International Journal of Agricultural Technology 2023 Vol. 19(2):781-804
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Mycobiome in soils from irrigated, rice-based farming systems in Apalit, Pampanga and Banaue, Ifugao, Philippines: Diversity and potential agro-biotechnological applications determined using targeted metagenomics

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Tabao, N. S. C., Mendoza, B. C., Pedro, M. S. and Monsalud, R. G. (2023). Mycobiome in Soils from irrigated, rice-based farming systems in Apalit, Pampanga and Banaue, Ifugao, Philippines: Diversity and potential agro-biotechnological applications determined using targeted metagenomics. International Journal of Agricultural Technology 19(2):781-804.

Abstract Mycobiomes of soils from two irrigated, rice-based farming systems in the Philippines, one in Apalit, Pampanga and another in Banaue, Ifugao, were assessed and compared through targeted metagenomics. The different phyla identified in this study were Aphelidiomycota, Ascomycota, Basidiomycota, Chytridiomycota, Entorrhizomycota, Glomeromycota Monoblepharomycota, Mortierellomycota, and Rozellomycota. All except Aphelidomycota were found in both sites. The most abundant fungal genera observed were *Arnium*, *Fusarium*, *Neurospora*, *Talaromces*, unidentified Pleosporales (Ascomycota), *Saitozyma*, *Westerdykella*, Massariosphaeria, *Boothiomyces*, *Nakataea*, *Penicillium*, unidentified Sordariales (Ascomycota), unidentified Sordariomycetes (Ascomycota), unidentified (Rozellomycota), unidentified Chytridiomycota, and the unidentified fungal genus. These phyla and genera had varying relative abundances and dominance across rice cultivations stages in the sites. The unidentified fungal phylum in the sites had sizeable abundances across rice cultivation stages in both sites that indicate its possible importance in these areas. Samples from Banaue, Ifugao had greater species richness and evenness compared to those from Apalit, Pampanga based on Chao1, Fisher, Shannon, and Simpson alpha diversity indices. The fungal diversities of both sites were different based on their weighted and unweighted Unifrac distances and PCoA ordination plot. Soil chemical characteristics did not correlate to fungal diversity collectively in each site but correlated well with specific taxonomic compositions at the phylum and genus levels. The uncovered composition and variation of mycobiomes of soils in these sites may help provide information for those seeking potential solutions to challenges faced in the sites and similar irrigated, rice-based farming systems such as low grain yield, pests, or diseases.

Keywords: Fungal community structure, Internal transcribed spacer (ITS) sequence, Next-generation sequencing (NGS), Rice-based farming systems, Edaphic factors

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Introduction

Rice (*Oryza sativa* L.) is a world food staple and Filipinos depend on it daily. In the past years, the Philippines produced an average of 4.5 million MT of rice with an average yield of 3.85 MT per hectare (PSA, 2019). Although the Philippine population growth rate has significantly slowed down at 1.72% (PSA, 2020), the current population estimated at 110 million is among the largest in the Southeast Asian region. The estimated annual per capita (per person) consumption of rice in the country is at 109.5 kg (Palanog *et al*., 2019), which means the country needs at least 12 million MT of rice to support the population. The current average production of rice that remains almost stably at its current rate is significantly insufficient to feed the current population. This situation could be worsened by climate change, particularly increasing temperatures that could reduce yield by 3.2 ± 3.7% (Zhao *et al*., 2017) and production figures may likely suffer in the next ten years if not mitigated. This will force the Philippines to depend heavily on rice imports to supplement the gap amidst growing local and global demand. Thus, it is relevant to find smarter and sustainable ways to improve rice production and yield in the Philippines, aside from increasing the conversion of forest land to rice production (Tilman *et al*., 2011).

Maximizing rice yield potential has long been the objective of many agricultural scientists (Li *et al*., 2019). Rice production and yield depend largely on cultivar and soil health, which is affected by various factors such as soil characteristics, water and nutrient availability, fertilizer inputs or organic amendments, pest and disease control, and microbial diversity, among others. Microbial diversity in agricultural soils had long been recognized by many soil scientists as a critical factor in maintaining soil health as it is assumed to promote stability and resilience towards disturbances, anthropogenic and natural alike, especially now in the age of climate change (Garbeva *et al*., 2004).

The beneficial impact of soil fungi in crop production and yield had already been reported (Dellagi *et al*., 2020), however, the fungal diversity that is specific to the different rice-based farming systems in the Philippines has yet to be documented. Specifically, the fungal diversities of fully irrigated rice-based farming systems in Apalit, Pampanga and Banaue, Ifugao have not yet been uncovered. To date, there are no published reports on the mycobiomes of these sites. Determining the taxonomic compositions of the fungal communities in these sites can yield information that may be beneficial for rice farming in the sites and at large. The study provided initial information on the edaphic characteristics and composition, variation of mycobiomes of soils in these sites

and their relationships, and the potential of the taxonomic compositions for use in agriculture and rice farming.

Materials and methods

Study site characterization

The characterization of the farming system and selection of the study sites were done through a series of key informant interviews (KII) at the regional, provincial, municipal, and village (barangay) levels of the study sites. The Global Positioning System (GPS) coordinates and altitude of both sites were taken and recorded using GPS & Maps: Location Tracker (iOS version 2.8) and confirmed with Polaris Navigation GPS (Android version V.9.22), while the Philippine standard geographic codes (PSGC) of both sites were taken from the PSGC database (https://psa.gov.ph/classification/psgc/) of the Philippine Statistics Authority. The average rainfall and temperatures were taken from the nearest Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) agrometeorological or synoptic station from the site during the duration of the project. Data were requested from and provided by the Department of Science and Technology (DOST)-PAGASA.

