Community Structure of Arbuscular Mycorrhizal Fungi in Saline Alkaline Soil of Northwest China

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Key Words: Arbuscular mycorrhizal fungi; Saline alkaline soil; Soil type; Soil properties

Abstract Community structure of arbuscular mycorrhizal fungi (AMF) was investigated in saline alkaline soil of northwest China. Soil samples were collected from the rhizospheres of 25 common plant species and were analyzed for soil properties and AMF composition. In total, twenty-eight species of AMF were identified, belonging to 4 genera, i.e. 4 *Acaulospora* species, 2 *Archaeospora* species, 2 *Diversispora* species and 20 *Glomus* species. *Glomus*, *Diversispora* and *Archaeospora* were dominant genera, *G. constrictum*, *G. geosporum, G. intraradices*, *D. versiforme* and *Ar. leptotichum* were dominant species.AMF species richness of saline soil, alkaline soil and salinized irrigation soil was 24, 18 and 17 species respectively. *Ar. leptotichum, D. versiforme, G. constrictum*, *G. geosporum* and *G. intraradices* were dominant in all soil types, whereas, some degree of specialization in AMF according to soil types was recorded. AMF community structure was correlated with changes in soil properties.

Introduction

In nature, communities of arbuscular mycorrhizal fungi (AMF) have different species composition and exert different symbiotic functions depending on their community structure (öepik et al., 2006). Thus, many researchers have studied the structure and function of AMF communities, and the relationship between AMF and ecological factors (Schenck, 1987; Gai and Liu, 2003; Wu et al., 2007; Moreira et al., 2007; Wolfe et al., 2007). AMF occurred in a wide variety of ecosystems, such as farmland, forestland, grassland, desert, saline alkaline soil and so on (Wang et al., 2003). Preliminary researches have indicated that AMF widely occurred and formed associations with most of the plants growing in saline alkaline soil (Marson, 1928; Kim and Webber, 1985), being thought to play a particularly important role in saline alkaline soil through enhancing plant absorption of P and other elements and improving water uptake and its transport to plants to withstand the stressful conditions (Berta et al., 1990; Cantrell and Linderman, 2001; Ruiz-Lozano and Azcón, 1995). The population of AMF varied greatly and the distribution of AMF was affected by various biotic and abiotic factors in saline alkaline conditions (Liu et al., 1999). Cooke et al. (1993) and Mathur et al.

(2007) reported AMF spore density had a strong correlation with soil pH, organic carbon content, Olsen's P content and the salinity of soil, however, Aliasgharzadeh et al. (2001) pointed out that the spore density of AMF in saline soil was not correlated with soil salinity but suffered adverse effects of the accumulation of some anions and cations; Other studies also reported the variance of AMF communities was partly attributed to host plant species (Mathur et al., 2007), irrigation, water salinity and rootstock (Levy et al., 1983).

Soil salinization is a widespread problem, approximate 7% of the global land surface is covered with saline soil (Ruiz-Lozano et al., 1996). Out of 1.5 billion ha cultivated land, about 77 million ha (5%) are affected by excess salt content mainly induced by irrigation with ground water of high salt content (Munns et al., 1999). These problem soils also occur in China, where it was estimated that about 3693 hm^2 land was under salt stress conditions (Zhao and Li., 1999). Over the past 20 years, surveys of AMF community composition and species distribution were conducted widely in China (Gai et al. 2006), but no study was done on saline alkaline soils in northwest China. Liu et al. (1999) and Wang et al. (2003; 2004) investigated AMF distribution in saline alkaline soils of the Yellow River Delta of China, and found AMF widely occurred in saline alkaline soil and its community was affected by organic matter content, soil depth, soil type and soil alkalinity. In general, knowledge of the relationship between AMF community and soil properties is lacking in saline alkaline soil of China, especially in northwest China.

The objectives of this study were (1) to investigate AMF community, and (2) determine whether soil type and soil properties have relation to AMF community in saline alkaline soil of northwest China.

