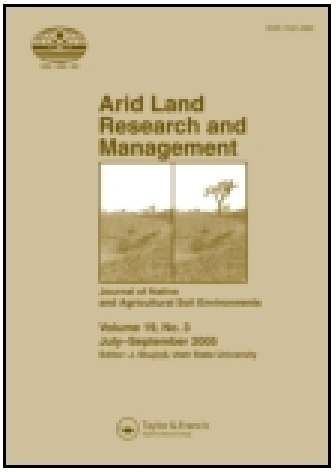

Arid Land Research and Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uasr20

Changes in Soil Ergosterol Content, Glomalin-Related Soil Protein, and Phospholipid Fatty Acid Profile as Affected by Long-Term Organic and Chemical Fertilization Practices in Mediterranean Turkey

Oğuz Can Turgay [a], David Buchan [b], Bram Moeskops [b], Bart De Gusseme [c], İbrahim Ortaş [d] & Stefaan De Neve [b]

[a] Department of Soil Science, Faculty of Agriculture, Ankara University, Dıskapı, Ankara, Turkey

[b] Department of Soil Management, Gent University, Gent, Belgium

[c] Department of Environmental Technology, Gent University, Gent, Belgium

[d] Department of Soil Sicence, Faculty of Agriculture, Çukurova University, Adana, Turkey

Published online: 07 Nov 2014.

To cite this article: Oğuz Can Turgay, David Buchan, Bram Moeskops, Bart De Gusseme, İbrahim Ortaş & Stefaan De Neve (2015) Changes in Soil Ergosterol Content, Glomalin-Related Soil Protein, and Phospholipid Fatty Acid Profile as Affected by Long-Term Organic and Chemical Fertilization Practices in Mediterranean Turkey, Arid Land Research and Management, 29:2, 180-198, DOI: 10.1080/15324982.2014.944246

To link to this article: http://dx.doi.org/10.1080/15324982.2014.944246

Arid Land Research and Management, 29:180–198, 2015

ISSN: 1532-4982 print/1532-4990 online
DOI: 10.1080/15324982.2014.944246

Taylor & Francis
Taylor & Francis Group

Changes in Soil Ergosterol Content, Glomalin-Related Soil Protein, and Phospholipid Fatty Acid Profile as Affected by Long-Term Organic and Chemical Fertilization Practices in Mediterranean Turkey

Oğuz Can Turgay[1], David Buchan[2], Bram Moeskops[2], Bart De Gusseme[3], İbrahim Ortaş[4], and Stefaan De Neve[2]

[1]Department of Soil Science, Faculty of Agriculture, Ankara University, Dıskapı, Ankara, Turkey
[2]Department of Soil Management, Gent University, Gent, Belgium
[3]Department of Environmental Technology, Gent University, Gent, Belgium
[4]Department of Soil Sicence, Faculty of Agriculture, Çukurova University, Adana, Turkey

*The present study examines the effects of different fertilization treatments (chemical fertilization, farmyard manure, plant compost, and mycorrhiza-inoculated compost) on the soil fungi under a crop rotation of wheat (*Triticum aestivum L.*) and corn (*Zea mays *L.) in a long-term field experiment established in Mediterranean Turkey in 1996. Soil samples were collected in May, August, and October 2009. Soil pH, organic carbon, plant-available nitrogen and phosphorus, mycorrhizal colonization, and a series of biochemical markers (phospholipid and neutral lipid fatty acid [PLFA and NLFA] profiles, soil ergosterol content, and glomalin related soil protein [GRSP] as indicators of abundance of bacteria, saprotrophic, and arbuscular mycorrhizal [AM] fungi) were assessed. No significant difference was observed in soil organic C and plant available N in relation to long-term fertilization treatments, but plant available P in soil changed significantly in relation to the fertilization treatment used and the sampling season (between 11.5–33.8 $mg \cdot kg^{-1}$ in spring, 10.4–28.6 $mg \cdot kg^{-1}$ in summer, and 10.5–33.2 $mg \cdot kg^{-1}$ in autumn). Mycorrhizal colonization patterns were similar for both plants. However, mycorrhiza-inoculated compost treatment exhibited higher root colonization (77.3%) over control (16.3%), chemical fertilization (10.0%), farmyard manure (19.3%), and plant compost (20.0%). No statistically significant change was observed in ergosterol content. The effect of long-term organic treatments on soil PLFA structure was statistically prominent; whereas seasonality only affected bacterial PLFAs. Organic fertilization increased GRSP (mean annual ranging from 0.91 to 2.46 $mg \cdot g^{-1}$ total GRSP) but long-term annual mycorrhizal inoculation had no significant effect on the soil GRSP pool.*

Received 10 June 2014; accepted 9 July 2014.
Address correspondence to Oğuz Can Turgay, Department of Soil Science, Faculty of Agriculture, Ankara University, 06110, Dıskapı, Ankara, Turkey. E-mail: turgay@agri.ankara.edu.tr

Keywords arbuscular mycorrhiza, ergosterol, glomalin, organic fertilization, phospholipid fatty acids, soil fungi

Mediterranean and Aegean Turkey have a pioneer role in covered cultivation and greenhouse agriculture. The regions provide 95% of total domestic vegetable production and harbor a great potential to export 50 different kinds of fresh products to many other countries (Kacira et al., 2004). However, in such regions, soil productivity is low due to unfavorable climatic effects and soil characteristics, that is, high lime and clay content. The depletion of soil organic matter in Mediterranean climates is exacerbated by the burning of crop residues, excessive tillage and continual cropping of arable land. These practices adversely affect soil biological properties, including fungal hyphal networks. Consequently, soil quality decreases under such conditions (Del Mar Alguacil et al., 2009). In recent years, sustainable agricultural practices such as organic fertilization have been found to enhance soil biological activities, stimulating turnover of organic matter and the release of plant available nutrients in soil (Carpenter-Boggs et al., 2000). Furthermore, the advantages of long-term organic applications with regard to soil microbial ecology and fertility have been extensively documented by several researchers in varied regions of the world (Böhme et al., 2005; Raupp et al., 2006; Liu et al., 2010). Fungal biomass play a dominant role in vital soil processes and thrive through a spatial hyphal network that develops throughout the soil. Such vital processes include soil organic matter decomposition, nutrient turnover and availability, and aggregate stability in agricultural soils, which can be markedly affected by agricultural management practices such as intensive tillage (Al-Kaisi et al., 2005), no tillage (Frey et al., 1999), fertilization (Bardgett et al., 1993), crop rotation (Alvey et al., 2003), and residue burning (Turgay et al., 2002). In terms of fungal community structure and community dynamics in agricultural soils, the ecology and functions of arbuscular mycorrhizal (AM) fungi have gained increasing scientific attention due to their potential role in soil quality (Rillig, 2004). It was reported that the majority of principal crop fields such as wheat and corn were colonized by AM fungi (Smith and Gianinazzi-Pearson, 1988). As a result of these interactions, important benefits such as growth promotion and increases in stress resistance were noted (Sanchez-Diaz and Honrubia, 1994).

