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REVIEW ARTICLE

Exploring microbial diversity in the rhizosphere: a comprehensive review of metagenomic approaches and their applications

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Abstract

The rhizosphere, the soil region influenced by plant roots, represents a dynamic microenvironment where intricate interactions between plants and microorganisms shape soil health, nutrient cycling, and plant growth. Soil microorganisms are integral players in the transformation of materials, the dynamics of energy flows, and the intricate cycles of biogeochemistry. Considerable research has been dedicated to investigating the abundance, diversity, and intricacies of interactions among different microbes, as well as the relationships between plants and microbes present in the rhizosphere. Metagenomics, a powerful suite of techniques, has emerged as a transformative tool for dissecting the genetic repertoire of complex microbial communities inhabiting the rhizosphere. The review systematically navigates through various metagenomic approaches, ranging from shotgun metagenomics, enabling unbiased analysis of entire microbial genomes, to targeted sequencing of the 16S rRNA gene for taxonomic profiling. Each approach's strengths and limitations are critically evaluated, providing researchers with a nuanced understanding of their applicability in different research contexts. A central focus of the review lies in the practical applications of rhizosphere metagenomics in various fields including agriculture. By decoding the genomic content of rhizospheric microbes, researchers gain insights into their functional roles in nutrient acquisition, disease suppression, and overall plant health. The review also addresses the broader implications of metagenomic studies in advancing our understanding of microbial diversity and community dynamics in the rhizosphere. It serves as a comprehensive guide for researchers, agronomists, and policymakers, offering a roadmap for harnessing metagenomic approaches to unlock the full potential of the rhizosphere microbiome in promoting sustainable agriculture.

Keywords Rhizosphere · Metagenomics · PGPM · Bioremediation · Biofuel production · Agriculture

Introduction

The rhizosphere, the narrow zone of soil that surrounds plant roots, represents a dynamic and highly intricate ecosystem where plants and microorganisms engage in intimate interactions. This belowground realm is teeming with life, harboring a remarkable diversity of microorganisms that thrive in the vicinity of plant roots. There is a need for an ecologically compatible and environment-friendly method in the agriculture system that helps to increase the quality and quantity of agricultural products inorder to supply enough essential nutrients for the increasing rate of population growth. The rhizosphere of plant is the most intricate microhabitat comprising a diverse consortium of bacteria, fungi, eukaryotes and archaea (Hakim et al. 2021). They possess the properties of plant growth promotion, disease suppression, removal of toxic compounds and incorporating of nutrients into plants. The rhizosphere is a region that is governed by essential interactions between host plants and microorganisms that are closely associated with plant roots. In addition, nutrient-rich rhizosphere environments stimulates crop improvement. It is characterised by intense biological activity due to the release of root exudates by the host plant, which promotes or inhibits rhizosphere organisms (Ayangbenro et al. 2022). In plant–microbe interactions, microbe provide nutrients to plants through the acceleration of nutrient cycling. Many studies have reported, identification and manipulation of specific plant-beneficial microbes, disease antagonist agents and hormone producers (Binyamin et al. 2019). However, the importance of rhizosphere microbes and rhizosphere

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microbial diversity remains poorly understood (Liu et al. 2020).

Soil microorganisms play an important role in organic and inorganic conversion, energy flows and biogeochemical cycles. A significant amount of research has been done on the quanitity, diversity and complexity of the various microbe-microbe interactions and plant–microbe relationships found in the rhizosphere (Philippot et al. 2024).To understand the genesis of microorganisms in the rhizosphere, one has to interpret their species and functional diversity. Even though there has been significant progress, there is still much work to be done because just 1% of all soil microorganisms are—found to be culturable (Martiny 2019). Scientists now have a better understanding of the rhizosphere microboiota due to the advent of omics and culture-independent approaches (Pramanik et al. 2020). Using high throughput techniques, estimates of up to 10^{11} microbial cells per gram of roots have been reported, including at least 30,000 prokaryotes. It may vary according to the plant species, genotype and age (Bandyopadhyay et al. 2017).

Microbial diversity in the rhizosphere refers to the variety and interactions of microorganisms within the soil ecosystem surrounding plant roots. The microorganisms, collectively known as the rhizospheric microbial community, play multifaceted roles in plant–microbe interactions (Bhattacharyya et al. 2021). They exhibit symbiotic relationships, where microbes provide plants with essential nutrients or help protect them from pathogens and the plant provides nutritions to the microbes, while plants supply the microbes with necessary organic compounds and nutrients. For instance, nitrogen-fixing bacteria like *Rhizobium* spp. establish mutualistic associations with leguminous plants, providing a direct source of nitrogen (Mahmud et al. 2020). Additionally, mycorrhizal fungi, such as those in the genus *Glomus*, form mutualistic symbioses with the majority of terrestrial plants, enhancing nutrient uptake, particularly phosphorus (Sun and Shahrajabian 2023). Moreover, some rhizospheric microorganisms, known as Plant Growth-Promoting Rhizobacteria (PGPRs), actively enhance plant growth and health through various mechanisms such as biological nitrogen fixation and phosphate solubilization, stress alleviation through the modulation of ACC deaminase expression, and production of phytohormones and siderophores (Chandran et al. 2021). Previous research studies on potatoes, sugarcane (Javed et al. 2020) and certain plants like *Arabidopsis* (Müller et al. 2016) and barley (Walters et al. 2016) have shown genotype-dependent variation in microbes present in rhizosphere suggesting diversity in community (Shankar et al. 2023). Recently, studies on rice genotype have shown relative abundance in the rhizosphere compared with bulk soil (Breidenbach et al. 2016; Wu et al. 2022; Xiong et al. 2021). Understanding the composition and functions of rhizospheric microbial communities has traditionally relied on culture-based techniques, but recent advancements in metagenomics have revolutionized our ability to explore this microbial world in depth. Metagenomics, a discipline that involves the direct sequencing of DNA from environmental samples, enables researchers to decipher the genetic potential and functional diversity of these communities. By applying omics approaches, researchers can gain a comprehensive understanding of microbial diversity, functions, and interactions within various environments, offering insights that are not possible through traditional culturing methods alone. Shotgun metagenomics is an advanced high-throughput sequencing technique used to achieve a comprehensive understanding of microbial communities, such as those found in soil. This approach enables the study of microbial diversity, functions, and interactions within the soil ecosystem, offering valuable insights into its ecological dynamics (Fadiji and Babalola 2020).

In this review we will discuss the microbial diversity of the rhizosphere and will try to undercover the functionality of this microbial diversity. We will delve into the multifaceted interactions between plants and rhizospheric microorganisms, emphasizing their ecological and agricultural significance. Moreover, we will explore how metagenomics has transformed our ability to study these communities, offering insights into their structure and functional capabilities. By examining the importance of microbial diversity in the rhizosphere and its connection to metagenomics, we aim to shed light on the intricate and vital relationships shaping subterranean ecosystems and inform strategies for sustainable agriculture and ecosystem management. The rhizospheric microbial community refers to the diverse population of microorganisms that inhabit the soil directly surrounding plant roots, known as the rhizosphere. These microorganisms include bacteria, fungi, archaea, viruses and other microbes. The rhizosphere is the most active micro-domain for plant–microbe interactions and contains microbes that play important roles in plant growth and the maintenance of plant health. Rhizosphere microbes are of paramount importance for several reasons such as nutrient cycling, disease suppression, enhanced nutrient uptake, bioremediation, plant growth promotion, stress tolerance, soil structure and erosion control. Understanding and managing the rhizopsheric microbial community are crucial for sustainable agriculture, ecosystem functioning and environmental health. In natural ecosystems, most nutrients such as N, P, and S are converted to inorganic form by soil microbes and therefore are the preferred nutrient forms for plants (Jacoby et al. 2017). Certain rhizospheric microbes can act as biocontrol agents, protecting plants from pathogens by producing antimicrobial compounds or outcompeting harmful organisms. Some microbes form symbiotic relationships with plants, such as mycorrhizal fungi, which can extend the roots reach for nutrient uptake, enhancing the plants ability to access nutrients.

