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Different microbial communities in paddy soils under organic and nonorganic farming

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Abstract

Organic agriculture is a farming method that provides healthy food and is friendly to the environment, and it is developing rapidly worldwide. This study compared microbial communities in organic farming (Or) paddy fields to those in nonorganic farming (Nr) paddy fields based on 16S rDNA sequencing and analysis. The predominant microorganisms in both soils were *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, and *Nitrospirota*. The alpha diversity of the paddy soil microbial communities was not different between the nonorganic and organic farming systems. The beta diversity of nonmetric multidimensional scaling (NMDS) revealed that the two groups were significantly separated. Distance-based redundancy analysis (db-RDA) suggested that soil pH and electrical conductivity (EC) had a positive relationship with the microbes in organic paddy soils. There were 23 amplicon sequence variants (ASVs) that showed differential abundance. Among them, g_B1-7BS (*Proteobacteria*), s_*Sulfuricaulis limicola* (*Proteobacteria*), g_GAL15 (p_GAL15), c_*Thermodesulfovibrionia* (*Nitrospirota*), two of f_*Anaerolineaceae* (*Chloroflexi*), and two of g_S085 (*Chloroflexi*) showed that they were more abundant in organic soils, whereas g_11-24 (*Acidobacteriota*), g__Subgroup_7 (*Acidobacteriota*), and g_*Bacillus* (*Firmicutes*) showed differential abundance in nonorganic paddy soils. Functional prediction of microbial communities in paddy soils showed that functions related to carbohydrate metabolism could be the major metabolic activities. Our work indicates that organic farming differs from nonorganic farming in terms of microbial composition in paddy soils and provides specific microbes that might be helpful for understanding soil fertility.

Keywords Organic agriculture · 16S rDNA · Rice field · Microbial community

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Introduction

At present, there are an increasing number of trends in the analysis of soil microbiota by overall genomics or overall transcriptomics, such as the application of soil microbiota in Arctic peat [1], grassland soil microbiota [2], dry soil microbiota in Antarctica, and changes in water and organic matter microbiota [3]. The study of the bacterial flora of wetland sedimentary soil shows that *Proteobacteria* is the main bacterial phylum, and the next most prominent phyla are *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia*. In *Proteobacteria*, *Gamma-*, *Delta-*, and *Betaproteobacteria* are the representative classes [4]. Comparing different soil management practices, such as forest and agricultural soil, the results show that the bacteria in forest soil are more abundant than those in agricultural soil [5]. It is possible that land preparation, fertilization, and spraying

of pesticides in agricultural management may cause the bacterial phase of the soil to tend to be monotonous.

The addition of organic fertilizers (alfalfa straw, glucose, compost manure, and wood biochar) helps to promote the growth of *Acidobacteria* and *Gemmatimonadetes* bacteria, whereas synthetic fertilizers may promote an increase in *Actinobacteria* and *Proteobacteria* bacteria in rocket (*Eruca sativa*) cultivation [6]. In paddy soil, the application of inorganic fertilizers increased the abundance of bacteria *Bacteroidetes* and *Acidobacteria*, but the application of organic fertilizers (composted pig manure and rice straw) increased the abundance of eutrophic bacteria (such as *Proteobacteria*) [7]. Moreover, studies on the long-term effects of organic and inorganic fertilizers on the functioning of soil microbial communities have shown that the application of nitrogen fertilizer alone reduces microbial diversity, while farmyard manure application alone or in combination with chemical fertilizers improves soil fertility [8]. However, farmyard manure application might not be the major cause, but balanced fertilizer containing nitrogen, phosphorus, and potassium would result in the highest microbial diversity in paddy soil [9]. Indeed, paddy soil fertility is affected by microbial communities, and high-yield soil microbes are likely more active in modulating soil fertility for rice production [10]. The positive correlation of specific soil bacteria with grape-growing regions in the United States shows that the identification of specific microbes is helpful for understanding viticulture and flavor [11]. Overall, it is not yet clear the microbial communities between organic and nonorganic farming that certain bacteria could be applied as indicators to distinguish organic paddy soil.

In this study, we collected soils from organic and nonorganic farming of paddy fields in Changhua County, Taiwan. Soil microbial DNA was prepared, and 16S rDNA was sequenced, followed by microbe comparison and biodiversity analysis. This study aims to use next-generation sequencing to comprehensively compare microbial communities between organic and nonorganic paddy fields and further apply bioinformatics tools for functional analysis.

Material and methods

Soil sample collection

The soils used in this study were collected from six paddy fields with organic farming (Or1-5, Or6-8, Or9-11, and Or12-14) and nonorganic farming (Nr1-5, and Nr6-8) in Changhua County, Taiwan (Fig. 1). The soil collection periods for Or1-5 and Nr1-5 samples were during Oct. 2018, and for Or1-5, Or6-8, Or9-11, and Nr6-8 samples were during Oct. 2019. Five or three soil samples collected from each paddy field were applied as the replicates. The soil was
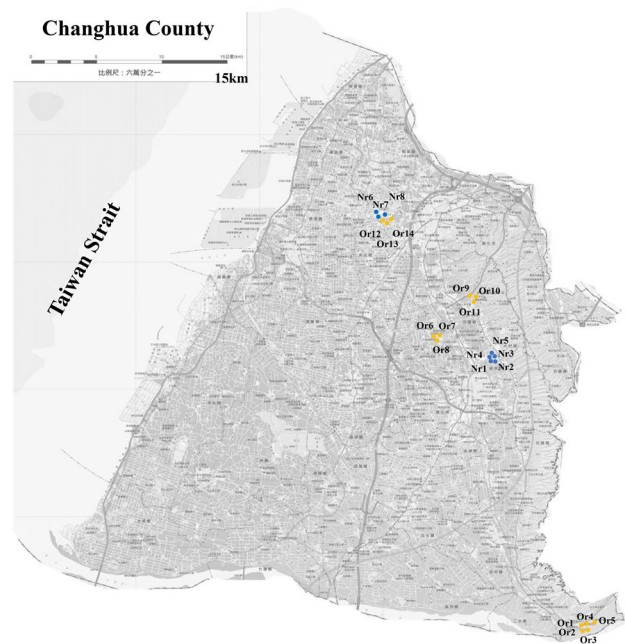
Fig. 1 Location and overview map of the researched area. Nr indicates nonorganic farming. Or indicates organic farming

collected with a soil sampler at a depth of 10–20 cm. After collection, samples were placed in labeled sterile polyethylene centrifuge tubes, brought to the laboratory, and stored at −20 °C. The study information includes samples, farming types, collection date, collection location, soil pH, and electrical conductivity (EC), which are listed in Table 1.

