

Emerging Technologies and Management of Crop Stress Tolerance

Volume II *A Sustainable Approach*



Edited by
Parvaiz Ahmad
and Saiema Rasool



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A Sustainable Approach

Volume 2

Edited by

Parvaiz Ahmad

Saiema Rasool



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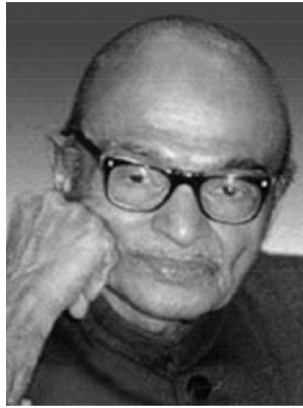


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This book is dedicated to



Hakim Abdul Hameed
(1908–1999)
Founder of Jamia Hamdard (Hamdard University)
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Sustainable Soil Management in Olive Orchards: Effects on Telluric Microorganisms

20

Adriano Sofo, Assunta Maria Palese, Teresa Casacchia and Cristos Xiloyannis

20.1 Introduction

Obtaining top yields of high quality and preservation of environmental sustainability is possible by maintaining microbiological soil fertility using innovative, sustainable agricultural techniques (Kushwaha et al., 2000; Ding et al., 2013). In particular, the first layers of the pedosphere are the habitat for a high number of bacterial and fungal communities that play a key role in the pedogenetic processes and in soil fertility improvement (Brady and Weil, 2008; Jagadamma et al., 2008). On this basis, changes in the structure and dynamics of soil bacterial and fungal communities, as a response to different soil management in agricultural systems, represent an interesting assessment index of soil status with respect to its quality and complexity (Visser and Parkinson, 1992; Anderson, 2003).

The use of microbiological techniques has allowed the isolation of important physiological groups of bacteria related to soil fertility, such as the microorganisms involved in important steps of the carbon cycle (e.g., actinomycetes, *Pseudomonas* spp., and *Bacillus* spp.), the major decomposers of complex polymers (e.g., lignocelluloses and chitin), and the nitrogen cycle (i.e., nitrogen fixer, proteolytic, ammonifying, nitrifying, and denitrifying bacteria) (Zaitlin et al., 2004; Ding et al., 2013). Nitrogen-fixer microorganisms are able to reduce $\text{N}\equiv\text{N}$ to NH_3 for the biosynthesis of organic nitrogen compounds (Brady and Weil, 2008). Proteolytic bacteria are responsible for soil protein degradation in peptons, peptic acids, and in aminoacids, whereas ammonifying bacteria release ammonium ions (NH_4^+) from nitrogen-containing organic compounds (Brady and Weil, 2008; Ding et al., 2013). Moreover, fungi and actinomycetes are able to colonize rhizosphere and use root exudates as a carbon source, supply roots with easily assimilable nitrates, and play a key role in the biological control of root pathogens and in the maintenance of soil health (Govaerts et al., 2008).

The olive is the emblematic tree of the Mediterranean Basin where it is an integral and significant part of the landscape and culture (Loumou and Giourga, 2003; Castillo-Llanque and Rapoport, 2011; Ehrenberger et al., 2012; Gómez-del-Campo and García, 2012; Rodrigues et al., 2012; Sanzani et al., 2012; Cuevas et al., 2013); however, its ecological importance has only recently been acknowledged. Olive oil was and is the major source of nutritional fats for the residents of the Mediterranean area and the most valuable export product from this region. Olive trees have been cultivated for centuries mainly in the hilly and marginal parts of the Basin, becoming one of the

most representative and stable fruit crops in the world occupying around 9.5 Mha in 2010 (Barlett et al., 2012; FAOSTAT, 2012). In such areas soil degradation processes (e.g., erosion, soil organic impoverishment, groundwater contamination, soil salinization, biodiversity losses) are very intense because of a lack of conservative soil management practices (tillage, no organic matter input) and the abandonment of nonproductive olive groves and/or their overgrazing (Ben-Gal, 2011; Hammami et al., 2011; Perez-Martin et al., 2011; Fernández-Escobar et al., 2012; Carr, 2013; Caruso et al., 2013; Gómez-del-Campo, 2013; Morales-Sillero et al., 2013; Palese et al., 2013). Therefore, in the semiarid Mediterranean olive orchards, the loss of soil fertility needs to be avoided by using innovative and optimized agricultural techniques with low environmental impact (Dag et al., 2011; Rewald et al., 2011a,b; Diaz-Espejo et al., 2012; Gracia et al., 2012; Gucci et al., 2012a,b).

