



FRUIT CROPS

Diagnosis and Management
of Nutrient Constraints

Editors

A. K. Srivastava
Chengxiao Hu

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Diagnosis and Management of Nutrient Constraints

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Microbial ecology in sustainable fruit growing: Genetic, functional, and metabolic responses

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1 Introduction

Microorganisms comprise the majority of soil biological diversity (Riesenfeld et al., 2004). Soil microbial communities influence soil fertility and plant growth, and changes in their structure and dynamics in response to different soil management practices can give information about soil status, in terms of its quality and biological complexity (Bai et al., 2018) (Fig. 22.1). Particularly, under Mediterranean climates, a sustainable soil management aimed at increasing soil organic carbon and microbiological fertility is of key importance. In these environments, the need for a new approach in orchard management is urgent for improving or maintaining soil quality, health, and fertility (Sofo et al., 2019a). Particularly, in fruit crops, the positive influence of sustainable management systems on soil physical, biochemical, and microbiological characteristics has been described (Sofo et al., 2019b). Sustainable soil management (S_{mng}) of fruit orchards can have positive effects on both soils and crop yields due to increases in microbial biomass, activity, and complexity. In Mediterranean orchards, a S_{mng} aimed at increasing soil organic carbon (SOC) stocks (e.g., by no-tillage, increased C and N inputs through recycling of pruning residuals, cover crops, and compost addition) can affect many soil parameters, such as physical characteristics (Palese et al., 2014), chemical parameters (Sofo et al., 2010a; Montanaro et al., 2010), water content (Celano et al., 2011), and CO₂ fluxes (Montanaro et al., 2012).

Soil management, if not well planned and conducted, can provoke decreases in soil organic matter (SOM), mainly due to SOM mineralization (Montanaro et al., 2010). For instance, in Mediterranean olive orchards, high temperature and water shortage, via stimulated microbial metabolism and respiration, cause decreases of SOC and other nutrients (primarily soil N; Pascazio et al., 2018). In these agroecosystems, a S_{mng} that includes a localized and evapotranspiration-based irrigation can improve plant physiological status, yield, and fruit quality in the midterm (Palese et al., 2009; Sofo et al., 2010a). The application of a S_{mng} also gives considerable economic advantages to the farmers and provides efficient ecosystemic and sociocultural services (Montanaro et al., 2017). In addition, the S_{mng} applied to fruit crops can reduce the negative repercussions on the environment linked to nutrient leaching/runoff, particularly weighty for nitrates (Palese et al., 2015).

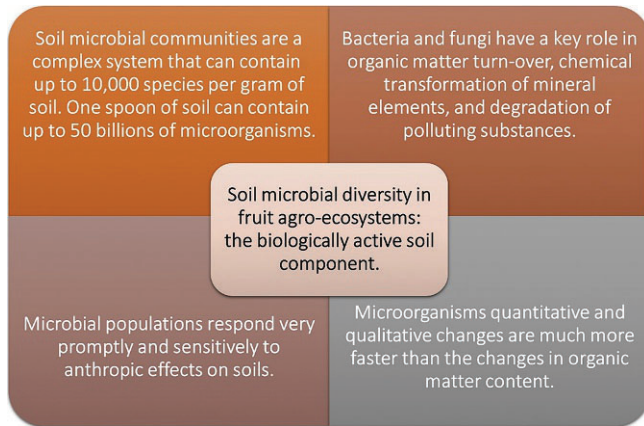


FIG. 22.1 Characteristic of soil microbial communities in fruit agroecosystems.

