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Research Article

Metagenomic Insights into Rhizosphere Fungal Communities Across Different Rice Cultivation Systems

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Abstract

Background and Objective: Rhizosphere fungi play a crucial role in nutrient cycling and plant protection, yet most are difficult to cultivate using conventional methods. Consequently, their ecological functions remain largely unknown. Therefore, metagenomic approaches allow for comprehensive and accurate mapping of fungal taxonomic profiles without the need for cultivation and this study investigated the variation of rhizosphere fungi across different rice cultivation systems to elucidate their diverse potentials. **Materials and Methods:** A metagenomic approach was employed to identify fungi originating from the rhizosphere of rice cultivated in various field conditions, including irrigated, rainfed and organic rice fields. The diversity of fungi from rhizosphere samples was assessed to comprehend the relationships and metrics within the rice cropping systems utilized by farmers. **Results:** The findings indicated that the rhizosphere fungal index from organic rice fields exhibited the highest Shannon and Simpson index values compared to those from irrigated and rainfed rice fields. **Conclusion:** Metagenomic analysis revealed that the most dominant fungal diversity at the family level was Trichocomaceae, at the genus level was Talaromyces and at the species level was *Talaromyces wortmannii.*

Key words: Rhizosphere, fungi, rice, cultivation, metagenomic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice is a fundamental crop and serves as the primary food source for nearly half of the global population[1]. It is a nutritious food, offering substantial amounts of complex carbohydrates, vitamins and minerals[2]. As a crucial food commodity, rice is particularly significant for the majority of the world's population, especially in Asia[3]. Rice cultivation has significantly influenced landscapes across various regions. Terraced rice fields, particularly prevalent in parts of Asia[4]. Exemplify the adaptability of rice cultivation practices. Rice is capable of being cultivated in diverse environments, ranging from inundated rice paddies to arid mountainous regions. Different rice cultivation methods, including irrigated rice, upland rice, rainfed lowland rice and direct-cropping rice, play a crucial role in shaping the composition of the rice microbiome. The systems of rice cultivation encompass irrigated, rainfed and organic rice paddies. The characteristics of irrigated rice fields include the availability of water throughout the year, allowing for up to three harvests annually. In contrast, rain-fed rice fields rely on rainfall, are generally infertile due to poor nutrient content[5]. and often experience drought conditions, resulting in only one planting cycle per year[6]. Both systems remain conventional, relying on chemical inputs, whereas organic rice fields do not utilize chemical inputs. The cultivation systems of lowland rice have a significant impact on the diversity and density of the soil microbiome. The type of irrigation system employed can alter the number and composition of microbial communities by modifying oxygen levels and soil structure[1]. The interactions among plants, soil and microbes ultimately influence agricultural yields.

The microbiome exhibits adaptability to various agricultural practices and geographic locations, thereby facilitating the growth of rice plants across diverse climatic conditions[7]. It serves as the primary occupant of the rice rhizosphere, which is the soil region surrounding plant roots. This rhizosphere is a dynamic and highly interactive habitat that supports diverse microbial communities. Plant roots selectively recruit specific microbial communities from the soil to form these root-associated communities[8]. The rhizosphere of rice is notably abundant in microbial populations, exhibiting approximately twice the microbial density compared to unfertilized soil. The presence of roots actively stimulates microbial proliferation within the rhizosphere[9]. Microorganisms in the rhizosphere are integral to a range of ecological and agricultural functions, such as the decomposition of organic matter, nutrient cycling and the enhancement of plant growth and crop yield[10]. The role of biogeochemical cycles is pivotal in influencing soil health, quality and fertility[11]. These cycles also contribute to the tolerance of abiotic stress[12]. and are instrumental in nutrient supply and plant protection against pathogens[13]. The interaction between plants and their roots is characterized by the production of exudates, including carbohydrates, amino acids and secondary metabolites, which serve to attract a diverse array of microorganisms. These microorganisms encompass functional groups such as phosphate decomposers, iron decomposers, nitrogen fixers, methane decomposers and fermenters[14]. Microbial communities exhibit significant variability and diversity within the root zone during the growth stages of rice plants[15]. Various factors, including soil pH, salinity, texture, organic matter content, nutrient concentration, seasonal effects and management practices such as irrigation, tillage, planting systems, fertilizer and pesticide applications and crop residue integratif substantially influence microbial diversity in the soil[16].

The application of metagenomics in the investigation of soil microbial communities has facilitated researchers in obtaining a comprehensive understanding of both the diversity and functional attributes of these communities, which is a crucial approach for assessing microbiological parameters. Analyzing functional diversity can also yield insights into how adaptive microorganisms may impact the environment. The swift progress in sequencing technology, coupled with bioinformatics tools, is enhancing the feasibility of conducting large-scale studies in microbial ecology, thereby offering a more profound comprehension of the composition and functions of microorganisms across various ecosystems[17]. This method offers a comprehensive approach to examining the genetic diversity of rhizosphere microorganisms. Metagenomic techniques facilitate a holistic analysis of environmental interactions by investigating the ecological roles of microorganisms within their environments, thereby elucidating interactions within complex ecosystems. Consequently, this study investigates and elucidates the diversity of rhizosphere microbes across various rice cultivation systems. The findings of this research will establish a scientific foundation and offer insights into the potential application of rhizosphere microbes for sustainable rice plant health management.

