Entomology

Utilizing metagenomics and metabarcoding for assessing the diversity and abundance of entomopathogenic fungi in soil ecosystems

Michelle S. Guerrero

National Crop Protection Center, College of Agriculture and Food Sciences, University of the Philippines, Los Baños, 4031 Laguna, Philippines

Correspondence Author: Michelle S. Guerrero

Received 19 May 2023; Accepted 30 Jun 2023; Published 10 Jul 2023

Abstract

Entomopathogenic fungi play a crucial role in natural pest control and have received significant attention in the field of biological control. The application of metabarcoding and metagenomic techniques has revolutionized the study of microbial communities in various ecosystems by identifying and characterizing many microorganisms at once. These methods improve soil fungal community assessments for entomopathogenic fungi. These studies require fungal DNA extraction from complex environmental samples like soil. These methods improve soil fungal community assessments for entomopathogenic fungi. These studies require fungal DNA extraction from complex environmental samples like soil. Bead-beating, chemical extraction, and commercial kits extract fungal DNA while minimizing environmental DNA contamination. Shotgun sequencing for metagenomics can sequence all genetic material in a sample without targeting specific genes. This method shows the microbial community, including entomopathogenic fungi. Metagenomic data can reveal the soil microbiome's functional potential and interactions. Shotgun sequencing and ITS barcoding for fungal metabarcoding are popular. The ITS region of the fungal genome is variable, making it a good marker for species identification. Researchers can assess soil entomopathogenic fungi diversity and abundance by amplifying and sequencing the ITS region. In this review, the utility of these approaches for the identification and characterization of entomopathogenic fungi in soil samples was assessed. This review focused on the extraction of soil DNA, shotgun sequencing for metagenomics, and the use of Internal Transcribed Spacer (ITS) as a barcode for fungal metabarcoding. Insights into the potential applications and future directions in the study of entomopathogenic fungi were given. These insights were provided by highlighting the benefits and limitations of these methodologies.

Keywords: entomopathogenic fungi, metabarcoding, metagenomics, ITS, diversity assessment

Introduction

Entomopathogenic fungi are naturally occurring pathogens that infect and kill insects, making them valuable in pest management strategies (Vega *et al*., 2009) [33]. These fungi are commonly found in soil samples, where they form part of diverse fungal communities (Sánchez-Peña *et al*., 2011) [24]. However, traditional isolation and identification methods for entomopathogenic fungi are laborious and time-consuming, which limits our understanding of their ecological role and diversity (Vega *et al*., 2009) [33].

The emergence of metabarcoding and metagenomics has revolutionized the field by enabling high-throughput and culture-independent approaches for studying microbial communities (Vega *et al*., 2009) [33]. These techniques have been applied specifically for studying entomopathogenic fungi in soil samples. For example, shotgun metagenomic sequencing has been used to characterize the microbiota of mosquitoes (Chandler *et al*., 2015) [4]. This approach allows for discovering unknown members of the microbiota and provides insights into the composition and dynamics of entomopathogenic fungal communities.

Soil samples are an excellent environmental shelter for entomopathogenic fungi (Sánchez-Peña *et al*., 2011) [24]. The soil protects from UV radiation and other adverse abiotic and biotic influences, allowing these fungi to thrive (Sánchez-Peña *et al*., 2011) [24]. Studies have found entomopathogenic fungi in the genera *Beauveria*, *Conidiobolus*, *Metarhizium*, and *Isaria* commonly found in soil (Sánchez-Peña *et al*., 2011) [24]. These fungi have been isolated from various agricultural and natural ecosystems, highlighting their wide distribution (Sánchez-Peña *et al*., 2011; Asensio *et al*., 2003; Safitri *et al*., 2018; Suwandi, 2020; Budiarti & Nuryanti, 2022; Ramos *et al*., 2022) [24, 1, 23, 30, 2, 21].

Understanding the diversity and communities of entomopathogenic fungi in different ecosystems is essential for their management and conservation (Sevim *et al*., 2009) [27]. Native populations of entomopathogenic fungi in soil can be managed to facilitate the control of pest insect populations within agroecosystems (Sevim *et al*., 2009) [27]. Isolating and characterizing indigenous entomopathogenic fungi also provides insights into the naturally occurring fungal biodiversity and offers a pool of potential biological control agents for pest control (Sevim *et al*., 2009) [27]. The ecological role of entomopathogenic fungi in semi-natural habitats and communities is still poorly understood (Hesketh *et al*., 2009) [12]. The spatiotemporal distribution patterns of these pathogens may create selective pressures on the ecological traits of herbivorous insects (Gielen *et al*., 2022) [8].