Materials and Methods

Site Selection and Sample Collection

Gansu province, Ningxia Hui Autonomous Region and Inner Mongolia Autonomous Region were selected as sample areas, which located in northwest China and are characterized by warm and dry summers and cold and dry winters, only have 100-800 mm mean annual precipitation, and include all typical types of saline alkaline soil.

Details of the sample sites investigated are given in Table 1. Twenty-five common plant species (Table 1) were taken in April, 2005. Five soil samples were collected from the rhizospheres of five plants of the same species randomly distributed at each site. Each soil sample, containing 400 g of soil, was collected from 5-20 cm depth; the top 5 cm was removed to eliminate part of the top leaf litter. Soil samples were placed in sterilized cotton bags, labelled, and air-dried for 1 week. Then, they were subsequently ground, sieved through a 2-mm sieve, thoroughly mixed, divided into two portions to measure soil properties and identify AMF, stored at 4°C and processed within 4 months.

Table 1 Characteristics of the sample sites investigated and common plant species in alkaline soil, salinized irrigation soil and saline soil of northwest China.

Soil type	Sample site	Plant species	Altitude (m)	Longitude and latitude
Alkaline soil	Pingluo County, Ningxia Hui Autonomous Region	*Spartina anglica* C.E.Hubb. *Achnatherum splendens* (Trin.)Nevski. *Nitraria tangutorum* Bobr.	1099	38°48′N, 106°16′E
	Naiman Banner, Zhelimu League, Inner Mongolia Autonomous Region	*Carex duriuscula* C.A.Mey. *Agropyron cristatum* (L.) Gaertn. *Salicornia europaea* L. *Phragmites australis* (Cav.)Trin.ex Steud. *Iris lactea* Pall.var.*Chinensis* (Fisch.)Koidz.	513	42°51′N, 120°39′E
Salinized irrigation soil	Anxi County, Gansu Province	*Medicago sativa* L. *Triticum aestivum* L.	1135	40°29′N, 95°36′E
	Dunhuang County, Gansu Province	*Gossypium hirsutum* L.	1050	40°25′N, 94°41′E
	Yumen Town, Gansu Province	*Glycyrrhiza uralensis* Fisch.	1404	40°26′N, 97°00′E
	Pingluo County, Ningxia Hui Autonomous Region	*Zae mays* L. *Allium fistulosum* L. *Oryza sativa* L. *Lycium chinense* Mill.	1099	38°48′N, 106°16′E
	Ke̓erqin Left Central Banner, Inner Mongolia Autonomous Region	*Helianthus annuus* L.	259	45°03′N, 121°28′E
Saline soil	Dunhuang County, Gansu Province	*Alhagi sparsifolia* Shap.	1050	40°25′N, 94°41′E
	Yumen Town, Gansu Province	*Elaeagnus angustifolia* L. var. *I.lactea* Pall. var. *Chinensis* (Fisch.)Koidz. *A. splendens* (Trin.)Nevski.	1404	40°26′N, 97°00′E
	Pingluo County, Ningxia Hui Autonomous Region	*Populus euphratica* Oliv. *Ailanthus altissima* Mill. *Salix gordejevii* Chang et Skv. *A. cristatum* (L.) Gaertn.	1099	38°48′N, 106°16′E
	Tuoketok County, Hohhot City, Inner Mongolia Autonomous Region	*N. tangutorum* Bobr. *P. australis* (Cav.)Trin.ex Steud.	986	40°17′N, 111°09′E
	Naiman Banner, Zhelimu League, Inner Mongolia Autonomous Region	*Caragana korshinkii* Kom.	513	42°51′N, 120°39′E
	Ke̓erqin Left Central Banner, Inner Mongolia Autonomous Region	*Leymus chinensis* (Trin.)Tzvel. *Carex kobomugi* Ohwi.	259	45°03′N, 121°28′E

