



Arbuscular Mycorrhizal Fungi as a Bio-Indicator for Monitoring Soil Attributes in Zagros Semi-Arid Woodlands

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ABSTRACT

Aims The present study aimed to 1) determine the extent of arbuscular mycorrhizal fungi symbiosis with *Amygdalus scopariain* in Melah-Roteh region in Dareshahr City, western Iran, and 2) model the presence or absence of different arbuscular mycorrhizal fungi on Almonds (*A. scoparia*) in relation to soil physicochemical properties.

Materials & Methods In the study area (Melah-Roteh region in Zagros Woodlands), 57 soil samples were taken from the 0-30cm depth under *A. scoparia* in the Dareshahr City forest west of Iran. Soil samples were processed in the laboratory to extract and identify spores and fungi, determine spore frequency, and to measure physicochemical soil properties.

Findings The results of two-way indicator species analysis (TWINSPAN) classification showed that arbuscular mycorrhizal fungi symbiotic with *A. scoparia* in the study could be classified into two groups. The evaluation of indicator species in each group using the importance value method and significance of indicator values based on Monte Carlo tests ($p < 0.01$) showed that *Rhizophagus fasciculatus* and *Funneliformis caledonium* (Indicator value= 90 and 89, respectively) were prominent in the first group and *Funneliformis mosseae*, *Claroideoglomus drummondii*, and *Glomus gigantea* (Indicator value= 95, 93, and 81, respectively) were representative of the second group.

Conclusion The presence-absence, symbiosis rate and spore density of arbuscular mycorrhizal fungi species associated with Almonds changes with varying physicochemical soil properties that can be modeled using certain soil attributes.

Keywords Disturbance; Site Heterogeneity; Almond; AMF; Forest

CITATION LINKS

[1] Bioindication of different stresses in forest decline ... [2] Developments in aquatic insect biomonitoring: A ... [3] Sentinel soil invertebrate taxa as bioindicators for ... [4] Tree nuts: Production, processing, ... [5] Partial replacement of saturated fatty acids with ... [6] Almond ... [7] Effect of organic farming on spore diversity of ... [8] Arbuscular mycorrhizae and soil/plant water ... [9] Contribution of the arbuscular mycorrhizal symbiosis to ... [10] A survey of soils for aggregate stability and glomalin, a ... [11] Red list plants: colonization by arbuscular mycorrhizal ... [12] Drought, but not salinity, determines the apparent ... [13] Plant defence theory re-examined ... [14] Seasonal variation of arbuscular mycorrhizal ... [15] Decomposition of litter in ... [16] The potential of VA mycorrhizae for plant nutrition ... [17] Distribution of VA mycorrhizal fungi along a latitudinal ... [18] Phylogenetic distribution and evolution ... [19] Mycorrhizal diversity: Cause and ... [20] Abundance and diversity of AM fungi across a gradient ... [21] Spatial soil heterogeneity has a greater effect on symbiotic arbuscular ... [22] The effect of different land uses on arbuscular ... [23] Arbuscular mycorrhizal fungi species ... [24] In: American Society of Agronomy, Soil Science ... [25] In: American Society of ... [26] Test of an ascorbic acid method for determining ... [27] An examination of the Degtjareff method ... [28] Spores of mycorrhizal Endogone species ... [29] Manual for the identification of VA ... [30] The application of GIS-based logistic ... [31] Sand mining disturbances and their effects ... [32] Relationships between flora biodiversity ... [33] Soil and geography are more important ... [34] Mycorrhizal influence on nutrient uptake ... [35] Modelling the environmental and soil factors ... [36] Biodiversity of arbuscular mycorrhizal fungi ... [37] Indicator species and co-occurrence in communities ... [38] Differences in the species composition ... [39] Arbuscular mycorrhizal fungi changes by ... [40] Occurrence of arbuscular mycorrhizal ... [41] Arbuscular mycorrhizal fungal diversity ... [42] Effects of drought on non-mycorrhizal and ... [43] Effects of mycorrhizal inoculation of shrubs from Mediterranean ... [44] Distribution of three endangered medicinal plant ... [45] Arbuscular mycorrhiza enhance the rate ... [46] Mycorrhizas and nutrient cycling in ...

