Chapter 5
Impact of Agricultural Land Management on Soil Bacterial Community: A Case Study in the Mediterranean Area

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Abstract Soil is a complex and dynamic ecosystem whose functionality is related to the equilibrium existing among chemical, physical and biological parameters and the resident microbial communities. Soil microorganisms play a central role in decomposing organic matter, in determining the release of mineral nutrients, and in nutrient cycling, and have direct and indirect effects on both crop growth and quality, as well as on the sustainability of soil productivity. In addition, soil microorganisms substantially contribute to the resistance and resilience of agro-ecosystems to abiotic disturbance and stress. Therefore, changes in microbial communities may directly affect soil ecosystem function since microbes can respond rapidly to environmental changes because of the vastness of microbial biomass and diversity. An increasing number of studies have shown how environmental impacts that cause modifications in microbial community structure and diversity ultimately affect soil biological processes. Agricultural land management is one of most significant anthropogenic activities that substantially alter soil characteristics, including physical, chemical, and biological properties. The present chapter gives a picture of the effect of different agricultural management practices on soil microbial community structure and function. A case study on the effects of tillage and nitrogen fertilization on soil bacterial community structure is also reported.

Keywords Microbial diversity • Culture-based methods • Function • Mediterranean area • Land management • Nitrogen fertilization • Soil • Microbial community structure • Unculture-based methods

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5.1 Introduction

Soil is the outermost weathered layer of the earth's crust and supports all terrestrial life forms. It is a complex, heterogeneous and dynamic system where lives a large variety of microorganisms including bacteria, archaea, fungi, yeasts, microalgae and protozoa. The soil microbial community is highly heterogeneous with arguably the highest level of prokaryotic diversity of any environment (Delmont et al. 2011). The balance of chemical, physical and biological (including microbial) components contribute to maintaining soil health. In some instances, detectable changes in soil physical and chemical properties can follow changes in microbial populations and/or activity, thereby providing an early evidence/warning of soil improvement or degradation, and making soil microorganisms excellent indicators of soil health assessment (Nielsen and Winding 2002).

Soil biological activity is mainly concentrated in the topsoil, the depth of which may vary from a few to 30 cm. The biological components (plants roots and soil organisms) occupy a little fraction (<0.5%) of the total soil volume with soil microorganisms being responsible for a large part of biological activity. Soil microbes play an active role in maintaining soil fertility and recycling of nutrients, since they are responsible for the decomposition of the organic matter entering the soil and are involved in the main biogeochemical cycles, improving plant health and contributing to higher crop yield (Aislabie and Deslippeet 2013). In fact, certain soil microorganisms produce compounds that stimulate the natural defense mechanisms of plants, improving resistance to pathogens. The involvement of microorganisms in many soil processes makes them suitable to give an integrated measure of soil quality and health, which cannot be obtained with physical/chemical measures alone.

Anthropogenic activities such as those including agricultural management practices alter physical and chemical soil properties, which directly affect microbial life strategies and bacterial community composition. To detect the effects that differing land uses and management strategies undoubtedly have on soil microbial communities, several techniques are applied, ranging from culture-based to culture-independent methods that offer new insights into the phylogenetic and functional diversity of microbial assemblages (Tilston et al. 2010).

In this chapter, an emphasis is given to soil microbial composition and function, with a focus on the response of soil bacterial communities to different agricultural land management options. The ability of an ecosystem to withstand severe disturbances may partly depend on its microbial components. Then, the assessment of bacterial community composition and structure is at the basis of better understanding their resistance and resilience, and permits to predict the response of soil bacteria to disturbance and to manipulate ecosystem processes (Bevivino et al. 2014; Shade et al. 2012; De Vries and Shade 2013). Finally, a case study of changes in the eco-physiological diversity of soil bacteria communities in an agricultural soil under long-term tillage system and nitrogen fertilizer application is reported.

5.2 Composition and Function of Microbial Communities in Soil Ecosystems

Soils harbor highly diverse communities of microorganisms (microbiota) which include members of each of the three domains of life, *Bacteria* and *Archaea* (both Prokaryotes), and fungi among *Eukarya*. Soil microbes are the dominant and highly diverse form of life in the soil making up 75–90% of living biomass. It has been estimated that every gram of soil contains up to 10 billion microorganisms (Delmont et al. 2011) with an estimated value ranging from 10^3 to 10^8 (bulk soil) up to 10^{11} (rhizosphere) prokaryotic cells (Sikorski 2015). The numbers of *Archaea* may be one to two order of magnitude below the numbers reached by bacteria, which have so far been the most extensively studied.

