

DYNAMICS OF ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTIC FUNGI UNDER THE CANOPY OF *CARAGANA KORSHINSKII* KOM

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Abstract

Root colonization by arbuscular mycorrhizae (AM) and dark septate endophytes (DSE) fungi in *Caragana korshinskii* Kom were investigated in a desert ecosystem of northwestern China. AM fungi colonization was significantly correlated with sampling month and plot, but not with soil depth. AM spore density was significantly correlated with sampling month, plot and soil depth. DSE hyphal colonization was only significantly correlated with sampling plot. Besides, DSE fungal colonization was relatively lower compared with AM fungi. Temporal and spatial dynamics of AM and DSE fungi were analyzed, the results showed that DSE fungal dynamics was similar to AM fungi, and both hyphal colonization of AM and DSE fungi were highest in October and with no significant difference ($p < 0.05$) in soil depth. The results of correlation analysis between soil factors and fungi demonstrated that AM colonization and spore density were significantly (positively/negatively) correlated with edaphic factors, while DSE fungi were influenced by edaphic factors lower than AM fungi.

Introduction

Arbuscular mycorrhizae (AM) fungi are ubiquitous in desert ecosystems and may play an important role in plant establishment and growth by bridging between plant and soil (Zhang *et al.* 2016 and Wicaksono *et al.* 2017). Heijden *et al.* (1998) showed that belowground diversity of AM fungi was a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning. Mycorrhizal plants had a greater ability to absorb nutrients, soil water and to increase plant fitness, which might lead to better survival under stressed environmental conditions (Cheng *et al.* 2015, Zarik *et al.* 2016). AM fungi, especially, could form enormous hyphae network systems in the rhizosphere, which could enhance the stability of soil aggregates, fix dune and improve the physical and chemical conditions of the soil (Sun *et al.* 2017 and Yang *et al.* 2017). So, AM fungi can play an important role in ecological system protection, restoration and reconstruction.

Dark septate endophytes (DSE) had attracted extensive attention with its settlement of plant species (Berthelot *et al.* 2016 and Santos *et al.* 2017), nevertheless, it was difficult to know about the functions of DSE in a variety of ecosystem (Newsham *et al.* 2009, Andrade-Linares *et al.* 2011 and Huusko *et al.* 2017) and it needs to have a further study. Recent studies showed that DSE colonized as ubiquitous as or even more than AM fungi (Huusko *et al.* 2017) and the functioning of ecosystems much like mycorrhizal fungi (Gao *et al.* 2016, Santos *et al.* 2017). Some studies reported that lots of DSE were able to enhance host mineral nutrition and growth (Andrade-Linares *et al.* 2011, Wang *et al.* 2016, Jin *et al.* 2017) and even some DSE species could effectively inhibit the development of plant diseases (Su *et al.* 2013, Gao *et al.* 2016).

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Mandyam and Jumpponen (2008) and Panwar *et al.* (2011) have reported temporal dynamics of AM colonization within and between years. While AM seasonality is relatively well studied, month dynamics of DSE fungi and AM fungi, as well as, the comparison of AM and DSE influenced by soil edaphic factors are unknown (Mandyam and Jumpponen 2008, Huusko *et al.* 2017, and Xie *et al.* 2017). In addition to the colonization of DSE in different plants (Wu *et al.* 2009), there was little work has been done on temporal and spatial variation of DSE colonization in sandy area in northwest China. Prior to testing, that occurrence of DSE are similar to AM fungi was speculated.

Caragana korshinskii Kom, a desert deciduous shrub species, belonging to *Caragana* Fabr., Leguminosae is distributed in various sandy regions of northwest China. It has been adopted to fix nitrogen in the air, improve soil fertility and plays an important role in vegetation restoration. Due to its rapid growth and vegetative propagation characteristics, the plant is now widely cultivated in northwest of China. Regardless of molecular mechanism between DSE fungi and host plant, the aim of the present study was to describe the temporal and spatial dynamics of AM and DSE fungi under the canopy of *C. korshinskii*, and the effect of abiotic conditions under the shrub on AM and DSE fungal dynamics in a desert ecosystem.

