Chapter 5
Cutting-Edge Tools to Assess Microbial Diversity and Their Function in Land Remediation

Indra Mani

Abstract Soil contamination caused by pollutants has been a great challenge for us. However, soil provides a vast shelter, which allows a co-occurrence of millions of microorganisms. These microbes play a critical role in the remediation of such contaminated land. There are various techniques available to evaluate microbial diversity (DNA and rRNA-based profiling) and their functions (functional genes). In addition, isolation of pure culture, 16S rDNA (for bacteria), and 18S rDNA (for fungi)-based identification and characterization have shifted to omics. For example, it has transformed from genomics to metagenomics, transcriptomics to metatranscriptomics, proteomics to metaproteomics, and metabolites to metabolomics to study microbial diversity and their function. These various omics methods are used to understand the microbial diversity, biomass, mineralization, detoxification, and nutrient cycling phenomenon. Currently, culture-independent-based molecular techniques prevailing tools to isolate and identify functional genes from the uncultured microbes. Continuous development of sequencing technology and in silico tools, which has accelerated the identification and characterization of complex microbial communities from various environmental samples. Therefore, the advancement of these technology would deliver meaningful insight to evaluate the microbial diversity and their function for land remediation. This chapter highlights various techniques from culture-dependent to culture-independent, which are to be used to assess the microbial diversity and their functions.

Keywords Microbial diversity · Metagenomics · Metatranscriptomics · Metaproteomics · Soil · Sequencing

5.1 Introduction

Soil is the major source of a variety of microorganisms such as viruses, archaea, bacteria, fungi, and other parasites. Approximately a gram of soil might comprise

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1000–10,000 species of unidentified prokaryotes (Torsvik et al. 1990). Microbes in soil play a vital role in soil fertility (O' Donnel et al. 2007), soil structure (Wright and Upadhyay 1998), plant health (Dodd et al. 2000), plant nutrition (Timonen et al. 1996), biogeochemical cycle (Wall and Virginia 1999), degradation of xenobiotic compounds (Barakat 2011), and land management (Nacke et al. 2011). Due to such great importance and so much complexity of microorganisms, it is very challenging to identify and characterize them. Interestingly advancement of genomics to metagenomics is very much helpful to characterize them (Mocali and Benedetti 2010; Huson et al. 2011; Mani 2020a; Gangotia et al. 2021; Gupta et al. 2021). Due to rapid progress in technology, that has enhanced the identification and characterization of microorganisms from any ecological samples. Soil contains a very important strain of microbes, which need to identify and use for the remediation of soil. Before, it was totally dependent on culture-based methods, which provides very trivial information about microorganisms. It might be due to a lack of numerous growth associated knowledge such as pH, temperatures, humidity, chemicals, and tracer molecules. However, culture-independent approaches (Metagenomics) are helpful to census the microbes in any environments (Schloss and Handelsman 2004; Schloss et al. 2016; Mani 2020b). Further, an advancement in the DNA sequencing technology and availability of international nucleotides sequence database collaboration (INSDC) provides an opportunity to assess the microbial diversity as well as the specific function of microbes. It provides established genome references, which are very important to analyze the microbial communities (microbiota) and their functions.

Metabolic networks such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Clusters of Orthologous Group (COG) are available to understand the metabolic pathways involve in synthesis and degradation of particular molecules. In addition, various omics techniques like metatranscriptomics, metaproteomics, and metabolomics are very helpful to understand the microbial diversity and their function in soil environments. After exploring these omics, a particular stain can be identified, characterized, and utilized for the bioremediation of soil (Mani 2020c). To understand the diversity and functions of microorganisms in metals contaminated and non-contaminated soils, it would provide valuable information. Further, it can be utilized to analyze an abundance of the particular microorganisms and also helpful to discover potential pathways involve in the degradation of heavy metals.

5.2 Culture-Dependent Techniques

Cultivation of microorganisms for isolation, characterization, and identification is a gold standard approach for the detection of the pathogens (Rudkjøbing et al. 2016). There are various media used for isolation, characterization, and identification of microorganisms such as nutrient agar (NA), brain heart infusion (BHI) agar, Salmonella-Shigella (SS) agar, macConkey agar, mannitol salt agar, eosin methylene blue (EMB) agar, potato dextrose agar (PDA), trypticase soy agar, sabouraud dextrose agar, and many more selective, differential, enriched, and enrichment media. Several

studies suggest that <0.1% of the microorganisms in soil are culturable using classical methods (Torsvik et al. 1990, 1994, 1996; Handelsman et al. 1998). However, molecular methods have the advantages to identify rapidly and cover more microbes, which may skip through a culture-based approach.

DNA markers are an appropriate tool in order to obtain information about gene flow, allele frequencies, and other parameters that are important in population biology (Neigel 1997). Ribosomal DNA (rDNA) is useful for phylogenetic analysis because different regions of the rDNA repeat unit evolve at very different rates. Therefore, regions of rDNA arrays that are particularly possible to generate informative data for almost any systematic question can be selected for analysis (Hillis and Dixon 1991). In addition, the islands of highly conserved sequences within most rRNA genes are very helpful for constructing "universal" primers, which can be used for sequencing either rRNA or rDNA from several species, for amplifying regions of interest by use of the polymerase chain reaction (PCR), or for use as probes in restriction enzyme analyses (Hillis and Moritz 1990). Remarkably, sequences of 16S rRNA gene uncover an information of microbial diversity "black box" that guide analysis of the previously unknown bacterial life and their function (Nelson et al. 2011; De Sundberg et al. 2013; De Vrieze et al. 2018). There are several molecular methods used for the analysis of identification and characterization of microorganisms from the soil.

5.2.1 Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Amplified ribosomal DNA restriction analysis (ARDRA) is utilized to investigate the microbial diversity on the basis of DNA polymorphism (Deng et al. 2008). In this method, 16S rDNA is amplified by either genus specific primer or universal primer and processed with restriction endonucleases, followed by agarose gel electrophoresis or polyacrylamide gel electrophoresis (PAGE). DNA band profiles are used to genotyping the microbial community (Tiedje et al. 1999). ARDRA has been used to evaluate the microbial diversity in soil with changes in land use in Hawaii, USA (Nüsslein and Tiedje 1999), the Karst forest, China (Zhou et al. 2009), and arsenic affected Bangladesh soils (Sanyal et al. 2016). In addition, ARDRA-based study has isolated 358 isolates, which clustered into 35 groups from glacier foreland soils. These groups belong to 20 genera and six taxa such as *Betaproteobacteria, Actinobacteria, Alphaproteobacteria, Bacteroides, Deinococcus-Thermus*, and *Gammaproteobacteria* (Wu et al. 2018). The finding shows that ARDRA techniques could characterize the glacier foreland soils culturable microbial communities.