Taxonomic diversity of soil microbial communities is mirrored by the diversity of their protein-encoded functions, encompassing a seemingly limitless array of physiologies and life history strategies (Fierer et al. 2012). The high heterogeneity of soil environment represents the primary factor driving the enormous diversity of soil microbial life; i.e., the different components of the solid fractions in soil (sand, silt, clay, and organic matter) provide myriads of different microhabitats (niches) that differentially select bacteria and/or fungal types. Also, horizon development with soil depth provides a readily observable change in important soil characteristics that are known to have a profound effect on soil properties and in microbial community composition (Michel and Williams 2011). Microbial communities change along the soil depth profile due to the different environment of each horizon concerning nutrient and water availability, soil structure, organic matter content, pH, temperature, and oxygen. Even if microbes exist throughout the soil profile, they are mostly abundant in surface soils, plants rhizosphere, and around macropores.

Bulk soil is characterized by oligotrophic, carbon-limited environments, whilst rhizosphere soil is rich in organic nutrients. The higher microbial biomass of rhizosphere soil in respect to bulk soil is likely due to the greater availability of substrates for microbial growth. In fact, actively growing roots secrete a diverse array of organic root exudates that stimulate the growth of microbial populations present at the soil-root interface (i.e., rhizosphere). Then, the structure of soil microbial community is distinct from that in the bulk soil, with an increased presence of bacteria establishing positive interactions with plant roots, such as plant growth promoting rhizobacteria (PGPR). The vast majority of soil microorganisms are heterotrophic bacteria which rely on organic matter for energy and nutrients (Aislabie and Deslippeet 2013). Based on life strategies, they can be divided into *r*-strategists or zymogenous (microorganisms that rapidly grow in response to availability of a resource/substrate, being able to colonize unstable ecosystem) and *K*-strategists or autochthonous (microorganisms that use the resources slowly but more efficiently, being persistent in stable/mature ecosystems) (Pianka 1970).

Basically, the *r*-strategists are Gram-negative bacteria and also considered to be opportunistic since they require access to readily available organic matter, while *K*-strategists, which are able to survive with a low supply of nutrients, are mostly Gram-positive bacteria (Van Elsas et al. 2007). It is well known that many oligotrophic microorganisms (such as many members of the *Acidobacteria*) may be *r*-strategists, while many copiothrophic microorganisms (such as many members of the *β-Proteobacteria* and *Bacteroidetes*) may be *K*-strategists. This classification is useful for understanding soil C dynamics but cannot be related directly to a particular taxon since microorganisms can switch growth strategies in a complex environment such the soil. The same soil bacteria can alternate between these two states, depending on the nutrient supply resulting in a reversible transition between the *r* and *K* states (Stenström et al. 2001). As suggested by Dorodnikov et al. (2009), changes in growth rates of the whole microbial community after amendment with easily available substrates can reflect the shift toward *r* or *K* types. To provide new insights into the taxonomic and functional diversity of soil microorganisms it is necessary to analyze in depth the population structure, determining the number of species (taxa) present (richness) and their relative abundance, i.e. the distribution of individuals within the various species (evenness), and their physiological role in connection with the environment and other microorganisms (Tiedje et al. 1999).

Currently, the majority of soil microorganisms cannot be cultured via traditional laboratory techniques and can only be investigated by using molecular methods. Before the exploring of the taxonomic diversity and composition of soil microbial communities using polymerase chain reaction (PCR)-based approaches, *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Micromonospora*, *Nocardia*, *Pseudomonas* and *Streptomyces* were considered to be the principal genera of soil bacteria, based on cultivation studies. Following the application of molecular ecological methods, that have allowed cultivation-independent investigations of soil microbial communities, members of the above nine genera together make up only 2.5 to 3.2% of soil bacteria (Janssen 2006). The dominant phyla in the libraries were *Proteobacteria*, *Acidobacteria, Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes, Chloroflexi, Planctomycetes*, *Gemmatimonadetes* and *Firmicutes*, with members of the two first phyla representing the most abundant soil bacteria (making-up an average of 39% and 20% of libraries derived from soil bacterial communities, respectively). Otherwise, members of *Bacteroidetes, Firmicutes,* and *Planctomycetes,* are less abundant, with an average of 7%, 5% and 2% respectively (Janssen 2006). The majority (79 to 89%) of 16S rRNA gene sequences were from bacteria that were not affiliated with known genera, and some of these were associated with well-studied lineages of bacteria, such as *Actinobacteridae, Flavobacteria*, *Sphingobacteria*, *Bacilli*, *Clostridia*, *a-Proteobacteria*, *β-Proteobacteria*, *γ-Proteobacteria* and δ-*Proteobacteria.*

Soil microbes play diverse and often critical functions in soil ecosystems. The roles of soil microbes are highlighted in the cycling of the main biological elements (C, N, P), in the recycling of wastes, and the detoxification of environmental pollutants. Soil microbes contribute to soil formation through nutrient cycling and organic matter production, can support plant growth through increasing nutrient availability

and by outcompeting invading pathogens, maintain soil fertility trough recycling nutrients, modulate the carbon storage capacity by mineralizing soil carbon and nutrients, and serve as repositories of genetic information, providing ecosystem services which are fundamental for human persistence (Aislabie and Deslippeet 2013).

