Arid Land Research and Management

ISSN: 1532-4982 (Print) 1532-4990 (Online) Journal homepage: http://www.tandfonline.com/loi/uasr20

Arbuscular mycorrhizal fungal community of wheat under long-term mineral and organic amendments in semi-arid Mediterranean Turkey

Takuya Buto, Kazuki Suzuki, Tomoyuki Kaidzu, Taishi Narisawa, Oguz Can Turgay, Ibrahim Ortas, Naoki Harada & Masanori Nonaka

To cite this article: Takuya Buto, Kazuki Suzuki, Tomoyuki Kaidzu, Taishi Narisawa, Oguz Can Turgay, Ibrahim Ortas, Naoki Harada & Masanori Nonaka (2016): Arbuscular mycorrhizal fungal community of wheat under long-term mineral and organic amendments in semi-arid Mediterranean Turkey, Arid Land Research and Management, DOI: 10.1080/15324982.2016.1177749

To link to this article: http://dx.doi.org/10.1080/15324982.2016.1177749

ARID LAND RESEARCH AND MANAGEMENT
http://dx.doi.org/10.1080/15324982.2016.1177749

Taylor & Francis
Taylor & Francis Group

Arbuscular mycorrhizal fungal community of wheat under long-term mineral and organic amendments in semi-arid Mediterranean Turkey

Takuya Buto[a,*], Kazuki Suzuki[a,*], Tomoyuki Kaidzu[a], Taishi Narisawa[a], Oguz Can Turgay[b], Ibrahim Ortas[c], Naoki Harada[d], and Masanori Nonaka[d]

[a]Graduate School of Science and Technology, Niigata University, Niigata, Japan; [b]Faculty of Agriculture, Ankara University, Diskapi, Ankara, Turkey; [c]Faculty of Agriculture, Cukurova University, Adana, Turkey; [d]Institute of Science and Technology, Niigata University, Niigata, Japan

ABSTRACT

A minimal amount of information is currently available concerning arbuscular mycorrhizal (AM) fungal associations with crops in semi-arid zones on Leptosols in Turkey. Therefore, using molecular ecological techniques, we studied the effects of different management practices (without fertilization, chemical fertilization, farmyard manure, and plant compost amendments) on AM fungal communities associated with wheat roots. Experiments were conducted in a field established in 1996 in southern Mediterranean Turkey where soil productivity is low owing to unfavorable climatic effects and soil characteristics. We determined 201 partial sequences of AM fungal nuclear ribosomal large subunit genes. The higher AM fungal richness was found in the control treatment without fertilization and plant compost treatments compared with the chemical fertilization and farmyard manure treatments. Clones related to *Rhizophagus* were found in all treatments and accounted for 37% of the total AM fungal clones, whereas those of *Funneliformis* were dominant under chemical fertilization. Redundancy analysis based on the frequency of operational taxonomic units revealed that AM fungal communities were divided into three groups, namely, the control treatment, the chemical fertilization treatment, and the organic treatments (farmyard manure and plant compost treatments). Although different organic amendments supported relatively similar AM fungal communities, plant compost induced higher AM fungal richness than farmyard manure fertilization.

ARTICLE HISTORY
Received 3 June 2015
Accepted 9 April 2016

KEYWORDS
Arbuscular mycorrhizal fungi; community analysis; large ribosomal subunits (LSU rDNA); Leptosol; organic fertilization; southern Mediterranean Turkey

Introduction

Arbuscular mycorrhizal (AM) fungi are the most common group of mycorrhizal fungi interacting with the roots of most terrestrial plants in nature, including many crops (Allen 1991). The major benefit of AM symbiosis for plants is that AM fungi collect water and minerals, particularly phosphorus (P), in moisture-deficient or nutrient-poor soils, and transport them through the extraradical hyphae to the root (Smith and Read 2008).

Turkey is one of the major wheat (*Triticum aestivum* L.) producing countries in the world and wheat is the most important cereal produced in Turkey, accounting for 64.5%

CONTACT Naoki Harada naharada@agr.niigata-u.ac.jp Institute of Science and Technology, Niigata University, 8050 Ikarashi-2, Nishi-ku, Niigata 950-2181, Japan.
*Takuya Buto and Kazuki Suzuki contributed equally to this work.
Supplemental data for this article can be accessed on the publisher's website.

of total cereal production (Ozkan, Akcaoz, and Fert 2004). Wheat is known to be a facultatively mycorrhizal plant (Molina, Massicotte, and Trappe 1992). Research on the associations between AM fungi and wheat-AM fungal associations in Turkey has mainly focused on the effect of AM fungal inoculation on nutrient uptake, plant yield, and soil fertility (Ortas 2003, 2012). Some previous studies indicated that the indigenous AM fungi isolated from semi-arid Mediterranean Turkey could significantly contribute to plant growth and P uptake (Almaca and Ortas 2010; Ortas 2010). However, these works used morphological tools such as AM fungal infection rates, spore morphology, and spore densities, which might not necessarily reflect the total AM fungal populations associated with the roots.