However, only a few studies have addressed the potentially important role of general and AM fungi under semi-arid conditions such as those found in Mediterranean Turkey. The effects of soil management practices on the survival of major fungal groups (i.e., saprophytes and mycorrhizae) have been extensively studied by different authors but the results have been contradictory. For example, intensive soil management practices were reported to have a negative effect on the saprotrophic fungal biomass (Donnison et al., 2000), measured as ergosterol concentration. However, more recent works have recorded no significant change in the content of saprotrophic fungi, as indicated by the ergosterol measurements under conventional and organic farming activities (Joergensen and Wichern, 2008; Joergensen et al., 2010). Similarly, recent studies comparing arbuscular mycorrhizal (AM) fungi under different management practices revealed contradictory results in that AM fungal community was found to be depleted in intensively managed agricultural systems (Johnson and Pfleger, 1992; An et al., 1993; Douds et al., 1997; Galvez

et al., 2001; Jansa et al., 2002). Only small differences were observed in some other cases (Kurle and Pfleger, 1996; Franke-Snyder et al., 2001). This may be due to regional differences in soil characteristics and microbial diversity. It may also be the result of selected methodologies and qualitative/quantitative fungal measurements exhibiting various constraints as summarized by several authors (Oehl et al., 2004; Joergensen and Wichern, 2008; Frostegård et al., 2011).

Therefore, in the present work, the effects of long-term fertilization practices on a variety of soil properties, especially fungal-biochemical soil characteristics are examined under Mediterranean conditions. A field experiment established in May 1996 in the Çukurova Region in Southern Turkey was selected. There, soil ergosterol content, glomalin related soil proteins (GRSP), and phospholipid and neutral lipid fatty acid (PLFA and NLFA) profiles were compared in soils applied with chemical fertilization, farmyard manure, plant compost, and mycorrhiza-inoculated plant compost. It was hypothesized that (1) soil fertility, the abundance of soil fungi (saprotrophic and AM), and bacterial biomass would increase under long-term organic fertilizations and that (2) the development of AM fungi would be higher under the long-term application of mycorrhiza-inoculated compost; as compared to that of traditional chemical fertilization treatment. In order to verify these hypotheses, ergosterol content, GRSP, and PLFA and NLFA profiles were monitored seasonally. Root-mycorrhizal colonization was also determined after first harvest.

Materials and Methods

Study Site

The experiment was established in April 1996 for a range of crops suited to the Menzilat soil series (Typic Xerofluvents Fluvents, Entisols) located on the Research Farm of Çukurova University (37° 00′ 54.31″ N longitude, and 35° 21′ 21.56″ E latitude and 34 m above mean sea level) in the eastern part of the Mediterranean region of Adana, Turkey. The regional climate is typically Mediterranean with a long-term average annual air temperature of 19.1°C (ranging from 14.2°C January–February to 25.5°C July–August) and precipitation of 670 mm (TSMS 2009).

Preparation of Compost and Mycorrhizal Inoculum

The compost used in the experiment was prepared according to Rynk (1992) by composting equal portions of grasses, wheat stubbles and corn leaves on a dry weight basis under atmospheric conditions for 8 months. The chemical characteristics and macro- and micronutrient contents of the compost were as follows: pH(1: 2,5 w/v) of 7.91, C/N ratio of 15.4, total C, N, P, K, Ca, Mg of 86.0, 5.60, 1.79, 9.8, 25.0, and 2.7 $g \cdot kg^{-1}$, respectively, and total Zn, Fe, Cu, Mn of 40.0, 1005.0, 12.0, and 141.0 $mg \cdot kg^{-1}$, respectively. Mycorrhizal inoculum (mixture of sand: soil: spores, in the ratio of 6:3:1, *v/v/v*, respectively) was produced from sorghum (*Sorghum bicolor L.*) roots in pot culture (Ortaş 1996). A cocktail mycorrhizal inoculum (*Funneliformis mosseae*) (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *F. caledonium* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, *Glomus clarium* (Nicolson and Schenk), and *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.)

C. Walker & A. Schüßler) equivalent to 500–600 kg ha^{-1} was mixed with the compost material prior to sowing the sorghum seeds. Approximately 1000 spores were used per plant at sowing stages. AM fungal species were provided by International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), Morgantown, West Virginia.

Field Experiment

The field experiment initiated in November 1996 is a complete randomized field experiment with three replicates (10×20 m plots). The treatments were (1) control; (2) annual applications of a chemical fertilization of 160 kg N ha^{-1} year^{-1} as $(NH_4)_2SO_4$, 83 kg K ha^{-1} year^{-1} as K_2SO_4 and 26 kg P ha^{-1} year^{-1} as $Ca(H_2PO_4)_2 \cdot H_2O$; (3) farmyard manure (organic matter = 58% and C/N ratio = 66.7) at 25 t dry weight ha^{-1} year^{-1}; (4) plant compost at 25 t dry weight ha^{-1} year^{-1}; and (5) mycorrhiza-inoculated compost at 10 t dry weight ha^{-1} year^{-1}. The plant rotation was wheat (*Triticum aestivum L.*) and corn (*Zea mays L.*) per year. Annually, the organic fertilizers were uniformly spread on the soil surface just before sowing and incorporated into the surface layer with a disc harrow.

Soil Sampling

In 2009, soil samples were taken from 0–5 cm depth four weeks after fertilizer incorporation, gently sieved to 2 mm and transferred to the laboratory. A portion of soil samples were immediately frozen at −40°C overnight, freeze-dried and subsequently stored at −20°C prior to soil biochemical analysis. This sampling protocol was carried out 3 times during the year in the early stage of the wheat growth in May, after the wheat harvest in August and before the corn harvest in late October 2009 representing spring, summer, and autumn, respectively.

General Soil and Plant Analysis

The particle size distribution, measured according to Bouyoucos (1951), Soil pH was measured on a soil:water ratio of 1:2.5 volume basis (Richards, 1954). Total %C was measured according to the Dumas method using a Variomax CNS elemental analyzer (Elementar GmbH, Hanau, Germany). Inorganic C was determined using a modified pressure-calcimeter method (Sherrod et al., 2002) and organic C was calculated by subtracting inorganic C from total C. Plant available nitrogen (NH_4-N plus NO_3-N) was measured according to steam distillation method using Kjeldhal apparatus and cation exchange capacity (CEC) using the ammonium acetate method described in Soil Survey Staff (1996). Plant available P was measured as indicated by Olsen and Sommers (1982). Grain yield was collected by harvesting the entire plot at physiological maturity. Plant root samples were washed, sub-sampled, and then stained with trypan blue (Koske and Gemma, 1989). The percentage of root length colonization was determined by the grid-line intersection method (Giovannetti and Mosse, 1980). All chemical analyses were carried out on three subsamples per field plot.

Soil Biochemical Analysis

Soil ergosterol was extracted and determined according to Gong et al. (2001). GRSP was extracted from soil samples as indicated by Wright and Upadhyaya, (1998).

Total GRSP and easily extractable GRSP were determined according to Bradford (1976). Changes in general microbial community composition under different management practices were measured by phospholipid and neutral lipid fatty acid (PLFA and NLFA) analysis according to a modified procedure of Balser (2001).