In addition to their role in pest management, entomopathogenic fungi have been found to have non-entomopathogenic roles in promoting plant health and growth (Dara, 2019) [6]. Several studies have provided insights into the relationship between

entomopathogenic fungi and plants, soil, and plant pathogens (Dara, 2019) [6]. These fungi can contribute to integrated pest management strategies and offer environmentally sustainable pest suppression (Dara, 2019) [6]. Meta barcoding and metagenomic methods are crucial in assessing the abundance and discovery of entomopathogenic fungi in soil habitats.

Soil DNA extraction techniques

Accurate identification of entomopathogenic fungi from soil samples is crucial for studying these organisms and their potential applications in pest control. However, the efficient extraction of high-quality DNA from soil can be challenging due to the complex nature of soil matrices and various inhibitors. Several DNA extraction methods have been developed and used for this purpose, including the CTAB-based method, bead-beating, and commercial kits (Feinstein *et al*., 2009; Särkinen *et al*., 2012; Yeates *et al*., 1998) [7, 25, 35]. Figure 1 shows the genomic DNA of the soil visualized in 1.2% agarose gel.

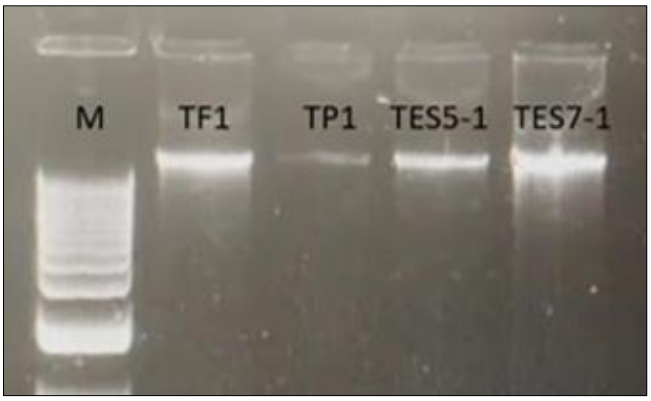

Fig 1: Visualization of genomic DNA extracted from soil samples using DNeasy PowerMax soil kit

The CTAB-based method is a commonly used DNA extraction method for soil samples. It involves using cetyltrimethylammonium bromide (CTAB) to lyse cells and remove contaminants. This method effectively extracts DNA from different types of soils, including clay, sand, and organic soils (Feinstein *et al*., 2009) [7]. However, it is essential to note that the CTAB-based method may have limitations in terms of DNA yield and purity and potential extraction biases (Feinstein *et al*., 2009) [7].

Bead-beating is another DNA extraction method that has been used for soil samples. This method involves the mechanical disruption of cells using beads and a bead-beating device. It effectively extracts DNA from soil samples, with higher DNA yields than other methods (Yeates *et al*., 1998) [35]. However, bead-beating efficiency can vary depending on the soil composition and the type of beads used (Yeates *et al*., 1998) [35].

Commercial DNA extraction kits are also commonly used for soil DNA extraction. These kits provide standardized protocols and reagents for efficient DNA extraction. They are designed to overcome the challenges associated with soil DNA extraction, such as removing inhibitors and isolating high-quality DNA (Särkinen *et al*., 2012; Mahmoudi *et al*., 2011) [25, 17]. However, it is essential to choose the appropriate kit based on the study's specific requirements and the soil samples' characteristics.

Each DNA extraction method has its advantages and limitations. The choice of method depends on various factors, including the type of soil, the desired DNA yield and purity, and the downstream applications. It is essential to consider these factors when selecting a DNA extraction method for studying entomopathogenic fungi in soil samples.

Shotgun sequencing for metagenomics

Shotgun metagenomics is a powerful approach that provides a comprehensive view of the genetic diversity within a microbial community, including entomopathogenic fungi. This method involves sequencing all the DNA present in a sample, allowing for the identification of known and novel taxa, functional gene analysis, and community profiling (Kang *et al*., 2015)[14]. The workflow of shotgun metagenomics includes several steps, such as sample collection, library preparation, sequencing platforms, and bioinformatics analysis (Kang *et al*., 2015) [14] (Figure 2).

Sample collection is a critical step in shotgun metagenomics. It involves obtaining a representative sample from the environment of interest, such as soil samples, for studying entomopathogenic fungi. The samples should be collected using appropriate techniques to minimize contamination and preserve the microbial community structure (Kang *et al*., 2015) [14].