On this basis, the chapter's aim is to present some results about the effects of sustainable management systems on soil microbial, genetic, functional, and metabolic diversity in Mediterranean olive orchards. We place particular attention on the most important groups of microorganisms. Among the agronomic sustainable practices, the input of soil organic matter as compost is an important factor that affects soil fertility. For this reason, the system of in situ compost production in olive groves is thoroughly discussed.

20.2 Sustainable management systems

Suitable management practices for fruit growing—that is, conservation tillage, cover crops, compost amendments, incorporation of cover crops (green manure), pruning residues into the soil, and adequate irrigation and fertilization—are recommended to save conventional water, restore soil organic matter, and reduce environmental pollution (Lal, 2004; Fernández et al., 2011a,b; Gomiero et al., 2011; Rapoport et al., 2012; Rodríguez-Domínguez et al., 2012; Sánchez-Alcalá et al., 2012; Fernández et al., 2013). As a matter of fact, sustainable soil management can determine optimal plant nutrition equilibrium, avoid nutrient accumulation in soils and leaching risks, improve irrigation efficiency, and prevent soil erosion and root asphyxia. Further, these sustainable practices can have positive effects on the activities and complexity of soil microbial communities (Govaerts et al., 2008; Gomiero et al., 2011). The optimization and innovative use of agricultural techniques with a low negative environmental impact have positive effects on both soil, yield, and quality because they increase microbial biomass activity and complexity (Gruhn et al., 2000; Kushwaha et al., 2000; Widmer et al., 2006).

In the semiarid Mediterranean agricultural lands, a new approach in fruit orchard management has been imposed by environmental emergencies such as soil degradation and water shortage (Lal, 2004; Hochstrat et al., 2006; Graniti et al., 2011; Larbi et al., 2011; Rosati et al., 2011; Searles et al., 2011; Moriana et al., 2012; Prieto et al., 2012; Proietti et al., 2012; Diaz-Espejo et al., 2013; Lobet et al., 2013). Therefore, the use of agronomical techniques that may be able to improve or preserve soil quality, health, and fertility is particularly recommended (Kushwaha and Singh, 2005; Govaerts et al., 2008; Machado et al., 2013; Pierantozzi et al., 2013). Especially in olive orchards, a positive influence of sustainable orchard management systems on soil biochemical characteristics and soil microbial genetic diversity has been observed (Hernández et al., 2005; Benitez et al., 2006; Moreno et al., 2009; Sofó et al., 2010, 2013).

Metabolic microbial community diversity in the structure of soil bacterial and fungal communities can be estimated by different methods and techniques. One of the most reliable and interesting is the Biolog[®] metabolic assay based on the ability of microbial isolates to oxidize different carbon and nitrogen sources (Zak et al., 1994; Insam, 1997). The community-level physiological profiles (CLPPs) obtained by the Biolog method are used to differentiate microbial populations from various soil environments or from soils subjected to different treatments (Calbrix et al., 2005; Gelsomino et al., 2006; Singh et al., 2006).

It is also true that these important data should be interpreted and accompanied by the use of culture-dependent methods in order to obtain the correct characterization of the microorganisms tested, and by the determination of the activities of some soil enzymes that are important markers of soil fertility status (e.g., glucosidase, dehydrogenase, protease, and fluorescein diacetate hydrolase). As in other agroecosystems, the response of telluric microorganisms to sustainable soil practices in olive orchards depends on the duration of the treatments. In the rest of this section, we describe three examples (short-, medium-, and long-term) based on recent field research.

Sofo et al. (2010) studied the effects of two soil management systems, known as “sustainable” (ST) and “conventional” (CT), on the composition and on the genetic, functional, and metabolic diversity of soil microbial communities in an olive orchard. The research was carried out during a seven-year period (short-term) in a mature olive orchard located in southern Italy under semiarid conditions (Figure 20.1). The ST system included no-tillage, integrated chemical fertilization, and organic matter input from drip irrigation, spontaneous cover crops, and pruning material shredding. In the experiment, microbial analyses were done using an integrated approach of culture-dependent (microbial cultures and Biolog) and culture-independent methods (i.e., denaturing gradient gel electrophoresis, DGGE).

After seven years of treatments, the average olive yield was 8.4 and 3.1 t ha⁻¹ yr⁻¹ for ST and CT systems, respectively. CT had a significantly higher number of total culturable bacteria and actinomycetes when compared to ST, whereas fungi were significantly lower. With ST, the number of ammonifying bacteria, proteolytic bacteria, and *Azotobacter* in the wetted areas under the drippers (ST-WET) was much higher than along inter-rows (ST-INTER). The DGGEs of microbial 16S/18S rDNA showed differences between ST and CT, whereas 16S/18S rRNA DGGEs of



FIGURE 20.1 Comparison of sustainable (left) and conventional (right) management of a mature olive orchard (cv. Maiatica) located in southern Italy.