Many rhizospheric microbes produce growth-promoting substances like phytohormones, siderophores and enzymes stimulating plant growth and development (Chandran et al. 2021). Microbes in the rhizosphere can help plants cope with various stresses, such as drought, salinity and heavy metal toxicity by improving the plants stress tolerance mechanisms. The rhizosphere is a hotspot for microbial genetic diversity. These microorganisms can have a direct or indirect impact on the genetic makeup of the plants they interact with, contributing to overall genetic diversity in plant populations. The genes *nifH* (nitrogenase iron protein gene), *ACC* Deaminase gene (*acdS*), mycorrhizal genes (various), and rhizobial nodulation genes (*nod* genes) are well-studied genes of microbes that impact the gene expression pattern of plants (Swarnalakshmi et al. 2020).

Plant microbe interaction in rhizosphere

The relationship between plants and microbes in the rhizosphere can be classified as associative, symbiotic, neutralistic, or parasitic. Symbiotic and associative interactions with beneficial microbes like endo- and ecto-mycorrhizal fungi, nitrogen-fixing bacteria, and plant growth-promoting rhizobacteria (PGPR) are examples of positive interactions. Negative interactions include association with parasitic plants, pathogenic bacteria, fungi, oomycetes, nematodes, and invertebrate herbivores.

Agricultural research has extensively investigated the molecular pathways governing species-specific cooperation between legume plants and soil rhizobia, emphasizing the critical relevance of their symbiotic relationship (Shankar et al. 2021). Host-specific flavonoids that are secreted in the root exudates influence interactions between legumes and rhizobia. These substances belong to a large family of aromatic chemicals that are produced from plants secondary metabolism. Flavonoids trigger a series of transcriptional events that lead to the synthesis of the primary rhizobial nodulation signals known as Nod factors (NF) or lipochito oligosaccharides (LCOs), depending on the host and the bacteria (Ghantasala and Roy Choudhury 2022). Nod factors cause many developmental processes in the plant partner that result in morphological changes in legume root hairs, the creation of infection threads, the growth of nodules, and symbiotic nitrogen fixation (Yang et al. 2022). More than 80% of plant species have symbiotic relationships with *arbuscular mycorrhiza* fungus (AMF) (Khaliq et al. 2022). It is reported that strigolactone signals in the rhizosphere may be detected by AMF using the ancestral receptor (Wang et al. 2024). Therefore, discovering the strigolactone receptors in fungi could be an intriguing area of study in the future. Nitrogen-fixing bacteria, such as those in the genera *Rhizobium* and *Frankia*, establish symbiotic relationships with specific plant species (Xu and Wang 2023). Leguminous plants, like soybeans and clover, commonly engage in nitrogen-fixing symbiosis (Goyal et al. 2021). Methanotrophic bacteria in wetland soils and the rhizosphere of certain plants help mitigate methane emissions, a potent greenhouse gas (Li et al. 2024). Wetland plants, such as rice, foster methane-oxidizing bacteria in their rhizosphere to reduce methane fluxes (Knief 2015). Rhizosphere can be divided into 3 zones: ectorhizosphere, rhizoplane and endorhizosphere. The inner realm of the rhizosphere encompasses the root cortex and endodermis, along with the intercellular space known as the apoplast. This region serves as a habitat for microbes and cations. The endophyes are microorganisms that live in the endorhizosphere. Endophytic bacteria and fungi inhabit plant tissues, often conferring benefits such as enhanced nutrient uptake, stress tolerance, and defense against pathogens (Santoyo et al. 2016). For instance, endophytic *Bacillus spp.* can promote plant growth and protect against diseases (Miljaković et al. 2020). Various microbial genera such as *Pseudomonas, Bacillus, Achromobacter, Burkholderia, Enterobacter, Azotobacter, Methylobacterium,* and *Trichoderma* have been extensively investigated in the context of plant growth promotion, due to their ability to improve plant resistance to abiotic stress (Meena et al. 2017). In earlier research, it was demonstrated that *Pseudomonas sp.* improved capacity to colonize roots and ability to generate exopolysaccharides resulted in increased tolerance to salinity (Fatima and Arora 2021). A thermotolerant *Pseudomonas putida* strain, NBR10987, was identified by (Mishra et al. 2022) from the drought-stressed rhizosphere of chickpea. The strain was able to overcome stress by generating exopolysaccharides with distinct water-holding properties.

Plant growth-promoting microbes (PGPMs)

Plant Growth-Promoting Rhizobacteria (PGPR) are a group of soil bacteria that inhabit the rhizosphere, the region of soil surrounding plant roots. They have gained considerable attention in recent years due to their ability to enhance plant growth and health (de Andrade et al. 2023). Some PGPR, such as nitrogen-fixing bacteria like *Azospirillum* and *Bradyrhizobium,* convert atmospheric nitrogen into ammonia, which plants can readily absorb (Etesami and Adl 2020). PGPR can solubilize insoluble forms of phosphate, making this essential nutrient more available to plants (Pradhan et al. 2017). In addition, PGPR produce plant hormones like auxins, gibberellins, and cytokinins, which stimulate plant growth and development (Khan et al. 2020). PGPR encompass a wide range of bacterial species, including *Bacillus, Pseudomonas, Rhizobium* and many others. The choice of PGPR strain often depends on the specific plant and environmental conditions (Khatoon et al. 2020). Among the diverse rhizospheric microorganisms, a subset of bacteria, fungi (endo- and ectomycorrhizal), cyanobacteria and archaea referred to as Plant Growth-Promoting Microbes (PGPMs) have emerged as central players in plant–microbe interactions (Meena et al. 2017). PGPR are divided into two groups based on their relationship with the host plants: (1) nonsymbiotic rhizobacteria (free living) that exist outside plant cells are known as extracellular PGPR, and (2) symbiotic rhizobacteria, which live inside the cells and invade the interior of the cells (intracellular PGPR), such as nodule bacteria *Rhizobium* (Reinhold-Hurek et al. 2015). A diverse assemblage of PGPMs encompasses rhizobacteria such as *Pseudomonas* and *Bacillus*, mycorrhizal fungi like *Glomus, Actinomycetes* including *Streptomyces,* endophytic bacteria and fungi such as *Enterobacter* and *Trichoderma*, alongside other taxa such as cyanobacteria, algae, nitrogen-fixing bacteria like *Azotobacter*, phosphate-solubilizing bacteria such as *Bacillus*, methanotrophic bacteria including *Methylococcus,* and siderophore-producing bacteria such as *Pseudomonas* (Naamala and Smith 2021). Molecular markers like nosZ (Florio et al. 2019), tdfA, nirK (Pothier et al. 2008), amoA (Hamada and Soliman 2023), and nifH (Li et al., 2017) are found in microbial communities associated with roots, soil, or rhizosphere settings (Florio et al. 2019).