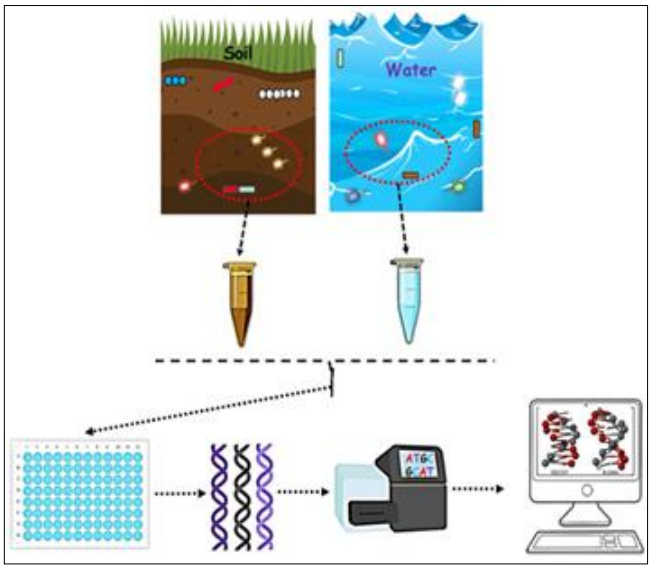

Fig 2: Process flow of soil metagenomics and metabarcoding

Library preparation is the next step in the workflow. It involves extracting DNA from the sample and preparing it for sequencing. DNA extraction kits and enrichment procedures can be used to obtain high-quality DNA for shotgun metagenomics (Buytaers *et al*., 2020) [3]. Once the DNA is extracted, it is fragmented into smaller pieces, and adapters are added to facilitate sequencing (Kang *et al*., 2015) [14].

Sequencing platforms play a crucial role in shotgun metagenomics. High-throughput sequencing technologies, such as Illumina and PacBio, are commonly used. Depending on the technology used, these platforms generate millions of short or long DNA reads (Kang *et al*., 2015) [14]. The choice of sequencing platform depends on factors such as the desired sequencing depth, read length, and cost (Kang *et al*., 2015) [14].

After sequencing, the generated reads are subjected to bioinformatics analysis. This involves several steps, including quality control, read assembly and metagenome binning. Quality control ensures that only high-quality reads are used for downstream analysis (Shay *et al*., 2023) [28]. Read assembly involves merging overlapping reads to reconstruct longer DNA fragments called contigs (Kang *et al*., 2015) [14]. Metagenome binning is a process that groups contigs into individual genomes, allowing the study of particular organisms within the microbial community (Kang *et al*., 2015) [14].

Shotgun metagenomics has been used to explore entomopathogenic fungi's diversity and functional potential in soil samples. Studies have employed this approach to identify known and novel taxa, analyze active genes, and characterize the community structure of entomopathogenic fungi (Kang *et al*., 2015) [14].

Shotgun metagenomics is a valuable tool for studying the genetic diversity within microbial communities, including entomopathogenic fungi. It allows identifying known and novel taxa, functional gene analysis, and community profiling. The workflow of shotgun metagenomics involves sample collection, library preparation, sequencing platforms, and bioinformatics analysis. This approach has been successfully employed in various studies to explore the diversity and functional potential of entomopathogenic fungi and other microbial communities in different environments.

Sequence analysis for shotgun metagenomics

Shotgun metagenomics is a powerful approach for studying microbial communities and generating vast sequence data. However, analysing this data requires robust bioinformatics tools and pipelines to extract meaningful insights (Pasolli *et al*., 2016) [21]. The typical sequence analysis pipeline for shotgun metagenomics includes several steps: quality control and preprocessing, read assembly or mapping, taxonomic and functional annotation, comparative analysis, and integration with other omics data (Pasolli *et al*., 2016) [21].

Quality control and preprocessing are essential steps in shotgun metagenomic analysis to ensure the accuracy and reliability of the data. This includes removing low-quality reads, adapter trimming, and filtering out contaminants (Pasolli *et al*., 2016) [21]. After preprocessing, the reads can be assembled into contigs or mapped to a reference genome for further analysis (Sczyrba *et al*., 2017) [26]. Assembly and mapping methods are crucial for reconstructing the genomes of individual microorganisms present in the metagenomic sample (Sczyrba *et al*., 2017) [26]. However, the performance of these methods can be affected by the presence of related strains and the parameter settings used (Sczyrba *et al*., 2017) [26].

Taxonomic and functional annotation can be performed once the reads are assembled or mapped. Taxonomic annotation involves assigning taxonomic labels to the assembled contigs or mapped reads, which helps identify the microorganisms present in the sample (Sczyrba *et al*., 2017) [26]. Functional annotation involves predicting the functions of genes and gene products in the metagenomic data, which provides insights into the functional diversity of the microbial community (Pasolli *et al*., 2016) [21]. These annotations can be performed using various bioinformatics tools and databases (Gupta *et al*., 2022) [9]. Figure 3 presents a taxonomic profiling result from shotgun metagenomics.

