Spatial dynamics of dark septate endophytes and soil factors in the rhizosphere of Ammopiptanthus mongolicus in Inner Mongolia, China

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Abstract Improved understanding of the spatial patterns of desert soil resources and the role of dark septate endophytes (DSE) is required to measure plant growth in desert areas. Spatial dynamics of DSE and soil factors were investigated in Wuhai, Urad Back Banner and Alxa Left Banner, located in Inner Mongolia, China. Soil samples in the rhizosphere of Ammopiptanthus mongolicus were collected. Sampling sites and soil depth had a significant influence on the morphology, distribution and infection of DSE. Hyphae, microsclerotia and total root infection of DSE reached their maxima in the 0– 20 cm soil layer. Microsclerotial infection at Wuhai and Alxa Left Banner was higher than that at Urad Back Banner. Hyphal infection was significantly positively correlated with amounts of organic matter and available nitrogen, and activities of soil alkaline phosphatase, acid phosphatase and urease. Microsclerotial infection was significantly positively correlated with amounts of soil organic matter and available nitrogen.

Colonization of DSE are useful indicators for evaluating soil quality and function of desert ecosystem.

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Root infection had no significant correlation with soil factors. We concluded that the dynamics of DSE have a highly spatial pattern, and were influenced by nutrient availability and enzymatic activity. This study suggests that the morphology and infection of DSE are useful indicators for evaluating soil quality and function of desert ecosystems.

Keywords Dark septate endophytes \cdot Soil factor \cdot Spatial dynamics . Ammopiptanthus mongolicus . Desert ecosystem

1 Introduction

Ammopiptanthus mongolicus (Maxim. ex Kom.) S. H. Cheng is the only evergreen broad-leaf legume shrub found in the desert area of northwestern China (Feng et al. [2001;](#page-8-0) He et al. [2006\)](#page-8-0). This species is usually used as a windbreak to protect soil from water loss or wind erosion. In addition, extracts from A. mongolicus are the source of valuable material for anti-freezing protein research (Wei and Wang [2005](#page-9-0)). Ammopiptanthus mongolicus is particularly well suited for the revegetation of degraded lands to maintain soil structure and reduce erosion and desertification.

Plant roots are colonized by different fungi, including saprotrophic or weakly pathogenic fungi. These fungi have symptomless endophytic or biotrophic phases in their life cycles that are not apparent to casual observation (Parbery [1996](#page-9-0)). Jumpponen and Trappe ([1998\)](#page-8-0) reviewed reports of dark septate endophytes (DSE) that colonized a wide range of plant species in stressful ecosystems and included common soil, saprotrophic and rhizoplane fungi, as well as known pathogens. DSE are often mitotic or sterile, differ morphologically from conventional mycorrhizal symbionts, and are identified primarily by stained or pigmented hyphae. Microsclerotia of DSE grow inter- and intra-cellularly within

Highlights A.mongolicus is a tertiary relict and the only evergreen broadleaf legume shrub in desert of China.

It is particularly suited for the revegetation of degraded lands, which can reduce desertification.

A strong symbiosis exists between A.mongolicus and DSE in Inner Mongolian desert of China.

Dynamics of DSE have a highly spatial pattern, and are influenced by soil factors in desert ecosystem.

the cortex. The characteristic dark color of DSE is the result of the incorporation of melanin, a natural dark pigment and common fungal wall component (Addy et al. [2005;](#page-8-0) Grünig et al. [2008;](#page-8-0) Sieber and Grünig [2006\)](#page-9-0).

DSE have been found in many ecosystems stressful to plants, such as deserts and arid grasslands (Jiang et al. [2014](#page-8-0); Newsham [2011;](#page-9-0) Smith and Read [2008\)](#page-9-0), neotropical cloud forests (Muthukumar and Tamilselvi [2010;](#page-9-0) Rains et al. [2003](#page-9-0)), frigid environments (Bjorbækmo et al. [2010](#page-8-0); Kytöviita [2005;](#page-8-0) Newsham [2011;](#page-9-0) Upson et al. [2009\)](#page-9-0), acidic organic soils (Wurzburger and Bledsoe [2001](#page-9-0)), peat bogs and fen meadows (Fuchs and Haselwandter [2004\)](#page-8-0). In subarctic alpine regions (Kauppinen et al. [2014;](#page-8-0) Ruotsalainen et al. [2002\)](#page-9-0), plant roots are frequently colonized by DSE. The ecological role of DSE fungi is currently unresolved. However, their widespread occurrence in cold or drought-prone ecosystems, their potential to function as mycorrhizal fungi and the extensive internal infection by active structures suggests that these endophytes are significant components of stressful ecosystems (Jumpponen [2001;](#page-8-0) Takeshi et al. [2012\)](#page-9-0).