In the last decade, the application of metagenomic approaches permitted to analyze microbial diversity and identify metabolic pathways and catalytic potential of the complex soil microbial communities, providing insight into the long-standing questions of "who's there?", "what are they doing?", "how sensitive are soil microbial communities to changing agriculture management and/or land use?", and "what are the dynamics of microbial communities in space and time?" (Myrold et al. 2014; Nesme et al. 2016). However, the large majority of metagenomic studies often measures the abundance and diversity of functional groups or genes associated with few relevant functions but this information is not always accompanied by the characterization of the new species' niche spaces. Microbial systems are responsible for the provision of a wide range of crucial ecosystem services, but little is known about the role of diversity in maintaining this function (Jurburg and Salles 2015). Furthermore, the link between phylogeny and function is often cut for prokaryotes, where horizontal gene transfer allows for the acquisition of functions associated with adaptability to new environments. Indeed, the ecosystem function of soil microbial communities demands much attention.

5.3 Agricultural Land Management Land Use and Soil Microbiota

Agricultural land management includes some of the most significant anthropogenic activities that alter soil physical, chemical, and biological properties, profoundly affecting microbial metabolism and survivor. The different members of a defined microbial community respond to these stresses with changes in population structure that further influence the ecosystem so that, ultimately, modification will be permanent with repercussions on the whole system, including plants and mammalians. In this scenario, the knowledge of structure dynamics of soil microbiota makes them useful as early indicators of soil quality and ecosystem stability concerning different soil uses.

5.3.1 Different Land Uses and Agriculture Managements

Land-use intensification includes the main drivers of biodiversity changes in soil ecosystems. Different managements and land use changes, such as the conversion of forest and pasture into cropped land, have ecosystem-scale impacts on soil cycling of organic compounds, biodiversity and soil nutrient dynamics (Bevivino et al. 2014; Dupouey et al. 2002; Houghton and Goodale 2004; Parfitt et al. 2003)

that result in shifts in composition and function of soil microbial communities. Agricultural practices regarding soil nutrient management such as fertilization, tillage, agronomic practices and land uses are below described.

Chemical fertilizers (nitrogen, phosphorus, and potassium) enhance crop yield but also may result in shifts in the functionality and quality of soils by directly or indirectly changing their physical and chemical properties, and structure and diversity of microbial populations. A strict connection between soil microbial communities and the levels of phosphorus and soil moisture as well as their enzymatic activities has been widely described. Luo et al. (2016) suggested that microbes play important roles in determining the fertility of nitrogen-free fertilizer rice soils. In addition, functional diversity and evenness of soil bacterial communities were significantly higher in organically cultivated land than in chemically fertilized soil and fallow grassland, suggesting an improvement in soil quality (Chaundry et al. 2012). Although previous studies have examined well the response of soil microbial communities to single chemical fertilizer, only very few studies have explored the impact of the combination of chemical fertilizers on soil microbial biomass and composition suggesting that both N and P additions had different effects on soil microbial community and, thus, probably altered ecosystem functioning (Li et al. 2015).

Tillage is a common agricultural practice affecting soil structure -increases in bulk density and decreases in porosity-, and therefore biological properties. So, intensive tillage reduced the bacterial diversity due to the interruption of physical diversity of the soil environment (Acosta-Martínez et al. 2011), with negative consequences on the recycling of nutrients and proper balance among organic matter, soil organisms and plant diversity, that are necessary components of a productive and ecologically balanced soil environment (Hendrix et al. 1990). The ecological impact of management practices has been demonstrated as being a consistent source of disturbance on soil ecosystems (Hobbs and Hueneke 1992). Cropping system profoundly affects soil microbial biomass. So, microbial C and N and enzyme activities can be more affected by crop rotation than tillage management, as observed with sorghum in sandy soil in a semi-arid region where dry-land cropping system was required for a sustainable agricultural production (Acosta-Martínez et al. 2011).

