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Arbuscular mycorrhizal fungi changes by afforestation in sand dunes

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A B S T R A C T

This study was conducted to assess the effects of afforestation and canopy coverage on arbuscular mycorrhizal fungi (AMF) diversity and determination of the effects of soil factors on AMF community changes in sand dunes. To this end, two afforested dunes with canopy coverage of 25–50% and 75–100% and control dune (no afforestation practice) were selected in the southwest region of Iran. The results indicated that there were significant differences between the studied AMF diversity indices in the studied dunes. Sixteen AMF species belonging to four families and six genera were identified in the studied dunes. The most frequent AMF genera were *Funneliformis* and *Glomus* with five and four species, respectively. *Funnelisformis verruculosom* appeared only in the stand with 75–100% canopy coverage while *Glomus pansihalos* was not observed in this class of canopy. Furthermore, *Archaeosporatrappei* only appeared in afforestation without control dunes. The AMF diversity changes in the studied dunes were due to the significant effects of afforestation on the soil physiochemical properties and moisture content. These effects resulted in improvement in soil conditions for AMF spore production. Furthermore, soil electrical conductivity, phosphorus, bulk density and moisture content were the soil factors with the most effect on AMF community in sand dunes. Summarily, it was concluded that sand dunes afforestation produced a better soil condition and consequently higher AMF richness and spore production.

1. Introduction

Sand dunes are tenuous ecosystem recognized for their low water and nutrient content, shifting sand, and harsh environmental conditions (Zhimin and Wenzhi, 2001; Elhadi et al., 2016). The negative effects of sand dunes on rural community, which includes destruction of farms, houses and being a source of pollution through formation of dust and suspended particles makes it imperative to confront this challenge headlong (Pahlavanravi et al., 2012a,b). Afforestation is one of the most efficacious ways for sand dunes fixation. Afforestation provides long-lasting fixation mechanisms and improvement in ecosystem conditions (Yang et al., 2012).

More than half of the landmass of Iran is located in arid and semi-arid areas (Modarres and de Paulo Rodrigues da Silva, 2007) with harsh climate, low soil fertility and water deficiency (Saad et al., 2011). Despite the above mentioned difficulties, successful afforestation programs have been carried out to fix the sand dunes and improve the soil physiochemical properties and humidity (Singh, 2004).

One of the key factors for successful afforestation could be the existence of arbuscular mycorrhizal fungi (AMF) in the soil. AMF form a mutualistic symbiosis with more than 80% of terrestrial plants. These fungi provide water and nutrient absorption mechanisms for plants (Smith and Read, 2008), improves plant ability to tolerate environmental stresses (Barea et al., 2011) and hard soil condition (Doubková et al., 2013). Furthermore, they could be affected by environmental factors (Moradi et al., 2014). AMF plays an important role in the establishment of pioneer plant species (Allen and Allen, 1988) and could induce changes in plants community (van der Heijden et al., 1998).

Studies have shown that the presence of many plants species in sand dunes are highly associated with AMF symbiosis (Gemma and Koske, 1997). Consequently, AMF could indirectly affect sand dunes fixation (Koske and Polson, 1984). Therefore, their important role in the fixation of sand dunes with lack of water, nutrient and environmental stress cannot be ignored.

To the best of the authors' knowledge, no specific information is

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available on the effects of afforestation and trees canopy coverage on AMF diversities and communities in sand dunes. Furthermore, no information is available on AMF community changes in sand dunes of Iran. Therefore, the objectives of this study were to evaluate the effects of afforestation canopy coverage on AMF diversity and spore production as well as determine the effects soil physiochemical factors on AMF community changes in sand dunes and to assess how canopy coverage differ from soil physiochemical properties.

2. Materials and methods

2.1. Study site

The study site was located in Susa, north of Ahvaz from Khouzestan province, Iran, (latitude: 48° 17′ 16″ and longitude: 31° 37′ 60″). The elevation ranged from 25 to 49 m a.s.l with 2% slope. The average temperature, relative humidity, annual precipitation and evapotranspiration were 24 °C, 54%, 251 mm and 2044 mm, respectively. In 1991, petroleum mulch was employed in the fixation of shifting sand dunes and consequently afforested by *Prosopis juliflora* in 1993.