The main objectives of this work were to study the spatial status of DSE in the rhizosphere of A. mongolicus in Inner Mongolia, China, and assess the effects of soil factors on these DSE. The data will help elucidate the natural history of these fungi. The utilizing of DSE resources for managing desert ecosystems is also discussed.

2 Materials and methods

2.1 Study sites

The sampling sites were located in the arid and semi-arid region of northwest China. The climate is temperate mid-continental, with the lowest monthly mean temperature of −7.5 °C in January and the highest monthly mean of 21.8 °C in July. The annual average temperature is 5° C and the annual average precipitation reaches 280 mm, with 60 % occurring during July–September. The studied soils were Entisols and Aridisols (Eswaran et al. [2002](#page-8-0)). The three selected plots were Wuhai, Urad Back Banner and Alxa Left Banner – all on the southwestern edge of the sandy region in Inner Mongolia, China(Table 1), where A. mongolicus is abundant. Distances between sites were <50 km.

2.2 Collection of soil and root samples

Four patches of 1 m^2 were selected randomly in natural populations of A. mongolicus in the sandy region. The soil and fine root samples in the rhizosphere of A. mongolicus were collected in 1 for each patch for four replicates in June 2012. The distance between patches was ≥200 m. Soil samples were collected from a depth of 50 cm at each patch and divided into sections corresponding to depths of 0–10, 10–20, 20–30, 30– 40 and 40–50 cm. Soil samples were placed in sealed plastic bags and transported to the laboratory in an insulated container. Before processing, all samples were sieved (<2 mm mesh size) to remove stones, coarse roots and other litter. Then, the fine roots were collected from each sample. Soil samples for enzyme analyses were dried under cool conditions (15– 25 °C), and stored in sealed plastic bags at 4 °C until analysis. Other subsamples were air-dried and used for determination of soil physico-chemical properties. The root samples were immediately processed for DSE morphological observation and infection measurement.

2.3 Soil analysis

Soil pH was determined with a digital pH meter (PHS-3C, Shanghai Lida Instrument Factory, China) on soil:water

Table 1 Environmental condition in the three sampling sites in Inner Mongolia, China

	Wuhai	Urad back banner	Alxa left banner	
Longitude/latitude(E/N)	39°43' N, 106°51' E	41°41' N, 106°59' E	38°54' N, 105°45' E	
Altitude(m)	1134	1422	1561	
Precipitation(mm/year)	162	141	150	
Average temperature(${}^{\circ}$ C)	9.55	3.9	8.3	
Soil type	sandy gray desert	sandy brown calcium	subalpine meadow, grey brown	
Vegetation type	xeric shrubs, semi shrubs	desert grassland, desert shrubs	xerophytic, super xerophytic shrub, saline, half shrubs, small shrubs	
Soil pH	7.36A	7.27A	7.22B	
Soil organic carbon (mg/g)	0.78C	1.54A	1.08AB	
Soil Available N (mg/g)	23.67B	21.08B	35.06A	
Soil Available P (mg / g)	2.05A	1.84A	1.35B	

The data within the same line followed by different capital letters are significantly different in different sites $(P<0.05)$

(1:2.5) suspension. Soil organic carbon was calculated from the percent organic carbon estimated by oxidization in the presence of sulfuric acid (Rowell [1994](#page-9-0)). Olsen phosphorus (P) was determined by chlorostannus-reduced– molybdophosphoric blue color method by extraction with 0.5 M sodium bicarbonate for 30 min (Olsen et al. [1954](#page-9-0)). Available nitrogen (N) was measured using the alkaline hydrolysis diffusion method. Soil acid phosphatase and alkaline phosphatase activity were determined by the method reported by Tarafdar and Marschner [\(1994\)](#page-9-0). The unit of phosphatase activity (Eu) was µmol p-nitrophenyl phosphate (pNPP) g^{-1} soil h^{-1} that was released by phosphatase. Soil urease activity was determined using the method of Hoffmann and Teicher [\(1961\)](#page-8-0), and the results of urease activity were expressed as μg of NH₄⁺-N released during 3 h from 1 g of soil.

