

Soil Health Management and Microorganisms : Recent Development

Detection, Diagnosis and Management of Soil-borne Phytopathogens

Manda, Raghavendra Reddy; Addanki, Venkata Avinash; Giabardo, Anita; Benjamin, Joshua; Hossain, Mohammad Jonaid et al

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Raghavendra Reddy Manda, Venkata Avinash Addanki,
Anita Giabardo, Joshua Benjamin, Mohammad Jonaid Hossain,
Sayesha Khanna, Manoj Gaddam, Ravindra Kumar,
and Seweta Srivastava

Abstract

One of the essential components for sustaining life on Earth is soil. It provides a diverse range of ecosystem services that are supported by soil processes and tasks

Raghavendra Reddy Manda, Venkata Avinash Addanki, Anita Giabardo, Joshua Benjamin, Mohammad Jonaid Hossain, Sayesha Khanna, Manoj Gaddam, and Ravindra Kumar authors have contributed equally and shared first authorship.

R. R. Manda

Wageningen University & Research, Wageningen, The Netherlands

V. A. Addanki

Università degli Studi di Padova, Legnaro, PD, Italy

A. Giabardo

University of Georgia, Athens, GA, USA

J. Benjamin

Georg-August-Universität Göttingen, Göttingen, Germany

M. J. Hossain

Universitat Politècnica de València, València, Valencia, Spain

S. Khanna

L'Institut Agro Montpellier, Montpellier, France

M. Gaddam

Citrus Center, Texas A & M University Kingsville, Weslaco, TX, USA

R. Kumar

Division of Crop Protection, ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India

S. Srivastava (✉)

School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

e-mail: seweta.21896@lpu.co.in

carried out by soil biodiversity. One of the key elements in maintaining plant health and biomass output is the soil microbiome in particular. The control of soil microbial populations, both targeted and untargeted, seems to hold promise for enhancing food crop productivity, nutritive value, and sustainability over the long term. The acquisition of indicators that can be employed to assess the soil's existing status and afterwards create sustainable agricultural systems is one of the main goals of assessing soil health. This is because during the past few years, tremendous progress has been achieved in the creation of particular biomarkers and macromolecular probes, allowing for quick and accurate assessments of soil microbial populations. Recent years have witnessed an increase in the use of omics techniques, which enable the assessment of microbial phylogenetic diversity and functional information, to research changes in soil microbial diversity brought on by agronomic practices and environmental conditions. The study of soil microbial diversity, plant health, and the quality of derived raw materials will benefit from the application of these high-throughput technologies, strengthening the relationship between soil health, food quality, food safety, and human health.

Keywords

Soil microbiome · Functional microbial diversity · Sustainability · Soil health management

18.1 Introduction

Soil is fundamental to the functioning of the Earth's ecosystems and to human life. In fact, in addition to constituting the base of the food chain, healthy soils also provide several ecosystem services that are essential to our survival, e.g., nutrients cycling, bioremediation, provision of clean drinking water, pest control, and contribution to plant growth (Wall et al. 2004). It is nowadays clear that soil is to be considered not only a substrate that is physically supporting the growth of naturally growing plants and cultivated crops but rather as a living ecosystem (Ponge 2015) to preserve and keep functional. Over the last decades, the scientific community, as well as international institutional bodies, have recognized the importance of promoting soil health at local, national, and international levels and have engaged in soil health awareness actions. The Council of Europe (1972) clearly stated the importance of soil as a fundamental asset to anthropic activities, the need to protect and monitor soil resources, and called for a soil conservation policy. Another, more recent example of international initiatives oriented to soil health awareness promotion the World Soil Day, which was instituted by the Food and Agriculture Organization of the United Nations (FAO 2022), with the aim of fostering human well-being and functioning ecosystems through soil health improvement.

The realization that soil health is a fundamental component to secure ecosystems stability and food security has also driven the demand for a set of internationally standardized measures of soil quality (Nortcliff 2002) to compose a common framework to be used as a reference and to produce effective indicators that could serve as soil health monitoring tools also for policymakers (Stone et al. 2016; Paleari 2017). In order to achieve this objective, a common definition of “soil health”—a matter that has been an object of research and debate—was necessary. Some argue that soil health and soil quality should be considered distinct concepts, in that soil health encompasses a broad range of sustainability components that include planetary health as a whole, while soil quality more strictly refers to ecosystem services important to humans (Lehmann et al. 2020). Within the present manuscript, following Doran and Zeiss (2000), the term “soil quality” will be used in relation to the ability of a given soil to absolve to a specific function, while “soil health” will be used, in a broader sense, to refer to the capacity of soil to ensure biological productivity, promote environmental quality through ecosystem services, and maintain plant and animal health (Doran and Parkin 1994). Soil health has also been defined as a function of ecological characteristics, such as disease suppression capability (Van Bruggen and Semenov 2000). One of the main drivers of soil quality and fertility is soil organic matter (SOM) content. In the last few decades, there strong evidence supporting the major role of the soil microbiome in the synthesis of SOM has been provided (Kallenbach et al. 2016). Hence, the approach to soil health assessment has to necessarily keep the soil microbiome in due consideration. How soil microbiome responds to different agricultural management systems (Mann et al. 2019) is also of high interest in that the agricultural practices farmers decide to implement should take that into account (Nunes et al. 2020), in that improper agricultural practices can be detrimental to soil health and jeopardize future harvests (Manda et al. 2020a).

In order to evaluate the effect of different agricultural practices on the soil microbiome, it is necessary to develop analytical tools and indicators that can provide information on the soil microbiome status and evolution in response to those practices. This information is supposed to not only enrich the shared scientific understanding of the mechanisms underlying soil fertility and plant–microorganism interaction, but also to help farmers, extension islands, and farming consults making better management decisions. The aim of the present work is to offer an overview on the recent developments on soil health management within agricultural systems, with a specific focus on the role of microorganisms and analytical tools and indicators used to monitor soil microbiome and on strategies for soil microbial diversity management in agricultural settings.

18.2 Biological Indicators and Standard Analytical Procedures Used to Determine Soil Health

In the attempt to provide international standards for the assessment of soil quality, a Technical Committee of the International Organization for Standardization (ISO) was set up (Hortensius and Nortcliff 1991). The ISO/TC 190 started their activities in 1985 and dealt with six topics: terminology, sampling, chemical analysis methods, soil quality and biological systems (including the effects on microorganisms), physical investigation of soil, and radionuclides and radioactivity determination methods. It is important to observe that those were not meant to be indicators of soil health but rather “a set of proven, widely used standard methods which can be reliably used by those seeking to evaluate soil quality” (Nortcliff 2002). As discussed earlier, soil health is a broad concept that may be defined in different ways. This poses a challenge to standard measurement method identification. Nevertheless, there has been some effort to identify a shared set of soil health indicators. The present chapter will discuss physical indicators, chemical indicators, and molecular techniques measuring microbial biomass and genetic and functional biodiversity that have emerged as informative and effective in providing information on soil health and quality. Lastly, an overview of -omics approaches will be provided. -omics approaches allow to assess microbial diversity and functionality and have increasingly been used in recent years. These approaches make use of promising technologies that could help studying the soil microbiome in a faster and more thorough way. Genomics, transcriptomics, proteomics, enzymomics, and metabolomics (Bertola et al. 2021) are the main -Omics approaches currently used to study soil microbial communities.

Although the pivotal role of microbial communities in the achievement of soil health has emerged as prominent (Sherwood and Uphoff 2000), microbial and biological indicators are not always able to provide the entire picture (Fierer et al. 2021). Moreover, the search for effective biological indicators, as highlighted by Bruggen and Semenov (2000), has not been systematic.

In fact, one of the major challenges in the use of biochemical properties to assess soil quality is the lack of reference values to interpret the proposed biochemical indicators (Bünemann et al. 2018a, b), as well as the contradictory behaviour shown by some of these indicators and regional variations in expression levels (Gil-Sotres et al. 2005). Therefore, standard analytical procedures, such as water infiltration rates, bulk density, pH, electrical conductivity, ion-exchange capacity, aggregate stability, and soil slaking which have been used and studied for decades, are well known among soil scientists, and have been tested under different environmental conditions in different areas of the world have to be kept as a valid reference and integrated with the newest analysis techniques with the aim to compose a complete picture of the health status of any particular soil.

18.3 Role of Soil Physical and Chemical Indicators for Microbial Sustainability

Daniel (2004) documented that 1 g of soil nearly contains 10 billion microorganisms with hundreds of various microbial communities. The presence of numerous microbes within side the soil is crucial for balanced plant growth as a maximum of the nutrient cycles are managed with the aid of using those microorganisms such as natural remember decomposition, nitrogen fixation, and conversion of ammonia to available plant nitrate. The physical, as well as chemical, signs of soil play a major role in the detection of microbial sustainability in a particular soil type. Polysaccharides and polyuronides launched at some point of decomposition allow for fostering the aggregation of soil particles and hence affect the physical condition of the soil in a particular location. Besides, the chemical compounds produced by fungi mycorrhizal are also crucial in the promotion of soil aggregation (Wright et al. 1999).

18.4 The Effect of Soil Water Infiltration Rate on Microorganisms

18.4.1 Water Infiltration in the Soil

Normally, a huge amount of water is stored in soils. Crops and microbial populations in the soil depend extremely on the presence of water in the soils, besides water is necessary for nutrient cycling. The infiltration rate of water is more in uncompacted soil rather than in a soil which is highly compacted though of the same type (Hamza and Anderson 2005), whereas soil water infiltration rate differs both in time and in space, thus controlling dual effects: availability of water to microbes and plants; and has a dominant effect on the rate of diffusion of solutes and gases (Adl 2003). The infiltration rate of a soil is the highest rate at which the soil of a region under a given set of specific conditions can absorb rain (Richards and Moore 1952). Besides, a quantitative definition for infiltration rate could be explained as the amount of water percolating into the soil per unit area per unit time. The status of soil infiltration rate is explained in two major approaches: the soil water infiltration rate, which explains the quantity of water present in the soil, and soil water potential associated with the energy level by which the water is held in the soil including matric, osmotic, and gravitational potential (McKenzie et al. 2002). Processes dealing with water balance are more related to water infiltration rate whereas processes related to water movement are related to soil water potential (Warrick and Or 2007).

18.4.2 Effect of Water Infiltration Rate on Microbes

Water is an essential participant in hydrolysis processes, and it is also an important transport medium for the substrate. As a result, the water infiltration rate regulates

the activity of microbes and is a paramount component which regulates the rates of mineralization (Paul et al. 2003).

Low Water Infiltration Rate A decrease in water infiltration rate causes a reduction in the water that reached the living organisms in the soil, thus leading to a reduction in the activity and growth of microbes (Bottner 1985; Kieft 1987), mineralization of C and N (Sleutel et al. 2008), and also shifts the structure of microbial community (Hueso et al. 2012; Sorensen et al. 2013). By maintaining a higher osmotic potential (more negative) in the cytoplasm than that of the environment, cells are able to retain enough water for cell turgor and metabolism (Martin et al. 1999). Soil microorganisms can build up organic and inorganic compounds at low water infiltration rates (high water potential), which raises the osmotic potential within their cells. Therefore, deposition of osmolytes serves as the primary mechanism of tolerance for both high salinity and low water infiltration rate. Additionally, because the pores drain and water films present around the aggregate as soils dry up, the substrate reservoir shrinks dramatically in size, resulting in weaker and unconnected aggregates (Ilstedt et al. 2000).

High Water Infiltration Rate In this case, excess soil water infiltration rate leads to a decrease in oxygen diffusion because oxygen diffusion in water is much lower (about 104 times) than in air, which causes a reduction in the functioning of aerobic microbial communities (Skopp et al. 1990), whereas this environment also leads to the increase in the viability and activities of anaerobic microorganisms. Gram-negative bacteria are less able to withstand high matric potential than fungi, gram-positive bacteria, and archaea because they possess weaker cell walls (Fierer et al. 2003; Martin et al. 1999; Schimel et al. 2007; Vasileiadis et al. 2012). Variations in the pace of water infiltration's impact on soil microorganisms temperature, the length of irrigation periods (for farmlands), and seasonal cycles of rainfall all affect soil moisture and the distribution of water within a soil profile (Manda et al. 2021). In semi-arid and Mediterranean ecosystems, the soil on the topmost layer commonly experiences long dry periods, which are followed by relatively frequent and fast wetting (Fierer and Schimel 2002). Research on the effects of drying and rewetting on soil microbial populations and their functions has been conducted (Griffiths et al. 2003; Herron et al. 2009; Schimel et al. 2007; Xiang et al. 2008). The result was that the concentration of available substrate and microbial activity reach its highest point in the initial 24 h after rewetting (Fierer and Schimel 2003).