Soil pH and EC analysis

For soil pH analysis, 5 g of air-dried soil was put into a 15 mL sterile polyethylene centrifuge tube, followed by the addition of 5 mL deionized water (sample: water = 1:1), and placed on a reciprocal shaker for 30 min at 140 rpm [12]. For soil EC analysis, 5 g of air-dried soil was put into a 50 mL sterile polyethylene centrifuge tube, mixed with 25 mL of deionized water (sample: water = 1:5), and placed on a reciprocal shaker for 60 min at 140 rpm, followed by standing for 30 min [13]. The solution or supernatant was recorded for the soil pH or EC, respectively, on a multifunctional water detector meter, WA-2017SD (LUTRON ELECTRONIC ENTERPRISE CO., LTD., Taipei, Taiwan).

DNA extraction, polymerase chain reaction (PCR), and sequencing

Bacterial genomic DNA (gDNA) was extracted from 0.5 g of soil by using a NucleoSpin Soil DNA isolation kit (MACHEREY–NAGEL, Dueren Germany) according to the manufacturer's instructions, and DNA sequencing was performed

Table 1 Metadata. Study information includes samples, farming types, collecting date, collecting location, soil pH, and EC

Sample	Farming	Date	Location	pH	EC (mS/m)
Nr1	Nonorganic	Oct. 2018	23°58′47.8″N, 120°35′09.0″E	6.11	134.3
Nr2	Nonorganic	Oct. 2018	23°58′48.0″N, 120°35′09.7″E	6.39	81.1
Nr3	Nonorganic	Oct. 2018	23°58′48.5″N, 120°35′09.7″E	6.56	86.1
Nr4	Nonorganic	Oct. 2018	23°58′48.4″N, 120°35′08.9″E	6.48	98.7
Nr5	Nonorganic	Oct. 2018	23°58′49.3″N, 120°35′09.8″E	5.81	324
Or1	Organic	Oct. 2018	23°47′52.8″N, 120°39′12.2″E	7.11	135.4
Or2	Organic	Oct. 2018	23°47′51.8″N, 120°39′12.6″E	6.88	234
Or3	Organic	Oct. 2018	23°47′52.1″N, 120°39′14.3″E	6.6	173.1
Or4	Organic	Oct. 2018	23°47′52.8″N, 120°39′14.3″E	6.6	173.1
Or5	Organic	Oct. 2018	23°47′53.7″N, 120°39′16.6″E	6.6	173.1
Nr6	Nonorganic	Oct. 2019	24°05′00.1644″N, 120°30′05.9076″E	6.5	124.3
Nr7	Nonorganic	Oct. 2019	24°05′00.1644″N, 120°30′05.9076″E	6.52	116.5
Nr8	Nonorganic	Oct. 2019	24°05′00.1644″N, 120°30′05.9076″E	6.51	112.7
Or6	Organic	Oct. 2019	23°59′52.3068″N, 120°32′28.914″E	6.88	194.2
Or7	Organic	Oct. 2019	23°59′52.3068″N, 120°32′28.914″E	7.07	170
Or8	Organic	Oct. 2019	23°59′52.3068″N, 120°32′28.914″E	7.14	186
Or9	Organic	Oct. 2019	24°01′38.352″N, 120°34′12.4356″E	6.42	275
Or10	Organic	Oct. 2019	24°01′38.352″N, 120°34′12.4356″E	6.6	149
Or11	Organic	Oct. 2019	24°01′38.352″N, 120°34′12.4356″E	6.33	163.2
Or12	Organic	Oct. 2019	24°04′57.756″N, 120°30′9.23″E	6	247
Or13	Organic	Oct. 2019	24°04′57.756″N, 120°30′9.23″E	6.49	197.7
Or14	Organic	Oct. 2019	24°04′57.756″N, 120°30′9.23″E	6.52	184

at BIOTOOLS Co., Ltd. (New Taipei, Taiwan). For 16S rRNA gene sequencing, the V3-V4 region was amplified by a specific primer set (319F: 5′ CCTACGGGNGGCWGCAG 3′, 806R: 5′ GACTACHVGGGTATCTAATCC 3′—according to the 16S Metagenomic Sequencing Library preparation procedure (Illumina, San Diego, CA, USA)). PCR and sequencing were performed as described [14]. Nucleotide sequences determined in the study have been deposited into the NCBI Sequence Read Archive database, and the BioProject accession number is PRJNA997222.

Processing and analysis of sequence data

The demultiplexed 16S rDNA sequences were primers removed and denoised with DADA2 [15] to obtain the amplicon sequence variants (ASVs), and the derived table indicated the number of times each ASV was observed in each sample by QIIME 2 [16]. For DADA2 denoising steps, the qiime dada2 denoise-paired command was applied, and the parameters were –p-trim-left-f 10, –p-trim-left-r 10, –p-trunc-len-f 240, and –p-trunc-len-r 200 based on the quality profile and amplicon length. The quick phylogeny with fast tree, rarefaction curve generation, and taxonomy assignment were performed with QIIME 2 [16]. The Silva 138 ribosomal RNA database was assigned for taxonomy [17]. The phyloseq project was built for microbial community analysis [18] as described [19] in R version 4.3.0 [20].