Data Evaluation and Statistical Analyses

Repeated measures analysis was carried out for statistical evaluation of the effects of different agricultural applications and seasonal changes in pH, organic carbon, plant-available nitrogen and phosphorus, plant yield GRSP, and ergosterol content. The analysis was performed using IBM-SPSS statistics 20 software (SSPS, Chicago, IL) and Duncan's multiple range tests as a multiple comparison test of the treatments (Duncan, 1955). To compare the relative composition of the soil microbial community under different agricultural treatments, PLFA data were calculated as percentages of the total PLFA concentration of the soil sample. Fatty acids contributing less than 1% to the pool of fatty acids were not included in subsequent statistical analysis. In order to discriminate different field treatments, canonical discriminant analysis (CDA) was applied to the percentage distribution of PLFA using SSPS 20.0 software. The high correlation between the fatty acids a15:0 and a16:0 ($r = 1$, $p < 0.001$) affected CDA negatively; therefore, a16:0 was disregarded in the analysis.

Among 18 fatty acids included in CDA, the sums of the following marker fatty acids selected according to Bossio and Scow (1998) and Kozdrój and van Elsas (2001), were used to evaluate different microbial groups. G+ bacteria were represented by the sum of i15:0, a15:0, i16:0, i17:0, and a17:0. G− bacteria were considered represented by 16:1ω7c, 18:1ω7c, and cy17:0, and actinobacteria by 10Me16:0 and 10Me18:0. The sum of the marker PLFAs for G+ and G− bacteria and for 15:0, 17:0, and cy19:0ω11,12c was regarded as the indication of the total bacterial community; PLFA 18:2ω6,9c was used as signature for soil saprotrophic fungal biomass; and 16:1ω5c was used as signature fatty acid for AM fungi.

In order to distinguish AM fungal and bacterial 16:1ω5c, the ratio between NLFA and PLFA 16:1ω5c was evaluated according to the fact that the NLFA/PLFA ratio changes between 1 to 200 in AM fungi while it is less than 1 in bacteria (Olsson, 1999; Vestberg et al., 2012). Bacteria/fungi ratios and G+/G− ratios were calculated by dividing the respective sums of marker fatty acids.

Results

Changes in Mycorrhizal Colonization, Soil, and Plant Yield Characteristics Under Different Treatments

CEC, $CaCO_3$ percentage, and organic C content of control soil were 75.4 $\mu mol_c\, g^{-1}$, 3.68% and 12.26 $g \cdot kg^{-1}$, respectively. The particle size distribution was 37.5, 31.0, and 31.5 (clay/silt/sand, respectively). Statistically, neither field treatments nor seasonal changes had any effect on the content of soil organic C. Similarly, long-term chemical and organic fertilizations did not affect soil pH, and available N content significantly. However, soil pH and plant available N (NH_4-N plus NO_3-N) significantly differed between spring and autumn from 7.78 to 8.04 ($p < 0.001$) and from 38.97 to 44.94 $mg \cdot kg^{-1}$, ($p < 0.05$) respectively. The result of the repeated-measures analysis of variance also showed that treatment × season interaction was significant

Table 1. Soil available phosphorus content, plant yield, and mycorrhizal colonization under different field treatments

	Soil available P ($mg \cdot kg^{-1}$)			Yield ($t \cdot ha^{-1}$)		Mycorrhizal colonization (%)	
	Spring	Summer	Autumn	Wheat	Corn	Wheat	Corn
Treatment							
Control	11.5 Da	10.4 Da	10.5 Da	2.680 C	8.050 D	18.7 BC	16.3 B
Chemical fertilization	25.2 Ba	23.7 Ba	25.2 Ba	7.207 A	11.317 B	11.3 C	10.0 B
Farmyard manure	33.8 Aa	28.6 Ab	33.2 Aa	6.200 AB	10.300 C	19.3 BC	19.3 B
Plant compost	25.1 Bb	20.6 Bc	33.2 Aa	4.733 B	10.683 BC	24.3 B	20.0 B
Mycorrhiza inoculated Compost	15.9 Cab	14.2 Cb	19.0 Ca	6.493 AB	12.717 A	73.3 A	77.3 A
Probability values							
Treatment		**<0.001**		**0.003**	**<0.001**	**<0.001**	**<0.001**
Season		**<0.001**					
Season × Treatment		**0.001**					
CV (%)		37.44		34.74	15.23	80.45	88.22

Note: Capital letters represent significant differences among field treatments, whereas lowercase letters denote significant differences between seasons; data are means of three replicates; Values in bold are significant *p*-values; CV = mean coefficient of variation between the replicates.

for plant available P in soil. It generally followed the order of manure > chemical fertilization > plant compost > mycorrhiza-inoculated plant compost > control throughout each season (Table 1). Maximum wheat and corn grain yields were obtained from both chemical fertilization and mycorrhiza-inoculated compost treatments. The treatment effect on plant yield was statistically more pronounced for corn than for wheat (Table 1). Mycorrhizal colonization patterns were similar in wheat and corn after harvest. Comparing different treatments, the highest root colonization was observed under mycorrhiza-inoculated compost treatment (Table 1).

Soil Ergosterol Content and GRSP

Neither long-term chemical fertilization nor organic treatments caused a remarkable change in soil ergosterol content. Seasonal change itself was statistically more prominent ($p < 0.001$) than either individual treatment effect or treatment × season interaction ($p < 0.05$) (Table 2). The individual effects of field treatments and seasonality on total and easily extractable GRSP were significant ($p < 0.001$ and $p < 0.004$,

Table 2. Changes in soil ergosterol and glomalin related soil protein (GRSP) contents under different field treatments

	Soil ergosterol ($\mu g \cdot g^{-1}$)			Total GRSP ($mg \cdot g^{-1}$)	Easily extractable GRSP ($mg \cdot g^{-1}$)
	Spring	Summer	Autumn	Mean seasonal[§]	Mean Seasonal
Treatment					
Control	0.63 Ab	0.43 Bc	0.79 Ba	0.91 B	0.140 C
Chemical fertilization	0.69 Ab	0.64 Ab	1.15 Aa	1.34 B	0.219 AB
Farmyard manure	0.81 Ab	0.68 Ab	1.10 Aa	2.46 A	0.276 A
Plant compost	0.62 Ab	0.62 Ab	1.14 Aa	2.37 A	0.281 A
Mycorrhiza inoculated Compost	0.64 Ab	0.60 ABb	0.85 Ba	1.36 B	0.202 B
Probability values					
Treatment		**0.023**		**<0.001**	**0.001**
Season		**<0.001**			
Season × Treatment		0.039		0.081	0.348
CV (%)		30.20		48.36	29.88

Note: Total and easily extractable GRSP data are mean values of three seasonal measurements for each field treatment with $n = 3$; Capital letters represent significant differences among field treatments, whereas lowercase letters denote significant differences between seasons; Significant p-values were shown in bold; CV = mean coefficient of variation between the replicates.