Materials and Methods

Three study sites were selected, Ordos Sandy Land Ecological Station of the Institute of Botany, the Chinese Academy of Sciences (Yanjiuzhan), Shanxi Yulin Rare Sandy-plants Conversation Field (Yulin) and Ningxia Shapotou (Shapotou). Yanjiuzhan and Yunlin in the northeast of Mu Us Sandland, while Shapotou located at the southeast edge of Tengger Desert. Detailed information about the sites is described in Table 1.

Table 1. List of environmental conditions in the sampling sites in the Mu Us sandy land of China.

Site	Latitude/Longitude (N/E)	Altitude (m)	Soil type	Annual rainfall(mm)	Annual temperature (°C)
Yanjiuzhan	39°29'40"N/110°11'22"E	1270	Sandy chestnut	370	7.8
Yulin	38°20'07"N/109°42'54"E	1088	Original chestnut	412.4	10.7
Shapotou	37°32'37"N/105°03'21"E	1280	Original gray-brown	296.4	7.7

Soil samples were collected in four replicates from the rhizosphere of *C. korshinskii* with a random sampling method. Surface soil (humic matter) was removed and 50 cm soil core was divided into layers of ten cm each and all cores were taken from under the plants in May, August and October 2016. Simultaneously, soil temperature and moisture were measured. Each layer from each replicate was placed in an individual plastic bag and transported to the laboratory. Air-dried soil samples were sieved (2 mm mesh size) and root segments were collected from each sample (Du *et al.* 2017).

Subsamples from each replicate were used for soil chemical analysis. pH was measured in water (1/5, soil/water method). Organic matter content was assessed by using the Walkey-Black method and available nitrogen (N) was measured using alkali hydrolysis diffusion method. Available phosphorus (P) was assessed by the method of NaHCO₃-extractable. Results of urease activity were expressed as µg NH₄⁺-N released during 3 hrs from 1 g soil. Soil protease activity was determined by using the method of modified ninhydrin colorimetry, and results of protease activity were expressed as µg glycine released per 1 g of dry soil after being cultured in 10 ml 1% white gelatin for 24 hrs.

Fresh roots were cut into 0.5 to 1.0 cm long segments and processed by washing them free of soil and clearing in 10% (w/v) KOH at 90°C in a water bath for 15 to 30 min depending on the degree of lignification of the roots. The root subsamples were cooled, washed and stained with 0.5% (w/v) acid fuchsin (Zhao and He 2007). Fifty root fragments were examined at 100 to 400 × magnification using a Nikon YS100 microscope with an automatic photomicrographic system for the presence of AM fungi and DSE structures. Percent colonization of AM (total colonization, hyphae, vesicles and arbuscules) and DSE (hyphae and microsclerotia (MS)) were expressed as the percentage of root segments colonized for each root sample (Mandyam and Jumpponen 2008, Hu *et al.* 2015).

Spores or sporocarps were extracted from 20 g air-dried soil of each soil sample by wet sieving followed by flotation-centrifugation in 50% sucrose. The spores were collected on a filter paper, washed several times with distilled water, and counted by using a dissecting microscope at 75 × magnification. A sporocarp was counted as one unit.

A statistical comparison of means was examined with ANOVA, Duncan's multiple range tests and Pearson correlation coefficient available in the SPSS (Version 22.0) statistical package. Significance was set at * $p < 0.05$ and ** $p < 0.01$.

Results and Discussion

The results showed that the roots of *C. korshinskii* were co-infected by AM and DSE all of which had a higher occurrence rate. Total colonization of AM was the highest (90.15%) in Yanjiuzhan and the colonization of DSE was the highest (72.44%) in Yulin. The lowest of total colonization of AM and DSE was 80.76 and 31.76%, respectively in Shapotou. Morphology and different structure of AM and DSE were very rich in the roots of *C. korshinskii*. Higher spore density of AM fungi existed in rhizosphere of *C. korshinskii* with a mean of 53.33 spores per 10 g soil.