Soil sampling

Soil samples (~1kg) were taken using a spade from a depth of 0-20 cm and diagonally from three sampling points in each of three plots within two distinct rice-based farming systems in Apalit, Pampanga (Site 1) and Banaue, Ifugao (Site 2). The samples were taken during the three cultivations stages of rice planted in the site, particularly the before-planting (BP), full-booting (FB), and harvest (HA) stages. These were placed in autoclavable plastic bags and sealed tightly with rubber bands to minimize moisture loss while being transported to the Philippine National Collection of Microorganisms (PNCM) laboratory at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), where the samples were immediately processed.

Fungal community analysis of soils from the study sites

Metagenomic DNA (gDNA) was extracted from soil preparations using a DNeasy PowerSoil (Qiagen, Germany) kit following the manufacturer's instructions. The quality of the eluted gDNAs were checked by electrophoresis

at 50 V for 2 h in a 0.8% (w/v) agarose gel pre-stained with Gel Red in 1X TAE buffer and visualized under UV light. The gDNA were also quantified with nanophotometer (Implen, Germany) and then stored at −80°C until further use. The gDNA samples were submitted to the sequencing facility of 1st BASE/Apical Scientific (Malaysia) for next generation sequencing and bioinformatic analysis. Amplification of the ITS region was performed using pair-end (PE) Illumina MiSeq platform that generated 300bp raw reads. Sequence adapters and low-quality reads were trimmed and removed from the raw reads using BBDuk (version 38.76) (http://jgi.doe.gov/data-and-tools/bb-tools/). The raw reads were aligned and merged using QIIME2 (version 2019.10) (Bolyen *et al*., 2019). The Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline (version 1.14) (https://benjjneb.github.io/dada2/) was used to remove and/or correct error reads (denoising), remove low quality regions and chimeric errors to obtain amplicon sequence variant (ASV) (Nearing *et al*., 2018). The taxonomic classification of the resulting ASVs was done by using scikit-learn (https://scikit-learn.org/stable/) and Naive Bayes classifier against database UNITE ITS version 8.2 (Nilsson *et al*., 2018; Kõljalg *et al*., 2020). The alpha diversity of the samples was measured using Chao1 (Chao and Chiu, 2014), Fisher (Fisher *et al*., 1943), Shannon (Shannon, 1948), and Simpson (Simpson, 1949) diversity indices. The beta diversities were measured using quantitative (weighted UniFrac) and qualitative (unweighted UniFrac) distance metrics, which were illustrated in a PCoA plot using RStudio (2021.09.1 Build 372 "Ghost Orchid" Release (8b9ced188245155642d024aa 3630363df611088a, 2021-11-08) for Windows.

Soil Physico-Chemical analysis

Soil samples were submitted to the Analytical Service Laboratory of the Agricultural Systems Institute, UPLB for the determination of Soil pH (pH-H_2O), Total organic carbon (OM), Total nitrogen (N), Available phosphorus (P), Exchangeable potassium (K), Cation exchange Capacity (CEC), Electrical Conductivity (EC) (for Apalit, Pampanga only), and Available Zinc (Z). Soil moisture content determination was conducted at the PNCM.

Data analysis

Downstream analyses were performed at the PNCM. Among these were statistical analyses that included F-Test (Two-Sample for Variances) to determine sample variances, t-Test:Paired Two Sample for Means, Two-sample assuming equal variances, and two-sample assuming unequal variances) to

compare paired means, Analysis of Variance (ANOVA): Single Factor and to compare within and in between group means, and Spearman's Correlation Analysis to determine the relationships among the different chemical characteristics of the site soils and their fungal compositions. Post-hoc testing was also done through Least Significant Difference Tests (LSDT). Downstream bioinformatic work included literature and database comparisons.

Results

Profile of the study sites

The geographical, climatic, farming system, and edaphic (physico-chemical and nutrient) profile of the sites are presented in Tables 1-3.

Table 1. Geographical, climatic, edaphic, and farming systems profile of the study sites in Apalit, Pampanga (Site 1), and Banaue, Ifugao (Site 2), Philippines during the study period in 2019

Parameter	Site 1	Site 2
Province	Pampanga	Ifugao
Municipality	Apalit	Banaue
Barangay	Colgante	Gohang
Geocode	035402007	142701011
GPS Location	14 °55.820' N, 120 °44.170' E	16 °55.342' N, 121 °02.956' E
Altitude (masl)	5.00	1,228.00
Climatic Conditions*		
Average rainfall (mm)	2,073	3,933
Temperature Range (°C)	25.9 – 30.0	18.0 – 21.1
Average Temperature (°C)	27.6	19.5
*Season***	Dry Season (4 dry months)	Dry spanning wet (4 dry months, 4 wet months)
Farming System	Fully Irrigated, Lowland	Fully Irrigated, Lowland
Rice variety	Hybrid (Commercial brand)	Heirloom (*Tinawon*)
Cropping pattern	Rice-Rice	Rice-Taro
Rice Cropping period	4 months (January – April 2019)	8 months (January – August 2019)
Average yield	7.4 t ha^{-1}	0.6 t ha-1
Fertilization program	Inorganic (High input) 15-15-10 kg ha^{-1} of N-P_2O_5-K_2O	Rice straw incorporation
Pest management		
Insect Control	No	No
Weed Control	Water management	Manual (pulling of weeds)

*Data taken from PAG-ASA Synoptic Stations in Clark City and Baguio City for Site 1 and 2, respectively, from 2008-2020. **Covered by study period. Dry and wet months were based on the Oldeman Climate Zoning for the Agricultural Area (Maru *et al*., 2016).