MATERIALS AND METHODS

Study site and duration: This research was conducted in July-November 2024 in the rice cultivation center and in the Plant Disease Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. The research sample location

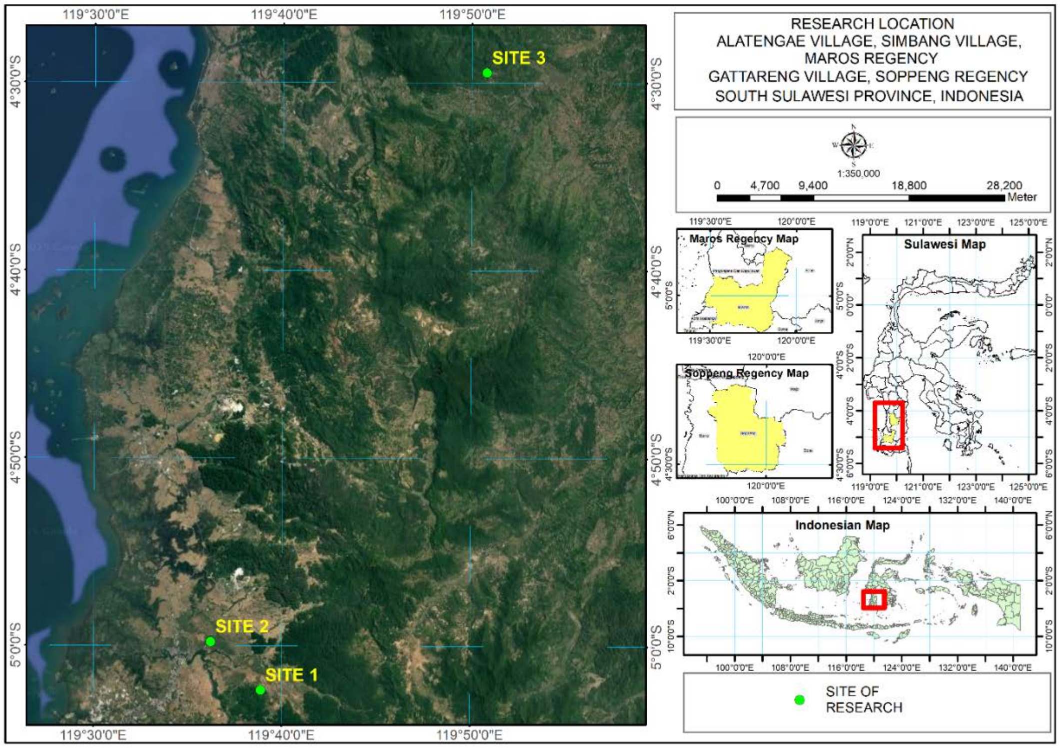

Fig. 1: Map illustrating the locations of the research sample points: Organic Rice Fields (ORF) in Gattareng Village, Soppeng Regency; Irrigated Rice Fields (IRF) in Alatengae Village; and Rainfed Rice Fields (RRF) in Simbang Village, Maros Regency, South Sulawesi, Indonesia

was determined using purposive sampling with specific criteria. Organic rice field soil (ORF) samples were taken from Gattareng Village, Marirowawo District, Soppeng Regency (S 4°29'27.9" E119°50'47.9"). Irrigated rice field soil samples (IRF) were taken from Alatengae Village, Bantimurung Subdistrict, Maros Regency (S-4.996607 E119.603697) and rainfed rice field soil samples (RRF) from Simbang Subdistrict, Maros Regency (S-5.039359 E119.64787). South Sulawesi, Indonesia. The locations are indicated on the map (Fig. 1).

Rhizosphere soil sampling: Rhizosphere soil samples from three types of rice fields (organic rice fields, irrigated rice fields and rainfed rice fields) were taken at five points in each plot. Each sampling point yielded 500 g of soil at a depth of 10 cm from the soil surface. The collected soil samples were combined and put into zip-lock plastic bags. The samples were then stored in a cool box for analysis in the laboratory.

DNA extraction: Genomic DNA for metagenomic analysis was extracted directly from the rice soil samples. DNA was extracted from rice soil using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082) and Genomic DNA Quantification by Nanodrop and Qubit. The extracted DNA was stored at 20°C for further metagenomic analysis.

Library preparation: DNA libraries were prepared using the Oxford Nanopore Technology (ONT) kit, adding adapters and barcodes for multiplexing. The completed libraries were loaded into the ONT flow cell for sequencing (Fig. 2)

Metagenomic analysis: This stage utilises the internal transcribed spacer (ITS) region of nuclear DNA (rDNA), which has been the most frequently sequenced region for identifying fungal taxonomy at the species level, even within species. DNA concentrations were determined using a NanoDrop spectrophotometer and Qubit fluorometer. Library preparation was performed using a kit from Oxford Nanopore Technology. Nanopore sequencing was operated using MinKNOW software version 23.04.5. Basecalling was performed using Guppy version 6.5.7 with a high accuracy model[18] . The quality of the FASTQ files was visualized using NanoPlot and quality filtering was performed using NanoFilt[19,20]. The filtered reads were classified using Centrifuge classification[21]. A fungal index was created using the NCBI ITS RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/).