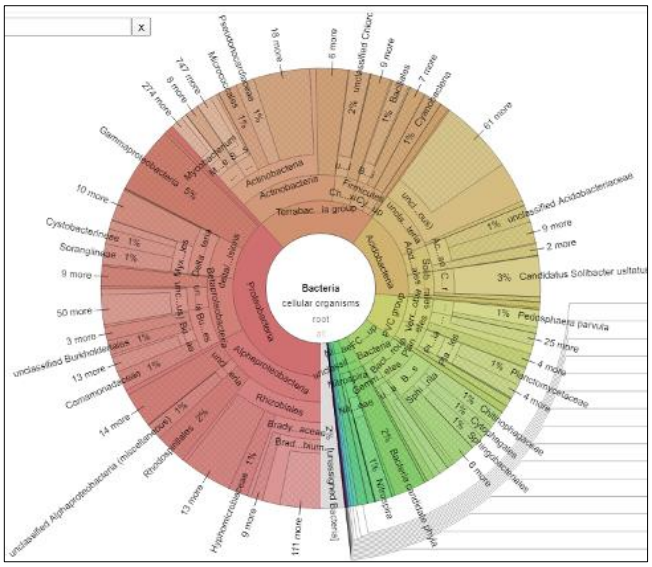

Fig 3: Shotgun metagenomic result of soil DNA

Comparative analysis is another critical step in the shotgun metagenomic analysis. It involves comparing different samples' taxonomic and functional profiles to identify differences and similarities between microbial communities (Nayfach & Pollard, 2016) [20]. This analysis can provide insights into the ecological roles of microorganisms and their functional diversity within the soil microbiome (Pasolli *et al*., 2016) [21]. Comparative metagenomics requires accurate and quantitative data summaries comparable across samples and studies (Nayfach & Pollard, 2016) [20]. However, using abundance statistics and biases introduced by experimental protocols and data-cleaning approaches can hamper comparability (Nayfach & Pollard, 2016) [20].

Integration with other omics data is a valuable approach to comprehensively understanding microbial communities. Shotgun metagenomics can be combined with other omics techniques, such as meta transcriptomics, metaproteomic, and metabolomics, to study the gene expression, protein composition, and metabolite profiles of microbial communities (Pasolli *et al*., 2016) [21]. This integration allows for a more holistic analysis of microorganisms' functional potential and activity in their environment.

Shotgun metagenomics generates vast sequence data that require robust bioinformatics analysis to extract meaningful insights. The typical sequence analysis pipeline includes quality control and preprocessing, read assembly or mapping, taxonomic and functional annotation, comparative analysis, and integration with other omics data. These analyses provide insights into microorganisms' ecological roles and functional diversity within microbial communities. However, challenges such as benchmarking datasets, parameter settings, and comparability of data need to be addressed to improve the accuracy and reproducibility of shotgun metagenomic analysis (Sczyrba *et al*., 2017; Nayfach & Pollard, 2016) [26, 20].

Using ITS as barcode for fungi metabarcoding

Metabarcoding is a powerful technique that utilizes short DNA markers, such as the Internal Transcribed Spacer (ITS) region, to identify and quantify multiple fungal taxa simultaneously (Tedersoo *et al*., 2014) [31]. The ITS region is highly variable and widely used for fungal identification (Tedersoo *et al*.,

2014) [31]. It has been shown that fungal richness is decoupled from plant diversity, and climatic factors, followed by edaphic and spatial variables, constitute the best predictors of fungal richness and community composition globally (Tedersoo *et al*., 2014) [31].

ITS metabarcoding involves several steps, including PCR amplification, library preparation, sequencing platforms, and bioinformatics analysis (Tedersoo *et al*., 2015) [32]. The choice of primer pairs in PCR amplification can affect the taxonomic resolution and richness of operational taxonomic units (OTUs) (Tedersoo *et al*., 2015) [32]. It has been recommended to use ITS2 or the whole ITS region for metabarcoding and to carefully choose primer pairs based on the relative proportion of fungal DNA and expected dominant groups (Tedersoo *et al*., 2015) [32].

PCR-based methods, including metabarcoding, have been widely used to quickly characterize microbial communities in complex environmental samples (Sun *et al*., 2021) [29]. However, these methods can exhibit technical shortcomings that may introduce biases in the analysis (Tedersoo *et al*., 2015) [32]. For example, the choice of forward primer in ITS metabarcoding can explain a significant amount of variation in OTU-level analysis (Tedersoo *et al*., 2015) [32]. It is essential to consider these biases and limitations when interpreting metabarcoding data.

Case studies have applied ITS metabarcoding to uncover entomopathogenic fungi's diversity and distribution patterns in soil ecosystems (Majchrowska-Safaryan *et al*., 2017) [18]. Entomopathogenic fungi infect and kill insects, playing an essential role in the biological control of insect pests (Majchrowska-Safaryan *et al*., 2017) [18]. The use of metabarcoding has allowed researchers to identify and quantify entomopathogenic fungi in soil samples, providing insights into their diversity and distribution (Hallouti *et al*., 2017; Majchrowska-Safaryan *et al*., 2017) [10, 18].