Rewetting causes sensitive bacteria to lyse, which causes this to happen (Manda et al. 2020b). Meanwhile, other microbial communities release the organic solutes that these strains have gathered during the dry phase (Halverson et al. 2000). Additionally, the soil aggregates disintegrate, exposing the organic material that was previously shielded and allowing for further decomposition. An increase in the quantity of dry and rewetting cycles leads to a decline in the microbial biomass, activity, and nitrification (Mikha et al. 2005; Wu and Brookes 2005). Because of faster microbial biomass turnover and carbon loss during the flush in respiration

upon rewetting, the microbial biomass declines as the number of drying and rewetting cycles increases (Fierer and Schimel 2003). According to Jin et al. (2013), the interaction of soil moisture and soil type, aggregation, and the concentration of potentially accessible soil organic matter affects how microbial activity responds to drying and rewetting (Anderson and Ingram 1993). Nevertheless, drying and rewetting kill microbiome and change the makeup of the microbial community, which may have an effect on nutrient cycling (Fierer et al. 2003; Schimel et al. 2007). The study found that rewetting and drying resulted in an increase in Gram-positive bacteria and a decrease in fungus (Butterly et al. 2009).

18.4.3 Effect of Bulk Density on Soil Microbes

Bulk density reflects the ability of the soil to function for structural support, nutrient and microbial life movement, and water and soil aeration as a very compact soil has few large pores which are less hospitable to various organisms like springtails, mites, and earthworms. In contrast, lower levels of oxygen present in compact soils may influence the forms of nutrients and their availability; e.g., significant quantities of NO_3 may be lost under anaerobic conditions (Wai et al. 2020).

18.5 Effect of Bulk Density on Bacterial Population

18.5.1 Bacteria

Pupin et al. (2009) concluded that soil microbial biomass is adversely affected by the increase in bulk density. Average bacterial cell density was observed to be 174 cells mm^{-2} and 99 cells per square metre with a bulk density of 1.3 and 1.5 g cm^{-3} in the soil, respectively (Juyal et al. 2021). The propagation of bacteria and their colonization of the pore space at lower bulk density are influenced by soil porosity and solid–pore interfaces, leading to substantially higher bacterial populations in bigger pore spaces. At lower bulk density, soil porosity and solid–pore interfaces affect the spread of bacteria and their colonization of the pore space which leads to relatively higher bacterial densities in larger pore spaces (Juyal et al. 2021). For example, there was a decline in the rate of the spread in *Pseudomonas* with an increased bulk density of soil (Juyal et al. 2021). The compaction caused by an increase in bulk density leads to a reduction in the soil aeration of because of the decline in the air-filled porosity by 13–36% which further results in the reduction of microbial biomass nitrogen and microbial biomass carbon (Tan and Chang 2007). In addition to this, Tan et al. (2008) found the decline of microbial biomass phosphorus with the increase in soil bulk density, thus leading to an increase in compaction. Shestak and Busse (2005) concluded that the soil strength values ranging from 75 to 3800 kPa change the physical properties of the soil but cause no effect on the biological indicator of the soil including microbial biomass and enzymatic activity.

18.5.2 Enzymatic Activity

Any disturbance or stress to the soil can impact the enzymatic activities of the soil (Buck et al. 2000). The increase in bulk density alters the physical as well as chemical attributes of the soil and induces a reduction of phosphatase, amidase, and urease. Anaerobic conditions in the soil lead to changes in the microbial community, thus favouring the microbial populations which are capable of tolerating these conditions. Lower the eukaryotic/prokaryotic ratios, more is the iron and sulphate reducers, and thus, higher methanogens were found in compacted soils in comparison with uncompacted soils (Schnurr-Pütz et al. 2006).

To conclude, a high soil bulk density can adversely affect soil physical properties and can limit microbial activity and biochemical processes which are crucial for nutrient availability.

18.5.3 Soil pH

The pH of any soil determines its acidity or alkalinity as it represents the concentration of hydronium ions [H_3O^+] available in the soil. Sources of H^+ ions in soil include carbonic acid formed when carbon dioxide (CO_2) from root respiration, decomposing organic matter and the soil atmosphere is dissolved in the soil water. Nitrification of ammonium (NH_4^+) from fertilizers and organic matter mineralization, the reaction of aluminium ions (Al^{3+}) with water, rainwater, the reaction of sulphur compounds, and acid rain are the other sources of H^+ ions. Lauber et al. (2009), Andrew et al. (2012), and Zhalnina et al. (2015) observed that pH is one of the biggest influencers affecting the soil microbial community. Furthermore, pH was currently reported to be the best predictor of microbiome diversity at the phylum level (Geyer et al. 2014). Hence, there are numerous studies conducted globally to focus on the effect of pH at different scales. To illustrate, continent-wide research clearly demonstrated an association between soil pH and the presence of certain microbial communities (Fierer and Jackson 2006; Lauber et al. 2009), demonstrating that pH was the key factor accountable for this variation of diversity and richness of the soil bacterial communities (Fierer and Jackson 2006). This is because it greatly controls the abiotic factors, namely carbon availability (Andersson et al. 2000), availability of nutrients (Kemmitt et al. 2006), and metal solubility (Flis et al. 1993). Besides, soil pH may possibly impact biotic factors, for instance, the biomass composition of bacteria and fungi (Fierer et al. 2006), in both forest (Bååth and Anderson 2003) and agricultural soils.

The beneficial microorganisms present in the soil prefer an approximate pH scale of 6–7; hence, alteration in the soil acidity could lead to shifting in the species, quantities, structure, and functions of various microbes living in the soil. The effects on two principal microbial decomposer groups—bacteria and fungi, when there are changes from the neutral pH are as follows:

Decrease in pH Acidic soil indicates high H^+ ion concentration, which impacts the microbial community in numerous processes, namely decline in the reproductive ability, cell membrane distortion, and fluctuation in the release of enzymes. The overall microbial function decreased in the health and productivity of soils due to these reasons (Birgander et al. 2014). Besides, soil fungi prefer a low pH environment to flourish; hence, soil with a low pH has an unbalance between the fungi and bacterial concentrations; the fungi population being the dominant (Rousk et al. 2010). This could further lead to the high probability of fungal pathogen infections due to the favourable environment and decrease the mineralization of nutrients at pace by soil microbes into plant-available forms. The reason for the latter is the imbalance in the microbial composition because various microbes release different nutrients after decomposition, potentially limiting plant mineral uptake. Therefore, this imbalance causes the immobilization of soil carbon and nutrient release (Rousk et al. 2009). Generally, Fungi show a wider range of pH tolerance in comparison with bacteria.

18.5.4 Bacteria

Nodulation of Legumes Leguminous crops fix their own nitrogen through a symbiosis from the air with specialized bacteria (Manda et al. 2020c). Under favourable conditions, nitrogen-fixing bacteria maintain a symbiotic relationship with crops and pasture legumes in root nodules (Weese et al. 2015). Acidic soils limit both root growth and rhizobia survival, which reduces the chances of roots contacting the bacteria to form a nodule which results in inhibition of the performance of nodules (Weese et al. 2015). Essentially, low pH leads to failure in the formation of nodules. In the case of acidic soils, the failure of a functioning symbiotic relationship results in a deficiency of plant nitrogen (Weese et al. 2015). Species of rhizobia bacteria have variable tolerance to soil acidity like medic rhizobia are very sensitive to low pH and may fail to survive in such soils. In acidic soil, grass-dominated pastures can result from the failure of pasture legumes (Weese et al. 2015).

When soil pH is around neutral (6 or 7), roots of the leguminous plants naturally form an alliance with rhizobia bacteria in the soil and fix nitrogen symbiotically, which was earlier in an unavailable form to the plants. Whereas the use of ammonia-based fertilizers decreases the efficiency of this symbiotic relationship effectively and increases the availability of Nitrogen (N) to their host plant (Weese et al. 2015). Even though some rhizobia can survive in an acidic environment, it can drastically diminish the number of nodules, their functions, and the ability of leguminous plants like lentils, chickpeas, and soybeans to fix Nitrogen (Tang and Thomson 1996). This causes a decline in the plant vigour and productivity and consequential yield loss in a region where soil pH plummets. In soils, where the pH remains below 5, nodules per soybean crop can decline by an average of 50%, in comparison with a soil with a pH of more than 6 (Lin et al. 2012). The lower value of pH can inhibit nodulation by

limiting the legume's ability to secrete the signals required into the rhizosphere which attracts the rhizobia and the formation of the root nodules (Hungria and Stacey 1997). Besides, calcium (Ca^{2+}) and molybdenum (Mo) ions become unavailable below pH 5, as both are known to be necessary for root nodule formation and nitrogen fixation thus limiting rhizobia N fixation. Moreover, as pH lowers in soil solution metals like aluminium (Al) and manganese (Mn) become increasingly available, these are toxic to the legume–rhizobia symbiosis (Bordeleau and Prévost 1994).

Fungi There is a higher proportion of fungi in acidic soil communities because numerous strains of soil bacteria are not able to survive in acidic conditions well (Sylvia et al. 2005). Research by Ritz (2011) concludes that hyphal length can be as long as approximately 176 miles per ounce when fungi predominantly account for nearly 75% of the soil microbial biomass in agroecosystems. Many of these soil fungi microbiome function primarily in decomposition processes as well as nutrient cycling, but they may also aid with remediation of metals such as Al in acidic conditions. Fungi assist in ameliorating soil and plant health in the acidic environment by fungal-driven binding of aluminium (Gadd 2007). Plant–symbiotic fungi, known as mycorrhizas, have been found to protect plant root systems against stresses ranging from nutrient depletion to drought and disease, as well as metal toxicity (Seguel et al. 2013). These fungi escalate the access to limiting nutrients, including phosphorus (P), which is predominantly essential in low pH soils because of the reduced P availability in acidic conditions (Seguel et al. 2013).

Increase in pH Bacterial growth is favoured by soil having neutral or high pH, in contrast to acidic soil, which prefers fungal growth (Rousk et al. 2010). To illustrate, in a study it was found that treatment of forest soils with lime and ash resulted in an increase of pH from about pH 4 to 7, which further escalated bacterial growth by about five times, as measured by TdR incorporation (Bååth et al. 1995). Similarly, another research that included more than 15 different soils from regions with different land uses, spanning a pH range from 4 to 8, demonstrated that there was a rise in bacterial growth by four times at higher pH as measured by Leu incorporation (Bååth et al. 1998).

In conclusion, by regulating the chemical forms of the soil components, soil pH is highly correlated with the availability of nutrients for plants (Reddy et al. 2020). This has also been seen as an indirect limiting factor for the populations of soil-borne microorganisms (Zhalnina et al. 2015). While acidic soils typically exhibit lower diversity indices, neutral soils generally have a greater diversity of bacteria or microbes (Fierer and Jackson 2006; Lauber et al. 2009; Rousk et al. 2010).

In light of this, soil pH would only affect a few microbial species' survival and is not a universal determinant for all species. Instead, numerous studies have not discovered a connection between soil pH and the ecosystem's bacterial diversity. For instance, numerous abiotic factors, including soil pH, were examined in a biogeographic study of the nitrogen-fixing rhizobacterium *Sinorhizobium meliloti*

across various regions of Croatia; however, only soil type and other geographical characteristics were recognized as being necessary for defining the genetic diversity of the 128 isolates analysed. Surprisingly, soil pH did not have to be present in order for genetic diversity to exist. The conclusion is that pH is therefore essential in affecting the nutrients.