Comparative assessment of rhizospheric and bulk soil microbial community dynamics

Soil microbiomes are dynamic and complex, contributing to vital ecosystem processes. Two prominent microbial habitats in soil are the rhizosphere (the soil surrounding plant roots) and the bulk soil (non-rhizospheric soil). These communities differ significantly in composition and function (Kumawat et al. 2022). The rhizosphere often harbors higher microbial diversity than bulk soil due to the availability of carbon-rich substrates and plant–microbe interactions (Sharma et al. 2021). In addition, rhizospheric microbes engage in intricate interactions with plant roots, aiding in nutrient acquisition, disease suppression, and stress tolerance (Khan et al. 2021). Bulk soil microbes are key players in organic matter decomposition, nutrient cycling, and the mineralization of organic compounds (Bhattacharyya et al. 2022). (Ulbrich et al. 2022). The rhizosphere communities tend to be more stable over time, reflecting the core microbiome of a given ecosystem. Amplicon and shotgun metagenomic sequencing have been used in a number of studies that have expanded our knowledge of microbial diversity in recent years. (Brumfield et al. 2020). For instance, Xu et al.(2018) conducted a comprehensive analysis delving into the structural and functional composition of the microbiome in citrus bulk soil and rhizosphere. Additionally, Alzubaidy et al. (2016) provided valuable insights into the taxonomic and functional aspects of the rhizosphere microbiome of gray mangroves (*Avicennia marina*) in the Red Sea. *Proteobacteria, Bacteroidetes, and Firmicutes* were the dominant taxa, with substantial concentrations of methanogens and sulfate reducers. Functional examination of the rhizosphere microbiomes revealed considerable enrichment in metabolism of aromatic compounds, mobile genetic elements, potassium metabolism and pathways that utilize osmolytes. Moreover, (Bakker et al. 2015) demonstrated how various bacterial and archaeal taxa react to chemical resource amendments, leading to a reduction in microbial diversities within bulk soil. Consequently, the composition of the bulk soil microbial communities within a specific soil type could impact the structure of the rhizosphere microbial community in *Zea mays*. Table 1 summarizes the recent metagenomics studies on the rhizospheric soil and the representative microbiota present in that soil.

Microbial communities in plant rhizospheric soil: diversity and commonalities

Understanding the diversity and commonalities of microbial communities inhabiting the rhizospheric soil of different plant species is essential for elucidating plant–microbe interactions and their impact on ecosystem functioning. Metagenomic studies have provided valuable insights into the composition and functional potential of these microbial communities across various plant species (Table 1). Metagenomics, a powerful molecular approach, has revolutionized our ability to study microbial communities by allowing the comprehensive analysis of microbial diversity and functional potential directly from environmental samples. Various studies employing molecular approaches such as molecular fingerprinting, 16S rRNA gene sequencing, shotgun metagenomic sequencing ((Bulgarelli et al. 2015), and ITS rRNA (Yang et al. 2020) sequencing have shed light on the microbial diversity in the rhizosphere of different plant species. These studies have identified a wide range of microbial taxa, including bacteria, fungi, archaea, and other microorganisms, and have highlighted the influence of plant species on shaping the rhizosphere microbiome.

Diversity of microbial communities

Metagenomic analyses have revealed a wide diversity of microbial communities in the rhizosphere, influenced by factors such as plant species, soil characteristics, and environmental conditions. For instance, *Sorghum bicolor* (L) Moench exhibits a microbial community dominated by *γ-Proteobacteria, Firmicutes,* and *Actinobacteria*. In contrast, *Lactuca sativa* hosts *Bacteroidetes,* along with *Proteobacteria, Actinobacteria,* and *Firmicutes* in its rhizospheric soil (Kalu et al. 2022). Similarly, *Hordeum*

Table 1 The most prevalent rhizospheric microbial profiles identified by rhizosphere metagenomic analysis of some common plants

Plant Name	Molecular approach	Identified microbial profiles	Ref
Sorghum bicolor (L) Moench	Molecular fingerprinting (T-RFLP/DGGE)	γ-Proteobacteria, Firmicutes, Actinobacteria	Ramond et al. (2013)
Lactuca sativa	Shotgun metagenomic sequencing	Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes	Krober et al. (2014)
Hordeum vulgare	16S rRNA gene and shotgun metagenomic sequencing	Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes, Chloroflexi	Bulgarelli et al. (2015)
Welwitschia mirabilis	16S rRNA gene Sequencing	Proteobacteria, Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria	Valverde et al. (2016)
Vigna radiate	16S rRNA gene sequencing	Proteobacteria, Actinobacteria, Deltaproteobacteria, Gammaproteobacteria, Alphaproteobacteria	Iyer and Rajkumar (2017)
Solanum lycopersicum	Metagenomic sequencing	Bacteroidetes, Firmicutes, Verrucomicrobia, Ignavibacteriae, Proteobacteria, BRC1	Kwak et al. (2018)
Paspalum scrobiculatum	Metagenomic sequencing	Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, Acidobacteria, Bacteroidetes, Verrucomicrobia, Planctomycetes, Cyanobacteria	Prabha et al. (2019)
Zea mays	Shotgun metagenomic sequencing	Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Fungi, Archaea	Enebe and Babalola (2020)
Glycine max, Trifolium, Zea mays, Oryza sativa, Brassica napus, Brassica oleracea	16S and ITS rRNA sequencing	Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Mortierellomycota, Ascomycota, Mucoromycota, Olpidiomycota	Yang et al. (2020)
Calotropis procera	16S rRNA gene sequencing	Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Gemmatimonadetes, Proteobacteria, Thermomicrobia, Cyanobacteria, Verrucomicrobia	Ramadan et al. (2020)
Helianthus annuus	Shotgun metagenomic sequencing	Actinobacteria, Proteobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia	Alawiye and Babalola (2021)
Oryza sativa	16S rRNA gene sequencing	Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Verrucomicrobia, Gemmatimonadetes, Firmicutes, Nitrospirae, TM7	Oliveira et al. (2022)
Lantana camara L	Metagenomic sequencing	Pseudomonadota, Actinomycetota, Bacillota, Acidobacteriota, Planctomycetota, Cyanobacteria, Bacteroidota, Euryarchaeota, Thermoproteota	Gola et al. (2023)
Brachypodium distachyon	16S rRNA gene sequencing	Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi, Planctomycetota, Verrucomicrobiota, Bacteriodota, Myxococcota, Firmicutes	Acharya et al. (2023)

vulgare shows a diverse microbial profile consisting of *Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes,* and *Chloroflexi* (Bulgarelli et al. 2015).

Other plant species, such as *Welwitschia mirabilis, Vigna radiate*, and *Solanum lycopersicum*, also exhibit distinct microbial communities in their rhizospheric soil, reflecting the specificity of plant–microbe interactions (Valverde et al. 2016; Iyer and Rajkumar 2017; Kwak et al. 2018). Moreover, shotgun metagenomic sequencing studies have identified diverse microbial taxa, including *Actinobacteria, Proteobacteria, Chloroflexi, Firmicutes, Acidobacteria, Bacteroidetes,* and *Verrucomicrobia*, in the rhizospheric soil of *Paspalum scrobiculatum* (Prabha et al. 2019).

Common microbial communities

Despite the variability in microbial community composition among different plant species, certain microbial groups are commonly found across various rhizospheric soils. *Proteobacteria, Actinobacteria, Bacteroidetes,* and *Firmicutes* are among the most frequently encountered bacterial phyla in the rhizosphere of diverse plant species (Na et al. 2018).

Proteobacteria, a diverse group known for its metabolic versatility, is often abundant in plant-associated microbiomes (Uroz et al. 2019). *Actinobacteria*, renowned for their ability to produce bioactive compounds and participate in nutrient cycling, are also commonly found in the rhizospheric soil of different plant species (Mitra et al. 2022). Similarly, *Bacteroidetes* and *Firmicutes* contribute to organic matter decomposition, nutrient cycling, and plant growth promotion in the rhizosphere (Gu et al. 2020). Furthermore, other microbial groups such as *Chloroflexi, Planctomycetes, Acidobacteria,* and *Verrucomicrobia* are frequently encountered in rhizospheric soils, indicating their widespread presence and potential ecological roles across diverse plant–microbe systems (Kalam et al. 2022).