2.4 Isolation, identification and infection quantification of DSE fungi

Fresh roots were separated from soil, washed in tap water and cut into 0.5-cm long segments. The segments were cleared with 10 % (w/v) potassium hydroxide and stained with 0.5% (w/v) acid fuchsin solution (Phillips and Hayman [1970\)](#page-9-0). Assessment of fungal infection was conducted on each sample by the glass slide method, in which 50 randomly selected 0.5 cm root segment units were examined microscopically (Giovannetti and Mosse [1980](#page-8-0)). Root infection (%), hyphal infection $(\%)$ and microsclerotial infection $(\%)$ were expressed respectively as the percent of fine root segments infected for each root sample.

Endophytic fungi were isolated according to Liu [\(2011\)](#page-8-0), classified and identified according to colony morphology, including colony size, color, surface features, characteristics of matrix and individual characteristics, such as hyphal and spore morphology (Addy et al. [2005](#page-8-0); Barneit and Hunter [1977](#page-8-0); Grünig et al. [2009](#page-8-0); Reblova et al. [2011](#page-9-0); Wei [1979](#page-9-0)).

2.5 Statistical analysis

The effects of spatial changes on measured variables were tested by one-way analysis of variance, and comparisons among means made with the Least Significant Difference (LSD) test $(p<0.05)$. Stepwise regression analysis was used to test the correlation and principal component analysis of soil factors on DSE fungal infection. Principal components analysis (PCA) was used to extract comprehensive index of multiple index system, so as to select the main influencing factor of the soil desert habitat. Correlations were considered significant at $p<0.05$ and $p<0.01$. Statistical procedures were carried out with the software package of SPSS 19.0 for Windows.

3 Results

3.1 Morphological characteristics and identification of endophytic fungi

Microscopic and colonial morphology of endophytic fungi are shown in Fig. [1](#page-3-0).

Figure [1a](#page-3-0), a: At the beginning, a white colony turned green gray, loose colonies, powder, uplift with entire margins. Hyphae almost completely entered into the culture medium. The outstanding characteristic of the endophytic fungi is the conidiophores formed directly from vegetative hyphae, shape of different sizes, 2×5 μm short stakes like to clavate $5 \times$ 15 μm, a typical conidiophores and conidia chain, as well as some conidia powder spores. Spores were relatively small, colorless or light brown, with 0–1 septa and were nearly spherical or ovate in single droplets or clustered into chains. As a result, this was identified as Exophiala.

Figure [1b, b:](#page-3-0) Colonies were black, middle uplift, density with the surface granular and the margin was entire and white. The top produced conidia, conidiophores were erect stems, short or reduced to small terrier, and were light brown. Spores were solitary, fascicled or in chains, ovoid, dark, with 0–1 septa, were simple or branched, top has obvious collar, produced its own mucus in small terrier. This was identified as Phialophora.

Figure [1c, c:](#page-3-0) Colony was olive green in color, loose, blanket, flat, margins were entire, formed a round collar bottle terrier, bottle-shaped stems were often white or transparent, long cylindrical, head of the trunk spore production, primary spores ovoid, oblong, spherical $1-5.2 \times 1-2.5$ µm, secondary spore spherical, set into the big head of mucus. It was identified as Phialocephala.

According to the relevant literature, Exophiala, Phialophora and Phialocephala are typical DSE (Addy et al. [2005](#page-8-0); Grünig et al. [2009;](#page-8-0) Reblova et al. [2011\)](#page-9-0).

3.2 Morphology of DSE association in A. mongolicus roots

Acid fuchsin staining microscopy confirmed the ubiquity of DSE hyphae and microsclerotia-like structures in root tissues (Fig. [2a, b and e\)](#page-3-0). Dark hyphae of DSE colonized the epidermis and cortex of roots, and hyphae grew along the epidermis or cortex parallel to the longitudinal axis of the roots (Fig. [2c](#page-3-0) [and f\)](#page-3-0). Hyphae had dark red-brown to dark brown color, thick lateral walls and frequent septa (Fig. [2a, b and d\)](#page-3-0). Microsclerotia could fill single cortical cells or were distributed in more than one cell (Fig. [2d, e and g](#page-3-0)). Swellings of adherent hyphal tips were able to develop appressorium-like structures and aggregated chlamydospore-like structures were also observed wellw (Fig. [2c and h](#page-3-0)). These features indicated that a symbiotic system was formed between DSE and roots of host plants.