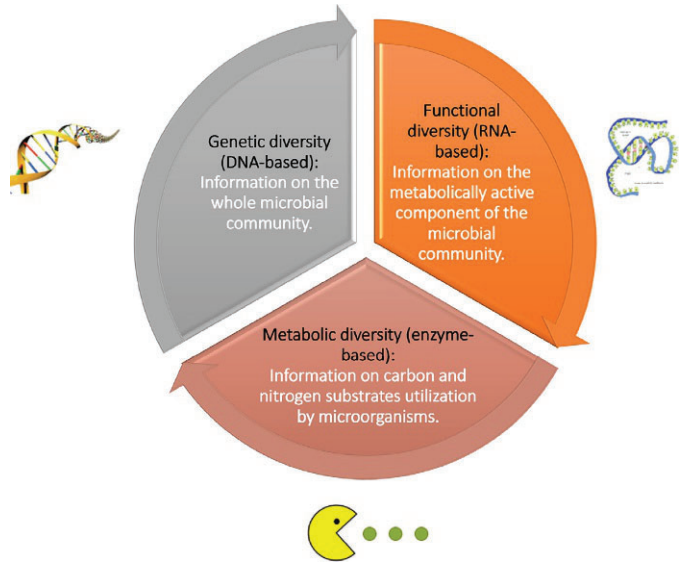


FIG. 22.2 The three major components of the microbial diversity.

In view of circular economy principles and to capitalize on natural potential of soils, sustainable strategies have to be developed for land use practices that optimize nutrient and energy use in fruit groves. The application of these strategies can not only reduce soil organic matter decline, soil erosion, and degradation but also promote ecosystem services and foster all the components of microbial biodiversity (Fig. 22.2). Through an innovative multidisciplinary approach, with a particular emphasis on microbial ecology, region-specific land management practices should be proposed. In this chapter, we present a survey on the researches carried out in several experimental fruit groves in the last two decades by our research group. In particular, the Metapontino area of Basilicata Region (Italy), where most of the researches and experimental trials here presented have been conducted, is listed as a “nitrate vulnerable zone” (NVZ), and for this reason, a steady microbiological and chemical monitoring is continuously needed. We here discuss the changes in the structure, dynamics, complexity, and genetic diversity of soil microbial communities and their relationships with soil health status and soil fertility.

2 Soil microbial dynamics in orchard agrosystems

Soil microorganisms' dynamics (e.g., mobility, growth, nutrient absorption, and respiration), the major responsible of soil fertility and quality (Bünemann et al., 2018), are strongly affected both by the type of soil management and irrigation, and this has been widely demonstrated in fruit crops (Palese et al., 2009; Sofo et al., 2012, 2014a,b; Pascazio et al., 2018). Soil microorganisms influence and, at the same time, are influenced by the soil C and N contents, being bacteria an essential part of C and, even more, of N-cycling processes (Zhang et al., 2014; Li et al., 2018).

Because of the complexity and site specificity of soils, defining soil quality is not an easy task (Bünemann et al., 2018). Qualitative soil profile descriptions and the analysis of C/N ratios and pH values both in topsoil and subsoil can be very useful when interpreting soil C and N data for understanding the general context of the environment soil microorganisms live in (Li et al., 2018). Particularly, the characteristics of the litter layer are a very informative addition to the soil profile description, as they reflect the equilibrium between litter production, litter microbial decomposition, and interaction with the mineral soil (Zanella et al., 2018). Among microbiological techniques, one of the easiest and reliable techniques for defining soil microbiological status is the determination of microbial metabolic diversity by the Biolog method, having a high discriminating power between microbial soil communities from different soil environments and from soils subjected to various agronomic treatments (Sofo et al., 2010a,b). Culture-based and genetic techniques have been used successfully in olive orchards to ascertain the presence of C- and N-cycling functional bacteria (Sofo et al., 2010a,b, 2014a). The study of the soil C- and N-cycling bacteria and of the C and N dynamics could help to understand how soil management can affect soil status (Pascazio et al., 2018; Li et al., 2018). This is particularly important for Mediterranean orchards, where N is often a limiting factor, even when soils are managed sustainably and have high organic C inputs (Montanaro et al., 2010; Celano et al., 2011).

