Long-Term Consequences of Tillage, Organic Amendments, Residue Management and Localized Irrigation on Selected Soil Micro-Flora Groups in a Mediterranean Apricot Orchard

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Abstract

In semi-arid Mediterranean environments, the loss of soil fertility can be avoided through the optimization and innovation of low environmental impact agricultural techniques. Such 'innovative', suitable agricultural management practices can have positive effects on soil microbial communities. The aim of the present study was to explore the effects of two different agricultural systems ('innovative' and 'conventional') on the diversity of important groups of microorganisms related to soil fertility, such as fungi, actinomycetes, *Pseudomonas* spp., *Bacillus* spp., *Azotobacter* spp., proteolytic and ammonifying bacteria. The study was carried out in an apricot orchard located in Southern Italy. Since 2003, half of the orchard was managed adopting no-tillage, cover crops, compost application, drip irrigation, winter pruning and limited mineral fertilization, whereas the other half was subjected to 'conventional' management. Soil samples were randomly collected from the superficial soil layer in four different orchard positions: innovative along the inter-rows (IIR) and under drip emitters (IUE), and conventional along the inter-rows (CIR) and under drip emitters (CUE). Higher populations of total bacteria, actinomycetes and proteolytic bacteria were induced by IIR treatment, whereas *Pseudomonas* spp., *Azotobacter* spp. and ammonifying bacteria showed increased populations in IUE. No differences between the treatments were found for total fungi and *Bacillus* spp. The higher inputs of organic matter of innovative plots caused an increase in the microbial groups responsible for nitrogen metabolism in well-watered zones (IUE), and higher activities of decomposer and humus-forming microorganisms in IIR. Results show that suitable agricultural practices can have positive effects on microbial activities and complexity, which in turn influence soil fertility.

INTRODUCTION

In semi-arid Mediterranean apricot orchards, the loss of soil fertility can be avoided through the optimization and innovation of low environmental impact agricultural techniques. In these environments, agricultural systems based on 'conventional', non-sustainable, techniques often cause impoverishment in soil organic matter, groundwater contamination, soil accumulation of mineral elements (in particular phosphorus and nitrogen), soil salinization and nutritional imbalances in plants (Lal, 1997). In this scenario, suitable agricultural management practices, such as conservation tillage, cover crops, compost amendments, incorporation of crop and pruning residues into the soil, and adequate irrigation, fertilization and pruning, can have positive effects on the activities and complexity of soil microbial communities (Govaerts et al., 2007).

The first layers of the pedosphere are the habitat for a high number of bacterial and fungal communities with a key role in pedogenetic processes and in soil fertility improvement (Brady and Weil, 2008). The use of culture-based microbiological

techniques with specific cultural media allows the isolation of important physiological groups of bacteria related to soil fertility, such as micro-organisms involved in important steps of carbon cycle (such as actinomycetes, *Pseudomonas* spp. and *Bacillus* spp., the major decomposers of complex polymers, such as lignocelluloses and chitin) and nitrogen cycle (nitrogen fixer, proteolytic, ammonifying, nitrifying and denitrifiying bacteria) (Zaitlin et al., 2004). Nitrogen-fixing micro-organisms are able to reduce N≡N to NH_3 for the biosynthesis of organic nitrogen compounds (Brady and Weil, 2008). Proteolytic bacteria are responsible for soil proteins degradation in peptons, peptic acids and, finally, in aminoacids, whereas ammonifying bacteria release ammonium ions (NH_4^+) from nitrogen-containing organic compounds (Brady and Weil, 2008). Moreover, fungi and actinomycetes are able to colonize rhizosphere and use root exudates as 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., 2007).

The aim of the present study was to explore the effects of two different management systems (so called 'innovative' and 'conventional') on the diversity of important groups of microorganisms, on soil microbial communities by using culture-dependent methods. We propose that the correct utilization of 'innovative' and suitable, agricultural techniques and soil management, important for fruit production and quality, can also improve soil quality and fertility.

