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Arbuscular mycorrhiza, rhizospheric microbe populations and soil enzyme activities in citrus orchards under two types of no-tillage soil management

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Abstract

The arbuscular mycorrhizal (AM) status [total AM colonization (RLT), percentage of root length with arbuscules (RLA) and vesicles (RLV), spore density and hyphal length density], microbial populations and soil enzyme activities were investigated in citrus (Satsuma Mandarin grafted on *Poncirus trifoliata* L. Raf) orchards. Two types of no-tillage soil management, natural grass cover and use of herbicides, were employed in these orchards. The citrus AM colonization (37.26-70.09%) was high in all the experimental orchards sampled. The highest RLA (43.83%), spore density (384.63 spores/100 g soil), hyphal length density (4.09 m g^{-1} soil), rhizospheric microbial populations and enzyme activities were observed in the orchards with a natural grass cover, and the lowest values, except urease activity, were found in the orchards treated with herbicides. Spore density, hyphal length density, catalase activity and phosphatase activity varied notably between no-tillage/natural grass and no-tillage/herbicides treated orchards in the soil layers above 40 cm. A correlation analysis showed that the hyphal length density and organic matter were significantly positively correlated. Soil enzyme activities, except phosphatase, were strongly correlated with the bacteria populations. The data presented here demonstrates that the RLA, spore density, hyphal length density, rhizospheric microbe populations and enzyme activities were significantly better in the soil layers above 40 cm of orchards with a natural grass cover than herbicide-treated soils. So, the establishment of a natural grass cover benefits soil quality in citrus orchards in Southern China.

Additional keywords: AM colonization; AM hyphal length density; AM spore density; natural grass cover; soil bacteria, fungi and actinomycetes; soil catalase, invertase, urease and phosphatase.

Resumen

Micorrizas arbusculares, poblaciones de microbios rizosféricos y actividades enzimáticas del suelo en huertos de cítricos bajo dos tipos de manejo de suelos sin labranza

Se investigó en huertos de mandarino Satsuma injertado sobre *Poncirus trifoliata* L. Raf el estado de las micorrizas arbusculares (AM) [colonización total de AM (RLT), % de longitud de la raíz con arbúsculos (RLA) y vesículas (RLV), densidad de esporas y de hifas], las poblaciones microbianas y las actividades enzimáticas del suelo. En estos huertos se emplea dos tipos de gestión del suelo sin labranza, uno mediante cubierta de césped natural y otro mediante uso de herbicidas. La colonización de AM en la raíz de los cítricos fue alta (37,26-70,09%) en todos los huertos muestreados. Se observó el mayor RLA (43,83%), densidad de esporas (384,63 esporas/100 g de suelo), densidad de longitud de hifas (4,09 m g^{-1} de suelo), poblaciones microbianas rizosféricas y actividades enzimáticas en los huertos con cubierta de césped natural. En las capas del suelo hasta los 40 cm, la densidad de las esporas y de las hifas, y la actividad de la catalasa y de la fosfatasa variaron notablemente entre los dos tratamientos. Un análisis de correlación mostró que la

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densidad de las hifas y la materia orgánica estaban significativamente correlacionadas. Las actividades enzimáticas del suelo, a excepción de la fosfatasa, estuvieron fuertemente correlacionadas con las poblaciones de bacterias. Por lo tanto, la no labranza y el establecimiento de una cobertura de césped beneficia la calidad del suelo en estos huertos de cítricos en el sur de China.

Palabras clave adicionales: bacterias, hongos y actinomicetos del suelo; catalasa, invertasa, ureasa y fosfatasa del suelo; cobertura de césped natural; colonización de micorrizas; densidad de esporas de micorrizas; densidad de hifas de micorrizas.

Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous components of most agroecosystems and inhabit both plant roots and surrounding soils. They can benefit their host plants in several ways, including better uptake of phosphorus nutrition (Toro *et al*., 1998), improving host plant growth and photosynthesis (Shrestha *et al*., 1995; Meir *et al*., 2010), increasing the tolerance to adverse biotic and abiotic factors (Ruíz-Lozano and Azcón, 1995; Pozo *et al*., 1999; Wu *et al*., 2006; Goicoechea *et al*., 2010) and improving soil environment, fertility and quality (Wright and Upadhyaya, 1998; Gaur and Adholeya, 2004; Li *et al*., 2007). The rhizosphere (*i.e*., zone of soil influenced by plant roots and characterized by an important microbiological activity) represents a highly dynamic region governed by a complex mosaic of interactions between plants and micro-organisms (Kennedy and De Luna, 2004). The so-called plant-growth-promoting rhizobacteria (PGPR) can influence plant development not only directly via hormone production, P solubilization or asymbiotic N fixation (Khan, 2005), but also indirectly through influence on other plant-microbe interactions, such as the mycorrhiza or the rhizobium symbioses (Garbaye, 1994; Linderman, 1994; Barea *et al*., 1996). Simultaneously, the formation of the AM symbiosis can change the composition of the microbial community in the rhizospheric soil (Schreiner *et al*., 1997) and its activity (Olsson *et al*., 1996). This change might be a consequence of the competition for energy-rich carbon compounds (Christensen and Jakobsen, 1993) or to an indirect influence through the quantity and quality of plant root exudates and soil structure (Johansson *et al*., 2004).

