

# Relationships between flora biodiversity, soil physiochemical properties, and arbuscular mycorrhizal fungi (AMF) diversity in a semi-arid forest

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**Background and aim** – Arbuscular mycorrhizal fungi (AMF) communities are a main component of soil. They could be an indicator of fertility of semi-arid ecosystems. We aimed to analyse the biodiversity of AMF and understorey plants in Zagros forest (Iran), and to find their relationships with environmental conditions (physiography and soil).

**Methods** – Seventy-five plots (20 m × 20 m) were randomly divided from 1 700 to 1 900 m a.s.l. In each plot, four 1.5 m × 1.5 m microplots were established and all trees, shrubs, and herbaceous species coverage were recorded. Furthermore, four soil samples were taken from a depth of 0–20 cm in each microplot (one pooled sample for each main plot) by auger in spring. These soil samples were used for AMF assessment and soil physiochemical properties.

**Results** – Seventeen AMF species (in Glomeraceae, Claroideoglomeraceae, Diversisporaceae, Gigasporaceae, and Acaulosporaceae) and 47 plant taxa (in Poaceae, Asteraceae, Fabaceae and fourteen other families) were identified. Tests determined that the factors soil P, slope, tree coverage, litter thickness, and soil texture affect AMF and plant communities. Soil physiochemical properties have an effect on AMF, plant diversity, and evenness diversity indices. Furthermore, there is a high correlation between plants and AMF diversity indices and any change in the plant diversity will result in AMF changes. The results also showed positive correlations between plant diversity and AMF diversity.

**Conclusions** – AMF and plant diversity indices are highly correlated, but this correlation could be affected by soil physiochemical properties and environmental factors. Moreover, canopy coverage and litter thickness are considered as strongly influencing both plants and AMF.

**Key words** – Zagros forest, mycorrhiza, semi-arid, soil.

## INTRODUCTION

Iran is located in the arid and semi-arid world belt and less than 8 % of its total land area is covered by forests of different kinds. One of these types which is located in the western part of Iran is known as Zagros forest and contains most of the country's green cover. These forests are characterized by diverse topographic, climatic, and other environmental conditions (Sagheb Talebi et al. 2014). Vigorous topographical relief and a variety of climatic conditions provide many diverse ecosystems in small areas and contain diverse plant species (Jazirehi & Rostaaghi 2003, Mohadjer 2012). Plant diversity is a valuable resource, particularly in terms of Carbon (C) sequestration, nitrogen (Diaz et al. 2009, De Deyn et al. 2009), microbial biomass (De Deyn et al. 2011, Hiiesalu et al. 2014), nutrient retention and enzyme activities (Zhang et al. 2010), nutrient absorption (Li et al. 2014) and ecosys-

tem services like erosion control and water quality (Quijas et al. 2010).

Effective recognized factors that affect plant diversity and communities are an important aspect of ecological studies (van der Heijden et al. 2008).

Arbuscular mycorrhizal fungi (AMF) are important symbiotic microbes, and have symbiosis with 80 % of terrestrial plants (Smith & Read 2008). AMF are important for nutrients and water absorption (Simard & Durall 2004, Smith & Read 2008) as well as other ecological aspects such as diversity of plant communities and productivity (Hartnett & Wilson 2002, van der Heijden et al. 2008).

Nevertheless, the identification of effective factors on both AMF and plant diversity is important because plants and fungi diversity maintenance is crucial for biodiversity (Johnson et al. 2012). Research has suggested that there might be a

positive correlation between plant diversity and AMF diversity (Hiiesalu et al. 2014). It has also been reported that AMF has the potential to either increase or decrease plant species diversity and composition (Hartnett & Wilson 2002).

There are many factors affecting mycorrhiza and plant diversity. For instance, it has been reported that soil organic matter (SOM), total Nitrogen (N) (Yang et al. 2009), disturbance (Forey et al. 2008), and environmental factors (Ihaddaden et al. 2013) all affect plant diversity. Nevertheless, there is little information available on correlations between AMF diversity, the plant community, and environmental factors in arid and semi-arid regions.

Recent studies have confirmed that information on plant and AMF interactions in natural ecosystems is scarce (Hiiesalu et al. 2014). Besides plants and AMF, we have added soil and physiographical data in our analysis in this study. However, information about all of these factors together is rare and does not cover all ecosystems. The aim of this study was to determine the biodiversity of flora and AMF diversity, environmental conditions (physiography and soil), and its relation with flora and AMF in Zagros forest. We hypothesized that there is a high correlation between plants and AMF diversity indices.

## MATERIAL AND METHODS

This study was carried out in the Kabir koh region located in Dareshahr district, in the south-western part of Iran, (46°90'25"N 33°39'07"E) (fig. 1). The parent material was limestone and soil textures consisting of silt-loam and clay-loam. The dominant tree and shrub species were *Amygdalus scoparia* Spach, *Quercus brantii* Lindl., *Crataegus pon-*

*tica* K.Koch and *Astragalus adscendens* Boiss. & Hausskn. ex Boiss. The average annual precipitation was 320 mm and maximum and minimum temperatures were 28 °C and 0.58 °C respectively (Mirzaei 2015).

## Plant and soil sampling

Seventy-five main plots (20 m × 20 m) were established randomly at 200 m intervals in different topographic positions ranging from 900 to 1700 m a.s.l. All tree and shrub species were recorded based on absence and presence of species and their coverage percentage (Mirzaei 2015). To determine the herbaceous diversity, four 1.5 m × 1.5 m microplots were established in each main plot and the data for these microplots were gathered together as one microplot. Furthermore, four soil samples were taken from each microplot and pooled to take one soil sample from each main plot (Mirzaei 2015). Soil sample was taken from a depth of 0–20 cm by auger in spring 2016 (König et al. 2010).