Hierarchical clustering was calculated using the MicrobiotaProcess package [21] with the get_clust function in methods, Jaccard (distance method for dissimilarity), Hellinger (distance method with standardization) and average (average distance between two clusters), and plotted with the ggclust function. Alpha diversity was generated with the plot_richness function, and beta diversity of nonmetric multidimensional scaling (NMDS) was calculated with Jaccard distance, followed by the analysis of similarities (ANOSIM) test, and generated with the plot_ordination function [19]. Distance-based redundancy analysis (db-RDA) was performed using the Microeco package [22] in the Jaccard method with cal_ordination, trans_ordination, and plot_ordination functions.

Functional prediction of microbial communities was performed with the Functional Annotation of Prokaryotic Taxa (FAPROTAX) [23], Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) [24], and Tax4Fun2 [25] as described previously [26].

Statistical analysis

The statistical analysis of metagenomic profiles (STAMP) was applied to compare the nonorganic and organic framing groups (Table 1) using White's nonparametric t test ($p < 0.05$) with difference between proportions parameter (0.1) for FAPROTAX and Tax4Fun2 analyses and difference between proportion (0.05) for PICRUSt [27]. For ASV

analysis, Storey false discovery rate (FDR) correction was applied with a q-value < 0.05, implying that the false-positive rate was 5% and that the difference between proportions was 0.2%. The White's nonparametric t test is a permutation method for non-normal distributions. For features with statistical significance, we further applied the difference between proportions filter to present widely differing features for comparison. The selection of parameters will vary according to different methods and is subjective.

Results

Phylogenetic and diversity analysis

All organic rice fields were certified by the agency, and mixed organic fertilizer containing peanut meal, rapeseed meal, castor meal, sesame meal, corn meal, red bran, palm ash, soybean meal, rice bran, fish meal, bone powder, plant ash, and weathered lignite was applied (personal communication). We collected soils from paddy fields of organic (Or) and nonorganic (Nr) farming, and the microbial communities were examined. Figure 2 compares two different farms based on the percent composition of different bacterial varieties. Both Or and Nr soils showed the same top five dominant microorganisms, but the order of relative abundance was different, which were *Proteobacteria* (22.5%), *Chloroflexi* (14.8%), *Acidobacteria* (12.8%), *Nitrospirota* (4.7%), and *Actinobacteria* (4.5%) in Or soils and *Proteobacteria* (19.8%), *Acidobacteria* (17.6%), *Chloroflexi* (15.2%), *Actinobacteria* (5.6%), and *Nitrospirota* (4.6%) in Nr soils. After comparing the community richness (Chao1 and Observed_features) and diversity (Shannon and Simpson), we found that there was no significant difference between Or and Nr soils (Fig. 3).

Microbial community analysis

NMDS, a nonparametric ordination analysis based on Jaccard distance, revealed two separated groups of microbial communities from Or and Nr soils (Fig. 4A), and the ANOSIM test showed a statistically significant difference ($p < 0.001$), suggesting that both microbial communities of paddy fields were distinct after organic and nonoganic farming. Hierarchical clustering of the soil microbial communities showed that the microbial communities in Or12, Or13, and Or14 soils were grouped together with the microbial communities in Nr6, Nr7, and Nr8 soils (Fig. 4B). The possible reason is that these soils were collected in proximity. However, the microbial communities in organic farming soils were different from those in nonorganic farming soils.

We applied db-RDA to test the relationship between the environmental factors (pH and EC) and microbial community structures, and the results showed that the angle between the pH and EC variables was smaller than 90°, suggesting that both are positively correlated (Fig. 5A). Most nonorganic soils were located in the opposite direction of the EC arrow, suggesting a negative relationship between the EC variable and the microbes, and the EC of organic paddy soils appeared to be higher than that of nonorganic paddy soils (Fig. 5B). The microbes of organic paddy fields had a positive relationship with the pH variable, and the pH of organic paddy soils was higher than the pH of nonorganic paddy soils (Fig. 5B). The results highly suggested that the microbial community composition in organic paddy soil was associated with higher pH and EC environment compared to the microbial community in nonorganic paddy soil. The RDA at the phylum level showed that *Acidobacteria* bacteria were positively correlated with pH, and *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Nitrospirota*, and *Firmicutes* bacteria were negatively correlated with pH. For EC, bacteria *Chloroflexi*, *Actinobacteria*, and *Firmicutes* showed a
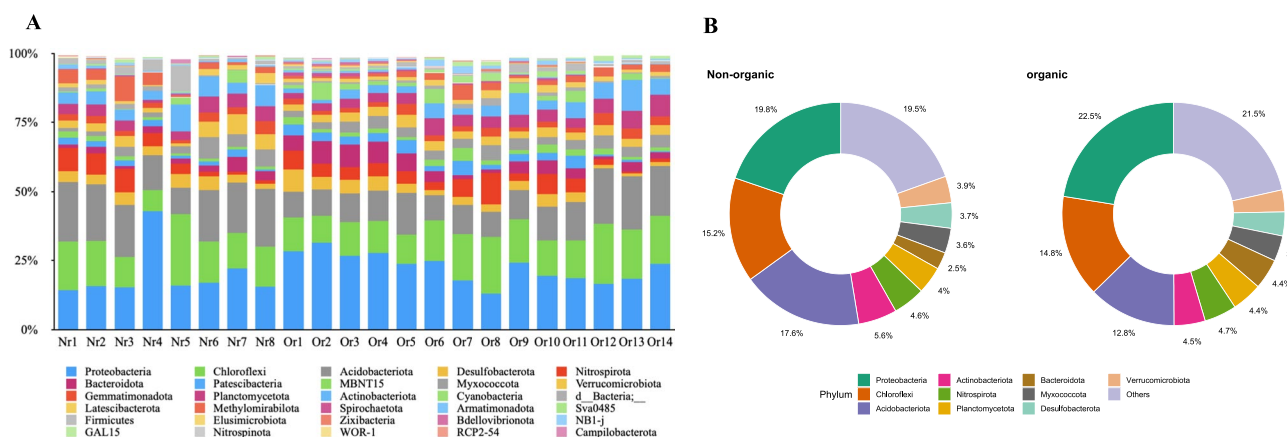