Molecular ecology methods have been developed that allow direct detection of AM fungal species in plant roots and, along with the development of molecular classification of AM fungi, have become promising tools to overcome the bottlenecks in AM fungal community analysis (Schussler, Schwarzott, and Walker 2001; Kruger et al. 2012; Redecker et al. 2013). Suzuki et al. (2014) investigated for the first time the composition of AM fungal phylotypes based on the nuclear ribosomal large subunit RNA genes in the roots of various agricultural plants such as tea, clover, wheat, corn, apple, apricot, sunflower, soybean, and alfalfa collected from the East Black Sea, Mediterranean, and Central Anatolian regions of Turkey. Their results showed that AM fungal communities in the Mediterranean region tended to be more diverse than those in the Central Anatolian region. It was also reported that indigenous AM fungi in the Mediterranean region improved the growth and nutrient uptake of citrus (Ortas and Ustuner 2014a, 2014b). However, no information is currently available regarding the community compositions of AM fungi in the wheat rhizosphere under different land managements in semi-arid soils of Mediterranean Turkey.

In this study, we hypothesized that wheat-associated AM fungal community responds to differences in fertilization practices and source materials of organic fertilizer in a long-term field experiment established in the Cukurova Region, Mediterranean Turkey. The soil productivity in the field is low owing to xeric climate conditions (i.e., strong seasonal winter/summer rainfall contrast resulting in soil root zone drying during the summer) and exacerbated carbon and nitrogen mineralization in soil (Turgay et al. 2015). The soil type is classified as a Leptosol. A minimal amount is known about AM fungal community in arable land on Leptosols around the world, although soil type is one of the key factors determining AM community composition (Oehl et al. 2003). The aims of this study were to: (1) elucidate using molecular methods AM fungal community in Turkish semi-arid arable land on a Leptosol, and (2) compare the impacts of different soil management practices, including organic management, on AM fungal community.

Materials and methods

Site description

The study was conducted in an experimental field on the Research Farm of Cukurova University (37°0′54″N, 35°21′21″E, 34 m above mean sea level) in the eastern part of the Mediterranean region of Adana, Turkey. The regional climate is typical Mediterranean with 19.1°C long-term average annual air temperature (ranging from 14.2 to 25.5°C) and 670 mm precipitation. Winter wheat was grown under rainfed conditions where the mean humidity and temperature were 73.2% and 17.8°C, respectively, and total

Table 1. Soil and yield characteristics in the experimental site[a].

Treatment	Soil properties						Wheat yield in 2009
	CEC ($\mu mol_c\ g^{-1}$)	$CaCO_3$ ($mg\ g^{-1}$)	$pH(H_2O)$[b]	Organic C[c] ($mg\text{-}C\ g^{-1}$)	Available N[d] ($mg\text{-}N\ kg^{-1}$)	Available P[e] ($mg\text{-}P\ kg^{-1}$)	($kg\ ha^{-1}$)
CO	7.54 ± 0.43	3.68 ± 0.05	7.81 ± 0.10	10.8 ± 0.15	37.8 ± 2.4	11.47 ± 0.28	3127 ± 785
CF	6.87 ± 0.50	3.92 ± 0.12	7.72 ± 0.01	15.1 ± 0.37	39.2 ± 0.7	25.17 ± 0.60	7207 ± 1075
FM	6.89 ± 0.60	3.67 ± 0.06	7.77 ± 0.07	16.3 ± 0.43	39.2 ± 4.3	33.76 ± 0.57	6200 ± 1325
PC	7.78 ± 0.10	3.67 ± 0.33	7.79 ± 0.05	12.5 ± 0.37	39.0 ± 1.8	25.05 ± 0.43	4733 ± 1209

Note: CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.
[a]Average $\pm$ standard deviation ($n = 3$).
[b]Measured on a soil:water ratio at 1:2.5 (*w/v*).
[c]Organic carbon (C) was calculated by subtracting the inorganic C, which was determined using a modified pressure-calcimeter method (Sherrod et al. 2002), from the total C, which was measured according to the Dumas method using a Variomax CNS elemental analyzer (Elementar GmbH, Hanau, Germany).
[d]Available nitrogen (N) means sum of NH_4 and NO_3, measured according to a steam distillation method adding Devarda's alloy.
[e]Available phosphorus (P) was measured as indicated by Olsen and Sommers (1982).

precipitation was 86.3 mm during the wheat growth period. According to the classification of the World Reference Base for Soil Resources, the soil at the experimental site was classified as Molli-Lithic Leptosol (Jones, Montanarella, and Jones 2005). The particle size distribution was 37.5, 31.0, and 31.5% for clay, silt, and sand, respectively. Other soil chemical characteristics under different amendments and wheat yield in 2009 are summarized in Table 1.

