

Research Article

Effects of Organic Farming on Soil Probiotics of Double Rice Cropping System in South China

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Abstract

Organic management practices of double rice cropping can contribute to soil health and crop productivity. However, the impact of organic versus conventional farming practices (CK) on the diversity and functionality of soil probiotics remains poorly understood, despite growing interest in sustainable agricultural methods. To this end, this study evaluated the effects of these farming systems in double rice production on soil microbial communities using metagenomic sequencing and the PROBIO database. Our study accessed the Shannon and Simpson diversity indices of soil probiotics between the two farming systems. Specifically, the Shannon index values were 93.82 for CK and 93.51 for organic farming, showing no statistically significant difference. Similarly, the Simpson index values were 6.18 for CK and 6.46 for organic farming, also demonstrating no significant variance. However, distinct variations in microbial community compositions were observed. Organic farming significantly increased ($P < 0.05$) the abundance of probiotics that benefit plant growth and nitrogen supply, but reduced those associated with plant nutrient supply and yield. The results show that while organic farming can positively influence certain microbial functions beneficial for sustainable agriculture, it also presents challenges that may affect crop productivity and ecosystem services. These findings suggest that organic farming practices need to be carefully managed to harness the benefits of enhanced microbial functions without compromising crop yields.

Keywords

Organic Farming, Conventional Farming, Soil Probiotics, Microbial Diversity, Soil Health

1. Introduction

The exploration of soil probiotics, specifically beneficial microbes in soil environments, is a crucial frontier in sustainable agriculture. Soil probiotics are instrumental in promoting plant growth, enhancing soil fertility, and providing biological control against pathogens. These microorganisms

significantly contribute to nutrient cycling by facilitating processes like nitrogen fixation and phosphorus solubilization, enriching the soil naturally without the reliance on chemical fertilizers [1]. Moreover, they bolster plant health through the production of growth-promoting hormones and enzymes, thus

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improving crop yields and resilience [2]. Additionally, soil probiotics are also vital in disease suppression, maintaining a balanced soil microbiome essential for preventing soil-borne diseases [3]. Their role in improving soil structure — enhancing water retention and root penetration—is critical for sustaining crop production under the coming global warming [4].

Despite the initial benefits associated with the use of agrochemicals in enhancing agricultural productivity over past decades, their extensive and frequent application has precipitated significant environmental issues. Notably, such practices have detrimentally impacted soil microbial biodiversity, soil health and ecosystem stability [5, 6]. Research has demonstrated that the prolonged use of mineral fertilizers is particularly deleterious, leading to marked reductions in soil microbial biomass and enzymatic activity, which are critical indicators of soil vitality and fertility [7, 8]. The extensive use of these chemicals often alters the natural soil chemistry and disrupts the ecological balance, inhibiting the growth and function of essential microbial communities. These microbes play a pivotal role in organic matter decomposition, nutrient cycling, and the formation of soil structure. They also contribute to the bioavailability of nutrients crucial for plant growth, thereby influencing agricultural productivity and plant health directly. Moreover, the adverse effects of agrochemicals extend beyond the immediate degradation of soil properties. They can lead to the accumulation of harmful residues in the soil, which may leach into water bodies, contributing to pollution and affecting aquatic life [9, 10]. Furthermore, the decline in microbial diversity and function can lead to increased soil pathogen prevalence [6], as the natural microbial checks and balances are disturbed. This disruption often necessitates even greater chemical inputs, such as fungicides and pesticides, to manage new or exacerbated pest and disease issues, perpetuating a cycle of dependency on chemical solutions [11]. Given these challenges, there is a pressing need for agricultural practices that minimize reliance on synthetic fertilizers and pesticides while promoting more sustainable and ecologically viable farming methods.

Organic rice production is increasingly becoming an attractive alternative to conventional farming methods, addressing both consumer demands for safer, more nutritious, and ethically produced food products and environmental concerns associated with modern agricultural practices [12]. According to Wijesinghe and Nazreen [13], the shift towards organic products is not just a trend but a response to growing health and environmental awareness among consumers. This transition is further supported by its potential to conserve water resources, an essential consideration given the extensive water usage required for traditional rice farming. Johannes, *et al.* [14] have documented how organic practices, through more efficient water use and improved soil structure, significantly reduce water runoff and increase the water-holding capacity of soils. Moreover, Nunes, *et al.* [15] highlighted that organic rice cultivation plays a critical role in

mitigating climate change impacts. This is achieved through substantial reductions in greenhouse gas emissions, primarily because organic farming reduced the synthetic nitrogen fertilizers, which are major sources of nitrous oxide, a potent greenhouse gas. Instead, organic farming utilizes organic fertilizers like compost and green manure, which not only supply nutrients in a more sustainable manner but also contribute to carbon sequestration in soil [16]. Beyond environmental benefits, organic farming practices have a profound impact on soil ecology. Hartmann, *et al.* [17] showed the positive effects of organic farming on soil microbial diversity and activity. By avoiding synthetic chemicals and employing organic matter as soil amendments, organic practices foster a rich microbial ecosystem. These microbial communities are fundamental to nutrient cycling, natural soil fertility, and the suppression of soil-borne diseases, ultimately enhancing plant health and crop resilience [18]. The diverse microbial life found in organically managed soils can transform and mobilize nutrients previously inaccessible to plants, thereby improving crop nutrition and growth. The significance of studying and implementing organic rice farming extends beyond immediate agricultural outputs. It encompasses broader objectives such as sustainability, biodiversity conservation, and ecological health. These practices not only align with the principles of environmental stewardship but also offer a viable model for future agricultural practices globally.