Fig. 2 The relative abundance of phyla of the microbial communities in paddy soils. **A** Individual soil sample comparison. **B** Nonorganic and organic group comparison. Nr indicates nonorganic farming. Or indicates organic farming

Fig. 3 Observed_features, Chao1, Shannon, and Simpson diversity indices of the soil microbial community in nonorganic and organic paddy soils
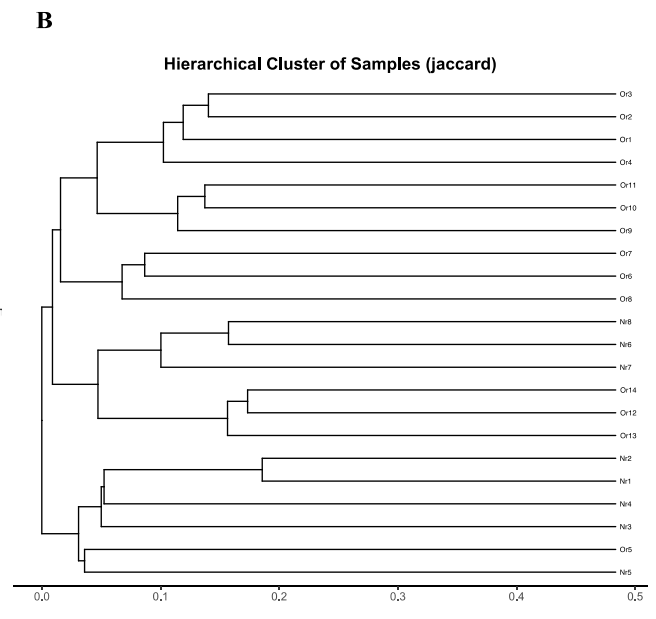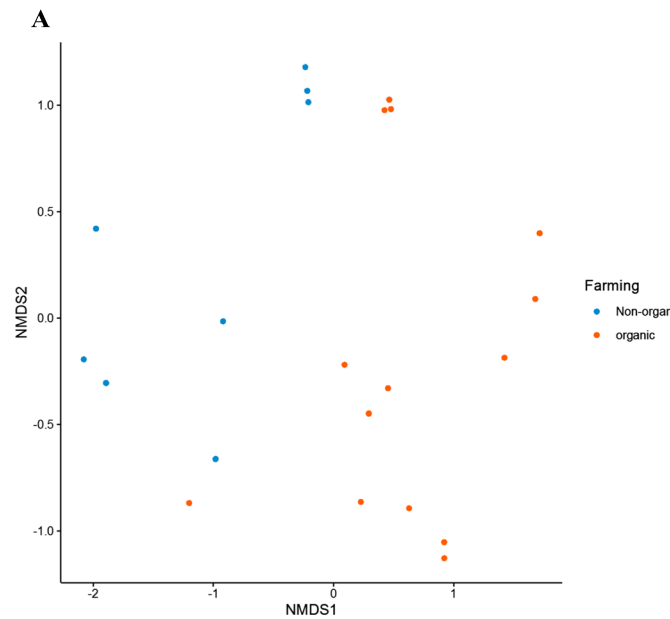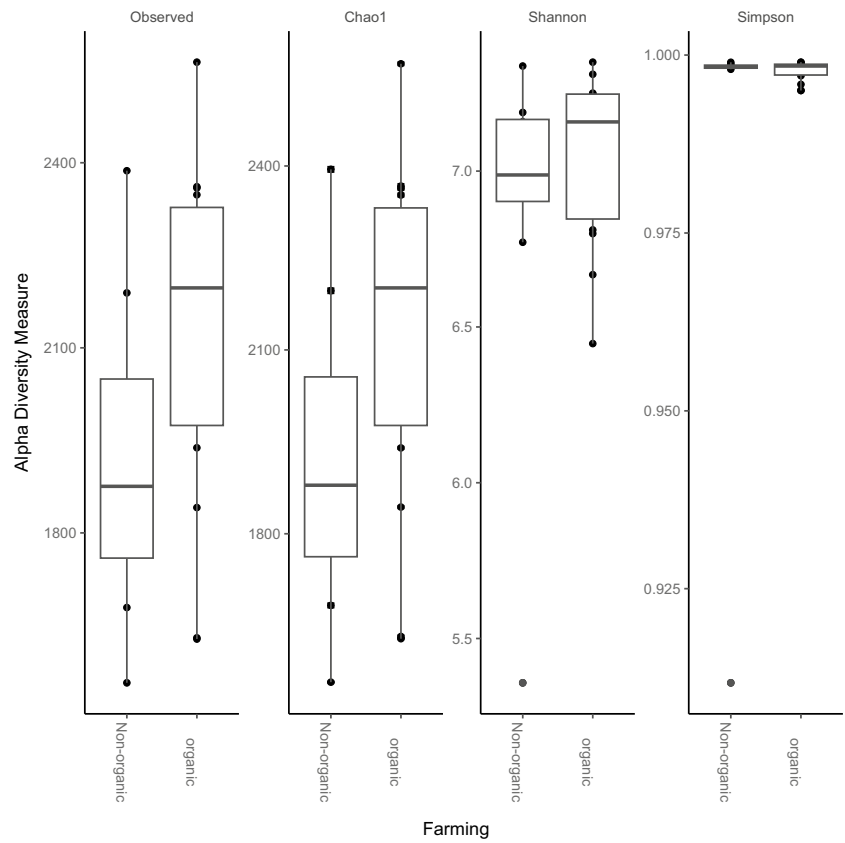