In addition to ITS metabarcoding, other DNA regions, such as the 16S region for bacteria, have also been used in metabarcoding studies (Sun *et al*., 2021) [29]. The choice of marker region depends on the target organisms and research objectives (Sun *et al*., 2021) [29]. Long-read sequencing technologies, such as those offered by Pacific Biosciences and Oxford Nanopore Technologies, have also been used in fungal identification (Hoang *et al*., 2022) [13]. These technologies generate ultra-long reads and can provide a promising approach for accurately identifying and diagnosing fungal species (Hoang *et al*., 2022) [13].

ITS metabarcoding is a valuable tool for studying entomopathogenic fungi's diversity and distribution patterns in soil samples. It allows for the simultaneous identification and quantification of multiple fungal taxa, providing insights into their ecological roles and potential applications in the biological control of insect pests. However, it is essential to consider the biases and limitations associated with PCR-based methods and to carefully choose primer pairs to optimize the taxonomic resolution and richness of the analysis. Future research should continue to explore the use of metabarcoding in uncovering the hidden diversity of entomopathogenic fungi and improving our understanding of their ecological functions in soil ecosystems.

Sequence processing for metabarcoding

Metabarcoding studies utilize high-throughput sequencing technologies to generate large volumes of short DNA sequences from multiple samples simultaneously. This approach has proven to be a powerful tool for assessing biodiversity and community structures in various ecosystems, including aquatic environments (Yamamoto *et al*., 2017; Lobo *et al*., 2017) [34, 15]. The sequence processing workflow for metabarcoding typically involves several steps, which we will discuss in the context of QIIME 2, a widely used bioinformatics tool (Curd *et al*., 2019) [5].

The first step in the sequence processing workflow is quality control and denoising. This involves removing low-quality reads, trimming adapter sequences, and filtering out contaminants to ensure the accuracy and reliability of the data (Curd *et al*., 2019) [5]. QIIME 2 provides various methods and algorithms for quality control and denoising, such as DADA2, a widely used tool for error correction and denoising of amplicon sequence data (Curd *et al*., 2019) [5].

The next step is sequence alignment and clustering. In this step, the processed reads are aligned to a reference database to identify similar sequences and group them into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) (Curd *et al*., 2019) [5]. QIIME 2 offers several sequence alignment and clustering algorithms, including VSEARCH and Deblur, which can handle large datasets efficiently (Curd *et al*., 2019) [5].

After sequence alignment and clustering, taxonomic classification is performed to assign taxonomic labels to the OTUs or ASVs. This step involves comparing the sequences against a reference database containing known taxonomic information (Curd *et al*., 2019) [5]. QIIME 2 integrates with various taxonomic classification tools, such as SILVA and Greengenes, to provide accurate taxonomic assignments (Curd *et al*., 2019) [5].

Abundance filtering and rarefaction are essential steps to normalize the data and account for differences in sequencing depth between samples. Abundance filtering removes low-abundance OTUs or ASVs that may be noise or artifacts. At the same time, rarefaction subsamples the data to an even sequencing depth to ensure comparability between samples (Curd *et al*., 2019) [5]. These steps help to reduce biases introduced by variations in sequencing depth and improve the accuracy of downstream analyses.

Diversity analysis is a crucial step in metabarcoding studies, as it provides insights into the composition and structure of the microbial or organismal communities. Various diversity metrics, such as alpha diversity (within-sample diversity) and beta diversity (between-sample diversity), can be calculated to assess the richness, evenness, and similarity of the communities (Curd *et al*., 2019) [5]. QIIME 2 offers a range of diversity analysis tools, including alpha rarefaction, beta diversity metrics (e.g., Bray-Curtis dissimilarity), and visualization techniques (e.g., principal coordinate analysis) (Curd *et al*., 2019) [5].

Visualization is an essential component of the sequence processing workflow, as it allows for the exploration and interpretation of the results. QIIME 2 provides interactive and customizable visualization tools, such as Emperor, which enables the visualization of beta diversity results in three-dimensional space (Curd *et al*., 2019) [5]. These visualization

techniques aid in identifying and characterizing entomopathogenic fungi in soil samples, as they allow for the visual representation of community structures and patterns. Figure 4 shows a taxonomic abundance profile from a soil sample resolved from metabarcoding.

The sequence processing workflow for metabarcoding studies using QIIME 2 involves quality control and denoising, sequence alignment and clustering, taxonomic classification, abundance filtering and rarefaction, diversity analysis, and visualization. These steps enable the identification and characterization of entomopathogenic fungi in soil samples and the assessment of biodiversity and community structures in various ecosystems. QIIME 2 provides a comprehensive suite of tools and algorithms to facilitate each workflow step, ensuring accurate and reliable analysis of metabarcoding data (Curd *et al*., 2019) [5].