Based on a well-managed field experiment of double rice cropping system from 2021 to 2023 in south China. This study hypothesizes that organic rice cultivation significantly influences the abundance and activity of soil probiotics due to the distinct field management (e.g., fertilizer management and weed control). This study anticipates that the environmentally friendly practices of organic farming create an optimal environment for beneficial microorganisms such as nitrogen-fixing bacteria like *Bradyrhizobium* and phosphorus-solubilizing bacteria such as members of the *Azospirillum* genus. By fostering a richer and more diverse soil microbiome, organic rice cultivation is expected to improve nutrient cycling, increase soil fertility, and boost plant health, contributing to higher productivity and enhanced crop resilience against environmental stresses. This hypothesis will be tested by comparing the soil probiotic populations and their functional impacts on plant growth and health between organically and conventionally managed rice fields.

2. Materials and Methods

2.1. Experimental Site Description

Our study spanned three years (2021 to 2023), taking place in Huangjiashan village, Luoping town, Yunfu City, Guangdong Province, China (22.62°N, 111.57°E) [12]. Characterized by a subtropical monsoon climate, this location experiences annual temperature fluctuations with highs averaging

27 °C and lows around 18 °C, alongside annual rainfall approximately 1026.4 mm. The site focuses on the double-cropping of 'Xiangyaxiangzhan', a popular aromatic rice variety in Southern China. The early rice cycle starts with sowing in March, transplanting in April, and harvesting in July; the late rice follows with sowing in July, transplanting in August, and harvesting in November. Initial soil assessments classified it as medium loam with an organic matter content of 30.1 g kg⁻¹, total nitrogen at 0.891 g kg⁻¹, phosphorus at 0.45 g kg⁻¹, and potassium at 19.2 g kg⁻¹ [12].

2.2. Experimental Design and Sampling Approach

This study contrasts two rice cultivation methodologies: conventional (CK) and organic farming [12]. The CK regimen incorporates a chemical-intensive protocol, including synthetic fertilizers (15% urea N, 5% P₂O₅, 15% K₂O at 900 kg ha⁻¹), pest control (acetamiprid and avermectin), alongside disease and weed management chemicals (fluroxypyr-methyl, cyhalothrin, hymexazol, kasugamycin). Alternatively, the organic method utilizes sustainable practices, with slow-release eco-friendly fertilizers (10% N, 3% K, 55% organic matter at 1500 kg ha⁻¹), manual weeding, and natural pest control agents (*Bacillus subtilis*, matrine, *Bacillus thuringiensis*, and *Mamestra brassicae* NPV). The experimental layout was a randomized complete block design, with three replications, across 40 m² plots.

2.3. Metagenomic Sequencing and Analytical Techniques

Following DNA extraction from soil samples, verified by 1% agarose gel electrophoresis, this study used the Covaris M220 for DNA fragmentation, and libraries were constructed with the NEXTFLEX™ Rapid DNA-Seq Kit. Sequencing was executed on the Illumina platform (either NovaSeq or HiSeq X), with sequences submitted to NCBI under PRJNA995040. Preprocessing included trimming via Fastp and assembly with Megahit and Newbler for sequences ≥100 bp. Open Reading Frames (ORFs) predictions utilized Prodigal, with clustering by CD-HIT. SOAPaligner facilitated alignment to a non-redundant gene set, assisting in gene abundance analysis.

In our study, the ProBio probiotics database played a pivotal role [3], offering comprehensive information on 448 commercial probiotic strains, encompassing their functions and integrated data. Among these, 167 strains have been subjected to clinical or field trials, 382 have been featured in research publications, with 329 identified as human probiotics, 89 as animal probiotics, and 52 as plant probiotics. The abundance calculation method employed was based on Reads Number, providing a detailed insight into the probiotic communities within our experimental plots.

2.4. Data Interpretation Strategies

The analysis incorporated Venn diagrams (VennDiagram package) for data visualization, community composition was depicted using ggplot2 and phyloseq packages, and Principal Component Analysis (PCA) was conducted using the stats package. The Wilcoxon rank-sum test compared the significance level of probiotic abundance between organic and CK farming practices, with the False Discovery Rate (FDR) applied for multiple testing correction and confidence intervals calculated using the bootstrap method.