Fig. 4 A Nonparametric multidimensional scaling (NMDS) analysis with Jaccard distance on bacterial communities of paddy soil. Each point represents the bacterial community of a given sample. **B** Hierarchical cluster analysis of the soil microbial communities by the Jaccard, Hellinger, and average methods. Nr indicates nonorganic farming. Or indicates organic farming
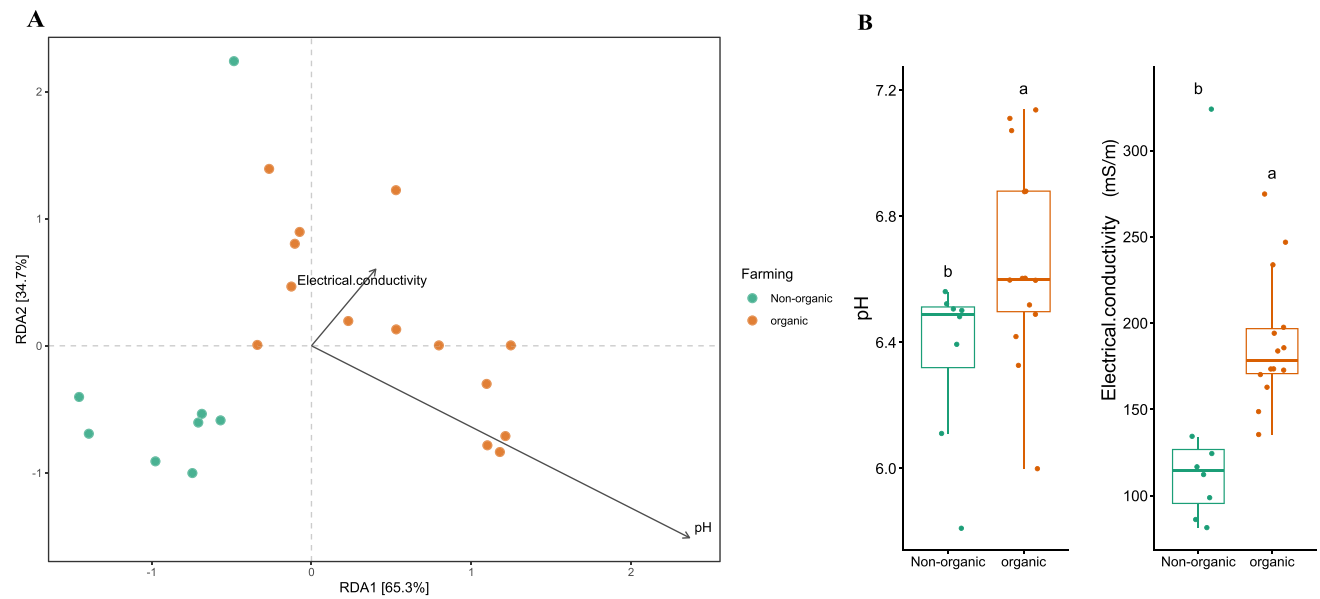
Fig. 5 **A** Distance-based redundancy analysis (db-RDA) of pH and electrical conductivity (EC) on microbial composition with the Jaccard method. **B** Effects of nonorganic and organic farming on paddy soil pH and EC. Different letters indicate significant differences by ANOVA with the cal_diff function in R

positive correlation, and bacteria *Acidobacteria* showed a negative correlation (Fig. S1).

In order to analyze the significant ASVs in different soils, a differential abundance test of ASVs was applied by the STAMP software with White's nonparametric *t* test to organic and nonorganic farming groups, followed by the Story FDR correction with the parameters of *q*-value < 0.05 and the difference between proportions of

0.2%. Twenty-three of 23937 ASVs showed differential abundance with statistical significance (Fig. 6A). There were 13 ASVs from Or soils that showed higher abundance than the ASVs in Nr soils. Among them, 5 ASVs were assigned to *Chloroflexi* bacteria, and 4 were assigned to *Proteobacteria* bacteria. The other 10 ASVs showed a higher abundance in Nr soils, 3 were *Acidobacteria* bacteria, and 3 were *Nitrospirota* bacteria (Fig. 6B). In the heatmap (Fig. S2) and
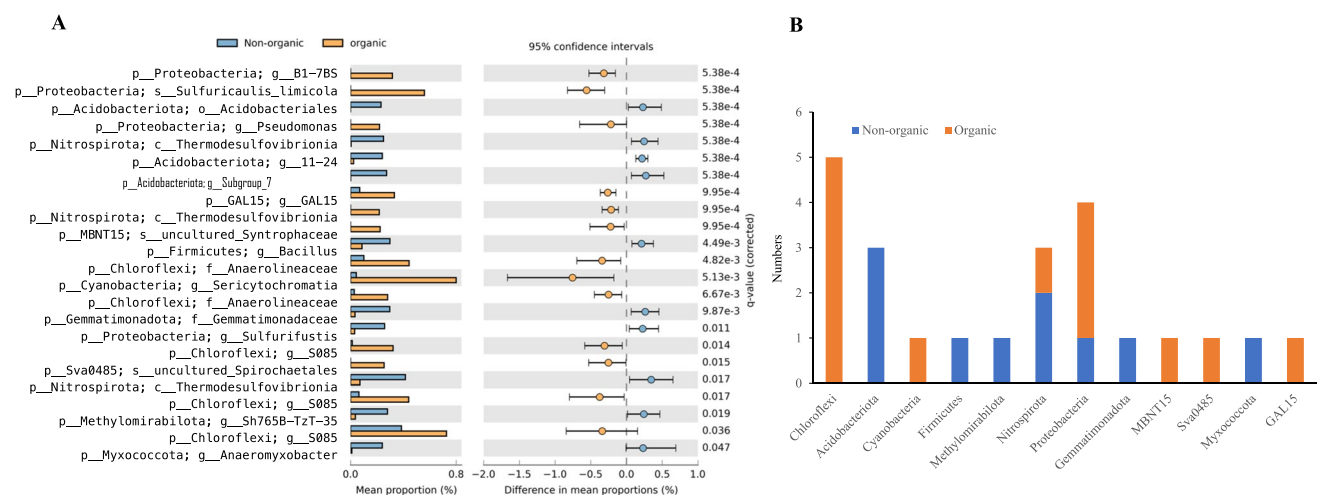