MATERIALS AND METHODS

The study was carried out in an apricot (*Prunus armeniaca* L.) orchard located in Southern Italy. The soil was a silty-clay loam. Since 2003, half of the orchard ('innovative') was managed adopting no-tillage, cover crops (*Lolium multiflorum* and *Medicago* spp.), compost application (15 t ha^{-1} $year^{-1}$ along the rows), drip irrigation (two drip emitters per plant discharging 10 L h^{-1} each), winter pruning aimed at vegeto-productive equilibrium of plants, limited mineral fertilization and incorporation of pruning residues into the soil. On the contrary, the agronomic practices adopted for the other part of the orchard ('conventional') included soil conventional tillage, chemical fertilization (about 100, 10 and 20 kg ha^{-1} $year^{-1}$ for N, P and K, respectively), complete removal of pruning residues from the field, and empirical irrigation and pruning.

The compost used in the innovative plots (22.2 C/N; Eco-Pol SpA, Verona, Italy) was manually applied in the autumn periods. Endogenous (cover crops, pruning residues) and exogenous (compost) amendments were incorporated yearly into the soil by ploughing (depth 30 cm) in October of each year. Composite samples of bulk soil (20 cm diameter cores per plot, mixed on site) were randomly collected in February 2007 from the upper soil layer (0-15 cm) in four different orchard positions: innovative along the inter-rows (IIR) and under drip emitters (IUE), and conventional along the inter-rows (CIR) and under drip emitters (CUE). Soil samples were immediately stored in sterilized plastic containers at 4°C before analysis.

Soil extracts were obtained by mixing 500 g of soil sample in 1 L of sterile water, incubated at 25°C for 7 days and diluted up to 10^{-9}. For total bacterial count, an aliquot of 1 ml of the dilution was added to 25 ml of a sterilized basic cultural medium containing 1 L soil extract, 1 g D-glucose, 3 g peptone, 1 g yeast extract and 15 g Agar Bactericological. For total fungal count, an aliquot of 1 ml of the dilution (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5}) was added with Yeast Peptone Dextrose medium containing 1 g yeast extract, 2 g D-glucose, 2 g peptone, 15 g agar in 1 L bidistilled water, supplemented with 150 ppm chloramphenicol. Actinomycetes were isolated by using Casein Starch Agar modified (Table 1) supplemented with 120 ppm of cycloehximide. The isolation of *Azotobacter* was carried out with Brown's substrate modified (Table 1), whereas the proteolytic bacteria were identified by MPN method in a cultural medium containing gelatin (Table 1). Ammonifying bacteria were isolated in a liquid cultural medium (Table 1) containing asparagine and incubated at 28°C for 15 days, and the presence of ammonium was revealed by Nessler's reactive. *Pseudomonas* were cultured on Pseudomonas Agar Base medium with the addition of Pseudomonas C-N (Oxoid Ltd., Hampshire, UK). The

isolation of *Bacillus* was carried out in PCA nutritive medium supplemented with KH_2PO_4 and K_2HPO_4, and was performed in diluted suspensions placed in water-bath at 80°C for 10 min, in order to kill termolable non-*Bacillus* microorganisms.

RESULTS AND DISCUSSION

The study of the response of soil microbiota to different agricultural management and the quantitative and qualitative description of soil microbial communities can help to evaluate whether the practices adopted improve soil quality and fertility (Borken et al., 2001; Govaerts et al., 2007). In this experiment, higher populations of total bacteria, actinomycetes and proteolytic bacteria were induced by IIR treatment, whereas *Pseudomonas* spp., *Azotobacter* spp. and ammonifying bacteria increased significantly in IUE plots, if compared to 'conventional' plots (CIR and CUE) (Figs. 1 and 2). The importance of these microbial groups was recently demonstrated in some recent studies. The actinomycetes of the genus *Streptomyces* are much diffused in the soil and synthesize vitamins, siderophores, aminoacids and organic acids, useful for plant growth, and antibiotics, such as streptomycin and chloramphenicol, against some soil-borne root pathogens. Most bacteria of *Pseudomonas* spp. are able to produce siderophores to chelate iron, so promoting its availability for plants (O'Sullivan et al., 1990), and induce resistance against root infections caused by nematodes, pathogenic bacteria and fungal soil-borne pathogens (Knox et al., 2004).