Table 2. Physico-chemical characteristics of soil samples taken during the before planting and harvest stages of rice from the study sites in Apalit, Pampanga (Site 1) and Banaue, Ifugao (Site 2), Philippines

Study Site	Textural grade		Soil pH		Electrical conductivity (mS cm^{-1})		Cation exchange capacity (cmol kg^{-1})		Moisture content (%)	
	BP	HA	BP	HA	BP	HA	BP	HA	BP	HA
Site 1	Loam	n.d.	6.50	5.10	1.32	3.01	25.36	25.67	66.20	31.38
Site 2	Loam	n.d.	5.90	4.40	n.d.	n.d.	33.49	32.32	108.40	194.70

Legend: BP – Before Planting, HA – Harvest

Table 3. Nutrient content of soil samples taken during the before planting and harvest stages of rice from the study sites in Apalit, Pampanga (Site 1) and Banaue, Ifugao (Site 2), Philippines

Study Site	Total Organic Carbon		Total Nitrogen		Available Phosphorus (ppm)		Exchangeable Potassium (cmol/kg)		Available Zinc	
	BP	HA	BP	HA	BP	HA	BP	HA	BP	HA
Site 1	Loam	n.d.	6.50	5.10	1.32	3.01	25.36	25.67	66.20	31.38
Site 2	Loam	n.d.	5.90	4.40	n.d.	n.d.	33.49	32.32	108.40	194.70

Legend: BP – Before Planting, HA – Harvest

Fungal taxonomic profile of study site 1: Apalit, Pampanga

The most abundant soil fungal phyla (≥1% relative abundance or r.a.) in Site 1 at BP were Ascomycota (80%), Unidentified Phylum (13%), Entorrhizomycota (4%), and Basidiomycota (2%). At FB these were Ascomycota (60%), Unidentified Phylum (36%), Chytridiomycota (2%), Rozellomycota (1%), and Basidiomycota (1%). At HA these were Ascomycota (85%), Unidentified Phylum (11%), and Basidiomycota (4%).

On the other hand, the rare phyla (<1% r.a.) at BP were Glomeromycota (0.4%), Chytridiomycota (0.2%), Rozellomycota (0.2%), Monoblepharomycota (0.1%), and Mortierellomycota (0.04%). At FB these were Mortierellomycota (0.4%), Glomeromycota (0.2%), Entorrhizomycota (0.2%), and Aphelidiomycota (0.02%). At HA these were Rozellomycota (0.2%) and Chytridiomycota (0.1%). Together, the most abundant and the rare phyla made up 100% of the abundance for each stage in the site.

The top ten (10) or the most abundant soil fungal genera in Site 1 at BP were: 1. *Stachybotrys* (38%), 2. unidentified Fungi (16%), 3. *Westerdykella* (7%), 4. *Apiosordaria* (6%), 5. *Zopfiella* (5%), 6. *Talbotiomyces* (5%), 7.

Talaromyces (2%), 8. unidentified (Sordariales, Ascomycota) (2%), 9. *Serendipita* (2%), and 10. unidentified (Pleosporales, Ascomycota) (2%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 16% of the remaining abundance.

At FB, the top 10 genera were: 1. unidentified fungi (42%), 2. *Stachybotrys* (7%), 3. *Zopfiella* (7%), 4. *Monocillium* (6%), 5. unidentified (Chaetomiaceae, Ascomycota) (4%), 6. *Westerdykella* (4%), 7. *Ochroconis* (3%), 8. *Talaromyces* (3%), 9. unidentified (Sordariomycetes, Ascomycota) (3%), and 10. *Penicillium* (2%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 19% of the remaining abundance.

At HA, the top 10 genera were: 1. unidentified (Sordariales, Ascomycota) (37%), 2. unidentified Fungi (13%), 3. *Achroiostachys* (8%), 4. *Zopfiella* (Ascomycota) (7%), 5. unidentified (Sordariomycetes, Ascomycota) (6%), 6. *Westerdykella* (3%), 7. *Tremella* (3%), 8. *Talaromyces* (3%), 9. *Ascosacculus* (Ascomycota) (2%), and 10. *Cirrenalia* (2%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 16% of the remaining abundance.

Fungal taxonomic profile of study site 2: Banaue, Ifugao

The most abundant soil fungal phyla (≥1% r.a.) in Site 2 at BP were Unidentified Phylum (40%), Chytridiomycota (37%), Ascomycota (18%), Rozellomycota (3%), and Basidiomycota (2%). At FB these were Unidentified Phylum (79%), Ascomycota (18%), Rozellomycota (2%), and Basidiomycota (1%). At HA these were Unidentified Phylum (72%), Rozellomycota (12%), Ascomycota (10%), Chytridiomycota (3%), and Basidiomycota (3%). On the other hand, the rare phyla (<1% r.a.) at BP were 1. Monoblepharomycota (0.15%), Glomeromycota (0.12%), and Entorrhizomycota (0.04%). At FB these were Chytridiomycota (0.14%), Monoblepharomycota (0.05%), Glomeromycota (0.02%), and Entorrhizomycota (0.02%). At HA the rare phylum was Glomeromycota (0.04%). Together, the most abundant and the rare phyla made up 100% of the abundance for each stage in the site.

The top ten or the most abundant soil fungal genera in Site 2 at BP were: 1. unidentified Fungi (42%), 2. unidentified (Chytridiomycota) (38%), 3. unidentified (Sordariales, Ascomycota) (4%), 4. unidentified (Sordariomycetes, Ascomycota) (3%), 5. unidentified (Rozellomycota) (3%), 6. *Penicillium* (2%), 7. *Saitozyma* (1%), 8. *Westerdykella* (1%), 9. *Talaromyces* (1%), and 10. *Fusarium* (1%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 6% of the remaining abundance.

The top ten soil fungal genera in Site 2 at FB were: 1. unidentified Fungi (83%), 2. unidentified (Sordariomycetes, Ascomycota) (3%), 3. unidentified (Rozellomycota) (2%), 4. *Nakataea* (2%), 5. *Westerdykella* (2%), 6. *Massariosphaeria* (1%), 7. unidentified (Sordariales, Ascomycota) (1%), 8. unidentified (Pleosporales, Ascomycota) (1%), 9. *Neurospora* (1%), and 10. *Saitozyma* (1%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 3% of the remaining abundance.