Table 3. Changes in the concentrations of marker PLFAs and fatty acid ratios under different agricultural managements

	Biomarker PLFAs ($nmol \cdot g^{-1}$)								Fatty acid ratios	
						AM fungi				
Treatment	G+ Bacteria	G− Bacteria	Total bacteria	Actino-bacteria	Saprotrophic fungi	Spring	Summer	Autumn	NLFA/ PLFA 16:1ω5c	Fungi/ Bacteria
Control	13.12 D[§]	7.83 C	22.10 C	4.94 D	1.62 D	3.33 Ca	2.41 Bb	3.48 Ba	4.30 A	0.073 A
Chemical fertilization	15.75 C	10.50 B	27.53 B	5.87 C	2.00 BC	3.99 BCa	3.10 ABa	3.77 Aba	1.96 C	0.073 A
Farmyard manure	20.95 A	12.40 A	34.87 A	7.31 A	2.64 A	6.68 Aa	3.91 Ab	4.45 Aa	3.39 B	0.076 A
Plant compost	18.48 B	9.67 B	28.51 B	6.54 B	2.26 B	4.63 Ba	3.06 ABb	4.38 Aa	3.69 B	0.080 A
Mycorrhiza inoculated compost	14.10 D	8.22 C	23.52 C	5.31 C	1.77 CD	3.89 BCa	2.67 Bb	3.51 Bab	3.79 B	0.075 A
Probability values										
Treatment	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**		**<0.001**		**<0.036**	0.053
Season							**<0.001**			
Season × treatment	0.159	0.542	0.225	0.542	0.703		**0.003**		0.410	0.447
CV (%)	25.75	19.41	22.01	19.41	21.88		28.08		36.91	7.25

Note: Data are mean values of marker PLFAs for three seasonal measurements with $n = 3$ except AM fungi (PLFA 16:1ω5c); Capital letters represent significant differences among field treatments, whereas lowercase letters denote significant differences between seasons; Values in bold are significant p-values; CV = mean coefficient of variation between the replicates.

respectively). However, no significant treatment × season interaction occurred in different GRSP pools (Table 2). Generally speaking, soil GRSP production was significantly higher under manure and plant compost treatments than under chemical fertilization and mycorrhiza-inoculated compost treatments. On the other hand, total GRSP tended to decrease while easily extractable GRSP content seemed to increase from spring to autumn (Table 2).

PLFA Profiles

Repeated measures analysis revealed that, excepting AM fungal PLFA 16:1ω5c, biomarker PLFAs representing major microbial communities (i.e., G+, G−, total bacteria, actinobacteria, and saprotrophic fungi) were significantly higher under manure than under other treatments ($p < 0.001$) (Table 3). The treatment × season interaction was significant only for AM fungal PLFA 16:1ω5c ($p < 0.05$), but there was no statistically marked differences in the concentrations of AM fungal PLFA 16:1ω5c in relation to chemical and organic field treatments (Table 3). NLFA/PLFA 16:1ω5c ratio was lowest under chemical fertilization and did not significantly differ between organic treatments. The fungal-bacterial PLFA ratio was unafffected by long-term chemical and organic treatments (Table 3). Seasonal shifts in biomarker PLFAs, except saprotrophic fungal PLFA 18:2ω6,9c, were statistically significant and pointed to a general decrease in summer but increase in autumn. NLFA/PLFA 16:1ω5c ratio was not significantly affected by seasonality (Table 4).

The CDA identified four significant canonical functions explaining a total of 95.7% of the data set variance. The first canonical discriminant variable (CD1) accounted for 53.5% of the total variance, clearly distinguishing chemical fertilization, manure, and plant compost treatments ($p < 0.001$). The second canonical discriminant variable (CD2) accounted for 27.0% of the total variance and was able

Table 4. Seasonal changes in the concentrations of marker PLFAs and glomalin related soil protein (GRSP) fractions

Marker PLFAs ($nmol \cdot g^{-1}$)	Sampling Season			Statistical significance (p-values)
	Spring	Summer	Autumn	
Total bacteria	30.12 a[§]	24.87 b	26.93 b	**0.002**
G+	11.61 a	8.49 b	9.07 b	**<0.001**
G−	6.21 a	5.38 b	9.07 a	**0.003**
Saprotrophic fungi	2.15	1.95	2.07	0.182
Actinobacteria	6.21 a	5.38 b	6.39 a	**0.003**
NLFA/PLFA	4.04	3.72	2.51	0.156
Fungi/Bacteria	0.072 a	0.078 b	0.077 b	**<0.001**
GRSP ($mg \cdot g^{-1}$)				
Total	1.99 a	1.85 a	1.22 b	**<0.001**
Easily extractable	0.198 b	0.241 a	0.232 a	**0.004**

Note: [§]Data are mean values of marker PLFAs and GRSP values for all field treatments with $n = 3$; Lowercase letters denote significant differences between seasons; significant p-values are shown in bold.

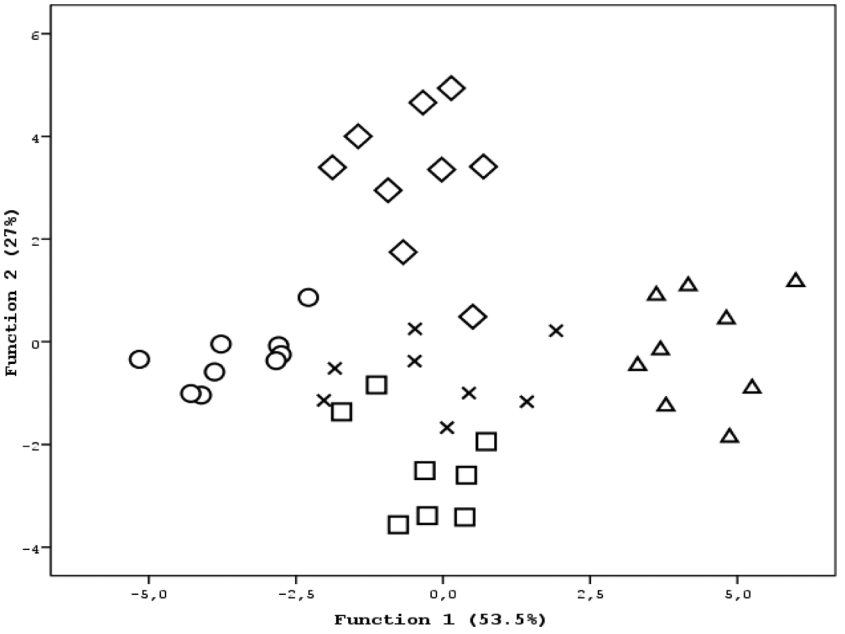

Figure 1. Canonical discriminant functions separating different soil managements (□: Control; ○: Chemical fertilization; ◇: Farmyard manure; △: Plant compost; X: Mycorrhiza inoculated compost).

to distinguish only manure from all other treatments ($p < 0.001$). However, CDA was not able to discriminate between the treatment of mycorrhiza-inoculated compost and that of control (Figure 1). AM fungal PLFA 16:1ω5c showed no significant loading score in CDA analysis (data not shown).

Discussion

Effect of Different Treatments on Soil Chemical Characteristics and Yield

The results from the present study revealed low significant changes in soil C pool and steady state conditions in soil N stocks. In 1999, three years after the initialization, Çelik et al. (2004) examined how organic fertilization and mycorrhizal inoculation could improve soil physical properties in the same field experiment. They noticed that soil organic matter only slightly changed at depth of 0–15 cm, while no significant change was observed at depth 15–30 cm under chemical fertilizer and different organic treatments. In 2008, nine years later, organic matter accumulation between the treatments became more distinct and significantly increased under manure and compost applications at both soil depths (Çelik et al., 2010). These observations seems compatible with the general view that few years of contrasting soil management are not usually sufficient to produce a permanent difference in soil organic matter content as indicated by Marinari et al. (2006) who also observed no consistent increase in total organic carbon between seven years of conventional and organic farming systems in Italy. Despite longer organic amendment history, insignificant changes of soil organic carbon content in the present work can be related to a higher

carbon mineralization in semi-arid conditions resulting in low soil organic carbon sequestration (Srinivasarao et al., 2012) and thus vulnerability to soil degradation (Tejada and Gonzáles, 2008) in Mediterranean Zones. In the same region with the present work, Gok et al. (2000) conducted a study to investigate the effects of organic substrate on organic matter accumulation in annual applications. They did not observe a significant organic matter increase in soils even with high rate of organic substrate application and attributed their results to the high mineralization rate in the study area.

