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Profile of microbial community of organic and conventional rice field using metagenomic analysis

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ABSTRACT. Organic farming can increase the diversity of soil bacterial. This research aimed to compare the profile of microbial community of organic and conventional rice fields in early (0 Day After Planting/DAP), mid (15 DAP), and late (45 DAP) fertilizer application period. The total DNA genome from the soil sample was extracted then analyzed metagenomically using Next Generation Sequencing (NGS). There was nine genus of bacteria found in high relative abundance, 95.28%, while 4.72% included in Domain of Archaea (genus *Methanosaeta*). Phylum of Firmicutes (genus *Clostridium* has 24.50% relative abundance, *Bacillus* 11.90%, *Lactobacillus* 9.69%); Proteobacteria (genus *Defluviicoccus* 12.10%, *Buchnera* 18.46%, *Rosenbergiella* 2.46%); and Actinobacteria (genus *Nocardioides* 12.21%, and *Streptomyces* 3.96%). Meanwhile, the average plant height of organic rice fields was shorter than conventional rice fields got directly measured coincided with soil sampled. Based on alpha and beta diversity analysis, the highest community diversity and abundance were found in organic rice field soil samples taken at 45 DAP, i.e., at the end of the fertilizer application period. However, in both organic and conventional rice field soils, there was almost no significant difference in the bacterial community, so it impacts that organic and conventional systems do not make a real difference in the total N, P available, CEC, and pH values. It makes a significant difference in organic C and organic matters.

Keywords: conventional farming; metagenomics analysis; microbial community; Next Generation Sequencing; organic farming

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INTRODUCTION

Conventional agricultural systems in the long term implementation harmed the environment due to synthetic chemical fertilizers and pesticides (Issaka *et al*., 2016). Realizing this phenomenon, the change in motion occurs among farmers to return to organic farming. Organic farming is an agricultural management system to improve ecosystem health, such as biodiversity and biological cycles, using non-synthetic materials (Minister of Agriculture RI, 2013). Applying organic fertilizer is the same as invading the microbial community in bulk that can increase soil microbial diversity by providing complex nutrients and organisms. Previous studies suggest that soil microbial abundance and diversity under organic management are higher than conventional practices (Hartmann *et al*., 2015; Xiong *et al*., 2015), where high microbial diversity has an association with high functional diversity in soils, causes several ecological processes that play a role for plants growth (Wagg *et al.*, 2014). The relationship between community structure and soil quality impacts the soil environment, accompanied by increasing microbial biomass and diversity, thus generally considered to indicate good soil quality (Wang *et al.*, 2019).

The metagenomic analysis identifies bacteria in a community through gene and genomic information in samples (Nalbantoglu *et al*., 2014; Forster *et al*., 2019). Genetic material is isolated directly from environmental samples in the metagenomic approach and capable to produce the same result as culture methods, while culture sometimes appears to have been dropped (Hilton *et al*., 2016; Amrane & Lagier, 2018). Hence, the thousands of bacterial species cannot be cultured in the laboratory. The metagenomic approach also provides a fast and cost-effective way to generate genomic images and explore bacterial diversity (Neelakanta & Sultana, 2013; Alves *et*

al., 2018). Next Generation Sequencing (NGS) provides a cheaper and higher throughput alternative to traditional Sanger sequencing. NGS has specific advantages for criteria: read length, accuracy, run time, and throughput.

In this study, metagenomic analysis was performed using the NGS technique to determine the profile of organic and conventional soil microbial community at Gempol Village, Karanganom District, Klaten Regency that had organic farming areas since 2012 and had issued a nationally certified product, Gempol Organic Rice. The organic farming area is still side by side with the conventional rice fields. The diversity and composition of soil microbial communities are closely related to agroecosystem productivity and stability (Francioli *et al*., 2016), so that they can be important information in sustainable agricultural management. This research aimed to compare the profile of microbial community of organic and conventional rice fields in the early (0 DAP), mid (15 DAP), and late (45 DAP) fertilizer application period.

MATERIALS AND METHODS

Soil treatment of the farming. The study site is organic and conventional rice fields in Gempol Village, Karanganom District, Klaten Regency, Central Java. Organic fertilizer is made from a mixture of cow dung, rice bran, dolomite lime, sugar drops, and moretan/MOL (local microorganisms). Plant growth promoting Rhizobacteria (PGPR) is also used in the dosage determined by the farmer group. Then urea, TS, and Phonska fertilizers are used for conventional rice fields. Organic insecticides use bitter, spicy, smelly, and hot plants such as neem leaves, chili, garlic, papaya leaves, soursop leaves, and others, while conventional agriculture uses various chemical insecticides such as Fitera. In organic rice fields, fertilizer application is made at 0 DAP, 7 DAP, 21 DAP, 35 DAP, and 40 DAP. Meanwhile, in the conventional rice field, fertilizer application is 7 DAP, 21 DAP, 30 DAP, and 35 DAP. Likewise, the provision of insecticides in organic rice fields was carried out at the age of 7 DAP, 15 DAP and 21 DAP, while conventional rice field was given insecticide at the age of 7 DAP and 21 DAP. The basis for determining sampling time is adjusted to the sidelines of applying organic and synthetic fertilizer, which is almost the same time.