Field experiment

The long-term field experiment started in April 1996 using a completely randomized design with four different fertilizer treatments in triplicate (each plot was 10×20 m), as follows: control without fertilization, traditional chemical fertilization of 160 kg-N $ha^{-1}\ y^{-1}$ as $(NH_4)_2SO_4$, 83 kg-K $ha^{-1}\ y^{-1}$ as K_2SO_4 and 26 kg-P $ha^{-1}\ y^{-1}$ as $Ca(H_2PO_4)_2 \cdot H_2O$, farmyard manure at 25 Mg $ha^{-1}\ y^{-1}$, and plant compost at 25 Mg $ha^{-1}\ y^{-1}$. Preparation of the plant compost material was described previously by Turgay et al. (2015). The organic fertilizers (farmyard manure and plant compost) were uniformly applied annually on the soil surface and were incorporated into the plow layer with a disc harrow. A double cropping system with wheat and maize (*Zea mays* L.) was introduced to the experimental field. Each plot was moldboard-plowed up to 20 cm depth after each harvest.

DNA extraction and cloning

Three soil samples were collected and mixed well from each plot in May 2009 during the wheat cropping season. Fine wheat roots were obtained using a 2-mm sieve and were washed thoroughly using sterile water and then stored in a refrigerator at 4°C until used. Total DNA was extracted from 50 mg of the root samples using Isoplant (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Using the extracted DNA as the template, AM fungal 28S large subunit (LSU) rRNA genes were amplified with a nested polymerase chain reaction (PCR) protocol. The first PCR amplification was performed using the fungal universal primers LR1 and FLR2 (van Tuinen et al. 1998; Trouvelot

et al. 1999), followed by the second PCR amplification using the Glomeromycota-specific primers FLR3 (van Tuinen et al. 1998; Trouvelot et al. 1999) and FLR4 (Gollotte, van Tuinen, and Atkinson 2004). Both reactions were performed using PCR Thermal Cycler Dice Gradient TP600 (Takara, Otsu, Japan) under the same conditions as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 40 s, polymerization at 72°C for 80 s, and final elongation at 72°C for 10 min. The amplicons generated from each root sample were cloned into the pGEM-T Easy Vector System (Promega, Fitchburg, USA) and were transformed into Takara *Escherichia coli* DH5α cells. Plasmids were purified using the High Pure Plasmid Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany). The nucleotide sequences were determined by the Fasmac DNA sequencing service (Atsugi, Japan) and have been deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB727771 to AB727848. The sequence results from triplicate plots were combined into one treatment for subsequent analyses. The sequence results were compared with those available in the GenBank database by the Basic Local Alignment Search Tool (BLAST) search engine.

Analysis of sequences

The nucleotide sequences determined above and known mycorrhizal 28S rDNA sequences of related species from GenBank were aligned and edited to the same length in BioEdit 7.0.5.3. All gaps and missing data were deleted. Subsequently, a neighbor-joining phylogenetic dendrogram was constructed in MEGA5 using the Kimura-2 method and bootstrap analysis with 1,000 replications.

Distance matrices were generated with the Jukes-Cantor model using the DNADIST program from Phylip version 3.6.9. Using the data as an input file, we clustered sequences into operational taxonomic units (OTUs) at 99, 97, and 95% similarity levels using the Mothur program version 1.25.1. Rarefaction curves and nonparametric species richness estimators ACE and Chao1 were generated in Mothur software. Based on the results, the frequency of each OTU was calculated and compared among treatments with soil chemical properties using redundancy analysis (RDA) in XLSTAT software.

Results and discussion

Arbuscular mycorrhizal fungal taxa and richness

We obtained from the wheat root samples a total of 201 clones that were highly similar to sequences from taxa belonging to the phylum Glomeromycota. Of the total sequences obtained, 52, 48, 49, and 52 clones were from the control, chemical fertilizer, farmyard manure, and plant compost treatments, respectively. Detailed results of sequences obtained are shown in Supplemental Table 1.