Fig. 6 Differential abundance analysis of amplicon sequence variants (ASVs) in paddy soils. **A** Significant ASVs in organic and nonorganic farming groups were calculated by the statistical analysis of metagenomic profiles (STAMP) software with White's nonparametric *t* test, followed by the Storey false discovery rate (FDR) correction with the parameters of *q*-value < 0.05 and the difference between proportions of 0.2%. The ASVs are shown in the assigned taxon. **B** The numbers of ASVs assigned to phyla

bar plot (Fig. S3) analyses, the differential abundances of ASVs were used to create a graphical representation for individual soil samples. If the uniformity of ASV abundance differences is considered, rather than being limited to only a few samples, these ASVs, g_B1-7BS (*Proteobacteria*), s_*Sulfuricaulis limicola* (*Proteobacteria*), g_GAL15 (p_GAL15), c_*Thermodesulfovibrionia* (*Nitrospirota*), two of f_*Anaerolineaceae* (*Chloroflexi*), and two of g_S085 (*Chloroflexi*), show that they are indeed more abundant in organic soils (Fig. S3A), whereas these ASVs, g_11-24 (*Acidobacteriota*), g__Subgroup_7 (*Acidobacteriota*), and g_*Bacillus* (*Firmicutes*), show differential abundance in nonorganic paddy soils (Fig. S3B). These significant ASVs can be used as the potential quality parameters to differentiate the two systems.

Functional feature prediction

There were 3, 17, and 17 functional features shown to be statistically significant from the programs FAPROTAX, PICRUSt2, and Tax4Fun2, respectively, and the differential abundance of the functional features is shown in Fig. 7A, B, C. According to functional characteristics, we referred to the KEGG pathway maps to classify manually and compare these functional features (Table S1). Among these features, functions related to carbohydrate metabolism were the only functions included in all three calculated programs, and the sum of the percentage was 62.8%. Subsequently, the functions of energy metabolism were included in the two programs, and the sum percentages were 72.6% (Fig. 7D).

Cellulolysis, bifidobacterium shunt, and pyruvate fermentation to isobutanol (engineered) of the functions relative to carbohydrate metabolism were upregulated in nonorganic paddy soils, whereas glycolysis II (from fructose 6-phosphate), butanoate metabolism, and amino sugar and nucleotide sugar metabolism were upregulated in organic paddy soils, suggesting carbon nutrient levels in both soils (Fig. 7A, B, C). Again, methanol_oxidation, nitrite_respiration and oxidative phosphorylation of the functions relative to energy metabolism were upregulated in organic soils, suggesting that the bacteria involved in these metabolic pathways are differentially abundant in organic soils (Fig. 7A, C). Among the metabolic pathways of the functional features, 8 pathway categories showed a higher differential abundance in Or soils, and 5 pathway categories showed a higher differential abundance in Nr soils (Table S1).

Discussion

Bacteria *Proteobacteria* (33.0%), *Acidobacteria* (12.0%), *Actinobacteria* (5.8%), *Bacteroidetes* (5.4%), *Firmicutes* (3.9%), and *Verrucomicrobia* (3.6%) have been shown to be the dominant phyla in paddy soils [28]. The relative abundances of bacterial phyla in a 47-year-old long-term fertilizer experiment under a rice−rice cropping system were *Proteobacteria* (43.3%), *Acidobacteria* (18.8%), *Actinobacteria* (7.9%), *Chloroflexi* (6.3%), and *Firmicutes* (3.9%) in nitrogen (N) + phosphorus (P) + potassium (K) fertilizer, whereas in farmyard manure, as the organic fertilizer, they
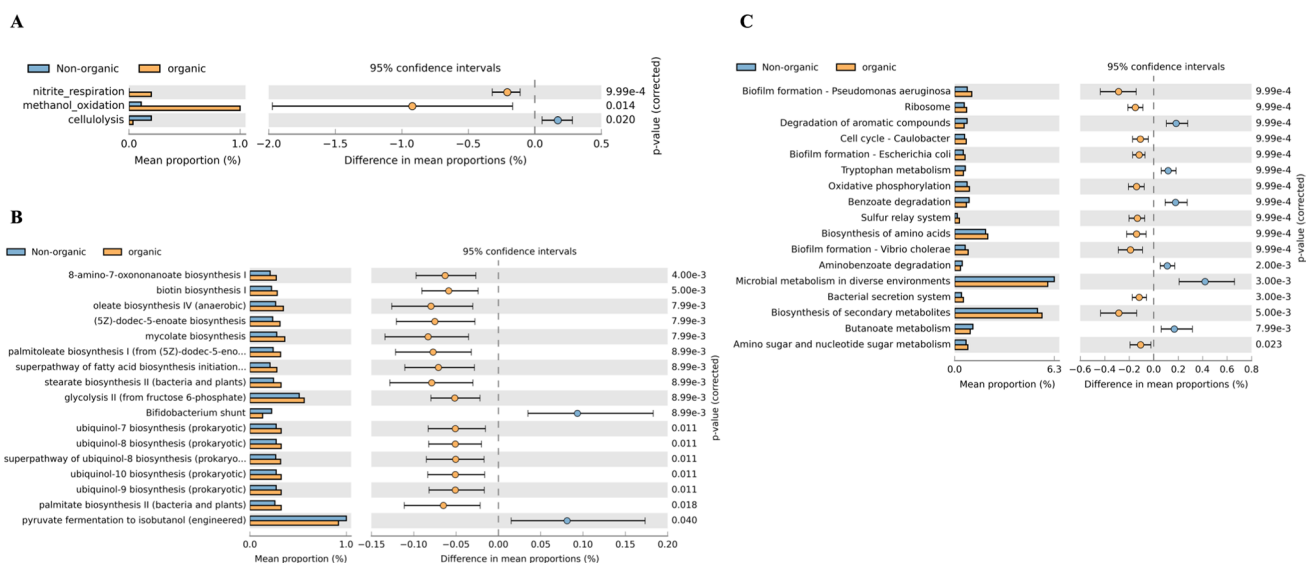