3. Results

3.1. Effects of Organic Farming on α -Diversity and Compositions of Soil Probiotics

The research findings presented here aim to explore the impact of organic farming on soil probiotics compared to conventional practices (CK). Analysis of biodiversity indices such as the Shannon and Simpson indices indicated similar levels of microbial diversity in both farming systems (Figure 1). Specifically, the Shannon index values were 93.82 for CK and 93.51 for organic farming, showing no statistically significant difference. Similarly, the Simpson index values were 6.18 for CK and 6.46 for organic farming, also demonstrating no significant variance.

Further investigation using a Venn diagram revealed a substantial overlap in the soil probiotic effects between the two farming methods (Figure 2), with 106 shared probiotic effects. However, the conventional farming system exhibited 2 unique probiotic effects not found in the organic system. In terms of the diversity of probiotic names, CK had 111 different probiotics, whereas organic farming had 107, with 5 unique to CK and both systems sharing 106 names.

Advanced multivariate analyses, including Principal Coordinates Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS), along with Principal Component Analysis (PCA), provided deeper insights. The PCoA highlighted that the first two principal coordinates (PC1 and PC2) explained 31.04% and 22.73% of the variation, respectively (Figure 3A). Additionally, both NMDS and PCA suggested that organic farming influences the composition of soil probiotics, indicating distinct microbial community structures between the two farming practices (Figures 3B and 3C). These results suggest that while overall biodiversity might be similar between organic and conventional systems, the composition and specific probiotic effects can differ, potentially leading to varied impacts on soil health and ecosystem functioning.

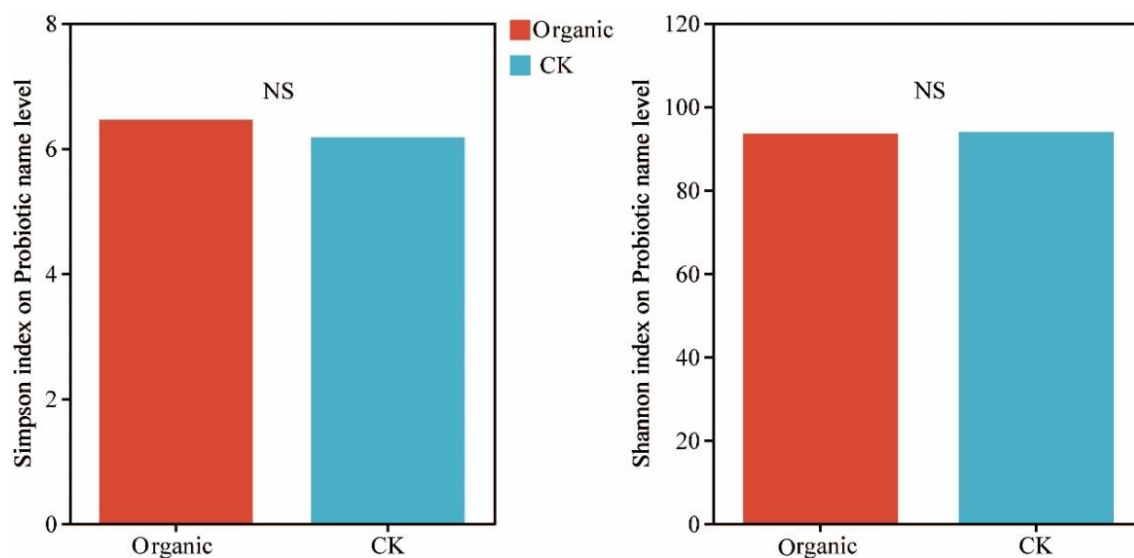


Figure 1. Effects of organic farming on Simpson and Shannon index of soil probiotics. Organic represents organic rice production; CK represents the conventional rice production.