Soil enzyme activity is often used as an index of soil microbial activity and related to its fertility (Dhruva Kumar *et al*., 1992). Most soil enzymes originate from soil bacteria, fungi, and plants roots (Lee *et al*., 2004) and are closely related to microbial biomass (Frankenberger and Dick, 1983). Changes, accumulation and decomposition of substances in the soils are complicated processes, in which soil enzymes play an important role (Wang *et al*., 2007). Catalase is a common enzyme found most in microorganisms exposed to oxygen (Weigand *et al*., 1995), which can mediate protection against activated oxygen species, like hydrogen peroxide. Invertase reported in plant, bacteria, yeast and filamentous fungi, such as *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus japonicus*, *Aspergillus caespitosus* and *Thermomyces lanuginosus* (Alegre *et al*., 2009) can decompose sucrose into fructose and glucose used as nutrients for plants and soil microorganisms. Urease associated with the soil N cycle bacteria such as ammonifying bacteria (Liu *et al*., 2010) is a nickel-containing enzyme decomposing urea to carbon dioxide and ammonia involved in nitrogen metabolism (Burne and Chen, 2000). Phosphatase, a key enzyme of P-solubilizing bacteria and fungi, hydrolyses organic phosphorus compounds, increasing the pool of available inorganic P that can be absorbed by plants (Pascual *et al*., 2002).

In an agricultural soil, the soil type, the crop's growth stage, management practices (*e.g*. crop rotation, mulching, tillage, and application of fertilizers and pesticides), and other environmental factors (pH, temperature, humidity, etc.) can influence the composition of the microbial community and therefore have an effect on the various enzyme activities in the rhizosphere (Ajwaa *et al*., 1999; Giri *et al*., 2005). Soil disturbance has been shown to reduce the density of AMF spores, species diversity and the length of extraradical

Abbreviations used: ACT (actinomycete); AM (arbuscular mycorrhizal); AMF (arbuscular mycorrhizal fungi); BAC (bacteria); CAT (catalase); FAA (formalin/aceticacid/ethanol); FUN (fungi); HLD (hyphal length density); INV (invertase); NH (no-tillage/herbicide treated); NN (no-tillage/natural grass); OM (soil organic matter); PHO (phosphatase); RLA (percentage of root length with arbuscules); RLT (total AM colonization); RLV (percentage of root length with vesicles); SD (spore density); URE (urease).

mycelium of AMF in comparison to undisturbed soil (Boddington and Dodd, 2000).

Most varieties of citrus have short or even rare root hairs, and are thus dependent on AMF (Wu and Xia, 2006) to achieve a good development and growth. Intercropping citrus trees with a leguminous herb, *Stylosanthes gracilis*, is widely practiced in organic production systems in Southern China to improve soil quality and productivity of citrus plants. AMF have been proposed as a potent biofertilizer in organic agriculture (Yao *et al*., 2008). However, the information concerning AMF and rhizospheric microbes is scarce in cultivated orchard systems, particularly in different types of no-tillage soil management of citrus orchards. In our study, effects of two types of no-tillage soil management, natural grass cover and use of herbicides, on AM colonization in citrus roots, spore density, hyphal length density, microbe populations and enzyme activities in citrus rhizospheric soils were investigated, in order to propose a beneficial soil management practice which can be employed in the citrus orchards in Southern China.

Material and methods

Field experiment

The investigation was conducted in citrus orchards located in hill slopes of Huazhong Agricultural University, Wuhan (113°41′-115°05′ E, 29°58′-31°22′ N) city. This area has a semi-tropical monsoon climate with annual sunlight of 1810-2100 h, frost-free period of about 211-272 days, mean precipitation of 1269 mm, mean temperature of 15.8-17.5°C, mean temperature of 28.8°C and 3°C in July and January, respectively. The no-tillage soil management practice of natural grass cover was done (eliminating competitive weeds, cutting grass to control the grass height and mulching under citrus trees) and was applied continuously for 5 years, while another no-tillage soil management practice of herbicides (*e.g.* Phenmedipham and Paraquat) treatment to suppress weeds was also employed for 5 years as a second treatment. The soil was classified as yellow sandy clay soil (Acrisols in FAO Taxonomy) and biological organic fertilizers (7% N, 4% P_2O_5, 4% K_2O and 20% organic matter) were used in all experimental orchards to preserve the basic soil fertility. The vertical profile of the citrus root distribution was drawn using a modified Oskamp method (1932). Three citrus trees were selected randomly from each type of orchard and a trench was digged (100 × 60 × 60 cm) along the dripping line at a distance of 130 cm from the trunk. The percentage of different diameter roots at each soil depth was calculated based on the formula: (Number of different diameter roots /Number of total roots) × 100 %.