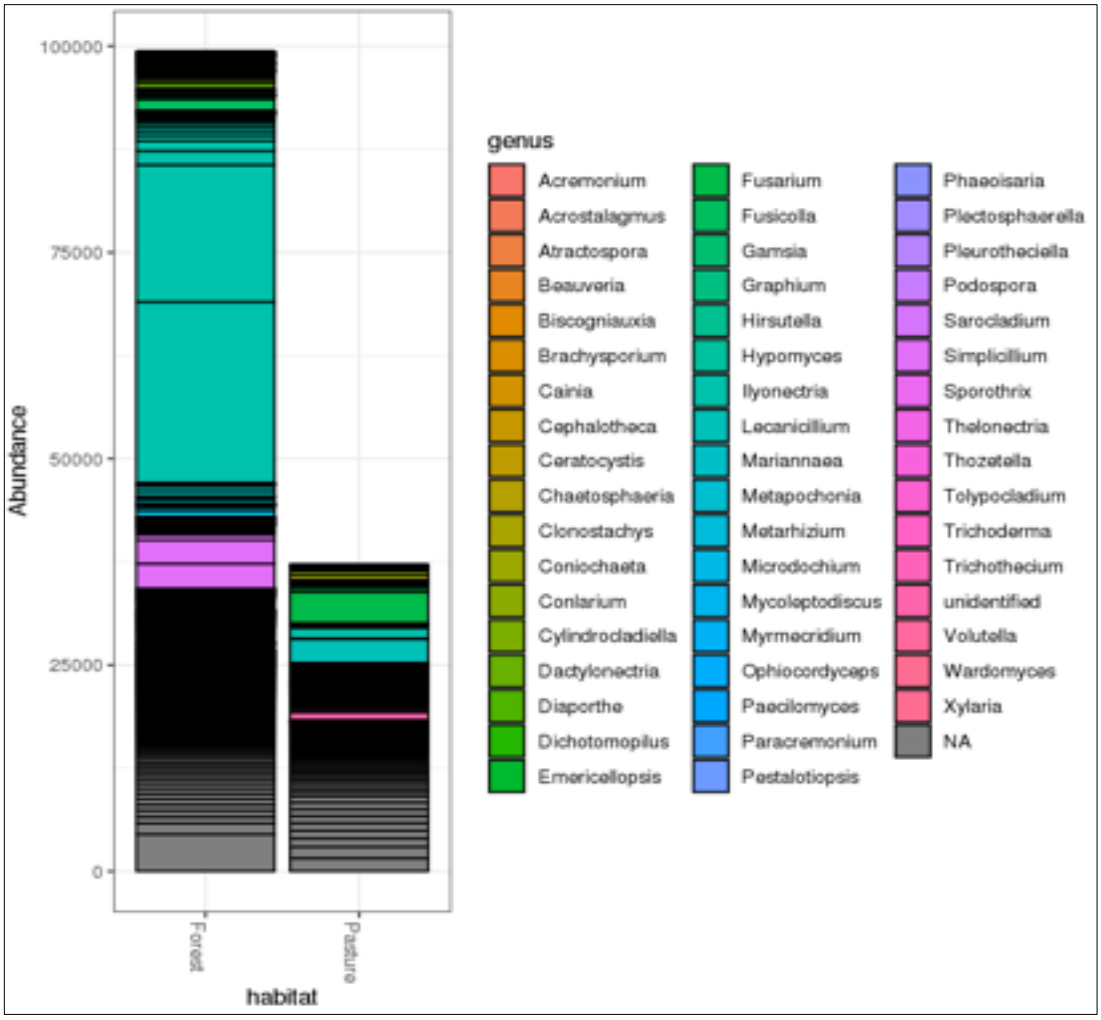

Fig 4: Metabarcoding result of the fungal community from a pasture and forest soil sample

Conclusions

Metabarcoding and metagenomics techniques have revolutionized the study of entomopathogenic fungi in soil samples. Soil DNA extraction techniques enable efficient recovery of high-quality DNA, while shotgun metagenomics provides a comprehensive view of microbial communities, including entomopathogenic fungi. ITS, a barcode for metabarcoding, offers a powerful tool for identifying and quantifying fungal taxa in diverse environments. These approaches and robust sequence analysis pipelines have improved our understanding of the diversity, ecology, and potential applications of entomopathogenic fungi. Future advancements in these methodologies hold great promise for further unravelling the complex dynamics and interactions involving entomopathogenic fungi in soil ecosystems.