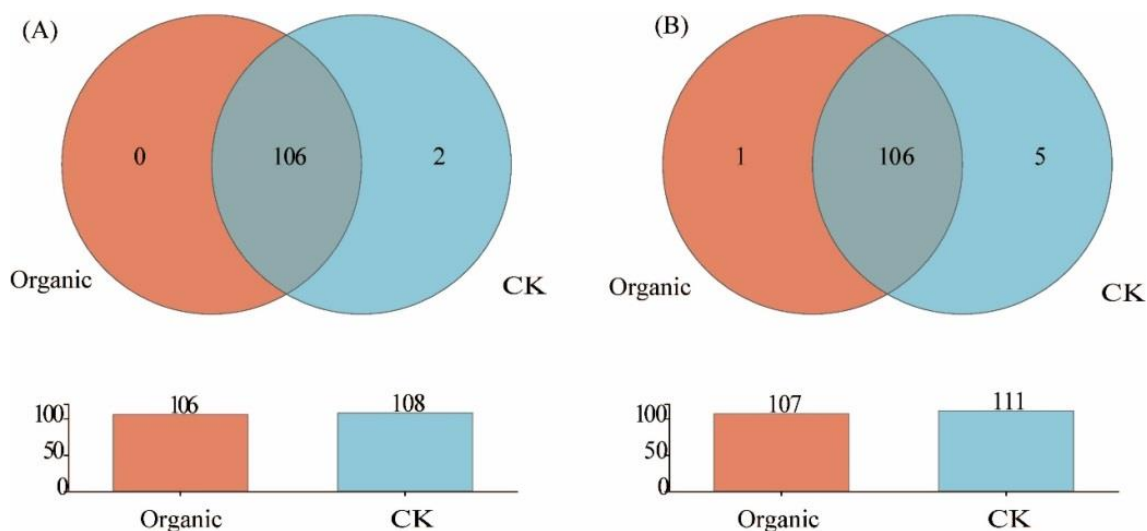
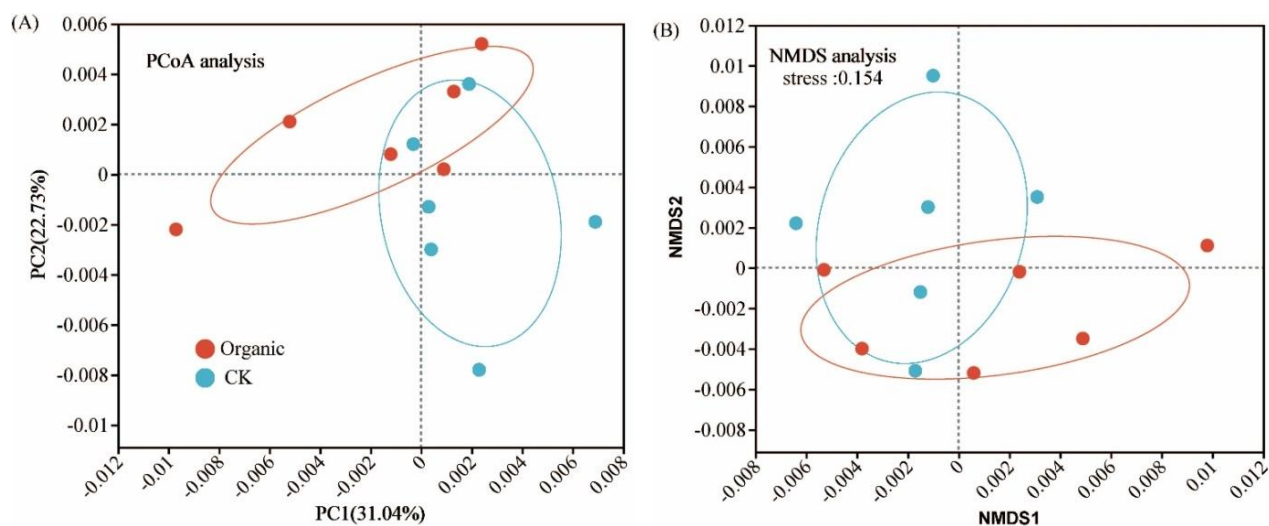


Figure 2. Effects of organic farming on the types of soil probiotics. (A) at the probiotic effects levels; (B) at probiotic name levels.



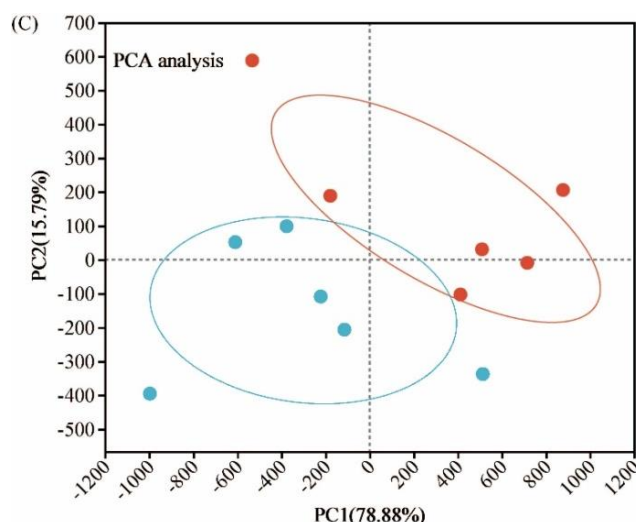


Figure 3. Effects of organic farming on the composition of soil probiotics.

3.2. Effects of Organic Farming on Soil Probiotics at Different Classification Levels

The research findings further demonstrate distinct differences in the abundance and functional impacts of soil probiotics under organic and conventional farming systems (CK). The analysis revealed that the abundance of probiotics beneficial to plant health was significantly higher in organic farming, constituting 50.4% compared to 47.8% in CK (Figure 4). Conversely, probiotics beneficial to animals and humans exhibited a higher prevalence under CK, with a statistically significant increase noted specifically for animal-benefiting probiotics.