The top ten or the most abundant soil fungal genera in Site 2 at HA (Figure 10) include: 1. unidentified Fungi (72%), 2. unidentified (Rozellomycota) (12%), 3. *Boothiomyces* (Chytridiomycota) (2%), 4. *Saitozyma* (Basidiomycota) (2%), 5. *Penicillium* (Ascomycota) (2%), 6. *Massariosphaeria* (Ascomycota) (2%), 7. *Westerdykella* (Ascomycota) (1%), 8. *Talaromyces* (Ascomycota) (1%), 9. *Arnium* (Ascomycota) (1%), and 10. unidentified (Chytridiomycota) (1%). Other fungal genera including the rare fungal genera (<1% r.a.) made up 4% of the remaining abundance.

Fungal diversity of the study sites

The alpha diversity indices of the soils of the study sites were computed to compare the differences in fungal diversity of the sites (Table 4).

Table 4. The alpha diversity indices of the soils under the different cultivation stages of rice in the study sites in Apalit, Pampanga (Site 1) and Banaue, Ifugao (Site 2) during the study period in 2019

Site	Alpha Diversity Index*			
	Species Richness		Evenness	
	Chao1	Fisher	Shannon	Simpson
Site 1	426^{B}	61^{B}	4.35^{A}	0.9434^{B}
Before Planting (BP)	$561^{A,a}$	$83^{A,a}$	$4.26^{B,b}$	$0.9350^{A,b}$
Full Booting (FB)	$466^{B,b}$	$66^{B,b}$	$5.06^{A,a}$	$0.9854^{A,a}$
At Harvest (HA)	$251^{B,c}$	$34^{B,c}$	$3.73^{B,c}$	$0.9098^{B,c}$
Site 2	441^{A}	63^{A}	4.34^{A}	0.9532^{A}
Before Planting (BP)	$449^{B,b}$	$62^{B,b}$	$4.34^{A,b}$	$0.9357^{B,c}$
Full Booting (FB)	$492^{A,a}$	$74^{A,a}$	$4.37^{B,a}$	$0.9599^{B,b}$
At Harvest (HA)	$381^{A,c}$	$54^{A,c}$	$4.31^{A,c}$	$0.9639^{A,a}$

*The mean values are shown. Means across stages with same letter designations are not significantly different by are not significantly different by t-Test (uppercase letters) and Least Significant Difference Test (lowercase letters) at α=0.05.

Site 2 (Banaue, Ifugao) was generally found to have greater species richness than Site 1 (Apalit, Pampanga), based on Chao 1 ($p= 0.003$) and Fisher ($p=0.004$) indices at $\alpha=0.05$. Site 2 (Banaue, Ifugao) also had greater evenness than Site 1 (Apalit, Pampanga), based on their Simpson indices ($p=0.04$) at

α=0.05. When the evenness indices disagree, the Simpson takes precedence as it also predicts dominance.

A Principal Coordinate Analysis (PCoA) plot based on the computed weighted UniFrac distances was constructed to further illustrate the relatedness and dissimilarity of the fungal communities during the different stages of rice in the two sites (Figure 1).

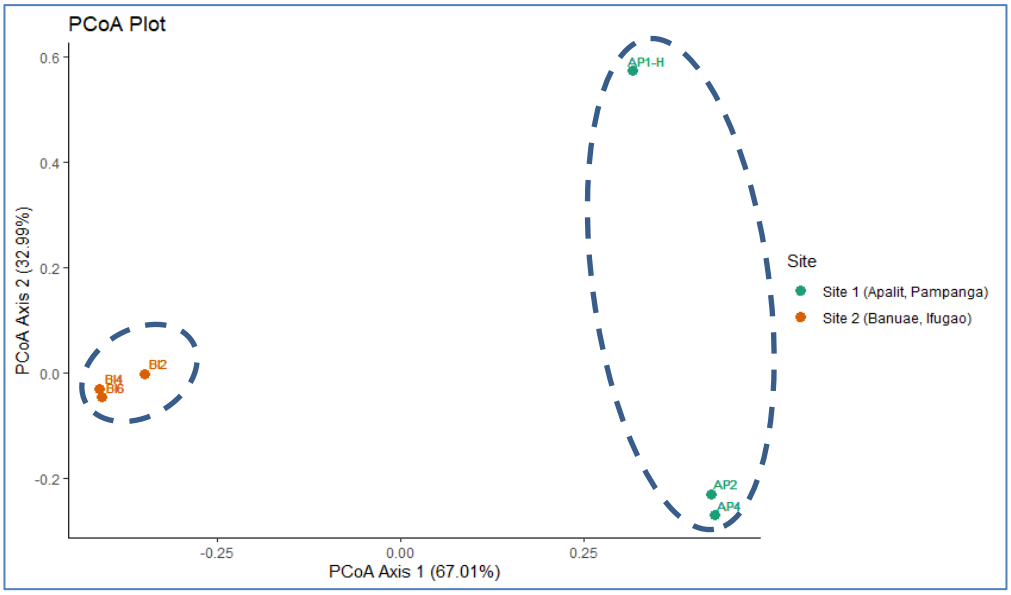

Figure 1. Principal coordinate analysis (PCoA) plot of the weighted UniFrac distances of fungal communities of soils sampled before planting (2), at full booting (4) and at the harvest (1-H, 6) stages of rice planted in Site 1 in Apalit, Pampanga (AP) and Site 2 in Banaue, Ifugao (BI) during the study period in 2019

The variation between the fungal communities can be explained by the two axes, wherein PCoA Axis 1 explains the variation by 67.01% (with respect to location) as opposed to PCoA Axis 2, which explains the variation by 32.99% (with respect to sampling stages).

Correlation of the different factors affecting fungal diversity

The results showed that the different factors and fungal taxa at the phyla levels are significantly correlated at α=0.05 (Table 5). No chemical characteristics correlated with the alpha diversity indices computed for each site.