ANOVA results for the comparison of AM and DSE colonization are presented in Table 2. AM hyphal colonization was significantly affected by sampling period. While, vesicular and arbuscular colonization were significantly affected by sampling period and location. Besides, total colonization was not significantly affected by sampling period, location and soil depth. While, spore density was significantly affected by sampling period, location and soil depth. In addition, DSE hyphal colonization was only significantly affected by location.

Table 2. Two-way analysis of variance (ANOVA) results for the AM and DSE colonization.

Index	Month		Location		Depth	
	F-test	p-value	F-test	p-value	F-test	p-value
Hyphae	10.325	0.000**	4.938	0.009	1.697	0.154
Vesicule	47.899	0.000**	19.542	0.000**	0.703	0.591
Arbuscule	57.702	0.000**	14.944	0.000**	0.712	0.585
Total colonization	4.882	0.009	5.522	0.005	1.958	0.104
Spore density	27.723	0.000**	14.828	0.000**	10.901	0.000**
DSE hyphal	5.276	0.006	39.742	0.000**	2.226	0.069
MS	0.257	0.744	4.257	0.016	2.890	0.025

**Means significant difference between two factors at $p < 0.01$ level.

Results of spatial distribution of soil factors (Fig. 1a) showed that soil temperature in Yanjiuzhan and Yulin was significantly lower than that of Shapotou and had no significant

difference between Yanjiuzhan and Yulin. Soil moisture in Yulin was significantly higher than that of Yanjiuzhan which was significantly higher than that of Shapotou. Yanjiuzhan and Shapotou had no significant difference ($p < 0.05$) in the aspect of pH, while it was higher than that of Yulin. Organic matter content, available P and available N in Yulin were significantly higher than those in other sites. Proteinase in Yulin and Shapotou was significantly higher than that in Yanjiuzhan, and urease in different sites had no significant differences ($p < 0.05$). Besides, the present data (Fig. 1b) also indicated that there was no significant difference ($p < 0.05$) in soil temperature, moisture pH urease and proteinase between the different soil depths. Available N in 0 - 10 cm soil layer was higher than that in 30 - 40 cm. Available P in 0 - 10 cm soil layer was higher than that of 20 - 30 cm and 40 - 50 cm soil layers. Organic matter in 0 - 10 cm was higher than that of other soil layers.