The numbers of AM fungal sequences, OTUs (based on 97% sequence similarity), and estimated phylotype richness (ACE and Chao1) are shown in Table 2. A total of 69 unique sequences were found. Rarefaction curves for the pooled data were constructed at 99, 97, and 95% levels to assess the sizes on the AM fungal clone libraries. The curves for 97 and 95% levels showed saturation, suggesting that the number of clone sequences was sufficient to estimate the AM fungal communities based on 97% OTU level (Figure 1A). Finally, the clones were ascribed to 26 OTUs at the 97% sequence similarity level.

Table 2. Description of clone libraries and richness estimators for AM fungal communities in wheat (*Triticum aestivum* L.) roots collected in this study.

			ACE		Chao1	
Treatment	No. of clones	No. of OTUs (A)	Estimation (B)	Coverage (A/B)	Estimation (C)	Coverage (A/C)
CO	52	12	13.8	0.870	13.0	0.923
CF	48	9	12.1	0.744	10.5	0.857
FM	49	5	5.4	0.926	5.0	1.000
PC	52	11	13.1	0.840	12.0	0.917

Note: ACE and Chao1 estimators were generated in MOTHUR software at 97% similarity. CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.

Rarefaction curves were determined at the 97% OTU level for the different agricultural management treatments (Figure 1B). A flattened curve was obtained from the manure amendment treatment, indicating that the number of OTUs approached saturation, and no more additional OTUs could be expected even if the number of samples had been increased considerably. The curves for the control, chemical fertilization, and plant compost treatments showed a clear leveling off. Therefore, we recognized that the sample sizes were sufficient to recover most potential OTUs that occurred under the different long-term agricultural management treatments.

The plant compost treatment and the control treatment had higher OTU numbers compared with the chemical fertilizer and farmyard manure treatments (Table 2). This suggests

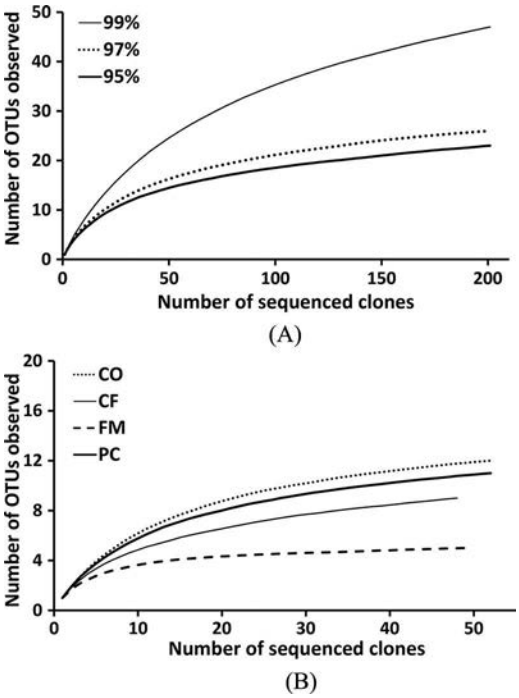

Figure 1. Rarefaction curves of the AM fungal large ribosomal subunit (LSU) rDNA libraries obtained from wheat roots in this study: for the pooled data at 99, 95, and 97% similarity levels (A) and each treatment at 97% similarity level (B). OTU, operational taxonomic unit; CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.

that environmental conditions due to different fertilization practices can shape AM fungal community in different ways. Tanu, Prakash, and Adholeya (2004) reported that the abundance of indigenous AM fungal propagules was higher in leaf compost-amended soil than in poultry manure-amended soil. Muthukumar and Udaiyan (2002) showed that mycorrhizal response was highly correlated with the type of organic manure applied. They also suggested that cowpea growth and yield in response to organic amendment were influenced by the type of amendment, resulting in proliferation of different AM fungi within the community.

The farmyard manure treatment showed the least number of AM fungal OTUs and the highest coverage of estimated richness. This may be because of high nutrient contents in the soil, especially P (Table 1), since higher soil P availability is known to suppress AM fungal colonization (Sharma and Adholeya 2001) and spore density (Menge et al. 1978).

Phylogenetic analysis of arbuscular mycorrhizal fungi and their distribution

An alignment was conducted between the different Glomeromycota sequences derived from all treatments and twenty-nine reference sequences. Clones that produced the same sequence were represented just once per plot in the alignment. A neighbor-joining tree was generated from the AM fungal phylotypes (Figure 2). Eight and one AM fungal sequence types were grouped in the Glomerales and Diversisporales orders, respectively, on the basis of bootstrap values >70%. The AM fungal sequence types belonging to Glomerales were subdivided into *Rhizophagus, Funneliformis*-1, *Funneliformis*-2, Glomeraceae-1, Glomeraceae-2, Glomeraceae-3, *Claroideoglomus*-1, and *Claroideoglomus*-2, all of which were in family Glomeraceae. In the Diversisporales group, one sequence type belonged to *Scutellospora*.