An examination of the most prevalent soil probiotics included *Rhodopseudomonas* sp., *Bradyrhizobium japonicum*, *Azospirillum brasilense*, *Azospirillum* sp., and *Bradyrhizobium pachyrhizi* (Figure 5). While there was no significant

difference in the abundance of *Rhodopseudomonas* sp. between the two farming systems, organic farming significantly enhanced the abundances of *Bradyrhizobium japonicum* and *Bradyrhizobium pachyrhizi*. In contrast, the abundance of *Azospirillum brasilense* was significantly reduced under organic farming, with no notable changes observed in *Azospirillum* sp.

Regarding the functional aspects of these probiotics, the top functions identified were related to plant growth, plant nitrogen supply, maintenance of aquatic conditions for animals, plant nutrient supply, and plant product yield. Organic farming practices were found to significantly increase ($P < 0.05$) the abundance of soil probiotics associated with plant growth and plant nitrogen supply (Figure 6), although the latter showed a trend towards significance ($P = 0.06$). However, it negatively impacted the probiotics related to plant nutrient supply and plant product yield, both of which showed a significant reduction ($P < 0.05$).

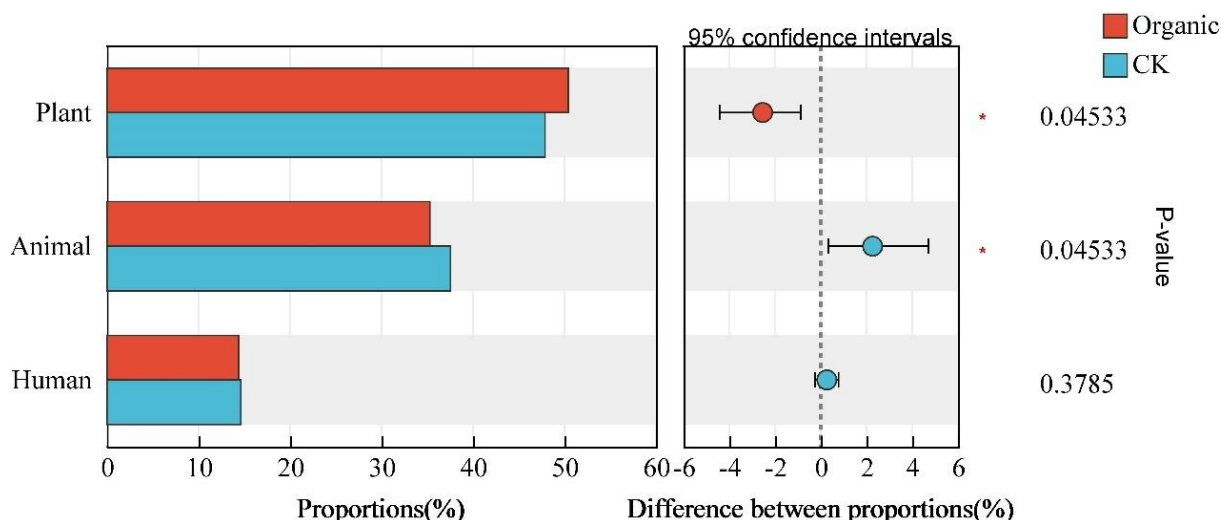


Figure 4. Effects of organic farming on the soil probiotics with the functions on plant, animal and human.