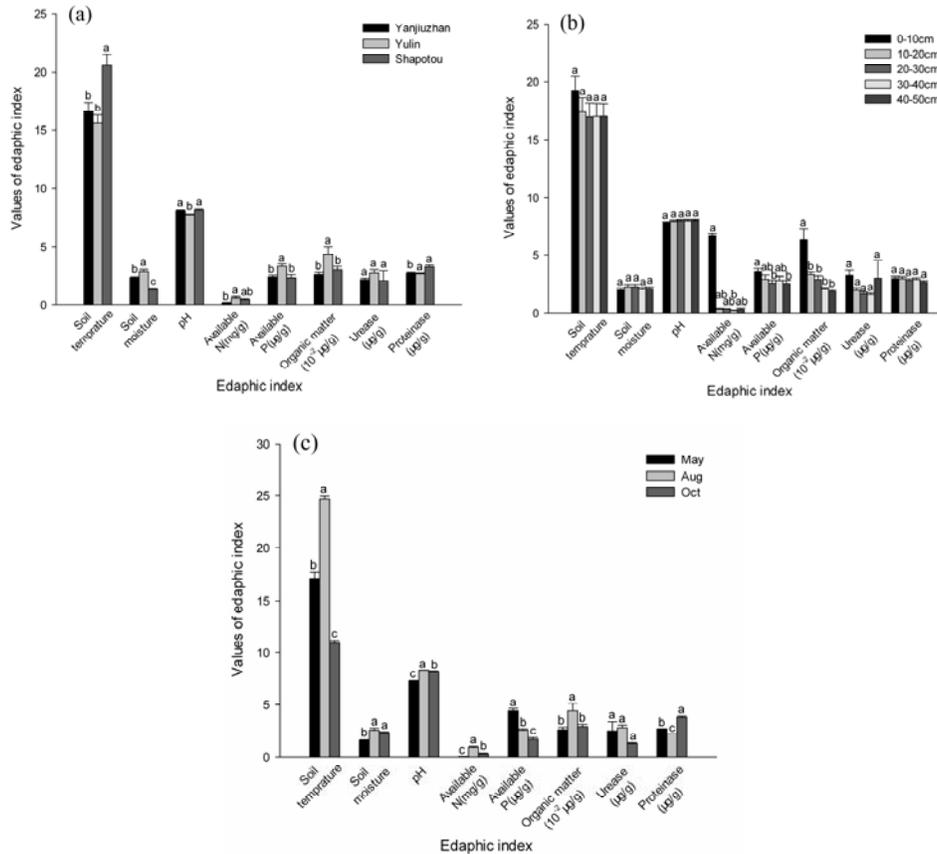


Fig. 1. Spatial distribution and month dynamics of soil factors. Different letters indicated significant difference between the same factors at $p < 0.05$ level.

Results of soil factors at different period (Fig. 1c) showed that soil temperature in August was significantly higher than that in May, while soil temperature in May was significantly higher than that of October. Soil moisture in August and October was higher than that of May. Soil pH in August was higher than that of October, while soil pH in October was significantly higher than that of May (Fig. 1c). Available N in August was significantly higher than that of October, and available N in October was significantly higher than that of May. Available P in May was

significantly higher than that of August, and which in August was significantly higher than that of October. Organic matter in August was significantly higher than that of May and October. Soil urease had no significant difference ($p < 0.05$) between different months. Soil proteinase in October was significantly higher than that of May, and which in May was significantly higher than that of August (Fig. 1c).

Study of location distribution of AM and DSE (Fig. 2a) showed that the colonization of hyphae and total colonization in Yanjiuzhan was significantly higher than that in Yulin and Shapotou, and there was no significant difference ($p < 0.05$) found of Yunlin and Shapotou. Vesicular colonization in Yanjiuzhan was significantly higher than that of Shapotou, while vesicular colonization in Shapotou was significantly higher than that of Yulin. Arbuscular colonization in Yanjiuzhan and Yulin was significantly higher than that of Shapotou, and there were no significant difference ($p < 0.05$) between that in Yanjiuzhan and Yulin. Spore density in Yulin was significantly higher than that in Shapotou, while in Shapotou it was significantly higher than that of Yanjiuzhan. Besides, results also showed that colonization of DSE and MS in Yulin were significantly higher than that of Yanjiuzhan, and which in Yanjiuzhan was significantly higher than that of Shapotou. In addition, soil depth dynamics of AM and DSE (Fig. 2b) showed that there was no significant difference ($p < 0.05$) between in colonization of hyphae, vesicle, arbuscule, total colonization and DSE between the different soil layers. Spore density in 0 - 10 cm soil layer was significantly higher than that in 10 - 20 cm soil depths and decreased from 20 - 30 cm soil depth to 30 - 40 cm soil depth with no significant difference ($p < 0.05$), meanwhile, spore density in 10 - 20 cm soil layer was significantly higher than that of 40 - 50 cm soil layer. Colonization of MS in 0 - 20 cm soil layer was significantly higher than that of 40 - 50 cm.

The dynamics of AM and DSE at different period (Fig. 2c) showed that hyphal colonization in August and October was significantly higher than that of May, without any difference ($p < 0.05$) between August and October. Meanwhile, both spore density and arbuscule were significantly higher in August than that of May and October, without any difference ($p < 0.05$) between May and October. Besides, vesicle in August was significantly higher than that of May and October, and which in May was lower. In addition, both DSE and total colonization were significantly higher in October than that of May, while there was no difference ($p < 0.05$) between months of MS.