18.5.5 Electrical Conductivity (EC)

The capacity of water present in the soil to carry electrical current quantifies as the electrical conductivity (EC) of the soil. Electrical conductivity is an electrolytic process which occurs chiefly through water-filled pores in the soil. The major soluble salts are cations: K^+ (potassium), Na^+ , Ca^{2+} (calcium), Mg^{2+} (magnesium), and NH_4^+ (ammonium) as well as anions, namely Cl^- (chloride), NO_3^- (nitrate), HCO_3^- (bicarbonate) and SO_4^{2-} (sulphate), transfer electrical charges and conduct the electrical current because these salts are dissolved in water-filled pores (Shi and Wang 2005). Therefore, the EC of soils is determined by the concentration of ions. EC is expressed in Deci Siemens per metre (dS/m). Electric conductivity is mainly used to measure soil salinity while in non-saline soils it can be used to evaluate soil attributes such as soil moisture and soil depth. The soil of an area is considered to be saline when the electrical conductivity (EC) is 15 dS/m, according to World Soil Resources Reports (2007) in contrast to Soil Taxonomy (2010) which considers the reference value at 30 dS/m. Soil that has excessive salts is known as salt-affected soil. The USDA system classifies the soils into three distinct categories as saline, sodic, and saline-sodic soils.

The size and the activity of soil microbes in biomass are impacted by the salinity of the soil (Rietz and Haynes 2003), which in turn plays an important role in biogeochemical cycles. Most of the microbes are sensitive to high electrical conductivity. Bacteria, except for halophytes (salt-tolerant bacteria), are more susceptible in comparison with Actinomycetes and fungi. Microbial processes, such as respiration and nitrification, decline as the salinity increases.

There are two main mechanisms namely the osmotic effect and specific ion effects that occur in the soils having a high concentration of soluble salts, affecting soil microorganisms.

Osmotic Effect The soluble salts (cations and anions) increase the negative osmotic potential of the soil water, thus leading to plasmolysis by desiccating the cells of water which may kill microbes and plant roots. Salinity reduces microbial biomass (Rietz and Haynes 2003) predominantly because the osmotic stress results in drying and cell lysis (Yuan et al. 2007). Low osmotic potential makes it challenging for roots and microbes to draw water from the soil, on the other hand (Oren 1999). The synthesis of osmolytes requires a lot of energy, which inhibits the growth and functioning of flora and microfauna. Plants and microorganisms can adapt to low osmotic potential by storing osmolytes (Wichern et al. 2006).

Specific ion effects: A high concentrations of certain ions, namely sodium, chlorine, and bicarbonate, lead to a toxic environment for numerous plants. There are specific ions that will affect a certain species of organisms. The sensitivity of soil enzyme activities to salinity (EC) may alter the activities of urease, β -glucosidase, and alkaline phosphatase (Pan et al. 2013). However, catalase and dehydrogenase were less affected (Garcia and Hernandez 1996).

Research by Andronov et al. (2012) showed that salinity decreased microbial biomass, and microbial activity and changes microbial community structure (Setia et al. 2011). Besides, an increase in soil EC declines soil respiration rate (Adviento-Borbe et al. 2006; Wong et al. 2009). To illustrate, it was reported that soil respiration may be reduced by more than 50% at EC (1 ratio 5) more or nearly equal to 5.0 dS 1/m (Setia et al. 2010). However, in 2003, Rietz and Haynes found out that soil respiration was not remarkably correlated with EC. As EC increased, the metabolic quotient which is respiration per unit biomass found to be increased. There are microbes that occur in naturally saline (with higher EC) habitats that are supposedly meant to share a strategy for resisting high salt concentrations (Sagot et al. 2010). They have developed a number of adaptations for maintaining their population active while surviving in extreme environmental conditions. According to the aforementioned information, microbes have the ability to adapt or tolerate salinity stress (Sagot et al. 2010), by accumulating osmolytes (Zahran et al. 1992). Proline and glycine betaine is the prime organic osmolytes while potassium cations are the most common inorganic solutes which are used as osmolytes accumulated by salinity tolerant microbes (Oren et al. 2001), whereas the synthesis of the organic osmolytes requires high amounts of energy, thus causing a decline in the activities of microbes and plants (Killham 1994). Accumulation of osmolytes as inorganic salts can be very toxic; therefore, it is confined to halophytic microbes evolving salt-tolerant enzymes to survive in highly saline environments (Gros et al. 2003). From the genetic point of view, the salt-tolerant species which display an under, overexpression of peculiar genes and metabolites allow them to cope with osmotic stress (Dion and Nautiyal 2008). It is important to understand the elusive dynamics of microbial communities in saline soil as it would shed light on the depth of selection mechanisms operative in the environment (Parkin 1993). The bacterial communities opting to wait for favourable conditions rather than developing metabolic adaptations for survival in a niche of higher salinity and instability represent the structure of microbial heterogeneity and taxa spatial composition in these soils (Pereira e Silva et al. 2012).

The bacteria/fungi ratio seems to be higher in saline soils as fungi tend to be more salt-sensitive than bacteria (Sardinha et al. 2003; Wichern et al. 2006). Therefore, the community structures in saline soils are impacted due to the difference in tolerance of salinity among microbes (Gros et al. 2003) in comparison with proportionate populations in non-saline soils (Pankhurst et al. 2001). According to the recent meta-analysis conducted on microbes, salinity has a major role in impacting the global composition of microbes in saline soils than any other chemical attributes like pH or temperature (Ma et al. 2013).

In conclusion, the understanding of the relationship of microbial communities in soils with varying salt concentrations will help to harness the biotechnological potential of these microbes which could be used in the conservation or restoration of the saline environments apart from being a genetic reserve for further applications. This would also help in exploring the diversity and mechanisms operative at the level of soil in limiting conditions (Nacke et al. 2011; Roesch et al. 2007)

18.5.6 Effect of Ion-Exchange Capacity on Soil Microbes

Ion-exchange capacity is an inherent soil chemical property that estimates the total capacity of a soil to clutch exchangeable cations. It impacts the soil's ability to grip important nutrients and acts as a buffer against the acidification of soil. The ion-exchange capacity is directly related to the amount of organic matter present in the soil. Besides, the changes in pH and salt concentrations affect the ion-exchange capacity, which is specially referred to as cation-exchange capacity.

Virus There is a diversity of viruses in the soil. According to a research (Lipson and Stotzky 1983), the addition of cations (as chloride salts) to distilled water enhanced the adsorption of reoviruses, with divalent cations being more effective than mono-valent cations and 10^{-2} M resulting in more adsorption than 10^{-3} M. Potassium ions suppressed the adsorption of such viruses to montmorillonite, probably by collapsing the clay lattices and preventing the expression of the interlayer-derived cation-exchange capacity. Higher quantities of the virus were adsorbed by montmorillonite, which converted homo-ionic to various mono-, di-, and trivalent cations than by comparable concentrations of kaolinite clay which are homo-ionic to the same cations. The sequence of adsorption amount to homo-ionic montmorillonite was $\text{Al} > \text{Ca} > \text{Mg} > \text{Na} > \text{K}$ while the sequence of adsorption to kaolinite clay was $\text{Na} > \text{Al} > \text{Ca} > \text{Mg} > \text{K}$.

Fungi Das et al. (1991) stated that actinomycetes and fungi in soil showed a positive correlation with available K^+ , exchangeable Ca^{2+} , Mg^{2+} and the cation-exchange capacity of the soil.

Bacteria Ciccolini et al. (2016) explored the composition of microbial communities involved in nitrogen cycling in Mediterranean peaty soils drained for farming activity and found that ammonia-oxidizing communities like AOA (ammonia-oxidizing archaea) were shaped by clay content, AOB (ammonia-oxidizing bacteria) were shaped by bulk density, and both AOA and AOB were controlled by exchangeable calcium content.

18.6 Aggregate Stability and Soil Slaking

Soil structure is an essential soil property as it controls numerous biological and physical soil processes. Soil structural properties in addition to soil aggregation are affected by changes in agricultural management practices (Six et al. 2006; Tiemann et al. 2015). Numerous techniques have been recommended to fragment soil into various aggregates with each fragment having its own inherent positive and negative attributes (Schutter and Dick 2002; Lützow et al. 2006; Dorodnikov et al. 2009). It was found that microbial diversity and community structure are more affected by particle size than other factors like pH or organic nutrient content (De Fede et al. 2001). Aggregate stability is a measure of the soil aggregates' vulnerability to outer destructive forces. The plenty of microbial groups and their functional diversities in soils are strongly influenced the type and amount of available organic substrates (Grayston et al. 2001). Besides, recent progress in isotopic, spectroscopic, and ecogenomic (DNA/RNA) techniques assists in measuring the changes and distribution in specific microbial genera (including active and total as well as functional and taxonomic groups) in different parts of aggregates and their pore spaces. These techniques also help in determining the role of these microbes in soil functional processes with reference to organic matter composition (Davinic et al. 2012). Slaking is the fragmentation process that occurs when soil aggregates are suddenly immersed in water (Chan and Mullins 1994) due to their resistance to withstand the stresses of rapid water uptake. At fast rates of wetting, internal stresses developed from differential swelling and the air entrapment in the soil aggregate. Soil slaking is affected by the rate of wetting, texture, water content, clay mineralogy, and organic matter content.

Soil structure regulates soil physical and chemical heterogeneity, therefore playing an important role in the distribution of microbial communities, and their activities among different aggregates (Vos et al. 2013). Aggregates of different sizes and their stability in the soil produce niches with different physicochemical and structural characteristics. These niches foster the colonization and maintenance of various microbial assemblages in each aggregate (Davinic et al. 2012; Vos et al. 2013; Tiemann et al. 2015).

According to research by Ling et al. (2014) and soil aggregates have a significant impact on the composition and structure of the microbial community (2014). It was shown that when aggregate size reduced, the enzymatic processes associated to carbon breakdown increased (Qin et al. 2010; Lagomarsino et al. 2011; Ling et al. 2014; Nie et al. 2014). Previous research that found that soil aggregate size mostly influences soil microbial activity and carbon dynamics supports this finding (Elliott 1986; Schutter and Dick 2002).

According to a study by Trivedi et al. (2015), farm management only altered the enzymatic activities of soil fractions in macroaggregate and cultivation methods that led to an increase in soil fertility. Enzymatic activity was greatly increased by carbon. The distribution of bacteria in different aggregates and subsequent effects on microbiological activities and diversity at small scales can be influenced by soil structure (Six et al. 2006; McCarthy-Neumann and Ibáñez 2013; Vos et al. 2013;

Ling et al. 2014). According to Nannipieri et al. (2012), variations in microbial populations' enzymatic activity can also depend on the type of plant inputs, including humic substances in the soil. Microbial mucilage and polysaccharides released by some species of bacteria (e.g., Acidobacteria and Actinobacteria) and several fungi play a major function in the stabilization of various aggregates (Tripathi et al. 2015).

18.6.1 Bacteria

Ranjard et al. (2000) and Sessitsch et al. (2001) and illustrated differences in bacterial community structure and composition by utilizing microbial community profiling methods in various aggregate size classes. Normally bacterial proportion within soil changes with aggregate size. Though a larger population of bacteria is connected with microaggregates, a smaller population is with macroaggregates (Monreal and Kodama 1997; Neumann et al. 2013). Interaction between the organic matter, microbe, and clay particles is necessary for the survival of bacteria as they provide nutrients and habitat to bacteria (Van Gestel et al. 1996; Sessitsch et al. 2001). For example, silt clay fractions demonstrate higher bacterial populations as compared to other aggregates (Trivedi et al. 2015). Besides, crop management practices influenced carbon content and microbial biodiversity more prominently in the larger-sized aggregate fractions than in fine clay silt fractions (McCarthy-Neumann and Ibáñez 2013). Poll et al. (2003) observed that differences in the bacterial community abundance were very less for the similar particle size fractions as compared to coarse sand fractions. The reason could be the lower C content in macroaggregates as compared with microaggregates because there is an increase in enzymatic activities related to carbon decomposition for smaller aggregate sizes (Nie et al. 2014).