Sample collection

There were five experimental replicated plots where five 15-17 year old uniform and healthy citrus trees (Satsuma Mandarin grafted on *Poncirus trifoliata* L. Raf) with similar growth vigor were selected to sample in each no-tillage type orchard. Fine roots and soils from one tree were sampled at four directions (east, south, west and north) using a soil auger (Φ = 6.8 cm) from three different soil layer depths (0-20, 20-40 and 40-60 cm) in October, 2008; then soils and roots collected from the same plot were separately mixed well. Rhizosphere soils were gathered according to the method of John and Leo (2003). After a gentle shake, soils adhering to roots were considered as rhizosphere soil samples and were stored in sealed sterile plastic bags at 4°C before analysis processing. The AMF spores extraction and soil enzymes soil samples were air-dried for 2 weeks, and then stored at 4°C until analysis. Roots ($\Phi \leq 1$ mm) were carefully washed with tap water to remove soil, chopped into 1 cm long pieces and fixed in formalin/aceticacid/ethanol [(FAA, 13:5:200 (v/v/v)] solution for 24 h, and then stored at 4°C.

Soil assessment

Chemical properties of soils were analyzed using the methods described by Tan (1996). Soil pH was determined by a potentiometric titration method, organic carbon was determined by humid oxidation with K_2CrO_7, total N by the K_2CrO_7-H_2SO_4 digestion method, and the alkali-hydrolyzable N by the alkaline hydrolysis diffusion method. Available P was determined by Olsen method, and available K was extracted with NH_4HCO_3 + DTPA (diethylenetriaminepentaacetic acid) and analyzed using an ICP-AE spectrometer.

Determination of mycorrhizal colonization

The colonization of various AM fungal structures were examined according to Phillips and Hayman

(1970) and Koske and Gemma (1989) under a compound-light microscope (Olympus-BH-2). Fungal colonization was estimated using the magnified intersection method (McGonigle *et al*., 1990). The RLT, RLA, and RLV were quantified by examining 200 intersections per sample.

Quantification of spores and hyphae in the rhizospheric soils

The spores were isolated from soils using the wet sieving and sucrose gradient centrifugation technique (Gerdemann and Nicolson, 1963). The number of AMF spores with intact surface and contents and without parasitism of each sample was counted with the stereoscopic microscope (Tech-XTS-30, Beijing, China). Spores that were tightly grouped in a sporocarp were considered as one unit, as it was difficult to count the number of spores in this case. The spore densities were expressed as the numbers of spores and sporocarps per 100 g dry weight of soil. Soil hyphal length density was determined as described by Bethlenfalvay and Ames (1987). Mycorrhizal and non-mycorrhizal hyphae were distinguished under a compound-light microscope (Olympus-BH-2) according to Miller *et al*. (1995) using similar criteria as for internal hyphae. Before external hyphae stained with 0.05% trypan blue were quantified, considerable time was spent on observing AM hyphae and associated structures (*e.g*., auxiliary cells and spores) found with fresh and stained roots by using the compound microscopes. These hyphae served as valuable morphological references for the scoring of other hyphae. Evidence was provided from previous study that most of the hyphae recovered from a soil growing mycorrhizal plants were mycorrhizal hyphae (Kabir *et al*., 1996). Hyphal lengths were determined with the aid of an ocular micrometer, and the lengths of hyphae per gram of soil dry weight were calculated.

Test of culturable microbes in the rhizospheric soils

The populations of culturable microbes were determined by a plate dilution method (Johnson and Curl, 1972). Aliquots of soil (~ 1 g) were homogenized with 9 mL sterilized distilled water, and shaken for 30 min. Bacteria were grown on beef extract peptone agar (pH 7.2), actinomycetes enumerated using Gause's synthetic agar (pH 7.2), and fungi grown on Martin's agar (containing 30 mg L^{-1} streptomycin and 33.34 mg L^{-1} rose bengal). Fungi and actinomycete plates were incubated at 28°C for 5-7 days, and the bacteria plates at 30°C for 36 h.

