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Relationships Between land Use and Arbuscular Mycorrhizal (AM) Spore Abundance in Calcareous Soils

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ABSTRACT
This study was conducted to determine soil properties that correlate with the arbuscular mycorrhizal fungal (AMF) spore numbers in semiarid calcareous soils of Hamadan province in northwestern of Iran. Soil samples from six sites managed differently were collected from a 0 to 30 cm depth. The results showed that land use and management systems had a significant effect on AMF spore number in soils. The mean spore number was found to fluctuate from 87 $10g^{-1}$ to 172 $10g^{-1}$ in coniferous woodland and dry farmland soils, respectively. The AMF spore numbers in soils exhibited a positive correlation with soil silt content, organic carbon/available P ratio, total nitrogen/available P ratio, basal respiration and fungal population, but a negative correlation with soil available P and available P/available K ratio. It may be concluded that soil management system, plant cover, silt content, available P are the main factors affecting AMF spore numbers in calcareous soils.

Keywords: Land use, plant cover, soil properties, arbuscular mycorrhiza

INTRODUCTION

Mycorrhizal associations between a fungus and a plant root are ubiquitous in terrestrial environments. The most common type of association is that of the arbuscular mycorrhizal fungi (AMF). The AMF association is the most ancient and probably aided the first terrestrial plants to colonize by scavenging for phosphate (Simon *et al*., 1993). This association is much more important in desert ecosystems, and may play an significant role in plant establishment and growth by bridging between plant and soil (Allen, 1983; Skujins & Allen, 1986). However, the abundance of AMF and their spores may depend on physica1 characteristics of soil (Ortega-Larrocca *et al*., 2001).

Most of studies have focused on the interactions between soil microorganisms and a single AMF added to microcosm units. Such studies are a first approximation to understanding the complex interactions that can occur, using controlled conditions which would be impossible in the field. However, the ecology of AMF in the field may be quite different. It certainly is more complex, with diversity of AMF in the root systems of plants. It is evident that a wide range of associations of soil constituents with AMF may occur in soils and presumably affect the mycorrhizal symbiosis with plants (Johnson *et al*, 1992).

Nevertheless, there is relatively limited information about the relation between soil characteristics and the abundance AMF in calcareous soils. Thus, the objectives of this paper were 1) to study the effect of land use on the AMF spore numbers and 2) to study physical, chemical and biological factors correlating AMF spore numbers in calcareous soils. We also investigated the relationship between AM spore numbers and some physico-chemical, biological and biochemical characteristics, in order to distinguish among soil factors correlating AMF activities in diverse field condition.

MATERIALS AND METHODS

Site Description and Experimental Design

The experimental sites are in Hamadan province, the northwestern of Iran with different land use systems. They are located above 1700 m over sea level at 34 °, 48 ′ North and 48 °, 31 ′ East. The regional climate is cold and semiarid, with an annual precipitation of 306 mm, of which 25, 4, 29 and 41.2% falls in spring, summer, autumn and

winter, respectively. Mean daily maximum temperatures is 18.9 °C and with minimum of 2.2 °C. Mean relative humidity is 55.4% and mean annual evaporation is 1813 mm. The sites have been used as pastures, deciduous and coniferous forests, and dry and irrigated farms for many decades. Some farmlands have been irrigated with high quality river water and some with untreated municipal wastewater. The plant covers of pastures are dominantly Poaceae (Bromous species, Poa bulbosa, Hordeum murinum), and legumes (Medicago species, Astragalus species). The plant covers of deciduous and coniferous forests are mainly Pinus species and Populus nigra, respectively. The plant covers of dry farmlands are dominantly Triticum aestivum and Hordeum vulgar. The plant covers of irrigated farmlands are mainly Triticum aestivum, Medicago sativa, Solanum tuberosum, Allium sativum, and vegetables (Petroselinum crispum, Mentha pieperata, Foeniculum vulare, Lepidium sativum and …). In Hamadan chemical fertilizers (especially P-fertilizer) may be used in dry farmlands, chemical and organic fertilizers and other agronomic management and practices are used in irrigated farmlands, but not in pastures and forests. Soil sampling was carried out at a depth of 0-30 cm in the root zones of plants in May, 2003. Based on the heterogeneity in each site, the sampling plan was completely randomized with unequal numbers of repetitions (>3) and 105 soil samples were taken from the experimental sites.