Additionally, cloning-sequencing analysis procedures used by Momma et al. (2006) concluded that the colonies of Alpha-proteobacteria, Actinobacteria with subdivision rubro-bacteriaceae, and Gemmatimonadetes within micro-aggregates had a huge population; however, the population of acido-bacteria was comparatively more profuse in macroaggregate fractions. In addition, there are differences in bacterial diversity and population within micro-aggregates. Similarly, according to the research by Remenant et al. (2009) closely related bacterial genotypes/communities survived in rhizosphere aggregates in contrast to the non-rhizosphere aggregates. This also illustrates that plant roots may allow certain strains of soil bacteria to survive and grow in the soil matrix. Besides, the roots of distinct plant species release different exudates, and the composition of microbial communities surrounding those roots will be different. Moreover, the spatial heterogeneity, as well as complex soil structure, produces different habitats for bacterial diversity, thus sustaining various different microorganisms (Vos et al. 2013).

18.6.2 Fungi

In 1982, Tisdall and Oades generalized that plant roots and soil fungi bound the smaller aggregates into stable macroaggregates. The authors claimed that both entanglement and adhesion processes accumulate small roots and mycorrhizal hyphae accumulate small aggregates and soil particles. Hattori et al. (1976) worked on the distribution of bacteria and fungi in various aggregate size fractions and at different locations of an organism and its metabolic functions. In addition to this, culture-based research by McCalla et al. (1957) found that soil aggregates get stabilized by fungi.

Recently, in 2011, Ruamps et al. claimed that fungal use of carbon substrates present in small pores of soil has more fungal use as fungi can spread easily as compared to bacteria through the growth of hyphal and mycelial.

Effect of Management Practices Soil carbon and microbial communities are influenced by the various crop management practices because they are affected by aggregate size distribution. These impacts are more prominent in macroaggregate as compared to micro-aggregate sizes.

Ghimire et al. (2014) observed a mechanism by which crop management practices could affect the soil microbial community is the influx of easily changeable carbon. According to Tripathi et al. (2015), the quantity of labile carbon declines with the increase in aggregate size, thus causing an impact on the microbial community of soil. Carbonetto et al. (2014) and Ghimire et al. (2014) observed the microbial communities of copio-trophic utilize the higher amounts of labile Carbon, thus proliferating within such management practices thus further leading to the increase in the availability of easily degradable carbon in the soil system.

Microaggregates are distinguished by an increase in the amount of recalcitrant carbon and these environments effectively decline the microbial responsiveness to crop management practices (Lal and Kimble 1997). According to Pankaj et al. (2015), the research indicates that smaller aggregates are less affected by microbial responsiveness to crop management practices declined.

Cultivation disrupts aggregates, thus changing the proportion of macroaggregate and macroaggregates. Furthermore, an increase in the microaggregates leads to significant reductions in various chemical and biological properties like organic carbon, fungal biomass, respiratory activity, and enzyme activities like arylsulfatase and acid phosphatase in comparison with macroaggregates, whereas in macroaggregates aggregates became destabilized due to disrupted fungal hyphae were mineralized and their binding properties destroyed.

Soil architecture (aggregate hierarchy and slaking) imparts various habitats like aerobic and anaerobic microsites that are essential to support the survival and activities of a diverse microbiome. It is evident that soil cultivation affects soil microbial habitats due to the changes in particle size distribution and structure of pores. A major result of this disturbance in the soil initially with protected and

undisrupted organic matter becomes available for microbial metabolism and impacts populations and functions of microbial communities (Six et al. 2006).

Studies, activities, and research of microbial communities in different microenvironments including aggregate size are limited as this study has essential implications for increasing crop production and agricultural sustainability (Grundmann 2004). Besides, the researchers have limited understanding of the importance of soil aggregates in structuring microbial communities and not much is documented about the localization of microbial communities and their functions. The scientists need to study in-depth soil aggregate structure and the location of various microbial communities which have impacts on microbial community resilience to environmental stress.

18.7 Molecular Techniques to Measure Soil Health: Microbial Biomass

18.7.1 Fluorescence Microscopy

Estimating the populations of soil bacteria, as well as their biomass, cell volumes, and cell division frequencies can be done with the help of fluorescence microscopy and computerized image processing (Bloem et al. 1995). Some photoreactive molecules have a property known as fluorescence. This property is characterized by the absorption of energy at a specific wavelength (λ), which causes the electrons of the fluorescent molecule to move into an excited state. After a certain amount of time (also referred to as the fluorescence lifetime), a portion of the energy that was absorbed, is then emitted, which causes the electrons to return to their stable state (Herman 1998). Only at particular wavelengths, which are exclusive to a given molecule, can the fluorescence molecule's energy absorption and emission take place. Fluorescence microscopes are constructed with this very principle of the emitted wavelength fluorescence given off by the emitted fluorophores in mind.

For the purpose of researching and quantifying the microbial biomass in soil, intact soil samples are required. The use of procedures that involve resin embedding will preserve both the structure of the soil and the spatial link between the soil microorganisms and the matrix of the soil. Fixation, staining and de-staining, dehydration, resin embedding, and thin sectioning of the soil sample are the steps outlined in the technique for preparing a thin soil section (Altemüller and Van Vliet-Lanoe 1990). When imaging bacteria in thin sections, selecting an appropriate fluorochrome is the step that carries the biggest importance. Staining with fluorescein isothiocyanate can be utilized as a method for estimating the biomass of soil microbes. However, when using a fluorescence microscope, it might be challenging to differentiate between different types of bacterial cells in soil matrices because of autofluorescence and background staining. There are two different categories that emerge when fluorophores are categorized according to the functional features that they share.

The first group is responsible for staining individual components of the cell, such as nucleic acids, proteins, or lipids, whereas the second group is responsible for staining the entire cell. The second category of fluorochromes is vulnerable to fluorescent cell processes rather than fluorescing on their own (Tsuji et al. 1995; Riis et al. 1998). These fluorochromes do not fluoresce on their own. Studies on the spread of microorganisms do not usually focus on the activity of bacterial cells because it is not always a problem. There are many different fluorochromes that are based on different binding targets, such as acridine orang (DeLeo et al. 1997), ethidium bromide (Roser 1980), or DAPI for nucleic acid, FITC (Decho and Kawaguchi 1999), Mg-ANS (Mayfield 1975) for protein, and cellufluor (Hartmann et al. 1997) for polysaccharide. According to the findings of one study, the pathogenicity of resting spores of club root (*Plasmodiophora brassicae*) in soil was directly examined using a technique called fluorescence microscopy (Takahashi and Yamaguchi 1989). In addition, fluorescence microscopy is utilized in order to see nematophagous fungi in their natural habitats in soils (Saxena and Lysek 1993). Examination of the soil with a microscope has long been an essential part of the study of soil microbiology. The use of fluorescence microscopy allows for an ecological study of the many different kinds of microorganisms as well as the direct measurement of their population size. This direct insight into natural settings is made possible by the use of fluorescence microscopy.

18.7.2 DNA Measurement

It is essential to estimate the biomass of microorganisms in order to acquire an in-depth understanding of the roles that microbes play in the environments in which they are naturally found. Soil bacteria are crucially important to a number of processes, including the decomposition of organic matter, the mineralization of soil, and the creation of humus (Miltner et al. 2012; Semenov et al. 2018; Torsvik and Øvreås 2002; Van Den Hoogen et al. 2019; Veresoglou et al. 2015). Microorganisms in the soil are responsible for a number of important functions, including regulating the decomposition of organic matter and the cycling of nutrients. Consequently, this demonstrates the need for land management and soil fertility (Powlson et al. 1987). Agricultural soil management practices are responsible for a decrease in the fungal biomass, and the ratio of fungal biomass to bacterial biomass in agricultural soils is typically significantly lower than in natural soils (Bailey et al. 2002). Comparatively speaking, bacteria have a higher DNA concentration per unit of biomass compared to fungi. Because of this, ecophysiological indices such as qCO₂ (microbial community respiration per biomass unit) and the C_{mic}:C_{org} ratio (microbial biomass C to soil organic C) are utilized frequently. Both the chloroform fumigation-extraction (CFE) and the substrate-induced respiration (SIR) methods are dependent on chloroform, however, and both of these methods have limitations when applied to soil in more severe conditions (Semenov et al. 2018).

The DNA content of soil microbes has become an increasingly significant metric for measuring the biomass of soil microbes (Semenov et al. 2018; Yokoyama et al. 2017), and the same can be said for the RNA content of soil microbes. The DNA ratio can be used as a measurement tool to determine the amount of bacteria in the soil (Loeppmann et al. 2016). In addition, a correlation between soil DNA concentration with Cmic (Fornasier et al. 2014; Gangneux et al. 2011; Marstorp and Witter 1999; Semenov et al. 2018) and Nmic (Yokoyama et al. 2017; Bouzaiane et al. 2007) has been reported in a number of research studies. This correlation has been found to be significant. However, there are studies that contradict the correlation between soil DNA concentration and Cmic (Griffiths et al. 1997; Leckie et al. 2004). These studies were conducted when the soil was contaminated with heavy metals over a prolonged period of time and in forest humus, which included pH and conductivity. As of today, DNA-based measurement of soil microbial biomass has been demonstrated to be constant even in extremely harsh soil conditions. This allows for the estimation of soil microbial biomass (Semenov et al. 2018).

The evaluation of microbial biomass in the quantification of microbial double-stranded DNA (dsDNA) is based on the amount of universal cell compound that is present in the sample. dsDNA quantitative analysis makes use of a fluorescent dye with a high level of sensitivity, such as PicoGreen® (Fornasier et al. 2014; Terrat et al. 2012). A wide variety of independent research proposed a conversion factor (FDNA) of $\mu\text{g dsDNA (g soil)}^{-1}$ to $\mu\text{g SIR-Cmic (g soil)}^{-1}$. This factor ranged in a remarkably small range, ranging from 5.0 (Anderson and Martens 2013) to 5.4 (Blagodatskaya et al. 2003) to 5.6 (Anderson and Martens 2013; Lloyd-Jones and Hunter 2001). It is possible to use DNA content to assess microbial growth dynamics after substrate addition to soil (Nannipieri et al. 2003; Anderson and Martens 2013), and in ecophysiological indexes, metabolic quotient, and activity parameters (Blagodatskaya et al. 2003, 2014), which are essential for accessing nutrient cycling and organic carbon decomposition in arid or semi-arid environments. This is an additional advantage of using DNA content (Vishnevetsky and Steinberger 1997). A fluctuation in DNA concentration is produced as a result of the non-uniform extraction procedures, which is one of the major constraints (Gong et al. 2021). The efficiency of DNA extraction may change depending on the conditions of the setting in which it is carried out (Torsvik et al. 1996). There is also the possibility that plant residues and recalcitrant extracellular DNA could change the DNA concentration, which would be an additional possible limitation. The DNA of dead plants can be discovered in the soil for a number of months after they have decomposed (Yokoyama et al. 2017) despite the fact that the dsDNA level of the plant never went above 2.6% of the total dsDNA content for a variety of soils (Gangneux et al. 2011).

18.7.3 Fluorescence In Situ Hybridization

The method of fluorescence in situ hybridization, or FISH, involves staining and counting the microorganisms that are found in the soil using oligonucleotide probes

that have been fluorescently labelled. Using this method, it is possible to do in-depth research on the microbial communities that are present in environmental samples (Amann et al. 1995; Daims et al. 2004; Stein et al. 2005; Eickhorst and Tippkötter 2008a, b). Visually detecting soilborne pathogens using FISH is another successful method that does not require the extraction of DNA from the sample (Milner et al. 2019). The FISH method relies on the detection of rRNA as its foundation; nevertheless, the number of phylogenetically diverse target organisms that can be recognized in a single experiment places limitations on the method's detection capabilities. FISH results are affected by the metabolic state of the organism or cell as well as the activity level of the organism or cell (Poulsen et al. 1993; Kemp et al. 1993; DeLong et al. 1989; Hahn et al. 1992; Moter and Göbel 2000; Amann et al. 1997; Pernthaler et al. 2001; Amann 1995; Christensen et al. 1999). It is feasible, through the use of FISH, to link geographical information with the metabolic capacities of microorganisms that have not been grown in the lab (Berry et al. 2013). When performing an analysis of FISH signals, it is generally agreed upon that the ability to detect active cells has a direct connection to the metabolic rates of the bacteria being studied (Bouvier and Del Giorgio 2003). A few thousand rRNA molecules must be present in order for a discernible FISH signal to be generated using monolabelled fluorescent oligonucleotide probes. This signal can be observed using fluorescence microscopy (Amann et al. 1995). The conventional technique is only applicable to bacteria that have a significant number of ribosomes. Because the FISH technique is incapable of detecting microorganisms that have a low ribosome concentration or that are dormant (Wagner et al. 2003; Daims et al. 2004).