Frequency of the AM fungal groups in each plot was evaluated (Figure 3 and Supplemental Figure 1) and the sequence type *Rhizophagus* accounted for 37% of the AM fungal sequences detected in this study. *Rhizophagus*-related clones were found in all treatments, indicating that *Rhizophagus* spp. are the most common AM fungi in the area. The farmyard manure treatment showed higher frequency of *Rhizophagus*-related clones (53%) than the control, chemical fertilization, and plant compost treatments (31, 27, and 38%, respectively). After *Rhizophagus*, the next-most-frequent sequence type was *Funneliformis*-2, 38 clones of which were found from all treatments except for the control. *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe (currently *Funneliformis mosseae* (Nicolson & Gerdemann) Walker & Schuessler) and closely related taxa have been reported as common and typical AM fungal species in arable fields in Germany and Switzerland (Hijri et al. 2006). The sequence type *Funneliformis*-2 was most dominant under the chemical fertilization (44%), whereas the sequence type *Rhizophagus* was most dominant in the control, manure and plant compost treatments. The incidences of clones belonging to the sequence types *Funneliformis*-1, Glomeraceae-1, Glomeraceae-2, Glomeraceae-3, *Claroideoglomus*-1 and *Claroideoglomus*-2 were less than 10%. The sequence type *Scutellospora* was found only in the chemical fertilization treatment. This corresponds with results from Singh et al. (2008) who reported the absence of large-spored Gigasporaceae, including genus *Scutellospora*, in a cultivated ecosite in India. Some taxa in genus *Scutellospora* may be rare or absent in the arable field investigated in this study.