The importance of the adoption of 'innovative' and sustainable soil management systems was pointed out by several recent studies. In a long-term experiment (15 years), Govaerts et al. (2007) demonstrated that a cropping system that combines zero tillage and crop residue retention can increase overall biomass and micro-flora activity and diversity, create conditions favourable for the development of antagonists and predators, and foster ecological stability. Crop residues (shoot and root materials) and soil organic matter (SOM) quality can affect the functional diversity of the soil microbial community evaluated by Biolog® CLPP (Bending et al., 2002). Moreover, Liu et al. (2007) evaluated the positive impact of organic and sustainable management strategies on the diversity of soil bacterial communities and on soil physical, chemical, and biological properties.

The results of this study show that higher inputs of organic matter in innovative plots caused a significant increase in the microbial groups responsible for nitrogen metabolism (*Pseudomonas* spp., *Azotobacter* spp. and ammonifying bacteria) in well-watered zones (IUE), and higher activities of decomposer and humus-forming microorganisms (mainly actinomycetes and proteolytic bacteria) in IIR (Figs. 1 and 2). On the contrary, no differences between 'innovative' and 'conventional' treatments were found for total fungi and *Bacillus* spp. (Fig. 1). Both IUE and IIR plots were supplied with exogenous (compost) and endogenous sources (cover crops and pruning resides) of organic matter. Soil organic matter is a key factor for supplying nutrients, nutrient recycling, improving soil/plant available water reserves, increasing soil buffer capacity and stabilizing soil structure, so enhancing soil quality and fertility (Liu et al., 2007). For all these reasons, soil organic matter is considered as a major component of soil quality because it contributes directly or indirectly to soil physical, chemical and biological properties. Moreover, compost amendments strongly influence soil biological properties in a short term, at a global level as well as at a community level (Pérez-Piqueres et al., 2006). The significant increase in *Pseudomonas* spp., *Azotobacter* spp. and ammonifying bacteria observed in watered 'innovative' plots (IUE), if compared to drier plots (IIR) (Fig. 2), pointed out that soil moisture is another key factor influencing the development of specific microbial groups. Such information is of particular importance for semi-arid orchards with limited water resources, where correct irrigation management and irrigation technique allow to improve irrigation efficiency, save water and maintain top yields of high quality while avoiding nutrients leaching, soil erosion and root asphyxia (Xiloyannis et al., 2004; Dichio et al., 2007).

CONCLUSIONS

A better understanding of soil ecology could lead to identifying agricultural management practices that support and stimulate soil organisms for beneficial purposes in agriculture. Results show that the adoption of 'innovative' and sustainable agricultural practices have positive effects on soil microbiota, which in turn influence soil fertility and plant growth by increasing nutrients availability and turnover. Therefore, it is more convenient to adopt agricultural practices that preserve or restore soil bacterial functional diversity than to adopt practices that diminish it.

ACKNOWLEDGMENTS

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Tables

Table 1. Specific cultural media used for the isolation of bacterial groups.

Actinomycetes	g L^{-1}	Proteolytic	g L^{-1}	Ammonifying	g L^{-1}	Azotobacter	g L^{-1}
Starch	10	$MnSO_4$	0.5	$MnSO_4$	0.25	D-Glucose	5
Casein	0.5	K_2HPO_4	0.20	K_2HPO_4	0.20	K_2HPO_4	0.5
$NaNO_3$	1	KH_2PO_4	0.05	KH_2PO_4	0.05	KH_2PO_4	0.5
NaCl	1.5	$MgSO_4*7H_2O$	0.15	$MgSO_4*7H_2O$	0.10	$MgSO_4*7H_2O$	0.2
K_2HPO_4	1.1	$CuSO_4*5H_2O$	0.02	$CuSO_4*5H_2O$	0.02	$CaCl_2$	0.1
KH_2PO_4	0.5	MnSO4	0.25	NaCl	0.12	NaCl	0.05
$MgSO_4*7H_2O$	0.05	$CaCl_2$	0.1	$FeSO_4*7H_2O$	0.03	$FeSO_4*7H_2O$	0.01
$CaCl_2$	0.01	NaCl	0.12	Asparagine	0.25	Agar	15
Na_2CO_3	0.5	$FeSO_4*7H_2O$	0.03	pH = 6.8±2		pH = 6.8±2	
$FeSO_4*7H_2O$	0.01	Agar	15				
Agar	15	pH = 7.2 ± 2					
pH= 6.8 ± 2							