Correlation analysis (Table 3) demonstrated that soil temperature was positively correlated with vesicular ($p < 0.05$) and arbuscular ($p < 0.01$) colonization. Soil moisture was positively correlated with total, arbuscular, hyphal ($p < 0.01$) and vesicular ($p < 0.05$) colonization. Soil pH was positively correlated ($p < 0.01$) with hyphal, vesicular and arbuscular colonization. Available N was positively correlated with vesicular ($p < 0.05$) and arbuscular ($p < 0.01$) colonization. Available P was negatively correlated with total, hyphal, vesicular ($p < 0.01$) and arbuscular ($p < 0.05$) colonization. Soil protease was positively correlated ($p < 0.05$) with total and hyphal colonization and negatively correlated ($p < 0.01$) with arbuscular colonization. Spore density was positively correlated with soil moisture, available N, organic matter, soil urease ($p < 0.01$), soil temperature ($p < 0.05$) and soil pH ($p < 0.05$). DSE hyphae were positively correlated with soil moisture ($p < 0.01$) and organic matter ($p < 0.05$) and negatively correlated ($p < 0.01$) with soil temperature.

The present results showed high colonization ratio of AM and DSE in the roots of *C. korshinskii* suggesting that between them and their host plant, and even rhizobia, there might be a harmonious and symbiotic relationship in desert environment. Although DSE fungi had been isolated from the roots of *C. korshinskii* and had a high colonization, DSE fungal colonization was relatively lower compared with AM fungi. Hyaline hyphae and hyaline vesicles of DSE structures were not separated to analyze as results of a few structures of hyaline hyphae and hyaline vesicles in sampling roots or they were difficult to be visualized (Yu *et al.* 2001, Li *et al.* 2015, Xie *et al.* 2017).

Hyphal, vesicular and arbuscular colonization of AM fungi and AM fungal spore density were significantly correlated with the sampling month and plot (Table 2), suggesting a month pattern of AM fungal colonization and spore density in all sample plots of *C. korshinskii*. Variations in AM fungal colonization and spore density with different plots and months may be generated by a variety of potential mechanisms, including variations in host plant phenological events (Lugo *et al.* 2003, Hu *et al.* 2015), mycorrhizal dependency, host plant-mediated alterations of the soil microenvironment, or other unknown host plant traits (Yang *et al.* 2013 and Lara-Pérez *et al.* 2014, Zhang *et al.* 2017). Analysis of the results showed that AM fungal colonization was not significantly correlated with depth (Table 2). Thus, it may be suggested that AM fungal colonization had no significant difference ($p < 0.05$) with depth as a result of edaphic vertical characters, physical and chemical nature which in different soil depths were similar in sample plots.