Besides microbiological and genetic analysis, nowadays, next-generation sequencing (NGS), coupled with bioinformatic tools and metagenomic approach, has made it easier to comprehensively analyze microbial communities on any type of matrix, including soils (Jansson and Hofmockel, 2018). Using a metagenomic approach, Sofo et al. (2019a,b) evaluated the possible persistence of potential human pathogenic bacteria (HPB) in olive orchards sustainably irrigated with treated urban wastewater. Indeed, under suitable conditions, low-quality, urban wastewater is an additional water resource for irrigation in water-scarce environments, but its use in agriculture requires a careful monitoring of a range of hygiene parameters, including HPB. On the basis of the results obtained and from the general analysis of previous researches, the authors concluded that irrigation with urban wastewater, if adequately treated and applied, does not constitute health risks for consumers and farmers.

3 Sustainable systems versus conventional systems

Several works published by research group have evaluated the medium-term effect (approximately 20 years) of two different soil management systems, so-called sustainable (S_{mng}) and conventional (C_{mng}), on bacterial and fungal genetic, functional, and metabolic diversity in soils of different types of orchards. The S_{mng} system included no-tillage and endogenous and polygenic organic matter inputs deriving from spontaneous cover crops, pruning material left on the field and/or compost, while C_{mng} soils were tilled, without cover crops and pruning residues were removed. In many cases, the microbial analyses were carried out by culture-based (plating, spectrophotometry, and Biolog) and molecular-based approaches (Denaturing Gradient Gel Electrophoresis [DGGE] 16S DNA cloning/sequencing and metagenomic analysis) (Fig. 22.3).

Significant differences between S_{mng} and C_{mng} fruit systems, such as olive, peach, and kiwifruit groves (Sofo et al., 2010a,b, 2014a, 2019b), have generally been observed, in terms of number of the activities of microbial soil enzymes, Biolog carbon source utilization patterns and related indices, DNA abundance, and presence of the bacteria involved in soil dynamics, such as C and N biogeochemical cycles, lignin degradation, humification, and organic matter mineralization. In detail, in S_{mng} soils, significant increases were observed for the following parameters: (a) functional groups of culturable bacteria (e.g., actinomycetes, ammonifying, proteolytic, and free N-fixing bacteria); (b) activities of microbial enzymes (β -glucosidase and protease); (c) diversity indices (Shannon's diversity index, evenness, and richness) obtained from Biolog assay; and (d) number and type of bacterial taxa involved in C and N dynamics,