Fig. 1 Morphological characteristics of endophytic fungi isolated from the rhizosphere of A. monglicus (shown in longitudinal section, $bar=10 \text{ }\mu\text{m}$). a, b, c. Microscopic morphology of endophytic fungi. a,b,c. Colonial morphology of endophytic fungi

Fig. 2 Dark septate endophytic fungal association in A. monglicus roots (shown in longitudinal section, $bar=10 \mu m$) a, b. DSE hyphae and microsclerotia. c. Appressorium-like structures in root cortex. d. Superficial DSE hyphae and microsclerotia in epidermis and cortex of roots. e. Microsclerotia full of the whole or part of the organization of the

cell. f. Colonization of dark hyphae in epidermis and cortex, and hyphae growing along the epidermis or cortex parallel to the longitudinal axis of the roots. g. Initiation, development and formation of microsclerotia. h. Infection of dark hyphae in epidermal cells with some chlamydosporelike structures

Fig. 3 Spatial changes of DSE morphology in A. monglicus roots (shown in longitudinal section, $bar=10 \mu m$) a. DSE hyphae of Wuhai. b, c. DSE hyphae of Urad Back Banner. d. DSE hyphae of Alxa Left Banner darker

septate shorten and conspicuous. e.Microsclerotia of Wuhai, chlamydospore-like structures. f,g. Microsclerotia of Urad Back Banner. h. Microsclerotia of Alxa Left Banner, labyrinth shape

The changes of DSE morphology in A. mongolicus roots in the three sites are shown in Fig. 3. Hyphal color was shallow, separation was not obvious, with visible bending or random dendritic branching at Wuhai (Fig. 3a). Hyphal color deepened, thick and deformation, at the top of expansion at Urad Back Banner (Fig. 3b and c). Hyphal color was darker and septa were shorter and conspicuous at Alxa Left Banner (Fig. 3d). Microsclerotia were similar to the chlamydospore structures gathered, small and scattered at Wuhai (Fig. 3e). Microsclerotia were of leaf shape and annular cerebriform at Urad Back Banner (Fig. 3f and g). Microsclerotia were similar to labyrinth shape at Alxa Left Banner (Fig. 3h).

3.3 The spatial infection changes of DSE association

Hyphal and total root infection of DSE in the 0–40 cm soil layer were significantly higher than in 40–50 cm at Wuhai, and significantly higher in the 0–20 cm than the 30–50 cm layer, at Alxa Left Banner. Hyphal and total root infection were significantly higher in the 0–10 cm than the 30–50 cm layer, and significantly higher in the 10–30 cm than the 40– 50 cm layer, at Urad Back Banner. In comparing the same soil depths and different sites, total root infection in the 0–10 cm layer was significantly higher at Urad Back Banner than at Alxa Left Banner. Hyphal infection in the 30–40 cm layer was significantly higher at Wuhai than at Alxa Left Banner (Fig. [4a and c\)](#page-5-0).

Microsclerotial infection order in the three sites was: Alxa Left Banner $>$ Wuhai $>$ Urad Back Banner (Fig. [4b](#page-5-0)), and decreased with increasing soil depth. Maximum values were in the 0–10 cm soil depth (Fig. [4b](#page-5-0)). Microsclerotial infection was significantly higher in the 0–10 cm layer than in other layers at Wuhai, and it in 10–30 cm soil depth was significantly higher than that in 10–30 cm soil depth (Fig. [4b\)](#page-5-0) at Alxa Left Banner. In comparisons of the same soil depth for the three sample sites, microsclerotial infection in the 0–10 and 30–50 cm layers was significantly higher at Alxa Left Banner and Wuhai than at Urad Back Banner (Fig. [4b](#page-5-0)).

3.4 Spatial changes of soil parameters

Activities of soil urease, acid phosphatase and alkaline phosphatase were closely related to sample sites and soil depth (Fig. [4](#page-5-0)). Soil urease activity was significantly higher at Alxa Left Banner than Urad Back Banner and Wuhai, with the highest activity for the three sites in the 0–10 cm layer. There were no significant differences across sites in the other soil layers. The activities of soil acid phosphatase and alkaline phosphatase were the highest at Urad Back Banner. The activity of alkaline phosphatase was always highest in the 0– 10 cm layer for all sites, and activity decreased with increased soil depth. In addition, soil acid phosphatase activity was usually greater in the 0–30 than the 30–50 cm layer.

Fig. 4 Spatial changes of DSE association in A. monglicus roots. Figures followed by the same letter are not significantly $(P<0.05)$ different, according to the LSD test

Soil organic matter content differed significantly between the sample sites, with the highest at Urad Back Banner and the lowest at Wuhai. The content of soil organic matter in the 0– 10 cm layer was significantly higher than in the other layers at Wuhai and Alxa Left Banner; and was significantly higher in the 0–40 cm than the 40–50 cm layer at Urad Back Banner. Soil available N was significantly higher at Alxa Left Banner than at Urad Back Banner and Wuhai. Soil pH was the highest at Wuhai, and was significantly higher than that at Alxa Left Banner. However, the significant difference was not

found in different sample sites and different soil layers (Fig. [5](#page-6-0)) (Fig. [6\)](#page-6-0).