Enzyme activities in the rhizospheric soils

The catalase activity was determined by back-titrating residual H_2O_2 with $KMnO_4$ (Ya, 1988). The invertase activity was analyzed following the standard method reported by Zhao and Jiang (1986). The urease activity was determined by the method proposed by Kandeler and Gerber (1988) and Wang *et al*. (2007). The urease activity was measured as mg NH_4-N released g^{-1} dry soil in 24 h with 20 min color development, and then was determined with UV-2450 spectrophotometer at 578 nm. The phosphatase activity was measured as μmol *p*-nitrophenol (PNP) hydrolysed g^{-1} dry soil h^{-1} (Nannipieri *et al*., 1980; Wang *et al*., 2007) using a UV-2450 spectrophotometer (Shimadzu, Japan) at 398 nm.

Statistical analysis

The experimental data were statistically analyzed using an ANOVA and Pearson correlation test with SAS 8.1 software. Data on percentage of AM colonization and citrus roots were transformed by arcsin $x^{1/2}$. Means were compared by least significant difference (LSD) at the 0.05 level. Pearson correlation coefficients were employed to assess the relationships between the total AM colonization (RLT), spore density (SD), hyphal length density (HLD), rizhospheric bacteria (BAC), actinomycetes (ACT) and fungi (FUN) count values, activities of catalase (CAT), invertase (INV), urease (URE), phosphatase (PHO) and soil organic matter content (OM).

Results

Soil chemical properties and citrus root vertical distribution

The OM content at the 0-20 cm soil layer was significantly higher in the NN orchards than in the NH orchards, while the available P and available K contents

Table 1. Chemical properties of the experimental soils at three soil depths (0-20, 20-40, 40-60 cm) in citrus orchards under two different types of soil management: NH, citrus orchards under no-tillage/herbicide treated; NN, citrus orchards under no-tillage/natural grass.

Properties	NH			NN		
	0-20	20-40	40-60	0-20	20-40	40-60
pH value	5.23c	5.41b	5.69a	5.20bc	5.36bc	5.67a
Organic matter (g kg^{-1})	19.35b	15.58c	11.91d	21.73a	15.97c	9.90e
Total N (%)	0.14a	0.12b	0.10c	0.13ab	0.10c	0.07d
Alkali-hydrolyzable N (mg kg^{-1})	140.18a	105.42b	78.66d	136.46a	93.11c	51.91e
Available P (mg kg^{-1})	57.41a	42.97b	14.77d	26.96c	13.43d	4.91e
Available K (mg kg^{-1})	135.27a	104.42b	102.42b	86.28c	77.20d	57.24e

Values in each column followed by different letters are significantly different ($p < 0.05$) according to LSD test.

at the 0-20 cm soil layer were notably lower in the NN orchards than in the NH orchards. The OM, total N, alkali-hydrolyzable N, available P and available K contents decreased with the increase of the soil depth in both types of orchards, while the pH value was in contrary manner (Table 1).

The percentage of citrus roots ($\Phi < 2$ mm) decreased with the increase of the soil depth in both types of orchards (Figs. 1A and 1B). The largest percentage (57.33%) of citrus roots ($\Phi < 2$ mm) found in the 0-20 cm soil layers in the NN orchards was significantly ($p < 0.05$) more than that (45.13%) in the NH orchards. However, in the 40-60 cm soil layers, the percentage of citrus roots ($\Phi < 2$ mm) showed the opposite status which it was significantly ($p < 0.01$) larger in the NH orchards than in the NN orchards. The percentage of citrus roots (2 mm $< \Phi <$ 5 mm) was largest (5.38%) in the 20-40 cm soil layers in the NH orchards, however, there were no significant differences in the corresponding soil layers between the NN orchards and NH orchards. The largest percentage (2.64%) of citrus roots ($\Phi > 5$ mm) was found in 20-40 cm soil layers in NH orchards, and the lowest (0%) in 40-60 cm soil layers in NN orchards (Figs. 1A and 1B).

Arbuscular mycorrhizal colonization, spore density and hyphal length density

Table 2 shows that the RLT ranging from 37.26% to 70.09% was high in all the experimental orchards sampled. The highest RLT was always detected in the 20-40 cm soil layer, and the lowest RLT in the 0-20 cm soil layers in both types of orchards. The RLA changed along with the soil depth with a similar trend to the