Table 5. Spearman's correlation of soil chemical characteristics and fungal phyla in the study sites during the study period in 2019

Chemical Characteristics	Apalit, Pampanga (Site 1)		Banaue, Ifugao (Site 2)	
	Positive	Negative	Positive	Negative
pH	OM, P, K, Mon.	EC, CEC, Zn	OM, P, K, CEC	N, Zn
EC (mS/cm)	N, CEC, Zn	pH, OM, P, K, Mon.	N/A	N/A
OM (%)	pH, P, K, Mon.	EC, N, CEC, Zn	pH, P, K, CEC	N, Zn
N (%)	EC, CEC, Zn	pH, OM, P, K, Mon.	Zn	pH, OM, P, K, CEC
P (ppm)	pH, OM, K, Mon.	EC, N, CEC, Zn	pH, OM, P, K, CEC	N, Zn
K (cmol/kg)	pH, OM, P, Mon.	EC, N, CEC, Zn	pH, OM, K, CEC	N, Zn
CEC (cmol/kg)	EC, N, Zn	pH, OM, P, K, Mon.	pH, OM, P, K	N, Zn
Zn (mg/kg)	EC, N, CEC	pH, OM, P, K, Mon.	N	pH, OM, P, K, CEC
MC (%)	-	Ent. Glom.	Unidentified phylum	Chy. Glom.

*Based on computed Spearman's correlation coefficient (r_s or ρ), where a very strong to perfect positive relationship: ρ = +0.90 to +1, or a very strong to perfect negative relationship: ρ = -0.90 to -1 between variables exists (Akoglu, 2018), degrees of freedom=1, α=0.05, N/A – not applicable, parameter not determined. Legend: EC – electrical conductivity (mS/cm), OM – total organic carbon (%), N – total nitrogen (%), P – available phosphorus (ppm), K – exchangeable potassium (cmol/kg), CEC – cation exchange capacity (cmol/kg), Zn – available zinc (mg/kg), MC - moisture content (%), Mon. – Monoblepharomycota, Ent. – Entorrhizomycota, Chy – Chytridiomycota, Glom. – Glomeromycota

For significant correlations involving fungal genera in the sites the Spearman's correlation coefficient, ρ, ranged from +0.90 to +1. In Site 1, fungal genera that positively correlated with pH, OM, P, and K, negatively correlated with EC, N, CEC, and Zn. These genera were: *Arcuadendron*, *Boubovia*, *Chaetomium*, *Keratinophyton*, *Leiothecium*, *Leucosphaerina*, *Magnaporthiopsis*, *Minutisphaera*, *Myrothecium*, *Nakataea*, *Paratrimmatostroma*, *Periconia*, *Phoma*, *Plectosphaerella*, *Pyrenochaetopsis*, *Roussoella*, *Savoryella*, *Schizophyllum*, *Schizothecium*, *Scytalidium*, *Spiromastix*, *Xylaria*, *Zygosporium*, and unidentified genera each belonging to Halosphaeriaceae, Lasiosphaeriaceae, Microascaceae, Pyronemataceae, and Pezizales (Ascomycota); *Candelabrochaete*, *Exidiopsis*, *Fibrodontia*, *Flavodon*, *Phellinus*, *Phyllozyma*, and *Pluteus* (Basidiomycota); *Monoblepharis* (Monoblepharomycota); and unidentfied genera each belonging to Glomeromycota, Paraglomerales, and Glomeraceae.

Fungal genera that positively correlated with MC in Site 1 were: *Apiosordaria*, *Aspergillus*, *Nigrospora*, *Stachybotrys*, *Westerdykella*, and *Zopfiella* (Ascomycota); unidentified ascomycete genera belonging to

Pleosporales, and Sordariomycetes; *Serendipita* (Basidiomycota); *Talbotiomyces* (Entorrhizomycota); and *Paraglomus* (Glomeromycota) while those that negatively correlated with it were *Ascosacculus*, *Candida*, *Cirrenalia*, *Cladosporium*, *Podospora*, *Scedosporium*, and *Trichoderma* (Ascomycota).

Fungal genera that positively correlated with pH, OM, P, K and CEC, negatively correlated with N and Zn in Site 2. These were: *Ascosacculus*, *Cercospora*, *Engyodontium*, *Meyerozyma*, *Neodevriesia*, *Ophiosphaerella*, and *Tetracladium* (Ascomycota); *Cintractia*, *Fomes*, *Hygrocybe*, *Peniophora*, *Rhizoctonia*, *Sterigmatomyces*, *Tritirachium*, and an unknown genus belonging to Auriculariales (Basidiomycota); *Acaulospora* and an unidentified genus under Archaeosporaceae (Glomeromycota).

Fungal genera that positively correlated with MC in Site 1 were: *Aphanoascus*, *Roussoella*, and *Westerdykella* (Ascomycota); a genus belonging to GS07 (*Rozellomycota*), and an unknown fungal genus, while those that negatively correlated with it were: *Aspergillus*, *Fusarium*, *Conioscypha*, and *Zopfiella* (Ascomycota); *Ganoderma* (Basidiomycota) and an unidentified chytrid fungus.

Potential agro-biotechnological applications of top fungal taxa

To determine the potential benefits of the fungal phyla that were consistently the most abundant across stages and the identified genera that were abundant in at least two stages in the study sites, bioinformatic searches and database mining were done. Some of the potential agro-biotechnological applications of the top fungal taxa present in the study sites are summarized in Table 6.