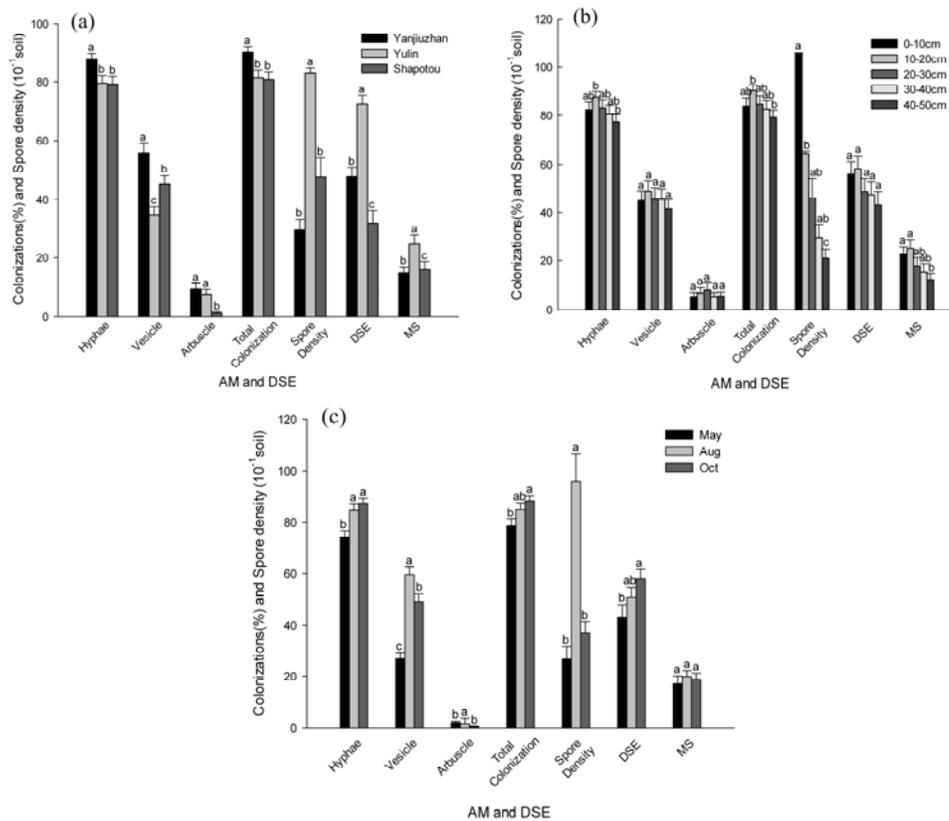


Fig. 2. Spatial and month distribution of AM and DSE. Different letters indicated significant difference between the same factors at $p < 0.05$ level.

In different sample plots, DSE hyphal and MS colonization in Yulin were significantly higher than that of Yanjiuzhan, and DSE hyphal and MS colonization in Yanjiuzhan were significantly higher than that of Shapotou, meanwhile, the variation of DSE hyphae was consistent with MS. These results may be due to MS function as vegetative propagules was able to germinate in suited conditions, and to produce hyphae (Yu *et al.* 2001). In different soil depths, there was no significant

difference ($p < 0.05$) in DSE hyphal colonization, and MS colonization in 0 - 20 cm soil layer was significantly higher than that of 40 - 50 cm. These results were similar to AM hyphal colonization and spore density, respectively. DSE hyphal colonization in October was highest and significantly higher than that of May. No significant difference ($p < 0.05$) was found in MS colonization between different months. Similarly, Mandyam and Jumpponen (2008) reported an increase in DSE colonization during the growing season in 2002, while DSE colonization did not vary in 2003.

Table 3. Correlation analysis between soil factors and fungi.

	Hyphae	Vesicule	Arbuscule	Total colonization	Spore density	DSE hyphae	MS
Soil temp.	-0.053	0.177*	0.360**	-0.07	0.187*	-0.291**	-0.079
Soil moisture	0.274**	0.181*	0.364**	.252**	0.289**	0.280**	0.094
Soil pH	0.199**	0.474**	0.193**	0.141	0.190*	0.017	0.049
Available N	0.054	0.168*	0.296**	0.035	0.715**	0.017	0.031
Available P	-0.324**	-0.404**	-0.178*	-0.260**	0.079	-0.107	0.006
Organic matter	0.034	0.014	0.133	0.035	0.838**	0.157*	0.094
Soil urease	0.109	0.084	0.115	0.101	0.202**	-0.047	-0.041
Soil protease	0.155*	-0.018	-0.366**	0.148*	-0.099	0.078	0.058

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Correlation analysis demonstrated that AM colonization and spore density were significantly (positively/negatively) correlated with edaphic factors (Table 3). According to Liu *et al.* (2009) and Huusko *et al.* (2017), not only plant phenology influenced AM fungal colonization and the whole AM fungal lifecycle, but also soil properties had a significant effect on fungal biology, besides variation in the soil environment might result in the variation of AM colonization and spore density.

Jumpponen and Trappe (1998) citing the report of Trappe in 1962, suggested that the strongly melanized DSE hyphae and MS might protect DSE fungi from extreme temperatures and drought and broaden their ecological niche. So edaphic characters might have less impacts on DSE fungi. From the analysis of the present results it appears that (Table 3), DSE hyphal colonization was only positively correlated with soil moisture and organic matter, and negatively correlated with soil temperature, while MS was not significantly correlated with edaphic characters. Extensive studies are required to assess whether both fungi have synergetic effects in promoting plant growth and plant disease prevention.

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