5.2.2 Ribosomal Intergenic Spacer Analysis (RISA)

Ribosomal intergenic spacer analysis (RISA) is another technique, which is based on ribosome DNA sequences. It is a culture-dependent technique, which used for the microbial community analyses (Sigler and Zeyer 2002). But in this technique, information coming from the spacer region of rDNA. In this technique, a pair of oligonucleotides primers (one from 16S and other from 23S rDNA) are required to amplify the internal transcribed spacer (ITS) (Borneman and Triplett 1997). The size of ITS ranges between 150 and 1500 bp, and it is a good candidate for an analysis of bacterial diversity (Sigler et al. 2002).

The ITS regions evolve rapidly and, hence, are useable as "high-resolution marker" in populations genetics (van Oppen et al. 2002). Although in the few cases, polymorphisms have been detected in these non-coding regions (Nichols and Barnes 2005). The ITS region has progressively been utilized for discrimination among bacterial species or strains, including *Mycobacterium* species (Roth et al. 1998), cyanobacteria (Boyer et al. 2001), acetic acid bacteria (Trcek 2005), and *Escherichia coli* strains (Gibreel and Taylor 2006), which cannot be easily distinguished by the 16S rRNA gene. Similarly, the identifications of closely related species based on only morphological characters are difficult in the case of the multi-species genus.

5.2.3 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is a molecular technique that used a decamer (10 nucleotides) primer for PCR amplification and followed by agarose gel electrophoresis. Comparative amplified fragments are used for the analysis of microbial diversity. These short primes randomly bind anywhere in genomic DNA at low melting temperature (Tm) (Franklin et al. 1999). RAPD has been used to analyze microbial diversity in the soil of arid zone plants (Sharma et al. 2013), viral diversity in soils (Srinivasiah et al. 2013), Panax ginseng rhizosphere, and non-rhizosphere soil (Li et al. 2012). Due to a limited resolving ability of RAPD and massive microorganisms, it needs to integrate with other advanced approaches.

5.3 Culture-Independent Techniques

There are numerous culture-independent methods, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP), and fluorescent in situ hybridization (FISH) have been utilized to investigate microbial diversity (Hwang et al. 2008; Rademacher et al. 2012; Klang et al. 2015).

5.3.1 Denaturing Gradient Gel Electrophoresis (DGGE)

Denaturing gradient gel electrophoresis (DGGE) is a method that has been exploited for species identification. There are various steps involved in this method, such as genomic DNA extraction, amplification of 16S rDNA sequences, and separation of amplified products by PAGE. The electrophoretic mobility of DNA fragments depends upon the melted double-stranded DNA in gel contains the linear gradient of DNA denaturant, formamide, and urea (Muyzer et al. 1993) or a linear temperature gradient (Muyzer and Smalla 1998). DGGE has been used to assess the microbial diversity for the sulfate-reducing bacteria (Kleikemper et al. 2002), *Gamma* and *Betaproteobactera* (Fahrenfeld et al. 2013), and for functional diversity in different contaminated sites (Ferris et al. 1996; Geets et al. 2006; Orlewska et al. 2018). It has been extensively used for the assessment of various microorganisms in different environmental samples.

5.3.2 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Terminal restriction fragment length polymorphism (T-RFLP) technique is based on PCR and restriction endonuclease digestion. After extraction of genomic DNA from any environmental sample, fluorescence labelled primers are utilized for amplification of 16S rDNA followed by restriction digestion. Analysis of separated fragments carried out by automated DNA sequencer, which provides the patterns of the peaks in the form of electropherogram (Thies 2007; Stenuit et al. 2008). The electropherogram peaks are identified through an available database for analysis of microbial diversity (Marsh et al. 2000). T-RFLP has been used to estimate the microbial diversity for the different groups such as eubacteria (Brunk et al. 1996), planctomycetes (Derakshani et al. 2001), methylotrophs and methanotrophs (Allen et al. 2007), aerobic and anaerobic hydrocarbon-degrading communities (Tipayno et al. 2012), and microbial diversity in anaerobic digestion (De Vrieze et al. 2018). This technique has been replaced with 16S rRNA gene sequencing because of its time-consuming and complex nature (De Vrieze et al. 2018). Another disadvantage of the method, it covers limited phylogenetic analysis due to short sequence reads (Marzorati et al. 2008). This technique facilitates the detection of different haplotypes from any environmental samples.

5.3.3 Fluorescence in situ Hybridization (FISH)

Fluorescence in situ Hybridization (FISH) is a molecular tool, which was developed by Langer-Safer et al. (1982). In this technique, a fluorescence dye labelled probes (DNA or cDNA) are used, which bind to the complementary region of the

DNA. The probes are prepared either by nick translation or PCR or tagged with biotin. After denaturation of DNA and probes, both allow for hybridization. After hybridization, followed by post-hybridization, samples examined under the fluorescence microscope (Amann et al. 1995; Mani et al. 2011). FISH, which can be used as a cultivation-independent approach for visualization, identification, and quantification of microorganisms in the medical and environmental sample. FISH has used to evaluation of microbial diversity in contaminated environments (Richardson et al. 2002), s-triazine herbicides treated soils (Caracciolo et al. 2010), methane-rich gas field in the Cook Inlet basin of Alaska (Dawson et al. 2012), and activated sludge from a nitrifying-denitrifying tank at the municipal wastewater treatment plant (WWTP) of Klosterneuburg, Austria (Lukumbuzya et al. 2019). Due to advancement in the FISH technique, multicolor FISH can be more suitable as compared to a classical FISH.

5.4 Cutting-Edge High-Throughput Culture-Independent Approach for Microbial Diversity

Presently, omics techniques like metagenomics, metatranscriptomics, metaproteomics, and metabolomics are very helpful to understand the microbial diversity and their function in soil (Fig. 5.1). For the bioremediation of soil, multi-omics approach can be utilized to screen potential microbial strain (Mani 2020c). These multi-omics are discussed in detail.