Despite the fact that fluorescent particles in the soil were the most important factor in determining whether or not FISH-stained cells were found (Hahn et al. 1993; Zarda et al. 1997), this impact could be prevented by employing laser scanning microscopy in conjunction with Nycodenz in order to eliminate bacteria prior to FISH (Bertaux et al. 2007). Using horseradish peroxidase (HRP)-labelled oligonucleotide probes during tyramide signal amplification (TSA) is a promising prospective technique to solving this problem. There are many more potential approaches as well. This improved approach is known as the CARD-FISH method (catalysed reporter deposition). According to Eickhorst and Tippkötter (2008a, b), the high signal intensities of the tyramides utilized in the CARD-FISH process and the utilization of fluorescein-dyes in a double filter excitation allowed stained cells to be easily differentiated from the background autofluorescence. It is ideally suited for identifying bacteria that have a low rRNA content, which is a result of low physiological activity (Ferrari et al. 2006). There is also a method known as multicolour DOPE-FISH, which makes use of oligonucleotide probes that are double-labelled with various fluorophores at their 5'- and 3'-ends in order to induce fluorescence signal intensities (Stoecker et al. 2010; Behnam et al. 2012).

CLASI-FISH is another method that uses dye combinatorial labelling and spectrum imaging to concurrently detect several different species for the purpose of phylogenetic research (Valm et al. 2012; Mark-Welsh et al. 2016). Each microbial taxon is tagged with a specific combination of two or more individually monolabelled probes. This allows the taxa to be differentiated from one another

based on the spectrum features of the combined fluorophores. Since it was developed, it has been used to successfully differentiate between as many as 15 separate target organisms (Valm et al. 2011; Mark-Welsh et al. 2016).

In a nutshell, fluorescence in situ hybridization (FISH) is a useful instrument for microbiologists working in all fields since it allows them to visualize, identify, count, and precisely locate individual microorganisms.

18.7.4 RNA Measurement

The molecular method known as 16S rRNA analysis makes it possible to do more in-depth research on the microorganisms that are found in the soil. The study of 16S rRNA genes has brought about a significant change in the field of bacterial systematics. This has resulted in greater comprehension of the microbial variety found in the natural world (Duineveld et al. 2001). Methods of biomass estimation that are generally acknowledged as competent are sufficient for high-biomass systems that have been thoroughly researched; nevertheless, these methods are typically insufficiently sensitive for systems with extremely low amounts of biomass. Even in low-biomass settings, one may produce physiologically relevant results by quantifying the biomass of prokaryotes by using the 16S rRNA gene and the biomass of fungi by utilizing the 28S rRNA gene (Knox et al. 2017; Mueller et al. 2016). In addition to this, a recent study sequenced the bacterial 16S rRNA gene as well as the fungal 28S rRNA gene in order to conduct an analysis of the taxonomic profile. In order to conduct an analysis of extremely low levels of microRNA (mRNA), the reverse transcriptase polymerase chain reaction (RT-PCR) technique, followed by the polymerase chain reaction technique, is required (PCR). Real-time RT-PCR with SYBR Green I detection as the method of choice for the detection step allows for the generation of data that are both prompt and accurate (Pfaffl and Hageleit 2001). The choice of RT-PCR quantification method is determined by a number of parameters, some of which are the target sequence, the predicted range of mRNA concentrations, the desired level of precision, and the question of whether or not absolute or relative quantification is required (Freeman et al. 1999).

It is now possible to analyse the transcriptional activity of various soil microbial communities in real time by employing a technique known as metatranscriptomics. This technique makes use of whole RNA sequencing. In addition, total RNA sequencing has been shown to be effective in determining the functional roles of active microbial communities in soil (Urich et al. 2008; Hultman et al. 2015; Epelde et al. 2015; Geisen et al. 2015; Schostag et al. 2019) because of its capacity to explore regulatory responses to changes in the surrounding environment (Carvalhais et al. 2012). RNA viruses, unlike bacteria and fungi, have the capability to affect the carbon cycle in soil. This ability distinguishes them from bacteria and fungi. Phylogenetic analysis of soil metatranscriptomes from a variety of soil environments and time points reveals that fungi are the most prevalent hosts for RNA viruses in the grassland soil that was investigated (Starr et al. 2019). We have a very limited understanding of the magnitude of the effects that viruses have on the carbon cycle in

the soil at this time. Fungal viruses in the soil can have small but important effects on a variety of physiological processes, including toxin generation, reproduction, mating success, symbiosis, and other physiological consequences (Márquez et al. 2007; Zhang et al. 1998; Rodríguez-Cousiño et al. 2011). Through RNA-viromics, researchers have found that soil RNA viruses have the capacity to influence grass-land ecosystems at multiple trophic levels (Hillary et al. 2022). Studying the activity of soil microbes using a methodology that combines RNA biology with metagenomic and metatranscriptomic techniques has, on the whole, been shown to be a fruitful line of inquiry.

18.7.5 Stable Isotope Probing

The SIP method is a molecular strategy that involves treating bacteria with substrates that have been labelled with the heavier and more stable isotopes of common elements. Additionally, it is a way for understanding the variety of microbial communities in the soil and the role that they play in the soil (Dumont and Murrell 2005; Neufeld et al. 2007). It is possible to identify the individual molecular components that make up a complete cell, such as its DNA or RNA or its proteins or lipids, in order to specifically target one or several active bacteria. The nucleic acid isotopic marker that is most widely employed is ^{13}C . It is possible to separate ^{13}C -labelled molecules from unlabelled nucleic acid through the utilization of density gradient centrifugation. Because the rate of RNA synthesis is far higher than the rate of DNA synthesis, RNA is considered to be a superior biomarker for use in SIP investigations when compared to DNA. As a consequence of this, they are determined not by DNA replication but rather by copy number, which is a measurement of cell activity rather than a measurement of replication itself (Manefield et al. 2002). SIP of mRNA is more sensitive than that of DNA because the label can be rapidly incorporated into mRNA and does not depend on cellular replication (Franco Dias et al. 2013; Jakobs-Schönwandt et al. 2010). It may now be possible to include microorganisms in a sample that do not reproduce (Pratscher et al. 2011). In certain low-growth settings, such as marine silt and seawater, certain types of algae can be found. Because they do not replicate their DNA in significant amounts, the cells of microorganisms may actively turn over RNA (Frias-Lopez et al. 2009; Glaubitz et al. 2009; Vandieken et al. 2012). By utilizing mRNA-SIP, it is possible to identify the organisms and genes that are involved in the assimilation of a substrate. Although DNA-SIP combined with ^{18}O -water has been used to investigate the development and death of microorganisms in soils from a wide variety of ecosystems, including terrestrial, marine, and aquatic systems, the results have been inconclusive. Not to mention the fact that ^{18}O -water is also utilized in RNA-SIP research (Schwartz et al. 2016). In addition, phospholipid fatty acid (PLFA) SIP is utilized for the particular microbial activity, metabolic function, lipid biosynthesis, and carbon flow target (Hanson et al. 1999; Beulig et al. 2015).

Together, SIP and high-throughput sequencing are becoming an increasingly popular combination. The goal of this sequencing effort might be to create a big

amplicon database, or it might even be to sequence a full metagenome or metatranscriptome from soil (Dumont et al. 2006, 2013). mRNA molecules that have been tagged with stable isotopes can offer a powerful picture of the metabolic activity of the enzymes that are linked with them at any particular point in time. In addition to this, it can be utilized to acquire a specific metatranscriptome from a group of functional microorganisms (Dumont et al. 2013). In the subsequent stage, we are going to employ metaproteomics in concert with SIP labelling to determine which members of the community are involved in metabolically active processes. In addition, the accurate quantification of incorporation in protein-SIP enables us to recognize food webs within microbial communities throughout time-course investigations (Jehmlich et al. 2016). Interactions between rhizospheres and microorganisms are another aspect of microbial ecology that could benefit from the knowledge provided by SIP. It has been demonstrated that root exudation has a substantial impact on the microbial population dynamics surrounding plant roots (Das et al. 2021). This may be seen, for instance, in the transfer and cycling of carbon in the soil (Singh et al. 2004; Griffiths et al. 2004a, b). The future seems bright for SIP, particularly when combined with other cutting-edge technologies that are targeted at enhancing our understanding of microbiology and biogeochemistry.

18.8 Molecular Techniques to Measure Soil Health: Genetic and Functional Biodiversity

Soil health can be easily characterized by the genetic and functional biodiversity of various invertebrates and microorganisms within the soil (Rutgers et al. 2016). The functional biodiversity of a soil microbial community is indicative of the extent to which it occupies a given niche space (Yin et al. 2020). However, the cumulative estimate of the inherent quantity of genes in a soil microbe and its corresponding physiological expression within a biological community represents its genetic diversity within that unique soil ecosystem (Carolina 2018).

A comprehensive investigation and evaluation of the impact of both functional and genetic diversity on the health status of the “living” soil are indisputable. Molecular-based tools have found their application in analysing how genetically and/or functionally diverse a sampled soil microbiome is and this does not seem to be novel. These tools have been used to monitor soil microbial diversity through environmental-controlled experiments that study species composition under stress conditions (Leflaive et al. 2008).

Various tools have been considered and will be extensively explained in this review. While certain tools such as Biolog EcoPlates™ probe into how functionally diverse a soil microbial community might be, others rely on the polymerase chain reaction (PCR) dependent to properly investigate the relative gene composition of specific microbial species within the soil such as terminal fragment length polymorphism, denaturing gradient gel electrophoresis, and temperature gradient gel electrophoresis (Shawy and Burns 2005). By encoding for 16S rRNA in prokaryotes (18S rRNA for eukaryotes), these tools serve as gene fingerprints (Arias et al. 2005).

18.9 Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a molecular technique to work by the principle of DNA fragment separation from PCR products based on similar base-pair sizes but differing sequence patterns. To achieve such segmentation of DNA fragments, the polyacrylamide gel used is characterized by the presence of an increasing gradient of chemical denaturants. Urea and formamide are two denaturants that have been long considered and utilized (Strathdee and Free 2013; Zulfarina et al. 2018). This method dates back to the 1980s when it was primarily exploited in detecting mutations at specific points (e.g., single-nucleotide polymorphisms; SNPs) of the genes that were linked to very striking disease conditions. However, the first recorded application of the DGGE as an analytical tool to probe into microbial communities goes way back to the early 1990s (Valášková and Baldrian 2009). This was a remarkable milestone and consequently prompted its frequent application for explorative research into soil microbial community. However, it is quite important to note that the DDGE seems to face some limitations given how complicated a soil microbial community can be. Hence, researchers have had to intentionally select PCR primers only specific to the target microbe population. In many cases, the actinomycetes and NH_3 -oxidizing beta proteobacteria have been easily studied in this regard (Nakatsu 2007). Despite its known constraints, the DGGE technique is credited with the ability to efficiently generate individual species profiles for a studied soil microbe (e.g., bacteria). This is observable as separate bands each representative of the individual species and confirmation of the species after each band have been excised, sequenced, and compared to existing databases (Arias et al. 2005).

Summarily, the application of DGGE for soil microbial community analysis typically involves five main steps. First, as expected, the soil samples have to be collected from specific study locations followed by the DNA or RNA extraction procedure. Second, following the PCR protocol, the target gene of the microbe is amplified leading to the generation of a composite mix of gene fragments. Third, the PCR products are subject to separation via gel electrophoresis where, specifically unique to the DDGE, a denaturing gradient is utilized. After separation, the gel profile is visualized, as the fourth step using precise equipment. Fifth, further analysis is carried out for proper interpretation of the derived data (Shawy and Burns 2005).