More pronounced differences were recorded in plant available P levels, which were concordant with previous works carried out in Mediterranean soils (Lagomarsino et al., 2009; Caravaca et al., 2002). Lower plant available P content under mycorrhiza-inoculated compost treatment may likely be related to lower organic matter input ($10\,t\,ha^{-1}\,year^{-1}$) as compared to those of other organic treatments ($25\,t\,ha^{-1}\,year^{-1}$). The reason behind the lower compost rate in the combined application was to assess if mycorrhizal inoculation would affect soil and yield characteristics in comparison with a single compost treatment at a higher application rate. Despite this lower input usage and significantly lower plant available P, the mycorrhiza-inoculated compost treatment resulted in the highest corn yield of all the treatments. This could be attributed to a better association with AM fungi under a low level of plant available P, a relationship previously indicated by Del Mar Alguacil et al. (2010) in phosphorus-poor tropical savanna soils.

Effect of Different Treatments on Mycorrhizal Colonization

Corn has a medium mycorrhizal root colonization of around 50% while wheat varieties are considered to be less dependent, with values ranging from 13% to 50% (Nelson and Spanner, 2010). Therefore, similar colonization values under different field treatments (Table 1) can be explained by the closeness between mycorrhizal colonization characteristics of wheat and corn plants. In their work, Sharif et al. (2010) observed similar trends in AM fungal root intensity under wheat and corn crops in Malakand, Pakistan. The highest mycorrhizal colonization rates in the present study were obtained from mycorrhiza-inoculated compost treatment. Higher corn production under a low level of plant available P can be supported by the considerably higher colonization percentage (77.3%) obtained from mycorrhiza-inoculated compost treatment (Table 1). Using nonindigenous mycorrhizal species, Al-Karaki et al. (2004) also investigated the effects of mycorrhizal inoculation on growth, grain yield and mineral acquisition of two winter wheat (*Triticum aestivum L.*) under well-watered and water-stressed conditions and noticed that plants inoculated with mycoorhiza had higher colonization, grain yields, and biomass than in non-mycorrhizal plots. In another work done by Mardukhi et al. (2011), it was shown that inoculation with a mixture of native mycorrhiza species significantly increased the levels of esential nutrients in wheat plant. This investigation importantly showed that nutrient content of the plant was also related to wheat cultivar as well as AMF inoculation. In a series of studies in relation to re-establishment of native shrub species in degraded semiarid Mediterranean conditions, Caravaca et al. (2003, 2005) reported effective colonisation of both allochthonous and native AM species depending on the native shrub species. These findings indicated that both indigenous and non-indigenous AMF species can be effectively colonized on plant roots depending on plant species, type, cultivar, and so forth. Therefore, in

our study, it is likely that continous inoculation with a mixture of nonindigenous AM strains could provide better colonization beyond the generally more efficient indigenous population.

Changes in Soil Ergosterol Content Under Different Treatments

In the present study, soil ergosterol content did not change under different treatments in general (Table 2). Cavagnaro et al. (2006) also observed no significant change in ergosterol content and microbial biomass carbon in relation to plant genotype and fertilization practices under on-farm organic management. The steady state status of the fungi/bacteria ratio under different treatments (Table 3) also appears to be in line with this idea. Similarly, Joergensen et al. (2010) found no significant difference in saprotrophic fungi measured as soil ergosterol content but noted a shift toward bacterial residues under long-term organic and inorganic farming activities.

Changes in Total and Easily Extractable GRSP

In general, our results showed higher total and easily extractable GRSP under manure and plant compost treatments than under chemical fertilization and mycorrhiza-inoculated compost treatments (Table 2), which is in line with the amount of carbon provided to soil by these treatments. However, unchanging soil organic carbon versus different GRSP levels under different treatments in our study seems to be contradictory to earlier works reporting strong correlations between soil carbon and total GRSP (Del Mar Alguacil et al., 2009). This contradiction can be explained by the fact that glomalin is a matter produced by living hyphae of AM fungi, meaning its synthesis occurs through a different pathway rather than microbially decomposing nutrient sources such as dead plant residues (Rillig et al., 2001). Therefore, it is likely that GRSP can change regardless of fluctuations in soil carbon status. The incompatibility between soil carbon content and GRSP levels in the present study may also be related to another conflict that organic management has shown to enhance AM fungal richness (Oehl et al., 2004) and colonization levels (Bending et al., 2004), whereas some other authors noted a poor AM fungal performance in different organic systems (Dann et al., 1996; Scullion et al., 1998), probably due to variations in regional differences, that is, climate and soil characteristics.

The easily extractable GRSP was initially viewed as an indication of the recently deposited portion of GRSP (Steinberg and Rillig, 2003). As a result, it was commonly monitored in different studies as an important step towards defining GRSP with respect to age and/or function under different agricultural management (Nie et al., 2007) and environmental conditions (Bai et al., 2009). The results of this study, however, showed no remarkable difference in easily extractable GRSP pool under different treatments (Table 2). This was supported by previous findings indicating relatively stabile levels of easily extractable GRSP (Steinberg and Rillig, 2003). Moreover, total GRSP tended to decrease, whereas easily extractable GRSP increased from spring to autumn (Table 4). Hontoria et al. (2009) reported that total protein content increased more than the easily extractable protein content in a sample of the abandoned soil of olive cultivation. This finding suggests that changes in easily extractable GRSP pool may not necessarily be related to recently deposited proteins in soil. The contradiction between two GRSP data sets in our work supports this

theory. It challenges whether easily extractable GRSP can really reflect ephemeral changes in GRSP production originating from AM fungal activities in soil.

It is likely that methodological bottlenecks in GRSP measurements may obscure the actual AM fungal development under different soil treatments. GRSP extracts can include both AM fungi-associated proteins and other nonproteinaceous materials such as tannic and humic acids. This may contribute to a darker extract, causing inaccurate estimations of soil protein via Bradford reaction (Schindler et al., 2007). The contradiction between total and easily extractable GRSP results in our work can be associated with the extraction efficiency of these fractions by common GRSP measurement. This argument has been critically tested by Gillespie et al. (2011). They reported that GRSP procedure can remove some proteins with different molecular weight and modify similar proteins through more intensive glomalin extraction processes.

PLFA Profiles Under Different Treatments

Measurement of PLFA composition of soil microorganisms is also a sensitive tool for characterizing soil bacterial-fungal communities in terms of their microbial community structure and biomass size (Kaur et al., 2005). It has succesfully been used as an indication of general and AMF biomass (Olsson, 1999) under long-term fertilization experiments (Toyota and Kuninaga, 2006).