Introduction

Forest decline worldwide, increased the interest of environmental research in bio-indication [1]. Various indicators are used for monitoring different ecosystem conditions [2, 3]. These indicators can be useful to identify rapid ecosystem changes in an inexpensive manner. Although the effects of disturbances on forest ecosystems have been widely studied, little is known about the effects of site heterogeneity on arbuscular mycorrhizal fungi that are symbiotic with trees in many ecosystems such as Zagros oak woodlands of Iran.

Almonds (Rosaceae family) are among the most popular tree nuts worldwide [4], with high economic values derived from their use for human nutrition and health [5]. *A. scoparia* is a wild species of almond that occurs throughout large arid and semi-arid areas in Iran and adjacent countries. This shrub can persist in unfavorable environmental conditions (severe dehydration and thermal fluctuations) and various climates. Also, it occurs on a range of levels to steep landforms. Important ecological features of almond include that it does well in full sunlight, has resistance against dryness, wind, and heat, and can grow on saline soils. Wild populations of almond species vary in morphology over a wide geographical area in west and central Asia [6].

Arbuscular mycorrhizal fungi (AMF) in the phylum Glomeromycota are obligate symbionts with over 80% of all terrestrial plants, inhabiting plant roots and surrounding soils [7]. They provide benefits to the host plants by improving uptake of mineral nutrients, enhancing plant photosynthesis and water status [8], increasing tolerance to adverse environmental conditions [9], and improving soil fertility and quality [10]. Mycorrhizal symbiosis, symbiotic associations between fungi and plant roots, is one of the natural biological mechanisms by which plants are able to acclimate and tolerate sites characterized by environmental stress. Mycorrhizal relationships involve plants and fungi, whereby both organisms benefit through an exchange of nutrients at the root-soil interface. AMF expand the interface between plants and the soil environment and contribute to plant uptake of the macronutrients P and N as well as the micronutrients Cu and Zn [11]. AMF can also prevent or alleviate soil toxicity (for example toxic heavy metals in soils) in plants [12],

and provide plants protection against insect herbivores [13]. Soil characteristics, plant diversity and climate are the main factors that affect AMF diversity [14]. It has been shown that mycorrhiza presence is influenced by site-specific topographic variables such as terrain slope, and local geography [15]. Diederichs and Moawad [16] reported that infection by AMF is increased by high temperatures and Koske [17] found that under specific conditions, the spore density correlates with fluctuations in temperature. Wang and Qiu [18] generalized that the primary abiotic factors known to influence the abundance and distribution of AMF are water, nutrient, and oxygen availability.

Species compositions of AMF assemblages affect plant nutrient uptake and photosynthetic performance, but they are sensitive to changes in soil properties [19]. Several examples from Lakshmipathy *et al.* [20] include that (1) physico-chemical properties of the soil influenced the abundance and function of AMF, (2) bulk density had a positive influence on root colonization and spore density, (3) organic C had a significant positive influence on the formation of AMF in soil, and (4) total and available P had a negative influence on AMF formation. Further, disturbance and environmental heterogeneity are factors that can affect AMF in different ecosystems. Cheeke *et al.* [21] indicated that spatial variation and heterogeneity in the field has a significant effect on the structure of AMF communities. Palta *et al.* [22] studied the effect of different land uses on arbuscular mycorrhizal fungi in the northwestern Black Sea Region and showed that pasture lands had the higher mean value of AMF root colonization than other land uses.

However, little information exists to date regarding the association of AMF with *A. scoparia*, or on the species composition and diversity of AMF spores in *A. scoparia*.