3.5 Relationship between DSE and soil factors

3.5.1 Correlation analysis

The correlation analysis showed that DSE hyphae were significantly positively correlated with activities of soil alkaline phosphatase, acid phosphatase and urease and contents of organic matter and available N. Microsclerotia were significantly positively correlated with soil organic matter and available N. The percentage of root infection was not significantly correlated with the soil factors (Table [2](#page-7-0)).

3.5.2 PCA of soil factors

According to the eigenvalues of the correlation matrix was greater than 1, the principle of the accumulative variance contribution rate was greater than 85 %, selected the three principal components. The PCA results are shown in Table [3.](#page-7-0) The accumulative contribution rate was 89.92 %, the extraction of principal components could reflect all index information. On the first principal component, soil organic matter, alkaline phosphatase and available N had higher weightings (in the range of 0.935–0.962), acid phosphatase times (0.869). On the second principal component, soil pH and urease had higher weightings; and on the third principal component, available P had higher weightings. However, the first principal component accounted for a large amount of information. Thus, organic matter, phosphatase and available N were the main factors.

4 Discussion

4.1 DSE infection in A. mongolicus roots

Fungal symbionts, comprising mainly mycorrhizal fungi and fungal endophytes, are ubiquitous in terrestrial plant roots. They are beneficial to plants by regulating host nutrition, metabolites and stress response (Baldi et al. [2008;](#page-8-0) Rai et al. [2004;](#page-9-0) Redman et al. [2002\)](#page-9-0). Fungal endophytes colonize the internal tissues of living plants without causing any external disease symptoms. Such associations can go back millions of years (Krings et al. [2007](#page-8-0); Rodriguez et al. [2008](#page-9-0)), benefitting both fungus and host plant.

Among root-associated fungi, DSE are ubiquitous and found in a wide range of plant species. The abundance of DSE in arctic, alpine and temperate habitats has been investigated extensively (Mandyam [2008](#page-8-0); Newsham et al. [2009;](#page-9-0) Schmidt et al. [2008\)](#page-9-0). DSE are known to colonize the intraand extra-cellular tissues of host plants with no apparent

Fig. 5 Spatial changes of soil enzymatic activities in the rhizosphere of A. monglicus. Figures followed by the same letter are not significantly (P<0.05) different, according to the LSD test

negative effects (Jumpponen [2001](#page-8-0)). The dark hyphae, typical of these fungi, are considered to be important for the host to survive stressful conditions, because cell wall melanin can trap and eliminate oxygen radicals generated during abiotic stress (Richier et al. [2005](#page-9-0)). DSE, characterized by darkpigmented and septate hyphae, particularly confer traits that improve their hosts' tolerance to unfavorable environmental conditions (Hesse et al. [2003\)](#page-8-0). In this study, microscopy revealed that DSE were ubiquitous in A. mongolicus roots, and showed that the dominant infection by microsclerotia (large sclerotium-like structures inside the root cortex) and other small sclerotium-like structures co-existed with dark septate

Fig. 6 Spatial changes of soil factors in the rhizosphere of A.monglicus. Figures followed by the same letter are not significantly $(P<0.05)$ different, according to the LSD test

Table 2 Correlation coefficients between DSE and soil factors

The data in the table is correlation coefficients between DSE fungi and soil factor

* means the correlation is significant at $P<0.05$

** means the correlation is very significant at $P \le 0.01$

hyphae. Some DSE species occupy the whole cortical cell volume, and the chlamydospore-like structures of A. mongolicus roots by DSE might confer tolerance to environmental stress, and carbon absorption, or signal exchange with the host and important place.

Read and Haselwandter [\(1981\)](#page-9-0) found that the colonization of Austria alpine area including many plants sedge was DSE. In high altitude areas, colonizing by DSE might provide some advantages to plants. Casanova-Katny et al. [\(2011](#page-8-0)) investigated the colonization of 23 species of fungi in different altitude habitats in the Andes Mountains. Their results indicated that the DSE infections might have advantages for plant growth and resistance function. In the present study, DSE colonization in A. mongolicus roots followed the order of Alxa Left Banner > Wuhai > Urad Back Banner, and gave direct support to the above views. With changes in elevation and precipitation of the sampling environment, DSE hyphal color deepened gradually, changing from thin to thick and hyphae had more branches. The hyphae were deformed, apically dilated, and the diaphragm was more common and shorter. Microsclerotia showed diversity, a simple leaf-like form became a ring cerebriform and labyrinth. DSE behavior was based upon the intra- and/or extra-cellular colonization of host plant roots. DSE, characterized by dark-pigmented and septate mycelia, may confer traits that improve tolerance to unfavorable environmental conditions in the host (Hesse et al. [2003](#page-8-0);