CDA analysis in the present study clearly identified the functions separating different treatments (chemical fertilization, manure and plant compost) with the exception of mycorrhiza-inoculated compost versus control (Figure 1). In general, organic treatments seemed to increase the concentrations of the marker PLFAs but the highest and most consistently significant enhancement in the selected microbial groups was observed only under manure treatment (Table 3). This may be due to the stimulation of indigenous microbes by manure addition as it is comprised of a variety of microorganisms at time of application (Ngosong et al., 2009) and may develop a different microbial community structure through repeated long-term applications (Toyota and Kuninaga, 2006). Relatively high concentrations of G− bacterial PLFAs under organic treatments can be attributed to high substrate availability with increasing organic matter (Peacock et al., 2001). G+ bacterial and actinobacteria PLFAs also gave distinct responses to the individual organic and chemical fertilization treatments (Table 3). Actinobacteria are known to have a crucial role in the degradation process of complex, recalcitrant compounds (Goodfellow and Williams, 1983). Branched fatty acids, which were specific to G+ bacteria, are noted by Arao et al. (1998a, 1998b) as becoming higher in compost-amended soils than in non-amended soils. The present results are in line with the work of Elfstrand et al. (2007) who also found that both actinobacteria and G+ bacteria were more strongly associated with decomposing organic material.

NLFA to PLFA 16:1ω5c Ratio as an Indication of AMF Biomass in Soil

The PLFA 16:1ω5c exists in larger concentrations in the majority of AM fungi (Johansen et al., 1996; Olsson et al., 1999). While it has been found to correlate with the fungal hyphal length, NLFA 16:1ω5 correlated well with AM fungi spores (Olsson et al., 1997). However, PLFA 16:1ω5c can also be said to originate from soil bacterial biomass (Nichols et al., 1986). This suggests that caution must be taken

when assigning it to AM fungi (Frostegård et al., 2011). NLFA 16:1ω5 was determined to be more sensitive than PLFA 16:1ω5c as an indicator of AM fungi in soil due to the NLFA/PLFA 16:1ω5c ratio changes between 1 and 200 in AM fungi; whereas, it is lower than 1 in bacteria (Olsson et al., 1999).

Vestberg et al. (2012) reported higher ratios with mycotrophic crops such as barley (4.0), reed grass (7.0), and caraway (7.5), whereas they observed lower values with nonmycorrhizal plants such as buckwheat (0.42) and nettle (0.78) in nonrhizosphere soil samples. The authors mainly ascribed these changes to variation in NLFA 16:1ω5 as they observed no significant changes in PLFA 16:1ω5c. In parallel with these observations, PLFA 16:1ω5c did not indicate prominent differences between chemical and organic treatments, especially in summer and autumn. However, mean seasonal NLFA/PLFA 16:1ω5c ratios ranged between 1.96 and 4.3 under different treatments in our study (Table 3).

Both mycorrhizal colonization (measured in percentage of root length) and AMF PLFA 16:1ω5c followed a similar trend displaying insignificant changes between different treatments, except mycorrhiza-inoculated compost treatment. However, the remarkably higher NLFA/PLFA 16:1ω5c ratio in untreated as opposed to treated soils (Table 3) and the contradiction in considerably higher mycorrhizal colonizations (Table 1) versus insignificant changes in the level of AMF PLFA 16:1ω5c under mycorrhiza-inoculated compost treatment (Table 3) seemed to diminish the reliability of 16:1ω5c as an AM fungal PLFA marker. These contradictions may partly be attributed to a background 16:1ω5c, which can be produced by other soil microorganisms, varying from 30% to 60% of total PLFA 16:1ω5c (Olsson, 1999). It therefore obscures actual PLFA 16:1ω5c contained by AM fungi in soil. Our observations are compatible with the findings of Vestberg et al. (2012), who also reported unchanging PLFA 16:1ω5 levels. Another explanation for the contradictory results between root colonization-% and AM fungal PLFA/NLFA data may relate to the possibility that AM species can compete to colonize plant roots but are not capable of colonizing the soil. Cavagnaro et al. (2006) also noted an insignificant loading score for PLFA 16:1ω5c in their statistical analysis, which might be due to the preponderance of nonrhizosphere soil sampled in their work. Similarly, the discrepancies between PLFA and NLFA 16:1ω5c values and mycorrhizal colonization-% under mycorrhiza-inoculated compost treatment can be related to sampling strategy based on 2×2 m grid sampling in the present study.

Conclusions

The overall results obtained from semi-arid Çukurova soils of Mediterannean Turkey showed that shifting from intensive chemical fertilizers to organic soil management enhanced plant available phosphorus content of soil and increased plant yield; whereas, contrary to our initial hypothesis, no significant difference was observed in soil carbon and nitrogen status in relation to long-term organic fertilization treatments. Despite long-term organic matter additions, the steady-state levels of soil carbon and nitrogen is likely related to the climatic characteristics of the Çukurova Region, Mediterranean Turkey, where xeric moisture regime, high humidity, and temperature exacerbate carbon and nitrogen mineralization processes in soil. On the other hand, increasing wheat and corn yields under long-term mycorrhiza-inoculated compost treatment indicated that the use of AM inoculum can be

considered as a prefential strategy to increase plant productivity under such climatic and environmental conditions.

This field experiment produced some mixed results as well. There was an increasing abundance of bacterial PLFA markers contrasting with the steadiness of ergosterol concentration and fungal PLFAs in the present study. This suggests that the changes in soil microbial community structure under long-term fertilization practices were mainly achieved by bacteria rather than fungi. Higher mycorrhizal colonization and plant yield values obtained from mycorrhiza-inoculated conditions were compatible with our second hypothesis. However, soil fungal markers, GRSP and, most notably, PLFA 16:1ω5c, did not seem to be affected by mycorrhizal inoculation. Such inoculation might be highly efficient in terms of a plant growth promotion effect but not good enough to augment AM fungal biomass in soil. The steady seasonal values of PLFA 16:1ω5c under mycorrhiza-inoculated conditions were likely related to the uncertainties on this PLFA marker, also synthesized by various soil bacteria. Those of GRSP under the same condition can be related to problems in glomalin extraction and measurement in soil.

Acknowledgments

The authors wish to thank Mr. Uygar Türk and Zeynep Bozkurt (Faculty of Agriculture, University of Çukurova, Adana, Turkey) for their assistance during field work.

References

Al-Kaisi, M. M., X. Yin, and M. A. Licht. 2005. Soil carbon and nitrogen changes as affected by tillage system and crop biomass in a corn-soybean rotation. *Applied Soil Ecology* 30: 174–191.

Al-Karaki, G. N., B. McMichael, and J. Zak. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza* 14: 263–269.

Alvey, S., C. H. Yang, A. Buerkert, and D. E. Crowley. 2003. Cereal/legume rotation effects on rhizosphere bacterial community structure in West African soils. *Biology and Fertility of Soils* 37: 73–82.

An, Z. Q., J. W. Hendrix, R. S. Hershman, D. E. Ferriss, and G. T. Henson. 1993. The influence of crop-rotation and soil fumigation on a mycorrhizal fungal community associated with soybean. *Mycorrhiza* 3: 171–182.

Arao, T., S. Okano, and T. Kanamori. 1998a. Analysis of the phospholipid fatty acids of upland light colored Andosol and the relationship among the size of biomass based on phospholipid fatty acid analyses, microscopical counts and chloroform fumigation-incubation. *Japanese Journal Soil Science Plant Nutrition* 69: 38–46.

Arao, T., S. Okano, and T. Kanamori. 1998b. The analysis of phospholipid fatty acids of upland soils. *Japanese Journal Soil Science Plant Nutrition* 69: 47–52.