AMF Isolation and Identification

Soil samples were wet-sieved for spores using the method described by Gerdemann and Nicholson (1963), as modified by Daniels and Skipper (1982). Fifty grams of soil per soil sample was independently suspended in 250 ml water, stirred with a magnetic stirrer bar for 10 minutes and the suspension was sieved. Spores and debris were collected on 38.5-µm, 70-µm, 100-µm, 150-µm and 500-µm sieves with tap water, filtered onto a piece of filter paper, and then placed in a 9-cm Petri dish for examination under a binocular stereomicroscope. Each spore type was mounted in water, lactophenol, PVA and Melzer's reagent, respectively, for identification. The identification was based on spore colour, size, surface ornamentation and wall structure with reference to the descriptions provided by the International Collection of AMF (http://invam.caf.wvu.edu) and the originally published species descriptions of Schenck and Perez (1988), as modified by Morton and Redecker (2001). The permanent slides were mounted in polyvinyl-lacto-glycerol, sealed with nail varnish, and stored at the Microbiology Laboratory, College of Forestry, Northwest A&F University, Shaanxi province, China.

Chemical Analysis of Saline Alkaline Soil

Soil properties measured were pH (soil / water ratio of 1:2.5W/V), concentrations of organic matter, available N, available K, available P, water-soluble salt, CO_3^{2-}, HCO_3^-, K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^- using the methods described by Bao (2000).

Data Analysis

AMF composition was evaluated from the isolation frequency (IF) and species richness (SR).

IF was expressed as the percentage of samples from which spores of a particular genus or species were isolated. The degree of dominance was described as: dominant species or genus (IF >50%), most common species or genus (30% <IF≤50%), moderately common species or genus (10% <IF≤30%), and rare species or genus (IF≤10%).

SR was described as the quantity of the total AMF species in the sample.

To test the relation between soil properties and AMF community structure, a canonical correspondence analysis was generated using PC-ORD (Windows version 4.0).

Results

AMF Community

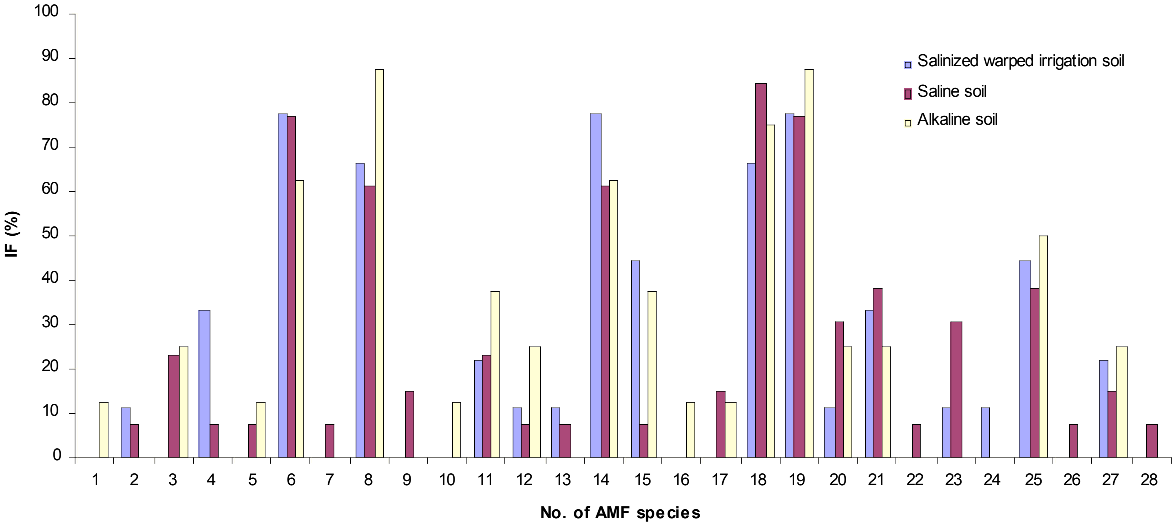
Table2 The isolation frequency (IF) of AMF species or genus in saline alkaline soil of northwest China