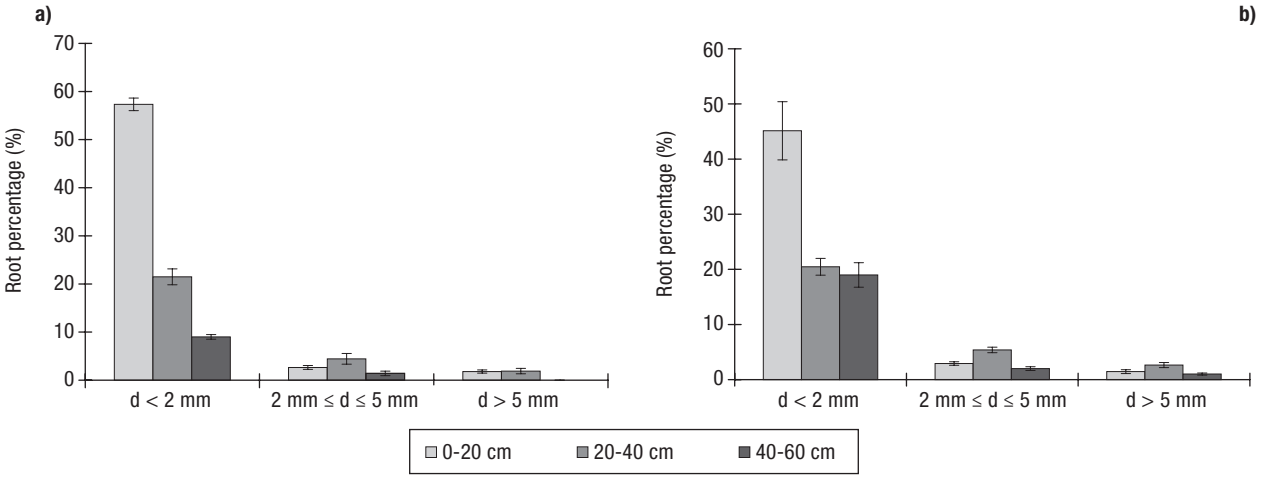

Figure 1. Percentage of different diameter roots at different soil depths in both types of orchards. a) Citrus roots vertically distributed in natural grass orchards; b) Citrus roots vertically distributed in herbicide treated orchards. d: diameter.

Table 2. The AM status (with respect to AM colonization, spore density and hyphal length density) at three soil depths in citrus orchards under two different types of soil management: NH, citrus orchards under no-tillage/herbicide treated; NN, citrus orchards under no-tillage/natural grass

Site abbreviation	Soil depth (cm)	AM colonization (%)			Spore density (spores/100 g soil)	Hyphal length density (m g^{-1} soil)
		Total	Arbuscules	Vesicles		
NH	0-20	37.26c	17.76c	0.52a	238.52c	2.05c
	20-40	70.09a	33.41b	0.51a	167.41d	1.38de
	40-60	51.27b	29.01b	0.33a	151.30d	0.90e
NN	0-20	39.60c	30.34b	0.51a	384.63a	4.09a
	20-40	51.82b	43.83a	0.43a	311.11b	3.05b
	40-60	43.00bc	30.83b	0.33a	148.89d	1.60cd

Values in each column followed by different letters are significantly different ($p < 0.05$) according to LSD test.

RLT observed in two different types of orchards, and the highest RLA (43.83%) was detected in the NN orchard. Additionally, the RLA in the soil layers above 40 cm was significantly higher in the NN orchards than in the corresponding layers in the NH orchards. Although no significant differences in the RLV were observed among the three layers, they decreased with increasing of soil depth in both types of orchards.

The spore density also decreased with the increase of soil depth in both types of orchards. In the NH orchards, the spore density in the 0-20 cm soil layer was significantly higher than in other soil layers. The spore density showed significant differences among the three soil layers in the NN orchards. The highest spore density (384.63 spores/100 g soil) detected in the 0-20 cm soil layer in the NN orchards was significantly higher than that in the corresponding layerChanges in the hyphal length density were similar to the results observed for the spore density in both types of orchards. The highest (4.09 m g^{-1} soil) hyphal length density was observed in 0-20 cm soil layer in the NN orchards, and the lowest (0.90 m g^{-1} soil) in the 40-60 cm soil layer in NH orchards. The hyphal length density in the 0-20 and 20-40 cm soil layers in the NN orchards were significantly higher than in the NH orchards. In synthesis, in our experiment, the AM associated with the citrus roots in the NN orchards were better established than in NH orchards, especially in the upper 40 cm soil layer.

Number of bacteria, actinomycetes and fungi

The number of colonies of culturable bacteria, actinomycetes and fungi decreased with the increase of soil depth in both types of orchards. The colony numbers of bacteria varied significantly among the three soil layers in each type of orchards. The largest number (43.27×10^6 cfu g^{-1} soil) of bacteria was found in the 0-20 cm soil layer in the NN orchards. However, the value was not significantly different from the value found in the corresponding layer in NH orchards. The number of bacteria in the top 20-40 cm soil layer in the NN orchards was significantly larger than in the NH orchards (Fig. 2A). The number of actinomycetes in the top 0-20 cm soil layer in the NN orchards was significantly larger than in NH orchards. However, there were no other significant differences in the number of actinomycetes when comparing the corresponding soil layers between both types of orchards (Fig. 2B). The largest number (30.67×10^3 cfu g^{-1} soil) of fungi was observed in the NN orchards. The number of fungi was high at the topsoil layer in both types of orchards, but it decreased rapidly in the NH orchards, while in the NN orchards a high number was maintained in the 20-40 cm soil layer (Fig. 2C). In the 40-60 cm soil layer, the number of bacteria was significantly larger in the NH orchards than in the NN orchards (Fig. 2A), which was paralleled to the relatively abundant citrus roots in this layer in NH orchards compared to the NN orchards.