Bai, C. M., X. L. He, H. L. Tang, B. Q. Shan, and L. L. Zhao. 2009. Spatial distribution of arbuscular mycorrhizal fungi, glomalin and soil enzymes under the canopy of *Astragalus adsurgens Pall*. in the Mu Us sandland, China. *Soil Biology and Biochemistry* 41: 941–947.

Balser, T. C. 2001. The impact of long-term nitrogen addition on microbial community composition in three Hawaiian forest soils. *Scientific World Journal* 1: 500–504.

Bardgett, R. D., J. B. Whittaker, and J. C. Frankland. 1993. The effects of agricultural practices on the soil biota of some upland grasslands. *Agriculture, Ecosystem and Environment* 45: 25–45.

Bending, G. D., M. K. Turner, F. Rayns, M. C. Marx, and M. Wood. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry* 36: 1785–1792.

Böhme, L., U. Langer, and F. Böhme. 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. *Agriculture, Ecosystem and Environment* 109: 141–152.

Bossio, D. A., and K. M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology* 35: 265–278.

Bouyoucos, G. J. 1951. A calibration of the hydrometer for making mechanical analysis of soils. *Agronomy Journal* 43: 9–11.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding. *Analytical Biochemistry* 72: 248–254.

Caravaca, F., M. M. Alguacil, J. M. Barea, and A. Roldan. 2005. Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. *Soil Biology and Biochemistry* 37: 227–233.

Caravaca, F., J. M. Barea, D. Figueroa, and A. Roldán. 2002. Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for reafforestation with *Olea europaea* subsp. sylvestris through changes in soil biological and physical parameters. *Applied Soil Ecology* 20: 107–118.

Caravaca, F., J. M. Barea, J. Palenzuela, D. Figueroa, M. M. Alguacil, and A. Roldán. 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *Applied Soil Ecology* 22: 103–111.

Carpenter-Boggs, L., A. C. Kennedy, and J. P. Reganold. 2000. Organic and biodynamic management—effects on soil biology. *Soil Science Society of American Journal* 64: 1651–1659.

Cavagnaro, T. R., L. E. Jackson, J. Six, H. Ferris, S. Goyal, D. Asami, and K. M. Scow. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. *Plant and Soil* 282: 209–225.

Çelik, I., H. Gunal, M. Budak, and C. Akpinar. 2010. Effect of long-term organic and mineral fertilizers on bulk density and penetration resistance in semi arid Mediterranean soil conditions. *Geoderma* 160(2): 236–243.

Çelik, I., I. Ortas, and S. Kilic. 2004. Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil Tillage Res.* 78: 59–67.

Dann, P. R., J. W. Derrick, D. C. Dumares, and M. H. Ryan. 1996. The response of organic and conventionally grown wheat to superphosphate and reactive phosphate rock. *Australian Journal of Experimental Agriculture* 36: 71–78.

Del Mar Alguacil, M., E. Diaz-Pereira, F. Caravaca, D. A. Fernandez, and A. Roldan. 2009. Increased diversity of arbuscular mycorrhizal fungi in a long-term field experiment via application of organic amendments to a semiarid degraded soil. *Applied and Environmental Microbiology* 75: 4254–4263.

Del Mar Alguacil, M., Z. Lozano, M. J. Campoy, and A. Roldán. 2010. Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system. *Soil Biology and Biochemistry* 42: 1114–1122.

Donnison, L. M., G. S. Griffith, J. Hedger, P. J. Hobbs, and R. D. Bargett. 2000. Management influences on soil microbial communities and their function in botanically diverse haymeadows of northern England and Wales. *Soil Biology and Biochemistry* 32: 253–263.

Douds, D. D., L. Galvez, M. Franke-Snyder, G. Reider, and L. E. Drinkwater. 1997. Effect of compost addition and crop rotation point upon VAM fungi. *Agriculture, Ecosystem and Environment* 65: 257–266.

Duncan, D. B. 1955. New multiple range and multiple F tests. *Biometrics* 11: 1.

Elfstrand, S., K. Hedlund, and A. Mårtensson. 2007. Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. *Applied Soil Ecology* 35: 610–621.

Franke-Snyder, M., D. D. Douds, L. Galvez, J. G. Phillips, P. Wagoner, L. Drinkwater, and J. B. Morton. 2001. Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA. *Applied Soil Ecology* 16: 35–48.

Frey, S. D., E. T. Elliott, and Paustian, K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biology and Biochemistry* 31: 573–585.

Frostegård, Å., A. Tunlid, and B. Erland. 2011. Use and misuse of PLFA measurements in soils. *Soil Biology and Biochemistry* 43: 1621–1625.

Galvez, L., D. D. Douds, L. E. Drinkwater, and P. Wagoner. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil* 228: 299–308.

Gillespie, W. A., R. E. Farrell, F. L. Walley, Ross, A. R. S., P. Leinweber, K. U. Eckhardt, T. Z. Regier, and R. I. R. Blyth. 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. *Soil Biology and Biochemistry* 43: 766–777.

Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhiza in roots. *New Phytologist* 84: 489–500.

Gok, M., I. Onac, A. Coskan, T. Saglamtimur, I. Inal, J. C. G. Ottow, and G. Benckiser. 2000. Influence of organic fertilization on N mineralization, denitrification and biological activity in soil under maize plantings. *Turkish–German Agricultural Research–6th Symposium*, pp. 85–90. 27 Sept–2 Oct. 1999. Stuttgart, Germany.

Gong, P., X. Guan, and E. Witter. 2001. A rapid method to extract ergosterol from soil by physical disruption. *Applied Soil Ecology* 17: 285–289.

Goodfellow, M., and S. T. Williams. 1983. Ecology of actinomycetes. *Annual Review of Microbiology* 37: 189–216.

Hontoria, C., R. Velasquez, M. Benito, J. Almorox, and A. Moliner. 2009. Bradford-reactive soil proteins and aggregate stability under abandoned versus tilled olive groves in a semi-arid calcisol. *Soil Biology and Biochemistry* 41(7): 1583–1585.

Jansa, J., A. Mozafar, T. Anken, R. Ruh, I. R. Sanders, and E. Frossard. 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza* 12: 225–234.

Joergensen, R. G., P. Mäder, and A. Fließbach. 2010. Long-term effects of organic farming on fungal and bacterial residues in relation to microbial energy metabolism. *Biology and Fertility of Soils* 46: 303–307.

Joergensen, R. J., and F. Wichern. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry* 40: 2977–2991.

Johansen, A., R. D. Finlay, and P. A. Olsson. 1996. Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus Glomus intraradices. *New Phytologist* 133: 705–712.

Johnson, N. C., and F. L. Pfleger. 1992. Vesicular-arbuscular mycorrhizae and cultural stresses. In: Mycorrhizae in sustainable agriculture, pp. 71–99, in G. J. Bethlenfalvay and R. G. Linderman, eds., *American Society of Agronomy special publication no. 54*. American Society of Agronomy, Madison, WI.

Kacira, M., S. Sase, O. O. Kacira, L. Okushima, M. Ishii, H. Kowata, and H. Moriyama. 2004. Status of greenhouse production in Turkey: Focusing on vegetable and floriculture productions. *Journal of Agricultural Meteorology of Japan* 60(2): 115–122.