Table 6. The potential agro-biotechnological application of the top taxa found in the study sites in Apalit, Pampanga (Site 1) and Banaue, Ifugao (Site 2) during the study period in 2019

Fungal Taxon	Study Site*	Rice Cultivation Stage*	Potential Agro-Biotechnological Application	Reference
Phy. Ascomycota	1	BP, FB, HA	Food, feed, medicine, plant symbiosis, soil nutrient-cycling, post-harvest management of food contaminants and mycotoxins	Guarro *et al*., 2012
Stachybotrys	1	BP, FB	Control of mycotoxins, disease control in farm animals and crops	Mostrom, 2011; Dalefield, 2017
Talaromyces	1	BP, FB, HA	Plant growth promotion and protection	Yamagiwa, *et al*., 2011; Naraghi *et al*., 2012; Zhao *et al*., 2021
	2	BP, HA		

Fungal Taxon	Study Site*	Rice Cultivation Stage*	Potential Agro-Biotechnological Application	Reference
Westerdykella	1	BP, FB, HA	Enzymes and soluble pigments production	Yilmaz *et al.*, 2016; Tian, 2020
			Post-harvest management of food contaminants and mycotoxins	Stošić *et al*., 2020
			Insect control, e.g., spotted wing drosophila, cocoa bugs, cotton leafworm, coffee borer beetle, and cotton aphid	Nicoletti and Becchimanzi, 2022
			Biofertilizers (phosphate solubilization)	Kanse *et al*., 2015
	2	BP, FB	-do-	-
Zopfiella	1	BP, FB, HA	Biocontrol of pathogens (*Pseudomonas syringae; Fusarium moniliforme, F. oxysporum, and Curvularia lunata,* and *Pythium*)	Yi *et al*., 2021
Penicillium	2	BP, HA	Biofertilizers (phosphate solubilization)	Whitelaw *et al.*, 1999
			Plant growth promotion	Khan and Lee, 2013; Radhakrishnan *et al*., 2014, Babu *et al*., 2015
			Biocontrol of pathogens	Radhakrishnan *et al*., 2014
			Antimicrobials, antivirals, antiparasitics, insecticidals	Toghueo and Boyom, 2020
			Bioremediation	
			Enzymes production	
Massariosphaeria	2	FB, HA	Bioremediation	Pietro-Souza *et al*., 2017
			Nutrient cycling	
			Plant protection from abiotic stressors (Hg and other metals)	
Phy. Basidiomycota	2	BP, FB, HA	Food, feed, medicine, plant symbiosis, soil nutrient-cycling; post-harvest management of food contaminants and mycotoxins	de Mattos-Shipley *et al*., 2016
Saitozyma	2	BP, FB, HA	Biofuel production	Gorte *et al*., 2020
Unidentified Phy.	1,2	BP, FB, HA	Potentially novel applications	-

Legend: * - Site and stage in which taxon belonged to those that had the most abundance, 1 – Apalit, Pampanga, 2 – Banaue, Ifugao, Phy. – Phylum

Discussion

The two study sites had unique characteristiscs despite belonging to the same fully irrigated, lowland farming system. The similarities and dissimilarities in the chemical characteristics of the soils of Site 1 and Site 2 revealed their varying potential effects to the fungal diversity. Both sites had good soil texture (loam) that may have increased fungal diversity (Tančić Živanov *et al.,* 2017) in both sites. Site 1 had moderately acidic while Site 2

had highly to moderately acidic soil, which could have negatively affected their fungal diversity (Liu *et al*., 2018). Site 1 had slightly saline soil but not Site 2. High EC increases microbial diversity but there may be a complex fungal response to it (Thiem *et al*., 2018). The OM in site 1 was mid-level while high to low-level in Site 2. High OM levels favor certain species of fungi to proliferate (Tančić Živanov *et al.*, 2017) and a decrease in OM may lead to a lower fungal diversity and functionality (Chaer *et al*., 2009). The soil nitrogen levels in both sites were considered low and this could indicate increased fungal biomass (de Vries *et al*., 2007) and a shift from bacterial to fungal dominance in the sites. The available P in Site 1 was very high to very low, while in Site 2, it was medium to very low. Soil P is significantly correlated to the abundance and diversity of fungi in soil albeit in a sub-tropical climate according to Luo *et al*., 2021 but Garcia *et al*. (2017) indicated that the opposite is true for arbuscular mycorrhizal fungi (AMF). The exchangeable potassium (K) levels in both sites were both high to low. Soil K is reportedly significantly correlated to the abundance and diversity of fungi in soil also in a sub-tropical climate (Luo *et al*., 2021). The CEC in both sites were considered high. In a recent study, abundance and richness of lichenized fungi was positively correlated with CEC, however, this was in an Antarctic soil (Canini *et al*., 2020). This means that the CEC in both site soils can affect the sub-components but not the whole fungal diversity. Lastly, the soil zinc levels in both sites were lower than the average of 2-25 ppm (Schulte, 2004). These are significantly lower than the inhibitory level of 4.99 ppm that could affect both fungal production and sporulation rates (Medeiros *et al*., 2010) and the toxic level (>100 ppm) to crops (Schulte, 2004).

On the bases of mean precipitation, the type of organic input, or the non-practice of straw burning, it was initially assumed that the site in Banaue, Ifugao can possess a greater fungal diversity than the site in Apalit, Pampanga. The higher alpha diversity in Site 2 compared to Site 1 supported this assumption and conformed with the results of the global study of Tedersoo *et al.* (2014) but on the basis that annual precipitation has the highest positive effect on fungal diversity. The non-correlation of any of the soil chemical characteristics in both sites to the alpha diversity indices (species richness) of each site also supports this and indicated that each of these characteristics affected instead the specific taxonomic compositions of the fungal communities within each site.

