Chapter 14
Soil Microbial Diversity and Metagenomics

Sandeep Sharma and Sukhjinder Kaur

Abstract The management of soil fertility for sustainable and productive agriculture embroils understanding of chemical, physical, and primarily biological components of soil. The soil microbiome ability to predict noticeable changes in soil properties as they are involved in nutrient cycling, soil structure formation, decomposition of organic matter, and plant growth promotion. The microbial diversity prevailing in the soil can be explored either through culture-based or recently through novel genomic approaches that proved to be powerful tool in microbe-centric studies and delivers more comprehensive assessment of microbial functions. Soil metagenomics holds unusual potential to enhance crop production and to discover several unexploited soil microorganisms, their functions and genes for diverse applications. In this book chapter, special emphasis has been highlighted on the role of metagenomics for unlocking the soil microbiome and its processes in different management practices.

Keywords Metagenomics · Management practices · Soil microbiome · Soil enzymes · Soil fertility

14.1 Introduction

Environmental soil degradation with long-term continuous cropping involving utilization of chemical fertilizers leads to the imbalance or reduction in nutrient availability and fertility of soil (Dong et al. 2012). The management of soil to ensure its long-term productivity, stability, and fertility is of paramount importance for plant growth. The maintenance of the physical and chemical soil fertility is driven by the metabolic repertoire of the soil microorganisms (Sabale et al. 2019). The soil biological fertility relies on the microbial community, which is termed as the

S. Sharma (✉) · S. Kaur
Department of Soil Science, Punjab Agricultural University, Ludhiana, Punjab, India
e-mail: sandyagro@pau.edu

C. Cruz et al. (eds.), *Soil Nitrogen Ecology*, Soil Biology 62,
https://doi.org/10.1007/978-3-030-71206-8_14

indicators of soil health that directly impacts the functioning of soil ecosystem. Thus, the soil health comprises biological, chemical, and physical properties of soil but is mostly dependent on the activity of microorganisms. The biological measurement of soil health can be inferred from certain robust indicators (e.g. microbial diversity, enzyme activity, and soil organic matter content) that can provide instant information about the current status of soil (Rincon-Florez et al. 2013). Among different soil health indicators, there is increased concern in studying soil microorganisms in their specific environments, as microbial diversity is closely linked to soil structure and function. Moreover, soil microorganisms rapidly respond to any kind perturbations (Jacoby et al. 2017).

14.2 Soil Microbiome

The soil microbiome is indispensable as it performs key soil services including organic matter decomposition, biogeochemical cycling, aggregates formation, gaseous exchange, and plant growth promotion (Christopher 2017; Naylor et al. 2020). The soil represents the most diverse habitats consisting complex assemblages of bacteria, archaea, viruses, fungi, and other microbial eukaryotes which are collectively referred as the "soil microbiome" (Fierer 2017; Jansson and Hofmockel 2020). The estimate suggests 1000–10,000 bacterial species in per gram of agricultural soils as inferred from the 16S rRNA gene phylotypes (Attwood et al. 2019). The reservoir of microbial communities in soil improves plant growth by affecting nutrients availability, aids in crop residue recycling along with determination of agroecosystems productivity (Van-Der Heijden et al. 2008). The sustainable agriculture depends on the diversity of soil microbes that influences soil fertility. Therefore, the present day research focuses more in managing soil microbiome (Dubey et al. 2019).

The characterization and classification of soil microbiome by typical cultivation approaches (plate count and most probable number) have underestimated the microbial diversity as largest proportion of soil bacteria still remain uncharacterized (Dupont et al. 2016). The majority of the soil isolated microbes belonged to the phyla, namely *Proteobacteria*, *Actinobacteria*, *Firmicutes,* and *Bacteroidetes*, as these are cultivated easily under laboratory conditions (Hirsch et al. 2010). Due to severe constraints in isolation methods, there is need for switching to the molecular and genetic level approaches that will unearth more comprehensive picture of soil microbiome by discovering new microbial players through in-depth characterization (Agrawal et al. 2015; Sabale et al. 2019). During the last few years, significant improvement has been seen in the development of certain biomarkers and macromolecular probes, rapid and reliable measurements of soil microbial communities (Arias et al. 2005). The measurement of microbial diversity can be classified into phenotypic and molecular based approaches. The determination of true microbial diversity using phenotypic techniques is difficult due to lesser accuracy of the extraction or detection methods (Agrawal et al. 2011). Thus, soil microbiologists

have attempted to ameliorate molecular methods. This book chapter emphasized on the recent methods adopted for evaluating soil fertility with focus on strategies for identifying microbial communities via metagenomics.

14.3 Molecular Approaches for Measuring Soil Microbiome

The molecular approaches to analyze soil microbiome are DNA-based methods, microscopic observation of root colonizing labeled microbes, and labeled nutrient substrates. These new molecular, enzymatic, and organism-based methods have complimented the existing physico-chemical properties and possess ability to evaluate the soil diversity and composition. All the techniques are properly evaluated for their potential to differentiate among various types of soils and their significance in the ecosystem. The current molecular strategies have led to the discovery of unusual microbial diversity, majority of which was uncharacterized so far because of non-culturable nature (Agrawal et al. 2015). Molecular techniques to determine microbial diversity in soil can be categorized into PCR-dependent and PCR-independent techniques. Nucleic acid re-association/hybridization, carbon source utilization profile/community level, physiological profile (CLPP)/BIOLOG, fatty acid methyl ester (FAME) analysis, phospholipids fatty acid (PLFA) analysis are PCR-independent approaches used for measuring microbial communities. Some of the limitations of the aforementioned techniques include dominance of culturable community and preferring microbes that can utilize the available carbon sources. These methods mainly signify metabolic diversity rather than microbial diversity (Fakruddin and Mannan 2013).