Table 3 Principal component loading matrix, eigenvalue and contribution rate

Physico-chemical factor	PC ₁	PC ₂	PC ₃
pН	-0.302	0.688	0.356
Organic matter	0.962	-0.085	0.359
Available P	0.612	0.501	0.561
Available N	0.935	-0.096	-0.058
Kaline phosphatase	0.956	-0.090	0.235
Acid phosphatase	0.869	0.285	-0.014
Urease	-0.256	0.612	-0.111
Eigenvalue(λ)	3.933	1.216	1.024
Contribution rate /%	67.025	15.661	7.231

Jumpponen and Trappe [1998](#page-8-0)). The results showed that A. mongolicus formed a symbiosis with DSE.

4.2 The spatial colonization of DSE and soil factors

The general vegetation analysis showed that the sampling sites were relatively similar, although the soil parameters indicated subtle differences. Generally, pH and nutrient availability strongly affects the colonization of fungi (Deacon [2006](#page-8-0)). Colonization of DSE hyphae and microsclerotia reached maxima in the 0–20 cm soil layer at the different sample sites, and then decreased with increasing soil depth. The contents of the surface soil available N, available P and organic matter in the A. mongolicus rhizosphere were higher than elsewhere, and plant roots were significantly positively correlated with DSE hyphae and microsclerotia. This correspondence showed that plant growth was closely related to soil nutrients and DSE. DSE have been reported to be prevalent in dry habitats (Mandyam and Jumpponen [2005](#page-8-0)), and some authors hypothesized that DSE might be involved in plant drought tolerance (Barrow [2003;](#page-8-0) Pennisi [2003\)](#page-9-0). The DSE fungal infection in A. mongolicus roots on the sand dunes in Inner Mongolia also supported this hypothesis.

DSE are a miscellaneous group of ascomycetes and colonize root tissues intra- and inter-cellularly without causing any apparent negative effects on the host plant (Jumpponen [2001;](#page-8-0) Silvani et al. [2008](#page-9-0); Wilson et al. [2004](#page-9-0)). DSE associations have been recognized in about 600 plant species of 320 genera in 114 families, including non-mycorrhizal species (Jumpponen and Trappe [1998](#page-8-0)). DSE might benefit their host plants by promoting absorption by plants of mineral nutrients (including N and P) and water (Caldwell and Jumpponen [2003\)](#page-8-0), and suppressing infection by plant pathogens (Narisawa et al. [2004\)](#page-9-0). One DSE, Heteroconium chaetospira, was reported to transfer N to host plants (Usuki and Narisawa [2007](#page-9-0)). In this study, PCA indicated that soil available N was one of the important soil factors affecting DSE in the desert ecosystem of Inner Mongolia. Correlation analysis also showed that DSE activity was closely related to soil factors, and colonization of hyphae and microsclerotia was highly positively correlated.

Soil enzymes are active organic components of soil, and are largely indicative of soil microbial metabolic processes

(Zhou [1987\)](#page-9-0). DSE infection, mediated through soil enzyme activity, can affect the host metabolic activity. The hyphal phosphatase catalyzes the hydrolysis of soil phosphate esters and phosphoric acid to esters, promoting hydrolysis of organic phosphate to plant-available inorganic phosphate. Plants can then take up this form of P (He et al. 2011). Urease regulates urea transformation to ammonium, which can be taken up by both mycorrhizal fungi and plants (Zhou [1987](#page-9-0)). In this study, the infection of hyphae had a significant positive relationship with activities of soil alkaline phosphatase, acid phosphatase and urease. This further illustrated the hypothesis that the DSE might be involved in changing soil composition and soil fertility through soil enzyme action, thus improving soil structure and health.

5 Conclusion

In this study, a symbiosis was found between A. mongolicus and DSE fungi in the Inner Mongolian desert. The dynamics of DSE fungi had a highly correlated spatial pattern, which further correlated with soil nutrient availability and enzymatic activity. DSE morphology and infection might be useful indicators for evaluation of soil quality and function of desert ecosystems. Future research should investigate the function of DSE associations in different plants and would be valuable to improve understanding of the role of DSE fungi in desert ecosystems.