Overall, the present study addressed the following hypotheses:

- 1) Soil organic carbon and bulk density are important soil properties on the presence or absence of AMF on Almonds (*A. scoparia*),
- 2) Almond habitat heterogeneity can be reflected by different arbuscular mycorrhizal fungi species, and
- 3) there are specific groups of arbuscular mycorrhizal fungi species that serve as indicator species on Almond that vary with changing soil properties.

Materials and Methods

Study area

The study was carried out on Melah-Roteh in Dareshahr City, which is in western Iran (33°39'07" N and 46°90'25" E, mean 950m a.s.l.; Figure 1). The study site is characterized by uniform physiographic conditions. This region is dominated by *A. scoparia* and other species such as *Quercus brantii*, *Astragalus sp.* and *Crataegus pontica*. The disturbances regimes in the studied region include sheep and goats grazing, land development for agriculture, and firewood removal that have caused heterogeneity of soils in the study region. The mean annual precipitation of this region is 450mm. The maximum temperature is 28°C, while the minimum is 0.58°C. The soil classification in the studies region is sandy-loamy.

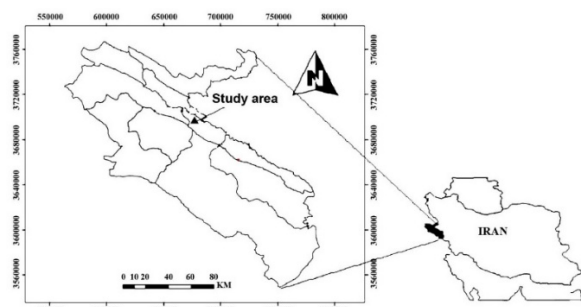


Figure 1) Location map of the studied area

Sampling

In the study area, 57 soil samples were taken from the rhizosphere of *A. Scoparia* in the spring and autumn of 2013. Ten transects (200 meters apart from each other) were established for selecting *A. scoparia*. Selected trees were 100-150 meters apart from each other. Soil samples were taken from the 0-30cm depth, after litter removal [23] and were passed through a 2mm sieve, from which subsamples were used for analyses. Fine root samples that were suitable for staining were taken from the rhizosphere in the same areas where soil samples were taken under a tree canopy. All root samples were transported to the lab and washed with tap water to remove all remaining soil particles. Washed fine roots were kept in FAA (formalin-acetic acid-alcohol) until they were stained.

A combined soil sample was taken from the root zone, i.e. from soil surface to a depth of 30cm to identify and extract the symbiotic mycorrhizal fungi. Characteristics of *A. scoparia* such as diameter and height were recorded. Soil samples were processed in the laboratory to

extract and identify fungi and spores, determine spore frequency, and to measure chemical and physical soil properties.

Physico-chemical analyses of soil

Soil electrical conductivity (EC) was determined in distilled water (1:5 soil/water ratio) based on the method described by McLean [24]. Soil bulk density was measured gravimetrically. The hydrometrical method was used to determine soil textures. Soil pH measured by pH meter (1:2.5 soil/water ratio). The Kjeldahl method was performed to determine available nitrogen (N) [25]. Method of Watanabe and Olsen [26] was used for available phosphorus (P) determination. Exchangeable calcium (Ca), potassium (K), and magnesium (Mg) were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Organic carbon (OC) was determined using the Walkley-Black method [27].

Spore extraction and identification

100 grams of soil from each of the soil sample were used for spore extraction. Wet sieving (mesh sizes were 400, 200, 80, and 50 microns) and the centrifuging method were used for spore extraction [28]. Extracted spore identified based on the morphological features of spores including spore size, color, number of cell walls, hyphal walls, and septum [29].