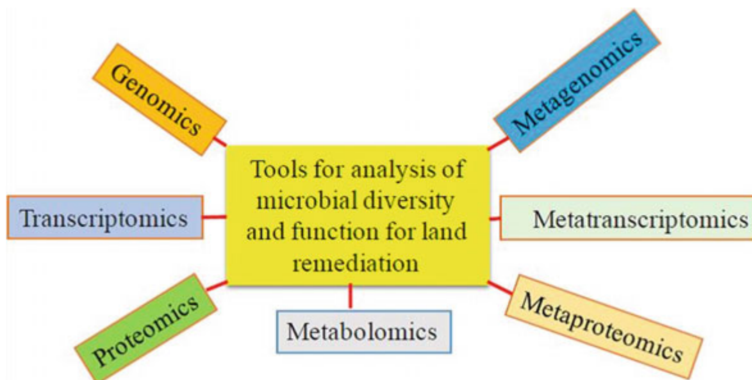

Fig. 5.1 A schematic diagram of different molecular tools that used for an assessment of the microbial diversity and their functions in the remediation of contaminated land

5.4.1 Metagenomics

Metagenomics is also known as environmental genomics or community genomics or population genomics. The term metagenomics was coined by Handelsman et al. (1998). A detail procedure of shotgun metagenomic sequencing (Regar et al. 2019) is given in Fig. 5.2. A shotgun metagenomic sequencing has been utilized to evaluate the microbial diversity from the pesticides contaminated and non-contaminated soil samples. Results have shown various abundance of microbes such as *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes,* and *Acidobacteria* in both samples. Interestingly, substrate specific pathway degradative gene analysis has shown the presence of many genes for both upper and lower pathways. However, a smaller number of degradative genes have identified for the degradation of atrazine, styrene, naphthalene, and bisphenol (Regar et al. 2019). These xenobiotic degradative genes carrying microbes can be utilized for remediation of such pesticides contaminated lands.

Another shotgun metagenomics-based study has reported that bacterial and fungal microbes were associated with vineyards and forest land in Chile. In both habitats, the most abundant bacteria *Candidatus*, *Bradyrhizobium,* and *Solibacter*, and the fungus *Gibberella* were identified. Interestingly, metabolic diversity was different in the vineyards associated microbes while no difference was observed at the taxonomic level (Castañeda and Barbosa 2017). Swenson et al. (2018) analyzed the metagenomics to understand microbial diversity from the biological soil crust (biocrust). The study demonstrated that microbial diversity was directly linked with environmental chemistry in biocrust (Swenson et al. 2018). Soil microbial diversity greatly affected

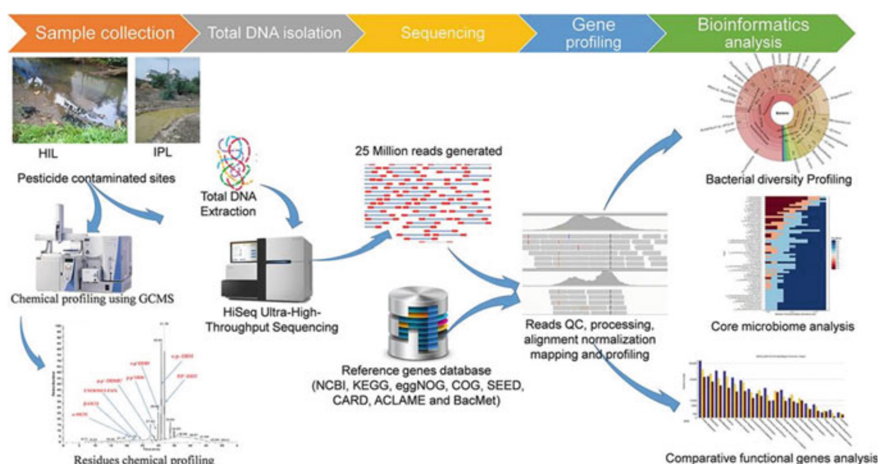

Fig. 5.2 A schematic presentation of a shotgun metagenomic approach for evaluation of the microbial diversity and their functions from the pesticides contaminated sites. Major steps such as sample collection, total DNA isolation, sequencing, gene profiling, and bioinformatics analysis have been shown (Regar et al. 2019. *Adapted with permission*)

at mining sites due to soil pollution. To understand the abundance of microbes, a study was conducted in zinc (Zn) and lead (Pb) contaminated soil using the 16S rDNA-based metagenomics approach. Results from the study, ten frequently detected bacteria, which included *Geobacter, Solirubrobacter, Edaphobacter, Gemmatiomonas, Pseudomonas, Xanthobacter, Sphingomonas, Ktedonobacter, Pedobacter*, and *Nitrosomonas* (Hemmat-Jou et al. 2018). This study demonstrates the bacterial profiling in Pb and Zn contaminated soils using a powerful tool like metagenomics.

To understand microbial diversity in the desert environment, a 16S rDNA sequence-based metagenomics approach has been utilized. For the analysis, soil samples were taken from the two quadrates desert environment (Thar Desert India) that face hot dry weather with fewer rain and intense temperatures. In this study, they utilized V3-V4 regions of 16S rDNA and Illumina next generation sequencing (NGS) to analyze bacterial diversity. They found the three phyla in abundance, *Actinobacteria*, *Proteobacteria*, and *Acidobacteria,* in both environments (Sivakala et al. 2018). Among these phyla, *Actinobacteria* is an important phylum based on their commercial value. A finding suggests that desert environments can be a good source to isolate an important microorganism to remediation of land.

A 16S rDNA-based high throughput sequencing method has been used to analyze alfalfa and barley rhizosphere microbial diversity in oil contaminated soil. A study reported that oil contaminated soil has higher abundance of oil-degrading microbes (*Alcanivorax* and *Aequorivita*) but reduced diversity as compared to oil non-contaminated samples. Moreover, two more phyla (*Thermi* and *Gemmatimonadetes*) were also present in the oil-contaminated soil (Kumar et al. 2018). These findings suggest that the presence of these oil-degrading microbes play a vital role in the degradation of hydrocarbon contamination in soil. The combination of metagenomics and in silico approaches have been used to identify novel genes, proteins, and enzymes from the diverse groups of microbes. With the help of the NGS and Sanger sequencing method, genome sequences are generated, and in silico method aids in the prediction of protein function. After all, it can be cloned and expressed in a particular host in in vitro. Such types of approaches can be used in any environmental samples (Calderon et al. 2019). Interestingly, this approach can be utilized to discover important enzymes from microbial diversity for the remediation of land.