In general, continuous monoculture systems tend to reduce soil organic matter due to low organic inputs and disturbance from tillage practices while crop rotations have positive effects on soil properties related to the higher C inputs and diversity of plant residues returned to soils in comparison with continuous systems (Miller and Dick 1995). For instance, integrated cotton (*Gossypiumhirsutum*) cropping and livestock production systems in West Texas were shown to provide more sustainable alternatives to the traditional continuous cotton system and improve soil quality (Acosta-Martínez et al. 2004). Organic and reduced-tillage management systems are aimed at favoring greater increases in soil microbial biomass and more diverse microbial communities with higher substrate utilization efficiency than with conventional management (Ghimire et al. 2014). In general, the root exudation produced by crops used in rotation systems varies with plant age and genotype, and consequently specific microorganisms respond and interact with different host

plants, so explaining the effects of crop rotation and change in land-use on soil on soil microbial communities (Bergsma-Vlami et al. 2005; Chiarini et al. 1998; Dalmastri et al. 1999; Di Cello et al. 1997; Ramachandran et al. 2011).

Changes in land-use practices impact soil microbial community structure (Lauber et al. 2013). When forests are converted to grasslands, and grasslands turned into agricultural lands, a significant effect on chemical and structural composition of soil organic matter, as well as a sharp switch from one type of soil microbial community to another one occurred. For instance, in a typical Mediterranean ecosystem, dominated by *Quercus suber L.*, the microbial composition was found to change in response to five different land uses along with seasonal changes (Bevivino et al. 2014). Change in land use not only alters the taxonomic structure of soil microbial communities, but also their functional gene composition, as also reported in a study on the Amazon rainforest (Paula et al. 2014). Forest to pasture conversion varied the diversity significantly across functional gene groups, with genes linked to carbon and nitrogen cycling mostly altered, raising concerns about impacts of land use change at an ecosystem scale. Then, land-use type and, in particular, differences in vegetation dynamics have a role in modulating the temporal variability in soil bacterial communities (Lauber et al. 2013). Microbial communities result to be very different between managed and unmanaged agricultural systems. Conversion of the tidal wetlands into agricultural land was followed by a significant increase in microbial biomass and changes in diversity patterns that were more pronounced than those in functional gene abundances (Bannert et al. 2011).

In summary, different land uses and agriculture managements can cause decreased microbial production and biodiversity changes in microbial community composition, which can be seen even decades after the anthropogenic disturbance (Atlas et al. 1991; Buckley and Schmidt 2003).

5.3.2 How Microbes Respond to Land Uses

Environmental disturbances in general, and in particular different land uses and agriculture treatments as seen above, alter the microbial community composition, and land-use change is considered one of the main drivers of biodiversity changes in grassland ecosystems. So, it is crucial to understand how soil microorganisms respond to anthropogenic disturbance.

Responses of microbial populations to disturbances may include resistance or resilience (Griffiths et al. 2001; Westergaard et al. 2001). When the microbial community is not altered after a disturbance, it is considered to be resistant. Otherwise, when the community changes, but recovers and returns to the original state, it is deemed to be resilient. Soil microbiota response to land-use changes is correlated to microbe growth strategy since *r*-strategists require access to readily available organic matter, while *K*-strategists are able to survive with a low supply of nutrients (Van Elsas et al. 2007).

When toxic stress occurs in an ecosystem, the functions can be maintained by the replacement of sensitive with tolerant populations. Indeed, microbial communities may change in composition without any effects on microbial processes, since different microbial groups carry out the same functions, a phenomenon known as "functional redundancy" (Allison and Martiny 2008). The fact that ecological functions of different bacteria may overlap in the community (Botton et al. 2006) favors the persistence of the same functions even if changes occur, thanks to the activity of microbes which are resistant to changes/stress. Indeed, it is not the species composition but the functional aspect of the microbial community that is critical to maintain (Øvreås 2000). Therefore, diversity represents a large resource in the soil as well as in any natural habitat, and diverse communities are considered to be more resistant to environmental disturbances. However, the great diversity does not necessarily ensure functional stability in microbial communities (Peterson et al. 1998). In most cases, perturbations can alter microbial community structure resulting in permanent changes in microbial processes like nutrient cycling, decomposition, and energy flow. Substantially, any shifts in population structure will have consequences on ecosystem function, when the tolerant microorganisms fail to compensate for biogeochemical functions usually carried out by inhibited or eliminated microbial groups (Widenfalk et al. 2008).

In general, bacterial diversity is reduced by agricultural and management practices such as continuous monoculture, which can reduce soil organic matter because of low organic inputs systems (Miller and Dick 1995), and tillage (Acosta-Martínez et al. 2010). Soil bacterial communities showed a more stable structure in soils subjected to low human inputs (cork-oak forest and pasture) than in those with high human inputs (vineyards and managed meadow) (Bevivino et al. 2014). Differences in class composition across the site were also observed suggesting that the microbial composition changes in response to land use. In fact, seven classes (α, β and γ-*Proteobacteria*, *Sphingobacteria*, *Flavobacteria*, *Actinobacteria* and *Bacilli*) were present in the vineyard, cork-oak forest and pasture soils, while all classes but α-*Proteobacteria* in tillage vineyard and all classes but *Sphingobacteria* in managed meadow were found.