Fig. 7 Differential abundance analyses of functional features calculated by **A** FAPROTAX, **B** PICRUSt2, and **C** Tax4Fun2 in nonorganic and organic paddy soils. Statistical analyses were performed with STAMP using White's nonparametric *t* test (*p* < 0.05) with a difference between proportions parameter (0.1) for FAPROTAX and Tax4Fun2 analyses and a difference between proportion (0.05) for PICRUSt2 without correction

were *Proteobacteria* (36.4%), *Acidobacteria* (24.5%), *Actinobacteria* (7.6%), *Chloroflexi* (9.9%), and *Firmicutes* (3%) [8]. Furthermore, with the addition of composted pig manure and rice straw, the bacteria *Acidobacteria*, *Verrucomicrobia*, and *Latescibacteria* were significantly decreased, but *Actinobacteria* and *Firmicutes* were significantly increased in paddy soils [29]. *Nitrospira* bacteria have been shown to increase in nitrogen fertilizer application alone over 47 years [8], suggesting that the presence of *Nitrospira* is related to nitrogen nutrients in soils. Biochar application in paddy soils significantly reduced *Nitrospirae* bacteria by 25%, thus depressing nitrification [30]. Our results showed the top five microorganisms were *Proteobacteria* (22.5%), *Chloroflexi* (14.8%), *Acidobacteria* (12.8%), *Nitrospirota* (4.7%), and *Actinobacteria* (4.5%) in Or soils and *Proteobacteria* (19.8%), *Acidobacteria* (17.6%), *Chloroflexi* (15.2%), *Actinobacteria* (5.6%), and *Nitrospirota* (4.6%) in Nr soils. Therefore, our results are consistent with previous studies, that is, the dominant bacterial phyla in rice fields are *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*, and the distribution of *Nitrospirota* could be related to nitrogen fertilizer in the soil. Interestingly, a study on soil bacterial communities in paddy fields under organic and conventional farming conditions reported similar results: *Proteobacteria* (25–30%), *Chloroflexi* (20%), *Acidobacteria* (15%), and *Actinobacteria* (10%) were the predominant bacteria in all treatments, and rice bran and rapeseed oil cake were used as the organic fertilizers [31]. Because different organic fertilizers were applied, such as farmyard manure, compost pig manure, green manure, rice straw, rice bran, rapeseed oil cake, biochar, and mixed organic fertilizer, the different nutrients drove soil microbes toward diverted adaptation.

The promising results have been shown to improve wildlife diversity in organic farming. Research on the impact of organic farming on wildlife in rice fields has shown that the number and richness of wildlife have increased, and the number and richness of waterbird have a positive correlation with organic farming [32]. Moreover, organic farming in paddy fields can improve terrestrial arthropod diversity but not underground soil eukaryotic diversity [33]. However, there were no consistent results for microbial diversity in paddy soil under organic farming. For example, organic fertilizers could increase bacterial alpha diversity [6, 8], while no significant differences were observed [7, 29, 31]. For beta diversity, organic fertilizers seem to produce distinct clusters separated from the microbial communities with chemical fertilizers [7, 29]. Therefore, organic fertilizer application should provide different nutrients to influence the growth of certain microbes. Furthermore, cultivation periods, such as elongation, booting, grain filling, or ripening stages, also affect soil microbial community composition [31, 34].

Our db-RDA showed that the microbial community composition in organic paddy soil was positively correlated with higher pH and EC compared to the microbial community in nonorganic paddy soil (Fig. 5A, B). Organic amendments, such as alfalfa straw, compost manure, or wood biochar, significantly increase soil pH and decrease soil EC compared to mineral applications [6]. A similar result was presented in which biochar application could elevate soil pH in paddy soil, and *Nitrospirae* bacteria were also found to decrease pH by 25% [30]. A previous study showed that the relative abundances of the *Proteobacteria* and *Bacteroidetes* phyla were positively correlated with soil pH, while the abundances of *Actinobacteria*, *Firmicutes*, and *Chloroflexi* were negatively correlated with soil pH [35]. Cesarano et al. [6] also reported that *Actinobacteria* bacteria were significantly negatively correlated with soil pH, whereas *Acidobacteria* bacteria were negatively correlated, and *Actinobacteria* bacteria were significantly positively correlated with soil EC. The soil pH indeed affects the composition and diversity of soil bacteria [8]. Furthermore, soil EC plays roles in the changes in soil texture, cation exchange capacity, and percentage of humic matter, presenting the specific relationships between EC and nutrient concentrations [36]. Further studies are needed to clarify the relationships between certain bacteria and soil pH and EC and the relationships between certain organic fertilizer applications and soil pH and EC.

The ASVs assigned to g_B1-7BS (*Proteobacteria*), s_*Sulfuricaulis limicola* (*Proteobacteria*), g_GAL15 (p_GAL15), c_*Thermodesulfovibrionia* (*Nitrospirota*), two of f_*Anaerolineaceae* (*Chloroflexi*), and two of g_S085 (*Chloroflexi*) are specifically present in organic soils (Fig. 6A, S3A). B1-7BS showed a relatively higher dispersion degree among the anaerobic, anoxic, and oxic zone in a carrousel oxidization ditch system for domestic wastewater treatment [37]. In a study of Deoxynivalenol (DON) degradation by microbes, B1-7BS was also reported to be abundant in wheat fields [38]. *Sulfuricaulis limicola* could oxidize thiosulfate, tetrathionate, and elemental sulfur as electron donors to support autotrophic growth [39]. Phylum GAL15 has been shown to increase in abundance with increasing soil depth [40–42] and might be related to wetlands, such as riparian soils [42] and floodplain soils [41]. The genus *Thermodesulfovibrio*, a thermophilic, gram-negative bacterium, can reduce sulfate, thiosulfate, and sulfite as electron acceptors for growth [43]. *Thermodesulfovibrio* was found to be frequently present in environments with sulfur, nitrogen, and methane cycling [44], suggesting that its abundance in soil is related to soil fertility, responsible for the energy metabolism pathway. Interestingly, we found one ASV assigned to c_*Thermodesulfovibrionia* specific to organic paddy soil and two ASVs specific to nonorganic paddy soil (Fig. 6A). The genus *Anaerolinea* contains gram-negative, nonmotile, and filamentous bacteria that strictly grow in anaerobic conditions [45]. *Anaerolineaceae* could syntrophically cooperate with *Methanosaeta* archaea in the process of methanogenic

degradation of alkanes [45, 46]. Furthermore, *Thermodesulfovibrio* spp. have been shown to be associated with *Anaerolineaceae* and *Methanoculleus* spp., and *Anaerolineaceae* initiates the activation of long-chain n-alkane biodegradation, followed by *Thermodesulfovibrio* metabolizing the intermidiates [47], implying in Or soils the potential cooperation between *Anaerolineaceae* and *Thermodesulfovibrio*. Genus S085 belongs to class *Dehalococcoidia,* which reduces dechlorinates tetrachloroethene to ethene [48]. S085 has also been shown to be present significantly in paddy soils with high arsenic levels compared to soils with low arsenic levels [49]. It is difficult to rationalize the bioremediation of S085 bacteria in Or soils.