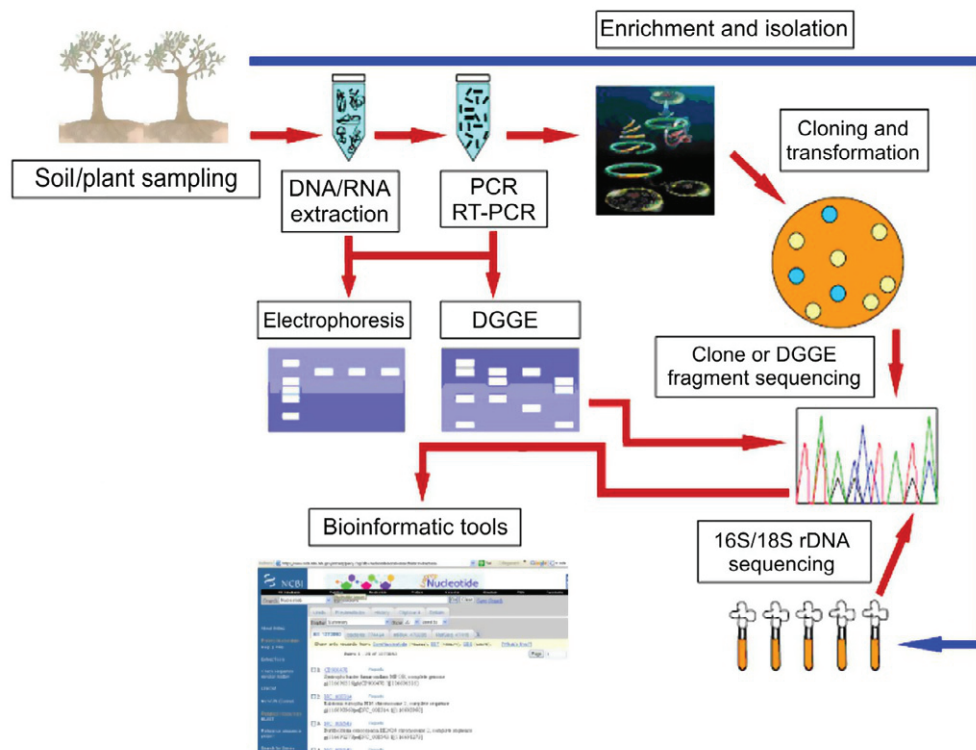


FIG. 22.3 An overview of the methods used for studying plant and soil microorganisms.

particularly symbiont N-cycling bacteria and the abundance of their related genes (*nifH* and *amoA*). Generally, the data revealed a significantly higher microbial abundance, activity, diversity, and complexity in S_{mng} soils. This higher biodiversity could lead to greater soil stability and multifunctionality, positively affecting also plant status and product quality. The results obtained in these papers highlighted that in Mediterranean orchards, under semiarid climatic conditions, the application of endogenous and/or exogenous organic matter can be a key factor to enhance soil quality/fertility and produce in a sustainable way, preserving natural resources and avoiding detrimental effects on the environment. The composition of the microbiota of S_{mng} and C_{mng} soils resulted to be significantly different, particularly for the bacteria involved in soil N cycle, lignin degradation, and humification, more abundant in the S_{mng} system.

The aim of the study of Casacchia et al. (2010) was to explore the effects of the application of S_{mng} system on the diversity of important groups of microorganisms (fungi and actinomycetes), bacterial species (*Pseudomonas* spp., *Bacillus* spp., and *Azotobacter* spp.), and functional groups (proteolytic and ammonifying bacteria) related to soil fertility. The culture-based analysis revealed that higher populations of total bacteria, actinomycetes, and proteolytic bacteria were induced by the S_{mng} application, whereas *Pseudomonas* spp., *Azotobacter* spp., and ammonifying bacteria showed increased populations in the C_{mng} system. Furthermore, the higher inputs of organic matter of the S_{mng} plot caused an increase in the microbial groups responsible for N metabolism in well-watered zones and higher activities of decomposer and humus-forming microorganisms in the drier areas. Similarly, in a recent paper, Pascazio et al. (2018) applied some microbial indicators of soil quality in drip-irrigated olive and peach orchards managed with sustainable agricultural practices, finding that irrigation plays a key role in the determination of soil quality. This latter was evaluated by the authors by measuring and following two parameters: (a) a biochemical soil indicator (N_c/N_k ratio) based on soil N/C turnover and soil enzyme activities and (b) the abundance of three important N-cycling genes (*nifH*, *amoA*, and *nosZ*). The N_c/N_k ratio exhibited all the attributes of a reliable soil fertility indicator. Both N_c/N_k and gene abundances gave a precise idea on N and C soil dynamics in the olive orchard examined.

The results of all these trials demonstrated that soil microorganisms respond significantly to a sustainable orchard management characterized by periodic applications of locally (cover crops and pruning residues) and externally (compost) derived organic matter. These sustainable practices caused an improvement in soil organic matter in the topsoil layers of the S_{mng} system, compared with the C_{mng} , with consequent increases in the abundance of soil N-cycling bacteria and of the values of the indices related to total microbial metabolic activity and diversity.

4 Microbial translocation from soil to plant

Microorganism-plant interactions, where both profit from each other, play an important role in orchards, positively affecting plant status and improving product quality (Pascazio et al., 2015). Indeed, microbial endophytes colonizing plant do not cause apparent damage and contribute to host plant's protection and survival. Plant microbiota forms a complex network with microbial diversity and community dynamically changing throughout the plant life cycle (Ying-Ning et al., 2017). The studies focused on epiphytic and endophytic microbiota of fruit trees grown under different agronomic systems can be useful for the promotion of plant growth and a higher crop quality (Pascazio et al., 2015). Many studies demonstrated that plant-associated microbes live either inside plant tissue or on the surface of plant organs (e.g., Berendsen et al., 2012; Bulgarelli et al., 2012). Particularly, the microbial inhabitants of the soil and phyllosphere (plant aerial surface) are considered epiphytes, whereas those residing within plant tissues (xylem sap) are the so-called endophytes. The relative abundance of microorganisms in different soil and plant compartments varies, as some ecological niches, like xylem vessels, are very selective and restrictive, whereas others, such as rhizospheric soils, are very rich of microbes (Fig. 22.4).