Investigation of bacterial and archaeal communities involved in inorganic nitrogen turnover in a study concerning the conversion of tidal wetlands into agricultural land for rice cultivation, showed higher abundances of ammonia-oxidizing microbes in the tidal wetland whereas fifty years of paddy management resulted in an increase of nitrogen-fixing bacteria (Bannert et al. 2011). Conversely, the functional diversity of denitrifying strains isolated from various rice paddy soils was minimally affected by crop rotation (Tago et al. 2011). The genus *Pseudogulbenkiania* was dominant at all locations, suggesting that *Pseudogulbenkiania* denitrifiers are ubiquitous in various rice paddy soils. Similarly, potential denitrifying activity was similar among the strains, regardless of the differences in taxonomic position and soil of origin (continuous cultivation of rice vs. rotational cultivation of rice and soybean), indicating that soil and other environmental factors, excluding cropping systems, could select for N_2-producing denitrifiers.

Also, organic amendments were found to influence the functional diversity and community structure in the soil enhancing soil suppressiveness and crop yield (Bonanomi et al. 2014; Pane et al. 2013). Diverse groups of microorganisms such as *Proteobacteria*, *Bacteroidetes* and *Gemmatimonadetes* were activated by its use (Chaudhry et al. 2012). Furthermore, an increase in *Proteobacteria* and *Ascomycota* groups and a reduction in *Acidobacteria* and *Mortierellales* were observed in soil subjected to composted almond shells as organic amendment. Also, genes related to the carbon cycle and other important soil processes had a higher significant relative abundance for amended soils as revealed by the functional GeoChip analysis (Vida et al. 2016). Interestingly, a group of specific probes included in the "soil benefit" category was found to be present only in almond shells-amended soils, corresponding to specific microorganisms described as potential biocontrol agents, such as *Pseudomonas* spp., *Burkholderia* spp., or *Actinobacteria.*

Finally, a decrease in both methanotroph (CH_4-oxidizing bacteria) diversity and methane consumption was observed in soils managed for row-crop agriculture when compared with native deciduous forests and never tilled soils managed as grasslands (Levine et al. 2011). This supports the fact that one of the most important drivers of species loss in terrestrial ecosystems worldwide is the simplification of ecosystem structure due to intensified land use (Sala et al. 2000). Conversely, a similar relationship between soil respiration and bacterial richness was not found, consistent with the prediction that microbial diversity is more likely to be important in specialized metabolic processes rather than in broadly distributed types of metabolism and that specialized processes are better targets for microbial mediation.

5.3.3 Detection of Microbial Response

Since the ability of soil ecosystem to withstand serious disturbances partly depends on its microbial components, characterizing bacterial community composition and structure will help to better understand and manipulate ecosystem processes.

Different approaches can be followed for studying diversity and community structure and evaluating dynamics processes at a global level or at the level of distinct taxonomic groups, for identification and typing, and for functional characterization. First of all, we need to distinguish between culture-based techniques and molecular assays which do not require cultivation. Culture-based techniques such as traditional plate counting can be used to enumerate microbial cells in a sample but they are limited by the fact that around 99–99.9% of the total microscopically countable bacterial cells in one gram of soil is not cultivable by standard culturing techniques (Hugenholz and Tyson 2008; Torsvik and Øvreås 2002). This is what has been described as "The Great Plate Count Anomaly" (Staley and Konopka 1985). The bacterial colony development method was used to determine eco-physiological differences in microbial communities in soil (De Leij et al. 1994) by using the concept of *r/K* strategy that attempts to explain changes in microbial community structure, including microbial shifts from *r*-strategists (copiotrophs) to *K*-strategists (oligotrophs).

To characterize the microbial communities, the ecophysiological index (EP, a modification of the Shannon diversity index) was calculated as described by De Leij et al. (1994). Indeed, based on these techniques, some studies on factors affecting soil communities were previously performed (Bevivino et al. 2014; Krzyżak et al. 2013; Papaleo et al. 2015).

One of the widely used culture-dependent methods is the community-level physiological profiles (CLPP) by the BIOLOG system, i.e. the Biolog Eco-Plates™ to measure the carbon substrate utilization patterns of microbial communities. Even if this culture-based method is more useful for comparing the general structure and the functional potential of soil microbial communities than for community characterization (Garland 1997; Preston-Mafham et al. 2002), it provides an exciting opportunity to overcome the drawbacks of conventional time consuming culture-based analyses or biochemical tests.