Statistical analyses

The normality of data distribution and homogeneity of variances were verified by Kolmogorov-Smirnov and Levene's tests, respectively. Samples were grouped using TWINSpan in PC-ORD 4 software. Indicator species analysis was used to analyze and determine indicator species based on the Monte Carlo test. Logistic regression was used to evaluate relationships among the presence and absence of mycorrhizal fungi species and various environmental factors. In each group, two species with the highest indicator value (IV) were included in the logistic regression analysis. Principal component analysis was used to determine the most important factors influencing the distribution of plots. Also, the efficiency of the logistic regression model was evaluated using the omnibus test. Two statistics coefficient of determination's Pseudo (Cox and Snell R Square and Nagelkerke R Square) were used to examine the explanatory power of the independent variables to changes in the dependent variable [30]. The coefficient of determination's Pseudo ranges between 0 and 1.

As the value of this statistic approaches one, it indicates that the independent variables have an increasing role in explaining the variance in the dependent variable. In addition, the Hosmer-Lemeshow test was used to determine the fitness of the logistic model. The general form of the model was as the following:

$$\log it(Y) = \text{natural } \log(\text{odds}) = \ln \frac{P}{1-P} = a + \beta X_k$$

Where P is the probability of an event with the presence of the independent variable X, a is the constant or parameters related to the coordinate axes, β is the regression coefficient and X_k are the independent or predictive variables. Statistical analyses were performed by SPSS 21.0.

Findings and Discussion

Two-way indicator species analysis (TWINSpan)

The results of TWINSpan classification are presented in Figure 2. According to the eigenvalue of each division, an arbuscular mycorrhizal fungus symbiotic with *A. scoparia* in the study area was classified into 2 groups. The evaluation of indicator species in each group using the IV method and significance of indicator values based on Monte Carlo tests showed that *Rhizophagus fasciculatus* and *Funneliformis caledonium* were prominent in the first group and *Funneliformis mosseae*, *Claroideoglossum drummondii*, and *Glomus gigantea* were representative of the second group (Table 1). Therefore, *R. fasciculatus* and *F. caledonium* in group 1, and *F. mosseae* and *C. drummondii* in Group 2 were included in the logistic regression model.

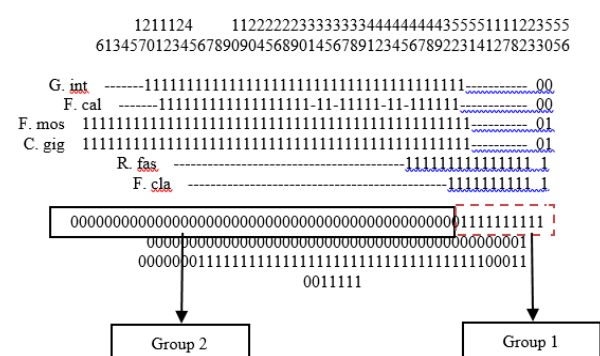


Figure 2) TWINSpan classification for arbuscular mycorrhizal fungi symbiotic with *A. scoparia* of the study area

Table 1) Indicator values (IV) for different species of arbuscular mycorrhizal fungi in each group

Species of arbuscular mycorrhizal fungi	Groups based on TWINSpan	Indicator value (IV)	Sig
<i>Rhizophagus fasciculatus</i>	1	90	0.001
<i>Funneliformis caledonium</i>	1	89	0.001
<i>Glomus intraradices</i>	2	41	0.002
<i>Glomus caledonium</i>	2	37	0.001
<i>Funneliformis mosseae</i>	2	95	0.002
<i>Claroideoglossum drummondii</i>	2	93	0.000
<i>Glomus gigantea</i>	2	81	0.001

Logistic regression model

The results showed that presence and absence of AMF can be explained with high probability (76, 77, 62, and 69% for *R. fasciculatus*, *F. caledonium*, *F. mosseae*, and *G. drummondii*, respectively) by the independent variables that included soil physical and chemical properties, the number of spores per 100g soil, litter depth (cm) and wild almond tree height.