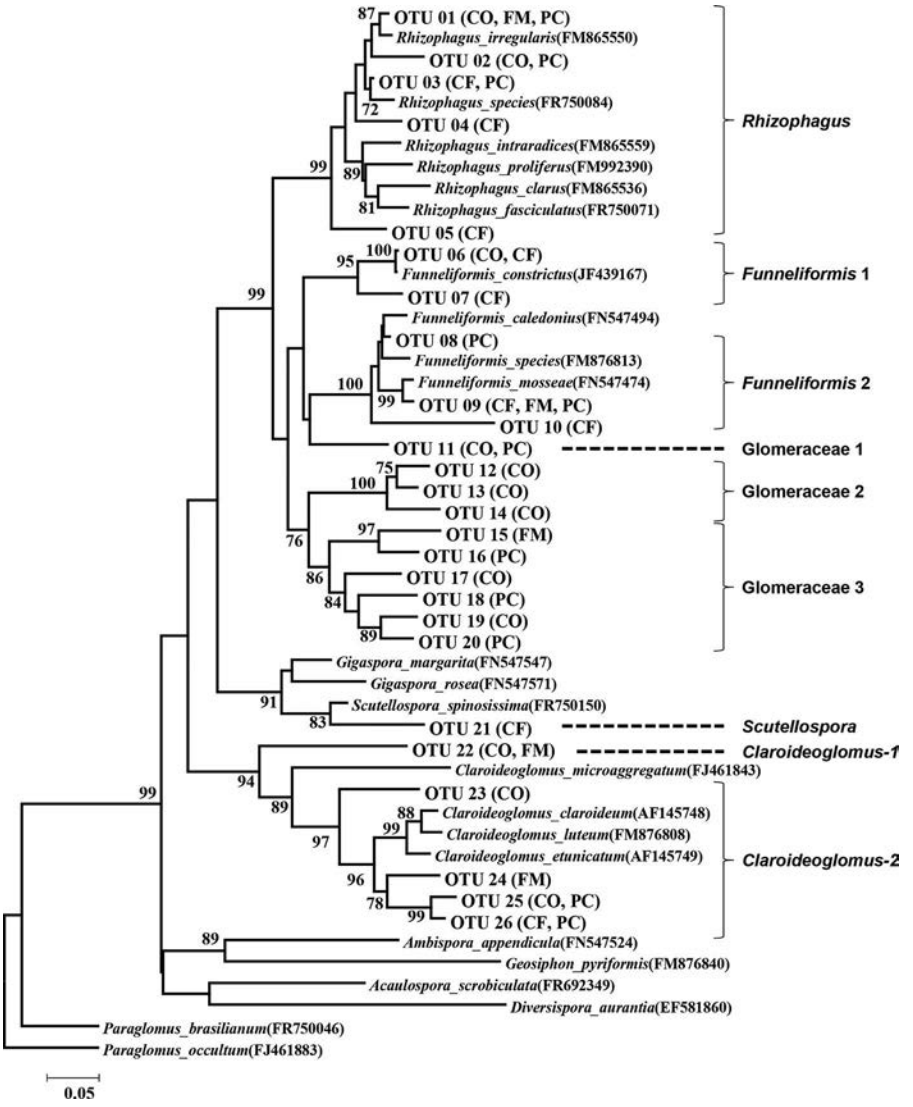

Figure 2. Neighbor-joining tree showing phylogenetic relationships of the AM fungal sequences obtained in this study and database sequences of known AM fungal species. The sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence similarity using Mothur software. Parentheses show the treatments from which each sequence was derived. The sequence of *Paraglomus occultum* was used as the outgroup. Numbers above branches denote bootstrap values more than 70% from 1,000 replications. Lines on the right delimit the phylotypes. The scale bar at the bottom left is proportional to branch length. CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.

RDA

RDA based on frequency of each OTU revealed that the AM fungal communities were divided into three groups, namely, the control group, the organic group (farmyard manure and plant compost treatments), and the chemical fertilization group along with the gradients of soil minerals such as available P, organic C, and available N (Figure 4). The first axis

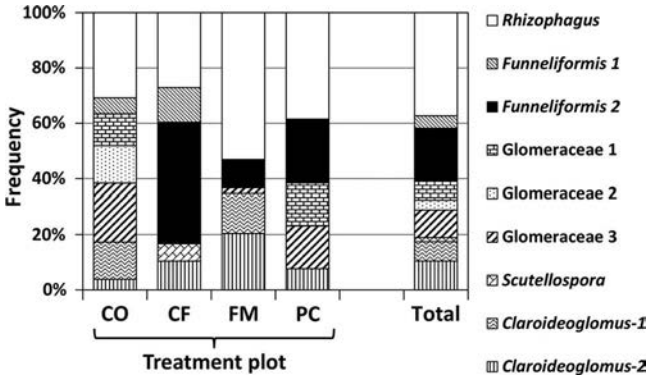

Figure 3. Frequency of the AM fungal groups detected in wheat root samples collected in this study. CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.

of RDA (60.28% of variance explained, Figure 4) indicated that the long-term chemical fertilization treatment altered the composition of the AM fungal community more than the organic treatments (farmyard manure and plant compost treatments). The result was similar to that of Verbruggen et al. (2010) who compared AM fungal community compositions under organic and conventional land managements using terminal restriction fragment length polymorphism community fingerprinting and reported that the difference in the AM fungal communities between conventionally managed farms and undisturbed, semi-natural grassland was larger than that between organically managed farms and grassland.

Sequence types *Funneliformis*-2 and *Rhizophagus* had the strongest axis1 and axis2 scores, respectively (Figure 4). This indicates that the first axis differentiated the treatments along the dominance gradient of sequence type *Funneliformis*-2 and the second axis differentiated treatments along that of *Rhizophagus*. The sequence types Glomeraceae-1, -2, and -3 also had strong axis2 scores, demonstrating the differences between the control from the farmyard manure and plant compost treatments. Bhadalung et al. (2005) showed different

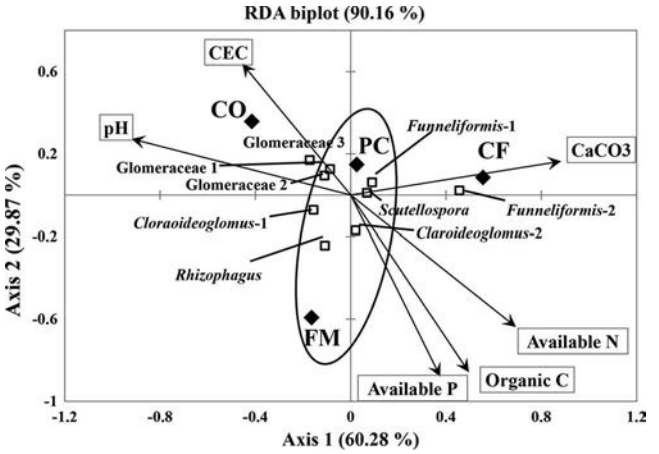

Figure 4. Redundancy analysis (RDA) between AM fungal communities associated with wheat roots collected in this study and environmental parameters. The percentages in the axes show the percentage of variation explained by the analysis. CO, control; CF, chemical fertilizer; FM, farmyard manure; PC, plant compost.

sensitivities among AM fungal species to fertilization based on an investigation of spore morphology. Our results suggested that the AM fungal species related to the sequence type *Rhizophagus* and *Funneliformis*-2 were relatively sensitive to organic fertilizer and chemical fertilizer, respectively.