To overcome problems associated with non-culturable bacteria, various methods have been developed (Kirk et al. 2004). Detection of soil microbiota changes is currently performed by means of genetic fingerprinting methods that generate a profile of microbial communities based on direct analysis of PCR products amplified from soil DNA (Rastogi and Sani 2011). These techniques include denaturing-gradient and temperature-gradient gel electrophoresis (DGGE/TGGE), single-strand conformation polymorphism (SSCP), random amplified polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (ARDRA), terminal restriction fragment length polymorphism (T-RFLP), length heterogeneity PCR (LH-PCR), and ribosomal intergenic spacer analysis (RISA), and produce a community fingerprint based on either sequence or length polymorphism. They all make possible to assess the association between molecular community fingerprints and environmental changes, and to follow the microbial community responses to a defined stress. Molecular analysis performed on total 16S rDNA present in the population furnishes a picture that is characteristic of defined conditions and permits to detect rapid changes as response to disturbances. Similarly to ecophysiological indexes, molecular indexes (Richness index, R; Shannon-Weaver index of general diversity, H'; Simpson index of dominance, D) are applied to elaborate profiles obtained in the different situations (Bevivino et al. 2014). The recent developments in new sequencing chemistries, bioinformatics, and instruments technologies have revolutionized the field of soil microbial ecology, providing researchers with the ability to assess bacterial diversity at lower costs, and quicker turnaround than prior 16S rRNA and sequencing methods (Shange et al. 2012). Barcode sequencing of 16S rDNA performed on total microbial DNA from soil samples enables to evaluate the diversity based on Operation Taxonomic Units (OTUs) composition as well as to assess their taxonomic status, and to further individuate molecular markers of changing.

Understanding how microbial communities function in natural environments is a central goal in microbial ecology. Given that metabolic function of indigenous microbes are essential to guarantee soil wellness and resilience, it is fundamental their knowledge also in the perspective of a correct soil management in agriculture. In addition to the conventional biochemical analysis of metabolites/enzymes,

investigation of functional gene composition can be performed in soil population (Paula et al. 2014). Indeed, the currently used enzymatic activity measurements are not able to provide either the taxonomic information from the microbial community or probe the function from a specific microbial group (Luo et al. 2016). Analysis of total RNA extracted from environmental samples enables to assess microbial populations reaction at the transcription level providing more valuable information than DNA in revealing active microbial communities versus dormant microbial communities (Torsvik et al., 2002). This kind of analysis put in evidence changes in the expression/production of metabolites/proteins but does not permit to assess if changes occurred at the taxonomic level. Therefore, different approaches are needed to discover if new/different bacteria (taxa) get the better of previous ones providing for maintaining functional processes (resilience).

Comparative analysis of results obtained by 16S rDNA and functional genes investigation will enable to assess shifts occurring in taxonomic composition and responsible of functional changes. The development in metagenomic approach (which investigate the collective microbial genomes retrieved directly from environmental samples) open the way to previously unknown scenarios to detect microbial activities in microbes without requiring their cultivation since gives the possibility to analyze the meta-community dynamics and to identify markers of soil microbiota changes in response to the different land uses and treatments (Hugenholtz and Tyson 2008). In spite of the trend to give up traditional methods for molecular approaches, their importance cannot be forgotten since a profound knowledge of microbial ecosystem requires the ability to cultivate the resident microorganisms for studying individual strains given their possible application as soil status indicators. So, data from microbiota analysis will be helpful also in the perspective to improve cultivation techniques.

5.4 A Case Study in the Mediterranean Area

5.4.1 Introduction

Tillage practice represents one of the main factor affecting soil microbial community structure and activity. The altered soil physical and chemical conditions under conservation tillage result in significantly higher soil organic matter contents compared with conventionally tilled soils, then increasing microbial population and activity as well as microbial biomass (Mathew et al. 2012).

In the present work, we aimed at assessing the impact of applying no tillage (NT) compared with conventional tillage (CT), along with two fertilizer treatments on silty clay soil bacterial communities in a rain-fed *Triticum durum* and *Zea mays* rotation under temperate sub-Mediterranean conditions in a silty clay soil, in which crop residues were left on the soil (NT) or incorporated (CT). We considered the relative abundance of *r*- and *K*-strategists to assess the resistance and resilience of microbial communities in respect to changes due to soil management. The central

hypothesis was that long-term use of no-tillage practices along with nitrogen fertilizer application would cause shifts in soil microbial community structure relative to conventional tillage practices.