Soil sampling. Samples were collected approximately 500 g of soil at a 5-10 cm depth, then put into sterile plastic bags and stored at -80°C for molecular work. Soil samples were taken on early 0 DAP, middle 15 DAP and final stages 45 DAP of fertilizer application. Soil sampling was carried out in three replications at three different locations in each type of rice field.

Soil DNA extraction and sequencing. Some of the soil samples were air-dried and crushed, then filtered through a 500-μmesh sieve for DNA extraction. Each soil sample about 250 μg used for DNA extraction. DNA of soil microbiota was extracted using ZymoBIOMICS® DNA Miniprep Kit, according to the instructions from the company (Zymo research, USA). Uniform mechanical lysis of all microbes is achieved by bead beating with the innovative, ultra-high density BashingBeads. DNA sequencing with the Next Generation Sequencing (NGS) technique according to the Illumina Inc. (USA) protocol and data analysis performed by NOVOGENE. The 16S rRNA (16SV3-V4) gene was amplified using a specific primer (16S V4: 515F-806R) 515F `GTGCCAGCMGCCGCGGTAA`; 806R `GGACTACHVGGGTWTCTAAT` (Caporaso *et al*., 2010) as a barcode. The genome library was obtained using the NEBNext ® Ultra DNA Library Pre-Kit for Illumina, and an index code was added. The quality of the genome library was assessed using the Qubit@ 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system, then sequenced on the Illumina platform to obtain a paired-end reading of 250 bp.

Chemical properties and plant height analysis. Meanwhile, some of the soil samples were air-dried at room temperature for one week, then filtered through a 100-μmesh sifter then stored at 4°C for chemical properties analysis (Feng *et al*., 2018). Determination of total N using the Kjeldahl method, available P

using the Bray method, cation exchange capacity (CEC) using the distillation method, ammonium acetate at pH 7, organic C, organic matter, and pH. Measurement of each parameter was carried out in triplicate of each soil sample. Soil chemical characterization was utilized at the Laboratory of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret. The data of soil chemical properties were analyzed using SPSS software analysis of variance (ANOVA) to determine the effect of treatment on the observed variables.

Plant height analysis. The rice plant character observed was plant height. The plant height was observed directly by using measuring tape at 0.15 and 45 DAP. Plant samples were taken from three locations of each organic and conventional rice field. From each location, three plants were measured.

Data analysis. The first step to data analysis is the preparation of paired-end readings and quality control. First, the paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Next, FLASH software applied for paired-end reads merging (Magoč & Salzberg, 2011), then the raw tags filtered according to the QIIME quality-controlled process (Caporaso *et al*., 2010) to obtain the high quality clean tags (Bokulich *et al*., 2013). In the second step, the tags were compared with database reference using the UCHIME algorithm (Edgar *et al.*, 2011) to detect chimera sequences. Then the chimera sequences were removed to obtain effective tags (Haas *et al*., 2011). The third step is OTUs clustering and annotated species using UPARSE software (Edgar, 2013), which assigns sequences with a similarity $\geq 97\%$ to the same OTUs. Representative sequence for each OTU was chosen for annotation of taxonomic information using GreenGene Database (DeSantis *et al*., 2006) based on the RDP classifier algorithm (Wang *et al*., 2007). The fourth step, the multiple sequence alignment, was conducted using MUSCLE software (Edgar, 2004) to study the phylogenetic relationship of different OTUs and the difference of dominant species in different samples. Finally, OTUs abundance was normalized using a sequence number standard corresponding to the samples with the least sequences. The fifth step is output normalized data were used to analysis of alpha diversity and beta diversity. Alpha diversity was applied on analyzing the complexity of species diversity for samples through six indices, including observed species, Chao1, Shannon, Simpson, ACE, Good-coverage. The indices were calculated by QIIME version 1.7.0 and displayed using R software Version 2.15.3. Beta diversity analysis on weighted and unweighted unifrac was calculated using QIIME (Caporaso *et al.*, 2010).

RESULTS AND DISCUSSION

Chemical Properties of Soil and Plant Height. Soil chemical properties such as total N, P available, CEC, pH, organic C, and organic matters are shown in Table 1.