Models with 4, 5, 6, and 5 variables, respectively for *R. fasciculatus*, *F. caledonium*, *F. mosseae*, and *C. drummondii* were able to best predict the variation in the presence-absence of arbuscular mycorrhizal fungi. The model for *R. Fasciculatus* contained the variables organic carbon (OC), bulk density (BD), clay and total nitrogen (N_{tot}), and for *F. caledonium* the set of dependent variables included OC, BD, clay, litter depth and N_{tot} . In contrast, the best model predicting the presence of *F. mosseae*, included OC, BD, clay, sand, litter depth and N_{tot} , whereas the model for *C. drummondii* had OC, BD, sand, litter depth, and N_{tot} .

The evaluation efficiency of the logistic regression model based on the Omnibus test showed that the model was acceptable and significant ($p < 0.01$) for all four species. Two statistics coefficient of determination's Pseudo, Cox and Snell R^2 , and Nagelkerke R^2 were high (Table 2) for *R. fasciculatus* and *F. caledonium* in the first group, and *F. Mosseae* and *C. drummondii* in the second group. This showed that the independent variables played an important role in explaining the variance in the presence of these indicator species.

Exp (B) in Table 3 is the exponentiated B coefficient. Exp (B) is >1 with increasing values of the independent variable indicating that the

arbuscular mycorrhizal fungi is more likely to be present with higher probability. BD and EC had a negative effect on the probability of the presence of arbuscular mycorrhizal fungi.

Table 2) Statistics coefficient of determination's Pseudo, Cox and Snell R² and Nagelkerke R²

Species of arbuscular mycorrhizal fungi	Statistics coefficient of determination's Pseudo	
	Cox and Snell	Nagelkerke
<i>R. fasciculatus</i>	0.711	0.792
<i>F. caledonium</i>	0.789	0.855
<i>F. mosseae</i>	0.818	0.823
<i>C. drummondii</i>	0.65	0.718

Table 3) Logistic model for presence and absence of arbuscular mycorrhizal fungi exponentiated coefficients or Exp (B), the significance level (Sig), Wald chi-square test (Wald) and logistic regression coefficient (B)

AMF	Exp (B)	Wald	B	Sig
<i>F. mosseae</i>				
Clay	1.29	3.8	-1.72	0.02
Organic carbon (%)	1.3	4	3.4	0.01
Bulk density (gr cm ⁻³)	1.49	3.11	-4.4	0.03
Total Nitrogen (%)	1.32	3.4	5.4	0.02
Litter depth (cm)	1.2	3.8	3.3	0.01
Sand	0.4	3	2.11	0.02
<i>C. drummondii</i>				
Organic carbon (%)M	1.3	6.3	5.3	0.01
Bulk density (gr cm ⁻³)	1.9	4.15	-3.6	0.02
Total nitrogen (%)	1.35	3.2	3.14	0.01
Litter depth (cm)	1.29	2.6	4.8	0.03
Sand	0.65	2.6	2.36	0.04
<i>R. fasciculatus</i>				
Clay	1.42	2.7	6.2	0.01
Organic carbon (%)	1.7	2.21	-0.41	0.04
Bulk density (gr cm ⁻³)	1.52	3.4	5.2	0.02
Total nitrogen (%)	0.5	6.5	-3.1	0.03
<i>F. caledonium</i>				
Clay	1.5	2.4	5.2	0.04
Organic carbon (%)	1.1	3.2	-0.8	0.02
Bulk density (gr cm ⁻³)	1.4	3.7	3.2	0.04
Total nitrogen (%)	0.41	4.3	-3.1	0.04
Litter depth (cm)	0.31	3.3	-2.1	0.04

Variables included in the logistic model for indicator species fungi

Indicator species belonging to the first group are as the following:

1) *R. fasciculatus*: The results showed that clay and BD with 1.42 and 1.52 exponentiated B coefficient had a positive effect on the probability *Rhizophagus fasciculatus*. Exp (B) is the exponentiated B coefficient. Exp (B)>1 i.e. with increasing values of the independent variable resulting in higher probabilities for the presence of *Rhizophagus fasciculatus*. OC and N_{tot} (Exp (B)= 0.5 and 1.7, respectively) had a negative effect on the probability of the presence

of *Rhizophagus fasciculatus*. In other words, decreasing OC and N_{tot} increased the probability that *Rhizophagus fasciculatus* would be present. 2) *F. caledonium*: Clay and BD with Exp (B) 1.5 and 1.4, respectively had a positive effect on the probability of the presence of *F. caledonium*, whereas depth of the litter, N_{tot} and OC had negative effects on it. In other words, by reducing these factors the probability of *Funnelformis caledonium* presence increases.