A metagenomics method has been extended to understand the effect of altitude on microbial diversity in soil. Features of high altitude ecosystems are low temperature, decreased atmospheric pressure, variable precipitation, and soil nutrient stress (Morán-Tejeda et al. 2013). It has found the most abundant phyla of *Acidobacteria*, *Proteobacteria,* and *Actinobacteria* at high altitude land, whereas *Fermicutes* and *Bacteroidetes* at low altitude. The high throughput sequencing data analysis helped to identify a novel bacterial diversity at high altitude, which was missed by conventional methods (Kumar et al. 2019). Due to better survival of microbes at high altitudes under various variable conditions including soil nutrient stress, it would be beneficial to explore them further. Therefore, a study suggests that these groups of microbes can be used to remediation of hill agriculture land. A metagenomic method has been utilized to examine the microbial diversity in effluent contaminated constructed wetlands and in rhizosphere soil. Interestingly, the rhizosphere soils have

shown the richness of microbial diversity as compared to wetlands. From functional analysis, it has been demonstrated that different xenobiotic degradation pathways are associated in the soils (Bai et al. 2014). The finding suggests that utilizing a recent tool to investigate the diversity of microbes on the sequence based as well as function based can be used to the remediation of effluents contaminated land.

A metagenomics-based study has analyzed the microbial diversity in Cadmium (Cd) contaminated soil. After comparison with non-contaminated soil, Feng et al. (2018) found that Cd-contamination significantly reduced the diversity of microorganism. Interestingly, they have found *Sulfuricella*, *Proteobacteria*, and *Thiobacillus* as major microbes which played an important role in the remediation of Cd-contaminated soil (Feng et al. 2018). These Cd resistant microbes can be further used in the remediation of Cd-contaminated land. Similarly, metagenomics study has performed in uranium contaminated soil to understand the functional and structural diversity of microbes. In uranium contaminated and non-contaminated soil, *Proteobacteria*, and *Actinobacteria* were common while *Alicyclobacillus*, *Robiginitalea*, and *Microlunatus* were present in the non-contaminated soil only. KEGG metabolic pathway database was used to analyze the metabolism of amino acids and signaling molecules (Yan et al. 2016). Common microbes such as *Proteobacteria* and *Actinobacteria* can be used in the remediation of uranium contaminated land.

A metagenomic study was reported from China utilizing mercury (Hg) contaminated soil. Analysis demonstrated the Hg affected microbial diversity, abundance, and functional aspects. In contaminated soil, *Firmicutes* and *Bacteroidetes* were abundance, and contamination of Hg also affected on different functional genes that involve in its transformation, such as methylation and reduction (Liu et al. 2018). Metagenomics methods have been used to evaluate the effects of natural groups of microbes and consortium microbes on the degradation of polycyclic aromatic hydrocarbon (PAH) in the contaminated soil. A study has demonstrated that the degradation of PAH was significantly higher by using microbial consortium as compared to other groups of microbes. At the gene level, variations in laccase, aromatic ring-hydroxylating dioxygenases (ARHD), salicylate, benzoate, and protocatechuate-degrading enzyme were found (Zafra et al. 2016). This study suggests that these potential gene producing microbes can be useful in remediation of PAH-contaminated land.

5.5 Cutting-Edge High-Throughput Culture-Independent Approach for Microbial Function

Assessments of functional characteristics of microorganisms are complex as compared to sequence-based study.

5.5.1 Metatranscriptomics

Metatranscriptomics is RNA-based methods used to analyze the taxonomic composition and profile of the microbial functions. There are various experimental steps involved in this approach, which need to be addressed while analyze through metatranscriptomics (Carvalhais and Schenk 2013; Jiang et al. 2016). The advancement in this technology is very promising to help to understand microbial function. A culture-independent method has been utilized to evaluate microbial diversity from halogen contaminated and non-contaminated German forest soils. Weigold et al. (2016) analyzed the genes encoding enzymes that are involved in halogenation and dehalogenation of the halogens. They determined that *Bradyrhizobium* and *Pseudomonas* genera were involved in these processes. Further, they found chloroperoxidases and haloalkane dehalogenases enzymes, which were responsible for the halogenation and dehalogenation of halogens in the contaminated forest soil (Weigold et al. 2016).

Metagenomics and metatranscriptomics methods have been used to examine microbial diversity and their functions in dissolved organic matter (DOM) from the soil samples. A study reported that there were great variations in microbial genera such as *Thermoleophilia, Syntrophobacterales, Spirochaeta, Geobacter,* and *Gaiella.* In this study, Li et al. (2018) found a correlation with the richness of microbial metabolic pathways lignolysis, methanogenesis, and fermentation in DOM of paddy soil samples. A metatranscriptomics-based study analyzed an environmental functional gene microarray (E-FGA) containing 13,056 mRNA microbial clones from different environmental samples. They have examined the E-FGA containing mRNA microbial clones by profiling the microbial activity of agricultural soils with a high or low flux of nitrous oxide (N_2O). Interestingly, 109 genes have been expressed and demonstrated significant variability with high and low N_2O emissions (McGrath et al. 2010). Such an approach may be useful to evaluate the functional activity of the microorganisms.

Shotgun metagenomic sequencing and metatranscriptomics studies have performed to analyze the rhizosphere microbial communities of *Archis hypogaea* (peanut plant), roots of plants grown in the soil of crop rotation, and peanut monocropping. Interestingly, in monocropping, an enrichment of different rare species occurred, but microbial diversity of rhizosphere had reduced. A further reduction occurred in the downregulation of genes in auxin and cytokinin and upregulation of genes related to other hormones (abscisic acid and salicylic acid) (Li et al. 2019). As the study suggested, plant rhizosphere microbiota and plant physiology were affected by land use history.

A metatrasncriptomics approach has been utilized to identify cadmium (Cd) tolerant genes from the contaminated sites. cDNA libraries of different sizes of yeast mRNA (from 0.1 kb to 4 kb) were developed. After screening of cadmium tolerant transcript through yeast complementation system, Thakur et al. (2018) have found that transformants ycf1ΔPLBe1 were capable to tolerate Cd in the range of 40–80 μM. Interestingly, a sequence of PLBe1 cDNA shown homology with AN1

type zinc finger protein of *Acanthameoba castellani.* In addition, it has also shown the tolerance against copper (Cu), cobalt (Co), and zinc (Zn) (Thakur et al. 2018). The finding suggests that PLBe1 can be a promising candidate for the multi-metal tolerant gene for remediation of the heavy metal contaminated lands. A metatranscriptomics study has identified an *Actinobacteria* as a most abundant family in hot desert soil samples. Interestingly, it found that chemoautotrophic carbon fixation genes were more expressed as compared to photosynthetic genes in these samples (León-Sobrino et al. 2019) indicating that chemoautotrophy could be alternative of photosynthesis in hot dessert soils.