Table 1. Chemical soil properties organic and conventional rice field soil.

Sample Name	N total (%)	P avail. (ppm)	CEC (me %)	Organic C (%)	Organic matters (%)	pH
Organic 1	0.32	14.56	0.41	2.15	3.72	6.35
Organic 2	0.38	20.05	0.34	2.38	4.11	6.57
Organic 3	0.30	11.54	0.22	1.45	2.50	6.20
Conventional 1	0.30	15.97	0.20	2.67	4.61	6.26
Conventional 2	0.35	14.03	0.24	3.16	5.45	6.63
Conventional 3	0.31	16.84	0.50	3.00	5.18	6.44

Notes: Organic 1= (DAP 0); Organic 2 = (DAP 15); Organic 3 = (DAP 45) at organic rice field; Conventional 1= (DAP 0); Conventional 2 = (DAP 15); Conventional 3 = (DAP 45) at conventional rice field.

The planting treatment with organic and conventional systems does not significantly differ in the total N, P available, CEC, and pH values. However, it makes a significant difference in organic C and organic matters indicated by ANOVA significant value below 0.05 that is 0.039 and 0.40 (Table 2). Planting organic and conventional systems indicates a

difference in organic C and organic matters due to organic fertilizers in organic rice fields, which contain organic matters more than the chemical fertilizer. Organic matters are the C organic storage (Rusdiana & Lubis, 2012), these two variables are interconnected.

Table 2. Standar deviation and ANOVA anlysis chemical soil properties organic and conventional rice field soil.

Chemical soil properties	Organic SD value	SD min	SD max	Conventional SD value	SD min	SD max	ANOVA analysis (∑)
N total	0.04163	0.30	0.38	0.02646	0.30	0.35	0.664
P available	4.31433	11.54	20.05	1.11330	14.63	16.84	0.875
CEC	0.09609	0.22	0.41	0.16289	0.20	0.50	0.931
Organic C	0.48439	1.45	2.38	0.24987	2.67	3.16	0.039
Organic matters	0.83990	2.50	4.11	0.4288	4.11	5.45	0.040
pH	0.18610	6.20	6.57	0.17034	6.26	6.63	0.668

Plant height that measured shows soil quality performance obtained are shown in Table 3. Observations of soil chemistry and plant height are needed as supporting data from this study, which shows the effect of soil bacterial communities on soil fertility through soil chemical parameters such as pH value, total N, available P, CEC, organic C and organic matter and also the effect on plant growth with plant height parameters. The higher the diversity and abundance of bacteria, the higher the functional diversity and interactions, positively correlated with critical ecological processes for plants and soil structure (Wagg *et al*., 2014).

Table 3. Plant height organic and conventional rice field soil.

	Organic rice field soil (DAP)			Conventional rice field soil (DAP)		
	0	15	45	0	15	45
Average plant height (cm)	15	22.6	52	15	31	60.3

Table 3 displaying the height of rice planted organically is a little shorter when compared to conventional rice produced because the pH, P available, CEC, and N total, which affect the quality of rice growth, does not show a significant difference. It is also caused by synthetic chemical fertilizers, which emphasize high productivity in the conventional rice field or perhaps due to rice fields still sided by the side.

The diversity and abundance of organic and conventional rice field soil bacteria. Sequences with similarities ≥ 97% are categorized in the same OTUs, then representative sequences in each OTUs are used for taxonomic marking. For example, in Fig. 1, the highest number of OTUs owned by organic3 is rice field soil at the end of the fertilizer application period (45 DAP), which is 3558. From the number of OTUs obtained, ten microbial genera were taken with the most found. Then the taxonomy was constructed as in Fig. 2.

A total of 9 genera were found in both organic and conventional rice fields, with a relative abundance of 95.28% included in the kingdom of bacteria (Fig. 2). In contrast, the rest are included in the domain Archaea, namely the genus *Methanosaeta*. Kingdom bacteria consists of three phyla, namely Firmicutes (genus *Clostridium* which has 24.50% relative abundance, genus *Bacillus* 11.90%, and genus *Lactobacillus* 9.69%); Proteobacteria (genus *Defluviicoccus* 12.10%, genus *Buchnera* 18.46%, and genus *Rosenbergiella* 2.46%; and phylum Actinobacteria (genus *Nocardioides* 12.21%, and *Streptomyces* 3.96%).