14.4 PCR-Based Approaches

The initial molecular approach for investigating biological community depends on the cloning of target genes isolated from environmental samples (DeSantis et al. 2007). Majority of the genetic fingerprinting techniques relies on PCR amplification which provides information regarding the genetic makeup of microbes. The prokaryotic diversity, identification, and phylogenetic relationships are provided by PCR-based 16S rDNA profile. PCR-based fingerprinting methods of microbial communities involves the extraction of DNA from a culture, a bioreactor, or an environmental sample, followed by the amplification of rRNA/rDNA using the Polymerase Chain Reaction (PCR), and finally an analysis of the DNA amplification products (Ngom and Liu 2014). The PCR-based approaches are distributed into two groups depending on the differential electrophoretic migration on agarose or polyacrylamide gels: (1) size-dependent migration, viz. T-RFLP, ARISA/RISA, RAPD,

SSCP, LH-PCR and (2) sequence-dependent migration which includes denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE). In all the above-mentioned techniques, community structure of microbial populations can be evaluated from the amplified fragments generated by selected primers. Thus, 16S rDNA-based PCR techniques such as DGGE, TGGE, single-strand conformation polymorphisms (SSCPs), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphisms (T-RFLPs), and ribosomal intergenic spacer analysis (RISA) offer comprehensive information regarding community richness, evenness, and composition present in a sample (Rawat and Johri 2014). All the PCR-based techniques are suitable for tracing the dominant members of the community in complex soil environment with selective amplification of shorter fragments comprising weaker secondary structures (Rincon-Florez et al. 2013). Moreover, these methods are time-consuming with low-throughput, PCR biased and prefer easily extractable DNA that usually leads to confusing and unsuitable results. Further, microarrays have also been developed with already known gene sequences from public databases with regular updating of new gene and genome sequences. But, the application of this technology for reviewing environmental sample still presents numerous limitations (He et al. 2012; Ngom and Liu 2014). Thus, to avoid the hindrance in evaluation of soil microbial communities, metagenomics combined with bioinformatics have been recently used and these new methods are more reliable in soil microbial diversity studies (Liu et al. 2006).

14.5 Concept of Metagenomics

Although several molecular approaches have been proposed but recently, the exploration of entire genomes existing in a soil sample, i.e. metagenomics, has provided a new strategy for studying microbial diversity bypassing the isolation and cultivation methods of individual species (Mocali and Benedetti 2010). The outgrowth of genomics and metagenomics demonstrated promising strategies that possess the ability to discover the hidden diversity of microbes along with their function in a well-defined manner. Further, advanced sequencing technologies recognized as the Next-Generation Sequencing (NGS) performs the analysis of soil-extracted microbial community DNA directly. The NGS resulted in the production of vast volume of data in a rapid and cost-effective manner. The ability to group the entire genome of any related organisms has permitted evolutionary and comparative studies on large scale that were impossible earlier (Weinstock 2012). The sequencing of soil by metagenomics offers understanding of microbial ecology that is beneficial or detrimental to crop production with the aim to improve agricultural sustainability (Petrosino et al. 2009). The concept of metagenomics and other associated strategies have become the prime technology in many research areas attributed to its efficiency for sequencing large volume of data. This technological advancement has generated a new direction for sequencing large-scale projects (Petrosino et al. 2009).

Metagenomics is basically community genomics which provides access to the genetic makeup of whole communities of organisms present in different ecosystems. It involves the isolation of soil DNA, fragmentation, and insertion of DNA into appropriate vectors followed by DNA cloning and transformation of suitable host cells and then delivering a metagenomic library and further screening of the clone library (Mocali and Benedetti 2010). In metagenomics, the combined genome is randomly sampled from simultaneously existing microbial communities and then sequenced (Ghazanfar et al. 2010). Through the direct assessment of collective genome, metagenomics possesses the potential to provide detailed insight about genetic diversity, species composition, development, and interactions with the microbial communities prevailing naturally in the environment (Fakruddin and Mannan 2013). Mass genome sequencing based shotgun analysis, genomic activity-driven studies aimed to find exact microbial functions, phylogenetic or functional gene expression analysis of genomic sequences, and next-generation sequencing strategies for evaluating entire gene content in environmental samples are the four sub-categories of unselective/untargeted and targeted metagenomics based on the various screening methods. The unselective/untargeted metagenomics involves shotgun analysis and next-generation sequencing, whereas targeted metagenomics includes activity and sequence-driven studies. Due to the cost-effectiveness and ease in DNA sequencing techniques, unselective metagenomic approach has been preferred widely (Neelakanta and Sultana 2013). Targeted metagenomics commonly sequences in parallel and extremely target genes, serving ribosomal RNA (rRNA) as evolutionary clocks. This biomarker relied on the massive databank of rRNA gene sequences (more than 200,000) collected for the reconstruction of the universal Tree of Life which increased exponentially due to targeted and untargeted sequencing. The rRNAs of all the organisms are sufficiently related to each other that they can be recognized as the same molecule but different enough that the differences are a good measure of evolutionary distance (Perito and Cavalieri 2018).