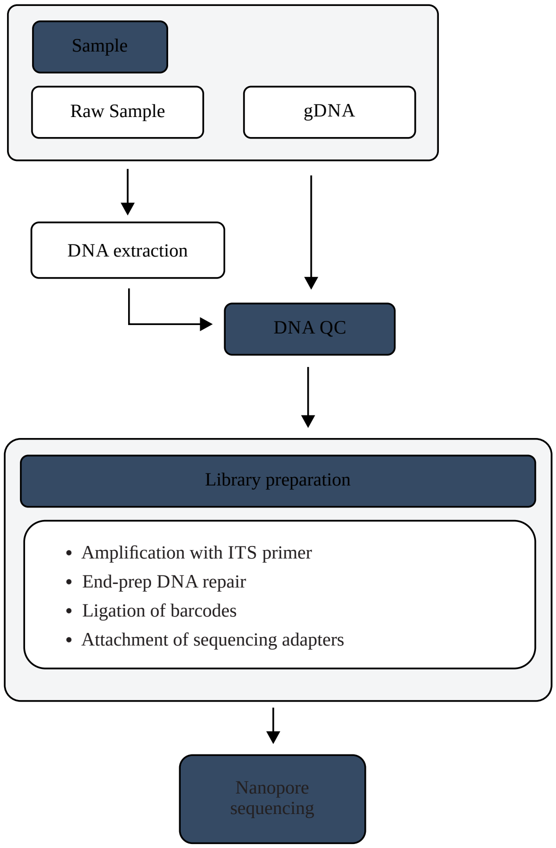

Fig. 2: Experimental workflow

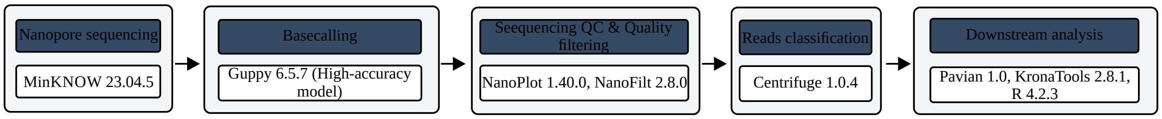

Fig. 3: Bioinformatic Workflow

Data analysis: Metagenomic analysis of soil microbes was performed using analysis and visualization tools such as Pavian (https://github.com/fbreitwieser/pavian), Krona Tools https://github.com/marbl/Krona) for interactive taxonomic representation (Fig. 3) and RStudio with R version 4.2.0 (https://www.R-project.org/).

RESULTS AND DISCUSSION

The crop cultivation system acts as a primary intervention that directly influences the quality of soil physical and chemical properties, which automatically determine the community structure and diversity of microbes. The physical and chemical properties of soil in Organic Rice Fields (ORF), Irrigated Rice Fields (IRF) and Rainfed Rice Fields (RRF) are shown in Table 1.

The pH values of organic rice fields, irrigated rice fields and rain-fed rice fields, which were recorded at 6.35, 5.92 and 5.85, respectively. The temperature and humidity levels across these rice fields were observed to be comparable. Both organic and irrigated rice fields exhibit a clay texture, whereas rainfed rice fields are characterized by a dusty clay texture, as detailed in Table 1. The nutrient content in Organic Rice Fields (ORF) surpasses that of Irrigated Rice Fields (IRF) and Fainfed Rice Fields (RRF). This disparity may be attributed to the distinct land use practices employed in rice cultivation. Organic rice fields (ORF) are indicative of cultivation practices devoid of chemical usage, in contrast to irrigated and rainfed rice fields, which continue to employ conventional methods reliant on chemical inputs, including fertilizers and synthetic pesticides. The conventional rice cultivation systems in irrigated and rainfed rice fields demonstrate a significant reliance on chemical inputs, particularly pesticides.

Table 1: Physical and chemical properties of soil in organic rice field (ORF), irrigated rice field (IRF) and rainfed rice field

Criteria	Organic rice field (ORF)	Irrigated rice field (IRF)	Rainfed rice field (RRF)
Temperature (°C)	27	27	27
Humidity (%)	75	83	83
pH	6.35	5.92	5.85
Texture	Clay	Clay	Dusty clay loam
Vegetation	Rice	Rice	Rice
C organic (%)	2.18	1.18	0.97
N total (%)	0.24	0.16	0.09
P_2O_5 (ppm)	11.20	10.48	8.93
Ca-dd/cmol/Kg	8.02	4.34	4.54
Mg-dd/cmol/Kg	1.65	1.46	0.91
K-dd/mol/Kg	0,30	0.13	0.24
Na-dd/cmol/Kg	0,37	0.16	0.21
KTK	24.85	25.82	18.95
KB (%)	42	24	31

Table 2: Diversity index of fungi in irrigated rice fields (IRF), organic rice fields (ORF) and rainfed rice fields (RRF)

Location of rice fields	Observed	Shannon	Simpson	InvSimpson
Irrigated	471	24.063.382.960.562	0.656887	291.449.506.823.417
Organic	574	486.308.777.550.616	0.981649	544.937.444.810.428
Rainfed	785	434.116.329.565.858	0.945295	182.798.021.845.957

Prolonged and excessive application of pesticides can alter soil acidity. In comparison to conventional farming systems, organic rice fields exhibit higher soil pH levels, approaching neutrality due to the incorporation of organic matter[21,22]. Organic rice fields (ORF) demonstrate the highest organic carbon content relative to irrigated rice fields (IRF) and rainfed rice fields (RRF). Elevated carbon content signifies enhanced activity of soil organisms, whereas the diminished organic carbon content in rainfed rice fields (RRF) suggests reduced soil organism activity, likely due to chemical input exposure. Conventional farming practices in irrigated and rainfed rice fields influence the activity of soil microorganisms

Diversity index of fungi in irrigated rice fields (IRF), organic rice fields (ORF) and rainfed rice fields (RRF): The fungal diversity index in different planting systems is shown in Table 2. Analysis of diversity indices revealed that fungal communities in organic paddy fields exhibited the highest Shannon and Simpson values, signifying greater species diversity and a more even distribution. In contrast, irrigated fields demonstrated the lowest Shannon and Simpson values, indicating reduced species diversity and distribution. The findings suggest that organic fields support higher biodiversity compared to irrigated and rainfed fields. Although rainfed fields recorded the highest observation values, their Shannon and Simpson values were lower than those of organic fields, possibly due to incomplete detection. This discrepancy may be attributed to the absence of chemical soil pressure in organic fields, which helps maintain and diversify organic matter content.

Analysis of relative abundance at the species, genus and family levels revealed variations in the population diversity of certain dominant fungal species, genera and families across different locations. The observed differences in diversity dominance highlight the top ten fungi at the species, genus and family levels. The abundance analysis at these taxonomic levels indicated that several taxa exhibited changes at each location.

Relative abundance at the species level: Relative abundance at the species level across different rice cultivation systems is shown in Fig. 4a. Clear differences in species composition were observed among irrigated, organic and rainfed rice fields. The irrigated rice field was strongly dominated by *Talaromyces wortmannii*, which accounted for approximately 75-80% of the total relative abundance, indicating a highly uneven fungal community structure. In this system, other species contributed only minor proportions, each representing less than 10% of the total community.