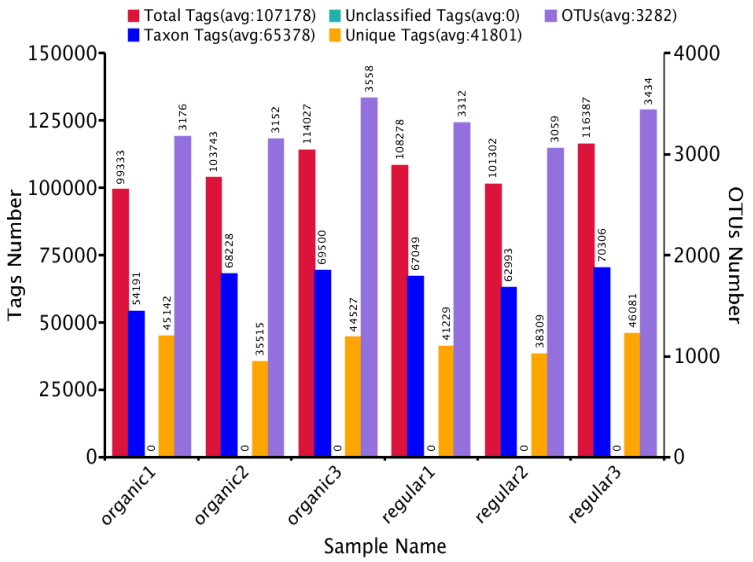

Fig. 1. Summarization of annotation. Organic1= organic soil (0 DAP); organic2= organic soil (15 DAP); organic3= organic soil (45 DAP); regular1= conventional soil (0 DAP); regular2= conventional soil (15 DAP); regular3= conventional (45 DAP).

Phylum microbial found in this study with the highest relative abundance are Proteobacteria, Actinobacteria, Firmicutes. On the other hand, relative abundances are less, i.e., Acidobateria, Euryachaeoa including *Methanosaeta*, *Verrucomicrobia*, *Cyanobacteria*, and *Nitrospirae*.

The most Proteobacteria found in this study consisted of several subphyla, including Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Alphaproteobacteria found in this study include the order Rhizobiales, and Rhodospirillales include *Defluviicoccus*. Gammaproteobacteria are included in Enterobacteriaceae, among others are *Buchnera*. Firmicutes members have *Clostridium* and *Bacillus*. Actinobacteria are seen, such as Nocardiaceae and also *Streptomyces*. This research also found Anaerolinaceae that include Chloroflexi phylum.

Alpha diversity analysis. The following analysis is alpha diversity and beta diversity which is analyzed based on normalized data output. Alpha diversity refers to the diversity within a particular area or ecosystem and is usually expressed by the number of species (i.e., species richness) in that ecosystem. If we examine the change in species diversity between these ecosystems, then we measure the beta diversity. Alpha diversity indices consist of six types, namely observed species; Chao1 and ACE, which show community abundance; Shannon and Simpson that show community diversity and Good's coverage that characterize the depth of sequencing shown in Table 4.

A diversity index is the measure of species diversity in a given community. Shannon's index accounts for both the abundance and evenness of the species present. Simpson's Diversity Index is a measure of diversity that considers the number of species present and the relative abundance of each species. As species richness and evenness increase, thus diversity increases.

Organic3 shows the most outstanding value in Shannon and Simpson parameters, among other fields. Therefore the bacterial community is the most diverse, while organic1 offers the greatest abundance of bacteria based on the value of Chao1 and ACE. Meanwhile, for each other type of rice field, it can be seen in each parameter as shown in table 4. The average value of the diversity and abundance parameter in organic rice field soil was higher than conventional rice field soil. Therefore, the Shannon and Simpson indices show the highest diversity of the community. Likewise, the Chao1 and ACE indexes show the highest community abundance.

This microbial diversity and abundance can happen because fertilizer makes microbial

metabolism faster (Ramirez *et al*., 2012; Leff *et al*., 2015), although it depends on the quality of organic matter constituent (Liu *et al*., 2014). Synthetic chemical fertilizers from conventional rice field systems can reduce total microbial biomass in the agricultural system (Lu *et al*., 2011).