In sequence-based metagenomics, the researcher's emphasis on finding the complete genetic sequence, i.e., the arrangement of all the nucleotide bases (A, C, G, and T) found in the DNA strands of a sample. The sequence obtained can then be analyzed in several ways which includes utilization of community's sequence in determination of entire genome of a specific organism or this sequence can also be used to analyze the genome of the community as a whole that offers insight about evolution and population ecology. Further, the function-based metagenomics involves screening of metagenomic libraries for several functions/products, such as genes involved in nutrients cycling and metabolic pathways, vitamins or antibiotics produced by microbes in a community. Scientists can recognize various functions through this method that was known in microbes. Recently through advances in function-based metagenomics technology, researchers can also directly extract novel proteins from a microbial community and identify their metabolites involved in cellular processes. Therefore, the study of soil fertility indicators through metagenomic approach will enhance the soil biological system, which in turn promotes soil fertility and improved productivity.

14.5.1 Metagenomic-Based Studies on Soil Microbiome

The advancement in high-throughput molecular biology methods over the last decades has resulted in significant increase in the understanding of the soil microbiome (Nannipieri et al. 2019). The metagenomic approach showed enormous potential in unlocking myriad of functions which include identification of uncultivated or new phyla possessing novel traits, understanding metabolic and biochemical activity of microbial players, functional diversity of microbes, finding shifts in microbial diversity associated with stress and disease tolerant plants (Köhl et al. 2014; Dubey et al. 2019). The additional target of metagenomic-based studies is to gain insights into biochemical cycling of nutrients (C, N, P, S, and other elements) summarized in Fig. 14.1 (Myrold et al. 2013). One international effort focusing on sequencing and interpreting the soil metagenome was proposed by combining the abilities of the global scientific community (Vogel et al. 2009) and named the project as the Terra Genome. This international sequencing consortium possesses primary objective of complete sequencing of a reference soil metagenome. The soil system selected for research is Park Grass, an internationally recognized agroecology field experiment that has been running for more than 150 years at the UK agricultural sciences institute, Rothamsted Research (Fujii et al. 2009).

The rhizospheric and phyllospheric bacterial population of Basmati rice in Pakistan were studied using metagenomic approach by Rasul et al. (2020). The results described the dominance of phylum *Proteobacteria*, *Chloroflexi*, *Actinobacteria,* and *Firmicutes* at different sites in the rhizosphere than

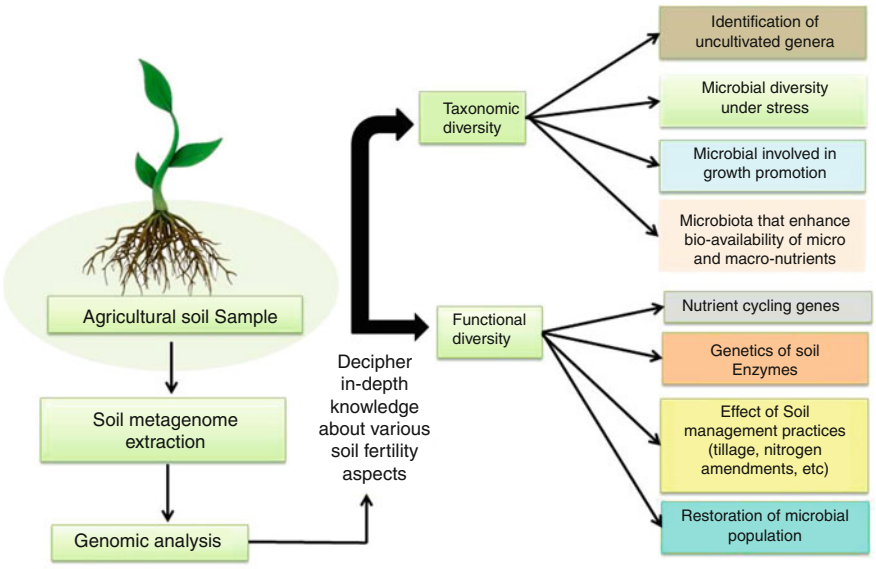

Fig. 14.1 Summary of the various soil aspects studied by metagenomics

phyllosphere. The plant growth promoting genera, *Azospirillum*, *Bacillus*, *Brevibacillus*, *Mesorhizobium*, *Paenibacillus*, *Streptomyces,* and *Sphingomonas* were also abundant in rhizosphere. Woźniak et al. (2019) compared rhizospheric and endophytic microbiome of *Paulownia* trees by Illumina MiSeq sequencing and described higher bacteria and fungi in endosphere samples. The abundant bacterial phyla reported were *Actinobacteria* and *Proteobacteria*. The rhizospheric fungal diversity includes *Ascomycota*, *Mortierellomycota*, and *Basidiomycota*, whereas the endophytic diversity involves *Olpidiomycota*, *Oomycota*, *Ascomycota,* and *Basidiomycota*. Hara et al. (2019) identified functional N_2-fixing bacteria associated with sorghum through omics approaches. Here, the roots extracted bacterial cells were studied by metagenome and proteome. Majority of the sequences were assigned to nif HDK of *Bradyrhizobium* species.

Ahmed et al. (2018) assessed the microbial diversity in the two rhizospheric saline soil samples through metagenomic approach and observed the dominance of halophilic/halotolerant phylotypes affiliated to *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Bacteroidetes, Firmicutes*, and *Acidobacteria*. Identification of osmotolerant clones SSR1, SSR4, SSR6, SSR2 harboring *BCAA_ABCtp, GSDH, STK_Pknb*, and *duf3445* genes confirmed their function in osmotolerance. The soil metagenomic libraries also reported the abundance and diversity of phosphatase genes using functional metagenome analysis. Similarly, Molina-Montenegro et al. (2018) compared the rhizospheric microbiome using shotgun metagenomic technology and found abundance of bacterial species (98%) followed by eukaryota (1.77%) and archaea (0.22%). The major genera reported in the rhizospheric soil were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria,* and *Firmicutes*. Metagenomic shotgun sequencing and functional annotation by means of eggNOG functional categories showed that metabolism was the highest represented category, followed by cellular process and signaling, and information storage and processing. In the category metabolism, the highest characterized terms were amino acid transport and metabolism, energy production and conversion, carbohydrate transport and metabolism, and inorganic ion transport and metabolism. Baeza et al. (2017) evaluated fungal sequences from Antarctica by amplicon metagenomic analysis and found 87 genera and 123 species, from which 37 genera were not reported previously. Lecanoromycetes and Eurotiomycetes were dominant the fungal classes.