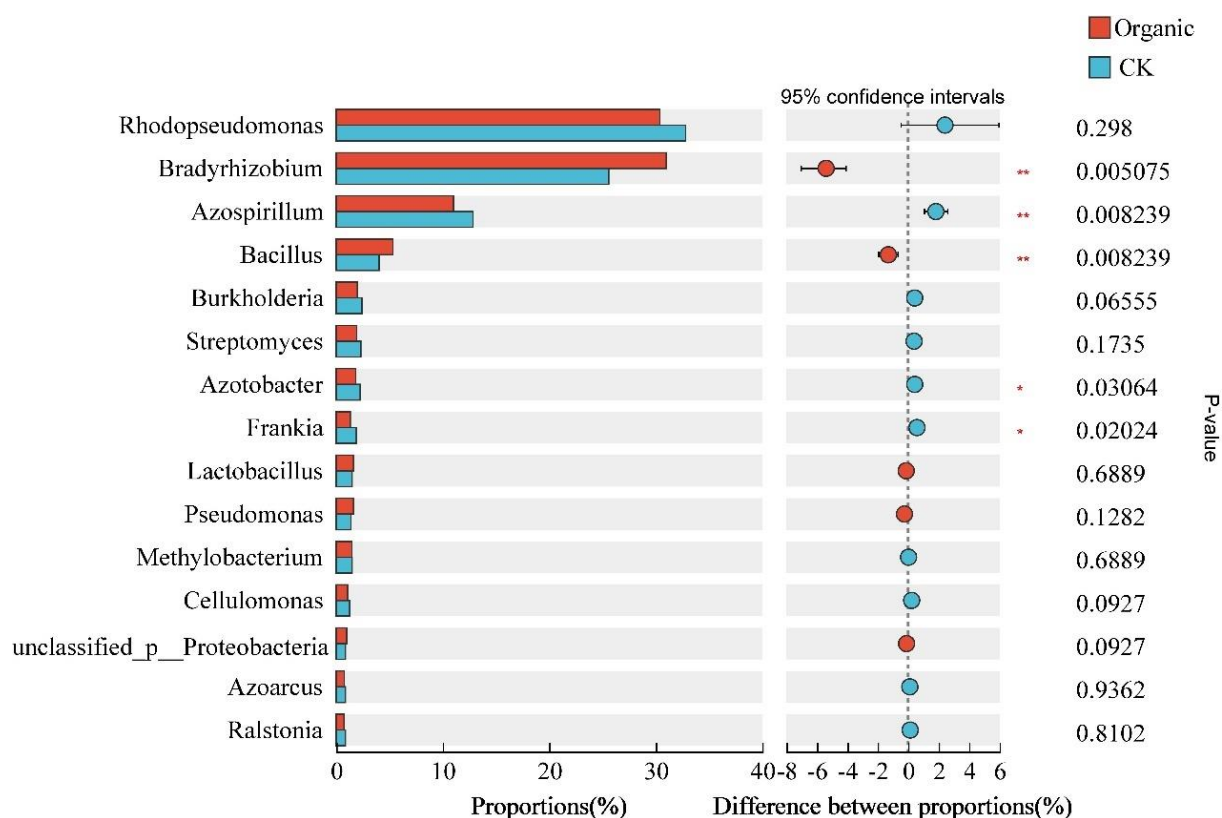


Figure 5. Effects of organic farming on the soil probiotics based on probiotic name.

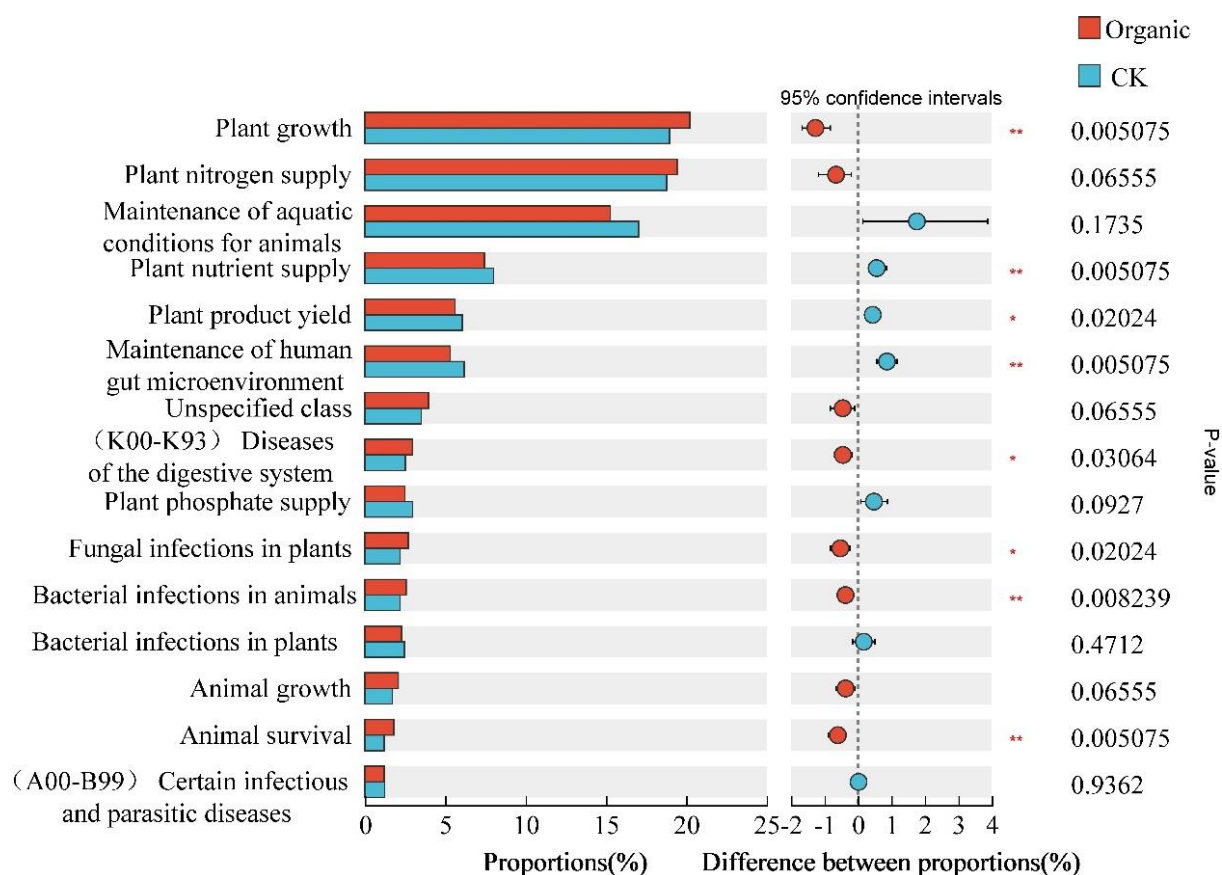


Figure 6. Effects of organic farming on the soil probiotics at disease class.