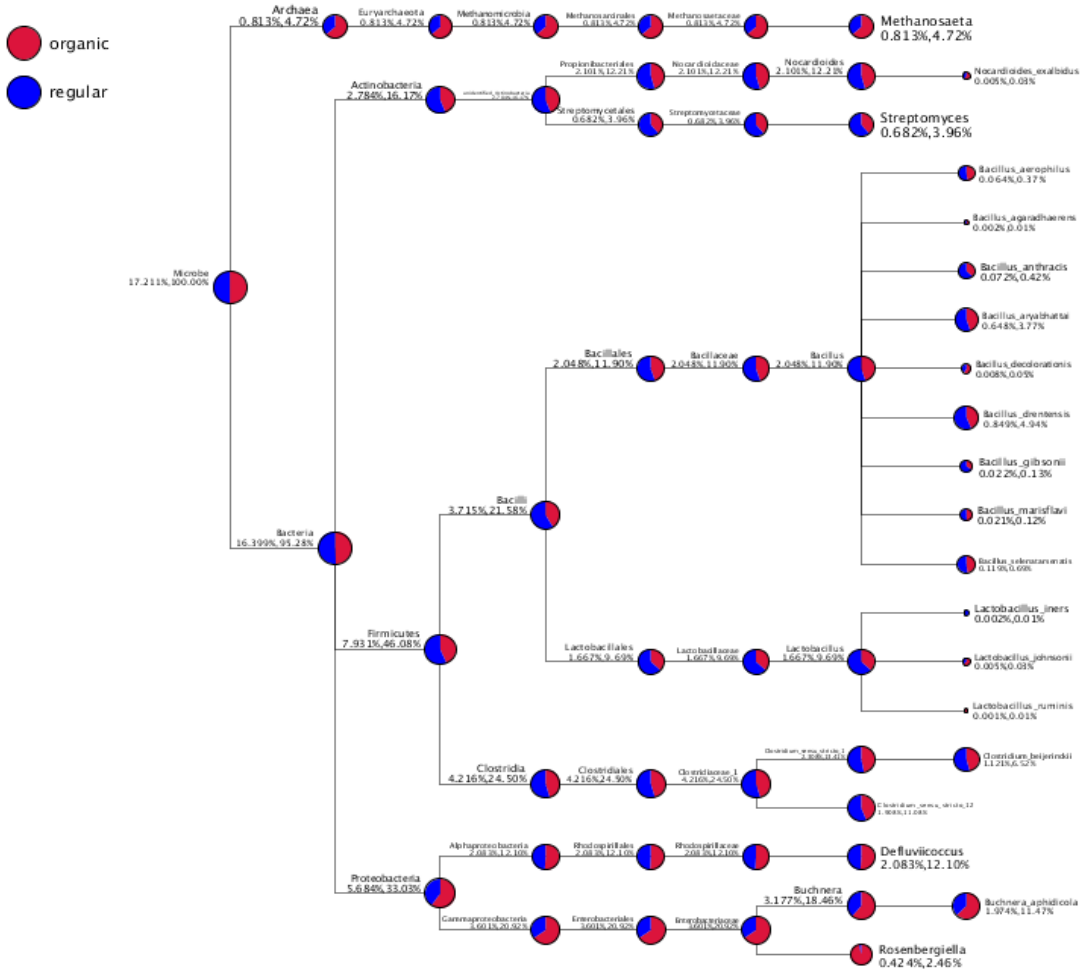

Fig. 2. Taxonomy tree microbe community in organic and conventional rice field. Organic1= organic soil (0 DAP); organic2= organic soil (15 DAP); organic3= organic soil (45 DAP); regular1= conventional soil (0 DAP); regular2= conventional soil (15 DAP); regular3= conventional (45 DAP).

Beta diversity analysis. Beta diversity analysis was used to evaluate the extent of diversity differences between samples analyzed. Weighted is a calculation that considers the abundance of taxa, while unweighted only assumes the presence or absence of taxa without considering its abundance. Furthermore, the data on the distance matrix is visualized by the UPGMA shown in Fig. 3 and 4.

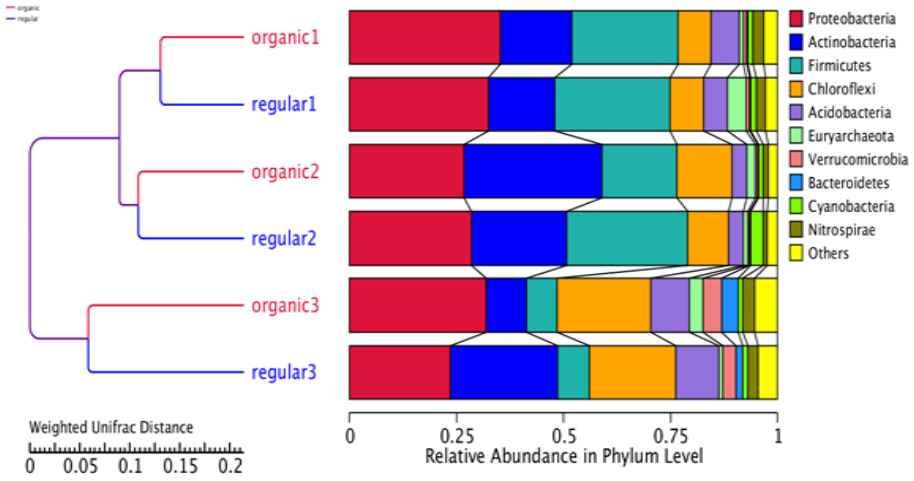

Fig. 3. UPGMA cluster tree based on Weighted Unifrac distance. On the left side of the diagram is the structure of the clustering tree. The right side is the relative abundance of the different phylum.