Soil physical and chemical analyses

Air-dry soil was subsequently crushed and sieved to pass a 2-mm mesh screen for particle-size analysis using the hydrometer method (Gee & Bauder, 1986). Calcium carbonate equivalents (CCE) were measured by back titration procedure (Leoppert. & Suarez, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil: water extract after shaking for 30 min (Hesse, 1971). Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley & Black, 1934). Total nitrogen in all samples was determined by the Kjeldahl method (Hinds & Lowe, 1980). Cation-exchange capacity (CEC) and available K were measured according to Bower *et al*., 1952. Available phosphorus was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Jackson, 1958).

Microbiological and biochemical analyses

Fresh soil samples were stored at 4 °C for microbiological analyses. Spores of VAM fungi were isolated from 50 cm^3 sub-samples by wet sieving (Gerdmann & Nicolson, 1963) and sucrose gradient centrifugation (Jenkins, 1964), and counted (Sylvia, 1994). Basal respiration was measured as CO_2 evolved in 5 days (Alef & Nannipieri, 1995). Substrate induced respiration (Anderson & Domsch, 1978), was determined in 72 h. Heterotrophic bacterial and Azotobacter populations were estimated by plate count method. Soil suspension and dilutions were prepared. Media of soil extract agar (SEA) (James, 1958; Parkinson, *et al*., 1971), rose bengal starch casein nitrate agar (RBSCNA) and modified potato dextrose agar (MPDA) were prepared in lab and used for determination of total soil bacterial, actinomycetes and fungi populations respectively (Alef & Nannipieri, 1995). Two media were prepared in lab for study of Azotobacter population in soil samples. The first one was Ashby's mannitol agar (Subba Rao, 2001). For inhibition of the growth of gram-positive bacteria and actinomycetes it was modified by addition of 1 ml crystal violet solution (5 g L^{-1} in ethanol). The second media for Azotobacter enumeration was LG medium (Alef & Nannipieri, 1995). Colony forming units on the solid media were numbered after a week of incubation at 27 °C (Alef & Nannipieri, 1995; Subba Rao, 2001). The activity of soil acid and alkaline phosphatases was analyzed according to the methods described by Eivazi and Tabatabai (1977). Soil cellulase activity was assayed by the improved method of Schinner, & Von Mersi (1990). The source of materials and substrates used in the culture media and enzymes assessments were Merck Co.

Statistical analyses

Data statistically analyzed for standard deviation, means were calculated and Duncan's new multiple range test was made to assess the land use and management system effects on the AMF spore numbers in

Table 1. Mean values of some soil properties in different land use and management.

Soil properties	Coniferous woodland	Deciduous woodland	Farmland Irrigated with Wastewater	Farmland Irrigated with river water	Dry farmland	Rang land
Sand (g kg^{-1})	545 a	522 a	526 a	470 a	495 a	469 a
Silt (g kg^{-1})	125 a	169 a	186 a	184 a	155 a	192 a
Clay (g kg^{-1})	330 a	309 a	288 a	345 a	350 a	339 a
CEC ($cmol_c$ kg^{-1})	25.1 a	21.9 a	17.7 a	18.0 a	17.9 a	17.8 a
CCE [a] (g kg^{-1})	165 ab	60 b	139 ab	174 ab	249 ab	260 a
OC [b] (g kg^{-1})	8 ab	15 a	16 a	13 ab	7 b	11 ab
EC (dS m^{-1})	0.32 a	0.37 a	0.52 a	0.45 a	0.21 a	0.22 a
pH	7.79 bc	7.62 c	7.98 abc	8.04 ab	8.19 a	8.03 ab
TN [c] (g kg^{-1})	0.72 c	1.3 ab	1.5 a	1.27 ab	0.82 bc	1.12 bc
Available P (g kg^{-1})	26 ab	33 ab	63 a	35 ab	11 b	13 b
Available K (g kg-1)	261 a	262 a	498 a	393 a	228 a	276 a
OC/TN ratio	11.3 a	10.2 a	10.6 a	10.0 ab	8.2 b	9.4 ab

Values in each raw followed by different letters are significantly different at the 0.05 probability level
a-CCE is equivalent calcium carbonate, b-OC is organic carbon and c-TN is total nitrogen of soils.

soils. Pearson correlations were performed to ascertain whether the investigated AMF spores in soil were related with soil physical, chemical and biological properties. The relationships between the AMF spore numbers and other soil properties were analyzed by correlation analysis. The computer programs used for data analysis were Ms-Excel and SPSS 9.0 for windows (SPSS Inc).