AMF species or genus (No.)	IF (%)	AMF species or genus (No.)	IF (%)
Acaulospora	33.33	(13) *G. clarum*	6.67
(1) *A. lacunosa*	3.33	(14) *G. constrictum*	66.67
(2) *A. rugosa*	6.67	(15) *G. coronatum*	26.67
(3) *A. scrobiculata*	16.67	(16) *G. diaphanum*	3.33
(4) *A. spinosa*	13.33	(17) *G. fasciculatum*	10.00
Archaeospora	76.67	(18) *G. geosporum*	76.67
(5) *Ar. gerdemannii*	6.67	(19) *G. intraradices*	80.00
(6) *Ar. leptotichum*	73.33	(20) *G. manihotis*	23.33
Diversispora	73.33	(21) *G. mosseae*	33.33
(7) *D. etunicatum*	3.33	(22) *G. pansihalos*	3.33
(8) *D. versiforme*	70.00	(23) *G. pustulatum*	16.67
Glomus	96.67	(24) *G. reticulatum*	3.33
(9) *G. aggregatum*	6.67	(25) *G. tenebrosum*	43.33
(10) *G. albidum*	3.33	(26) *G. trimurales*	3.33
(11) *G. australe*	26.67	(27) *G. verruculosum*	20.00
(12) *G. claroideum*	13.33	(28) *G. vesiculiferum*	3.33

Twenty-eight AMF species were distinguished from the rhizospheres of 25 plant species. Of the 28 AMF species, 20 belonged to the genus *Glomus*, 2 to *Diversispora*, 2 to *Archaeospora*, 4 to *Acaulospora* (Table 2).

Glomus, *Archaeospora* and *Diversispora* were dominant genera. *Acaulospora* was the most common genus. *G. constrictum*, *G. geosporum*, *G. intraradices*, *D. versiforme* and *Ar. leptotichum* were dominant species. *G. mosseae* and *G. tenebrosum* were most common species. There were 8 moderately common species, i.e. *A. scrobiculata, A. spinosa, G. australe, G. claroideum, G. coronatum, G. manihotis, G. pustulatum* and *G. verruculosum*. The others were rare species (Table 2).

Relationship between Soil Type and AMF Community



AMF species richness of saline soil, alkaline soil and salinized irrigation soil was 24, 18 and 17 species respectively. *Ar. leptotichum, D. versiforme, G. constrictum*, *G. geosporum* and *G. intraradices* were dominant in all soil types. *G. reticulatum* was particular for salinized irrigation soil, *D. etunicatum, G. aggregatum, G. pansihalos, G. trimurales* and *G. vesiculiferum* were for saline soil; *A. lacunosa, G. albidum* and *G. diaphanum* were for alkaline soil. This suggests some degree of specialization in AMF according to soil types.

Relationship between Soil Properties and AMF Community

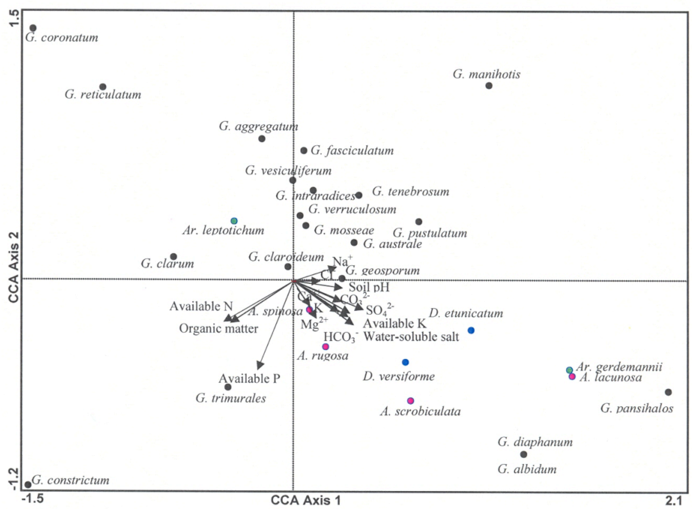


Fig.1 Canonical correspondence analysis (CCA) depicting the relation between AMF community structure and changes in available N, organic matter, available P, available K, Ca^{2+}, K^{+}, Mg^{2+}, HCO_3^-, water-soluble salt, SO_4^{2-}, Cl^-, Na^+, CO_3^{2-} and soil pH.