Among the long-term fertilization amendments, the farmyard manure and plant compost treatments, both of which possessed the dominant sequence type *Rhizophagus*, showed relatively similar AM fungal communities, whereas the chemical fertilization treatment was very different from all others, dominated by the sequence type *Funneliformis*-2 (Figures 3 and 4). These taxa are commonly found in arable soils (Öpik et al. 2006). The first axis of RDA in which soil available N had a strong axis1 score arranged the AM fungal communities according to the dominancy of the sequence type *Funneliformis*-2 (60.28% of variance explained, Figure 4). This suggests that AM fungi related to the sequence type *Funneliformis*-2 (including *F. mosseae*) can dominate under eutrophic conditions. Oehl et al. (2003) reported that spore abundance of *G. mosseae* (currently *F. mosseae*) was higher in mineral and/or mineral +organic fertilizer amendment sites compared with grassland and organic farming sites.

Along the second axis in which soil organic C and available P had a strong axis2 score (29.87% of variance explained), a shift in AM fungal community composition according to the dominancy of the sequence type *Rhizophagus* was detected. This suggested that the dominancy of *Rhizophagus* species was increased by the annual amendment with organic fertilizer, regardless of the type of organic matter, and decreased by the addition of chemical fertilizers. This finding corresponds to Toljander et al. (2008), who demonstrated that the occurrence of *Glomus intraradices* (currently *Rhizophagus irregularis*) was significantly affected by fertilization treatments.

The sequence types Glomeraceae-1, -2, and -3 were not detected (Figure 3) under the chemical fertilization amendment. This indicated that conditions in the long-term chemical fertilization treatment were inhospitable for symbiosis of AM fungi in family Glomeraceae with wheat, except for genera *Rhizophagus* and *Funneliformis* (i.e., sequence types Glomeraceae-1, -2, and -3).

Conclusions

In this study, AM fungal community compositions on a Leptosol under a long-term double cropping system with different fertilization practices were investigated in semi-arid Mediterranean Turkey. As with our hypothesis, different fertilization practices affected several ecological indices for the AM fungal communities. The AM fungal community richness under no-fertilization treatment and plant compost treatment was higher than those that were amended with farmyard manure or chemical fertilizers. Although different organic amendments supported relatively similar AM fungal communities, plant compost induced higher AM fungal richness than farmyard manure fertilization.

References

Allen, M. F. 1991. *The ecology of mycorrhizae*. New York, NY: Cambridge University Press.

Almaca, A., and I. Ortas. 2010. Growth response of maize plants (*Zea mays* L.) to wheat and lentil pre-cropping and to indigenous mycorrhizae in field soil. *Spanish Journal of Agricultural Research* 8:131–36. doi:10.5424/sjar/201008s1-1232

Bhadalung, N. N., A. Suwanarit, B. Dell, O. Nopamornbodi, A. Thamchaipenet, and J. Rungchuang. 2005. Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. *Plant and Soil* 270:371–82. doi:10.1007/s11104-004-1829-4

Gollotte, A., D. van Tuinen, and D. Atkinson. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* 14:111–17. doi:10.1007/s00572-003-0244-7

Hijri, I., Z. Sykorova, F. Oehl, K. Ineichen, P. Mäder, A. Wiemken, and D. Redecker. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology* 15:2277–89. doi:10.1111/j.1365-294x.2006.02921.x

Jones, A., L. Montanarella, and R. Jones. 2005. Soil Atlas of Europe. European Soil Bureau Network. European Commission, 128.

Kruger, M., C. Kruger, C. Walker, H. Stockinger, and A. Schussler. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist* 193:970–84. doi:10.1111/j.1469-8137.2011.03962.x

Menge, J. A., D. Steirle, D. J. Bagyaraj, E. L. V. Johnson, and R. T. Leonard. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. *New Phytologist* 80:575–78. doi:10.1111/j.1469-8137.1978.tb01589.x

Molina, R., H. Massicotte, and J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbiosis: Community-ecological consequences and practical implications. In *Mycorrhizal functioning: An integrative plant-fungal process*, ed. M. F. Allen 357–423. New York, NY: Chapman and Hall.