In contrast, the organic rice field exhibited a more balanced species composition, with no single species overwhelmingly dominating the community. Several taxa, including *Rhexocercosporidium microsporum*, Starmerella, *Loekoeslaszloa huriana* and members grouped as "Others", contributed moderate proportions to the overall abundance, reflecting higher evenness compared to the irrigated system. Similarly, the rainfed rice field showed a distinct pattern characterized by the dominance of Gorgonomyces haynaldii, accompanied by notable contributions from *Loekoeslaszloa huriana* and Starmerella, while *T. wortmannii* was absent.

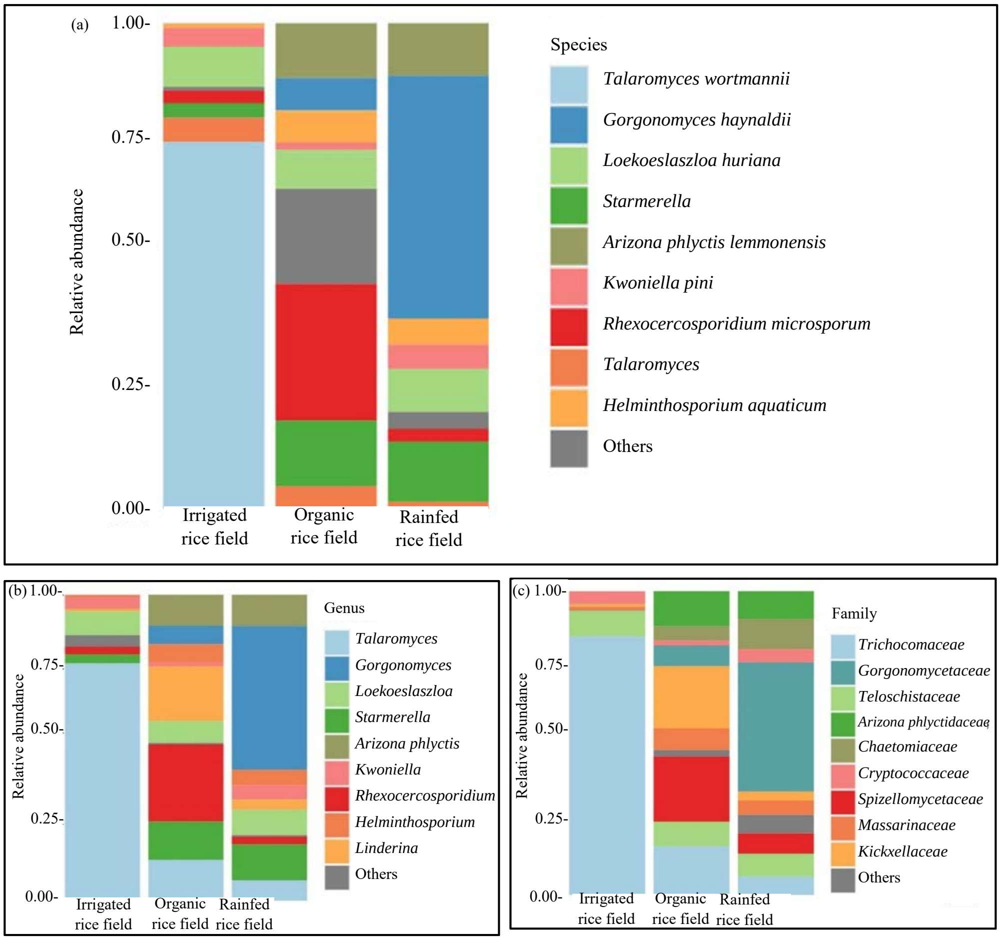

Fig. 4(a-c): Top 10 of relative abundance at (a) Species, (b) Genus, (c) Family level

The absence of *T. wortmannii* in both organic and rainfed fields suggests that this species is closely associated with irrigated conditions, likely due to sustained soil moisture availability. *Talaromyces wortmannii* (synonymous with *Talaromyces variabilis*[23]) is commonly reported in wet environments such as coastal soils[24] and water reservoirs[25]. This species is known to produce bioactive secondary metabolites, including wortmannin and wortmannilactones A-D[26,27], which exhibit antimicrobial activity and may influence rhizosphere microbial dynamics by suppressing competing fungi.

Meanwhile, *L. huriana* was consistently detected in organic and rainfed systems, indicating broader ecological adaptability under less water-intensive cultivation practices. Members of this group are associated with lichen-forming fungi and exhibit symbiotic characteristics with photosynthetic organisms. *L. huriana* has been reported to share similarities with *Yoshimuria spodoplaca*[28,29], suggesting potential functional roles in nutrient cycling and ecosystem stability under diversified or rainfed management systems.

Most other species contributed less than 10% each. Notably, *T. wortmannii* was absent in organic or rainfed locations. This species is synonymous with *Talaromyces variabilis*[23]. It is frequently found in wet environments, such as coastal soils[24] and water reservoir[25]. *Talaromyces wortmannii* is known for producing bioactive compounds, such as wortmannin, which possess significant biological properties, including anticancer activity and microbial growth inhibition. *Talaromyces wortmannii* can inhibit approximately 40-55% of the mycelial growth of *C. capsici, F. oxysporum f.sp. cubense* and *P. palmivora*[26]. The fungi in question have the potential to influence local microbial ecosystems through their production of antimicrobial compounds. Specifically, *T. wortmannii* is known to synthesize wortmannilactones A-D[27]. Additionally, certain species within the Talaromyces genus are capable of producing bioactive compounds, such as talaroderxines A and B from *T. derxii*, which exhibit activity against *Bacillus subtilis*[28].Under specific conditions, *T. wortmannii* may also serve as an indicator of ecosystem health. The species

Talaromyces wortmannii. Another species, *Loekoeslaszloa huriana*, comprising 671 species, was also identified in two other fields: Organic (57 species) and rainfed (435 species). These microbes are classified within the Liken species group due to their symbiotic characteristics and structures, as well as their association with photosynthetic organisms. *Loekoeslaszloa huriana* bears similarity to *Yoshimuria spodoplaca*[29].