Metagenomic studies have provided valuable insights into the diversity and commonalities of microbial communities inhabiting the rhizospheric soil of different plant species. Understanding the roles of these microbial communities in nutrient cycling, plant health, and ecosystem functioning is crucial for developing sustainable agricultural practices and ecosystem management strategies. Metagenomic studies have confirmed the high diversity of rhizosphere microbes, encompassing bacteria, fungi, archaea, and other microbial groups. While bacteria are often the most abundant and diverse microbial taxa in the rhizosphere, fungi and archaea also contribute significantly to overall diversity.

Metagenomic approach for microbial diversity analysis

Microbial abundance and diversity in soil ecosystem defines the health of soil and its productivity. Soil holds the major importance for plant growth and crop production as well as plays a significant role in life on the earth. Microbes that are present in the soil can be grown in the laboratory in various types of media and can be characterised and identified. Due to insufficient knowledge regarding the various conditions such as pH, temperature, media compositions necessary for the growth of the majority of soil microbes, we face challenges in cultivating them. The inability to culture the majority of soil microbes is attributed to their intricate ecological and physiological requirements, which often include complex nutritional dependencies, environmental conditions challenging to replicate in laboratory settings, and intricate interactions within microbial communities that are not fully understood. These factors present significant challenges to the successful isolation and cultivation of soil microbes in vitro. To identify the non-culturable microbes, metagenomics approach holds an upper hand. Metagenomic approach can again be subdivided into two main classes: one is culture dependent (Costa et al. 2020) and the other one is culture independent (Sabale et al. 2019). There are 4 major strategies available in culture-independent approach: PCR-dependent, PCR-independent, sequencing based and microbial lipid based (Fig. 1). Nevertheless, the primary focus of this review is on sequencing-based metagenomics techniques for unravelling the microbial ecology of the rhizospheric soil environment.

Uncultured microorganisms play a critical role in ecosystem functioning. Additionally, metagenomic approaches enable the exploration of microbial diversity at various taxonomic levels, from phyla to species, providing a holistic understanding of community structure (Srivastava et al. 2019). Furthermore, metagenomic analysis facilitates the prediction of functional pathways and metabolic capabilities within rhizosphere microbial communities (Wang et al. 2023). By annotating sequencing reads against reference databases of metabolic pathways, researchers can infer the potential biochemical processes occurring in the rhizosphere. This information is invaluable for understanding the contributions of microbial communities to nutrient cycling, plant growth promotion, and disease suppression (Pascale et al. 2020). The availability of sophisticated bioinformatics tools and software platforms further enhances the efficiency and accuracy of metagenomic analysis. Tools like QIIME 2, MG-RAST, MetaPhlAn, HUMAnN, and MetaCyc provide a comprehensive suite of functionalities for processing, analyzing,

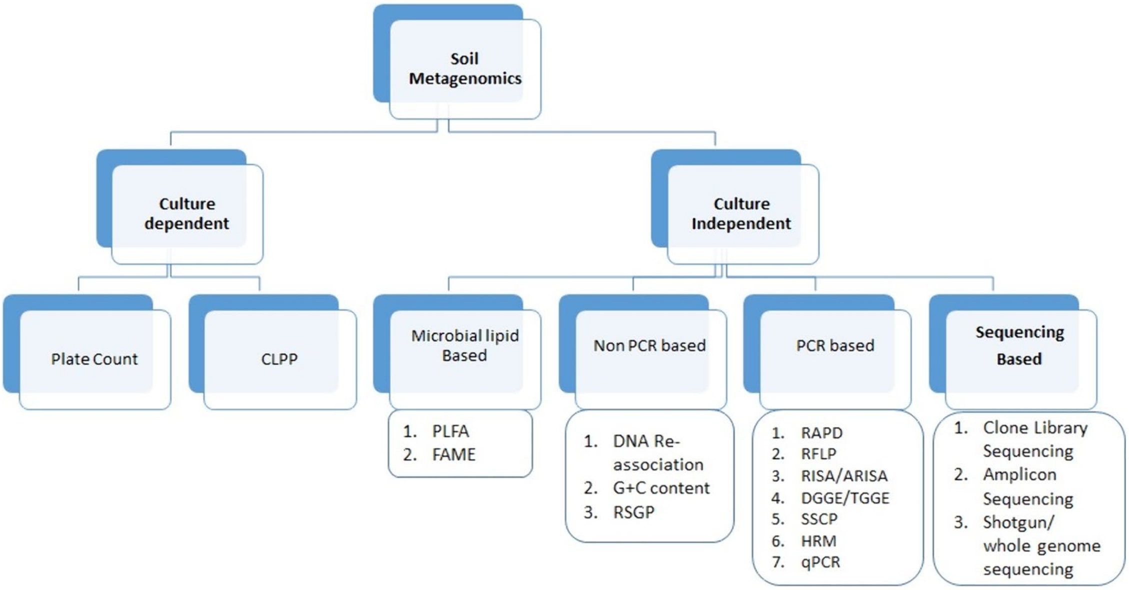

Fig. 1 Applications of soil metagenomic techniques for identifying the diversity of soil microbes

and interpreting metagenomic data (Woloszynek et al. 2018). These tools enable researchers to perform taxonomic classification, diversity analysis, functional annotation, and comparative studies across samples, facilitating robust and reproducible scientific investigations.

Amplicon sequencing targets specific marker genes, such as the 16S rRNA gene for bacterial communities or the ITS region for fungal communities (Walters et al. 2016). The workflow typically involves steps such as raw reads generation, merging, removal of barcodes and primers, quality control, and taxonomic profiling using tools like QIIME and USEARCH (Silva et al. 2023). Operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) are generated, followed by downstream analyses like denoising and functional prediction. Statistical analysis is crucial for understanding microbial community composition and dynamics (Trego et al. 2022). Tools like DESeq2, edgeR, and ALDEx2,MG-RAST, PICRUSt, and Tax4Fun identify taxa or OTUs with significant abundance differences between sample groups (Cappellato et al. 2022; Douglas 2020). The use of STAMP and PAST from a previous study (Hu et al. 2020), illustrating their roles in analyzing taxonomic and functional profiles of microbial communities, providing a strong foundation for our investigation into microbial diversity. Beta diversity analysis, using metrics like Bray–Curtis dissimilarity and UniFrac distances, reveals structural diversity and community similarity. Whereas, alpha diversity assesses the diversity within a sample, with its values compared using statistical tests like ANOVA or Mann–Whitney U test and visualized through methods such as box-plots (Kers and Saccenti 2022). Correlation analysis, with Spearman or Pearson coefficients, explores relationships between microbial taxa and environmental factors, investigating ecological interactions. Phylogenetic trees, generated by QIIME and mothur, show evolutionary relationships among taxa. In addition, heatmaps display OTU abundance across samples, revealing sample clusters and differential abundance. PCoA plots visualize beta diversity patterns and sample similarities, aiding in community structure exploration (Hugerth and Andersson 2017). Metagenomic sequencing involves the shotgun sequencing of total DNA extracted from a microbial community. The workflow includes steps such as library preparation, sequencing, assembly using tools like MEGAHIT or metaSPAdes, and taxonomic profiling using tools like MetaPhlAn2 or Kraken (Quince et al. 2017). Functional profiling can be performed using tools like HUMAnN2 or MEGAN, followed by prediction of gene functions using software such as metaGeneMark or Prokka (Walsh et al. 2023). These tools enable the examination of microbial communities through pathway enrichment analysis, functional annotation, and the discovery of genes and pathways that are differentially abundant. Shotgun metagenomic sequencing facilitates comprehensive visualization of microbial communities through diverse techniques. Functional profiles are typically portrayed using barplots or stacked barplots, presenting the relative abundance of functional categories or pathways. Taxonomic profiles utilize abundance plots to illustrate microbial community composition across taxonomic levels (Nagpal et al. 2019). Furthermore, network analysis tools like Cytoscape and Gephi allow visualization of microbial interactions and correlations inferred from metagenomic data (Tao et al. 2018).