Indicator species belonging to the second group are as the following:

1) *F. mosseae*: N_{tot}, sand, depth of the litter and OC with 1.32, 0.4, 1.2, and 1.3 exponentiated B coefficient that these variables have a positive effect on the probability of the fungi's presence, but clay and BD (Exp (B)= 1.29 and 1.49, respectively) had a negative effect on the probability of the presence of *F. mosseae*. In other words, decreasing clay and BD increases the probability of presence.

2) *Claroideoglossum drummondii*: For this species of fungi, N_{tot}, sand, depth of the litter and OC (Exp (B)= 1.35, 0.65, 1.29, and 1.3, respectively) were factors with positive effects on the probability of presence, but BD (Exp (B)= 1.9) had a negative effect.

Logistic model for presence-absence of fungi in this study was best explained by the following equations:

$P (Rhizophagus fasciculatus) = 12.1 + 6.2 \text{ Clay} - 0.41 \text{ OC} + 5.2 \text{ BD} - 4.1 \text{ N}$

$P (Glomus caledonium) = 8 + 5.2 \text{ Clay} - 0.8 \text{ OC} + 3.2 \text{ BD} - 3.1 \text{ N} - 2.1 \text{ Litter depth}$

$P (Funnelformis mosseae) = 13 - 1.72 \text{ Clay} + 3.4 \text{ OC} - 4.4 \text{ BD} + 5.4 \text{ N} + 3.3 \text{ Litter depth} + 2.11 \text{ Sand}$

$P (Claroideoglossum drummondii) = 10 + 5.3 \text{ OC} - 3.6 \text{ BD} + 3.14 \text{ N} + 4.8 \text{ Litter depth} + 2.36 \text{ Sand}$

Based on the above models, the effect of increasing or decreasing input variables on presence and absence of different fungi was not the same.

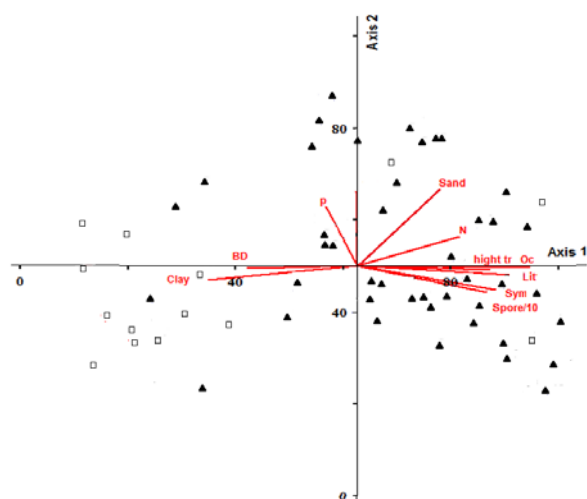
Principal component analysis (PCA)

The results of the PCA ordination are presented in Table 4 and Diagram 1. Broken-stick eigenvalues for the data set indicated that the first two principal components (PC1 and PC2) clearly captured more variance than could be expected by chance. The first two principal components together accounted for 43.5% of the total variance in the data set. Therefore, 33% and 10.5% of variance were accounted for by the first and second principal components, respectively (Table 4).