Bragalini et al. (2014) developed a solution hybrid selection (SHS) technique, which is very effective for the recovery of eukaryotes cDNAs from soil extracted mRNA. The authors utilized this technique on endo-xylanases of Glycoside Hydrolase (GH) 11 gene family. Approximately 25% cloned cDNAs sequences were expressed in *Saccharomyces cerevisiae* (Bragalini et al. 2014). This technique can be utilized to explore eukaryotic microbial communities to the prospecting of land remediation related genes. A 16S rDNA and metatranscriptomics methods were used to evaluate microbial diversity and their functions in the sandy loam soil, which was treated with various concentrations (60–2000 mg/kg) of silver nanoparticles (AgNPs). Analysis has shown that it was very much upregulation in genes, which are involved in the heavy metal resistance (Meier et al. 2020). Finding suggests that multi-level concentration-based studies are important to assess microbial functions in a particular land site.

5.5.2 Metaproteomics

Another powerful tool of omics is metaproteomics that includes the study of all proteins which are directly recovered from any environmental samples. Metaproteomic approaches are undertaking microbial functional characteristics more directly as compared to metagenomics and metatranscriptomics. This method is used to understand the functional diversity of microorganisms in any particular site. A metaproteomics-based study analyzed the maize rhizosphere soil, where 696 proteins were discovered from 244 genus and 393 species (Renu et al. 2019). These important results can be helpful in designing experiments for other rhizosphere soil samples. Metaproteomics and phospholipid fatty-acids analysis has performed in petroleum polluted semiarid soil samples to understand the phylogenetic and physiological response of the microbiome. A 2016 study illustrated that petroleum contamination increases proteobacterial proteins while reducing the richness of *Rhizobiales* as compared to non-contaminated soil (Bastida et al. 2016). A metaproteomics method has been utilized to understand the effect of chlorophenoxy acid-degrading bacteria on the soil sample, which was treated with 2, 4-dichlorophenoxy acetic acid (2,4-D) for 22 days. They have identified the chlorocatechol dioxygenases enzymes from

these samples (Benndorf et al. 2007). This enzyme can be further used for the treatment of 2,4-D contaminated land. Rotation of the plantation on a particular land may affect rhizosphere microbial diversity.

5.5.3 Metabolomics

Exometabolomics or metabolic footprinting is a sub-field of metabolomics, which is used to study extracellular metabolites (Allen 2003; Mapelli et al. 2008; Silva and Northen 2015). A detail procedure of metabolomics is given in Fig. 5.3. A study has analyzed exometabolome to understand the function of a microbial community of the biological soil crust (biocrust) (Swenson et al. 2018). Finding suggests that the microbial community is directly linked with environmental chemistry in biocrust. Gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS) were used to investigate the metabolites from the saprolite (chemically weathered rock) soil samples. In this study, 96 metabolites have been identified, including amino acids and their derivatives, nucleosides, sugar, alcohol, and carboxylic acids. After quantification of 25 metabolites, it has indicated an uneven quantitative distribution. There were two types of soil defined media (SDM 1 and SDM2) designed using these metabolites information. There were 30 different types of soil bacterial isolates grown on both media. However, a result has shown that SDM1 sustained growth of 13 isolates, and SDM2 supported the growth of 15 isolates (Jenkins et al. 2017). This information can be utilized to develop suitable media for the growth of promising microorganisms, which are potential candidates for land remediation.

5.6 Conclusion, Challenges, and Future Perspective

An increase of contamination in soil is a vast problem, and remediation of it a great challenge. Due to ubiquitous nature of the microbes in the environment, it plays an important role in the remediation of contaminated land. Further, microbes are an excellent source of enzymes that convert harmful metal into a neutral state. However, soil is a massive shelter of the diversity of culturable and unculturable microorganisms. Due to the complexity of microorganisms, it is very challenging to identify and characterize them. To understand the microbial diversity and their functions, various classical to advance techniques, including multi-omics are available. For the analysis of microbial diversity and their function, culture-dependent and culture-independent methods are being used. As metagenomics (culture-independent) molecular approach offers a powerful lens for viewing the microbial world and which is very promising to help to understand the questions like who are there? Or what are they doing? Therefore, the combined information of phylogenetic and functional aspects would provide thoughtful understandings about soil microorganisms. The