Fig. 3 and 4 show that the diversity of soil in the organic and conventional rice fields is almost the same, but there are abundant differences in each taxon. The profile of soil microbial community are not grouped based on differences in organic and conventional agricultural processing systems but are grouped according to the fertilizer application period with almost the same bacterial community composition. Based on the weighted and unweighted unifrac distance, the microbial community clustering of rice fields was separated based on the time of fertilizer application, not the type of rice field organic nor conventional. The clustering of the 0 DAP (organic1; conventional1) and 15 DAP (organic2; conventional2) were relatively close; while the 45 DAP (organic3; conventional3) were far apart.

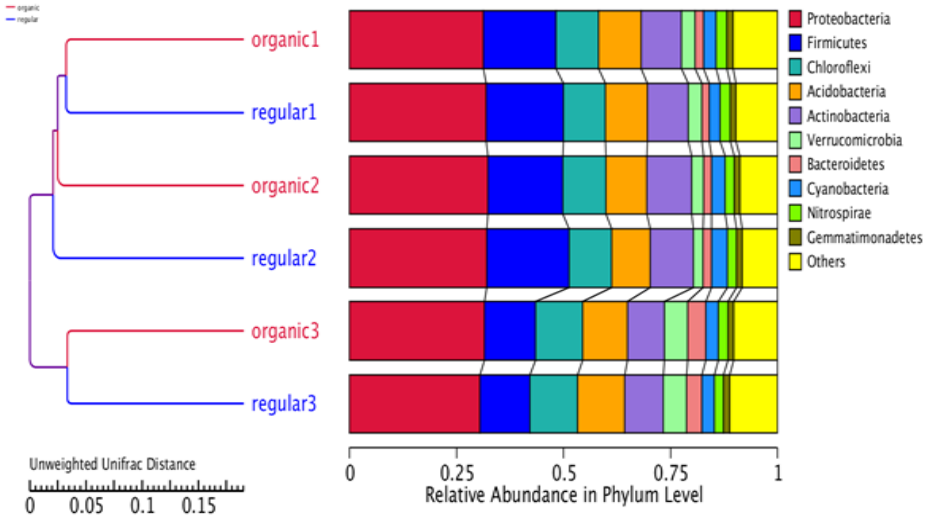

Fig. 4. UPGMA cluster tree based on Unweighted Unifrac distance. On the left side of the diagram is the structure of the clustering tree. The right side is the relative abundance of the different phylum.

Microbial found in this study have a beneficial role in soil fertility, such as Rhizobiales that play a role in fixing nitrogen (Shu *et al*., 2012). *Bacillus* also degrades many different carbon sources (Goldfarb *et al*., 2011). Actinobacteria could play a role in the C, N, and P (Hill *et al*., 2011; Zhang *et al*., 2019). Streptomyces cause soil texture strength

(Vetsigian *et al*., 2011; Maleki *et al.*, 2013). Acidobacteria plays a role in soil C cycling (Diamond *et al.*, 2019). Cyanobacteria contribute to N2 supply and produce IAA phytohormone through rhizosphere symbiotic association (Manjunath *et al*., 2013), fixes C in soil, and synthesize exopolysaccharides. It can increase soil fertility and water retention also improve soil structure and stability (Chamizo *et al*., 2018); also Nitrospirae that play a role in the nitrite-oxidizing process (Daims & Wagner, 2018).

Overall, this study shows the differences between organic systems and conventional rice fields which are influenced by organic C and organic matter, thus can be used as sustainable agricultural systems that are oriented towards productivity, low production costs, and safety for the community.

CONCLUSION

In summary, there was almost no significant difference in the microbial community in both organic and conventional rice field soils. It impacts that organic and conventional systems do not significantly differ in the total N, P available, CEC, and pH values. Still, it makes a real difference in organic C and organic matters. However, the implementation of organic farming in Gempol, whose performance is still sided by the side and less than ten years, can still positively signal a sustainable agriculture program. Alpha and beta diversity indices the highest community diversity and abundance found in organic rice field soil samples at 45 DAP in the last fertilizer application.

REFERENCES

Alves LDF, Westmann CA, Lovate GL, de Siqueira GM, Borelli TC, Guazzaroni ME. 2018. Metagenomic approaches for understanding new concepts in microbial science. *International Journal of Genomics.* vol 2018: 1–16. doi: https://doi.org/10.1155/2018/2312987.