Table 4) Eigenvalue, variance percentage, cumulative percentage of variance and Pearson's correlation coefficient of environmental variables and PC1 and PC2

Variables	Axis 1	Axis 2
pH (1:1 H ₂ O)	0.04 ns	0.29 ns
K (mg kg ⁻¹)	0.225 ns	0.195 ns
Ca (cmol c kg ⁻¹)	0.206 ns	-0.237 ns
Mg (cmol c kg ⁻¹)	-0.05 ns	0.9**
EC (ds/m ⁻¹)	0.22 ns	0.23 ns
Silt (%)	0.26 ns	-0.21 ns
Clay (%)	-0.7**	-0.24 ns
Sand (%)	0.55**	0.57**
BD (g cm ⁻³)	-0.640**	-0.07 ns
OM (%)	0.8**	-0.08 ns
N (%)	0.612**	0.37*
P (mg kg ⁻¹)	-0.342*	0.5**
Coexistence (%)	0.715**	-0.33*
Number of spores per 100g soil	-0.69**	-0.33*
Litter depth (cm)	0.7**	-0.19 ns
Almond tree height	0.789**	-0.12 ns
Eigenvalues	5.6	1.7
Percent of variance explained	33	10.5
Cumulative percent of variance explained	33	43.5

*Significant correlation at 0.05; **significance at 0.01; ns: not significant

**Diagram 1)** Distribution and ordination of soils sampled from 0-30cm depth within PCA in their similarity features;

□ : First group; ▲: Second group; OC: Organic carbon; Lit: Litter depth; BD: Bulk density; Sym: Symbiosis rate

The results showed that moving in a negative direction along the first axis is associated with factors such as BD and clay. In other words, these factors had a high correlation with negative direction of PC1. The first group (more than 90% of its samples were consistent with the first group based on TWINSpan) were correlated highly with a negative direction on the first axis. Moving in a positive direction along the first axis reflected a gradient of sand, N_{tot}, OC, tree height, the number of spores per 100gr soil,

litter depth and P_{tot} (more than 85% of its samples were consistent with the second group based on TWINSpan).

In the present study, 7 species of AMF associated with *A. scoparia* were found, which they were classified into two distinct groups of species based on their presence or absence. Therefore, they can be used as an indicator species. These two groups have strong associations and dependence with many environmental factors such as soil OC, BD, clay, litter depth, and sand. This finding is correspondence to the finding of other studies that reported these soil variables as important factors affecting AMF [31, 32]. AMF is soil microorganisms that are important for conservation of biodiversity and can be used as keystone indicator species in disturbance regimes. Because they are sensitive to both plant species and anthropogenic activity [31]. This finding proves hypothesis of the present study that AMF can be modeled by soil physicochemical properties and they can reflect the heterogeneity of habitat. Although, Jansa *et al.* [33] reported no AMF taxa, in Swiss agricultural soils, as biomarker but in semi-arid woodlands of the study site, some specific AMF taxa (*Rhizophagus fasciculatus*, *Funneliformis caledonium*, *Glomus intraradices*, *Funneliformis caledonium*, *Funneliformis mosseae*, *Claroideoglossum drummondii*, and *Glomus gigantea*) as a bioindicators were identified. AMF associated with host plant species may be due to a number of factors such as mycorrhizal dependency [34], specific habitat, ecological conditions and variations in seasonal conditions and ontogenetic characteristics. However, it seems that in the studied site with wild almond the local availability of AMF, determining the composition of AMF species. In the other hand, we might say that differences in soil heterogeneity resulted in AMF community changes. Therefore, the response of AMF to the host plant is related not only to the host but also to the soil properties.

AM fungi are obligate symbionts and thus rely on their host plant, therefore, the impact of any environmental factor may be partitioned into a direct effect of the environment on the AMF and an indirect effect through modification of the plant community [35].