Enzyme activities

Fig. 3 shows that the activities of catalase, invertase, urease and phosphatase declined with the increase in the soil depth in both types of orchards. The activities of the four soil enzymes determined all showed significant differences among the three soil

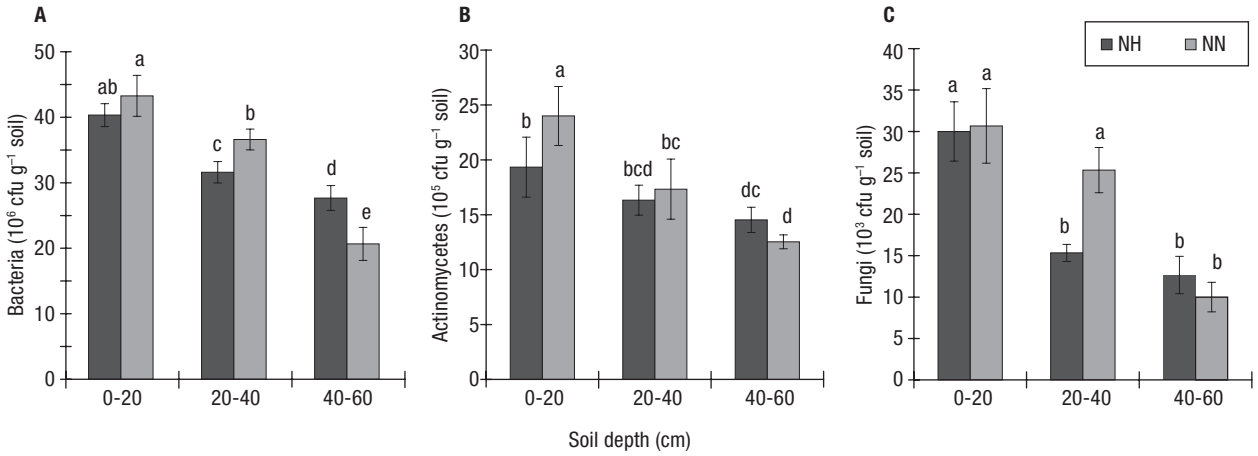

Figure 2. Effect of two different types of no-tillage soil management on the citrus rhizospheric microbe populations at three different soil depths. A: bacteria, B: actinomycetes and C: fungi. Different letters above columns designate significantly different means ($p < 0.05$).

layers in the NN orchards, while only the activities of urease and phosphatase exhibited significant differences among the three soil layers in the NH orchards. The highest enzyme activities were all detected in the 0-20 cm soil layer in the NN orchards, which were significantly higher than in the NH orchards. However, the lowest enzyme activities were also found in the 40-60 cm soil layers in the NN orchards. The activities of catalase and phosphatase in the 20-40 cm soil layer in the NN orchards were significantly higher than in the NH orchards. The variations of these enzymes activities followed a similar trend to the variations in the number of cultivable microorganisms.

Correlation analysis

Correlation analysis was done with both types of orchards considered together. The results indicate that no significant ($p > 0.05$) relationships were found among the RLT and the SD or HLD, and no significant ($p > 0.05$) relationship between the RLT and soil enzyme activities. The SD and HLD had a significant ($p < 0.001$) strong positive correlation. A significant ($p < 0.001$) positive correlation was also noted between the HLD and OM. The CAT and PHO were ($p < 0.001$) positively correlated with the BAC, ACT and FUN, the INV ($p < 0.001$) with the BAC and FUN, and the URE with the BAC at $p < 0.001$ level, and with FUN at the $p < 0.01$ level (Table 3).