Amrane S, Lagier JC. 2018. Metagenomic and clinical microbiology. *Human Microbiome Journal*. vol 9: 1–6. doi: https://doi.org/10.1016/j.humic.2018.06.001.

Bokulich, NA, Subramanian S, Faith JJ, Grevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. 2013. Quality filtering vastly improves diversity estimates from illumina amplicon sequencing. *Nature Methods*. vol 10 (1): 57–59. doi: https://doi.org/10.1038/nmeth.2276.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *In Nature Methods*. vol 7 (5): 335–336. doi: https://doi.org/10.1038/nmeth.f.303.

Chamizo S, Mugnai G, Rossi F, Certini G, De Philippis R. 2018. Cyanobacteria inoculation improves soil stability and fertility on different textured soils: Gaining insights for applicability in soil restoration. *Frontiers in Environmental Science*. vol 6: 1–14. doi: https://doi.org/10.3389/fenvs.2018.00049.

Daims H, Wagner M. 2018. Nitrospira. *Trends in Microbiology*. vol 26(5): 462–463. doi: https://doi.org/10.1016/j.tim.2018.02.001

DeSantis TZ, Hugenholtz, P, Larsen N, Rojas, M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology.* vol 72(7): 5069–5072. doi: https://doi.org/10.1128/AEM.03006-05.

Diamond S, Andeer PF, Li Z, Crits-Christoph A, Burstein D, Anantharaman K, Lane KR, Thomas BC, Pan C, Northen TR, Banfield JF. 2019. Mediterranean grassland soil C–N compound turnover is dependent on rainfall and depth, and is mediated by genomically divergent microorganisms. *Nature Microbiology*. vol 4(8): 1356–1367. doi: https://doi.org/10.1038/s41564-019-0449-y.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high acuracy and high throughput. *Nucleic Acid Research*. vol 32 (5): 1792–1797. doi: https://doi.org/10.1093/nar/gkh340.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. vol 27(16): 2194–2200. doi: https://doi.org/10.1093/bioinformatics/btr381.

Edgar RC. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*. vol 10 (10): 996–998. doi: https://doi.org/10.1038/nmeth.2604.

Feng G, Xie T, Wang X, Bai J, Tang L, Zhao H, Wei W, Wang M, Zhao Y. 2018. Metagenomic analysis of microbial community and function involved in cd-contaminated soil. *BMC Microbiology*. vol 18(1): 1–13. doi: https://doi.org/10.1186/s12866-018-1152-5.

Forster SC, Kumar N, Anonye BO, Almeida A, Viciani E, Stares MD, Dunn M, Mkandawire TT, Zhu A, Shao Y, Pike LJ, Louie T, Brown HP, Mitchell AL, Neville BA, Finn RD, Lawley, T. D. 2019. A human

gut bacterial genome and culture collection for improved metagenomic analyses. *Nature Biotechnology*. vol 37(2): 186–192. doi: https://doi.org/10.1038/s41587-018-0009-7.

Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T. 2016. Mineral vs. organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Frontiers in Microbiology*. vol 7: 1–16. doi: https://doi.org/10.3389/fmicb.2016.01446.

Goldfarb KC, Karaoz U, Hanson CA, Santee CA, Bradford MA, Treseder KK, Wallenstein MD, Brodie EL. 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Frontiers in Microbiology*. vol 2: 1–10. doi: https://doi.org/10.3389/fmicb.2011.00094.

Hartmann M, Frey B, Mayer J, Mäder P, Widmer F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME Journal*. vol 9(5): 1177–1194. doi: https://doi.org/10.1038/ismej.2014.210.

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Petrosino JF, Knight R, Birren BW. 2011. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Research*. vol 21(3): 494–504. doi: https://doi.org/10.1101/gr.112730.110.

Hill P, Krištůfek V, Dijkhuizen L, Boddy C, Kroetsch D, van Elsas JD. 2011. Land Use Intensity Controls Actinobacterial Community Structure. *Microbial Ecology*. vol 61(2): 286–302. doi: https://doi.org/10.1007/s00248-010-9752-0.

Hilton SK, Castro-Nallar E, Pérez-Losada M, Toma I, McCaffrey TA, Hoffman EP, Siegel MO, Simon GL, Johnson WE, Crandall KA. 2016. Metataxonomic and metagenomic approaches vs. culture-based techniques for clinical pathology. *Frontiers in Microbiology*. vol 7: 1–12. doi: https://dx.doi.org/10.3389%2Ffmicb.2016.00484.