Results and Discussions

The soils had higher than 288 g kg^{-1} clay content with a clay to clay loam texture (Table 1). The equivalent calcium carbonate (CCE) contents of the soils were higher than 6%. Except deciduous woodlands, the sampled soils were calcareous. The CCE contents of woodlands were lower than those of irrigated farmlands, dry farmlands and rangelands. Soil pH values ranged from 7.6 to 8.2. The mean pH was also found to be significantly higher in dry farmlands than in woodlands. The 2:1 extract of soils exhibited low EC (<0.52 dS m^{-1}). No significant differences were found between soil EC values among the sites. The mean total nitrogen (TN) percentage was found to be significantly higher in irrigated farmlands with wastewater (1.52 gkg^{-1}) than in rangelands (1.12 g kg^{-1}), dry farmland (0.82 g kg^{-1}) and coniferous woodlands (0.72 g kg^{-1}). The differences between total nitrogen of the other sites were not significant. The soil available P in these sites was higher than 10 mg kg^{-1}. The soil available P in rangelands and

Table 2. Arbuscular mycorrhizal fungal spore numbers in 10 g of soil in different land use and management.

Management practices	Minimum	Maximum	Mean	Std. Deviation
Coniferous forest	52	148	87 b	36
Deciduous forest	86	261	165 a	59
Farmlands irrigated with raw municipal wastewater	39	225	95 b	55
Farmlands irrigated with river water	37	273	123 a	63
Dry farmlands	123	248	172 a	34
Rangelands	39	284	129 a	60

Values followed by different letters are significantly different at the 0.05 probability level

dry farmlands was significantly lower than in irrigated farmlands with wastewater. There was no significant differences between soil available P of the other sites. Although soil available K in rangelands and dry farmlands was relatively lower than in wastewater irrigated farmlands, there were no significant differences between available K in soils. The mean C/N ratio was found to be significantly lower in dry farmlands (8) than in coniferous woodlands (11), wastewater irrigated farmlands (11), and deciduous woodlands (10). The differences between C/N ratio of the other sites were not significant.

The results of AMF spore numbers analysis showed that there were significant differences between some means of AMF spore numbers in the calcareous soils of Hamadan in northwestern Iran (Table 2). The highest AMF spore numbers counted in soils sampled from dry farmlands (172 spores 10 g^{-1} soil). These soils which were mainly covered with Poaceae had the lowest fertility (Table 1) and moisture contents naturally. After that, AMF spore numbers in soils sampled from deciduous woodlands (165 spores 10 g^{-1} soil), rangelands (129 spores 10 g^{-1} soil) and farmlands irrigated with river water (123 spores 10 g^{-1} soil) were relatively high. The AMF spore numbers in soils sampled from farmlands irrigated with raw municipal wastewater (95 spores 10 g^{-1} soil) and from coniferous woodlands (87 spores 10 g^{-1} soil) were significantly ($p<0.05$) lower than the numbers in other soils. Several reports have shown that increasing soil fertility especially concentrations of soluble phosphate in soils can decrease fungal colonization (Graham *et al*., 1981; Asimi *et al*., 1980; Plenchette *et al*., 1983; Schwab *et al* 1983; Guillemin *et al*., 1995). Wastewater irrigated farmlands had the highest fertility or N, P and K contents (Table 1) which are factors decreasing root colonization and AMF spore numbers. Although soil fertility of the soils of coniferous forest was relatively low, AMF spore numbers in these soils were markedly low. This situation might be related to their low plant diversity, their allelopathic compounds (tannins and aromatics) affecting on the activity of their microflora and microfuana. Although AMF are non-host-specific in their ability to infect a wide range of hosts the degree of benefit to each partner in any given AMF–host plant association can depend on the particular species involved (Sanders and Fitter 1992; Bever *et al*. 1996; Simon *et al*. 1993). Both host and AMF community structures may be influenced by such differential effects between individual AMF–host partners (Simon *et al*. 1993; Douds *et al*. 1996). The composition of the AMF community may be strongly influenced by the host species through differential effects on hyphal growth and sporulation (Bever *et al*. 1996; Daniels Hetrick & Bloom 1986; Eom *et al*. 2000; Johnson *et al*. 1992; Sanders & Fitter 1992). Root exudates of different species may differ, influencing the germination and growth of specific AMF species (Douds *et al*. 1996; Tsai & Phillips 1991). A large part of the differences between AMF spore numbers in sites might be related to their different plant cover and diversity.