Discussion

Arbuscular mycorrhiza is the most abundant underground symbiosis (Smith and Read, 2008). In our study, all citrus rootstocks were heavily colonized by native AMF, and the RLT levels varied significantly in the citrus roots between the different soil layers, especially in the NH orchards. The percentage of root length with arbuscules considered the major site of exchange between the fungus and host (Smith, 1995) was significantly higher in the soil layers above 40 cm in NN orchards compared to the NH orchards. This result shows that a natural grass cover is a better soil management system for improving citrus AM due to the changes in weeds populations in accordance with the earlier work by Jansa *et al*. (2003). In our experiment, significantly higher spore numbers and hyphal length densities in the soil layers above 40 cm were observed in the NN orchards which might be due to the direct effects of increased numbers of plant species and a higher density of plant roots available for colonization. This result is also in agreement with Burrows and Pfleger (2002), who reported that increasing plant species richness was correlated with increases in AMF sporulation and species numbers as well as changes in AMF community composition. The increase in spore numbers could, in turn, enhance AM colonization of cultivated plants. Therefore, weed species maintained in orchards might be favorable for AMF propagation and mycorrhizal symbiosis formation of cultivated plants (Chen *et al*., 2004). However, in our experiment, no relationship was found between the total citrus AM

colonization and the spore density. AM colonization in citrus roots was influenced by various abiotic and biotic factors such as physicochemical soil properties and soil management systems (Nadja *et al*., 2010). The changes observed in the orchards treated with herbicide could be due to the effects of these factors. The carbon requirements of the AMF must be supplied by the host photosynthate. Hence, any factor which modifies the photosynthetic products available for distribution might affect AM development. The herbicide used, Phenmedipham, inhibits photosynthesis when applied by foliar spray or through the soil (Ocampo and Barea, 1985). Consequently, less photosynthetic products are distributed in roots, and this could negatively affect AM development.

The higher OM content was found in the 0-20 cm topsoil layer in the NN orchards in comparison with NH orchards. This result is in accordance with that weeds kept in orchard increased the soil quality by returning back the carbon and nitrogen (Chen *et al*., 2004). The higher hyphal length density also contributed to the soil quality improvement in NN orchards, and in this study, the hyphal length density is positively correlated with the OM. Jastrow *et al*. (1998) and van der Heijden *et al*. (2006) showed that AMF improve aggregate stability, as their extraradical hyphae can bind soil particles together, both mechanically and chemically through the exudation of glomalin (Wright and Upadhyaya, 1998). However, the lower available P and K contents were found in soil layers above 40 cm in the NN orchards. This was probably due to the higher quantity of extraradical mycelium that could rapidly take up soil minerals from the broader rhizosphere and efficiently transport them to the host plants in the NN orchards. Li *et al*. (1991) reported that the high affinity of AM hyphae for specific ions such as phosphate results in depletion of the available pool of these ions.