The total number of species identified on organic land is 2,608. The species *Rhexocercosporidium microsporum* exhibited the highest prevalence, contributing 28.11% to the total. Other notable species include Starmerella, accounting for 13.57% and a category labeled as Others, comprising 19.58%. However, species within the genus Rhexocercosporium have demonstrated the ability to control pathogenic fungi such as *Colletotricum gloeosporioides*, *Fusarium solani* and *Alternaria panax* Whetzel on Panax notoginseng[30]. There is information that another Starmerella species, namely *Starmerella bacillaris,* can control *Botrytis cinerea* disease[31].

In contrast, the total number of species identified in rainfed rice fields was 8,047, with *Gorgonomyces haynaldii* being the most prevalent, contributing 49.98% to the total. Starmerella also made a significant contribution, accounting for 12.41%. *G. haynaldii* was classified within the phylum Chytridiomycota, which was generally acknowledged for its critical role in the decomposition and recycling of nutrients within aquatic ecosystems[32].

Relative abundance at the genus level: The relative abundance of fungal genera across rice cultivation systems is shown in Fig. 4b. The irrigated rice field was dominated by Talaromyces, which accounted for 77.56% of the total fungal community, whereas Arizonaphlyctis was present at a very low proportion (0.03%). In the organic rice field, Rhexocercosporidium was the most abundant genus, contributing 25.22% of the community, accompanied by several other genera at moderate levels, indicating higher genus evenness. The rainfed rice field showed a distinct composition characterized by the dominance of Gorgonomyces, with lower representation of Talaromyces, highlighting clear differences in genus-level community structure among cultivation systems.

Meanwhile, rainfed land is significantly influenced by the genus Gorgonomyces, which comprises 46.99% of the fungal community, suggesting its adaptability to rainfed conditions. Talaromyces is known to inhabit a variety of environments, with approximately 50 species isolated from diverse soil types, including forest, agricultural and swamp soils[33]. This genus belongs to the phylum Ascomycota and is noted for producing a range of secondary metabolites, such as esters, terpenes, steroids, alkaloids, polyketides and anthraquinones. Some of these compounds exhibit biological activities, including anti-inflammatory, bacteriostatic and antitumor properties.

Relative abundance at the family level: The relative abundance of fungal families across rice cultivation systems is presented in Fig. 4c. The irrigated rice field was dominated by Trichocomaceae, which accounted for approximately 67.95% of the fungal community, followed by the "others" group (20.25%). In contrast, the organic rice field was largely dominated by the "others" category, comprising 79.38% of the total family-level abundance, indicating a more heterogeneous fungal assemblage. Similarly, in the rainfed rice field, the "others" group represented the largest proportion (46.37%), while the remaining families contributed smaller relative abundances.

The Trichocomaceae family exhibits significant dominance on irrigated land compared to organic and rainfed land. The substantial presence of the "other" category in organic and rainfed systems suggests that it encompasses highly adaptive microorganisms. Notably, the Trichocomaceae family, particularly the genera Aspergillus and Penicillium, plays a crucial role in soil ecosystems as saprotrophs (decomposers), facilitating nutrient cycling and enhancing soil structure. The Trichocomaceae family promotes water-stable soil aggregation through the secretion of extracellular materials and the formation of hyphal networks during growth[34].

The distribution of reads across various taxonomic levels is visualized in the Sankey diagram. The Sankey diagram in (Fig. 5) depicts the flow of data across different taxonomic levels, based on the number of reads classified within the dataset, utilizing taxonomic references. In the irrigated field, the dataset was predominantly represented by the species Talaromyces wortmannii, which accounts for the largest number of reads (6.130 out of a total of 19.100 reads). Figure 5 indicates that the dataset is primarily composed of Fungi, with a significant presence of species within the phylum Ascomycota, family Trichocomaceae and genus Talaromyces.

On organic land, the predominant phyla are Ascomycota, with 6.01k reads and Chytridiomycota, with 1.420 reads. Within Ascomycota, the families Cyphelohoraceae and Aspergillaceae exhibit significant read counts, with 188 and 104 reads, respectively. In Chytridiomycota, the family Terramycetaceae records the highest number of reads at 245. The dominant species identified include *Rheoxocescosporium microsporum* from Ascomycota with 201 reads, *Terramyces subangulosum* from Chytridiomycota with 162 reads and