## Soil analysis

Electrical conductivity (EC) and soil pH were measured in deionized water (1:5 and 1:2.5 soil/water ratio, respectively) (McLean 1982). Soil texture was determined hydrometrically (Prihar & Hundal 1971), bulk density was measured gravimetrically, N was measured by the Kjeldahl technic (Bremner 1996), P (phosphorus) was determined using the NaHCO solution according to Watanabe & Olsen (1965), Exchangeable Ca, K and Mg were determined using inductively coupled plasma atomic emission spectroscopy (Kalra & Maynard 1991) and organic C was determined using wet oxidation technique (Walkley & Armstrong Black 1934).

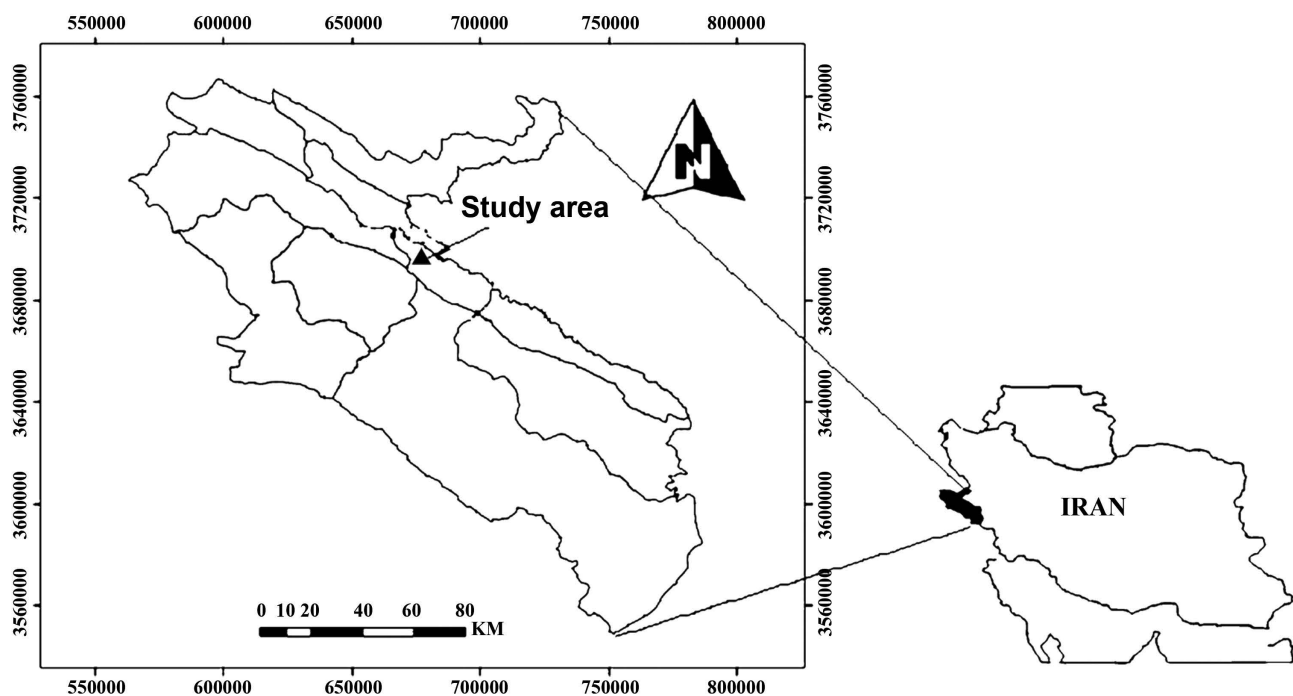


Figure 1 – Location of study area.

**Table 1 – Plant diversity list in the study site.**

Th: Therophytes; Cr: Cryptophytes; Ph: Phanerophytes; Ch: Chamaephytes; He: Hemicryptophytes.