Kaur, A., A. Choudhary, A. Kaur, R. Choudhary, and R. Kaushik. 2005. Phospholipid fatty acid-A bioindicator of environment monitoring and assessment in soil ecosystem. *Current Science* 89: 1103–1112.

Koske, R. E., and J. N. Gemma. 1989. A modified procedure for staining roots to detect VAM. *Mycol. Res.* 92: 486–505.

Kozdrój, J., and J. D. van Elsas. 2001. Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. *Journal of Microbiological Methods* 43: 197–212.

Kurle, J. E., and F. L. Pfleger. 1996. Management influences on arbuscular mycorrhizal fungal species composition in a corn-soybean rotation. *Agronomy Journal* 88: 155–161.

Lagomarsino, A., M. C. Moscatelli, A. D. Tizio, R. Mancinelli, S. Grego, and S. Marinari. 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecological Indicators* 9: 518–527.

Liu, E., Y. Changrong, M. Xurong, H. Wenqing, S. H. Bing, D. Linping, L. Qin, L. Shuang, and F. Tinglu. 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma* 158: 173–180.

Mardukhi, B., F. Rejali, G. Daei, M. R. Ardakani, M. J. Malakouti, and M. Miransari. 2011. Arbuscular mycorrhizas enhance nutrient uptake in different wheat genotyeps at high salinity levels under field and greenhouse conditions. *Comptes Rendus Biologies* 334: 564–571.

Marinari, S., R. Mancinelli, E. Campiglia, and S. Grego. 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. Ecol. Indicators 6, 701–711.conditions. *Geoderma* 160(2): 236–243.

Nelson, A. G., and D. Spanner. 2010. Cropping systems management, soil microbial communities, and soil biological fertility, pp. 217–242, in E. Lichtfouse, ed., *Genetic engineering, biofertilisation, soil quality and organic farming*. Springer, The Netherlands.

Ngosong, C., J. Raupp, S. Scheu, and L. Ruess. 2009. Low importance for a fungal based food web in arable soils under mineral and organic fertilization indicated by Collembola grazers. *Soil Biology and Biochemistry* 41: 2308–2317.

Nichols, P., B. K. Stulp, J. G. Jones, and D. C. White. 1986. Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria Vitreoscilla Flexibacter, Filibacter. *Archives of Microbiology* 146: 1–6.

Nie, J., J. M. Zhou, H. Y. Wang, X. Q. Chen, and C. W. Du. 2007. Effect of long-term rice straw return on soil glomalin, carbon and nitrogen. *Pedosphere* 17(3): 295–302.

Oehl, F., E. Sieverding, P. Mäder, D. Dubois, K. Ineichen, T. Boller, and A. Wiemken. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia* 138: 574–583.

Olsen, S. R., and L. E. Sommers. 1982. Phosphorus, pp. 403–430, in A. L. Page, R. H. Miller, and D. R. Keeney, eds., *Methods of soil analysis. Part 2.* 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Olsson, P. A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29: 303–310.

Olsson, P. A., E. Bååth, and I. Jakobsen. 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. *Applied and Environmental Microbiology* 63: 3531–3538.

Olsson, P. A., I. Tingstrup, I. Jakobsen, and E. Bååth. 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. *Soil Biology & Biochemistry* 31: 1879–1887.

Ortaş, I. 1996. The influence of use of different rates of inoculum on root infection plant growth and phosphorus uptake. *Communications in Soil Science and Plant Aanalysis* 27: 2935–2946.

Peacock, A. D., M. D. Mullen, D. B. Ringelberg, D. D. Tyler, D. B. Hedrick, P. M. Gale, and D. C. White. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry* 33: 1011–1019.

Raupp, J., C. Pekrun, M. Oltmanns, and U. Köpke, Eds. 2006. Long-term field experiments in organic farming. *ISOFAR Scientific Series No 1*. Verlag Dr. Koster, Berlin.

Richards, L. A. 1954. Diagnosis and improvement of saline and alkali soils. *USDA Handbook* 60: 160.

Rillig, M. C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian. Journal of Soil Science* 84: 355–363.

Rillig, M. C., S. F. Wright, K. A. Nichols, W. F. Schmidt, and M. S. Torn. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* 233: 167–177.

Rynk, R. 1992. *On-farm composting handbook*. Northeast Regional Agricultural Engineering Service, Ithaca, NY.

Sanchez-Diaz, M., and M. Honrubia. 1994. Water relations and alleviation of drought stress in mycorrhizal plants, pp. 167–178, in S. Gianinazzi and H. Schepp, eds., *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*. Birkhauser, Basel.

Schindler, F. A., E. J. Mercer, and J. A. Rice. 2007. Chemical characteristics of glomalin related soil protein (GRSP) extracted from soils of varying organic matter content. *Soil Biology and Biochemistry* 39: 320–329.

Scullion, J. S., W. R. Eason, and E. P. Scott. 1998. The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass-arable rotations. *Plant and Soil* 204: 243–254.

Sharif, N. M., K. Rubina, and T. Burni. 2010. Occurrence and distribution of arbuscular mycorrhizal fungi in wheat and maize crops of Malakand division of North West Frontier Province. *Pakistan Journal of Botany* 42(2): 1301–1312.

Sherrod, L. A., G. Dunn, G. A. Peterson, and R. L. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calcimeter method. *Soil Science Society of America Journal* 66: 299–305.

Smith, S. E., and V. Gianinazzi-Pearson. 1988. Physiological interactions between symbionts in vesicular arbuscular mycorrhizal plants. *Annual Review of Plant Physiology* 39: 221–244.

Soil Survey Staff. 1996. *Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report*, vol. 42. Ver. 3.0 USDANRCS. U.S. Govt. Printing Office, Washington, DC, p. 693.

Srinivasarao, Ch., A. N. Deshpande, B. Venkateswarlu, R. Lal, A. K. Singh, S. Kundu, K. P. R. Vittal, et al. 2012. Grain yield and carbon sequestration potential of post monsoon sorghum cultivation in Vertisols in the semi arid tropics of central India. *Geoderma* 175–176: 90–97.

Steinberg, P. D., and M. C. Rillig. 2003. Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. *Soil Biology and Biochemistry* 35: 191–194.

Tejada, M., and J. L. González. 2008. Influence of two organic amendments on the soil physical properties, soil losses, sediments and runoff water quality. *Geoderma* 145: 352–334.

Toyota, K., and S. Kuninaga. 2006. Comparison of soil microbial community between soils amended with or without farmyard manure. *Applied Soil Ecology* 33: 39–48.

Turkish State Meteorological Service (TSMS). 2009. http://www.meteor.gov.tr

Turgay, O. C., J. Lumbanraja, S. Yusnaini, and M. Nonaka. 2002. Effect of land degradation on soil microbial biomass in a hilly area of South Sumatra, Indonesia. *Japanese Soil Science and Plant Nutrition* 48(5): 769–774.

Vestberg, M., A. Palojärvi, T. Pitkänen, S. Kaipainen, E. Puolakka, and M. Keskitalo. 2012. Neutral lipid fatty acid analysis is a sensitive marker for quantitative estimation of arbuscular mycorrhizal fungi in agricultural soil with crops of different mycotrophy. *Agricultural and Food Science* 21(1): 12–27.

Wright, S. F., and A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* 198: 97–107.