The fungi that positively correlated with pH may have been sensitive to high acidity and may have favored higher or near neutral pH in soil. A case wherein the growth and mycotoxin production of the species *C. globosum* is favored in a neutral pH (Fogle *et al.*, 2008) supports the finding in this study for the genus *Chaetomium* that positively correlated with soil pH in Site 1 (Apalit,

Pampanga). Another is for *Magnaporthiopsis*, wherein a slightly acidic soil of pH 6.5 contributes to the highest degree of disease of *M. maydis*, which is a pathogen of corn (PPQ, 2021). Another genus that positively correlated with pH is *Cercospora*, which has species that are also pathogens of pine, commonly found growing in the vicinities of Site 2, *C. pini-densiflorae* (Kobayashi *et al.*, 1979) and corn, *C. zeae-maydis* (Khare *et al.*, 2012). According to Khare *et al.* (2012), *C. zeae-maydis* grow best in near neutral pH, hence, it is possible that the *Cercospora* found in Site 2 shares the same physiological requirement. Another pathogen in Site 2 is *Rhizoctonia*. A species of this genus, *R. solani*, a necrotrophic pathogen is the causative agent of Rice sheath blight (RSB) (Li *et al.*, 2021). Mycelial growth by this organism is reported to be optimal at pH 4.5-5.5 (Watanabe *et al.*, 2011), however, unlike *C. zeae-maydis*, the disease incidence of *R. solani* is not correlated to pH ranging from 4.5 to 7.2 (Watanabe *et al.*, 2011). This may indicate that the *Rhizoctonia* found in Site 2 may have responded to pH differently. It is unexpected, however, for *Arcuadendron* to favor higher pH values as a strain, *Arcuadendron* sp. (TS-4), had been reported to produce glycoprotein bioflocculants that have greater activities on high acid or lower pH level (pH 3.0) (Lee *et al.*, 1995). While some studies support or seemingly oppose the correlation results of this study with regard to the soil pH of both sites, according to Rousk *et al.*, (2009) there exists a challenge in studying pH in soil as it has different effects on multiple abiotic and biotic factors. In this study, it was observed that the same set of fungi that correlated positively to soil pH also correlated well with several other soil chemical characteristiscs such as OM, P, and K in both study sites, and additionally CEC in the soil of Site 2.

The set of fungi that correlated positively with OM in this study may possess a wide variety of extracellular enzymes that are utilized to break down different kinds of organic matter and convert these into fungal biomass (Žifčáková *et al.*, 2016). It is then presumed that the extracellular enzymes of these fungi that are responsible for breaking down organic matter are more active in increased pH levels (low acidity) as indicated by the correlation of these fungi with both pH and OM.

While specific responses to available P addition are poorly understood (Lekberg *et al.*, 2021), there are reports that phosphorus fertilization favors pathogenic over mutualistic fungi. In this study, it is presumed that not all mutualistic fungi follow this trend as two genera of AMF, albeit unidentified, were positively correlated to available P in Site 1 and *Acaulospora* and another unidentified genus of AMF were positively correlated with P in Site 2. Of the list of fungi positively correlated to available P in this study, however, some of these have species that were reported or are known pathogens of plants,

animals, or humans, e.g., in Site 1: *Magnaporthiopsis* (PPQ, 2021), *Phoma* (Deb *et al.*, 2020), *Chaetomium* (Jiang *et al.*, 2017), and *Xylaria* (Garcia-Aroca *et al.*, 2021) and in Site 2: *Cercospora* (Khare *et al.*, 2012) and *Rhizoctonia* (Li *et al.*, 2021).

Exchangeable potassium (K) is a plant requirement and mutualistic and other beneficial fungi help provide this element to plants via different mechanisms (Bhattacharjee *et al.*, 2021). It is possible that the AMF (e.g. *Acaulospora*) and other fungi that positively correlated to K in this study have direct access to it for their own nutrition via various mechanisms such as mineral weathering (Bhattacharjee *et al.*, 2021).

Salinity can reduce biomass through osmotic stress that results in drying and lysis of cells (Yan *et al.*, 2015) like the fungi found negatively correlated to this edaphic factor in Site 1. These may also have enzymes that are sensitive to salinity, such as β-glucosidase, ureases, and alkaline phosphatases were reportedly strongly inhibited by salinity in soil (Pan *et al.*, 2013).

Nitrogen addition in soil had been reported to affect abundance of paddy soil fungi (Cai *et al.*, 2019) and have negative effects on fungal biomass (Zhang *et al.*, 2018). In this study, only certain taxonomic compositions were affected by nitrogen levels in the soil in both sites. *Spiromastyx* (found in Site 1 that negatively correlated with N) was experimentally shown to be negatively affected by increasing N through fertilizer addition. The opposite was true for *Scytalidium* and *Pluteus* (Zhang *et al.*, 2018). This can only mean that the effects of N to *Scytalidium*, and *Pluteus* could be strain or species specific.

The fungi found in this study that correlated negatively with zinc in soils of both sites could be sensitive to increasing available zinc concentrations in soil, probably due to their own "zinc quota" (the cellular zinc content required for optimal growth) (Outten and O'halloran, 2001) and it is possible that these fungi when faced with excess zinc would have an imbalance in their oxidative metabolism (Pagani *et al.*, 2007). It should be noted that while the site zinc concentrations are considered low in both sites across cultivation stages, the estimated concentration of zinc in cells is in the picomolar to the nanomolar range (Eide, 2006), changes in the milligrams range are probably quite significant for them.

For the fungi that correlated negatively to CEC in Site 1 it is possible that the increase in concentration of cations such as zinc and other micronutrient cations such as copper, iron, and manganese due to the retention action of CEC, and coupled with the salinity of the site (Na^+ retention), could have negatively affected fungal growth (through imbalances in their oxidative metabolism) and consequently their abundance (Pagani *et al.*, 2007). On the other hand, for the fungi in Site 2 that positively correlated with CEC, it is

possible that these fungi favor the increasing concentrations of nutrient cations in the soil, especially in the absence of salinity.

The fungi that positively correlated with moisture content in soils in this study may be considered hydrophilic (water-loving or requiring Aw ≥ 0.9) (Flannigan and Miller, 2001) and the negatively correlated ones may either be considered xerotolerant or xerophilic (prefer dry places or grow in dry conditions) (Samson *et al.*, 2010). Some of the genera found positively correlated to MC in this study are reported to have species that are considered hydrophilic, e.g. *Aspergillus* and *Stachybotrys* (Park *et al*., 2008). On the other hand, xerotolerant species of *Candida* (Rodr ǵuez-Andrade *et al.*, 2019) and *Cladosporium* (Petrovič *et al.*, 2000), genera found to be negatively correlated to MC in Site 1, and *Aspergillus*, *Fusarium* (Petrovič *et al.*, 2000) genera found to be negatively correlated to MC in Site 2, have been reported.