Species	Life form	Family	Frequency (%)
<i>Aegilops triuncialis</i> L.	Th	Poaceae	100
<i>Agropyrum trichophorum</i> (Link) Richter	Cr	Poaceae	95
<i>Allium stamineum</i> Boiss.	Cr	Alliaceae	100
<i>Alyssum marginatum</i> Steud. ex Boiss.	Th	Brassicaceae	80
<i>Amygdalus lycioides</i> Spach	Ph	Rosaceae	70
<i>Amygdalus scoparia</i> Spach	Ph	Rosaceae	40
<i>Artemisia haussknechtii</i> Boiss.	Ch	Asteraceae	60
<i>Astragalus adscendens</i> Boiss. & Hausskn.	Ch	Fabaceae	15
<i>Astragalus neo-mozaffarian</i> Massimo	Ch	Fabaceae	5
<i>Astragalus ovinus</i> Boiss.	He	Fabaceae	5
<i>Avena wiestii</i> Steud.	Th	Poaceae	95
<i>Bromus danthoniae</i> Trin.	Th	Poaceae	100
<i>Bromus sterilis</i> L.	Th	Poaceae	90
<i>Bromus tectorum</i> L.	Th	Poaceae	100
<i>Bromus tomentellus</i> Boiss.	He	Poaceae	90
<i>Capparis parviflora</i> Boiss.	Ch	Capparaceae	10
<i>Centaurea behen</i> L.	He	Asteraceae	5
<i>Centaurea depressa</i> M.Bieb.	Th	Asteraceae	45
<i>Crataegus pontica</i> K.Koch	Ph	Rosaceae	40
<i>Echinops kotschyi</i> Boiss.	He	Asteraceae	60
<i>Euphorbia denticulata</i> Lam.	He	Euphorbiaceae	45
<i>Ferulago macrocarpa</i> (Fenzl) Boiss.	He	Apiaceae	95
<i>Fibigia suffruticosa</i> (Vent.) Sweet	He	Brassicaceae	75
<i>Galium setaceum</i> L.	Th	Rubiaceae	90
<i>Geranium stepporum</i> P.H.Davis	Cr	Geraniaceae	40
<i>Geranium tuberosum</i> L.	Cr	Geraniaceae	35
<i>Gundelia tournefortii</i> L.	He	Asteraceae	25
<i>Hordeum bulbosum</i> L.	Cr	Poaceae	40
<i>Hordeum spontaneum</i> C.Koch.	Th	Poaceae	20
<i>Malva neglecta</i> Wallr	Th	Malvaceae	65
<i>Marrubium vulgare</i> L.	He	Lamiaceae	45
<i>Medicago rigidula</i> (L.) All.	Th	Fabaceae	70
<i>Medicago radiata</i> L.	Th	Fabaceae	85
<i>Nepeta persica</i> Boiss.	Ch	Lamiaceae	40
<i>Onosma microcarpum</i> DC.	He	Boraginaceae	60
<i>Onosma rostellatum</i> Lehm.	He	Boraginaceae	55
<i>Phlomis olivieri</i> Benth.	He	Lamiaceae	60
<i>Poa bulbosa</i> L.	Cr	Poaceae	100
<i>Quercus brantii</i> Lindl.	Ph	Fagaceae	25
<i>Stachys kurdica</i> Boiss. & Hohen. var. <i>kurdica</i>	He	Lamiaceae	50
<i>Stipa hohenackeriana</i> Trin. & Rupr.	He	Poaceae	55
<i>Stipa pennata</i> L.	He	Poaceae	40
<i>Torilis tenella</i> (Delile) Reichenb.	Th	Apiaceae	90
<i>Trifolium scabrum</i> L.	Th	Fabaceae	95
<i>Viola modesta</i> Fenz L.	Th	Violaceae	80
<i>Xanthium spinosum</i> L.	Th	Asteraceae	100
<i>Zeugandra iranica</i> P.H.Davis	He	Campanulaceae	55
<i>Ziziphora capitata</i> L.	Th	Lamiaceae	95

### AMF spore density, identification, and root length colonization

Spores were extracted from 50 g of soil by wet sieving (sieve size ranged from 80 to 400 mesh) and the decanting method (Gerdemann 1963). All spores were mounted on a slide with PVLG (polyvinyl alcohol lactic acid glycerol) and PVLG with Melzer reagent. Identification was made according to evaluations of spore wall, colour, and size using a stereomicroscope (Olympus BH2) (Sasvári et al. 2012, Belay et al. 2013). Root samples were stained according to the method of trypan blue cited in Phillips & Hayman (1970) and the grid-line intersect method was used to determine root length colonization (Giovannetti & Mosse 1980). The root samples were washed in 10 % hot KOH. Then, these roots were bleached with 30 % H<sub>2</sub>O<sub>2</sub> and added to 1 % HCl. These root samples were stained in 0.05 % trypan blue and observed through a microscope. To determine root length colonization, ten samples of 1 cm stained root segments were randomly selected and mounted on a slide to check the absence or presence of AMF structures (mycelium, vesicles, and arbuscules) (Becerra et al. 2009).

### Plant and AMF diversity, richness, and evenness indices

Relative AMF diversity was determined using abundance (RA; spore number of a species per total number of spores), Shannon-Wiener diversity index

$$H = - \sum P_i \ln P_i$$

Simpson's diversity index

$$S_i = 1 - \sum P_i^2$$

where  $P_i$  is the relative abundance, species richness

$$R = \frac{S}{\sqrt{N}}$$

and evenness

$$E = H/H_{\max}$$

### Statistical analysis

Homogeneity of variances and normal distribution were determined using the Kolmogorov-Smirnov and the Levens tests respectively. Diversity indices were calculated using the above-mentioned formula and Microsoft Excel (ver. 12.0 for Windows). This data was subjected to logarithmic transformation to follow the normal distribution. For principal component analysis (PCA) and canonical correspondence analysis (CCA), all data was standardized to zero mean and unit variance and analyses was done on the correlation matrix. CCA was performed to study the relationship between AMF, flora, and environmental variables. Furthermore, PCA was performed to determine the main environmental factor affecting AMF and plant diversity. Ordination analyses were conducted using the statistical package PC-ORD (ver. 4) for Windows. Finally, assay correlations between parameters

were calculated using Pearson's coefficient. Pearson's correlations were performed using SPSS (ver. 16) for Windows.

## RESULTS

### Plant diversity and AMF species

Forty-eight taxa belonging to 36 genera and 17 families were identified. The Poaceae (12 species) was the dominant family in the studied site; whereas 8 families (Alliaceae, Campanulaceae, Capparaceae, Euphorbiaceae, Fabaceae, Malvaceae, Rubiaceae and Violaceae) had only one species identified (table 1). Therophyte (17 taxa) and Hemicryptophyte (16 taxa) were the most frequent life forms on the studied site; Phanerophyte (4 taxa) was the least frequent life form (table 1). Seventeen AMF taxa belonging to eight genera and five families were identified based on morphological characteristics (table 2). *Rhizophagus* was the most frequent genus with four species. *Claroideoglossum*, *Funneliformis* and *Glomus* had three species each while *Septoglossum*, *Diversispora*, *Gigaspora* and *Acaulospora* were the least frequent genera, with one species each (table 2).

### AMF and plant diversity

The mean of richness, evenness, Shannon-Wiener, and Simpson, as the diversity indices for AMF were 7.65, 0.87, 1.74, and 0.78 respectively (table 3), while the mean richness, evenness, Shannon-Wiener index of diversity and Simpson's index of diversity for plant were 12.08, 0.83, 2.05, and 0.86 respectively (table 3).