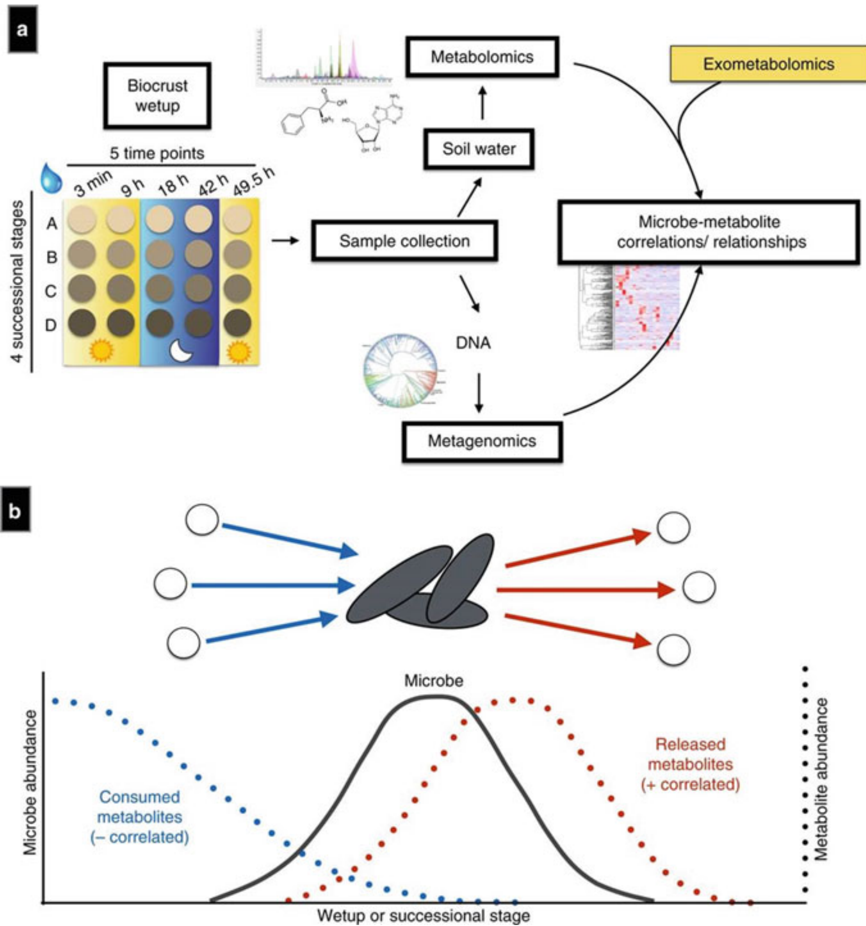

Fig. 5.3 Experimental workflow and biocrust microbe–metabolite relationship predictions. **a** Biocrust wetup metabolomics and metagenomics experimental setup and analysis. **b** Exometabolomics-based in situ microbe-metabolite relationship prediction (Swenson et al. 2018. *Adapted with permission*)

multi-omics methods such as metagenomics, metatranscriptomics, metaproteomics, and metabolomics are very helpful to screen potential microbes. Further, through the use of cutting-edge tools, a potential microbe can identify, characterize, modify, and construct microbial consortium to remediation of any contaminated land.

References

Allen J (2003) High-throughput classification of yeast mutants for functional genomics using metabolic footprinting. Nat Biotechnol 21(6):692–696

Allen JP, Atekwana EA, Atekwana EA, Duris JW, Werkema DD, Rossbach S (2007) The microbial community structure in petroleum-contaminated sediments corresponds to geophysical signatures. Appl Environ Microbiol 73(9):2860–2870

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Bai Y, Liang J, Liu R, Hu C, Qu J (2014) Metagenomic analysis reveals microbial diversity and function in the rhizosphere soil of a constructed wetland. Environ Technol 35(17–20):2521–2527

Barakat MA (2011) New trends in removing heavy metals from industrial wastewater. Arab J Chem 4:361–377

Bastida F, Jehmlich N, Lima K, Morris BEL, Richnow HH et al (2016) The ecological and physiological responses of the microbial community from a semiarid soil to hydrocarbon contamination and its bioremediation using compost amendment. J Proteomics 135:162–169

Benndorf D, Balcke GU, Harms H, von Bergen M (2007) Functional metaproteome analysis of protein extracts from contaminated soil and groundwater. ISME J 1(3):224–234

Borneman J, Triplett EW (1997) Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl Environ Microbiol 63(7):2647–2653

Boyer SL, Flechtner VR, Johansen JR (2001) Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in Cyanobacteria. Mol Biol Evol 18:1057–1069

Bragalini C, Ribière C, Parisot N, Vallon L, Prudent E et al (2014) Solution hybrid selection capture for the recovery of functional full-length eukaryotic cDNAs from complex environmental samples. DNA Res 21(6):685–694

Brunk CF, Avaniss-Aghajani E, Brunk CA (1996) A computer analysis of primer and probe hybridization potential with bacterial small-subunit rRNA sequences. Appl Environ Microbiol 62(3):872-879

Calderon D, Peña L, Suarez A, Villamil C, Ramirez-Rojas A et al (2019) Recovery and functional validation of hidden soil enzymes in metagenomic libraries. Microbiologyopen 8(4):e00572

Caracciolo AB, Bottoni P, Grenni P (2010) Fluorescence in situ hybridization in soil and water ecosystems: a useful method for studying the effect of xenobiotics on bacterial community structure. Toxicol Environ Chem 92:567–579

Carvalhais LC, Schenk PM (2013) Sample processing and cDNA preparation for microbial metatranscriptomics in complex soil communities. Methods Enzymol 531:251–267

Castañeda LE, Barbosa O (2017) Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. Peer J 5:e3098

Dawson KS et al (2012) Quantitative fluorescence in situ hybridization analysis of microbial consortia from a biogenic gas field in Alaska's Cook Inlet basin. Appl Environ Microbiol 10:3599–3605

Derakshani M, Lukow T, Liesack W (2001) Novel bacterial lineages at the (sub) division level as detected by signature nucleotide-targeted recovery of 16S rRNA genes from bulk soil and rice roots of flooded rice microcosms. Appl Environ Microbiol 67(2):623–631

De Vrieze J, Ijaz UZ, Saunders AM et al (2018) Terminal restriction fragment length polymorphism is an "old school" reliable technique for swift microbial community screening in anaerobic digestion. Sci Rep 8:16818

Deng W, Xi D, Mao H, Wanapat M (2008) The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. Mol Biol Rep 35:265–274

Dodd JC, Boddington CL, Rodriguez A, Gonzalez-Chavez C, Mansur I (2000) Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. Plant Soil 226(2):131–151

Fahrenfeld N, Zoeckler J, Widdowson MA et al (2013) Effect of biostimulants on 2, 4, 6-trinitrotoluene (TNT) degradation and bacterial community composition in contaminated aquifer sediment enrichments. Biodegradation 24:179–190

Feng G, Xie T, Wang X, Bai J, Tang L et al (2018) Metagenomic analysis of microbial community and function involved in cd-contaminated soil. BMC Microbiol 18(1):11

Ferris MJ, Muyzer G, Ward DM (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. Appl Environ Microbiol 62:340–346

Franklin RB, Taylor DR, Mills AL (1999) Characterization of microbial communities using randomly amplified polymorphic DNA (RAPD). J Microbiol Methods 35:225–235

Gangotia D, Gupta A, Mani I (2021) Role of bioinformatics in biological sciences. In: Advances in bioinformatics. Springer Nature, pp 37–57

Geets J et al (2006) DsrB gene-based DGGE for community and diversity surveys of sulfate-reducing bacteria. J Microbiol Methods 66:194–205

Gibreel A, Taylor DE (2006) Macrolide resistance in *Campylobacter jejuni* and Campylobacter coli. J Antimicrob Chemother 58:243–255

Gupta A, Gangotia D, Mani I (2021) Bioinformatics tools and softwares. In: Advances in bioinformatics. Springer Nature, pp 15–34

Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chem Biol 5(10):R245–R249

Hemmat-Jou MH, Safari-Sinegani AA, Mirzaie-Asl A, Tahmourespour A (2018) Analysis of microbial communities in heavy metals-contaminated soils using the metagenomic approach. Ecotoxicology 27(9):1281–1291

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic interference. Q Rev Biol 66(4):411–453