The microbial colonization of plants depends on some key factors, such as plant genotype, tissue, growth stage and physiological status, and on soil environmental conditions, as well as on some agricultural practices (Singh et al., 2009). It is known that the taxonomic diversity of plant-associated bacteria is tissue specific and that microorganisms exhibit a particular spatial compartmentalization within plants. Experimental supports provided that microorganisms reach the rhizosphere by chemotaxis toward root exudate components and that the preferred site of attachment and subsequent entry is the apical root zone (Bulgarelli et al., 2012). The differentiation zone and the intercellular spaces in the epidermis have been suggested to be preferential sites for colonization (Berendsen et al., 2012). Soil microenvironment, particularly in terms of temperature and humidity, affects the colonization of endophytes and their community structure.

Studies on olive plants revealed that soil sustainable management practices have positive effects on soil fertility and on fruit and leaves as concerns microbiological genetic diversity and metabolic activity (Sofa et al., 2014a; Pascazio et al., 2015). Particularly, the phyllosphere represents a niche with great agricultural and environmental importance,

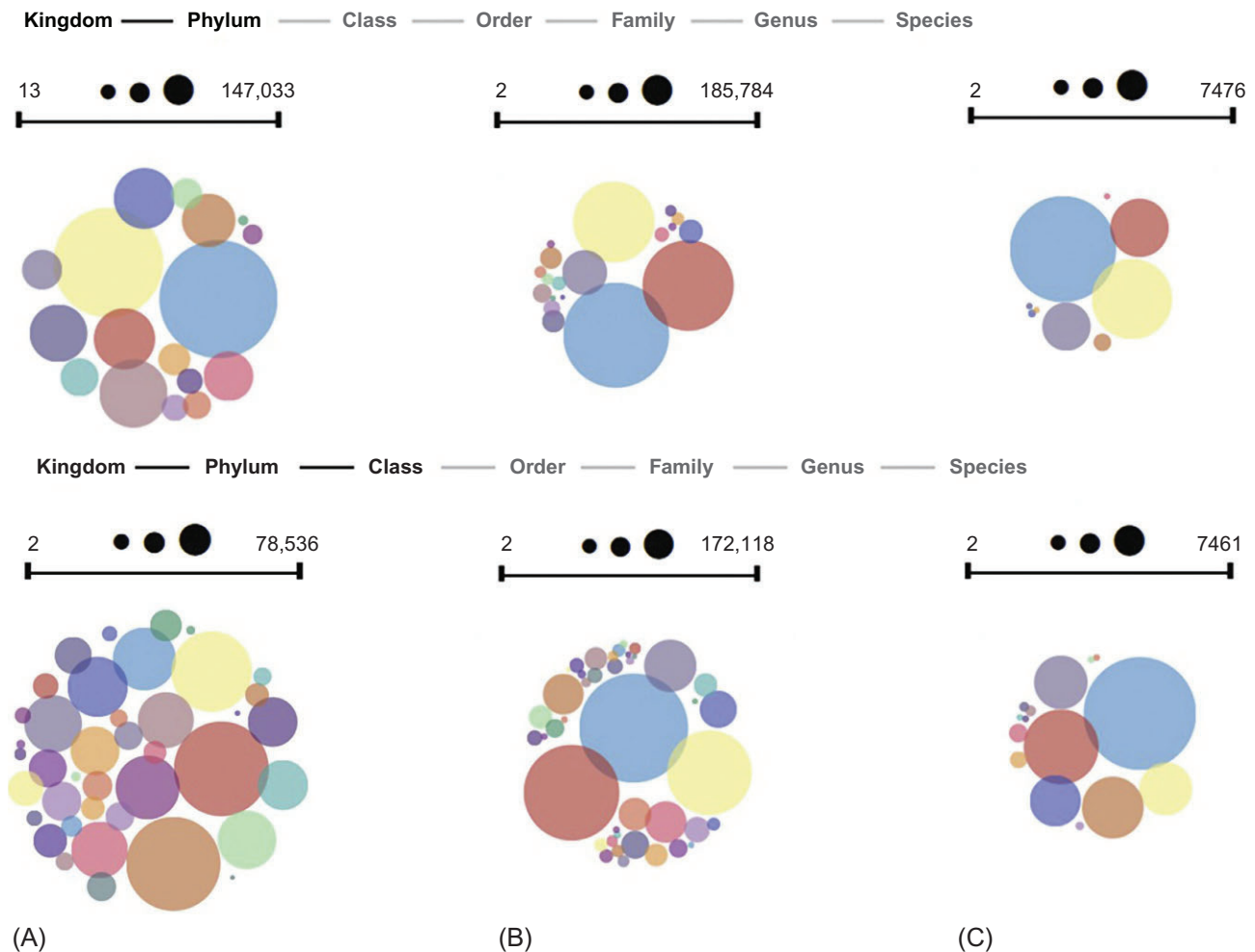


FIG. 22.4 Bubble charts of the values of bacterial relative abundance in (A) soil, (B) leaves, and (C) xylem sap at the phylum (row above) and class level (row below) in an olive orchard. Each bubble represents a bacterial taxon filled with a specific color, the size of which is proportional to the summary level of this taxon in the examined samples. The increasing scale of the total reads, represented by the smaller bubbles to the bigger ones, is displayed above each bubble chart. Xylem sap is the poorest in terms of microbial abundance and diversity.

