2). Following the research of Hu et al. (2013), in addition to methanogenesis, the Euryarchaeota phylum can also oxidize methane, fix nitrogen, reduce nitrate, and metabolize sulfur and iron. Bacteria from the Euryarchaeota phylum also play an important role in CH_4 emissions from paddy fields (Wu et al. 2022).

The abundance of the Crenarchaeota phylum in pesticide paddy fields (10.89%) and non-pesticide paddy fields (11.03%). While the phylum Thermoplasmatota in pesticide paddy fields (1.43%) and non-pesticide paddy fields (1.60%). As in the Halobacterota phylum, the abundance of Crenarchaeota and Thermoplasmatota is also influenced by the concentration of soil organic matter, especially fertilization, and plays an important role in CH_4 emissions from paddy fields (Wu et al. 2022). Non-pesticide paddy fields that applied organic fertilizers from animal manure and plant weathering (straw, stover, corn cobs) caused a higher abundance of Crenarchaeota and Thermoplasmatota in non-pesticide paddy fields. According to Li et al. (2021), the phylum Crenarchaeota may play an important role in soil ammonia oxidation. While Thermoplasmatota is one of the common methanogens in soil, it is very important for soil methane emission because some organisms in this phylum contain the essential gene mcrA (Brauer et al. 2020).

The diversity of microbial phylum PPF is higher than PF. The Shannon index value is higher in PPF (9.924) than in PF (9.800). Simpson's diversity index between PPF and PF has the same value (0.996). The number of species observed in PPF was more (4,055) than in PF (3,875). The highest 3 phylum dominance of Bacteria from PPF were Proteobacteria, Actinobacteriota, and Acidobacteriota, and in PF were Proteobacteria, Acidobacteriota, and Chloroflexi. The highest 3 phylum dominance of Archaea from PPF were Halobacterota, Euryarchaeota, and Crenarchaeota; in PF were Halobacterota, Crenarchaeota, and Euryarchaeota. The abundance of microbial phylum of PPF is higher than PF. The Chao1 index value in PF was smaller (4,100,860) than PPF (4,306,099), as well as the ACE value in PF was smaller (4,242,065) than PPF (4,435,911). The phylum that plays an important role in the biodegradation of organochlorine pesticides is Actinobacteria, which dominates in PPF; the low abundance of Nitrospirota in PPF is a bioindicator of organochlorine pesticide contamination.

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