5.4.2 Experimental Design and Methods

Sampling was performed at the "Pasquale Rosati" experimental farm of the Polytechnic University of Marche in Agugliano (AN) (43° 32′ N, 13° 22′E, 100 m a.s.l.) in the coastal hills of Marche, Italy (De Sanctis et al. 2012; Seddaiu et al. 2016). The Agugliano soil is a Calcaric Gleyic Cambisol with 20% slope, which in the first 30 cm has an Ap horizon (that is the homogeneous layer due to plowing) and a clay-silt texture (Lagomarsino et al. 2009).

The soil sampling was carried out in the frame of a National Research Project (SOILSINK Project) and soils used in this study were collected under consent of the landowners.

A split-plot with randomized blocks was designed to compare CT system (40 cm deep ploughing) with NT (sod seeding with chemical desiccation and chopping) and two fertilizer treatments (70 Kg/ha P_2O_5 with or without 90 Kg/ha NH_4NO_3) (Fig. 5.1). The soil samples were collected during wheat (*Triticum durum* L.) in autumn (October) in rotation with maize (*Zea mays* L.) in late spring (June). Five randomly replicates at each season and for each soil type (each one as a composite sample of 5 soil cores) were performed for a total of forty soil samples (5 replicates × 4 soil types × 2 seasons). After removal of litter layer, soil core samples (50 to 100 g; diameter, 5 cm) were taken at 20 cm depth, using a 5-on-dice sampling pattern

Fig. 5.1 The experimental area in the "Pasquale Rosati" experimental farm in Agugliano, Italy (gently provided by Prof. Pier Paolo Roggero and colleagues in the frame of the SOILSINK Project)

Table 5.1 Physical soil characteristics

Wheat-maize rotation[a]	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Soil texture	Soil (pH)	Field capacity (% vol)
NT-0	105	512	383	Silty-clay	8.4	41.9
NT-90	103	403	494	Clay	8.4	42.5
CT-0	248	373	379	Clay loam	8.1	41.1
CT-90	249	353	398	Clay	8.1	41.7

[a]*CT* conventional tillage, *NT* no tillage; 0, without nitrogen fertilization; 90, with nitrogen fertilization (Pastorelli et al. 2010; Landi et al. 2011)

Table 5.2 Chemical soil characteristics

Wheat-maize rotation	C org (g/kg)	N tot (g/kg)	Nitric N (mg/kg)	OM	C/N	CEC (Meq/100g)
NT-0	5.0	0.8	2.05	1.18	7.82	24.07
NT-90	7.1	0.9	2.43	1.13	8.09	22.51
CT-0	11.8	1.4	1.36	1.78	8.43	23.28
CT-90	12.7	1.4	1.41	2.01	8.67	21.7

C org total organic carbon, *N tot* total nitrogen, *OM* organic matter, *CEC* cation exchange capacity

with ca. 70 m distance between each sampling point. Soil samples were immediately sieved (<2 mm) to remove fine roots and large organic debris, air dried and transported to the labs for microbiological analysis. The soil physical and chemical characteristics of the experimental areas are reported in Tables 5.1 and 5.2.

All soils had similar texture (clay soil), pH soil (8.1-8.4) and low C/N ratio (7.82-8.67). Differences were present in organic matter and nitrogen amounts; in particular, NT plots had a higher amount of organic matter and N-tot (1.4 g/kg) than CT plots, while nitric N was higher in CT soils than NT soils.

Soil bacterial community analysis was performed on five soil replicates for each treatment. Bacterial cell extraction was performed according to the recommendations of Smalla et al. (2001) with minor modifications as reported in Bevivino et al. (2014).

To determine the changes in the structure of cultivable fraction of soil bacteria, the *r/K*-strategy concept proposed by De Leij et al. (1994) was used. Colonies were enumerated at 1, 2 and 6 days of growth on 0.1 Tryptic Soy Agar (TSA), generating three counts (classes) per sample (C1, C2, C3).

The biodiversity of soil bacterial populations was investigated by using the Eco-Physiological (EP) index, that is a measure of both richness (i.e. total number of species in the community) and evenness (i.e. how evenly individuals in the community are distributed over the different species) of groups of microorganisms with similar developmental characteristics (De Leij et al. 1994). Bacterial population data (CFU/g of soil) were log transformed and subsequently analysed by one-way ANOVA (GraphPad Prism 7 Software). Percentage data of EP index value were *logit*-transformed, as follows: Logit (p) = log [p/(1-p)] for the proportion *p*, and compared using one-way ANOVA (GraphPad Prism 7 Software).

5.4.3 Results and Discussion

The four differently managed have similar characteristics, such as clay texture, pH and low C/N ratio, while they differ in some parameters resulting from the field's agricultural use. In the two seasons examined, a high diversity of colony morphology was observed in no-tilled system (data not shown).