as there is growing evidence that phyllosphere microbial inhabitants could affect the quality and productivity of agricultural crops, as they promote plant growth and contribute to host plant protection and survival (Rasche et al., 2006). In the study of Pascazio et al. (2015), phyllosphere and carposphere bacterial communities of olive trees subjected to two different soil management systems (S_{mng} and C_{mng}) were characterized in a mature olive grove. From a genetic point of view, the 16S ribosomal RNA eubacterial genes (16S *rDNA*) separated by DGGE and belonging to the bacteria living on leaf and fruit surface, and in fruit pulp, clustered separately in the two systems. Moreover, a clone library of 16S *rDNA* amplicons extracted from the bacteria living in pulp homogenates and a phylogenetic analysis were performed. The medium-term sustainable orchard management resulted in a higher number of bacterial species from olive fruit pulp. Finally, phyllosphere and carposphere communities evaluated by DGGE were affected by the type of the agricultural practices adopted.

These results are of key importance, as many endophytic microorganisms have been appreciated for their capacity to protect their hosts against insects-pests and pathogens (Azevedo et al., 2000). Furthermore, they could confer other important characteristics to plants, such as greater resistance to stress conditions, alteration in physiological properties, and production of phytohormones and compounds of biotechnological interest (Azevedo et al., 2000). Epiphytes and endophytes establish a symbiotic relationship with the plant and colonize an ecological niche like that of phytopathogens and are possible candidates for biocontrol agents. Thus, the identification and quantification of endophytic microflora present in and on plants provide information and data that may also have potential practical implications for disease management in fruit trees, which can deeply impact crop quality and production.