In the past years until recently, the DGGE has found its prioritized application in different research studies that focused on undermining how diverse microbial communities of the soil can be. Here are a few, among many, of such scientific studies. In Indonesia, research desired to know how diverse the community of nitrifying bacteria could be within the tropical rain forests of the Bukit DuaBelas National Park and oil palm plantations of Sumatera and the tool employed was the DGGE (Zulfarina et al. 2018). Another research took a completely different investigatory path and attempted to understand how bacteria communities lived and interacted in *Pseudomonas putida*/Cephalosporin antibiotics-treated soils. Again, at the discretion of the scientists, the DDGE was considered the best tool for this

study (Orlewska et al. 2018). Similarly, Gelsomino and Cacco (2006) evaluated the changes in soil bacterial community composition after solarization practice and incorporation of biodegradable amendments on already cultivated fields of the Mediterranean University of Reggio Calabria in Southern Italy. Diagnosis of the presence of certain microbes within soil biomes has not been left out as seen in research by Jousset et al. (2010) where the DGGE was used to identify ciliate populations inhabiting soils polluted with polycyclic aromatic hydrocarbons. Likewise, a comparative study on the utilization of different 18S rDNA primers in DGGE explored the broad communities of fungi within the cultivated soils in farming regions of Japan (Hoshino and Morimoto 2008). Using the PCR-dependent DDGE protocol, microbial communities of different heavily-polluted locations have been characterized and tracked for sudden or expected changes in community makeup and interaction (Chen et al. 2016; Li et al. 2006; Wakase et al. 2008). These and many more highlight the unique role of DGGE in microbial studies of the soil.

18.10 Temperature Gradient Gel Electrophoresis

Another interesting technique for molecular analysis of soil microbial communities is the temperature gradient gel electrophoresis (TGGE). Similar to the entire procedure of the DGGE, its unique feature is the application of a temperature gradient for the denaturation phase of the gel electrophoresis process. The soil sample with the target DNA or rRNA is subjected to a PCR process for amplification after which it is passed through the usual polyacrylamide gel for separation into defined bands (Nocker et al. 2007; Rastogi and Sani 2011). Amplification is executed by using primers with 50-base pairs GC clamp followed by the incorporation of a regulated Peltier-based heating/cooling system for the generation of temperature gradients (Valášková and Baldrian 2009). Although, if compared to the DGGE, the TGGE has found less application in protein analyses, it, however, has been recommended as an efficient tool for PCR amplification specific to. Unlike DGGE is less commonly applied to proteins but can be very effective in PCR amplification of mutable regions of the 16S rRNA sequences (Bharagava 2019). Given the chemical homogeneity of TGGE, it is considered to have some advantage over the DGGE which stems from the fact that the analysis by TGGE is concluded in real time (6 h) in contrast to that of DGGE (14 h) (Valášková and Baldrian 2009).

Just like the DGGE, the TGGE has also been successfully applied in various scientific research sought to investigate microbial populations and compositional shifts in real time. It has been confirmed to offer proper detection of individual species within a complex bacteria population even at low levels in microbe communities (Fouratt et al. 2003; Likar and Regvar 2009). Given its highly reproducible ability, research studies carried out for a robust investigation into several bacteria species have employed the TGGE technique in developing *in situ* probes that are unique to individual species (Arias et al. 2005). Some research applied the temporary version of the TGGE method to characterize endophytic fungal populations that inhabit the soil microbial communities stressed by heavy metal

pollution in the presence of blooming *Salix caprea* L tress (Likar and Regvar 2009). Similarly, an attempt to do elaborate probation into the bacteria population that makes up a nitrifying bio-augmentation product was accomplished with the application of the TGGE (Fouratt et al. 2003). Hence, we conclusively say that the TGGE is a proven tool for the molecular evaluation of soil health.

18.11 Terminal Restriction Fragment Length Polymorphism (TRFLP)

Another very important molecular tool used to study microbial communities is the terminal restriction fragment length polymorphism. Specifically, it finds its use in profiling communities of microbes by considering the location of restriction sites that are closest in proximity to terminals of an amplified gene sequence labelled with fluorescent dye. As a result of being reproducible, the TRFLP effectively analyses genes that express polymorphic tendencies and consequently, it helps to unveil unique characteristic features of a specific microbial community (Arias et al. 2005; Bharagava 2019). The TRFLP works on the principle of creating T-RF patterns that result from the amplification of DNA fragments from bacteria assemblage. This is made possible by utilizing one or two primers marked with fluorescence for the PCR after which the products are digested by restriction enzymes (Zhang et al. 2008). The TRFLP technique is quite remarkable in its ability to assess very complicated microbial communities by exclusively spotting-out single ribo-types that indicate restriction fragments with fluorescently-branded terminals (Rastogi and Sani 2011).

Like the already mentioned DGGE and TGGE, the TRFLP has been applied in recent years to understudy various community structures of diverse soil microbes. Research studies that assessed the functional and genetic reaction of microbial communities, present in slightly contaminated sites, to lower concentrations of bioavailable anthracene, used the TRFLP to accurately achieve substantial results. Besides, the TRFLP was efficient to explore the complex mega communities of bacteria and archaea inhabiting composted soil ecosystems (Louati et al. 2013; Tiquia 2010). The dynamics of bacterial community interaction and their evolution, within soil ecosystems harbouring plants treated with industrial wastewater, have been extensively analysed using the TRFLP (Fredriksson et al. 2019). Similarly, another research that explored the impact of introducing novel microbes into an existing and dynamic community of actinobacterial endophytes in the roots of wheat had very amazing results by engaging the TRFLP tool (Conn and Franco 2004; Pavithra et al. 2020). Wu et al. (2015) exploited the power of the TRFLP in analysing how diverse and rich the bacterial communities were in soils of varying forms of vegetation (broad-leaf forest, coniferous forest, subalpine dwarf forest, and alpine meadow) within a mountainous national reserve location in China. In addition, the TRFLP found its relevant use in estimating microbial richness, abundance, and biodiversity in soils of forest regions already impacted by seasonal fires (Mabuhay et al. 2004).

Despite the extensive application of the TRFLP, it poses a great disadvantage—it generates a non-reliable underestimate of community diversity. This has been found to result from a limitation in the number of bands generated for each gel electrophoresis (up to 100) given that the diverse bacterial species being studied have similar T-RF lengths (Rastogi and Sani 2011). Regardless, the TRFLP has established its importance as a great molecular tool for estimating microbial abundance and biodiversity in different soil types.

18.12 BIOLOG™

The BIOLOG™ has become one of the most popular and fastest molecular tools uniquely suited for very extensive analysis of the functional biodiversity of whole microbial communities. Its primary use has been devoted to studying metabolic dynamics within very complex and mixed populations of microbes. By coupling with Ecoplates (Biolog Inc., Hayward, CA, USA), the BIOLOG™ works on the single principle of generating a real-time estimation of how much carbon substrates are used up to determine the dynamic constant interactions playing out in microbial communities of the soil (Checcucci et al. 2021). A proper analysis of the active metabolic reactions of microbes within different biological systems, such as soils and sediments (Anna et al. 2017), water plants and grains (Ge et al. 2018), has become simply realistic with the development of the BIOLOG™. The operation and utilization of the Biolog™ EcoPlates are considered basic yet elaborate enough to give a broad description of the physiological profiles of communities (Zheng et al. 2020). This is quite important because an understanding of the physiological profiles of microbial communities aids an adequate appreciation of the genetic and functional structure of soil (Gałązka et al. 2018).

Summarily, Biolog™ Ecoplates has found relevant application in also tracking time-space changes in the biochemical activity of microbial communities, labelling unique characteristic features of diverse communities as well as assessing carbon source utilization patterns (Ecoplate Brochure 2017).

A brief description of what makes up the Biolog™ EcoPlate is eccentric to understand how it works. Three recurring sets of 31 lyophilized carbon substrates make the 96-well microplates that form the entire component of the Biolog™ EcoPlate setup (Ecoplate Brochure 2017). Carbon substrates can be a mixture of different biomolecular sources such as carbohydrates, amino acids, and polymers. A blank well is left unfilled serving as a control. A tetrazolium redox dye plays a significant role as an indicator of metabolic activity (Sofa and Ricciuti 2019).

The Ecoplate Brochure (2017) gives a very detailed description of how the Biolog™ EcoPlate is used for assessing the functional diversity of a microbial community. First, soil samples are collected, suspended, diluted to a defined cell density, and directly pipetted into wells before incubation. Kinetic runs are made to generate specific patterns that indicate the metabolic activity of the microbial community under study (Checcucci et al. 2021). Certain key features are observed to assess the physiological profiles of soil microbial community and these include,

first, the rate of colour change in individual wells (activity), stability of the generated patterns, and richness of a positive reaction (diversity). The most striking indication of substrate utilization by the microbial community is a redox reduction of the tetrazolium violet dye resulting in a colour change in individual plate wells (Checcucci et al. 2021).

The application of the Biolog™ EcoPlate technology in various research studies has predominantly focused on an assessment of the metabolic dynamics of diverse bacterial communities. This is quite significant given that free-living, predatory, and parasitic bacteria account for an extremely large portion of whole microbial communities. Hence, single research was devoted to analysing contaminated soils of the Riyadh community for bacterial strains and their active utilization of biomolecules by using the Biolog™ EcoPlate tool (Al-Dhabaan and Bakhali 2017). Similarly, communities of heterotrophic bacteria, inhabiting various soils in the Netherlands, were investigated and profiled for their physiological components (Rutgers et al. 2016).

Very recent research studies reveal that sustainable olive orchards in Southern Italy were examined by the Biolog™ EcoPlate technology to determine the functional diversity of their resident soil bacterial community (Sofa and Ricciuti 2019). Besides, soils stressed by long-term pollution with petroleum hydrocarbons were analysed with the aid of Biolog™ EcoPlate to assess their bacterial microbiome in terms of genetic and functional biodiversity (Gałazka et al. 2018). Specifically, in East China, the Biolog™ tool found its eccentric use in tracking the physiological profiles of soil microbes in the Chaohu Lakeside Wetland (Zhang et al. 2014). This tool remains effective today in estimating the health status of different soil groups around the world despite the evolving climate change events.

18.13 Microbial Resilience

A soil's resilience capacity is directly related to its microbial biodiversity and hence microbial resilience can be thought to be an estimation of the population of a given microorganism living within a stressed soil (Mehta et al. 2022). Such stress could be drought, water logging, ground fires, and even contamination with non-biodegradable substances (Arias et al. 2005). The ability of the population of a defined soil microbial species to withstand adverse soil environmental conditions and still bounce back to its full physiological potential post-stress represents a significant component of soil stability (Griffiths et al. 2008). There are concrete assumptions that a microbe's physiology and the composition of its highly diverse community play peculiar roles in estimating its innate resilient capacity which is also a function of the soil's physicochemical characteristics (Griffiths et al. 2004a, b).

Unlike other tools that have been highlighted as indicators of soil health, the adoption of microbial resilience is unique yet quite unpopular. Its uniqueness stems from the fact that it focuses on providing a reliable estimate of how hardy a soil-residing microbial population can be under stress conditions. Therefore, several research studies have found microbial resilience applicable in investigating

microbial communities. To understand the behaviour of a soil-dwelling decomposer to differing conditions of the soil, research studies investigated how much time it took for *P. fluorescens* when grown first in sterile sandy or clay-loam soil and then in soil stress with copper and heat (Griffiths et al. 2008). Similarly, sites across Kadi, India, with oil-contaminated soils were sampled and examined using microbial resilience analysis to quantify the functional diversity of the communities of microbes present in them (Patel et al. 2016).

In some cases, examining the changes in extracellular enzyme activity (EAs) of soil microbes subjected to abiotic stress has been used as a strong indicator of microbial resilience. A scientific study observed a significant decrease in the inherent SOM content of the soils exposed to heat waves in the high plains of Texas. Such a conclusive finding was based on changes to the makeup of the evaluated communities of microbes stemming from a sharp increase in EAs (Acosta-Martínez et al. 2014). However, variations were found across different soils in Scotland in terms of how resilient their microbial communities were when exposed to stress conditions (Kuan et al. 2007). This is certainly indicative of the health status of such soils. Another research applied the concept of microbial resilience to understand the microbial dynamics of different cultivated soils under drought conditions (Pérez-Guzmán et al. 2020). Nevertheless, the use of microbial resilience as a tool in determining soil health is highly dependent on the application of previously mentioned techniques.