Figures

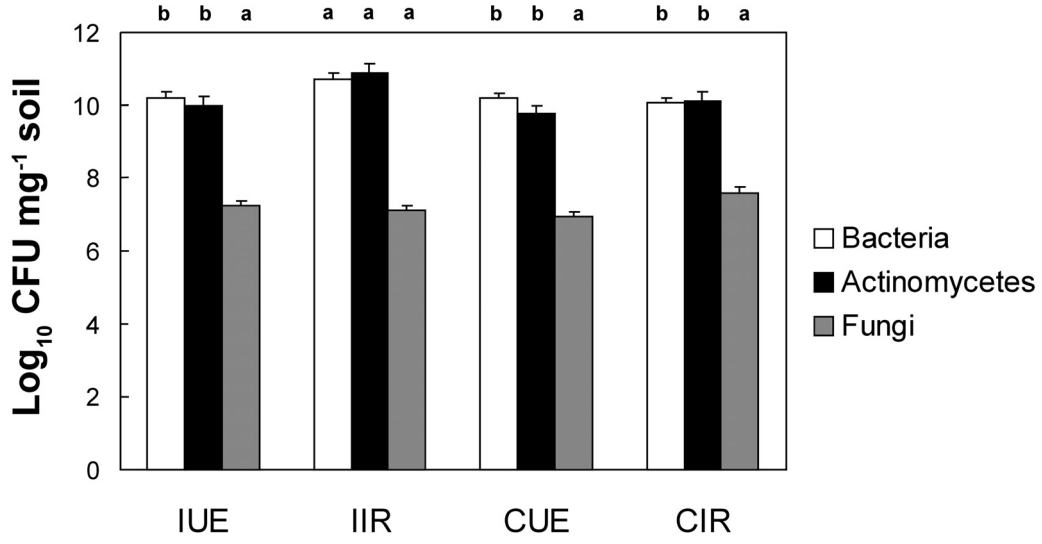

Fig. 1. Total microbial counts (CFU ± SE). Values with different letters (between columns with the same colour) are significantly different (Student's *t*-test; $P \leq 0.05$). IUE = Innovative Under Emitters; IIR = Innovative Inter-Rows; CUE = Conventional Under Emitters; CIR = Conventional Inter-Rows.

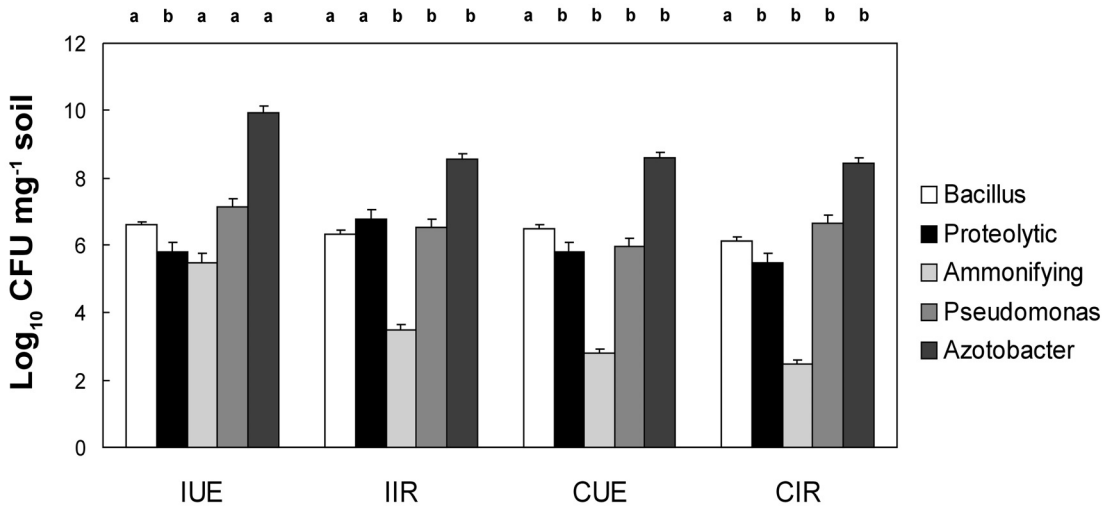

Fig. 2. Specific microbial counts (CFU ± SE). Values with different letters (between columns with the same colour) are significantly different (Student's *t*-test; $P \leq 0.05$). IUE = Innovative Under Emitters; IIR = Innovative Inter-Rows; CUE = Conventional Under Emitters; CIR = Conventional Inter-Rows.