4. Discussions

4.1. Effects of Organic Farming on α -Diversity and Composition of Soil Probiotics

Our study revealed that the alpha-diversity of soil probiotics, as measured by Shannon and Simpson indices, showed no significant differences between organic and conventional farming systems. This similarity suggests that organic farming does not necessarily enhance the overall diversity of soil microbial communities more than conventional managements. This finding contrasts with the commonly held view that organic practices, which avoid synthetic chemicals and incorporate more diverse crop rotations and organic matter inputs, would naturally lead to a more diverse microbial community [17]. The discrepancy between the findings of our study and the commonly held belief that organic farming enhances microbial diversity could be attributed to several factors, particularly the duration of the organic farming practices implemented. It is important to consider that microbial communities in soil are highly dynamic and can be deeply influenced by long-term agricultural practices [19]. Our experiment was conducted over a relatively short period of three years. Soil microbial communities are complex and can take several years to show noticeable changes in response to alterations in farming practices. Previous studies suggesting an increase in microbial diversity under organic systems often involve long-term comparisons where organic practices have been in place for many years or even decades [17]. The duration of these studies allows for the accumulation and stabilization of organic matter, which significantly influences microbial colonization and diversity. In contrast, three years may not be sufficient for these microbial communities to fully respond and exhibit detectable changes in diversity metrics.

The substantial overlap in soil probiotic effects between the two farming systems, as shown by the Venn diagram analysis, indicates a core microbiome common to both practices. However, the presence of unique probiotic effects in CK suggests that conventional practices may support specific functions not as prevalent under organic management. This could be due to differences in soil chemistry, crop management strategies, or the residual effects of chemical inputs [12]. Advanced multivariate analyses such as PCoA and NMDS further highlighted that while overall diversity was similar, the composition of the microbial communities differed significantly. This discrepancy emphasizes that organic farming may modify the microbial community structure in ways that are not captured solely by diversity indices.

4.2. Effects of Organic Farming on Soil Probiotics at Different Classification Levels

Organic rice cultivation significantly enhances the activity of soil probiotics and their impact on various "Disease Clas-

ses," largely due to the sustainable practices it incorporates. By utilizing organic fertilizers and avoiding synthetic chemicals, organic farming provides a nourishing environment that supports the proliferation and activity of beneficial microbes [20, 21]. These microbes, such as phosphorus-solubilizing bacteria and nitrogen-fixing bacteria, thrive in the natural and healthy conditions created by organic practices [22-24]. They contribute directly to plant growth by improving nutrient availability and enhancing root health. Additionally, the absence of chemical pesticides in organic rice systems allows for a richer soil microbiome, which might better support plant health and resilience against diseases [6, 25, 26]. The enhanced microbial activity not only boosts nutrient cycling, making essential nutrients more available to plants, but also promotes greater plant growth and productivity. This sustainable approach aligns with ecological balance, reduces environmental impact, and ultimately supports healthier crops in organic rice fields.

Our study revealed a distinct increase in the abundance of soil probiotics that are beneficial to plant health under organic farming systems, which shows the potential advantages of organic agricultural practices in enhancing microbial communities that support plant growth. This trend can largely be attributed to the soil management practices inherent to organic farming, which typically includes the application of organic matter such as compost and manure, and the avoidance of synthetic pesticides and fertilizers [12]. These practices not only improve soil structure and increase organic carbon content but also establish a more favorable soil environment for microbial activities involved in key ecological processes such as nutrient cycling, nitrogen fixation, and the synthesis of phytohormones that promote plant growth [27, 28]. Conversely, in conventional farming systems, where chemical fertilizers and pesticides are regularly used, there is often a notable shift in microbial community composition. The frequent application of these chemicals can create selective pressure that favors microbial strains with resistance mechanisms. This selection can lead to a higher prevalence of probiotics that are beneficial to animals and humans but may also be more robust against chemical disturbances. Such microbial communities might possess adaptive traits that allow them to survive in less favorable soil conditions, where chemical stressors are present. However, this can lead to a decrease in microbial diversity and a reduction in the population of microbes that are more sensitive but crucial for plant-specific interactions and soil health [29]. The dichotomy observed between microbial populations in organic and conventional systems suggests that agricultural management practices significantly influence the ecological balance within soil microbiomes. The implications of this are profound, impacting not only plant health and crop productivity but also the broader ecological services provided by soil organisms, including disease suppression, decomposition, and the support of higher trophic levels through the food chain [30].