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References

- Addy HD, Piercey MM, Currah RS (2005) Microfungal endophytes in roots. Can J Bot Rev Can Bot 83:1–13
- Baldi A, Jain A, Gupta N, Srivastava AK, Bisaria VS (2008) Co-culture of arbuscular mycorrhiza-like fungi (Piriformospora indica and Sebacina vermifera) with plant cells of Linum album for enhanced production of podophyllotoxins: a first report. Biotechnol Lett 30: 1671–1677
- Barneit HL, Hunter BB (1977) Illustrated genera of the imperfect fungi, 3rd edn. Science Press, Beijing
- Barrow JR (2003) A typical morphology of dark septate fungal root endophytes of Bouteloua in arid southwestern USA rangelands. Mycorrhiza 13:239–247
- Bjorbækmo MFM, Carlsen T, Brysting A, Vrålstadl T, Høiland K, Ugland KI, Geml J, Schumacher T, Kauserud H (2010) High diversity of root associated fungi in both alpine and arctic dry asoctopetala. BMC Plant Biol 10:244
- Caldwell BA, Jumpponen A (2003) Arylsufatase production by mycorrhizal fungi. Fourth International Conference on Mycorrhizae, Montreal, p 312

Casanova-Katny MA, Torres-Mellado GA, Palfner G, Cavieres LA (2011) The best for the guest: high Andean nurse cushions of Azorella madreporica enhance arbuscular mycorrhizal status in associated plant species. Mycorrhiza 21:613–622