Our results showed that *Rhizophagus* is the most frequent genus, with four species. On the other hand, *Septoglossum*, *Diversispora*, *Gigaspora* and *Acaulospora* were the least frequent genera with one species each. *Claroideoglossum*, *Funneliformis* and *Glomus* had three species each. *Funneliformis caledonium*, *Funneliformis mosseae*, *Rhizophagus intraradices* and some other fungi species were distributed in all parts of the study site, while *Diversispora trimurales* appeared in only 20 % of the studied plots (table 2).

Correlation analysis for plant diversity and environmental factors revealed that soil P and slope had a negative correlation ( $P < 0.05$ ) with plant richness. Evenness had a significant ( $P < 0.05$ ) negative correlation with total N and bulk density. Plant Shannon-Wiener index of diversity showed a negative correlation with bulk density and slope while, had a significant positive correlation with litter thickness. Furthermore, Simpson's index of dominance was negatively affected by total N and bulk density (table 4).

Our results revealed that AMF Shannon-Wiener index of diversity had a positive correlation with canopy coverage and a negative one with soil P. Furthermore, the richness index had a negative correlation with slope and a significant positive correlation with canopy coverage (table 5). Moreover, spore density was positively correlated with canopy coverage and litter thickness, and had a negative correlation with slope.

**Table 2 – List of arbuscular mycorrhiza fungi and their frequency in the study site.**

Family	Genus	AMF species	Frequency (%)
Glomeraceae	<i>Funneliformis</i>	<i>Funneliformis geosporum</i> (T.H.Nicolson & Gerd.) C.Walker & A.Schüßler	80
		<i>Funneliformis caledonium</i> (T.H.Nicolson & Gerd.) C.Walker & A.Schüßler	100
		<i>Funneliformis mosseae</i> (T.H.Nicolson & Gerd.) C.Walker & A.Schüßler	100
	<i>Rhizophagus</i>	<i>Rhizophagus intraradices</i> (N.C.Schenck & G.S.Sm.) C.Walker & A.Schüßler	100
		<i>Rhizophagus diaphanus</i> (J.B.Morton & C.Walker) C.Walker & A.Schüßler	80
		<i>Rhizophagus fasciculatus</i> C.Walker & A.Schüßler	100
		<i>Rhizophagus clarus</i> (T.H.Nicolson & N.C.Schenck) C.Walker & A.Schüßler	100
	<i>Septoglosum</i>	<i>Septoglosum constrictum</i> G.A.Silva & Oehl	100
	<i>Glomus</i>	<i>Glomus macrocarpum</i> Tul. & C.Tul.	100
		<i>Glomus aggregatum</i> N.C.Schenck & G.S.Sm.	60
<i>Glomus pansihalos</i> S.M.Berch & Koske		80	
Claroideoglomeraceae	<i>Claroideoglosum</i>	<i>Claroideoglosum drummondii</i> (Blaszk. & C.Renker) C.Walker & A.Schüßler	100
		<i>Claroideoglosum etunicatum</i> (W.N.Becker & Gerd.) C.Walker & A.Schüßler	80
		<i>Claroideoglosum walkeri</i> (N.C.Schenck & G.S.Sm.) C.Walker & A.Schüßler	60
Diversisporaceae	<i>Diversispora</i>	<i>Diversispora trimurales</i> (Koske & Halvorson) C.Walker & A.Schüßler	20
Gigasporaceae	<i>Gigaspora</i>	<i>Gigaspora gigantea</i> (T.H.Nicolson & Gerd.) Gerd. & Trappe	60
Acaulosporaceae	<i>Acaulospora</i>	<i>Acaulospora koskei</i> Blaszk	60

**Table 3 – Diversity indices for plant and arbuscular mycorrhiza fungi (mean ± SE).**

E: Evenness; H: Shannon-Wiener index; Si: Simpson index.

Diversity indicators	AMF	Plants
Richness	7.65 ± 2.15	12.08 ± 2.41
E	0.87 ± 0.04	0.83 ± 0.10
H	1.74 ± 0.30	2.05 ± 0.32
Si	0.78 ± 0.06	0.86 ± 0.11
Colonization	50.88 ± 9.82	–
Frequency	51.50 ± 12.79	–

### Correlation between AMF and plants diversity indices

Plant evenness, Shannon-Wiener and Simpson's indices of diversity had a significant correlation with AMF Shannon-Wiener indices of diversity, Simpson's diversity indices, evenness and spore density (table 6). Also, there was a significant correlation between AMF root colonization and plant richness (table 6).

The CCA result indicated that there is positive correlation between root colonization and slope. Moreover, litter thickness had positive correlation with plant richness and AMF evenness. Also, diversity, richness and frequency of AMF had positive correlation with canopy coverage, soil sand and silt (fig. 2).

**Table 4 – Plant diversity indexes and environmental factors correlation.**

PL-E: plant evenness, PL-H: Plant Shannon-wiener diversity; PL-Si: Plant Simpson index of diversity; EC: electrical conductivity (Decisiemens per meter). \* Correlation is significant at the 0.05 level (2-tailed).