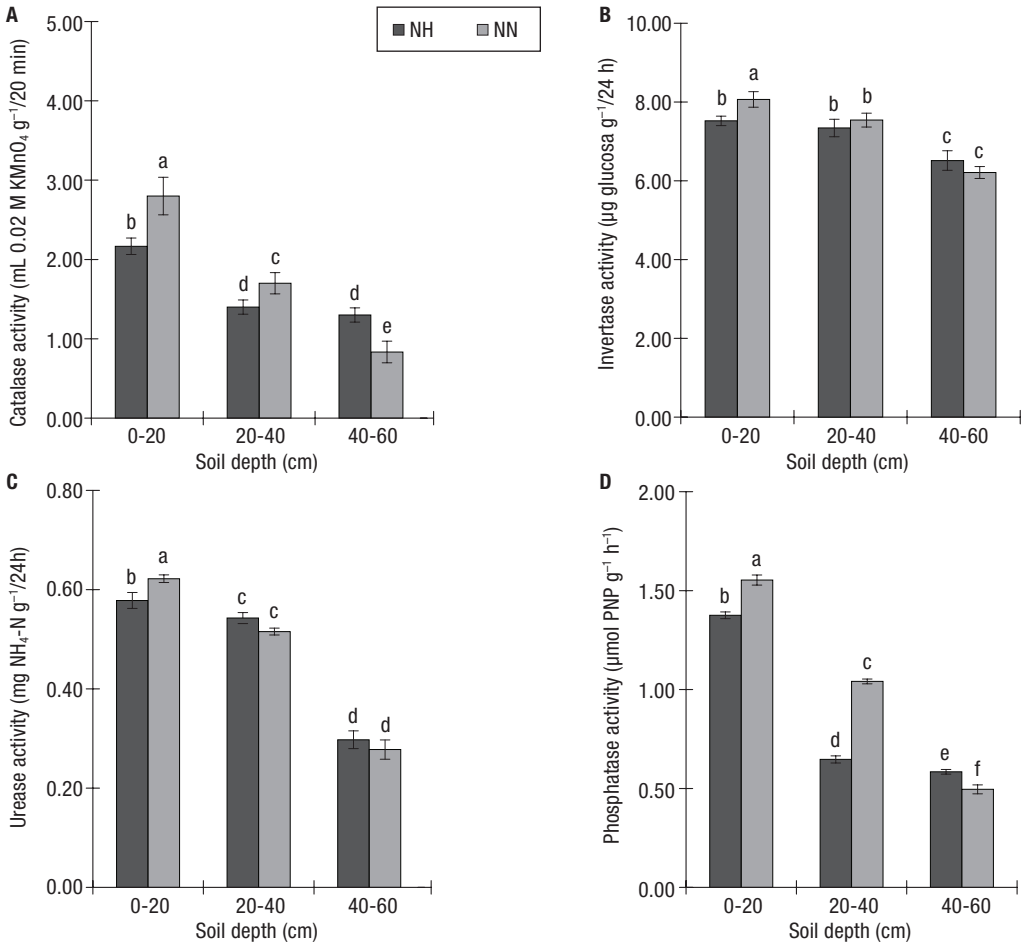

Figure 3. Effect of two different types of no-tillage soil management on enzyme activities at three different soil depths. A: catalase, B: invertase, C: urease and D: phosphatase. Different letters beside columns designate significantly different means ($p < 0.05$).

Table 3. Correlation coefficients between RLT (percentage root colonized with AMF), SD (AM spore density), HLD (AM hyphal length density), BAC (bacteria population), ACT (actinomycetes population), FUN (fungi population), CAT (catalase activity), INV (invertase activity), URE (urease activity), PHO (phosphatase activity) and OM (organic matter content) in a citrus orchard under two different types of no-tillage soil management

	RLT	**SD**	**HLD**	**BAC**	**ACT**	**FUN**	**CAT**	**INV**	**URE**	**PHO**	**OM**
RLT	1.000	–0.391	–0.453	–0.363	–0.458	–0.511	–0.377	–0.388	–0.517	–0.550	–0.518
SD		1.000	0.950**	0.791	0.747	0.822**	0.838**	0.833**	0.709	0.860**	0.787
HLD			1.000	0.724	0.708	0.721	0.791*	0.748	0.649	0.801*	0.929**
BAC				1.000	0.863**	0.862**	0.921**	0.898**	0.896**	0.920**	0.556
ACT					1.000	0.729	0.824**	0.781	0.786	0.836**	0.573
FUN						1.000	0.860**	0.834**	0.814*	0.936**	0.634
CAT							1.000	0.846**	0.817**	0.947**	0.628
INV								1.000	0.928**	0.843**	0.580
URE									1.000	0.819**	0.522
PHO										1.000	0.705
OM											1.000

* $p < 0.01$, ** $p < 0.001$.

The mycorrhizosphere is the zone of soil influenced by both plant roots and AMF with a high microbial activity (Barea *et al*., 2002). In this study, the highest microbial populations (bacteria, actinomycetes and fungi) were found in the NN orchards. The quantity and quality of the plant root exudates in the mycorrhizosphere in NN orchards might be different from the exudates found in NH orchards due to AMF colonization of grass roots besides the citrus roots, which might lead to a different composition of the microbial community (Barea, 2000; Linderman, 2000; Artursson *et al*., 2005). In our research, the highest rhizospheric soil enzyme activities were observed in the topsoil layers in the NN orchards. The correlation analysis showed soil enzyme activities, except phosphatase, strongly and positively correlated with the bacteria population and not with the total citrus AM colonization. Phosphatase activity was in its turn strongly correlated with fungi population. Thus, the larger bacteria and fungi populations and organic matter content play a main role in enhancing soil enzyme activities in the topsoil layers in the NN orchards, while the low organic matter content in the soil layers above 40 cm in the NH orchards lead to low bacteria and fungi populations and to low soil enzyme activities. Brady and Weil (1999) reported that organic matter stimulated soil microbe populations and soil biological activity. So, apart from the effect on the AM activity, the natural grass cover improved the microbial activity.

In our research, fine roots ($\Phi < 2$ mm) were more abundant in the topsoil in the NN orchards than in the NH orchards. The main reason was probably that the topsoil fertility and quality were heavily improved by the natural grass cover. Gregory (2006) reported that root distribution near the nutrient-enriched zone favored plant establishment. However, the citrus fine roots in the 40-60 cm soil layer were more abundant in the NH orchards than in the NN orchards. Consequently, the AMF and microbes could get more material and energy deriving from the root exudates to improve their development and biological activity, accordingly in our results, the number of bacteria and the catalase and phophatase activities in the 40-60 cm soil layer were significantly higher in the NH orchards than in the NN orchards.

This study reveals differences in AM colonization, spore density, hyphal length density, rhizospheric microbial populations and soil enzyme activities at different soil layers in the citrus orchards with two kinds of no-tillage soil management practices. The natural grass cover could improve the AM symbiosis in citrus roots, microbial populations and soil enzyme activities in citrus topsoil rhizosphere. So, it is reasonable to select the soil management of natural grass cover for promoting a low chemical input agriculture and protecting the orchard ecology environment.

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