Deacon J (2006) Fungal biology. Blackwell, Malden

- Eswaran H, Rice T, Ahrens R, Stewart BA (2002) Soil classification: a global desk reference. CRC Press, Boca Raton, pp 1–263
- Feng JCH, Zhou YJ, Zhou HY (2001) Physiological responses of Ammopiptanthus mongolicus (Maxira)to change of soilmoisture. J Desert Res 21(8):223–226
- Fuchs B, Haselwandter K (2004) Red list plants: colonization by arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza 14:277–281
- Giovannetti M, Mosse B (1980) An evaluation of technique for measuring vesicular-arbuscular mycorrhizaal infection in roots. New Phytol 84:489–500
- Grünig CR, Queloz V, Sieber TN, Holdenrieder O (2008) Dark septate endophytes (DSE) of the Phialocephala fortinii – Acephala applanata species complex in tree roots: classification, population biology, and ecology. Bot 86:1355–1369
- Grünig CR, Queloz V, Duo A, Sieber TN (2009) Phylogeny of Phaeomo Uisia piceae gen. sp. nov.: a dark, septate, conifer-needle endophyte and its relationships to Phialocephala and Acephala. Mycol Res 113: 207–221
- He HB, Hao YG, Ding Q, Jia GX (2006) Chamcteristics of plant community of *Ammopiptanthus mongolicus* and the diversity of its nodules. J Beijing For Univ 28(4):123–128
- He XL, Yang J, Zhao LL (2011) Spatial distribution of arbuscular mycorrhizal fungi in Salix psammophila soil in Inner Mongolia desert rootzone. Acta Ecol Sin 31(8):2159–2168
- Hesse U, Schöberlein W, Wittenmayer L, Förster K, Warnstorff K, Diepenbrock W, Merbach W (2003) Effects of Neotyphodium endophytes on growth, reproduction and drought stress tolerance of three Lolium perenne L. genotypes. Grass Forage Sci 58:407–415
- Hoffmann GC, Teicher K (1961) A colorimetric technique for determining urease activity in soil. Dung Boden 95:55–63
- Jiang Q, He XL, Chen WY, Zhang YJ, Rong XR, Wang L (2014) Spatial distribution of AM and DSE fungi in the rhizosphere of Ammopiptanthus nanus. Acta Ecol Sin 34(11):2929–2937
- Jumpponen A (2001) Dark septate endophytes are they mycorrhizal? Mycorrhiza 11:207–211
- Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295– 310
- Kauppinen M, Raveala K, Wäli PR, Ruotsalainen AL (2014) Contrasting preferences of arbuscular mycorrhizal and dark septate fungi colonizing boreal and subarctic Avenella flexuosa. Mycorrhiza 24:171– 177
- Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007) Fungal endophytes in a 400-million-yr-old land plant: Infection pathways, spatial distribution, and host responses. New Phytol 174:648–657
- Kytöviita MM (2005) Asymmetric symbiont adaptation to Arctic conditions could explain why high Arctic plants are non-mycorrhizal. FEMS Microbiol Ecol 53:27–32
- Liu JL (2011) Isolation and determination of antioxidant activity of endophytic fungi from Lycium barum. Lishizhen Med Mat Med Res 22(4):857–860
- Mandyam K (2008) Dark septate fungal endophytes from a tallgrass prairie and their continuum of interactions with host plants. Ph. D. thesis. Kansas State University, Manhattan, KS
- Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root colonizing dark septate endophytic fungi. Stud Mycol 53:173– 189
- Muthukumar T, Tamilselvi V (2010) Occurrence and morphology of endorhizal fungi in crop species. Trop Subtrop Agroecosyst 12: 593–604
- Narisawa K, Usuki F, Hashiba T (2004) Control of Verticillium yellows in Chinese cabbage by the dark septate endophytic fungus LtVB3. Phytopathology 94:412–418
- Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–793
- Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2:10–20
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture, Washington, p 939
- Parbery DG (1996) Trophism and the ecology og fungi associated with plants. Biol Rev 71:473–527
- Pennisi E (2003) Fungi shield new host plant from heat and drought. Science 301:1466
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscualr mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161
- Rai MK, Varma A, Pandey AK (2004) Antifungal potential of Spilanthes calva after inoculation of Piriformospora indica. Mycoses 47:479– 481
- Rains KC, Nadkarni NM, Bledsoe CS (2003) Epiphytic and terrestrial mycorrhizas in a lower montane Costa Rican cloud forest. Mycorrhiza 13:257–264
- Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. New Phytol 88:341–352
- Reblova M, Gams W, Slepanek V (2011) The new hyphomycete genera Brachyalara and lnfundichalara, the similar Bxochalara and species of 'Phidophom sect. Catemilatae' (Leoliomyceies). Fungal Divers 46:67–86
- Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298: 1581
- Richier S, Furla P, Plantivaux A, Merle PL, Allemand D (2005) Symbiosis- induced adaptation to oxidative stress. J Exp Biol 208: 277–285
- Rodriguez RJ, Henson J, Volkenburgh EV, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat- adapted symbiosis. ISME J 2:404–416
- Rowell DL (1994) Soil Science: Methods and Applications. Longman Group U.K. Ltd, London
- Ruotsalainen AL, Vare H, Vestberg M (2002) Seasonality of root fungal colonization in low-alpine herbs. Mycorrhiza 12:29–36
- Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt C (2008) Mycorrhizal and dark septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. Arctic Antarct. Alp Res 40:576–583
- Sieber TN, Grünig CR (2006) Biodiversity of fungal root-endophyte communites and populations, in particular of the dark septate endophyte Phialocephala fortinii s.l. In: Schulz B, Boyle C, Sieber T (eds) Microbial root endophytes. Springer- Verlag, Berlin, pp 107– 132
- Silvani VA, Fracchia S, Fernández L, Pérgola M, Godeas A (2008) A simple method to obtain endophytic microorganisms from field collected roots. Soil Biol Biochem 40:1259–1263
- Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Elsevier, Amsterdam Takeshi T, Hiroyuki U, Junichi K, Muneto H, Naoko M, Kenji F, Zhang GSH, Wang LH, Ken Y, Norikazu Y (2012) Colonization and community structure of root-associated microorganisms of Sabina vulgaris with soil depth in a semiarid desert ecosystem with shallow groundwater. Mycorrhiza 22:419–428
- Tarafdar JC, Marschner H (1994) Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol Biochem 26:387–395
- Upson R, Read DJ, Newsham KK (2009) Nitrogen form influences the response of Deschampsia antarctica to dark septate root endophytes. Mycorrhiza 20:1–11
- Usuki F, Narisawa K (2007) A mutualistic symbiosis between a dark septate endophytic fungus, Heteroconium chaetospira, and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99:175–184
- Wei JC (1979) Handbook of fungal identification. Science Technology Press, Shanghai
- Wei QSH, Wang JH (2005) A preliminary study on the distribution patterens and characeristics of Ammopiptanthus mongolicus populations in different desert environments. Acta Phytoecologica Sin 29(4):591–598
- Wilson BJ, Addy HD, Tsuneda A, Hambleton S, Currah RS (2004) Phialocephala sphaeroides sp. nov., a new species among the dark septate endophytes from a boreal wetland in Canada. Can J Bot Rev Can Bot 82:607–617
- Wurzburger N, Bledsoe CS (2001) Comparison of ericoid and ectomycorrhizal colonization and ectomycorrhizal morphotypes in mixed conifer and pygmy forests on the northern California coast. Can J Bot Rev Can Bot 79:1202–1210
- Zhou LK (1987) Soil enzymology. Science Press, Beijing