ST-WET clustered in a different way from those of CT and ST-INTER. Some Biolog metabolic indexes were significantly different between ST and CT. The results of this work revealed qualitative and quantitative changes of soil microbial communities in response to sustainable agricultural practices that stimulate soil microorganism activity.

The aim of another [Sofo et al. \(2013\)](#) study was to investigate the medium-term (12 years) effects of ST and CT on the soil microbial composition and metabolic diversity of a rain-fed mature olive orchard located in southern Italy. Although the ST system included no-till, spontaneous cover crops, and mulch derived from the pruning material, CT was managed by frequent tillage and included heavy pruning with residues removed from the orchard. Microbial analyses were carried out by culture-dependent methods (i.e., microbial cultures and Biolog). Molecular methods by light and electronic microscope were used to confirm the identification of the isolates of fungi and *Streptomyces* ([Figure 20.2](#)). A significantly higher number of total culturable fungi and bacteria

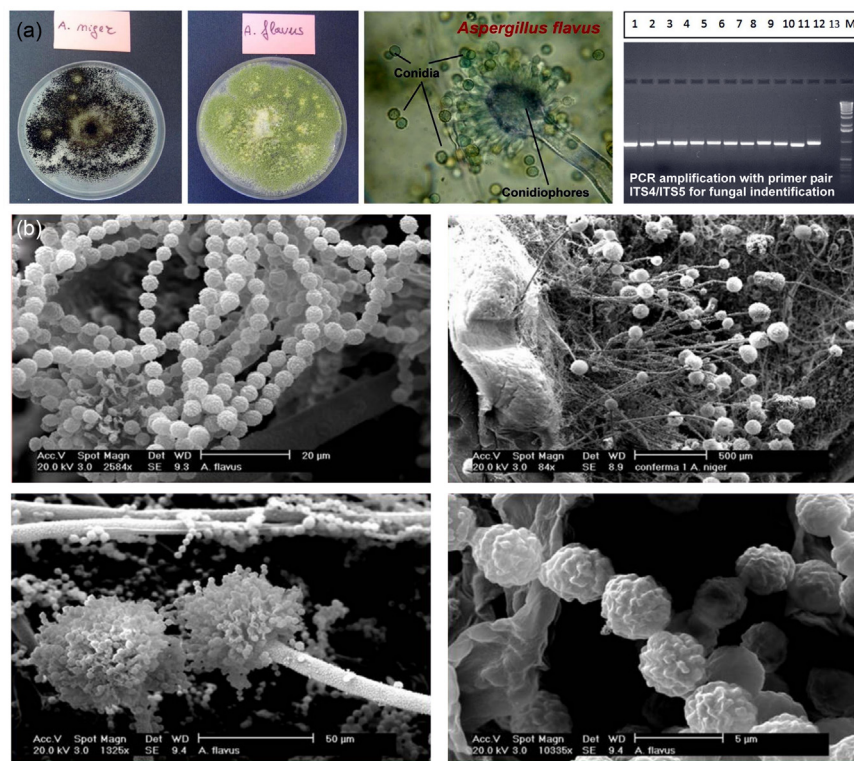


FIGURE 20.2 Examples of *Aspergillus* spp. identification in soils from an olive orchard located in southern Italy (cv. Maiatica).

The orchard was subjected to a sustainable management system for 12 years. The identification was carried out by means of cultural, molecular, and light microscopy techniques (a, top) and electronic microscopy (b, bottom).

Source: Thanks to Professor Ippolito Camele for some of these images.

were found using ST. The number of fungal groups found in ST was also much higher than when the CT system was used. Generally, overall and substrate-specific Biolog metabolic diversity indices of microbial communities and soil enzyme activities were greater with ST. The results of the study of [Sofo et al. \(2013\)](#) demonstrated that soil microorganisms responded positively to a sustainable orchard management characterized by periodic applications of endogenous sources of organic matter. This confirmed the necessity to guide olive orchard farmers toward soil management based on organic matter inputs associated with zero tillage to ameliorate soil functionality.