The metagenomic DNA from bulk soil of tomato, vegetables, and native forest extracted by Val-Moraes et al. (2013) represented uncultured fungi. The individual amplified sequences matched with Glomeromycota, Fungi incertae sedis, and Neocallimastigomycota. The tropical mangrove soil microbial diversity was characterized by Ismail et al. (2012) through the metagenome of a Malaysian mangrove soil sample and its microbial ecological roles via next-generation sequencing (NGS). Shotgun NGS data analysis revealed high diversity of ecologically essential microbes from bacteria and archaea domains. Also, an unusually high number of archaea was observed along with abundance of *Deltaproteobacteria*.

14.5.2 Metagenomic Insight of Soil Management Practices

Agricultural intensification for increased production resulted in severe food security and adverse impacts on soil fertility, nutrient leaching, and increased greenhouse gas emissions (Hartman et al. 2018; Souza et al. 2018). These conventional agriculture based strategies have affected the biodiversity and functionality of soil microbiome through curtailment of functions performed by microbes and reduction in their species (Souza et al. 2018). Moreover, these practices alter soil physico-chemical as well as biological properties which act as valuable indicators of soil quality and health (Carbonetto et al. 2014). Thus, the adoption of soil conservation practices is required to prevent soil degradation and to maintain active soil biota (Souza et al. 2013). Numerous conservation practices such as tillage, organic fertilization, crop rotations/successions and crop residues retention have resulted in improved sustainability and have promoted beneficial ecosystem services (Srour et al. 2020). Soil metagenomics also unravels the understanding of different soil management approaches (such as tillage, organic fertilizer amendments) aimed for enhancing plant productivity and nutrient acquisition (Attwood et al. 2019). Furthermore, the understanding of aforementioned functions associated with soil microbiome will play an essential role in management of soil fertility. Some of the recent studies highlighting diversity analysis among various soil management practices through metagenomics are summarized in Table 14.1.

14.5.3 Functional Metagenomic-Based Insight of Soil Enzymes

The soil enzyme activities (β-glucosidase, cellulose, protease, urease, and phosphatase) are directly involved in the nutrient cycling (such as carbon, nitrogen, and phosphorus) and reflect the metabolic requirements of soil microorganisms, which are important in the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter (Burns et al. 2013; Yang et al. 2017). Soil enzymes are crucial for the functioning of soil because of their role in decomposition and transformation processes (Jesus et al. 2016). The activity of soil enzymes is directly related to the metabolic requirements of the soil community and the available nutrients present in soil. The soil enzymes are categorized into hydrolases and oxidases that decompose substrates and release nutrients to the soil. Another enzyme, urease is associated with microbial N acquisition, as it catalysis the urea decomposition. Microbially produced hydrolytic enzyme, β-1,4-glucosidase decomposes polysaccharides whereas acidic and alkaline phosphatase are associated with P-acquisition (Hai-Ming et al. 2014). The most studied enzymes from the soil metagenome are esterase and lipase attributed to wide potential in industry (Lee and Lee 2013). The molecular methods deliver valuable information on expression and potential of enzymes targeting the abundance of enzyme-encoding genes or

Table 14.1 Metagenomic-based studies on diversity and function analysis of soil in different management practices

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
Tillage and fertilizer amendment in corn-soybean	USA	Long-term (1990–2015)	Mesic Udollic Endoaqualf	Metagenome Illumina sequencing	• Metagenome analysis revealed higher population of arbuscular mycorrhizae, mycoparasites and nematophagous fungi in no-till soils. • Fungal saprotrophs and plant pathogens dominated in tilled soils. • Copiotrophic bacteria and *Fusarium* species were favored under conventional tillage. • Higher abundance of pathways associated with energy metabolism, translation, metabolism of cofactors and vitamins, glycan biosynthesis and nucleotide metabolism in no-till	Srour et al. (2020)
Crop rotation	Germany	Long-term (1992–2015)	Chernozem	Whole metagenome shotgun sequencing	• Dominant genera were *Streptomyces, Bradyrhizobium, Mycobacterium, Nitrososphaera*, and *Nocardioides*. • Metagenomically assembled genomes revealed two important taxa present in large numbers, *Thaumarchaeota* and *Rhizobiales*. • Methylotrophic bacterium of unclassified Rhizobiales is involved in conversion of C1-components (carbon cycle). • Thaumarchaeota members contribute to ammonium oxidation and carbon dioxide fixation	Nelkner et al. (2019)

(continued)

Table 14.1 (continued)