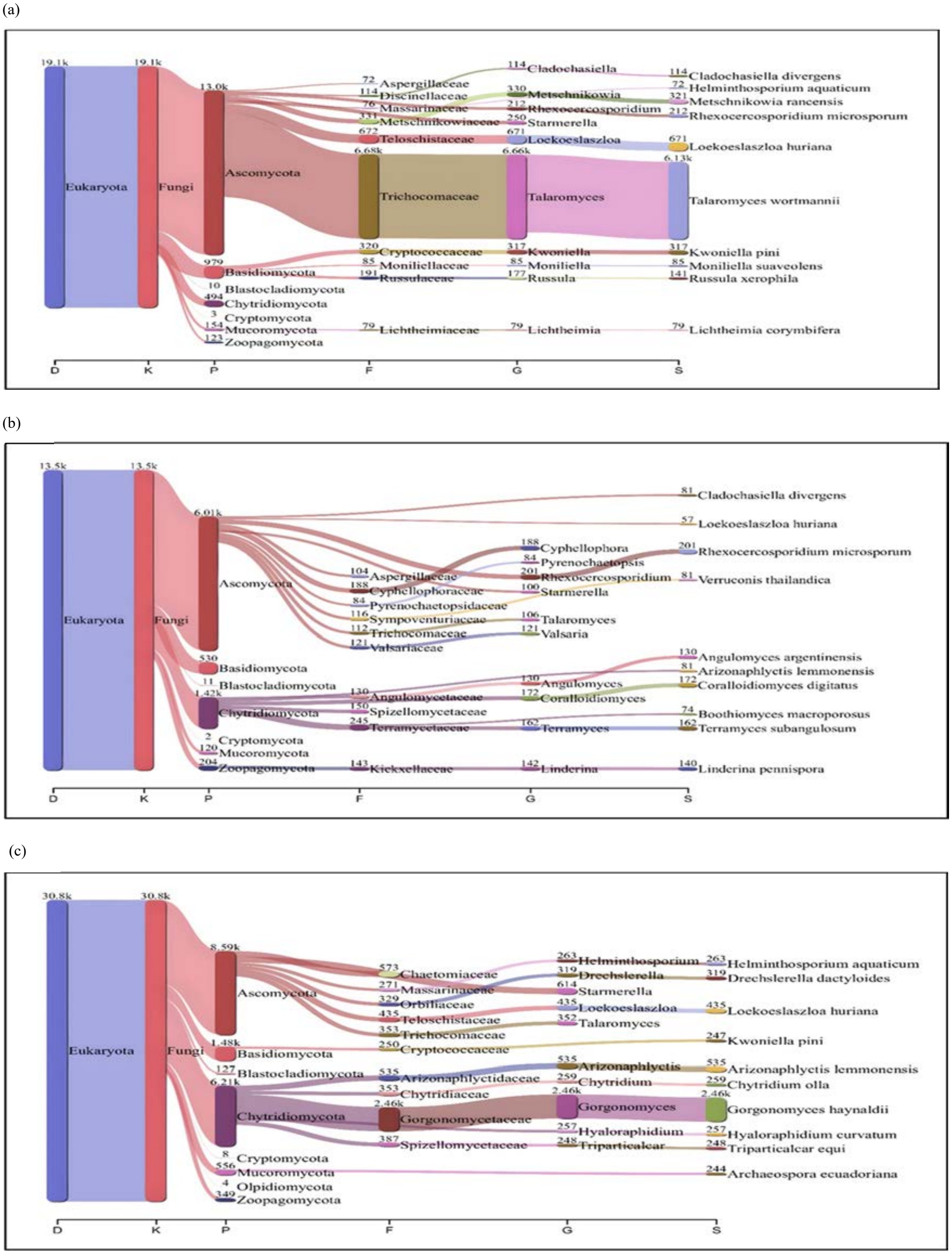

Fig. 5(a-c): Sankey diagrams illustrating the composition of microbial diversity across various taxonomic levels, including domain, kingdom, phylum, family, genus and species. The diagrams are categorized as follows: (a) Irrigated, (b) Organic and (c) Rainfed

Abbreviations: D: Domain, K: Kingdom, P: Phylum, F: Family, G: Genus and S: Species, Symbols: Rectangular nodes represent taxonomic ranks, while connecting flows indicate hierarchical relationships among taxa., Units: Numbers displayed on nodes and flows represent the total number of sequences (read counts) assigned to each taxon and The width of each flow is proportional to the relative contribution of taxa at each taxonomic level. Only dominant taxa are shown for clarity