Claroideoglossum drummondii and *Glomus gigantea* appeared in the second group in the analysis of the present study. This result suggested that these AMF species could be and

specific species as bio-indicator for monitoring soil attributes. This finding is in agreement with the finding of Mirzaei and Moradi [36] who reported these species as indicator species in plantation. Furthermore, *Funneliformis caledonium* and *Rhizophagus fasciculatus* appeared in the first group as indicator species. This finding is in line with finding of another study who reported *Funneliformis caledonium* as indicator species [37]. Although, soil clay, OC, BD, and N were the most important factors to determine the indicator species it should be noticed that litter depth and soil sand affecting on *Funneliformis caledonium*, *Claroideoglomus drummondii*, and *Funneliformis mosseae* but not *Rhizophagus fasciculatus*.

The present study, two distinguished separated groups with specific AMF species indicators, suggested the effects of soil variables as driving force on AMF community changes. Therefore, AMF species composition can be varied depends on soil variables and different AMF species have different requirements of soil nutrients.

These findings are in line with the finding of other studies who reported the changes of AMF composition and diversity to depend on the edaphic and environmental variables [31]. Unlike the report of Hempel *et al.* [38], these findings suggested that AMF composition does not just related to the host plant but also soil variables can be key factors when the host plants are the same. In fact, based on the result of two groups, AMF could have different requirements of soil nutrients and their presence could be vary depending on the soil variables such as OC, N_{tot}, and BD.

A negative correlation between the frequency of fungal spores with soil phosphorus is probably due to multiplication of fungi and spores that occurs in soils with a lack of phosphorus. Therefore, increasing fungal frequency permits plants to absorb more phosphorous. However, a significant and positive correlation has been observed between the number of spores isolated from the soil and AMF presence in some cases [39]. Soil type and organic matter content also influence AMF community patterns [40].

Spore production may be enhanced in sandy soils, which promotes better plant root proliferation through improved soil aeration; such observations have also been reported by Pande and Tarafdar [41]. In this study, soil was a mixture of clay and loam, hence it did not provide enough pore space for AMF

proliferation. However, clay has a higher capacity to retain water, prolonged moisture retention in the soil might affect AMF sporulation. This finding is in accordant with the finding of Moradi Behbahani *et al.* [31] who reported the negative correction of spore density with soil moisture content. Furthermore, Schellenbaum *et al.* [42] reported that the availability of moisture increased the growth of fungal mycelium, which is important for root colonization, leading to a decrease in AMF spore germination.

Arbuscular mycorrhizal fungi frequency of spores and the percentage colonization has a positive correlation with organic carbon that is consistent with the results of other researches [39, 43]. This indicated that the soil parameters directly affected the performance of mycorrhizae and AMF need some levels of soil nutrition for their hyphal growth and formation of spore [39]. There is a positive correlation between frequency of spores with soil nitrogen that is consistent with the results of Panawer and Tarafdar [44] indicating the needs of AMF for some soil nutrient level for their own use including spore production especially in harsh condition such as semi-arid woodlands.

There was a negative significant correlation between the frequency of spores and soil bulk density, which is in agreement with the findings of Mirzaei and Moradi [32]. Lower bulk density creates better conditions for the growth and activity of fungi. In the present study, results showed a positive correlation between litter and frequency of fungal colonization. This finding suggested the strong relation between AMF and litter that already has been reported by Gui *et al.* [45]. Who reported the significant effects of AMF in litter decomposition by preventing the other microorganisms.

Litter is a potential source of carbon and nitrogen to soil organisms. However, decomposition of litter and release of these resources must occur before fungi can benefit [46]. If the fungi are better able to utilize litter, then differences in performance can be related to positive feedbacks between host plants and their mycorrhizal partners.

Conclusions

The presence-absence, symbiosis rate and spore density of arbuscular mycorrhizal fungi species associated with wild Almonds changes with

varying soil conditions that can be modeled using certain soil attributes such as soil organic carbon, nitrogen, and bulk density. Arbuscular mycorrhizal fungi species can be suitable indicators for monitoring soil attributes in a heterogeneous semi-arid woodland. Furthermore, soil heterogeneity is a key factor to determine AMF community changes and consequently determines the AMF indicator species when the host plant is not changing.

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