18.14 Omics and Soil Microbial Diversity

18.14.1 Soil Nucleic Acid High-Throughput Sequencing Technologies

The quality, speed, and cost of high-throughput sequencing technologies are all rising quickly. As a result, it is increasingly being utilized to research entire communities of prokaryotes in a variety of fields (Di Bella et al. 2013). Prokaryotes are dominant in our globe. Estimates place the total number of microbial cells on Earth at 10^{30} (Turnbaugh and Gordon 2008). There are up to 100 trillion creatures in the human body, which is roughly ten times the amount of our own human cells (Savage 1977). There are literally millions of prokaryotic species, though most have not yet been cultivated (Jordan 2017). There are likely to be numerous enzymes and metabolic capacities encoded by these species' genes that have yet to be discovered. Bacteria have a vital part in the control of digestive, endocrine, and immunological systems in the human body. The makeup and diversity of the human microbiome are being discovered thanks to the development of more recent culture-independent sequencing-based technologies (Di Bella et al. 2013). The earliest direct cloning of environmental microbial DNA was proposed by Lane et al. (1985), while the term "metagenome" was proposed by Handelsman et al. (1998) to refer to "the genomes of the whole microbiota found in nature," which refers to the entire collection of genetic information for all bacteria in a given environment. Microbiomes, especially

those linked to human health and disease, have gained great insight thanks to advancements in technologies such as sequence- and function-based gene screening, high-throughput sequencing, and metatranscriptomics (Hess et al. 2011; Qin et al. 2010). Soil is a very complex environment containing huge microbial diversity (Torsvik and Øvreås 2002; Cameron et al. 2018). Its characteristics depend on physical and chemical but also biological factors (Marcote et al. 2001). The biotic component makes up about 0.2% of the soil, with microorganisms accounting for 20–40% of the total and influencing 80–90% of soil processes (Gregorich et al. 1997). Although there is a tremendous amount of micro- and meso-organism variety in soil, little is understood about the mechanisms that these creatures are engaged in (Geisen et al. 2019). A deeper understanding of soil biodiversity and its functions is urgently required given our limited understanding of the involvement of the biotic fraction in soil biochemical pathways. Since successful downstream analyses mainly depend on good-quality DNA, this is the approach's goal. There are a lot of inhibitory chemicals in soil, according to Bessetti (2007) and Huang et al. (2016), which stop or obstruct DNA amplification. The main challenge for the PCR amplification processes is these chemicals. Inhibitors can co-precipitate with DNA, adversely affecting the extract's quantity and quality (Demeke and Jenkins 2010). The most frequent inhibitors are humic chemicals, followed by heavy metals and aromatic compounds (Fornasier et al. 2014).

18.14.2 Soil Metaproteomics

Soil is a complex and dynamic network of biological processes that are intricately linked to allow ecosystems to function properly. Microbial diversity and function are essential for the proper functioning of ecosystems and their long-term survival. To date, metagenomic investigations have revealed the enormous diversity of both culturable and unculturable microbiomes in distinct ecosystems, but their precise significance in ecosystem functioning remains unknown. This can be done by looking at the ecosystem's protein repertoire, which are the direct and undeviating key participants in metabolic processes. Metaproteomics is a new discipline that attempts to capture all of the proteins present in a given environment at a defined time interval. Profiling microbial enzymes may be a sensitive indication of the soil ecosystem because it connects the phylogeny and functionality of soil microorganisms, describing not only at the level of the individual dominant organism, but also at the community level. The method to mining these functional complex soil microbiomes became viable with the advent of high-performance mass spectrometry; nevertheless, it is hampered by the presence of several interfering compounds in the soil samples (Abiraami et al. 2020). Environmental meta-omics in situ is quickly growing, offering a snapshot/profile of both cultivable and non-cultivable microbial populations present, as well as their functional functions in the environment. Soil is a huge, heterogeneous, and dynamic environment, and understanding its microbial life is crucial for biogeochemical cycling, restoration, and bioremediation. Although the active microbial community in the soil

is small, only about 1.8–2% (Bastida et al. 2009) the dead microbial biomass and dormant microflora, as well as their metagenome, can overture the relative number of phyla present. Metatranscriptome can be more useful in capturing community function and tracking active microbial diversity. Metatranscriptome approaches recurrently misrepresent community functioning because of the regulatory control of translation in alternative splicing of mRNA, codon bias, mRNA degradation along with post-translational modification of proteins, protein turnover rate, and low quality of transcript assembly obtained (Lau et al. 2018). These constraints can be circumvented by examining the proteins that are the ultimate functional participants in the cells that perform the function (Gutleben et al. 2018). The advent of high-throughput mass spectrometry, advances in protein identification platforms and separation techniques, concentrated efforts in extraction standardization, and the availability of a plethora of genomic databases have all resulted in significant advancements in the metaproteomics domain. Metaproteomics, by definition, is the study of aiding the identification of the protein repertoire at the community level in order to gain a better knowledge of how ecosystems work. It also assists in identifying the most active enzymes in the community as well as the phyla responsible for the function (Abiraami et al. 2020).

18.14.3 Soil Metabolomics

Metabolomics, the large-scale study of low molecular weight organic compounds in soil, offers one potential approach to characterize soils and evaluate the metabolic status of the soil biological community (Withers et al. 2020). Soils are central to a wide range of ecosystem services that are essential to earth system functioning (Bünemann et al. 2018a, b). As a result, it is critical that we keep an eye on our soils' health so that ecosystem services can continue to be provided (e.g., nutrient cycling, water purification, food provisioning, climate regulation). While a variety of soil quality indicators have been developed, the majority of them are focused on measuring conventional chemical features of the soil (e.g., pH, accessible P and K, organic matter content) as well as physical qualities of the soil (e.g., texture, structure, aggregate stability, bulk density) (Schloter et al. 2018). The creation of reliable markers of soil biological quality that may be widely used has eluded researchers despite numerous attempts (Schloter et al. 2018). Measurements of biological activity, such as baseline and substrate-induced respiration, enzyme activity, and the size and composition of the microbial population, such as CHCl_3 fumigation-extraction and fatty acid biomarkers, are a few examples of classic indicators (Bending et al. 2004). However, new approaches to evaluating soil biological function have been made possible by the advent of “omic”-based technologies for the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). While metagenomics and metabarcoding are becoming more common (Cameron et al. 2018; George et al. 2019) metabolomic analysis of soil microbial populations has received far less attention, untargeted metabolomics permits a worldwide

examination of the low molecular weight (1000 Da) metabolites present in a sample (Untargeted Metabolomics_Enhanced Reader.pdf n.d.). Recent advances in spectroscopy have made it possible to identify and quantify the relative abundance of thousands of metabolites present in biological samples (Patti et al. 2012). Metabolomics and its use in ecology (Enhanced Reader.pdf n.d.) and is not constrained by unknown levels of epigenetic control and post-translational modifications, respectively. A metabolomic technique is comparable to genomics and proteomics in terms of cost (Wilson et al. 2005) and allows for rapid sample processing (Patti et al. 2012). Furthermore, the approach can detect biochemical intermediates in interconnected metabolic pathways, potentially boosting our overall understanding of biological processes in soil and our ability to anticipate results (Withers et al. 2020).

18.15 Targeted and Untargeted Approaches to Soil Microbial Diversity Management

Soils act as a main habitat for many living organisms which includes fungi, bacteria, insects, and plants; however, soil microbes play an indispensable role in the ecosystem services such as nutrient cycling and waste recycling, maintaining soil structure, detoxification of harmful chemicals, reducing soil erosion, and reducing greenhouse gas emissions by carbon storage (Aislabie et al. 2013). It is believed that 1g of soil may accommodate billion of bacteria, and furthermore, higher diversity and population of microbes are found near the rhizosphere as microbes require water and nutrients for their survival and such requirement is met at rhizosphere (Terrence et al. 2021). Presence of functioning microbial community in the soil is an invaluable asset as it helps in achieving soil quality, fertility, and sustainable agriculture (Sun et al. 2016). Soil microbes play a significant role in regulating plant diversity and productivity; for instance, plant growth is promoted by rhizobium bacteria through nitrogen fixation, and likewise, diversity and productivity of plant community is improved with increased Arbuscular mycorrhizal fungi (AMF) richness and this is due to the reason that AMF improves plant uptake of resources from the soil and also protects the plants from disease-causing soil microorganisms (Schnitzer et al. 2011; Gray and Smith 2005; Vogelsang et al. 2006).

Plant roots emit exudates into the rhizosphere which provides nourishment to soil bacteria and as a result much higher population will be found in the rhizosphere when compared to other regions of the soil (Gray and Smith 2005). Crop plants which require sulphur for vitamin and protein synthesis are completely dependent upon soil for the sulphur uptake and on the other hand immobilization and mobilization of sulphur in the soil (which is organically bound) are believed to be carried out by soil microbes (Kertesz and Mirleau 2004). Activities related to management of soil microbes in the agricultural field can have beneficial impacts; however, farmers have limited tools/no tools to measure the impacts of implemented management practices and sometimes such management practices can also cause negative/unwanted impact (Terrence et al. 2021). There are two approaches (Targeted &

Untargeted) for soil microbial diversity management. Targeted approach includes zero tillage/conservation tillage and biofertilizer application. Untargeted approach includes organic farming and conservation agriculture.

18.16 Targeted Approach

18.16.1 Zero Tillage/Conservation Tillage

Tillage is performed for the following reasons: to prepare a seedbed suitable for sowing/planting; to minimize the soil compaction; to control the growth and spread of weeds; to incorporate crop residues, fertilizers; to follow the tradition learnt from ancestors (Gebhardt et al. 1985; Feng et al. 2003). Soils based on their structure, moisture level, and organic matter respond distinctively to the tillage, and as a matter of fact, soils are prone to erosion under conventional tillage (Gebhardt et al. 1985). Conventional tillage disrupts the basic structure of soil and lowers not only the crop residues on the surface of the soil but also the soil quality (Das et al. 2014). Zero tillage or conservation tillage helps the soil to preserve moisture and build organic matter, and thereby, it creates suitable habitat for soil microbial community, and moreover, many studies show that soils with zero tillage contain highest soil microbes when compared with soils which are conventionally tilled (Lauren Quinn 2016).

This may be due to the reason that tillage has an impact on soil microbes through altering the microclimate of the soil and organic matter content, and meanwhile, in the global perspective, zero tillage is being implemented only on 11% of total arable land (Zuber and Villamil 2016). In conservation tillage, chisel ploughs are used to cause least possible disturbance to the soil, and as a result, higher microbial community is associated with such soils than the soils which are tilled with disc ploughs or mouldboard ploughs (Lauren Quinn 2016). When the soil is less disturbed, fungal hyphae are not affected and play a vital role in nitrogen and carbon cycling (Zuber and Villamil 2016). Governments in the developing countries as far as concerned are likely to support conventional tillage and this may be due to lack of expertise to support them in switching from conventional system to conservational agricultural systems (Kassam et al. 2014). Soils which are not tilled for longer period of time showed increased levels of nitrogen, soil carbon, phosphatase activities, and total phospholipid fatty acids when compared to conventionally tilled soils, and on the other hand, crop residues which get accumulated on the soil surface in zero tillage systems are transformed into soil organic matter, and as a result, density of soil microorganisms gets increased (Mathew et al. 2012).

18.16.2 Biofertilizer Application

Biofertilizers are composed of living cells of microorganisms which promotes nutrient uptake by plants and improves the quality of the soil (Fasusi et al. 2021).

Especially in the last two decades, there seems to be a lot of growing interest in the research of microbial populations colonizing various habitats and their combined contribution to several parameters such as plant growth and health (Kumar et al. 2021). Biofertilizers have always had the capability to directly expand the beneficial soil microbes. For testing microbes as biofertilizers, they are first examined for higher colonization capacity and characteristics that promote plant development (Kumar et al. 2021; Pandey et al. 2019). Carbon requirement of soil microbes is met through the exudates emitted by the plant roots (Kumar et al. 2017). Crop yields are expected to rise by 10–40% with the usage of biofertilizers (Mahanty et al. 2017). Plants tend to absorb more phosphorus from the soil when the AMF are introduced into it (Duponnois et al. 2005). Mixed inoculation of *Azospirillum brasilense* and arbuscular mycorrhizal consortia resulted in higher quantity of *Azospirillum* colony-forming units (CFU) and arbuscular mycorrhizal (AM) spores in the soil (Mishra et al. 2008).