The abandonment of olive orchards is a phenomenon of great importance triggered mainly by economic and social causes. In such orchards, trees assume their original bushy form, canopies become dense and closed, and pioneer vegetation recolonizes free spaces according to ecological successions that tend to return, after a long time, to a natural formation (“climax”) where soil and vegetation components are *in equilibrium* ([Loumou and Giourga, 2003](#)). During the transition of an olive grove from a “disturbed” (cultivated) condition to a climax phase, soil properties progressively change, as found in similar agricultural systems ([Zornoza et al., 2009](#)). For this reason, the aim of a recent study by [Palese et al. \(2013\)](#) was to investigate some chemical, biochemical, and microbiological properties in soil of an olive grove located in southern Italy. To define the effect of long-term land abandonment (25 years) on soil properties, an adjacent olive grove, managed according to extensive practices, was taken as a reference (essentially minimum tillage and no fertilization) ([Figure 20.3](#)).

Soil organic matter, total nitrogen, and pH were significantly higher in the abandoned olive grove due to the absence of tillage and the natural input of organic matter at a high C–N ratio that, *inter alia*, increased the number of cellulolytic bacteria and stimulated the activity of β -glucosidase—an indicator of a more advanced stage of soil evolution. The soil of the abandoned olive orchard showed a lower number of total bacteria and fungi and lower microbial diversity, measured by means of the Biolog method, as a result of a sort of specialization trend toward low-quality organic substrates. From this point of view, [Palese et al. \(2013\)](#) concluded that extensive cultivation management did not seem to induce a disturbance to microbiological communities.



FIGURE 20.3 Comparison of a cultivated (left) and an abandoned (right) olive orchard located in southern Italy.

The trees (cv. Perenzana) were planted in 1970; in 1985, three-quarters of the orchard was completely abandoned, and its appearance has taken on the form of a Mediterranean coppice with shrubs, herbs, and weeds colonizing the space between the original trees and rows (left).

20.3 Using in situ compost production

Among the agronomic sustainable practices, the input of soil organic matter as compost in olive orchards is one of the most important factors affecting soil fertility in terms of enhancement of soil permeability and water retention, better endowment and availability of nutrients for plants, higher CO₂ uptake and carbon fixation, and reduction of soil erosion (Toscano et al., 2008; Toscano et al., 2009; Diacono and Montemurro, 2010; Boughalleb and Hajlaoui, 2011; Martín-Vertedor et al., 2011a,b; Nadezhdina et al., 2012; Tunahoğlu and Durdu, 2012; García et al., 2013; Rossi et al., 2013; Tomás et al., 2013; Torres-Ruiz et al., 2013a,b).

The olive pomace (OP), also called “*sansa vergine*” in Italian or “*orujo*” in Spanish, is defined as the residue that remains after the first oil extraction from olives (crude olive cake). The OP is a dry material that is 8 to 10% moisture. It is composed of ground olive stones and pulp with a high lignin, cellulose, and hemicelluloses content and a 3 to 5% oil content, depending on the olive mill typology (pressure or centrifugation) (Niaounakis and Halvadakis, 2006). This by-product is generally used for residual oil extraction using solvents, heating, animal feed supplements, or as an organic amendant for olive grove or other crop soils (Alburquerque et al., 2004). In terms of its agronomic value, the OP watered with olive mill wastewater (OMWW), another product of olive milling, or with another organic material, leads to a product that supplies nutrients to plants and is an efficient method for the disposal of olive mill residuals (Hachicha et al., 2008; Sellami et al., 2008). According to the Italian law 574/1996, it is possible to use not-composted OMWW and OP for agronomic purposes, as they are considered simple plant amendants with no limitation on the amount of OP to be applied to the soil; however, CEE Regulation 91/156 indicates that composting is one of the methods to recycle and recover organic wastes.

Olive mill wastewater is composed by the olives’ own water (vegetation water) and the water used in the different stages of oil elaboration (Niaounakis and Halvadakis, 2006). From an environmental point of view, OMWW is a hazard because it has a considerable organic polluting load, with a maximum biological and chemical oxygen demand of about 100 and 220 kg m⁻³, respectively, and an average concentration of volatile solids and inorganic matter of 15% and 2%, respectively; it has an organic matter fraction that includes sugars, tannins, polyphenols, polyalcohols, pectins, and lipids (Benitez et al., 1997). Therefore, a series of studies focused on the degradation of OMWW and its chemical components (Benitez et al., 1997; Vitolo et al., 1999; Beccari et al., 2002; Amaral et al., 2008), and many authors used specific microorganisms for OMWW treatment (Robles et al., 2000; Tsioulpas et al., 2002; D’Annibale et al., 2004; Dias et al., 2004; Lanciotti et al., 2005).