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
Napier grass amended with biochar	USA	2 years	Acidic oxisol	Shotgun metagenomics	• Biochar-amended soil microbiome exhibited enrichment in key metabolic pathways related to carbon turnover, such as the utilization of plant-derived carbohydrates and denitrification. • Increased soil carbon (labile and aromatic carbon compounds), available nutrients	Yu et al. (2016)
Tobacco (application of fertilizers incorporated with plant residues)	China	One year (2015)	Not specified	Metagenomic sequencing	• Functional annotation of metagenomic sequences revealed abundance of genes involved in metabolic pathways. • Predominant phyla were Proteobacteria, Actinobacteria, and Verrucomicrobia in 300 kg/mu straw. • Cyanobacteria, Basidiomycota, and Chlorophyta were abundant in soil samples with 200 kg/mu straw	Yang et al. (2017)
Nitrogen fertilization	USA	Long term	Sandy and silt loam	Short-gun Metagenomics	• Functionally assembled metagenomes revealed 6 deep-branching *Thaumarchaeota* and 3 ammonia oxidizer *Nitrospira.* • Also genomic analysis predicted its fivefold abundance in N fertilizer	Orellana et al. (2018)
Straw mulching in tobacco-rice rotation	China	1 year	Sandy loam	Illumina sequencing	• Abundance of Proteobacteria and Actinobacteria. • *Nitrospirae* was highest in the rice straw returning.	Lei et al. (2017)

					• Rice straw returning fire + quicklime and reduced fertilizer had the highest abundance of Firmicutes	
Forest and vineyards soils	Chilean Mediterranean	1 year	Not specified	Short-gun Metagenomics	• *Candidatus Solibacter*, *Bradyrhizobium* and the fungus *Gibberella* were most abundant. • Genes present in microbial diversity pertain to metabolism of amino acids, fatty acids, nucleotides as well as secondary metabolism	Castañeda and Barbosa (2017)
Tillage-crop residue management	Mexico	–	Hyposodic vertisol	Short-gun Metagenomics	• Population of degrading genera (*Promicromonospora, Bacillus, Agromyces, Streptomyces, Sinorhizobium*, and *Lysobacter*) was higher in retained treatments	Chávez-Romero et al. (2016)
Tillage effect on cellulose-degrading microbes	Germany	Long term (1992-2012)	Luvisol	Shotgun sequencing	• Abundance of cellulolytic enzymes and cellulolytic gene composition in reduced tillage. • Proteobacteria, Actinobacteria, and Bacteroidetes dominated in reduced tillage	DeVries et al. (2015)
Bacterial diversity under tillage	USA	Long term (52 years)	Typic Fragiudalf	Pyrosequencing	• No-till exhibited higher number of reads, bacterial richness, and five unique phyla. • Four unique phyla were observed in adjacent plow-till	Sengupta and Dick (2015)
No-till crop rotation in cultivated and uncultivated land	Argentina	15 years	Typic Argiudolls	Short-gun metagenomesequencing	• Phyla *Verrucomicrobia, Plactomycetes, Actinobacteria,* and *Chloroflexi* were more in non-cultivated soils while *Gemmatimonadetes, Nitrospirae* were abundant in cultivated soils. • The abundance of genes assigned to	Carbonetto et al. (2014)

(continued)

Table 14.1 (continued)

Cropping system/ experimental design	Location	Duration of experiment	Soil type	Genomic technique	Results	References
					transcription, protein modification, nucleotide transport and metabolism, wall and membrane biogenesis and intracellular trafficking and secretion were observed in cultivated fertilized soils	
Tillage practices+ crop rotation	Brazil	13 year	Oxisol	Short-gun sequencing	• Majority of the sequences were attributed to Bacteria (54%), and 0.3% and 0.2% to Archaea and Eucarya domains, respectively. • Significantly higher microorganisms associated with residue decomposition, carbon and nitrogen cycling, and xenobiosis were observed in conventional tillage (CT). • Eucarya were also abundant in CT, with possible relation in higher tolerance of environmental stresses. • No-till showed higher abundance of nitrogen-fixing Rhizobiales and Archaea	Souza et al. (2013)

transcribed sequences (Baldrian 2009). The soil microbiome harbors numerous novel enzymes which are identified by various metagenosmic studies and summarized in Table 14.2.

The application of metagenomic approaches for evaluating soil microbiomes and related functions has facilitated the better understanding of taxonomic, genetic, and functional characteristics of soil microbial community (Fierer et al. 2012). Still there are challenges that need to overcome by combining application of metatranscriptomics, metaproteomics, and metabolomics that are helpful to fill knowledge gaps about genes/protein expression and metabolic interactions (Jansson and Hofmockel 2018). Metatranscriptomics involves study of microbial RNA transcripts produced in a particular ecological sample (Baldrian et al. 2012). Metatranscriptomics approach immediately deciphers gene regulatory response as majority of bacteria exhibit transcriptional gene control that permits quick adaption to change altered environmental conditions at the sampling time (Moran 2009). The steps performed in this technique comprise extraction followed by reverse transcription, amplification, and lastly sequencing of transcripts. The transcript obtained is highly unstable and has shorter life span which is a major bottleneck to this technology (Cabellos-Ruiz et al. 2010). Meta-transcriptomic approach is widely preferred for unfolding microbial nutrient cycling (Barua et al. 2017).

Next, metaproteomics is the characterization of the microbial proteins (Ngom and Liu 2014) extracted from a sample, followed by fractionation, separation using liquid chromatography or two dimension polyacrylamide gel and then detection with tandem mass spectrometry (Zhang et al. 2010). Lin et al. (2013) conducted the metaproteomic profile of rhizospheric soil for elucidation of mechanism involved in yield decline of ratoon sugarcane. The results revealed 143 protein spots with high resolution and repeatability including 38 differentially expressed proteins involved in carbohydrate/energy, amino acid, protein, nucleotide, auxin and secondary metabolisms, membrane transport, signal transduction and resistance, etc. Thus, metagenomics is the predictive of community potential and combining it with the metaphenome will reveal the functional potential of soil communities and links between community genes and functions.