Environmental factors	PL-Richness	PL-E	PL-H	PL-Si
pH	-0.125	0.126	0.063	-0.092
EC (dsm <sup>-1</sup> )	0.008	-0.022	-0.030	-0.046
N (%)	0.032	-0.344*	-0.206	-0.344*
C/N	-0.208	0.102	0.068	0.195
K (mg/kg)	-0.168	-0.257	-0.160	-0.228
P (mg/kg)	-0.319*	-0.263	-0.265	-0.285
Bulk density (gr/cm <sup>3</sup> )	-0.253	-0.317*	-0.409*	-0.201*
Clay (%)	-0.188	0.001	0.015	0.120
Silt (%)	0.207	0.047	0.098	-0.014
Sand (%)	-0.207	-0.047	-0.098	0.014
Slope (%)	-0.356*	-0.244	-0.369*	-0.180
Elevation (m)	-0.212	-0.017	0.056	-0.013
Aspect	-0.019	-0.035	-0.084	0.150
Stone (%)	0.275	-0.035	0.085	-0.020
Canopy coverage(%)	-0.004	0.054	0.039	-0.050
Organic Carbon (%)	0.276	0.100	0.155	0.065
Litter thickness (mm)	0.420	0.200	0.365*	0.162

**Table 5 – Arbuscular mycorrhiza fungi diversity indices and environmental factors correlation.**

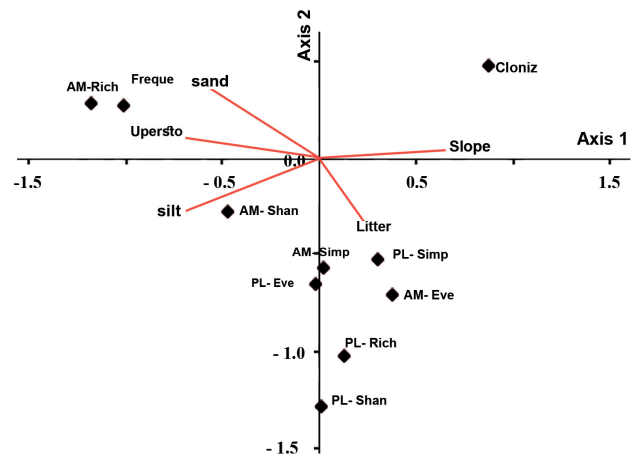
AM-H: Shannon-wiener diversity; AM-Si: AM Simpson diversity index; EC: electrical conductivity (Decisiemens per meter); N: nitrogen; CN: carbon/nitrogen; OC: organic carbon. \* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

Environmental factors	AM-H	AM-Si	AM-richness	AM-E	AM-colonization	Spore density
pH	0.057	0.071	0.080	-0.139	0.062	0.046
EC	0.067	0.062	0.095	-0.069	-0.033	0.216
N	-0.257	-0.285	-0.179	-0.260	0.124	-0.143
CN	-0.010	-0.014	-0.007	-0.079	-0.280	-0.037
K	-0.103	-0.146	-0.039	-0.162	0.116	0.048
P	-0.267	-0.332*	-0.222	-0.186	0.048	-0.214
Bulk density	-0.134	-0.151	-0.180	-0.087	0.098	-0.239
Clay	0.075	0.026	0.113	0.030	0.116	0.201
Silt	-0.167	-0.112	-0.224	0.012	-0.115	-0.256
Sand	0.167	0.112	0.224	-0.012	0.115	-0.256
Slope	-0.224	-0.230	-0.300*	0.048	-0.016	-0.460**
Elevation	-0.141	-0.142	-0.130	-0.117	-0.167	-0.049
Aspect	-0.050	0.006	-0.070	0.019	0.255	-0.009
Stone	-0.220	-0.186	-0.276	0.012	0.029	-0.297
Canopy coverage	0.315*	0.251	0.414**	0.008	0.017	0.388*
OC	0.146	0.131	0.178	0.005	-0.082	0.228
Litter	0.250	0.229	0.289	0.076	-0.018	0.336*

**Table 6 – Arbuscular mycorrhiza fungi and plant diversity indices correlation.**

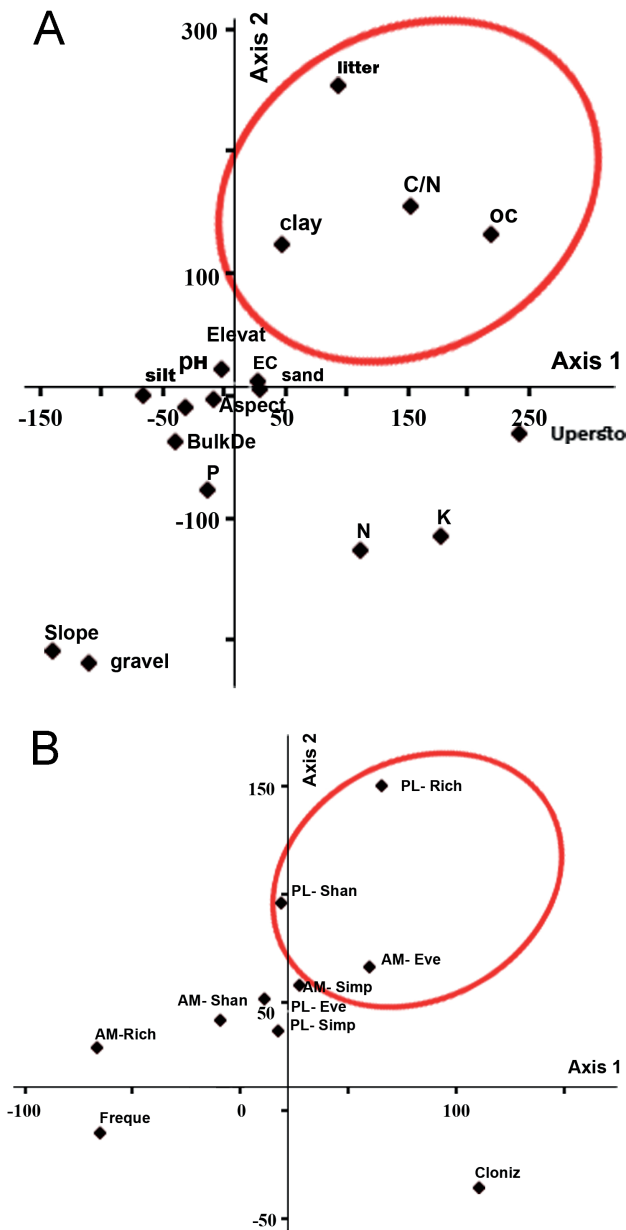
PL-E: plant evenness; PL-H: Plant Shannon-wiener index of diversity; PL-Si: Plant Simpson indexes of diversity; AM-H: arbuscular mycorrhizal Shannon-wiener index of diversity; AM-Si: arbuscular mycorrhizal Simpson index of diversity; AM-SD: arbuscular mycorrhizal spore density. \* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