Although, indicative of the possible actual relationships of edaphic factors and fungi found in the soils of both sites, the results of the correlations in this study still need to be experimentally validated. This will help establish whether specific edaphic factors indeed cause the changes in abundance and diversity of the fungal composition in both sites and vice versa.

The agro-biotechnological properties of the top fungal taxa in this study were determined to show their potentials in agriculture and rice farming. Ascomycota, for example, a top phylum in Site 1, has members that are plant symbionts but also plant pathogens and have various biotechnological applications (Guarro *et al.*, 2012) including in rice farming. On the other hand, categorical roles played by basidiomycetes, a consistent top phylum in Site 2, are model species, edible species, toxic species, medicinal basidiomycetes, symbionts, decomposers, and pathogens (de Mattos-Shipley *et al.*, 2016). It was observed that there was a sizeable amount of the unidentified phylum across stages in both sites. This could be due to the unclassified operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) from the estimated total fungal diversity of about 12 million species (Chethana *et al.*, 2020). The UNITE database that includes dark taxa, which are genomic or metagenomic sequence information that cannot be linked to any physical specimen (viable or not) or resolved taxonomic name (Ryberg and Nilsson, 2018), was used as the reference for the taxonomic assignments of sequences gathered in this study. It can be said that there is a wealth of unidentified and potentially novel fungi with novel properties inhabiting Philippine soils, particularly in the two study sites in Apalit, Pampanga and Banaue, Ifugao.

Species of *Talaromyces* had been reported to be food contaminants, mycotoxin producers, and human pathogens (Stošić *et al.*, 2020). However, species of this genus had also been reported to produce enzymes and soluble

pigments and had been implicated in biocontrol of pathogens (Tian, 2020), produce plant growth promoting compounds (Zhao *et al.*, 2021), have antagonistic relationships with agriculturally important insects such as spotted wing drosophila, cocoa bugs, cotton leafworm, coffee borer beetle, and cotton aphid (Nicoletti and Becchimanzi, 2022) and capable of phosphate solubilization in soil (Kanse *et al.*, 2015). The potential for use in agriculture is enormous for the *Talaromyces* found in both study sites as they may harbor these different characteristics.

Increased abundance of *Westerdykella* in soil amended with biochar was found to be beneficial to rice growth and TOC degradation by Wang *et al.* (2021). The potential for *Westerdykella* found in the study sites in rice farming is also promising. These can be analyzed for plant growth promoting properties and test these against rice.

The *Zopfiella* found abundant in Site 1 when cultured may be analyzed for antimicrobial and biocontrol potentials. A beneficial strain of the genus *Zopfiella* is reported to produce 3-Decalinoyltetramic acids (Yi *et al.*, 2021) and zopfiellasins A–D (Zhang *et al.*, 2021). These compounds are shown to have activities against *Pseudomonas syringae*, a well-known plant pathogen. An endophytic strain of *Zopfiella* sp. is being patented for the biocontrol of *Fusarium moniliforme*, *F. oxysporum*, and *Curvularia lunata*, and *Pythium* (Ortega *et al.*, 2020).

Stachybotrys, also abundant in Site 1, produces trichothecenes (TCTs) (mycotoxin) and zearalenone (mycotoxin) that are toxic to plants and animals (Mostrom, 2011; Dalefield, 2017). Given this information control strategies in the site may be considered.

Saitozyma was found to be abundant across stages in Site 2. While this genus is absent in Site 1, it is commonly found in cultivated soil (Yurkov, 2018) and had been reported to be correlated and enriched in soil with the accumulation of OM, Fe and Al oxides, and with sandy texture (Moreira and Vale, 2018), hence it is not surprising to find it in the soil of Site 2 which has high to mid-level OM. This is a basidiomycetous and oleaginous yeast that has been shown to produce lipids as potential source of biofuels (Gorte *et al.*, 2020).

The beneficial properties of *Penicillium* spp. include phosphate solubilization (Whitelaw *et al.*, 1999), plant growth promotion (Babu *et al.*, 2015), biocontrol of pathogens (Radhakrishnan *et al.*, 2014), production of antimicrobial, antiviral, antiparasitics and insecticidal compounds or secondary metabolites and enzymes, phytoremediation, and serving as biocatalysts (Toghueo and Boyom, 2020).

Some *Massariosphaeria* spp. have potential for heavy metals bioremediation, play an important role in nutrient cycling, and provide defense against the adverse effects of abiotic stressors such as mercury and other metals (Pietro-Souza *et al*., 2017).

This study revealed that both sites have unique and shared fungal taxonomic compositions collectively and at different rice cultivation stages during the study period. This study also confirms the correlations of soil chemical characteristics to different fungi at the phylum and genus levels. The uncovered composition and variation of mycobiomes of soils in these sites can help provide information for those seeking potential solutions to challenges faced in the sites and similar irrigated, rice-based farming systems such as such as low grain yield, pests, and diseases.

Acknowledgements

The authors would like to express their gratitude to the Department of Science and Technology (DOST) Accelerated Science and Technology Human Resource Development Program-National Science Consortium (ASTHRDP-NSC) program and the Department of Agriculture-Bureau of Agricultural Research (DA-BAR) of the Philippines for the research funds; the provincial and municipal agricultural offices, the farmer respondents in the study sites for their cooperation and assistance; and the Philippine National Collection of Microorganisms (PNCM) for providing the materials, facilities, equipment and additional funds.

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(Received: 21 October 2022, accepted: 30 December 2022)