Muthukumar, T., and K. Udaiyan. 2002. Growth and yield of cowpea as influenced by changes in arbuscular mycorrhiza in response to organic manuring. *Journal of Agronomy and Crop Science* 188:123–32. doi:10.1046/j.1439-037x.2002.00544.x

Oehl, F., E. Sieverding, K. Ineichen, P. Mäder, T. Boller, and A. Wiemken. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology* 69:2816–24. doi:10.1128/aem.69.5.2816-2824.2003

Olsen, S. R., and L. E. Sommers. 1982. Phosphorus. In A. L. Page R. H. Miller, and D. R. Keeney. *Methods of soil analysis. Part 2: Chemical and microbiological properties*, 581–893. Madison, Wisconsin: Soil Science Society of America Inc.

Öpik, M., M. Moora, J. Liira, and M. Zobel. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* 94:778–90. doi:10.1111/j.1365-2745.2006.01136.x

Ortas, I. 2003. Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in South Anatolia. *Journal of Plant Nutrition* 26:1–17. doi:10.1081/pln-120016494

Ortas, I. 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research* 8:116–22. doi:10.5424/sjar/201008s1-1230

Ortas, I. 2012. The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crops Research* 125:35–48. doi:10.1016/j.fcr.2011.08.005

Ortas, I., and O. Ustuner. 2014a. The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake. *European Journal of Soil Biology* 63:64–69. doi:10.1016/j.ejsobi.2014.05.007

Ortas, I., and O. Ustuner. 2014b. Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake. *Scientia Horticulturae* 116:84–90. doi:10.1016/j.scienta.2013.12.014

Ozkan, B., H. Akcaoz, and C. Fert. 2004. Energy input–output analysis in Turkish agriculture. *Renewable Energy* 29:39–51. doi:10.1016/s0960-1481(03)00135-6

Redecker, D., A. Schussler, H. Stockinger, S. L. Sturmer, J. B. Morton, and C. Walker. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23:515–31. doi:10.1007/s00572-013-0486-y

Schussler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the Glomeromycota: 398 phylogeny and evolution. *Mycological Research* 105:1413–21. doi:10.1017/s0953756201005196

Sharma, M. P., and A. Adholeya. 2001. Mycorrhizal dependency and growth responses of *Acacia nilotica* and *Albizia lebbeck* to inoculation by indigenous AM fungi as influenced by available soil P levels in a semi-arid alfisol wasteland. *New Forests* 21:89–104.

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. doi:10.2136/sssaj2002.0299

Singh, S., A. Pandey, B. Chaurasia, and L. M. S. Palni. 2008. Diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of tea growing in 'natural' and 'cultivated' ecosites. *Biology and Fertility of Soils* 44:491–500. doi:10.1007/s00374-007-0231-9

Smith, S. E., and D. J. Read. 2008. *Mycorrhizal symbiosis*, 3rd ed. New York, NY: Academic Press.

Suzuki, K., O. C. Turgay, M. O. Akca, N. Harada, and M. Nonaka. 2014. Molecular diversity of indigenous arbuscular mycorrhizal fungi in three different agricultural regions of Turkey. *Soil Science and Plant Nutrition* 60:367–76. doi:10.1080/00380768.2014.890916

Tanu, A. Prakash, and A. Adholeya. 2004. Effect of different organic manures/composts on the herbage and essential oil yield of Cymbopogon winterianus and their influence on the native AM population in a marginal alfisol. *Bioresource Technology* 92:311–19. doi:10.1016/s0960-8524(03)00198-6

Toljander, J. F., J. C. Santos-González, A. Tehler, and R. D. Finlay. 2008. Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. *FEMS Microbiology Ecology* 65:323–38. doi:10.1111/j.1574-6941.2008.00512.x

Trouvelot, S., D. van Tuinen, M. Hijri, and V. Gianinazzi-Pearson. 1999. Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by fluorescence in situ hybridization. *Mycorrhiza* 8:203–06. doi:10.1007/s005720050235

Turgay, O. C., D. Buchan, B. Moeskops, B. De Gusseme, I. Ortaş, and S. 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* 29:180–98. doi:10.1080/15324982.2014.944246

van Tuinen, D., E. Jacquot, B. Zhao, A. Gollotte, and V. Gianinazzi-Pearson. 1998. Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA–targeted nested PCR. *Molecular Ecology* 7:879–87. doi:10.1046/j.1365-294x.1998.00410.x

Verbruggen, E., W. F. M. Röling, H. A. Gamper, G. A. Kowalchuk, H. A. Verhoef, and M. G. A. van der Heijden. 2010. Positive effects of organic farming on below-ground mutualists: Large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist* 186:968–79. doi:10.1111/j.1469-8137.2010.03230.x