Acknowledgments

The author thanks her PhD supervisory committee for their support in the author's PhD studies that allowed her to gain insights into the metagenomics and metabarcoding of soil entomopathogenic fungi. The author also thanks the New Zealand Scholarship for providing financial assistance in her PhD studies.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Asensio L, Carbonell T, Lopez-Jimenez J, Lopez-Llorca L. Entomopathogenic Fungi in Soils from Alicante Province [Spain]. Span J Agric Res. 2003;3(1):37. https://doi org/10.5424/jar/2003013-33
2. Budiarti L, Nuryanti N. Entomopathogenic Fungus from Rice Rhizosphere Soil in Politeknik Negeri Lampung Land IOP Conf Ser: Earth Environ Sci. 2022;1(1012):012059. https://doi.org/10.1088/1755-1315/1012/1/012059.
3. Buyers F, Saltykova A, Denayer S, Verhaegen B,

Vanneste K, Roosens N. A Practical Method to Implement Strain-level Metagenomics-based Foodborne Outbreak Investigation and Source Tracking In Routine Microorganisms. 2020;8(8):1191. https://doiorg/10.3390/microorganisms8081191.
4. Chandler J, Liu R, Bennett S. Rna Shotgun Metagenomic Sequencing of Northern California (USA) Mosquitoes Uncovers Viruses, Bacteria, and Fungi Front Microbiol, 2015, 06. https://doi org/10.3389/fmicb201500185.
5. Curd E, Gold Z, Kandlikar G, Gomer J, Ogden M, O'Connell T. Anacapa Toolkit: An Environmental Dna Toolkit For Processing Multilocus Metabarcode Datasets Methods in Ecology and Evolution. 2019;9(10):1469-1475. https://doi org/10.1111/2041-210x 13214
6. Dara S. Non-entomopathogenic Roles of Entomopathogenic Fungi in Promoting Plant Health and Growth Insects. 2019;9(10):277. https://doi.org/10.3390/insects10090277.
7. Feinstein L, Sul W, Blackwood C. Assessment of Bias Associated with Incomplete Extraction Of Microbial Dna From Soil Appl Environ Microbiol. 2009;16(75):5428-5433. https://doi org/10.1128/aem00120-09.
8. Gielen R, Põldmaa K, Tammaru T. In Search of Ecological Determinants of Fungal Infections: A Semi-field Experiment with Folivorous Moths Ecology and Evolution, 2022, 5(12). https://doi org/10.1002/ece3 8926.
9. Gupta V, Bakshi U, Chang D, Lee A, Davis J, Chandrasekaran S, *et al*. Taxibgc: A Taxonomy-guided Approach For Profiling Experimentally Characterized Microbial Biosynthetic Gene Clusters And Secondary Metabolite Production Potential In Metagenomes Msystems, 2022, 6(7). https://doi.org/10.1128/systems 00925-22
10. Hallouti N, Zahidi N, Bouharroud N, Mousadik N, Aoumar N, Boubaker N. Diversity of Entomopathogenic Fungi in Argane Forest Soil and Their Potential to Manage Mediterranean Fruit Fly (Ceratitis Capitata). Journal of Pharmacy and Pharmacology, 2017, 10(5). https://doi.org/10.17265/2328-2150/201710007.
11. Hanora A, Amin M, Azab M, Shabayek S, Atwa K. The Vaginal Microbiota During Pregnancy Records of Pharmaceutical and Biomedical Sciences. 2023;2(7):80-92. https://doi org/10 21608/rpbs 2023 196524 1213
12. Hesketh H, Roy H, Eilenberg J, Pell J, Ails R. Challenges in Modelling Complexity of Fungal Entomopathogens in Semi-natural Populations of Insects Bio Control. 2009;1(55):55-73 https://doi org/10 1007/s10526-009-9249-2.
13. Hoang M, Rinyi L, Myer W. Long-read Sequencing in Fungal Identification Microbiology Australia. 2022;1(43):14-18. https://doi org/10 1071/ma22006
14. Kang D, Frula J, Egn R, Wan Z. Metabat, An Efficient Tool for Accurately Reconstructing Single Genomes from Complex Microbial Communities Peerj. 2015;3:e1165. https://doi org/10 7717/peerj 1165
15. Lobo J, Shokalla S, Hajiabaei M, Cota F. DNA barcoding for High-throughput Monitoring of Estuarine Macrobenthic Communities Scientific Reports, 2017, 1(7). https://doi org/10 1038/s41598-017-15823-6.
16. Łopusiewicz Ł, Mazurkiewicz-Zapałowicz K, Koniuszek M, Tkaczuk C, Bartkowiak A. The Influence of Tin Ions on Growth and Enzymatic Activity of Entomopathogenic Fungi Acta Mycol, 2019, 2(54). https://doi org/10 5586/am 1127.
17. Mahmoudi N, Slater G, Fulthorpe R. Comparison of Commercial Dna Extraction Kits for Isolation and Purification of Bacterial and Eukaryotic Dna from Pah-contaminated Soils Can J Microbiol. 2011;8(57):623-628. https://doi org/10 1139/w11-049.
18. Majchrowska-Safaryan A, Tkazuk C, Symnowicz B. Effects of the Application of A Mineral-and-organic Fertiliser Produced from Brown Coal on the Occurrence and Infectious Potential of Entomopathogenic Fungi in Soil Journal of Ecological Engineering. 2017;3(18):140-148. https://doi org/10 12911/22998993/70184.
19. Moreira C, Celetino D, Sobrnho T, Cardoo I, Ellio S. Agroforestry Coffee Soils Increase the Insect-suppressive Potential Offered by Entomopathogenic Fungi Over Full-sun Soils: A Case Proposing A "Bait Survival Technique" Ecol Evol. 2019;18(9):10777-10787. https://doi org/10 1002/ece3 5598.
20. Nayfach S, Pollard K. Toward Accurate and Quantitative Comparative Metagenomics Cell. 2016;5(166):1103-1116. https://doi org/10 1016/j cell 2016 08 007.
21. Pasolli E, Truong D, Malik F, Waldron L, Segata. Machine Learning Meta-analysis of Large Metagenomic Datasets: Tools and Biological Insights Plos Computational Biology. 2016;7(12):e1004977 https://doi org/10 1371/journal pcbi 1004977.
22. Ramos Y, Taibo A, Vega A, Lemes C, Castañeda-Ruiz R, Portal O. Abundance of Beauveria Spp and Metarhizium Spp in Maize and Banana Agroecosystems in Central Cuba Agron Colomb. 2022;1(40):141-146. https://doi org/10 15446/agron colomb v40n1 100886.
23. Safitri A, Herlinda S, Setiawan A. Entomopathogenic Fungi of Soils of Freshwater Swamps, Tidal Lowlands, Peatlands, and Highlands of South Sumatra, Indonesia Biodiversitas. 2018;6(19):2365-2373 https://doi org/10 13057/biodiv/d190647.
24. Sánchez-Peña S, Lara Medina R. Occurrence of Entomopathogenic Fungi from Agricultural and Natural Ecosystems in Saltillo, México, and their Virulence towards Thrips and Whiteflies Journal of Insect Science. 2011;1(11):1-10. https://doi org/10 1673/031 011 0101.
25. Särkinen T, Staats M, Richardson J, Cowan R, Bakker F. How to Open the Treasure Chest? Optimising Dna Extraction from Herbarium Specimens. PLoS ONE. 2012;8(7):e43808. https://doi org/10 1371/journal pone 0043808.
26. Sczyrba A, Hofmann P, Belmann P, Koslicki D, Janssen S, Dröge J, *et al*. Critical Assessment of Metagenome Interpretation–a Benchmark of Metagenomics Software Nature Chemical Biology. 2017;11(14):1063-1071. https://doi org/10 1038/nmeth 4458.
27. Sevim A, Demir Höfte, Humber R, Demirbag Z. Isolation and Characterization of Entomopathogenic Fungi from Hazelnut-growing Region of Turkey BioControl. 2009;2(55):279-297. https://doi org/10 1007/s10526-009-9235-8.
28. Shay J, Haniford L, Cooper A, Carrillo Blais B, Lau C. Exploiting a Targeted Resistome Sequencing Approach in Assessing Antimicrobial Resistance in Retail Foods