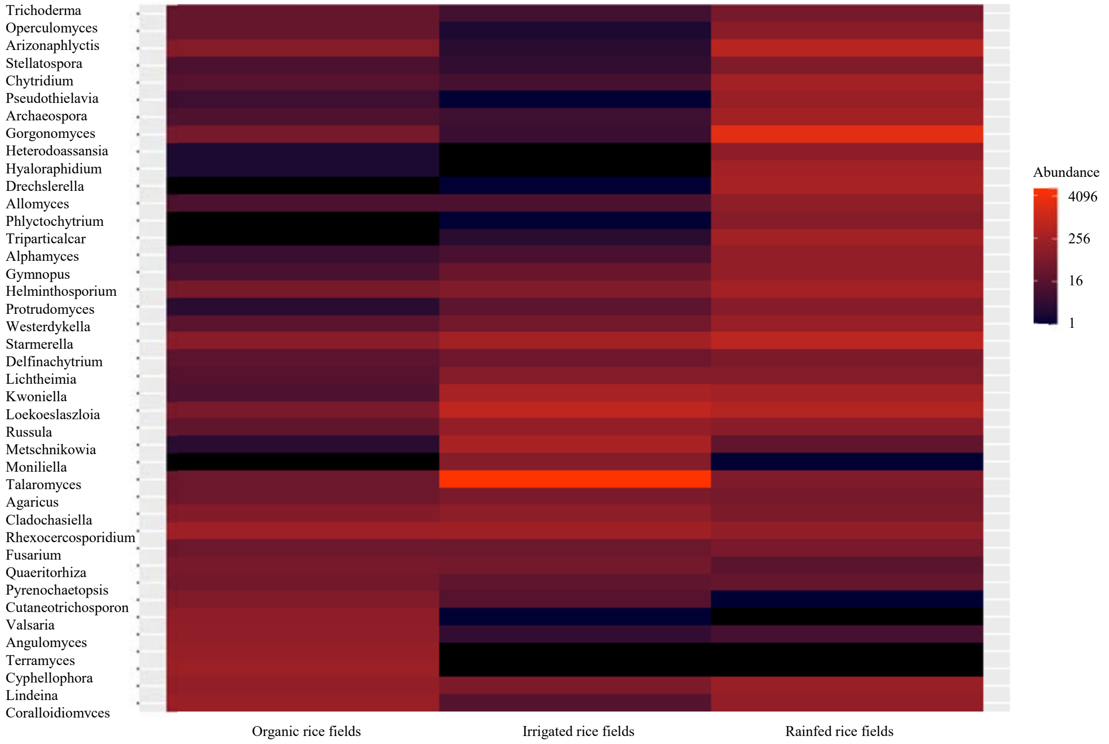

Fig. 6: Heatmap on family level

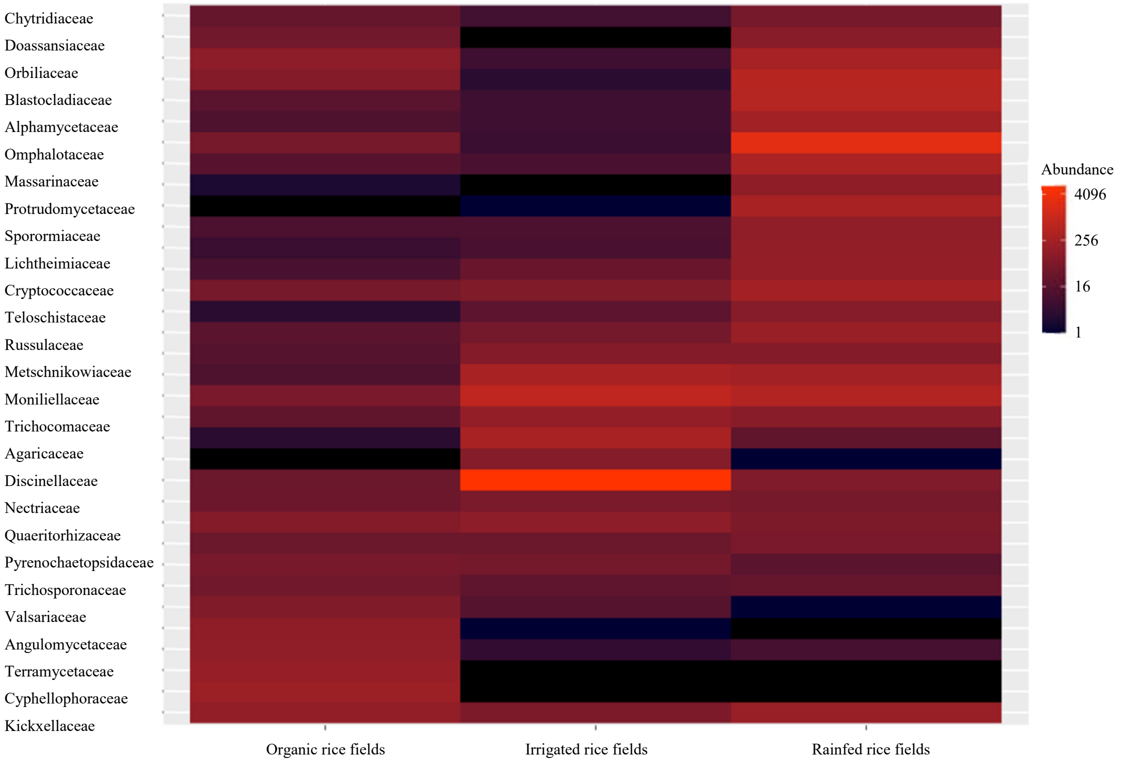

Fig. 7: Heatmap on genus level

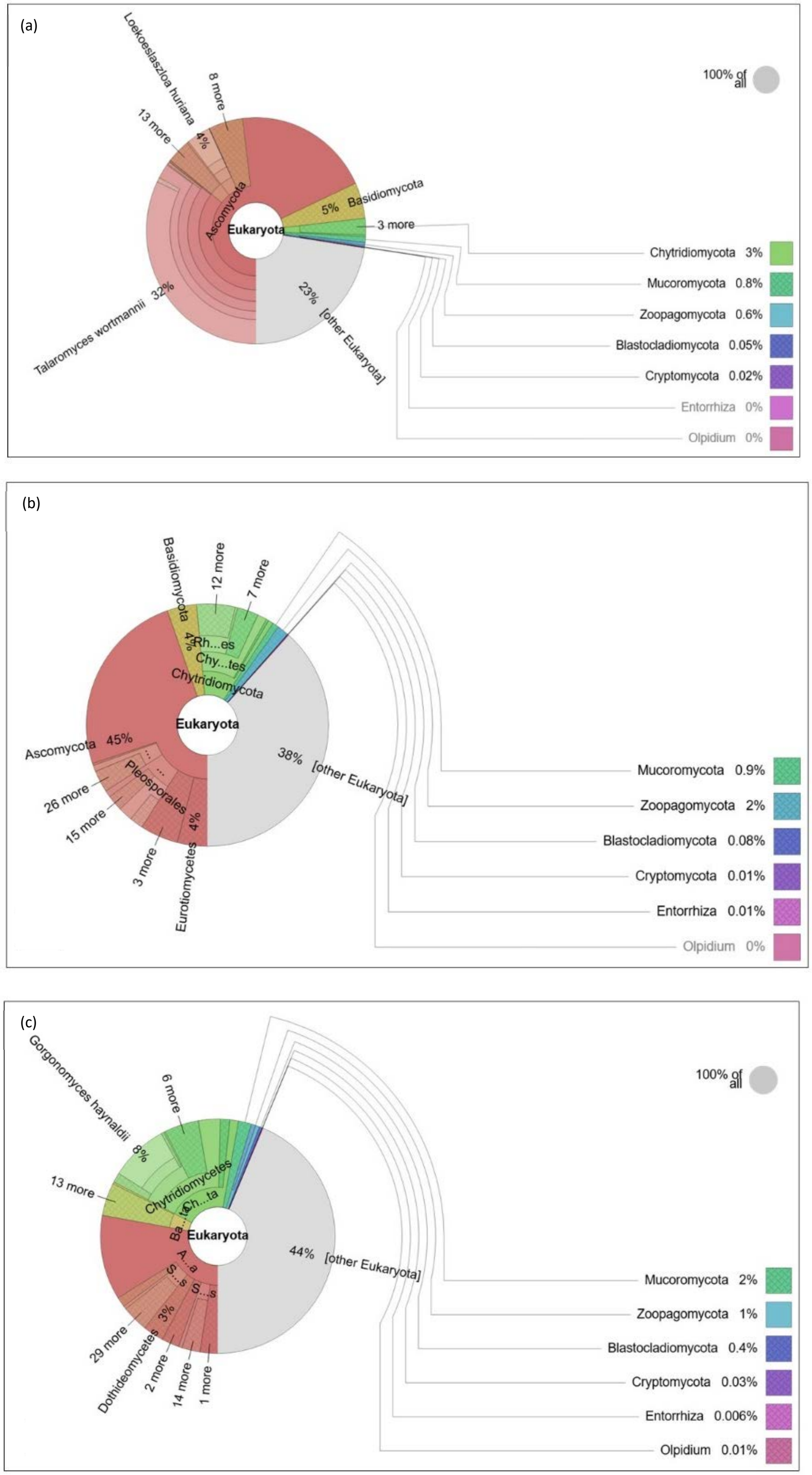

Fig. 8(a-c): Krona Visualization on (a) irrigated rice fields, (b) Organic fields and (c) Rainfed fields

Linderina pennispora from Zoopagomycota with 140 reads. The microbial community in this dataset is primarily dominated by the Ascomycota phylum, with notable contributions from the Cyphelohoraceae family and the Cyphellophora genus. Among the species, *Rheoxocescosporium microsporum* and *Terramyces subangulosum* have the highest read counts within their respective phyla.

In contrast, on rainfed land, the dominant phyla are Ascomycota, with 8.590 reads and Chytridiomycota, with 6.210 reads. Within Ascomycota, the families Trichocomaceae and Teloschistaceae exhibit high read counts. In Chytridiomycota, the family Gorgonomycetaceae records the highest number of reads at 2.460. The dominant species identified are Gorgonomyces haynaldii from Chytridiomycota and Loekoeslaszloa huriana from Ascomycota. The microbial community in this dataset is characterized by the dominance of the Ascomycota and Chytridiomycota phyla, with the distribution of reads extending to more specific taxonomic levels, including species such as Gorgonomyces *haynaldii* and *Loekoeslaszloa huriana*. This Sankey diagram provides a visual representation of the taxonomic diversity within the dataset.

The subsequent heatmap depicts the relative abundance of taxa from each sample through the use of color gradients, facilitating the identification of patterns among the samples. Red signifies taxa with high distribution, whereas black denotes low relative abundance. The heatmap presented here was at the family and genus level.

The heatmap illustrating fungal density reveals that certain phyla within the fungal domain, identified in rainfed rice fields, exhibit a predominance of red coloration compared to darker hues. Conversely, at the family level, these fungi tend to display darker colors. The darker coloration signifies a higher abundance of specific fungi in rainfed rice fields (Fig. 6-7). All diversity index parameters demonstrate that rainfed rice fields possess greater fungal diversity relative to irrigated and organic rice fields.

Krona visualization: Krona visualization facilitates an intuitive exploration of relative abundance within complex metagenomic classification hierarchies. The numerical data in Krona are derived from the num Reads of Centrifuge Reports (Fig. 8).

The Krona plot provides a hierarchical visualization of fungal taxonomic composition across different rice cultivation systems, displaying relative abundances from higher (phylum) to lower (genus/species) taxonomic levels. Across all field types, Ascomycota was the dominant phylum, with clear differences in the relative contribution of lower taxonomic ranks among irrigated, organic and rainfed systems.