Hillis DM, Moritz C (1990) An overview of applications of molecular systematics. In: Hillis DM, Moritz C (eds) Molecular systematics. Sinauer Associates, Sunderland, pp 502–515

Huson DH, Mitra S, Ruscheweyh HJ, Weber N, Schuster SC (2011) Integrative analysis of environmental sequences using MEGAN4. Genome Res 21:1552–1560

Hwang K, Shin SG, Kim J, Hwang S (2008) Methanogenic profiles by denaturing gradient gel electrophoresis using order-specific primers in anaerobic sludge digestion. Appl Microbiol Biotechnol 80:269–276

Jenkins S, Swenson TL, Lau R, Rocha AR, Aaring A et al (2017) Construction of viable soil defined media using quantitative metabolomics analysis of soil metabolites. Front Microbiol 8:2618. https://doi.org/10.3389/fmicb.2017.02618

Jiang Y, Xiong X, Danska J, Parkinson J (2016) Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. Microbiome 4:2

Klang J et al (2015) Dynamic variation of the microbial community structure during the long-time mono-fermentation of maize and sugar beet silage. Microb Biotechnol 8:764–775

Kleikemper J, Schroth MH, Sigler WV, Schmucki M, Bernasconi SM, Zeyer J (2002) Activity and diversity of sulfate-reducing bacteria in a petroleum hydrocarbon-contaminated aquifer. Appl Environ Microbiol 68(4):1516–1523

Kumar V, AlMomin S, Al-Aqeel H, Al-Salameen F, Nair S, Shajan A (2018) Metagenomic analysis of rhizosphere microflora of oil-contaminated soil planted with barley and alfalfa. PLoS ONE 13(8):e0202127

Kumar S, Suyal DC, Yadav A, Shouche Y, Goel R (2019) Microbial diversity and soil physiochemical characteristic of higher altitude. PLoS ONE 14(3):e0213844

Langer-Safer PR, Levine M, Ward DC (1982) Immunological method for mapping genes on Drosophila polytene chromosomes. Proc Natl Acad Sci USA 79(14):4381–4385

León-Sobrino C, Ramond JB, Maggs-Kölling G, Cowan DA (2019) Nutrient acquisition, rather than stress response over diel cycles, drives microbial transcription in a hyper-arid namib desert soil. Front Microbiol 10:1054

Li Y, Ying Y, Zhao D, Ding W (2012) Microbial community diversity analysis of Panax ginseng rhizosphere and non-rhizosphere soil using randomly amplified polymorphic DNA method. O J Gen 2:95–102

Li HY, Wang H, Wang HT, Xin PY, Xu XH et al (2018) The chemodiversity of paddy soil dissolved organic matter correlates with microbial community at continental scales. Microbiome 6(1):187

Li X, Jousset A, de Boer W, Carrión VJ, Zhang T, Wang X, Kuramae EE (2019) Legacy of land use history determines reprogramming of plant physiology by soil microbiome. ISME J 13(3):738–751

Liu YR, Delgado-Baquerizo M, Bi L, Zhu J, He JZ (2018) Consistent responses of soil microbial taxonomic and functional attributes to mercury pollution across China. Microbiome 6(1):183

Lukumbuzya M, Schmid M, Pjevac P, Daims HA (2019) Multicolor fluorescence in situ hybridization approach using an extended set of fluorophores to visualize microorganisms. Front Microbiol 10:1383

Mani I, Kumar R, Singh M, Nagpure NS, Kushwaha B, Srivastava PK, Rao DSK, Lakra WS (2011) Nucleotide variation and physical mapping of ribosomal genes using FISH in genus Tor (Pisces, Cyprinidae). Mol Biol Rep 38:2637–2647

Mani I (2020a) Metagenomic approach for bioremediation: challenges and perspectives. In: Bioremediation of pollutants. Elsevier, pp 275–285

Mani I (2020b) Current status and challenges of DNA sequencing. In: Advances in synthetic biology. Springer, Singapore, pp 71–80

Mani I (2020c) Biofilm in bioremediation. In: Bioremediation of pollutants. Elsevier, pp 375–385

Mapelli V, Olsson L, Nielsen J (2008) Metabolic footprinting in microbiology: methods and applications in functional genomics and biotechnology. Trends Biotechnol 26(9):490–497

Marsh TL, Saxman P, Cole J, Tiedje J (2000) Terminal restriction fragment length polymorphism analysis program, a web-based research tool for microbial community analysis. Appl Environ Microbiol 66:3616–3620

Marzorati M, Wittebolle L, Boon N, Daffonchio D, Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. Environ Microbiol 10:1571–1581

McGrath KC, Mondav R, Sintrajaya R, Slattery B, Schmidt S, Schenk PM (2010) Development of an environmental functional gene microarray for soil microbial communities. Appl Environ Microbiol 76(21):7161–7170

Meier MJ, Dodge AE, Samarajeewa AD, Beaudette LA (2020) Soil exposed to silver nanoparticles reveals significant changes in community structure and altered microbial transcriptional profiles. Environ Pollut 258:113816

Mocali S, Benedetti A (2010) Exploring research frontiers in microbiology: the challenge of metagenomics in soil microbiology. Res Microbiol 161:497–505

Morán-Tejeda EL, Moreno JI, Beniston M (2013) The changing roles of temperature and precipitation on snowpack variability in Switzerland as a function of altitude. Geophys Res Lett 40(10):2131–2136

Muyzer G, Smalla K (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek 73:127–141

Muyzer G, Waal ECD, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nacke H, Thürmer A, Wollherr A et al (2011) Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and glassland soils. PLoS ONE 6:e17000