Land use and plant density can change soil properties, controlling soil microbial population and activities (Subba Rao 2001). Correlation coefficients exhibited that there were no significant relationship between AMF spore numbers and soil sand and clay contents (Table 3). However the relationship between AMF spore numbers and soil silt content was positive and significant. The correlation coefficients between AMF spore numbers and soil CCE, CEC, EC, pH, organic carbon, total nitrogen, organic carbon/ available K ratio, and total nitrogen/available K ratio were negative but not significant. The relationships between AMF spore numbers and soil available K and organic carbon/ TN ratio were positive but not significant. However, the relationships between AMF spore numbers and soil organic carbon/ available P ratio and total nitrogen/available P ratio were positive and significant. On the other hand, there were negative and significant correlation coefficients between AMF spore numbers in soils and their available P and available P/ available K ratio.

The correlation coefficients between AMF spore numbers and soil bacteria, actinomycetes and Azotobacter numbers were negative. In contrast, the correlation coefficients between AMF spore numbers and soil cellulase, acid and alkaline phosphatase activities were positive. However all of these correlation coefficients were not significant. There were significant and positive correlation coefficients between AMF spore numbers and soil basal respiration and fungal numbers.

Table 3. Pearson correlation coefficients between AMF spore numbers in soil and some physical, chemical and microbial soil properties.

Soil Properties	Correlation Coefficients [a]	Soil Properties	Correlation coefficients
SAND	-0.161	TN/av.P ratio	0.211 *
SILT	0.292 *	TN/av.K ratio	-0.191
CLAY	-0.061	Av.P/av.K ratio	-0.393 **
CEC	-0.072	Basal respiration	0.353 **
CCE [b]	-0.038	Substrate induced respiration	0.001
Organic C	-0.011	Azotobacter in Ashby's medium [c]	-0.12
EC	-0.156	Azotobacter in LG medium	-0.146
pH	-0.011	Bacteria in SEA medium	-0.179
Total N	-0.028	Actinomycetes in RBSCNA medium	-0.119
Available P	-0.345 **	Fungi in MPDA medium	0.22 *
Available K	0.031	Acid phosphatase activity	0.131
OC/TN ratio	0.031	Alkaline phosphatase activity	0.147
OC/av.P ratio	0.208 *	Cellulase activity	0.132
OC/av.K ratio	-0.189		

a- Correlation coefficients marked by *, ** and *** are significant at the 0.05, 0.01 and 0.001 level respectively (2-tailed).

b- CCE is equivalent calcium carbonate, OC is organic carbon, TN is total nitrogen, av. P is available P and av. K is available K of soils.

CCE is equivalent calcium carbonate of soils

c- Ashby's m. is the N-free mannitol agar medium, LG M. is the N-free sucrose agar medium, SEA m. is the soil extract agar medium, RBSCNA m. is the rose bengal starch casein nitrate agar and MPDA is the modified potato dextrose agar medium.

Conclusion

Spore numbers are more frequently used and can serve as rough indicators of the reproductive capability of the AMF species present in soils. AMF spore numbers in the calcareous soils depend on the type of land use, management system, plant diversity and root exudates. The soil factors positively related to the AMF spore numbers were silt content, organic carbon/available P ratio, total nitrogen/available P ratio, basal respiration and fungal population, and the soil factors negatively related to the AMF spore numbers were available P and available P/available K ratio. It may be concluded that land use, plant cover, soil silt content and available P are the main factors that control AMF spore numbers in these calcareous soils.

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