Amplicon sequencing-based metagenomics

The ease of use, affordability, and time efficiency of Next-Generation Sequencing (NGS) has significantly simplified the process of uncovering microbial diversity within any ecosystem (Sabale et al. 2019). The initial step in amplicon-based metagenomics approach starts with the identification of the problems such as why do you wish to investigate the microbial diversity of soil? What plants whose rhizospheric soil you wish to examine and how does it affect crop yield? The collection of samples comes next after the location and plant have been determined. When gathering samples, one should exercise extreme caution. A successful metagnomic study requires a sufficient number and sample size. Amplicon-based metgenomics procedures are described in Fig. 1.

The diversity found in the 16S rDNA of bacteria and the 18S rDNA of eukaryotes is essential for amplicon-based sequencing metagenomics. 16S amplicon-based metagenomics is widely used. However, this strategy faces the problems of selecting the primers which are being used for the amplification of 16S rDNA. There are various primers available for the amplification of the different variable regions of the 16S rDNA. This problem could be resolved by a review of the literature for the same sample that you are going to study or electronic PCR (computational method that mimics PCR reactions using digital data) can be run on the basis of the SILVA database (Bonk et al. 2018). Sample sequencing is often performed on the Illumina MiSeq, HiSeq 2500, or NovaSeq 6000 platform in paired-end 250 bases (PE250) mode, which generates 50,000–100,000 reads per sample (Callahan et al. 2016). However, this technique can only reach genus-level resolution. Moreover, it relies on the specific primers and number of PCR cycles chosen, which may lead to some false-positive or false-negative results in downstream analyses (Johnson et al. 2019).

Whole genome sequencing based metagenomics

Whole genome metagenomics is a powerful approach used to study the collective genetic material of all microorganisms within a complex microbial community in a given environment. This technique involves sequencing the entire genomic content, including the genomes of bacteria, archaea, viruses, and eukaryotes present in a sample. Table 2 represents the comparison of amplicon-based and whole genome-based metagenomics. Table 3 represents advantages and disadvantages of both the metagenomics approaches.In recent years, research has increasingly shifted towards shotgun or whole genome metagenomics, which offers more comprehensive information compared to amplicon metagenomics (Bell et al. 2021). Shotgun metagenomics or whole genome metagenomics allows for the thorough analysis of the entire genomic content of microbial communities, offering insights into both taxonomic composition and functional potential (Purushothaman et al. 2022). The process of shotgun metagenomics for soil typically involves sample collection, DNA extraction, DNA fragmentation (achieved either physically, by

Table 2 comparison of comparison of amplicon-based and whole genome-based metagenomics

Aspect	Amplicon-based metagenomics	Whole genome metagenomics
Target genes	Amplifies specific marker genes (e.g., 16S rRNA, 18S rRNA)	Sequences the entire genomic content of all microorganisms
Information content	Provides taxonomic composition and diversity information	Offers both taxonomic and functional information
Resolution	Lower resolution as it focuses on specific genes	Higher resolution, capturing entire genomic diversity
Sequencing depth	Requires less sequencing depth	Requires deeper sequencing for comprehensive coverage
Cost	Generally less expensive due to targeted sequencing	Generally more expensive due to comprehensive sequencing
Bioinformatics complexity	Lower complexity in terms of bioinformatics analysis	Higher complexity, involving genome assembly and annotation
Applications	Community profiling and diversity studies	Detailed analysis of both taxonomy and functionality
Sample complexity	Suitable for less complex communities or specific taxonomic focus	Ideal for more complex microbial communities
Data analysis focus	Clustering sequences into OTUs or sequence variants	Genome assembly, gene prediction, functional annotation
Example technologies	16S rRNA gene sequencing, ITS sequencing (for fungi)	Shotgun metagenomic sequencing, Illumina, PacBio, Nanopore
Primary output	Operational Taxonomic Units (OTUs) or sequence variants	Assembled genomes, taxonomic and functional annotations
Typical research questions	What types of microorganisms are present?	What are the functional capabilities of the microbiome?
Commonly used in	Microbial community surveys, biodiversity studies	In-depth functional and taxonomic analysis of microbiomes

Table 3 Advantages and Limitations of Amplicon-based and whole genome-based metagenomics

Aspect	Amplicon-based metagenomics	Whole genome metagenomics
Advantages		
Focused sequencing	Cost-effective due to targeted sequencing	Comprehensive view of all genomic content
Taxonomic profiling	High taxonomic resolution for specific genes	Higher taxonomic resolution with potential strain-level identification
Computational efficiency	Lower computational complexity in data analysis	Provides more comprehensive information for detailed analyses
Ease of interpretation	Simpler data interpretation for taxonomic diversity	Allows for a deeper understanding of functional potential
Sample throughput	Suitable for high-throughput sequencing of many samples	Requires deeper sequencing and may be less suitable for high throughput
Community composition studies	Well-suited for studies focusing on community composition	Provides insights into both taxonomic composition and functional diversity
Limitations		
Limited functional insight	Primarily provides taxonomic information, limited functional insights	Although more comprehensive, may not capture all functions present in the community
Biased amplification	Susceptible to primer bias, leading to uneven representation of taxa	Minimizes primer bias but may have challenges with certain taxa or GC-rich regions
Limited strain-level resolution	Generally limited to genus or species level resolution	Offers higher resolution but may still have challenges with closely related strains
Impact of primer choice	Sensitivity to primer selection and sequence biases	Less dependent on primer choice, but other biases may still exist
Detection of novel microbes	May miss novel microbes not targeted by primers	Can identify novel microbes and reveal new functional potentials
Sequencing depth considerations	Requires less sequencing depth for taxonomic profiling	Requires deeper sequencing to capture the entire genomic content
Applicability to simple systems	Well-suited for less complex microbial communities	Ideal for more complex microbial communities
Cost efficiency for specific goals	Cost-effective for specific taxonomic profiling goals	Generally more expensive due to deeper sequencing for comprehensive analysis
Influence of library preparation	Susceptible to biases introduced during library preparation	Requires careful library preparation to minimize biases and artifacts
Interpretation of functional potential	Limited information on the potential functions of microbial communities	Provides more data for functional interpretation, but the complexity of analysis increases

sonication, or enzymatically), library preparation, adapter ligation, library amplification, and sequencing on platforms such as Illumina, PacBio, or Oxford Nanopore (Bell et al. 2021). Sequencing platforms generate millions to billions of short or long DNA reads, depending on the technology used, and manufacturers often provide kit-specific primers and adapters for library preparation, with researchers typically following the protocols provided by the kit (Fig. 2).

There is extensive research being conducted on rhizospheric soil using shotgun metagenomics. For example, in November 2023, (Akinola et al. 2023) conducted a study on the rhizospheric soil of maize in an attempt to identify the microbial diversity (archaeal, bacterial, and fungal) in the soil. In their study, the authors identified common genera in both rhizospheric and bulk soil, such as *Ascomycota, Thaumarchaeota, Euryarchaeota,* and *Crenarchaeota.* However, certain genera were found to be more prevalent in the rhizospheric soil of maize, including *Nitrosopumilus, Candidatus Nitrososphaera, Cenarchaeum, Chaetomium,* and *Neurospora.*

Functional potential of metagenomic analysis

The wealth of microbes in the soil is related to the quality of the soils. The more diversity and abundance of beneficial microbes in the soil results in the formation of healthy soil for growth of the plants. The microbes residing in the rhizosphere are actively engaged in carbon recycling, and other metabolic activities that either directly or indirectly affect the health of the soil and ultimately the growth of the plants (Jansson and Hofmockel 2020). Soil microbes play a crucial role in detoxifying heavy metal stress by immobilizing, transforming, and metabolizing