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. Annu Rev Ecol Syst 28:105–128
Nelson MC, Morrison M, Yu ZT (2011) A meta-analysis of the microbial diversity observed in anaerobic digesters. Bioresour Technol 102:3730–3739
Nichols S, Barnes PAG (2005) A molecular phylogeny and historical biogeography of the marine sponge genus Placospongia (Phylum Porifera) indicate low dispersal capabilities and widespread crypsis. J Exp Mar Biol Ecol 323:1–15
Nüsslein K, Tiedje JM (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl Environ Microbiol 65:3622–3626
O'Donnell GA et al (2007) Visualization, modelling and prediction in soil microbiology. Nat Rev Microbiol 5:9689–9699
Orlewska K, Piotrowska-Seget Z, Bratosiewicz-Wasik J, Cycon M (2018) Characterization of bacterial diversity in soil contaminated with the macrolide antibiotic erythromycin and/or inoculated with a multidrug-resistant Raoultella sp. strain using the PCR-DGGE approach. Appl Soil Ecol 126:57–64
Rademacher A, Nolte C, Schonberg M, Klocke M (2012) Temperature increases from 55 to 75 A degrees C in a two-phase biogas reactor result in fundamental alterations within the bacterial and archaeal community structure. Appl Microbiol Biotechnol 96:565–576
Regar RK, Gaur VK, Bajaj A, Tambat S, Manickam N (2019) Comparative microbiome analysis of two different long-term pesticide contaminated soils revealed the anthropogenic influence on functional potential of microbial communities. Sci Total Environ 681:413–423
Renu GSK, Rai AK, Sarim KM, Sharma A et al (2019) Metaproteomic data of maize rhizosphere for deciphering functional diversity. Data Brief 27:104574
Richardson RE, Bhupathi Raju VK, Song DL, Goulet TA, Alvarez-Cohen L (2002) Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques. Environ Sci Technol 36:2652–2662
Roth A, Fischer M, Hamid ME et al (1998) Differentiation of phylogenetically related slowly growing mycobacteria based on 16S–23S rRNA gene internal transcribed spacer sequences. J Clin Microbiol 36:139–147
Rudkjøbing VB, Thomsen TR, Xu Y et al (2016) Comparing culture and molecular methods for the identification of microorganisms involved in necrotizing soft tissue infections. BMC Infect Dis 16:652
Sanyal SK, Mou TJ, Chakrabarty RP et al (2016) Diversity of arsenite oxidase gene and arsenotrophic bacteria in arsenic affected Bangladesh soils. AMB Expr 6:21
Schloss PD, Handelsman J (2004) Status of the microbial census. Microbiol Mol Biol Rev 68:686–691
Schloss PD, Girard RA, Martin T, Edwards J, Thrash JC (2016) Status of the archaeal and bacterial census: an update. Mbio 7(3):e00201–e00216
Sharma G, Verma HN, Sharma R (2013) RAPD analysis to Study metagenome diversity in soil microbial community of arid zone plants. Proc Natl Acad Sci India Sect B Biol Sci 83:135–139
Sigler WV, Zeyer J (2002) Microbial diversity and activity along the forefields of two receding glacier. Microb Ecol 43(4):397–407
Sigler WV, Crivii S, Zeyer J (2002) Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb Ecol 44(4):306–316
Silva L, Northen T (2015) Exometabolomics and MSI: deconstructing how cells interact to transform their small molecule environment. Curr Opin Biotech 34:209–216
Sivakala KK, Jose PA, Anandham R, Thinesh T, Jebakumar SRD et al (2018) Spatial physiochemical and metagenomic analysis of desert environment. J Microbiol Biotechnol 28(9):1517–1526
Srinivasiah S et al (2013) Direct assessment of viral diversity in soils by random PCR amplification of polymorphic DNA. Appl Environ Microbiol 79(18):5450–5457
Stenuit B, Eyers L, Schuler L, Agathos SN, George I (2008) Emerging high throughput approaches to analyze bioremediation of sites contaminated with hazardous and/or recalcitrant wastes. Biotechnol Adv 26:561–575

Sundberg C et al (2013) 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol Ecol 85:612–626
Swenson TL, Karaoz U, Swenson JM et al (2018) Linking soil biology and chemistry in biological soil crust using isolate exometabolomics. Nat Commun 9:19
Thakur B, Yadav R, Fraissinet-Tachet L, Marmeisse R, Sudhakara Reddy M (2018) Isolation of multi-metal tolerant ubiquitin fusion protein from metal polluted soil by metatranscriptomic approach. J Microbiol Methods 152:119–125
Thies JE (2007) Soil microbial community analysis using terminal restriction fragment length polymorphisms. J Soil Sci Soc Am 71:579–591
Tiedje JM, Asuming-Brempong S, Nusslein K, Marsh TL, Flynn SJ (1999) Opening the black box of soil microbial diversity. Appl Soil Ecol 13:109–122
Timonen S, Finlay RD, Olsson S, Söderström B (1996) Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β-scanner. FEMS Microbiol Ecol 19(3):171–180
Tipayno S, Kim C-G, Sa T (2012) T-RFLP analysis of structural changes in soil bacterial communities in response to metal and metalloid contamination and initial phytoremediation. Appl Soil Ecol 61:137–146
Torsvik V, Gokseyr J, Daae FL (1990) High diversity in DNA of soil bacteria. Appl Environ Microbial 56:782–787
Torsvik V, Gokseyr J, Daae FL et al (1994) Use of DNA analysis to determine the diversity of microbial communitres. Beyond the biomass. John Wiley & Sons, Chichester, pp 39–48
Torsvik V, Sorheim R, Gokseyr J (1996) Total bacterial diversity in soil and sediment communities-a review. J Industr Microbial 17:170–l78
Trcek J (2005) Quick identification of acetic acid bacteria based on nucleotide sequences of the 16S–23S rDNA internal transcribed spacer region and of the PQQ-dependent alcohol dehydrogenase gene. Syst Appl Microbiol 28:735–745
van Oppen MJH, Wörheide G, Takahashi M (2002) Nuclear markers in evolutionary and population genetic studies of scleractinian corals and sponges. Proc 9th Int Coral Reef Symp. Bali 1:131–138
Wall DH, Virginia RA (1999) Controls on soil biodiversity: insights from extreme environments. Appl Soil Ecol 13(2):137–150
Weigold P, El-Hadidi M, Ruecker A, Huson DH, Scholten T et al (2016) A metagenomic-based survey of microbial (de)halogenation potential in a German forest soil. Sci Rep 6:28958
Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198(1):97–107
Wu X, Zhang G, Zhang W et al (2018) Variations in culturable bacterial communities and biochemical properties in the foreland of the retreating Tianshan No. 1 glacier. Braz J Microbiol 49(3):443–451
Yan X, Luo X, Zhao M (2016) Metagenomic analysis of microbial community in uranium-contaminated soil. Appl Microbiol Biotechnol 100(1):299–310
Zafra G, Taylor TD, Absalón AE, Cortés-Espinosa DV (2016) Comparative metagenomic analysis of PAH degradation in soil by a mixed microbial consortium. J Hazard Mater 318:702–710
Zhou J, Huang Y, Mo M (2009) Phylogenetic analysis on the soil bacteria distributed in karst forest. Braz J Microbiol 40(4):827–837