Figure 8a shows that the fungal community was dominated by taxa belonging to the phylum Ascomycota. Similarly, Ascomycota was also dominant in Fig. 8b, although at a lower relative abundance. In contrast, Fig. 8c exhibited the lowest relative abundance of Ascomycota among the three systems. The respective values were 85.8, 55.3 and 40.3%, respectively.

They create interpretations and demonstrate dominance patterns and how microbial taxa are interconnected within the rhizosphere[35]. Alpha diversity refers to the mean species diversity within a specific location or habitat at a local scale. The diversity of microbial communities in the rhizosphere is affected by the availability of nutrients, which is influenced by the activity of plant root exudates[36].The fungal community is primarily composed of Ascomycota, Mortierellomycota and Basidiomycota[37]. There exists a significant correlation between the abundance of fungi in the rhizosphere and the physico-chemical properties of the soil[38]. Complex microbial communities associated with plants play a crucial role in maintaining plant health[39]. Through metagenomic analysis, fungi from the rhizosphere have been identified as potential biocontrol agents that offer environmentally friendly alternatives and contribute to sustainable rice production systems.

CONCLUSION

Metagenomic analysis revealed significant variation in rhizosphere fungal diversity across different rice cultivation systems. Irrigated fields were dominated by *Talaromyces wortmannii* at the species level and the genus *Talaromyces*, while organic fields showed a higher representation of *Rhexocercosporidium microsporum*. In rainfed systems, *Gorgonomyces haynaldii* and the genus *Gorgonomyces* were the most prevalent. At the family level, *Trichocomaceae* dominated irrigated fields, whereas *Gorgonomycetaceae* was more prominent in rainfed systems and diverse fungal groups were observed in organic fields. These findings highlight the influence of cultivation practices on shaping rhizosphere fungal communities and provide insight into the ecological diversity associated with different rice production systems.

SIGNIFICANCE STATEMENT

This study demonstrates the effectiveness of metagenomic approaches in revealing the diversity of rhizosphere fungi associated with different rice cultivation systems. Understanding the variation in fungal communities under irrigated, organic and rainfed conditions provides valuable information for improving soil health, nutrient cycling and sustainable rice production. The findings contribute to better management of beneficial microbial communities that can support crop productivity and ecological balance in agricultural systems.

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