5 Future perspectives

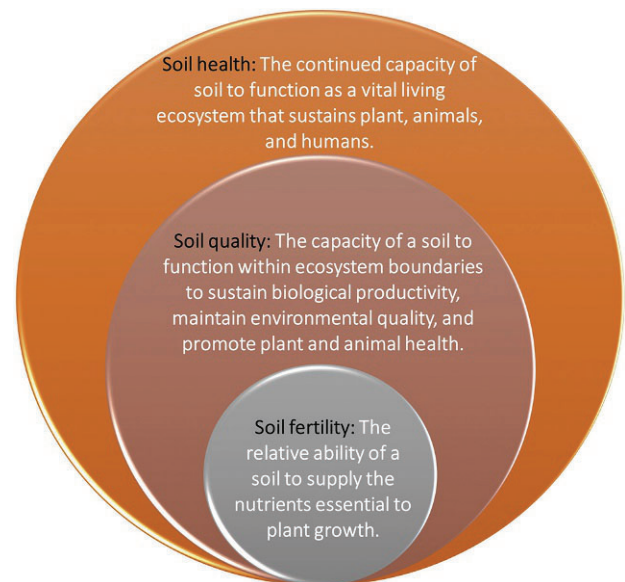
A sustainable orchard management is a key factor for increasing the functionality and genetic diversity of soil microbiota that in turn enhances soil health, quality, and fertility (Fig. 22.5). This amelioration positively affects plant physiological status and productivity. The studies mentioned here confirm the need for Mediterranean orchards to encourage farmers to practice soil management based on organic matter inputs associated with zero/minimum tillage to improve soil functionality and the genetic diversity of soil microorganisms. The results deriving from these researches could promote the development of new approaches for optimizing soil carbon cycling, managing nutrient transport, and sustaining and improving olive yield and quality.

The amount and type of C and N inputs, the composition of N-cycling bacterial community, the study of C/N and pH imbalances, and the kinetic of soil C dynamics and N cycling have a major role in the genetic, functional, and metabolic responses of soil microorganisms, according to the [Sofo et al. \(2012, 2014b, 2019b\)](#). Among the agronomic sustainable practices, the input of soil organic matter as compost is another relevant factor affecting soil fertility in orchards. All these issues should be taken into account for planning and establishing new orchards based on sustainable agronomic techniques, or for fruit growing conversion from conventional/traditional to sustainable/innovative. Particular attention should be paid on the most important groups of microorganisms, including many plant-growth-promoting fungi/bacteria and biocontrol agents. Finally, a better understanding of phyllosphere and carposphere microbiota of cultivated trees could be useful for the promotion of plant growth, a better plant protection and a higher crop quality.

Although studies of microbial community based on isolation/culture techniques have been conducted for a long time in fruit groves, in the immediate future, next-generation sequencing coupled with bioinformatic tools and metagenomic approach will made it easier to comprehensively analyze microbial communities of plant tissues. One of the main advantages of metagenomics compared with culture-dependent methods is the ability to theoretically detect all microorganisms that cannot or are extremely difficult to culture, which represent about 99% of the total estimated microbial diversity, as well as rare taxa that are usually missed by culturing techniques. For this reason, studies aimed at characterizing the microbial communities of groves and at determining the effects of different management systems on microbial diversity of soil and plant through a metagenomic approach will be needed.

The importance of soil microorganisms to ecosystem services of fruit groves is often overlooked and must be taken into account in future fruit growing management strategies. Nature-based solutions are required to facilitate sustainable use and conservation of soils, including adaptation and resilience to climate change. Scientific synthesis of the current understanding of soil microorganisms is needed, and guidelines for future experimentation and best regenerative practices to exploit soil multifunctionality have to be developed, tested, and validated. The application of sustainable agricultural practices in fruit groves can positively affect the variability and composition of soil and plant

FIG. 22.5 The definition of soil health, soil quality, and soil fertility. Soil fertility is a subset of soil quality, in turn a subset of soil health.



microbial communities in orchards, promoting plant growth and plant protection, enhancing fruit production and quality, restoring soil fertility, improving resource use efficiency, and providing orchard multifunctionality, with evident benefits to the whole agroecosystem stability. For sure, a sustainable orchard management is a key factor for increasing the genetic diversity of soil microbiota that in turn enhances soil microbiological fertility. The final target of these researches should be to transfer science to farmers, enterprises, policy makers, and related end users in the domain of microbial ecology in fruit productive systems, to promote cost-effective sustainable land use and soil restoration strategies.

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