	PL-Richness	PL-E	PL-H	PL-Si
AM-H	0.203	0.429**	0.367*	0.385*
AM-Si	0.246	0.461**	0.398*	0.424**
AM-Richness	0.168	0.474**	0.396*	0.411**
AM-Evenness	0.200	-0.066	0.001	0.006
AM-colonization	0.338*	0.024	0.245	0.051
AM-SD	0.196	0.633**	0.594**	0.544**



**Figure 2 – Result of CCA analysis for plant, arbuscular mycorrhiza fungi and environmental factors.** Cloniz: root length colonization; PL-Simp: Simpson diversity of plant; PL-Rich: richness of plant; PL-Shan: Shannon-Wiener diversity of plant; PL-Eve: evenness of plant; AM-Simp: Simpson diversity of arbuscular mycorrhiza fungi (AMF); AM-Rich: richness of AMF; AM-Shan: Shannon-Wiener diversity of AMF; AM -Eve: Evenness of AMF; upersto: canopy; Freque: frequency.

The results of PCA are given in fig. 3. These results indicate that litter thickness, clay, soil organic matter, and C/N had a positive significant correlation, while bulk density and slope had a negative significant correlation with axis 1 and 2 (fig. 3A). Furthermore, our results indicated that plant and AMF diversity indices had a negative significant correlation with bulk density and slope (fig. 3B).



**Figure 3** – Result of PCA for plant, arbuscular mycorrhiza fungi and environmental factors: A, soil and environmental factors; B, plant and AMF diversity indices. Cloniz: root length colonization; PL-Simp: Simpson diversity of plant; PL-Rich: richness of plant; PL-Shan: Shannon-Wiener diversity of plant; PL-Eve: evenness of plant; AM-Simp: Simpson diversity of arbuscular mycorrhiza fungi (AM); AM-Rich: richness of AM; AM-Shan: Shannon-Wiener diversity of AM; AM-Eve: evenness of AM; upersto: canopy; Freque: frequency; BulkDe: bulk density; EC: electrical conductivity; OC: organic carbon; C/N: carbon/nitrogen; Elevat: elevation.

## DISCUSSION

Although in this study the high level of correlation between plant diversity and AMF diversity indices was observed, it could not be only explained by plant and AMF specificity. As Montesinos-Navarro et al. (2015) stated, other ecological processes might affect it. In the present study, soil physicochemical properties and environmental factors affect both plants and AMF. The high correlation between AMF and plant species and the effect of AMF on plant diversity is also mentioned in the study of Klironomos (2003). AMF cause an increase in plant diversity. It is important to maintain AMF diversity because it maintains a diverse ecosystem and has an important effect on plant productivity and richness (Hiiesalu et al. 2014). Higher plant diversity will result in higher levels of AMF species diversity and spore density (Burrows & Pflieger 2002, Hiiesalu et al. 2014). An increase in plant diversity will provide more roots to be colonized by AMF (Burrows & Pflieger 2002). This will result in higher spore production than that observed in the present study.

In our study, plant diversity was affected by soil properties, which agrees with the findings of Lyaruu (2010). It simply shows that there should be a relationship between species richness and soil fertility (Nadeau & Sullivan 2015). An increase in soil fertility will result in plant diversity reduction (Borer et al. 2014). In less fertile soil, the competition between strong species to overcome other species is reduced and in this condition the richness of the species will increase (Rajaniemi 2002). In this study plant species diversity was negatively affected by slope. This is probably because of soil depth on steep slopes. This could result in lower nutrient and water maintenance capacity (Zhang et al. 2016). The result was the same for spore density; slope had a negative effect on it. There was a high level of correlation between plant coverage and spore density so the absence or reduction of plants could result in lower spore density. As discussed earlier, soil depth is lower on steeper slopes and this could result in lower plant density and consequently lower spore density (Zhang et al. 2016).

Although AMF species richness was correlated with plant diversity, which agrees with the findings of Castillo et al. (2006), the number of AMFs identified in the present study was not high. This might be caused by the lower plant species richness in the present study because of harsh climate conditions and anthropogenic disturbance in the study site (Mirzaei 2015). Furthermore, in this study there was a high correlation between spore density, soil organic matter, plant, and AMF diversity indices, which is in agreement with the reports of Verma et al. (2016) and Moradi et al. (2017). *Rhizophagus* was the dominant genus while *Diversispora trimurales*, *Gigaspora gigantea* and *Acaulospora koskei* were the least frequent species. This might be explained by development, since species such as *Gigaspora* need more time to produce spores (Wang & Jiang 2015).

Root length colonization could be correlated with spore density (Muthukumar et al. 2003, Khakpour & Khara 2012), available P and electrical conductivity (Khakpour & Khara 2012). In this study, we found out that root colonization was negatively correlated with silt while positively correlated with plant richness. This result is in line with the findings of

Carrenho et al. (2007) and demonstrates that soil texture is an important factor of AMF root colonization. The higher colonization rate was associated with increasing plant richness. This could be attributed to higher root colonization, which is in agreement with the findings of Spence et al. (2011) and could provide more roots to be colonized by AMF. Unlike the study of Moradi et al. (2015), in this study there was a positive correlation between root colonization and slope. This could be attributed to less soil compaction by domestic animals in areas with steeper slope rating.