Issaka YB, Antwi M, Tawia G. 2016. A comparative analysis of productivity among organic and non-organic farms in the West Mamprusi District of Ghana. *Agriculture*. vol 6(2): 1–10. doi: https://doi.org/10.3390/agriculture6020013.

Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America*. vol 112(35): 10967–10972. doi: https://doi.org/10.1073/pnas.1508382112.

Liu D, An SS, Cheng Y, Keiblinger K, Huang YM. 2014. Variability in soil microbial biomass and diversity among different aggregate-size fractions of different land use types. *Soil Science*. vol 179(5): 242–249. doi: https://doi.org/10.1097/SS.0000000000000064.

Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen J, Yang X, Li B. 2011. Responses of ecosystem nitrogen cycle to nitrogen addition: A meta-analysis. *New Phytologist*. vol 189(4): 1040–1050. doi: https://doi.org/10.1111/j.1469-8137.2010.03563.x.

Magoč T, Salzberg SL. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. vol 27(21): 2957–2963. doi: https://doi.org/10.1093/bioinformatics/btr507.

Maleki H, Dehnad A, Hanifian S, Khani S. 2013. Isolation and molecular identification of *Streptomyces* spp. with antibacterial activity from Northwest of Iran. *BioImpacts*. vol 3(3): 129–134. doi: https://doi.org/10.5681/bi.2013.01

Manjunath M, Taylor P, Prasanna R, Sharma P. 2013. Archives of agronomy and soil science developing PGPR consortia using novel genera Providencia and Alcaligenes along with Cyanobacteria for wheat. *Archives of Agronomy and Soil Science*. vol 57(8): 873–887. doi: https://doi.org/10.1080/03650340.2010.499902.

Minister of Agriculture RI. 2013. Regulation of Agricultural Ministry Number 64/Permentan/OT.140/5/2013 concerning organic agriculture systems. Jakarta: Minister of Agriculture Republic Indonesia. https://www.pertanian.go.id.

Nalbantoglu U, Cakar A, Dogan H, Abaci N, Ustek D, Sayood K, Can H. 2014. Metagenomic analysis of the microbial community in kefir grains. *Food Microbiology*. vol 41: 42–51. doi: https://doi.org/10.1016/j.fm.2014.01.014.

Neelakanta G, Sultana H. 2013. The use of metagenomic approaches to analyze changes in microbial communities. *Microbiology Insights*. vol 6: 37–48. doi: https://dx.doi.org/10.4137%2FMBI.S10819.

Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*. vol 18(6): 1918–1927. doi: https://doi.org/10.1111/j.1365-2486.2012.02639.x.

Rusdiana O, Lubis RS. 2012. Pendugaan korelasi antara karakteristik tanah terhadap cadangan karbon (carbon stock) pada hutan sekunder. *Jurnal Silvikultur Tropika*. vol 3 (1): 14–21.

Shu W, Pablo GP, Jun Y, Danfeng H. 2012. Abundance and diversity of nitrogen-fixing bacteria in rhizosphere and bulk paddy soil under different duration of organic management. *World Journal of Microbiology and Biotechnology*. vol 28(2): 493–503. doi: https://doi.org/10.1007/s11274-011-0840-1.

Vetsigian K, Jajoo R, Kishony R. 2011. Structure and evolution of streptomyces interaction networks in soil and in silico. *PLoS Biology*. vol 9(10): 1–12. doi: https://doi.org/10.1371/journal.pbio.1001184.

Wagg C, Bender SF, Widmer F, van Der Heijden MGA. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of Americ*a. vol 111(14): 5266–5270. doi: https://doi.org/10.1073/pnas.1320054111.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*. vol 73(16): 5261–5267. doi: https://doi.org/10.1128/AEM.00062-07.

Wang Z, Liu Y, Zhao L, Zhang W, Liu L. 2019. Change of soil microbial community under long-term fertilization in a reclaimed sandy agricultural ecosystem. *PeerJ*. vol 7: 1–21. doi: https://doi.org/10.7717/peerj.6497.

Xiong W, Li Z, Liu H, Xue C, Zhang R, Wu H, Li R, Shen Q. 2015. The effect of long-term continuous cropping of black pepper on soil bacterial communities as determined by 454 pyrosequencing. *PLoS ONE*. vol 10(8): 1–13. doi: https://doi.org/10.1371/journal.pone.0136946.

Zhang B, Wu X, Tai X, Sun L, Wu M, Zhang W, Chen X, Zhang G, Chen T, Liu G, Dyson P. 2019. Variation in Actinobacterial community composition and potential function in different soil ecosystems belonging to the Arid Heihe River Basin of Northwest China. *Frontiers in Microbiology*. vol 10: 1–11. doi: https://doi.org/10.3389/fmicb.2019.02209.