Microbiological and physicochemical parameters were used as indicators to study the kinetics of OMWW biodegradation such as chemical oxygen demand (COD), dissolved organic carbon, counts of heterotrophs, filamentous fungi and yeasts, and the K, P, and N content (Fadil et al., 2003; Amaral et al., 2008). Because OMWW does not generally contain sufficient N and P for an adequate aerobic purification process, its degradation may be performed by cocomposting, anaerobic digestion or enzymatic treatment (Paredes et al., 2002; Amaral et al., 2008). Some authors obtained satisfactory results, in terms of OMWW degradation and amelioration of soil physicochemical properties, by adding this liquid waste to agroindustrial and urban wastes and monitoring the physicochemical parameters during the composting process of the matrices (Paredes et al., 2000, 2001, 2005).

Angelidaki and Ahring (1997) studied a combined anaerobic digestion of OMWW together with manure, household waste, or sewage sludge; with this method, they managed to degrade OMWW

without previous dilution, without addition of external alkalinity, and without addition of an external nitrogen source. Over four months, [Hachicha et al. \(2008\)](#) efficiently monitored a compost made of OP, OMWW, and poultry manure by following temperature, pH, humidity, and C–N ratio to ascertain its maturity; after that, the authors tested its effectiveness in increasing potato agronomic production. Further, the cocomposting of exhausted olive cake with poultry manure and sesame shells was investigated by [Sellami et al. \(2008\)](#), who followed the process by studying some physicochemical parameters.

Generally, the study of the composting process of olive mill by-products was focused on their physicochemical aspects. In the near future, studies need to be performed to evaluate whether mixtures of olive mill pomace, olive mill wastewater, and olive pruning residues (OPR), without adding any other additives external to olive groves, can be efficiently composted under “*in farm*,” nonindustrial conditions—that is, based on spontaneous aerobic degradation by autochthonous microorganisms. This method of compost production needs limited resources, low energetic inputs, and uses machinery and equipment often already present at the farm. Indeed, to be really sustainable, the composting process should be carried out using the by-products available *in situ*.

In a study done by [Casacchia et al. \(2011\)](#), different mixtures of OP, OMWW, and OPR were aerobically cocomposted under natural conditions in an olive orchard located in southern Italy. During the experiment, compost temperature showed a sharp increase for the first 40 to 60 d, followed by stabilization at 60°C and a decline after 150 d; in contrast, compost water content ranged from 50 to 55% to 25 to 30%. The authors observed that *Pseudomonas* spp., anaerobic bacteria, actinomycetes, and fungi reached levels of 8, 7, 5, and 6 log CFU g⁻¹ compost, respectively, with a slight depression after 30 to 80 d. Total and fecal coliforms decreased significantly during composting, suggesting the lack of microbiological risk due to pathogenic microorganisms during this process. Considering that information on selective media for the microorganisms responsible for the spontaneous aerobic degradation of compost deriving from different olive materials and, in particular, from OP is lacking, [Casacchia et al. \(2011\)](#) also tested an innovative microbiological technique; it is based on microorganism cultivation using a broth extracted from the matrix to be composted in order to monitor the biomass degradation process during OP cocomposting.

In another recent work, [Casacchia et al. \(2012\)](#) performed a two-year experiment with two different soils from an Italian olive orchard—one managed traditionally and the other amended with *in situ* produced compost. The authors observed increases in total organic matter and total nitrogen and pH in the amended soil compared to that managed traditionally. Further, significant increases in total and specific microbial counts (*Pseudomonas*, *Bacillus*, and *Azotobacter*) were noted in the amended soil with a clear amelioration of microbiological soil quality. The results of this 2012 study demonstrated that soil amendment using composts derived from olive mill by-products (i.e., OP, OMWW, and OPR) can be an important agricultural practice for supporting and stimulating soil microorganisms and, at the same time, for reusing the by-products, thus avoiding their negative environmental impact.

20.4 Conclusion and future prospects

This chapter highlights the correct utilization of “innovative,” suitable agricultural techniques and soil management, which are important for fruit production and quality; they can also improve

orchards' soil quality and fertility. On the other hand, soil conservation is becoming a priority for sustainable soil management in rural areas due to the awareness of the deterioration of this natural resource and of the difficulty of efficiently recovering it (i.e., the cross-compliance concept of the European Union).

As a result of different traditions, climate conditions, soils, topography, water availability, and so on, there is substantial diversity regarding olive orchard management among the Mediterranean countries. Therefore, an essential objective in the near future may be to determine a set of standards and common operating principles based on scientific knowledge. Such standards and practical procedures need to be widely and effectively disseminated to growers in every olive producing country; they need to be adopted to design and adjust the local soil management practices in order to increase microbiological diversity. This is likely to lead to improvement in olive yield and product quality. The additional benefit may be to control environmental impact, while minimizing ground- and surface-water use and soil contamination through implementation of up-to-date soil management techniques.

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