## CONCLUSION

AMF and plant diversity indices are highly correlated. But, this correlation could be affected by soil physiochemical properties and environmental factors. Moreover, canopy coverage and litter thickness are considered strongly affecting both plants and AMF. Since soil silt was negatively correlated with root colonization, it might be concluded that this factor controls the AMF infection in roots.

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## REFERENCES

- Becerra A.G., Cabello M., Zak M.R., Bartoloni N. (2009) Arbuscular mycorrhizae of dominant plant species in Yungas forests, Argentina. *Mycologia* 101: 612–621. <https://doi.org/10.3852/08-176>
- Belay Z., Vestberg M., Assefa F. (2013) Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. *African Journal of Microbiology Research* 7: 5503–5515. <https://doi.org/10.5897/AJMR2013.6115>
- Borer E.T., Seabloom E.W., Gruner D.S., Harpole W.S., Hillebrand H., Lind E.M., Adler P.B., Alberti J., et al. (2014) Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508: 517–520. <https://doi.org/10.1038/nature13144>
- Bremner J.M. (1996) Nitrogen total. In: Sparks D.L. (ed.) *Methods of soil analysis, Part 3: chemical methods*: 1085–1121. Madison, Wisconsin, Soil Science Society of America & American Society of Agronomy.
- Burrows R.L., Pflieger F.L. (2002) Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Canadian journal of botany* 80: 120–130. <https://doi.org/10.1139/b01-138>
- Carrenho R., Trufem S.F.B., Bononi V.L.R., Silva E.S. (2007) The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Botanica Brasiliica* 21: 723–730. <https://doi.org/10.1590/S0102-33062007000300018>
- Castillo C.G., Borie F., Godoy R., Rubio R., Sieverding E. (2006) Diversity of mycorrhizal plant species and arbuscular mycorrhizal fungi in evergreen forest, deciduous forest and grassland ecosystems of Southern Chile. *Journal of Applied Botany and Food Quality* 80: 40–47.
- De Deyn G.B., Quirk H., Yi Z., Oakley S., Ostle N.J., Bardgett R.D. (2009) Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. *Journal of Ecology* 97: 864–875. <https://doi.org/10.1111/j.1365-2745.2009.01536.x>
- De Deyn G.B., Quirk H., Bardgett R.D. (2011) Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. *Biology Letters* 7: 75–78. <https://doi.org/10.1098/rsbl.2010.0575>
- Díaz S., Hector A., Wardle D.A. (2009) Biodiversity in forest carbon sequestration initiatives: not just a side benefit. *Current Opinion in Environmental Sustainability* 1: 55–60. <https://doi.org/10.1016/j.cosust.2009.08.001>
- Forey E., Chapelet B., Vitasse Y., Tilquin M., Touzard B., Michalet R. (2008) The relative importance of disturbance and environmental stress at local and regional scales in French coastal sand dunes. *Journal of vegetation science* 19: 493–502. <https://doi.org/10.3170/2008-8-18392>
- Gerdemann J.W. (1963) Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* 46: 235–244. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
- Giovannetti M., Mosse B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84: 489–500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>
- Hartnett D.C., Wilson G.W.T. (2002) The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant and Soil* 244: 319–331. [https://doi.org/10.1007/978-94-017-1284-2\\_31](https://doi.org/10.1007/978-94-017-1284-2_31)
- Hiiesalu I., Pärtel M., Davison J., Gerhold P., Metsis M., Moora M., Öpik M., Vasar M., Zobel M., Wilson S.D. (2014) Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. *New Phytologist* 203: 233–244. <https://doi.org/10.1111/nph.12765>
- Ihaddaden A., Velázquez E., Rey-Benayas J.M., Kadi-Hanifi H. (2013) Climate and vegetation structure determine plant diversity in *Quercus ilex* woodlands along an aridity and human-use gradient in Northern Algeria. *Flora - Morphology, Distribution, Functional Ecology of Plants* 208: 268–284. <https://doi.org/10.1016/j.flora.2013.03.009>
- Jazirehi M.H., Rostaaghi E.M. (2003) *Silviculture in Zagros*. Tehran, University of Tehran Press.
- Johnson D., Martin F., Cairney J.W.G., Anderson I.C. (2012) The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytologist* 194: 614–628. <https://doi.org/10.1111/j.1469-8137.2012.04087.x>
- Kalra Y.P., Maynard D.G. (1991) *Methods manual for forest soil and plant analysis*. Information Report NOR-X-319. Edmonton, Canada, Forestry Canada Northwest Region & Northern Forest Center.
- Khakpour O., Khara J. (2012) Spore density and root colonization by arbuscular mycorrhizal fungi in some species in the north-west of Iran. *International Research Journal of Applied and Basic Sciences* 3: 977–982.
- Klironomos J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84: 2292–2301. <https://doi.org/10.1890/02-0413>
- König S., Wubet T., Dormann C.F., Hempel S., Renker C., Buscot F. (2010) TaqMan real-time PCR assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: effects of soil characteristics, plant species richness,