14.6 Conclusion

Soil microbiome plays an imperious role in cycling of nutrients, mineralization, enzymes production, and improvement of indispensable soil processes that impacts soil fertility. Owing to drawbacks of traditional plate count techniques, molecular methods have offered a desired alternative for exploring soil microbiome. The taxonomists have developed various molecular techniques that permit rapid analysis of desired traits within microbial communities. Several PCR and non-PCR based techniques have been developed to explain functional profiling of natural microbial communities. The advancement in sequencing tools has resulted in the advent of novel and rapid molecular method known as integrated omic approaches that

Table 14.2 Soil enzymes studies performed by metagenomic strategies

Cropping system/soil sample	Location	Enzyme studied	Genomic technique	Results	References
Forest soil	Germany	Phosphatases/ phytases	Functional genomics	• Metagenome analysis revealed largest number and diversity of phosphatase genes. • two of the gene products carry domains which have never been associated with phosphatase activity before. • Also found previously unreported phytase activity of alkaline phosphatase and sulphatase superfamily and purple acid phosphatases from non-vegetal origin	Villamizar et al. (2019)
Solanum phureja soil	Columbia	_	Functional genomics	• Functional metagenome revealed the abundance of oxidoreductase activity (18%). • Also identified a protease and lipase/esterase domain	Calderon et al. (2019)
Crop succession (soybean/wheat), or crop rotation (soybean/maize/wheat/lupine/ oat) + tillage practices	Brazil	Hydrolases	Shotgun metagenomes	• The abundance order was lipases> laccases > cellulases > proteases >amylases>pectinases. • No-till showed five times more hydrolases than conventional tillage. • Majority of enzyme sequences belonged to fungi (*Verticillium* and *Colletotrichum* for lipases, laccases, and *Aspergillus* for proteases), and the archaea, *Sulfolobus acidocaldarius* for amylases	Souza et al. (2018)

Cabbage soil sample	Sweden	Chitinase	Functional metagenomics	• Bacterial chitinase, Chi18H8, with antifungal activity was identified. • Sequence analysis showed chi18H8 gene encodes a 425-amino acid protein of 46 kDa with an N-terminal signal peptide. • A catalytic domain with conserved active site and a chitinase insertion domain were also observed	Hjort et al. (2014)
Grassland soil	Germany	Cellulase and Xylanase	Functional metagenomics	• Novel cellulase-encoding gene (*cel01*) and two xylanase-encoding genes (*xyn01* and *xyn02*) were identified. • From sequence analysis, Cel01 (831 amino acids) belongs to glycoside hydrolase family 9 • Xyn01 (170 amino acids) and Xyn02 (255 amino acids) are members of glycoside hydrolase family 11	Nacke et al. (2012)
Forest soil	Korea	Lipolytic enzymes	Metagenomics	• Seven lipolytic enzymes were identified comprising lipase families II, IV, and V	Hong et al. (2007)
Soil and compost sample	Germany	Hydrolytic enzymes	Expression Metagenomics	• Active clones of lipolytic enzymes, amylases, phosphatases, and dioxygenases were identified. • Three genes encoding phosphatase or dioxygenase activity were also identified	Lammle et al. (2007)

constitutes metagenomics, transcriptomics, metaproteomics, and metabolomics. Metagenomics techniques are based on the direct analysis of DNA extracted from environmental samples and have circumvent the steps of isolation and culturing of microbes. No single technique till date can measure the whole microbial diversity. Biases are introduced at each treatment step as all of these techniques present advantages as well as drawbacks. Advanced screening approaches involving function-driven and sequence-dependent metagenomics will provide deeper insights of soil metagenome that will aid in sustaining crop management and soil fertility.

References

Agrawal PK, Agrawal S, Verma SK (2011) Methods for studying microbial diversity from soyabean rhizosphere by phenotypic and molecular approach. Adv Appl Sci Res 2:538–545

Agrawal PK, Agrawal S, Shrivastava R (2015) Modern molecular approaches for analyzing microbial diversity from mushroom compost ecosystem. 3 Biotech 5:853–866

Ahmed V, Verma MK, Gupta S, Mandhan V, Chauhan NS (2018) Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front Microbiol 9:159. https://doi.org/10.3389/fmicb.2018.00159

Arias ME, González-Pérez JS, González-Vila FJ et al (2005) Soil health-a new challenge for microbiologists and chemists. Int Microbiol 8:13–21

Attwood GT, Wakelin SA, Leahy SC et al (2019) Applications of the soil, plant and rumen microbiomes in pastoral agriculture. Front Nutr 6:107. https://doi.org/10.3389/fnut.2019.00107

Baeza M, Barahona S, Alcaíno J et al (2017) Amplicon-metagenomic analysis of fungi from antarctic terrestrial habitats. Front Microbiol 8:2235. https://doi.org/10.3389/fmicb.2017.02235

Baldrian P (2009) Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ 55:370–378

Baldrian P, Kolařík M, Stursová M et al (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISMEJ 6:248–258

Barua S, Hait A, Barua S (2017) Metatranscriptomic study of microbes in environment: a community function based approach. J Bacteriol Mycol 4:12–15

Burns RG, De Forest JL, Marxsen J et al (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Cabellos-Ruiz A, Janca H, Baena S et al (2010) Beyond metagenomics: integration of complementary approaches for the study of microbial communities. Metagenomics: theory, methods and applications. Caister Academic Press, Norfolk, UK, pp 15–37

Calderon D, Peña L, Suarez A et al (2019) Recovery and functional validation of hidden soil enzymes in metagenomic libraries. MicrobiologyOpen 8:e572. https://doi.org/10.1002/mbo3.572

Carbonetto B, Rascovan N, Álvarez R et al (2014) Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in argentine pampas. PLoS One 9(6):e99949. https://doi.org/10.1371/journal.pone.0099949