Most *Glomus* species were correlated with negative changes in concentrations of available N, organic matter and available P, whereas, most species of *Acaulospora*, *Archaeospora* and *Diversispora* were associated with positive changes in soil pH and concentrations of Ca^{2+}, K^{+}, Mg^{2+}, HCO_3^-, available K, water-soluble salt, SO_4^{2-} and CO_3^{2-}.

Discussion

Soil type was one of the most important factors linking to AMF composition and distribution in soil, changes in soil type could strongly affect species richness, quantity, sporulation, colonization and other physiological activities (Gai and Liu, 2003; Zhang et al., 1999). The results of this study indicated that AMF community and species richness differed in soil types, which could be linked to differences in the adapting ability of different AMF to soil types (Diaz et al., 1992). Some researchers reported that the dominant AMF species were different among soil types (Carvalho et al., 2001; Hildebrandt et al., 2001; Oliveira et al., 2005). However, in our study, the dominant AMF species were same among soil types. This may be because (1) the salinity and alkalinity inhibited the development of the majority of AMF and only benefited to the existence of a small part of AMF having a greater ability to adapt to saline alkaline conditions (Douds and Millner, 1999), (2) the developing processes of saline soil, alkaline soil and salinized irrigation soil had a little similarity with each other, and (3) though sample sites investigated in this study were respectively situated in three regions, however, all sites belonged to arid or semiarid zones, where the climate conditions and plant cover status were a little similar.

Liu et al. (1999) reported that an increase in organic matter concentration was related to an increase in AMF species richness when organic matter concentration was below 1.5%, whereas, and a decrease when organic matter concentration was over 1.5%. The data in this study also pointed out that changes in organic matter, available P and available N were correlated with AMF community structure, especially *Glomus* distribution, which may be mainly attributed to the effect of these ingredients on AMF sporulation and colonization (Liu et al., 1999). For example, available P, having the closest correlation with AMF development, negatively correlated with AMF spore density (Safari, 2006; Mathur et al., 2007), mainly being linked to the influence of available P on AMF development and function via changing root exudate of host plant (Tawaraya et al., 1994, 1996).

With regard to the relationship between AMF and both soil pH and concentrations of water-soluble salt, HCO_3^-, CO_3^{2-}, Ca^{2+}, Cl^-, Na^+, K^+, SO_4^{2-} and Mg^{2+}, most studies focused on the effect of these ingredients on AMF spore density (Cooke et al., 1993; Mathur et al., 2007; Aliasgharzadeh et al., 2001), but little on AMF community structure. Our data showed changes in soil pH and concentrations of Ca^{2+}, K^+, Mg^{2+}, HCO_3^-, available K, water-soluble salt, SO_4^{2-} and CO_3^{2-} were associated with AMF community structure, especially most species of *Acaulospora*, *Archaeospora* and *Diversispora*. This may be caused by the direct effect or by plant-mediated effect of soil properties on AMF (Juniper and Abbott, 2004, 2006), for instance, soil pH was related to the accumulation of AMF spores in soil since an increase in soil pH could reduce spore germination and hyphal development of AMF (Gai and Liu, 2003).

In conclusion, soil type and soil properties were related to AMF community in saline alkaline soil. Furthermore, since there are complex interactions among theses factors, any factor does not affect AMF singly, but as a whole. Thus, it is necessary to conduct further studies on the influence of the interaction among these factors on AMF community.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (Project 30225035), Key Program of NSFC (Project 30630054) and the Program of Changjiang Scholar and Innovative Research Team in China (IRT0748).

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