- and functional traits. *Applied and Environmental Microbiology* 76: 3765–3775. <https://doi.org/10.1128/AEM.02951-09>
- Li L., Tilman D., Lambers H., Zhang F.-S. (2014) Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. *New Phytologist* 203: 63–69. <https://doi.org/10.1111/nph.12778>
- Lyaruu H.V. (2010) The influence of soil characteristics on plant species diversity and their distribution patterns in Western Serengeti, Tanzania. *Agricultural Journal* 5: 234–241. <https://doi.org/10.3923/aj.2010.234.241>
- McLean E.O. (1982) Soil pH and lime requirement. In: Page A.L., Miller R.H., Keeney D.R. (eds) *Methods of soil analysis*. 2nd Ed. Part 2: chemical and microbiological properties: 199–224. Madison, Wisconsin, American Society of Agronomy.
- Mirzaei J. (2015) Impacts of two spatially and temporally isolated anthropogenic fire events on soils of oak-dominated Zagros forests of Iran. *Turkish Journal of Agriculture & Forestry* 40: 109–119. <https://doi.org/10.3906/tar-1406-61>
- Mohadjer M.R. (2012) *Silviculture*. Tehran, University of Tehran Press.
- Montesinos-Navarro A., Segarra-Moragues J.G., Valiente-Banuet A., Verdú M. (2015) Evidence for phylogenetic correlation of plant–AMF assemblages? *Annals of Botany* 115: 171–177. <https://doi.org/10.1093/aob/mcu228>
- Moradi M., Shirvany A., Matinizadeh M., Etemad V., Naji H.R., Abdul-Hamid H., Sayah S. (2015) Arbuscular mycorrhizal fungal symbiosis with *Sorbus torminalis* does not vary with soil nutrients and enzyme activities across different sites. *iForest* 8: 308–313. <https://doi.org/10.3832/ifor1236-008>
- Moradi M., Naji H.R., Imani F., Moradi Behbahani S., Ahmadi M.T. (2017) Arbuscular mycorrhizal fungi changes by afforestation in sand dunes. *Journal of Arid Environments* 140: 14–19. <https://doi.org/10.1016/j.jaridenv.2017.01.006>
- Nadeau M.B., Sullivan T.O. (2015) Relationships between plant biodiversity and soil fertility in a mature tropical forest, Costa Rica. *International Journal of Forestry Research* 2015: article ID 732946. <https://doi.org/10.1155/2015/732946>
- Phillips J.M., Hayman D.S. (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55: 158–161.
- Prihar S.S., Hundal S.S. (1971) Determination of bulk density of soil clod by saturation. *Geoderma* 5: 283–286. [https://doi.org/10.1016/0016-7061\(71\)90039-5](https://doi.org/10.1016/0016-7061(71)90039-5)
- Quijas S., Schmid B., Balvanera P. (2010) Plant diversity enhances provision of ecosystem services: a new synthesis. *Basic and Applied Ecology* 11: 582–593. <https://doi.org/10.1016/j.baae.2010.06.009>
- Rajaniemi T.K. (2002) Why does fertilization reduce plant species diversity? Testing three competition-based hypotheses. *Journal of Ecology* 90: 316–324. <https://doi.org/10.1046/j.1365-2745.2001.00662.x>
- Sagheb-Talebi K., Sajedi T., Pourhashemi M. (2014) *Forests of Iran – A treasure from the past, a hope for the future*. Dordrecht, Springer. <https://doi.org/10.1007/978-94-007-7371-4>
- Sasvári Z., Magurno F., Galanics D., Hang T.T.H., Ha T.T.H., Luyen N.D., Huang L.M., Posta K. (2012) Isolation and identification of arbuscular mycorrhizal fungi from agricultural fields of Vietnam. *American Journal of Plant Sciences* 3: 1796–1801. <https://doi.org/10.4236/ajps.2012.312A220>
- Simard S.W., Durall D.M. (2004) Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany* 82: 1140–1165. <https://doi.org/10.1139/b04-116>
- Smith S.E., Read D.J. (2008) *Mycorrhizal symbiosis*. 3rd Ed. London, Academic Press.
- Spence L.A., Dickie I.A., Coomes D.A. (2011) Arbuscular mycorrhizal inoculum potential: a mechanism promoting positive diversity–invasibility relationships in mountain beech forests in New Zealand? *Mycorrhiza* 21: 309–314. <https://doi.org/10.1007/s00572-010-0340-4>
- van der Heijden M.G.A., Bardgett R.D., van Straalen N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11: 296–310. <https://doi.org/10.1111/j.1461-0248.2007.01139.x>
- Verma N., Tarafdar J.C., Srivastava K.K., Sharma B. (2016) Correlation of soil physico-chemical factors with AM fungal diversity in *Ailanthus excelsa* Roxb. under different agroecological zones of Western Rajasthan. *International Journal of Life Sciences Scientific Research* 2: 316–323. <https://doi.org/10.21276/ijlssr.2016.2.4.3>
- Walkley A., Armstrong Black I. (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* 37: 29–38.
- Wang M., Jiang P. (2015) Colonization and diversity of AM fungi by morphological analysis on medicinal plants in southeast China. *The Scientific World Journal* 5: article ID 753842. <https://doi.org/10.1155/2015/753842>
- Watanabe F.S., Olsen S.R. (1965) Test of an ascorbic acid method for determining phosphorus in water and NaHCO<sub>3</sub> extracts from soil. *Soil Science Society of America Journal* 29: 677–678. <https://doi.org/10.2136/sssaj1965.03615995002900060025x>
- Yang Y.-H., Chen Y.N., Li W.-H. (2009) Relationship between soil properties and plant diversity in a desert riparian forest in the lower reaches of the Tarim river, Xinjiang, China. *Arid Land Research and Management* 23: 283–296. <https://doi.org/10.1080/15324980903231991>
- Zhang B., Li S., Chen S., Ren T., Yang Z., Zhao H., Liang Y., Han X. (2016) Arbuscular mycorrhizal fungi regulate soil respiration and its response to precipitation change in a semiarid steppe. *Scientific Reports* 6: 19990. <https://doi.org/10.1038/srep19990>
- Zhang C.-B., Wang J., Liu W.-L., Zhu S.-X., Liu D., Chang S.X., Chang J., Ge Y. (2010) Effects of plant diversity on nutrient retention and enzyme activities in a full-scale constructed wetland. *Bioresource Technology* 101: 1686–1692. <https://doi.org/10.1016/j.biortech.2009.10.001>

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