The total microbial density ranged from 7.89×10^5 to 1.16×10^7cfu g^{-1} of soil. No significant differences were found in total microbial density in respect to both season and soil management (Fig. 5.2). A significant variation of EP-Index was found between N-fertilized and unfertilized soil in conventional tillage (CT) system ($p < 0.05$) while no significant differences in EP-Index were found in respect to both season and soil management (tilled vs. no-tilled soils) ($p > 0.05$) (Fig. 5.2).

In June, conventional tillage system in fertilized soil seemed to favor slow growers (*K*-strategists, oligotrophic bacteria) whilst no-tillage treatment positively affected the fast growers (*r*-strategists, copiotrophic bacteria) (Fig. 5.3). Nitrogen fertilization

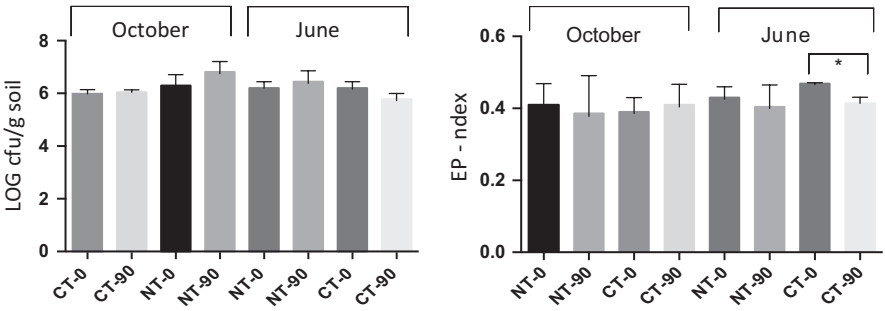

Fig. 5.2 Effect of tillage practices (CT, NT) and two fertilizer treatments [70 Kg/ha P_2O_5 with (90) or without (0) 90 Kg/ha NH_4NO_3)] on total bacteria (*left*) and EP index (*right*) in two seasons

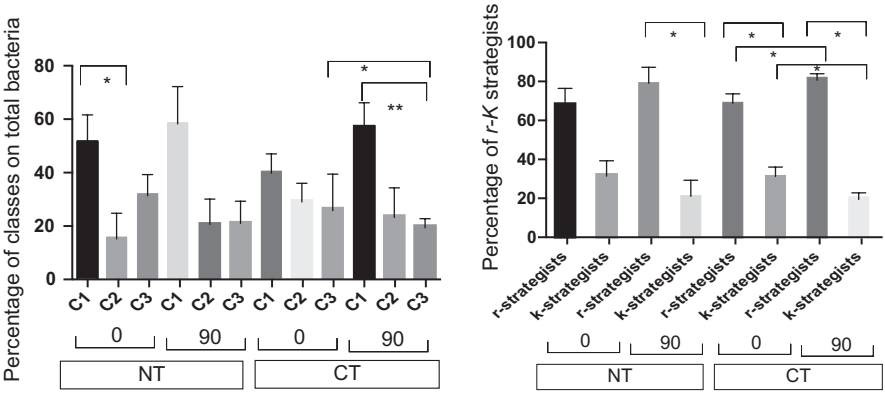

Fig. 5.3 Effect of tillage practices (CT, NT) and two fertilizer treatments (0, 90) on the percentage of C1, C2, C3 classes (left) and on the percentage of *r-K* strategists on total bacteria, in June

significantly affected the abundance of *r*-strategists in CT soils (P = 0.0002); in particular, N-fertilization reduced the abundance of *k*-strategists whilst increased abundance of *r*-strategists.

Overall, the investigation of the eco-physiological diversity of soil bacteria communities in relation to tillage system and nitrogen fertilizer revealed that only N-fertilization affected the relative abundance of *r-K* strategists as well as the evenness of population distribution and richness of the microbial community. We can conclude that N fertilization is able to increase the soil organic carbon level, particularly under conventional tillage, affecting soil microbial community structure. This study demonstrated that tillage systems along with different N fertilization rates influence soil microbial communities. Further research is needed to determine the impact of tillage-driven changes on soil microbial community composition and their dynamics.

5.5 Conclusions

In conclusion, diverse agricultural practices differently impact the microbial community resident in different soils. Given the role of soil microbes in soil safeness and function, there is the need to improve our knowledge about the ecology of microbial populations for proper agriculture management, at both levels of whole population structure and defined taxonomic/functional groups, such as those involved in inorganic nitrogen turnover and biogeochemical cycles. Clearly, a holistic approach taking into consideration all of potential factors and drivers is necessary when examining the structure–function relationships of soil microbial communities to improve understandings of controls and functioning of belowground processes.

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