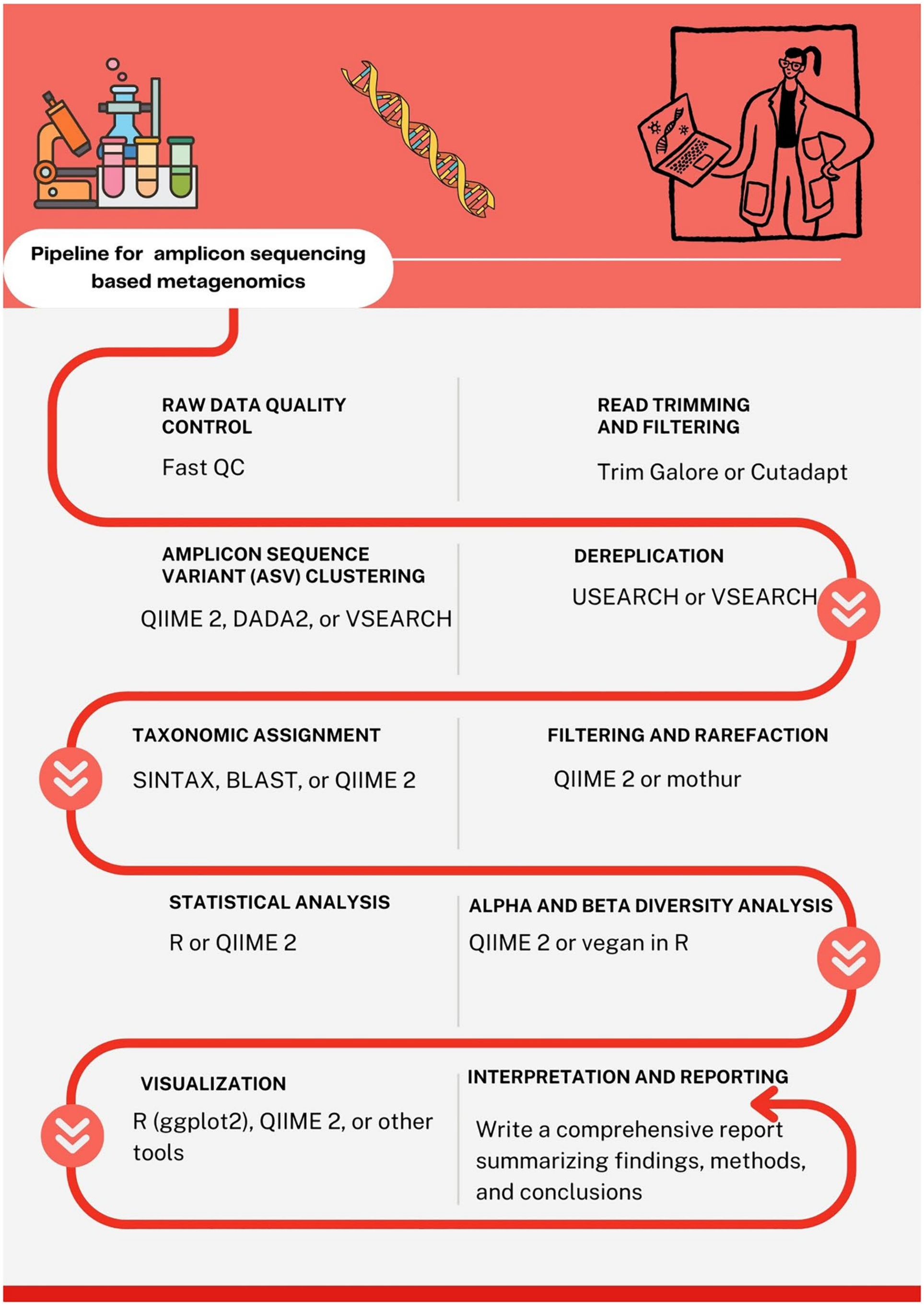

Fig. 2 Schematic overview of amplicon sequencing-based metagenomics pipeline

toxic metals, while also contributing to nutrient recycling and overall soil quality improvement (Lewin et al. 2024). The microbes in this area have novel metabolic pathways that might help in the discovery or formulation of bioactive compounds as antimicrobial compounds or they may possess some pathways directly related to the degradation of the pollutants present in the soil (Gaytán et al. 2020). The metagenomic analysis of the rhizosperic soil provides an era where the identification of microbes might help in various ways to help the growth and yield of the plants and thus could be beneficial to society (Subrahmanyam et al. 2020). In recent years various studies have been performed in the field of metagenomics to show the potential of differential microbes residing in the soil with numerous applications such as bioremediation (Jeffries et al. 2018), biofulel production, (Wang et al. 2019) as well as in sustainable agriculture (Mechan Llontop et al. 2020) Fig. 3 shows various aspects of application of metagenomics of rhizospheric soil.

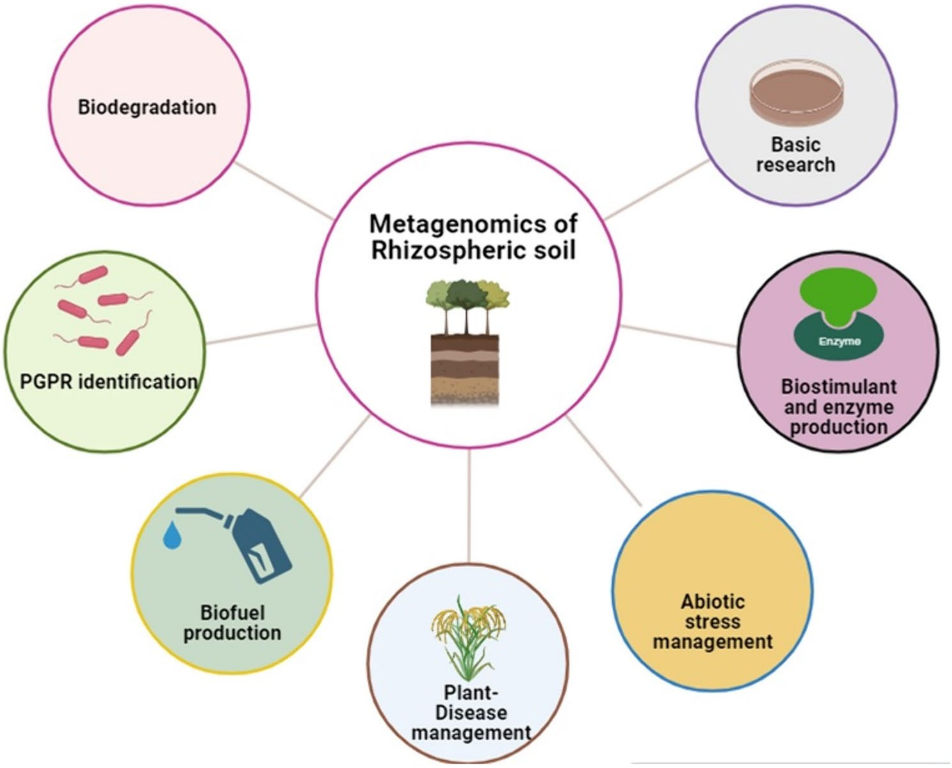

Fig. 3 Potential applications of metagenomics in rhizospheric soil

Metagenomic Insights into bioremediation

In the rhizospheric region plant roots secretes some biochemical compounds such as secondary metabolites, sugar and acids that serve as the signal for the microbial community and attracts them in the vicinity of the plant roots. These chemosignals have a very prominent role in the attraction and colonization of the microbes in the rhizophere. The microbes present in the soil contaminated with pollutants such as heavy metals, oil or hydrocabons, have the potential to either degrade the pollutants or with their physiological properties they can modulate the uptake of the pollutants by the plants (Asghar et al. 2016).