Castañeda LE, Barbosa O (2017) Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. PeerJ 5:e3098. https://doi.org/10.7717/peerj.3098

Chávez-Romero Y, Navarro-Noyab YE, Reynoso-Martíneza SC et al (2016) 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. Soil Tillage Res 159. https://doi.org/10.1016/j.still.2016.01.007

Christopher J (2017) Living soils: the role of microorganisms in soil health. In: Strategic analysis paper. Future Directions International, Nedlands. Available via. https://www.futuredirections.

org.au/wp-content/uploads/2017/06/Living-Soils-the-Role-of-Microorganisms-in-Soil-Health.pdf

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 3:371–383. https://doi.org/10.1007/s00248-006-9134-9

DeVries M, Schöler A, Ertl J et al (2015) Metagenomic analyses reveal no differences in genes involved in cellulose degradation under different tillage treatments. FEMS Microbiol Ecol 91:7. https://doi.org/10.1093/femsec/fiv069

Dong W, Zhang X, Wang H et al (2012) Effect of different fertilizer application on the soil fertility of paddy soils in red soil region of southern China. PLoS One 7:e44504. https://doi.org/10.1371/journal.pone.0044504

Dubey A, Malla MA, Khan F et al (2019) Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers Conserv 28:2405–2429

Dupont AÖC, Griffiths RI, Bell T et al (2016) Differences in soil micro-eukaryotic communities over soil pH gradients are strongly driven by parasites and saprotrophs. Environ Microbiol 18:2010–2024

Fakruddin M, Mannan KSB (2013) Methods for analyzing diversity of microbial communities in natural environments. Ceylon J Sci (Bio Sc) 42:19–33

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15:579–590. https://doi.org/10.1038/nrmicro.2017.87

Fierer N, Lauber CL, Ramirez KS et al (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J. https://doi.org/10.1038/ismej.2011.159

Fujii T, Morimoto S, Hoshino YT, Okada H, Wang Y, Chu H, Zhihua B, Yuko M, Tsushima S (2009) Metagenomic approach to soil microbial diversity and functions. Marco symposium 2009, Workshop 5: 1–9

Ghazanfar S, Azim A, Ghazanfar MA, Anjum MI, Begum I (2010) Metagenomics and its application in soil microbial community studies: biotechnological prospects. J Animal Plant Sci 6:611–622

Hai-Ming T, Xiao-Ping X, Wen-Guang T, Ye-Chun L, Ke W, Guang-Li Y (2014) Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. PLoS One 9:e100443. https://doi.org/10.1371/journal.pone.0100443

Hara S, Morikawa T, Wasai S et al (2019) Identification of nitrogen-fixing Bradyrhizobium associated with roots of field-grown sorghum by metagenome and proteome analyses. Front Microbiol 10:407. https://doi.org/10.3389/fmicb.2019.00407

Hartman K, van der Heijden MGA, Wittwer RA et al (2018) Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:14. https://doi.org/10.1186/s40168-017-0389-9

He Z, Deng Y, Zhou J (2012) Development of functional gene microarrays for microbial community analysis. Curr Opin Biotechnol 23:49–55

Hirsch PR, Mauchline TH, Clark IM (2010) Culture-independent molecular techniques for soil microbial ecology. Soil Biol Biochem 42(6):878–887

Hjort K, Presti I, Elväng A et al (2014) Bacterial chitinase with phytopathogen control capacity from suppressive soil revealed by functional metagenomics. Appl Microbiol Biotechnol 98:2819–2828

Hong KS, Lim HK, Chung EG et al (2007) Selection and characterization of forest soil metagenome genes encodinglipolytic enzymes. J Microbiol Biotechnol 17(10):1655–1660

Ismail Z, Sam CK, Yin WF, Chan KG (2012) Tropical mangrove swamp metagenome reveals unusual abundance of ecologically important microbes. Int Microbiol 8:13–21

Jacoby R, Peukert M, Succurro A et al (2017) The role of soil microorganisms in plant mineral nutrition-current knowledge and future directions. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.01617

Jansson JK, Hofmockel KS (2018) The soil microbiome from metagenomics to metaphenomics. Curr Opin Microbiol 43:162–168

Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18:35–46. https://doi.org/10.1038/s41579-019-0265-7

Jesus EDC, Liang C, Quensen JF, Susilawati E, Jackson RD, Balser TC, Tiedje JM (2016) Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. GCB Bioenergy 8:481–494

Köhl L, Oehl F, Van Der Heijden MGA (2014) Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Ecol Appl 24:1842–1853

Lammle K, Zippera H, Breuer M et al (2007) Identification of novel enzymes with different hydrolytic activities by metagenome expression cloning. J Biotechnol 127:575–592

Lee MH, Lee SW (2013) Bioprospecting potential of the soil metagenome: novel enzymes and bioactivities. Genomics Inform 11(3):114–120

Lei Y, Xiao Y, Li L (2017) Impact of tillage practices on soil bacterial diversity and composition under the tobacco-rice rotation in China. J Microbiol 55:349–356

Lin W, Wu L, Lin S, Zhang A, Zhou M, Lin R, Wang H, Chen J, Zhang Z, Lin R (2013) Metaproteomic analysis of ratoon sugarcane rhizospheric soil. BMC Microbiol 13:135. https://doi.org/10.1186/1471-2180-13-135

Liu B, Jia G, Chen J, Wang G (2006) A review of methods for studying microbial diversity in soils. Pedosphere l6:18–24

Mocali S, Benedetti A (2010) Exploring research frontiers in microbiology: the challenge of metagenomics in soil microbiology. Res Microbiol 161(6):497–505. https://doi.org/10.1016/j.resmic.2010.04.010