The ASVs assigned to g_11-24 (*Acidobacteriota*), g__ Subgroup_7 (*Acidobacteriota*), and g_*Bacillus* (*Firmicutes*) showed differential abundances in nonorganic paddy soils (Fig. 6A, S3B). Genus 11–24 belongs to class *Blastocatellia*. *Blastocatellia*, a gram-negative, spherical to rod-shaped and nonmotile bacterium, shows a broad tolerance range of pH for growth and is usually isolated from the soil environment [50]. A study on the prokaryotic communities from a lava tube cave on La Palma Island (Spain) reported 11–24 bacteria in the ochre stalactite [51]. In addition, 11–24 bacteria have also been shown to be the key player in the biofilm community regarding biological waste and wastewater treatment [52]. Genus Subgroup_7 belongs to class *Holophaga*. *Holophaga*, a gram-negative, nonspore-forming, obligate anaerobe, can ferment several aromatic compounds to acetate [53]. Subgroup_7 bacteria have a positive correlation with soil pH ($p < 0.05$), and the average abundance is 2% among 26 subgroups in 87 soils examined [54]. Subgroup_7 bacteria have also been shown to have a positive correlation with nutrient availability and a negative correlation with soil acidity [55]. *Bacillus* spp. isolated from the rice rhizosphere could promote plant traits and protect rice from bacterial blight disease [56].

These specific ASV sequences originate from the V3-V4 segment of the 16S rRNA gene. After taxonomy assignment, the taxonomic annotation cannot fully match to the species level; therefore, their metabolic functions in soil can only be inferred and discussed based on closely related microbes. The ASVs showed differential abundance with statistical significance (Fig. 6A), suggesting different agricultural farming, such as fertilizers, pesticides, or herbicides cause the differences. With the development of metagenomics, the taxonomic annotation of these ASVs might be clarified to better explain their potential roles in Or and Nr soils, and thus would aid in the evaluation and modulation of soil fertility in paddy fields.

We used FAPROTAX, PICRUSt2, and Tax4Fun2 tools to predict the functions of microbial communities based on 16S rDNA sequences. The carbohydrate metabolism features are the only function included in all three calculated programs.

They are cellulolysis (FAPROTAX, Fig. 7A), bifidobacterium shunt, glycolysis II (from fructose 6-phosphate), pyruvate fermentation to isobutanol (engineered) (PICRUSt2, Fig. 7B), butanoate metabolism, and amino sugar and nucleotide sugar metabolism (Tax4Fun2, Fig. 7C), suggesting that the carbon nutrients in both soils might be abundant. Interestingly, degradation of aromatic compounds, benzoate degradation, and aminobenzoate degradation, metabolisms related to aromatic compound degradation, showed differential abundance in nonorganic paddy soils from Tax4Fun2 analysis (Fig. 7C). The benzoate degradation pathway is related to folate synthesis, tyrosine metabolism, phenylalanine metabolism, aminobenzoate degradation, and chlorobenzene degradation [57, 58], suggesting that bacteria that degrade aromatic compounds might be capable of metabolizing aromatic pollutants [59]. At present, we do not have results to show that aromatic pollutants are present in nonorganic soils.

For functional prediction, the major concern is the limited reference databases for soil 16S rDNA, resulting in the low number of taxa assigned. The second problem is the different tools that would perform and derive different functional features [60]. We found that 79.63% of the ASVs were not assigned to any group in FAPROTAX. In PICRUSt2, the weighted nearest-sequenced taxon index (weighted NSTI) was used to evaluate the average distance for the ASVs in a given sample to a reference bacterial genome [24], and the weighted NSTI scores of this study were between 0.21 and 0.35 (> 0.15), suggesting few related references with low prediction quality (Table S2). Tax4Fun2 provides the fraction of taxonomic units that were unused (FTU) and the fraction of sequences unused (FSU) indices as quality indicators [25]. In this study, the FTU scores of all samples were larger than 0.90, whereas the FSU scores of 17 samples, among 22 samples, were larger than 0.90 (Table S2). Thus, although we used three tools to compromise the bias, the functional predictions were based on few sequences.

In conclusion, the beta diversity of NMDS revealed that microbial communities in organic and nonorganic paddy soils were significantly separated. The db-RDA analysis showed the microbial community composition in organic paddy soil was associated with higher pH and EC environment compared to the microbial community in nonorganic paddy soil. There were 23 of 23937 ASVs that showed differential abundance. Among them, g_B1-7BS (*Proteobacteria*), s_*Sulfuricaulis limicola* (*Proteobacteria*), g_GAL15 (p_GAL15), c_*Thermodesulfovibrionia* (*Nitrospirota*), two of f_*Anaerolineaceae* (*Chloroflexi*), and two of g_S085 (*Chloroflexi*) show that they are more abundant in organic soils, whereas g_11-24 (*Acidobacteriota*), g__Subgroup_7 (*Acidobacteriota*), and g_*Bacillus* (*Firmicutes*) show differential abundance in nonorganic paddy soils. These specific microbes are likely associated with soil nutrition, plants,

and environmental factors. Therefore, understanding the metabolic physiology of these microbes will contribute to assessing the impact of organic farming on the microbial community in paddy soil. The existence of these microbes may also aid in the evaluation and modulation of soil fertility in paddy fields.

Declarations

Competing interests The authors declare no competing interests.

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