Metagenomic studies of the microbes residing in the contaminated rhizospheric soil offer an approach of the identification of microbes with the potential to be used in the bioremediation of pollutants. Many of the oil biodegradation studies, however, focus solely on thee microbial community (Singha and Pandey 2021) or purely on oil compound decay dynamics (Hedgpeth et al. 2021). (Ribicic et al. 2018) performed microcosm experiments at a temperature of 5 °C, employing chemically dispersed oil in non-amended seawater. This study established a connection between biodegradation processes, microbial community dynamics, and metagenome changes, elucidating the succession pattern based on substrate specialization and identified Bermanella and Zhongshania as key agents responsible for initiating the degradation of n-alkanes.

Subsequently, *Colwellia* and microorganisms closely related to *Porticoccaceae* played crucial roles in secondary n-alkane breakdown and beta-oxidation. Additionally, *Cycloclasticus, Porticoccaceae,* and *Spongiibacteraceae* were associated with the degradation of mono- and polycyclic aromatics. These findings shed light on the intricate dynamics of microbial communities during oil biodegradation in cold environments, emphasizing the importance of understanding the specific roles played by different bacterial taxa in the process.

According to Yergeau et al. 2012, who used metagenomic analysis to try and determine which bacteria are responsible for the biodegradation of hydrocarbons in soil, the major groups involved in this process are *Actinobacteria, Gammaproteobacteria* and *Alphaproteobacteria*. RT-qPCR assays confirmed that *Pseudomonas* and *Rhodococcus* species actively expressed hydrocarbon degradation genes in Arctic biopile soils.

Metagenomic analysis has become instrumental in identifying microbial diversity and specific genes that hold promise as biomarkers for pollution in the context of bioremediation strategies. Numerous microbes play pivotal roles in remediating pollutants, leveraging bioactive compounds and enzymes chracterised through metagenomics approaches. Key microbial communities, including *Bacteroidetes, Chloroflexi, Proteobacteria, Firmicutes, Actinobacteria, Spirochetes, Acidobacteria* and *Patesibacteria* demonstrate a remarkable ability to tolerate elevated concentrations of metals. The application of metagenomics enables the exploration of the genetic repertoire of these microbial communities, unraveling their potential for pollutant degradation. The identification of specific genes associated with the synthesis of bioactive compounds and enzymes provides valuable insights into the mechanisms underlying bioremediation efforts. This nuanced understanding facilitates the development of targeted strategies harnessing the capabilities of diverse microbial communities for effective pollution mitigation (Sharma and Kumar 2021).

In marine environments, (Vigneron et al. 2017) conducted a comparative metagenomic study to uncover the diverse functions of microorganisms inhabiting deep cold seeps in the Gulf of Mexico. Their findings highlighted the potential of these microbes (present in the deep cold seeps in the Gulf of Mexico) to metabolize non-methane hydrocarbons, contributing to the ecosystem's bioremediation capabilities. The microbial community composition primarily comprised *Planctomycetes*, aligning with metabolic activities associated with the degradation of detrital marine snow. Conversely, in seep samples where methane served as the primary hydrocarbon, methane oxidation facilitated by prevalent ANME-1 members likely dominated the microbial processes. Samples from seep environments featuring fluids containing both methane and intricate hydrocarbons exhibited an abundance of *Chloroflexi (Anaerolinaceae)* and deltaproteobacterial lineages, suggesting a metabolic potential geared towards the degradation of complex hydrocarbons.

(Jeffries et al. 2018) employed metagenomic analysis to explore the functional potential and taxonomic composition of soil microbial communities exposed to organophosphorus pesticides. This approach enabled the prediction of chemical compound breakdown pathways, offering insights into pesticide degradation mechanisms. This study has a few key findings related with the importance of metagenomic analysis. The discovery of overrepresented taxa, such as *Candidatus Koribacter* and *Bradyrhizobium*, in soils with efficient organophosphate (OP) degradation suggests a direct link between microbial community composition and pesticide degradation capability. This highlights the importance of understanding microbial diversity and its functional potential in contaminated environments for effective remediation strategies. The identification of specific traits in key taxa, including metabolic versatility, membrane transport abilities, and roles in nutrient cycling, suggests their potential contribution to sustaining nutrient availability and supporting degradation processes in contaminated soils. This underscores the significance of microbial communities in maintaining ecosystem functions, even in polluted environments. The presence of genes associated with nutrient cycling and phosphorus metabolism in taxa enriched in rapid degradation clusters indicates their crucial role in facilitating pesticide degradation. This emphasizes the interconnectedness of microbial community structure, functional attributes, and pollutant degradation processes, highlighting the need for further research to isolate and characterize key OP-degrading taxa for a comprehensive understanding of biodegradation mechanisms. Furthermore, (Garrido-Sanz et al. 2019) investigated diesel degradation by analyzing metagenomic data from microbial consortia. Their study revealed bacterial genera, genes, and enzymes involved in diesel degradation, providing valuable information for bioremediation strategies. (Gaytán et al. 2020) integrated physical and chemical analyses with metagenomics to elucidate metabolic pathways associated with polyurethane degradation, aiming to address plastic and xenobiotic pollution.

Metagenomic insights into biofuel synthesis

Biomass stands out as a renewable energy source, with its conversion leading to the production of biofuels like biogas and liquid biofuels. Diverse biomass resources encompass forest products such as bark, shrubs, sawdust, trees, and wood residues, alongside agricultural waste from crops like alfalfa, grasses, soybean, sunflower, and starch crops including corn, barley, and wheat (Sarsekeyeva et al. 2015). Microbial-driven anaerobic digestion (AD) of plant materials serves as the primary mechanism for generating biogas, comprising methane (CH4) and carbon dioxide (CO2). Biogas plants (BGPs) utilize renewable agricultural resources like grass, maize, sugar beet, and biodegradable organic wastes for anaerobic digestion, leading to biogas production (Zhang et al. 2016). The process of methane production unfolds in distinct phases, starting with the hydrolysis of organic compounds such as carbohydrates, proteins, and lipids. This is followed by the production of butyrate, propionate, other short-chain volatile fatty acids (VFAs), and alcohols, subsequently leading to the production of acetic acid and ultimately resulting in CH4 production. Several bacteria, including *Clostridium bornimense, Fermentimonas caenicola, Herbinix hemicellulosilytica, Herbinix luporum, Herbivorax saccincola, Proteiniphilum saccharofermentans, Petrimonas mucosa,* and *Proteiniborus indolifex* play vital roles in the hydrolysis and acidogenesis phases (Maus et al. 2016). Metagenomics has been used to find enzymes such as lignases, xylanases, endoglucanases, amylolytic enzymes, glucosidase for bioethanol, and lipolytic enzymes for biodiesel production (Wang et al. 2019). According to (Leresche and Meyer 2006), bacteria seem to be the main prime source for these (Endoglucanase, Cellodextrinase β-glucosidase, Xylanase Mannanase, Xylosidase, Arbinofuranosidase, Lichenase, Glucanase, Amylase, Phosphophenol pyruvate, carboxykinase, Xylanase, Cyclophilin, Mannanase) enzymes. Metagenomic studies have been instrumental in isolating lignocellulose-degrading enzymes, such as β-glucosidase, which plays a crucial role in transforming cellulosic biomass into glucose. This enzyme has been identified in various environmental conditions, including alkaline polluted soil, hot springs, wastewater, marine water, mangrove soil, and agricultural soil (Solanki et al. 2021). Shotgun metagenomic analysis has revealed specific bacteria, including *Bacillus thermozeamaize, Caldibacillus debilis,* and *Geobacillus thermoglucosidasius*, possessing carboxymethylcellulose and glycosyl hydrolases (GHs) involved in lignocellulose degradation (Lemos et al. 2017). Functional metagenomic approaches have enabled the isolation of microbes with lignocellulolytic enzymes from diverse sources, such as corn stover, rice straw, sugarcane bagasse, and wheat straw (Batista-García et al. 2016). Xylanase, a key enzyme in xylan degradation for biofuel production, has been discovered in various environments. For instance, a soil-derived metagenomic library exhibited enhanced xylanase activity at lower temperatures and in a reduced alkaline environment (Verma and Satyanarayana 2020). Endoglucanase, derived from compost soils, and glycosyl hydrolase from a cow rumen metagenomic library have also been reported (Pabbathi et al. 2023). Thermophilic bacteria such as *Caldicellulosiruptor danielii, Caldicellulosiruptor morganii,* and *Caldicellulosiruptor naganoensis* NA10 utilize lignocellulose as a substrate (Lee et al. 2018). Additionally, specific genes involved in xylose metabolism have been identified in *Herbivorax saccincola A7* (Aikawa et al. 2018). Lignin-degrading enzymes like laccase have been explored for their potential in biofuel production. A novel bacterial laccase (LacM) with stability in organic solvents and high salt concentration was isolated from an acidic bog soil metagenome (Zhang et al. 2021). Pectinases, crucial for various applications including fruit ripening and biofuel production, have also been discovered through metagenomic approaches. A thermostable pectinase gene, active across a broad range of temperatures and pH, was identified in a soil metagenomic library (Zhou et al. 2017).