2.2. Sampling

Two stands with coverage of 25–50% and 75–100% were selected to assess the effects of canopy coverage on AMF. Each stand covering an area of about 10 ha was afforested by *P. juliflora*. At the vicinity of the stands, 10 ha of bare sand dunes was chosen as the control area. Unlike the afforested dunes, no practice of plantation was carried out on the control dunes. The studied sites (either control or afforested dunes) had the same soil condition in the past. Canopy classes were of the same age and range. Some of the seedlings were removed due to irregular irrigation and less attention at the initial time of planting. Therefore, different canopy classes in the range of 10–100% were formed.

In each site, 15 quadrat plots (20 × 20 m) were determined. Four soil samples were collected from four corners of each quadrat. The samples were collected at a depth of 15 cm. These four samples were then pooled and finally one soil sample in each quadrat plot was taken to the laboratory for further analysis. In the laboratory, soil samples were divided into two separate portions. One was used for spore extraction and the other for soil physiochemical analysis.

2.3. Soil physiochemical properties, spore extraction and identification

Soil phosphorus (P), total nitrogen, potassium (K), organic matter (OM), and pH were determined employing the methods described by Olsen et al. (1954), Bremner and Mulvaney (1982), Morwin and Peach (1951), Walkley and Black (1934) and Mclean (1982), respectively. The bulk density (BD) and soil texture were determined hydrometrically as well as by saturation (Prihar and Hundal, 1971). The soil electrical conductivity (EC) and pH were measured in deionized water (1: 5 and 1: 2.5 soil/water ratio, respectively) (Mclean, 1982). The moisture content (MC) was determined in the oven at 105 °C.

Spore extraction was performed by wet sieving (Gerdemann and Nicolson, 1963) followed by centrifuge. The sieve sizes were 500 and 38 µm. To do this, 100 g of soils were sieved; all AMF spores were extracted and counted. The spores were mounted on a slide with PVLG (polyvinyl alcohol lactic acid glycerol) and PVLG with Melzer's reagent. Morphological features of spore such as color, size and number of spore walls were used for AMF identification. The keys for identification were based on Schenck and Pérez (1990) and information on AMF spore characteristics from www.invam.cafe.wvu.edu and www.lrz.de/~schuessler/amphylo.phylogeny.com.

2.4. Biodiversity indices

Several diversity indices such as richness, Shannon-Wiener index of diversity (H), Simpson's index dominance (D), evenness (E), relative abundance (RA) and spore density (SD) were used to evaluate AMF diversity and composition in sand dunes. The indices were computed using equations (1)–(5):

$$H = -\sum P_i Ln P_i \tag{1}$$

$$E = \frac{H}{H_{\max}} \tag{2}$$

$$D = \sum [n_i(n_i - 1)/N(N - 1)] \tag{3}$$

$$RA = \frac{(spore\ number)of\ species}{the\ total\ number\ of\ spore} \times 100 \tag{4}$$

$$SD = \frac{Total\ Number\ of\ spore}{100\ gram\ soil} \tag{5}$$

Where, P_i is the relative abundance of each identified species per sampling site; n_i is the number of spores of a species; N is the total number of identified spore samples, and RA is the relative abundance (%).

2.5. Data analysis

The diversity indices and soil physiochemical properties data for the three sites were subjected to One-way analysis of variance test. If the test showed significant differences, Duncan test would be applied to compare means at the probability level of 95% ($p < 0.05$). Pearson's correlation coefficient was used to measure the correlation between diversity indices and soil physiochemical properties. SPSS® (V. 16) for Windows was used for the analysis. To assess the effect of soil physiochemical properties and AMF diversity indices on the studied sand dunes, canonical correspondence (CCA) ordination analysis was employed. Monte Carlo permutation test was carried out to assess the significance of soil physiochemical properties and AMF diversity indices. This analysis was performed by Canoco (V. 4.5) for Windows.

3. Results and discussion

3.1. Identified AMF in studied sites

In total, 16 AMF species were identified in the studied sites. These species belonged to four families and six genera. The most frequent AMF family was from Glomeraceae with two genera of *Funneliformis* and *Glomus* each with five and four species, respectively. *Rhizophagus* and *Acaulospora* with three and two species respectively were the most common genera. *Claroideoglomus* and *Archaeospora* each with one species were the least frequent species (Table 1).

The *Funnelisformis verruculosum* appeared only in canopy with 75–100% coverage. In contrast, *Glomus pansihalos* was not observed in this class of canopy coverage while *Archaeosporatrappei* appeared only in the afforestation site but not in the control. The most frequent species were *F. constrictum* and *F. badium*, respectively. The class with 75–100% coverage had the highest relative