When *Pseudomonas monteilii* strain HR13 is inoculated in the soil where Australian *Acacia* species is grown, ectomycorrhizal colonization with the plant roots was greatly improved (Duponnois and Plenchette 2003). When *Glomus intraradices* was introduced into the soil where *Acacia holosericea* is cultivated, the fluorescent pseudomonads community has increased dramatically (Duponnois et al. 2005). Few microorganisms which naturally occur in the rhizosphere are found to arrest the development of disease-causing microbes in the soil and also have the potential to influence the immune response of the plants (Sahu and Sindhu 2011; Wang et al. 2022). When *Glomus mosseae* was introduced into the soil, the biomass of the soil microbial community continued to rise (Zarea et al. 2009). Cytochemical and plating tests could be a good way to investigate the impact of bacterial fertilizers on the soil microbial community (Sharma et al. 2012).

The application of *Bacillus amyloliquefaciens* strain NJN-6 as a biofertilizer has decreased the prevalence of fusarium wilt disease in banana plantations by modifying the soil microbial community in a certain manner that *Bacillus*, *Cantharellus*, *Synchytrium* biomass has improved and has acted negatively with fusarium populations by significantly reducing their growth (Shen et al. 2015). Soils treated with two biofertilizers (one containing *Bacillus amyloliquefaciens* W19 and the other containing *Trichoderma guizhouense* NJAU4742) has shown greater phylogenetic diversity of microbes and showcased far more fungal and bacterial abundance than in the soils treated with chemical fertilizers and these biofertilizers has encouraged the growth of microorganisms in the soil which shows antifungal activity and suppressed the growth of *Fusarium oxysporum* which causes serious disease known as fusarium wilt (Xiong et al. 2017).

18.17 Untargeted Approach

18.17.1 Organic Farming and Conservation Agriculture

Long-term organic farming significantly enhanced microbial heterogeneity and richness under the plastic tunnel cultivation system (Liao et al. 2018) and also in the general agricultural fields and this may be due to the availability of organic carbon through organic manures and also the presence of few weed species creates suitable habitat for soil microbes (Lupatini et al. 2017). Presence of higher microbial diversity in the organic soils depends upon the type of management followed, for instance, organic fertilizer application and controlling pests using biological control agents (Chaudhry et al. 2012; Lupatini et al. 2017). Higher microbial diversity and evenness are seen in the soils of organic farms than conventional farms and this is due to the usage of plant protection products in conventional agriculture which creates unfavourable conditions and eventually results in death of few microbial groups present in the soil (Sugiyama et al. 2010; Lupatini et al. 2017). Organic fertilizers, crop rotation, cover cropping, and non-chemical pest and disease management are the main components of organic farming whereas permanent soil cover, crop rotation, and intercropping are the main components of conservation agriculture.

18.17.2 Organic Fertilizers/Manures

When compared to chemical fertilizer treatments, the densities of bacteria and fungi are relatively high in organic fertilizer treatments (Xiong et al. 2017). Farmyard manure application has altered the soil microbial community in a positive manner by increasing richness and lowering dispersion (Liao et al. 2018). Frequent organic manure application in agricultural fields has resulted in elevated concentrations of soil microbial biomass when compared to mineral (chemical) fertilizer application (Esperschütz et al. 2007). Compost made from sewage sludge has accelerated soil microbial activity (Bastida et al. 2008). Organic fertilizers not only promote the multiplication of soil microbes through their carbon and nitrogen but also lowers the microbial diversity in the soil if excess phosphorus is released through such manures/fertilizers (Ren et al. 2018; Wu et al. 2021). In rice fields, the richness of soil microorganisms is comparatively higher in the field treated with organic fertilizer than in chicken manure-treated field, and moreover, population of pathogenic *Pseudomonas* has expanded in the chicken manure-treated field (Li et al. 2020). Bacterial community richness and activity of few enzymes such as saccharase and urease have declined with increasing manure application (Sun et al. 2014). Vermicompost application to the soils increases iprodione concentration in the soils and which in turn causes reduction in microbial density and diversity in the soil (Verdenelli et al. 2012).

18.17.3 Crop Rotation

Soil microorganisms react differently to the root exudates released by different crops, and in addition, greater diversity of crop residues which are deposited on the farm as a result of crop rotation serves as a source of organic matter to the soil microbes and eventually their diversity gets increased (Costa et al. 2006; Venter et al. 2016). Crop rotations can lower the densities of soilborne plant pathogens and minimize the outbreak of soilborne diseases (Larkin et al. 2012). The infection percentage of *Rhizoctonia solani* (soilborne plant pathogenic fungus) was reduced more than half in potato when it is grown in rotation with *Vicia villosa* Roth, *Lupinus albus* L. ultra, *Avena sativa* Astro, and *Medicago sativa* L. Nitro (Honeycutt et al. 1996; Chosdon et al. 2021). When *Brassica napus* L. is used in crop rotation programme, not only microbial activity has improved but also culturable bacterial populations in soil has improved to a certain level (Bernard et al. 2012; Larkin et al. 2010).

Total microbial activity and culturable bacteria were improved when potato crop is grown under crop rotation programme with barley and canola when compared with potato monocropping, and moreover, the fluorescent pseudomonads and actinomycetes population is higher under potato–barley rotation than with canola and sweet corn rotations (Larkin 2003). The type of plant species also influences the soil microbial population as different plants release different kinds of organic compounds into the soil (Grayston et al. 1998; Larkin 2003). The bacterial population and diversity remained constant in soil whether it is maize monoculture or wheat-maize crop rotation (Navarro-Noya et al. 2013). When pulses are cultivated as rotation crop in wheat cropping system, they favoured the growth of plant pathogenic microbes (especially fungal populations) in the soil indicating the possibility of outbreak of soilborne diseases (Yang et al. 2021).

18.17.4 Cover Cropping/Permanent Soil Cover

Soil microbial richness is also determined by the type of cover crop grown as the crop residue which will be returned to the soil varies and therefore when Oat is used as a cover crop where organic farming practices are followed, the quantity of saprotrophic fungi was increased when compared with rye as a cover crop leading to a greater fungal: bacterial proportion (Martínez-García et al. 2018). Cover crops help in the stable development of beneficial microorganisms in the soil (Mercado-Blanco et al. 2018). When spring wheat, hairy vetch, and forage oat are cultivated as cover crops, more even population of AMF are linked with vetch and oat, and on the other hand, greater fungal richness is associated with wheat (Benitez et al. 2016).

The type of management practices followed in the farm and the cover crop together has the capacity to influence the catabolic nature of the soil microbiota (Martínez-García et al. 2018). Cover crops which yield high-quality residues support more bacterial growth, and accordingly, the ones which yield lower quality residues encourages fungal growth (Kramer et al. 2012; Muhammad et al. 2021). In organic

Zea mays L. fields, the colonization capacity of arbuscular mycorrhizal fungi has been improved by cover cropping (Njeru et al. 2014). When winter rye is cultivated as a cover crop in a potato-rapeseed/canola crop rotation, common scab and black scurf (soil-borne diseases) are decreased by 25–41% (Larkin et al. 2012). Planting *Flemingia macrophylla* as a cover crop for a period of 10 years enhanced bacterial populations and diversity in rubber plantations up to a depth of 60 cm inside the soil (Liu et al. 2019).

18.18 Future Prospects

Given the environmental challenges, current and future generations of scientists are and will be facing (e.g. climate change, soil erosion, water and soil pollution, salinization, loss of soil nutrients) in combination with the need to secure enough food production for a growing human population, it is fair to say that soil health measurement will continue to be a recurring topic among researchers and policymakers in the near future. Therefore, a more systematic approach to the exploration of soil health indicators and measurements is desirable. Some improvement areas are listed below.

- Making a distinction between general (that may be considered as universal) and specific (that depend on the geographic location, climate, soil type and history) biological indicators (Van Bruggen and Semenov 2000).
- Putting chemical and physical soil health indicators in relation with the newest biological indicators. In order to achieve this objective, a shared effort from scientists and researchers to fill knowledge gaps on the biochemical properties of soil is needed (Gil-Sotres et al. 2005). The fast development and validation of high-throughput -Omic technologies could speed up this process.
- Integrating different measurements to compose a complete picture of soil health (i.e. a soil health index) that can be used consistently across (or easily adaptable to) different environments and geographical areas. Rinot et al. (2019) suggested a multivariate-complex soil health approach with the aim of developing a new soil health index which could consider the connections between soil attributes and the Ecosystem Services provided (Fig. 18.1).
- Bridging the gap between scientific research and the agricultural sector (Doran 2002), following examples of best practices such as the Indian Soil Health Cards (Patel et al. 2017).

With regard to microbes as bioindicators, Fierer and Schimel (2002) gave some perspectives on how microbial data should be considered and microbial indicators adopted. Among those, they suggest to define microbial indices oriented to measure specific soil health outcomes rather than broadly profiling the microbial community, provide clear interpretation and guidance on how to interpret microbial measures also in specific geographical contexts, use microbial data to track how soils change over time (also as a consequence of agricultural management practices) rather than to

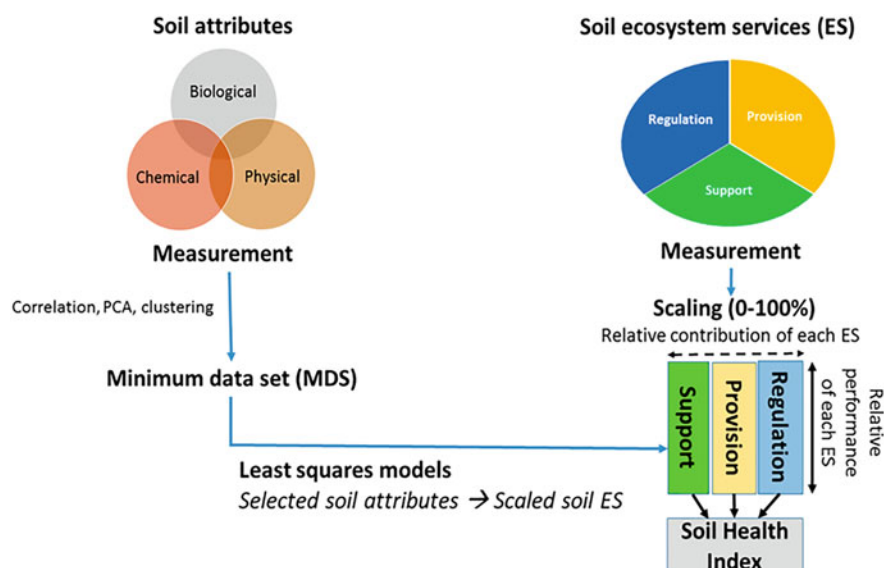


Fig. 18.1 The approach to soil health assessment proposed by Rinot et al. is depicted. By means of measurement and selection of specific soil attributes through monitoring and scaling of ecosystem services, a Soil Health Index is calculated. (From Rinot et al. 2019)

expect healthy soils to have a particular soil microbial community and use microbial measurements to infer soil characteristics only when other pre-existing methods are not sufficient, especially when the first ones are cheaper or easier to obtain.

18.19 Conclusion

Soil health is evidently an important component of sustainable development, especially with regard to the agricultural sector. In this review, we showed how soil health measurement evolved over time, following the discovery of its main biological drivers. We reviewed the most recent advancements on those measurement techniques and delineated targeted and untargeted approaches for a sustainable microbial community management of agricultural soils. We showed the importance traditional soil measurement techniques can still have, and the powerful information and progress the newest -Omics techniques can bring, despite the ongoing discussion on a shared definition of soil health. Overall, this article provided an overview on the currently used soil health measurement techniques accompanied by the most recent advancements on this topic, with the aim of giving a complete framework on the state of the art of this discipline. Soil health will surely benefit from soil scientists' efforts towards a more systematic, clearly interpretable set of traditional and biological indicators. It is clear that there are still knowledge gaps to be filled and methodological details to be discussed and to agree on among the scientific

community. Nevertheless, it is also evident that important advancements in this field of study will unravel the full potential of soil health measurement in securing a sustainable soil health management approach that will benefit the Earth's ecosystems.

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