Metagenomic insights in agriculture

Metagenomics has revolutionized agricultural research by providing detailed insights into the complex interactions between microbes and plants. Metagenomics studies have been utilized in various fields such as in studying pathogen and plant disease identification (Mechan Llontop et al. 2020), plant-pathogen interaction (Iquebal et al. 2022), Abiotic Stress Management (Nwachukwu and Babalola 2022).

Pathogen and plant disease identification

Agriculture is the backbone of country's economy. However, crop yield is significantly dependent on various biotic and abiotic factors. The biotic stress in the form of viral, bacterial fungal or any pathogen significantly reduces the crop yield. For better crop yield it is utmost required to early identify the disease and disease-causing agent in the crop. Plant disease control, identification and characterization of the microbes, and prevention are successful and efficient with early detection of plant disease outbreaks and accurate diagnostic methods. Various methods are employed in the diagnosis of plant diseases for species identification. These include gene sequence-based techniques with pure culture, the 16S rRNA gene method, and whole-genome sequencing with pure culture. Additionally, antibody-based assays like ELISA (Christopher-Hennings et al. 2019) are utilized. However, these methods come with limitations, such as a lengthy pathogen identification protocol to obtain a pure culture and the requirement for prior knowledge of pathogens. In the current context, all these limitations are overcome by leveraging metagenome sequencing and identification methods in agriculture.

Metagenomics has played a pivotal role in identifying bacterial strains responsible for tomato pathogenesis at a nuanced, strain-specific level (Mechan Llontop et al. 2020). The prevalence of *Fusarium* wilt, a widespread vascular fungal disease affecting numerous plants, prompted an investigation into endophytic communities in banana fields. This study, conducted on *Fusarium* wilt-infected banana fields, unveiled the influence of bacterial communities, notably *Flavobacteriales*, on disease progression (Kaushal et al. 2020). To enhance our understanding, simulated metagenome data analysis using environmental DNA (eDNA: genetic material that is extracted from environmental samples such as soil, water, or air, rather than directly from an organism) A was undertaken to detect fungal and oomycete plant pathogens (Espindola et al. 2015). The soil, a dynamic component in agriculture, acts as a disease-suppressive agent by shielding crops from detrimental microbes.

Abiotic stress management

Microbes associated with plants and crops, including those residing in the soil and rhizosphere microbiomes, play a crucial role in various functions essential for plant productivity. These functions encompass the mineralization of soil organic matter, nutrient cycling, stimulation of disease resistance, and response to abiotic stresses such as drought, salinity, and pollution. Notably, specific soil microbes have been extensively studied for their ability to influence abiotic stress tolerance in plants. For instance, the rhizospheric microbiome has been observed to impact the survival of certain plant species under extreme stress conditions (de La Fuente Cantó et al. 2020). Abiotic stress such as extreme temperature, drought, salinity, waterlogging, soil pollutants, and hazardous compounds like heavy metals, are non-living components, pose significant threats and create unfavorable conditions and challenges in agriculture. These stressors represent critical limiting factors in crop productivity. To cope with adverse environmental conditions, crop plants rely on their intrinsic biological mechanisms; failure to do so results in compromised plant growth, development, and productivity (Nwachukwu and Babalola 2022).

To understand the combined effects of plant and microbe interactions on managing abiotic stress, a thorough investigation like metagenomics is crucial. (Zolla et al. 2013) conducted research on Arabidopsis thaliana under drought stress conditions using the pyrosequencing approach. Their findings revealed a robust set of soil microbes capable of sensing abiotic stress, leading to increased plant biomass production and reduced expression of drought response marker genes. A similar study focusing on wheat rhizosphere for abiotic stress management highlighted the broad and diversified role of metagenomics. This study not only delves into the metagenomics of wheat but also explores its application in developing climate-resilient genotypes (Ahlawat et al. 2018).

Future prospects: navigating new frontiers in rhizospheric metagenomics

The horizon of rhizospheric metagenomics extends beyond the insights gained thus far, promising exciting avenues for exploration and application. In the realm of environmental sustainability, the continued investigation of microbial communities holds promise for novel bioremediation strategies. Harnessing the potential of specific microbial cohorts to tackle emerging pollutants and environmental challenges can lead to more targeted and efficient remediation approaches.

The field of bioenergy production stands on the brink of revolutionary transformations. Future prospects include the refinement of metagenomic techniques to identify microbial consortia with enhanced capabilities for biofuel synthesis. Understanding the intricate metabolic pathways within the rhizosphere can guide the development of bioenergy crops and facilitate sustainable practices in biofuel production. Agriculture is poised for a paradigm shift as metagenomic insights deepen. Future research may delve into precision agriculture, where tailored microbial interventions based on metagenomic analyses optimize crop health and productivity. The development of climate-resilient genotypes, guided by metagenomic approaches, could revolutionize the agricultural landscape, ensuring food security in the face of climate change. As technological advancements propel metagenomics into new frontiers, interdisciplinary collaborations will play a pivotal role. Integration with fields such as artificial intelligence and machine learning holds the potential for more efficient data analysis and pattern recognition, further enhancing our understanding of rhizospheric dynamics.

Conclusion

In this comprehensive review, the journey delves into the intricate microbial landscapes of the rhizosphere, exploring their diversity and the profound connections revealed by metagenomic approaches. The multidimensional significance of rhizospheric microbial communities has been underscored, highlighting their pivotal roles in supporting plant health, fostering sustainable agriculture, and addressing environmental challenges. The exploration began with an insightful introduction that laid the foundation for understanding the dynamic world of rhizospheric microorganisms and the transformative power of metagenomics in unraveling their intricacies. In the realm of bioremediation, metagenomic analyses have offered a treasure trove of information, guiding efforts to harness the potential of microbial communities in mitigating environmental pollutants. Similarly, the pursuit of biofuel synthesis and production has been enriched by metagenomic insights, paving the way for sustainable and eco-friendly energy solutions. Within the agricultural domain, metagenomics has emerged as a transformative force. The application of metagenomics in abiotic stress management has opened new avenues for enhancing crop resilience, ensuring food security in the face of changing environmental conditions. Future studies might explore precision agriculture, employing personalized microbial interventions informed by metagenomic analyses to enhance crop health and yield. Utilizing metagenomic methods to guide the development of climate-resilient plant varieties could transform agriculture, safeguarding food supplies amidst climate challenges. As metagenomics advances, interdisciplinary teamwork will be crucial, integrating disciplines like artificial intelligence and machine learning for improved data processing and pattern identification, deepening insights into rhizospheric dynamics.

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Author contributions VB conceptualized and finalized the manuscript. MS and BR carved the manuscript.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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