Environmental Microbiome, 2003, 1(18). https://doi org/10 1186/s40793-023-00482-0.
29. Sun X, Hu Y, Wan J, Fag C, Li J, Han & Suzuki S. Efficient and Stable Metabarcoding Sequencing Data Using a Dnbseq-g400 Sequencer Validated by Comprehensive Community Analyses Gigabyte, 2021, 1-15. https://doi org/10 46471/gigabyte 16.
30. Suwandi S. Exploring Entomopathogenic Fungi from South Sumatra (Indonesia) Soil and Their Pathogenicity Against a New Invasive Maize Pest, Spodoptera Frugiperda Biodiversitas, 2020, 7(21). https://doi org/10 13057/biodiv/d210711.
31. Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou N, Wijesundera R. Global Diversity and Geography of Soil Fungi Science, 2014, 6213(346). https://doi org/10 1126/science 1256688.
32. Tedersoo L, Anslan S, Bahram M, Põlme S, Riit T, Liiv I. Shotgun Metagenomes and Multiple Primer Pair-barcode Combinations of Amplicons Reveal Biases in Metabarcoding Analyses of Fungi Mycokeys. 2015;10:1-43. https://doi org/10 3897/mycokeys 10 4852.
33. Vega F, Goettel M, Backwell M, Handler D, Jakson M, Kellr S. Fungal Entomopathogens: New Insights on their Ecology Fungal Ecology. 2009;4(2):149-159. https://doi org/10 1016/j funeco 2009 05 001.
34. Yfun Ecoo S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M. Environmental Dna Metabarcoding Reveals Local Fish Communities in a Species-rich Coastal Sea Scientific Reports, 2017, 1(7). https://doi org/10 1038/srep40368.
35. Yeates C, Gillings M, Dvison A, Altvilla N, Val D. Methods for Microbial Dna Extraction from Soil for Pcr Amplification Biol Proced Online. 1998;1(1):40-47. https://doi org/10 1251/bpo6.