The differential impacts observed on specific probiotic

strains such as *Bradyrhizobium japonicum* and *Bradyrhizobium pachyrhizi* in our study are particularly significant, as these bacteria were found to thrive in the organic farming systems characterized by low-chemical input conditions. Both of these strains are well-documented for their capability to fix atmospheric nitrogen [31, 32], a process that is critical for natural soil fertility and for reducing the agricultural dependency on synthetic nitrogen fertilizers. Nitrogen fixation by *Bradyrhizobium* species plays a pivotal role in sustainable agriculture because it converts inert atmospheric nitrogen into ammonia, which plants can readily absorb and utilize. This biological process not only supports plant growth and productivity but also contributes to the nitrogen cycle in ecosystems, enhancing soil health over time. In organic systems, the absence of synthetic nitrogen sources puts a greater ecological premium on the natural processes of nitrogen fixation, making the roles of these bacteria even more crucial. The enhanced presence of *Bradyrhizobium japonicum* and *Bradyrhizobium pachyrhizi* in organic systems can be attributed to several factors. Firstly, organic farming practices such as the use of compost, manure, and other organic soil amendments can increase the organic carbon content of the soil, which provides additional energy sources for nitrogen-fixing bacteria. Secondly, the reduced use of chemical pesticides and fertilizers minimizes disturbances to soil microbiomes, allowing more sensitive microbial species to establish and maintain their populations effectively. Furthermore, the ability of *Bradyrhizobium* strains to form symbiotic relationships with leguminous plants can be particularly advantageous in organic farming systems, which often employ crop rotations that include legumes as a strategy to enhance soil nitrogen levels naturally [33]. This symbiosis not only benefits the immediate leguminous host by providing it with essential nitrogen but also improves overall soil fertility for subsequent crops in the rotation.

The thriving of these nitrogen-fixing bacteria in organic systems highlights the potential of integrated soil and crop management strategies that leverage biological processes for fertility and plant health. By fostering environments that support beneficial microbes like *Bradyrhizobium*, organic farming practices can contribute to more resilient agricultural ecosystems capable of sustaining high productivity without the environmental costs.

The findings suggest a compelling case for rethinking conventional agricultural practices to enhance the compatibility between microbial ecological functions and crop production goals. Conversely, the decrease in *Azospirillum brasilense* under organic farming, a strain known for its role in nitrogen fixation and growth hormone production, highlights the complex interplay between soil chemistry and microbial preferences. This reduction may point to specific nutrient or pH conditions under organic farming that are less favorable for this particular species, or it might indicate competition with other microbes that are more adapted to the organic environment.

4.3. Broader Implications and Future Directions

These findings suggest that while organic farming can enhance certain beneficial microbial functions, it may also suppress others, potentially leading to trade-offs between enhancing soil fertility and achieving high crop yields. The significant reduction in probiotics related to plant nutrient supply and yield under organic practices raises important questions about the long-term sustainability and productivity of these systems. Further research should explore the long-term dynamics of these microbial communities and their functional implications for soil health and plant productivity.

Future studies could benefit from integrating metagenomic and metabolomic approaches to better understand the functional capabilities of soil microbial communities under different farming practices. Additionally, extending these studies across different climatic and soil types could elucidate more general patterns and provide deeper insights into the mechanisms through which farming practices influence soil microbiomes. In conclusion, while organic farming practices generally support a shift towards more sustainable agricultural systems, our findings underscore the need for a nuanced understanding of how these practices affect soil microbial communities and their functional roles in the ecosystem. This knowledge is crucial for optimizing organic farming practices to support both environmental sustainability and agricultural productivity.

5. Conclusions

This study systematically investigated the effects of organic farming on the diversity and functionality of soil probiotics compared to conventional farming practices, specifically analyzing variations in microbial community structure and probiotic functions. Our findings confirm the hypothesis that while organic and conventional farming systems support similar levels of microbial diversity, as indicated by Shannon and Simpson indices, they differ significantly in community composition and the impact on specific probiotic functionalities. Organic farming enhanced the abundance of probiotics beneficial to plant growth and nitrogen supply, thereby supporting the anticipated benefits of organic practices on soil health and ecosystem functioning. However, the reductions observed in probiotics related to plant nutrient supply and yield under organic farming underscore the complexity and potential trade-offs of these agricultural systems. Thus, while organic farming can foster certain beneficial soil microbial functions, it also requires careful management to balance all aspects of soil health and plant productivity, ensuring sustainable agricultural success. These findings prompt further investigation into the mechanisms through which organic farming influences microbial diversity and functionality. Future research should focus on long-term studies to observe the cumulative effects of organic practices on soil health and crop yields. Additionally, exploring the interaction between

microbial communities and different organic management practices could provide deeper insights into optimizing organic farming for both environmental sustainability and agricultural productivity.

Abbreviations

CK	Conventional Farming
NMDS	Non-metric Multidimensional Scaling
PCoA	Principal Coordinates Analysis
PCA	Principal Component Analysis
FDR	False Discovery Rate
ORFs	Open Reading Frames
NCBI	National Center for Biotechnology Information
PRJNA	NCBI Project Identifier
PROBIO	Probiotics Database

Conflicts of Interest

The authors declare no conflicts of interest.

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