Molina-Montenegro MA, Ballesteros GI, Castro-Nallar E, Meneses C, Torres-Díaz C, Gallardo-Cerda J (2018) Metagenomic exploration of soils microbial communities associated to Antarctic vascular plants. Peer J Preprints 6:e26508v1. https://doi.org/10.7287/peerj.preprints.26508v1

Moran MA (2009) Meta transcriptomics: eavesdropping on complex microbial communities–large–scale sequencing of mRNAs retrieved from natural communities provides insights into microbial activities and how they are regulated. Microbe 4:329

Myrold D, Zeglin L, Jansson J (2013) The potential of metagenomic approaches for understanding soil microbial processes. Soil Sci Soc Am J 78:3. https://doi.org/10.2136/sssaj2013.07.0287dgs

Nacke H, Engelhaupt M, Brady S et al (2012) Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. Biotechnol Lett 34:663–675

Nannipieri P, Penton CR, Purahong W (2019) Recommendations for soil microbiome analyses. Biol Fertil Soils 55:765–766. https://doi.org/10.1007/s00374-019-01409-z

Naylor D, Fansler S, Brislawn C et al (2020) Deconstructing the soil microbiome into reduced complexity functional modules. mBio 11. https://doi.org/10.1128/mBio.01349-20

Neelakanta G, Sultana H (2013) The use of metagenomic approaches to analyze changes in microbial communities. Microbiol Insights 6:37–48

Nelkner J, Henke C, Lin TW et al (2019) Effect of long-term farming practices on agricultural soil microbiome members represented by metagenomically assembled genomes (mags) and their predicted plant-beneficial genes. Gen Dent 10(6):424. https://doi.org/10.3390/genes10060424

Ngom B, Liu X (2014) Techniques for tracking microbial community structure and function in natural environment and engineered systems. Int J Sci Res 3:800–807

Orellana LH, Chee-Sanford JC, Sanford RA (2018) Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Appl Environ Microbiol 84(2):e01646–e01617. https://doi.org/10.1128/AEM.01646-17

Perito B, Cavalieri D (2018) Innovative metagenomic approaches for detection of microbial communities involved in biodeteriorattion of cultural heritage. IOP Conf Series: Mater Sci Eng 364:012074. https://doi.org/10.1088/1757-899X/364/1/012074

Petrosino JF, Highlander S, Luna RA, Gibbs RA, Versalovic J (2009) Metagenomic pyrosequencing and microbial identification. Clin Chem 55:856–866

Rasul M, Yasmin S, Hakim S et al (2020) Metagenomic analysis of bacterial community associated with rhizosphere and phyllosphere of basmati rice. bioRxiv:4. https://doi.org/10.1101/2020.04.09.034009

Rawat S, Johri BN (2014) Thermophilic fungi: diversity and significance in composting. KAVAKA 42:52–68

Rincon-Florez VA, Carvalhais LC, Schenk PM (2013) Culture-independent molecular tools for soil and rhizosphere microbiology. Diversity 5:581–612

Sabale SN, Suryawanshi PP, Krishnaraj PU (2019) Soil metagenomics: concepts and applications. In: Hozzein WN (ed) Metagenomics – basics, methods and applications. IntechOpen. https://doi.org/10.5772/intechopen.88958

Sengupta A, Dick WA (2015) Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microb Ecol 70:853–859

Souza RC, Cantão ME, Vasconcelos ATR et al (2013) Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Appl Soil Ecol 72:49–61

Souza RC, Cantãoc ME, Nogueira MA, Vasconcelo ATR, Hungria M (2018) Outstanding impact of soil tillage on the abundance of soil hydrolases revealed by a metagenomic approach. Braz J Microbiol 49:723–730

Srour AY, Ammar HA, Subedi A et al (2020) Microbial communities associated with long-term tillage and fertility treatments in a corn-soybean cropping system. Front Microbiol 11:1363. https://doi.org/10.3389/fmicb.2020.01363

Val-Moraes SP, Pedrinho EPN, Lemos EGM et al (2013) Molecular identification of fungal communities in a soil cultivated with vegetables and soil suppressiveness to Rhizoctonia solani. Appl Environ Soil Sci. https://doi.org/10.1155/2013/268768

Van-Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Villamizar GAC, Nacke H, Boehning M et al (2019) Functional metagenomics reveals an overlooked diversity and novel features of soil-derived bacterial phosphatases and phytases. mBio 10(1):e01966-18. https://doi.org/10.1128/mBio.01966-18

Vogel TM, Simonet JJK, Hirsch PR, Tiedje JM, JElsas JD, Bailey MJ, Nalin R (2009) TerraGenome: a consortium for the sequencing of a soil metagenome. Nat Rev Microbiol 7:252

Weinstock GM (2012) Genomic approaches to studying the human microbiota. Nature 489:250–256

Woźniak M, Grządziel J, Gałązka A et al (2019) Metagenomic analysis of bacterial and fungal community composition associated with Paulownia elongata × Paulownia fortune. BioRes 14(4):8511–8529

Yang Y, Dong M, Cao Y, Wang J, Tang M, Ban Y (2017) Comparisons of soil properties, enzyme activities and microbial communities in heavy metal contaminated bulk and rhizosphere soils of Robinia pseudoacacia L. in the northern foot of Qinling Mountain. Forests 8:430. https://doi.org/10.3390/f8110430

Yu H, Si P, Shao W, Qiao X, Yang X, Gao D, Wang Z (2016) Response of enzyme activities and microbial communities to soil amendment with sugar alcohols. Microbiol Open 5:604–615

Zhang W, Li F, Nie L (2010) Integrating multiple omics analysis for microbial biology: application and methodologies. Microbiology 156:287–301