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ARTICLE

Arbuscular Mycorrhizal Fungal Colonization at Different Succession Stages in Songnen Saline-Alkali Grassland

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi can form symbiosis with 90% of the vascular plants and play important roles in ecosystem. To realize the AM fungal colonization at different succession stages in saline-alkali land and screen AM fungi species with great functions, roots and soil samples were collected from the three succession stages of Songnen saline-alkali grassland. The soil properties and AM fungal colonization were measured, and the fungus distributed extensively in three stages was annotated by sequencing for AML1/AML2 target, subsequently, maize was selected as the host to verify its colonization. The results showed that the soil properties improved with the succession of saline-alkali grassland. The plants' communities of the three stages could be colonized by AM fungi, and the colonization rate of *Leymus chinensis* (the third stage) ranged from 66.67% to 100%, *Puccinellia tenuiflora* (the second stage) ranged from 50% to 80%, while the *Suaeda glauca* (the first stage) was only 35%–60%. Glomeraceae sp1 was identified as the dominant AM fungi species which occurred frequently in the succession of saline-alkali land with the isolation frequency, relative abundance, and importance value of 100%, 18.1%, and 59.1%, respectively. The colonization rate of Glomeraceae sp1 in maize ranged from 80% to 87% and similar mycorrhizal characteristics were detected in the roots of *P. tenuiflora*, *S. glauca*, and *L. chinensis*, indicating that Glomeraceae sp1 colonized the samples in the field. The correlation matrix indicated that colonization rate, colonization intensity, and vesicle abundance were closely related to soil conditions most, and they were related significantly to all the soil properties except cellulase activity. Besides, redundancy analysis (RDA) showed that soil properties drove the changes of AM fungal colonization and sporulation. These results will provide theoretical support for realizing the relationship between AM fungal colonization and soil conditions, and also for the exploration of AM fungi species with great functions.

KEYWORDS

Succession of saline-alkali land; soil properties; AM fungi; colonization characteristics

1 Introduction

Soil salinization is one of the most serious problems of land degradation [1], UNESCO and FAO have reported that 954 million hectares of worldwide land was disturbed by salinization [2]. Salinization is not only considered as an environmental issue but has developed to an economic problem [3], it gradually becomes a great challenge restricting the development of agriculture and husbandry [4]. Songnen plain, which is situated in the Northeast of China, is one of the three soda salinized soil areas in the world and

the salinity types here are mainly $NaHCO_3$ and Na_2CO_3 [5]. Previous studies suggested that planting halophytes is one of the most effective ways to restore the salinized grassland, and it can significantly improve the soil properties and bring some economic benefits [6,7]. In addition, biomass accumulation of pastures will absorb the salt on soil surface [8]. Therefore, it is necessary to carry out research on promoting pasture growth.

The halobiotic communities of *Suaeda glauca* Bunge (Chenopodiaceae), *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. (Poaceae) and *Leymus chinensis* (Trin.) Tzvel. (Poaceae) are the three stages of succession occurring in Songnen saline-alkali grassland, they appear in turns as the salinity decreases [9], and the three communities arrange in concentric circles that do not cross each other. Among them, the transition from *S. suaeda* to *P. tenuiflora* was affected by precipitation, and the early rainy season (before July) could promote the succession. After *P. tenuiflora* grew for a while, *L. chinensis* invaded and gradually became the dominant grass species [10] so that cause the recovery of degraded grassland. Existing research proved that all of the three halophytes could be colonized by arbuscular mycorrhizal (AM) fungi to improve salt tolerance, and the AM fungal communities in the rhizosphere differed among the three plants [11]. It is well known that AM fungal communities are affected by various factors, and the relevant studies have confirmed that plant community coverage, aboveground biomass, plant richness, plant diversity, soil pH, total nitrogen content (Total N), ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), organic carbon (OC), phosphorus (P), C/N, N/P, soil moisture, soil temperature and warming all affected the survival and distribution of AM fungi [12–16]. The distribution of AM fungi differed among various environments [17–19]. And previous studies conducted by our research team showed that AM fungal colonization and community composition varied in habitats with different salinity levels, and the spore density, etc. were highly correlated with environmental factors [11,20]. AM fungi will play a series of ecological functions after the colonization, such as exchanging nutrients, regulating soil properties, and improving the relationships between plants and other microorganisms [21]. However, AM fungal ecological function was closely related to its source and habits, and local adaptability existed in the symbiosis of AM-plants [22]. Besides, AM fungi, which with the same ecological source as hosts, worked even better than some commercial fungi [23]. Therefore, exploring fungi with extensive symbiotic functions in saline-alkali habitats is crucial for the application of halophytes-AM in salty ecological remediation.

The dominant AM fungi in halophytic environment may have evolved the ability to improve the growth and saline-alkali resistance of hosts. Previous studies showed that *Claroideoglomus etunicatum* and *Rhizophagus intraradices* were common in Songnen saline-alkali grassland [24–26]. To explore other species with great functions, the rDNA should be extracted from the dominant fungus in three succession stages to accurately annotate the information [27]. However, many spores occurring in the rhizosphere soil do not necessarily colonize the roots of hosts to perform ecological functions [28]. It was important to determine the colonization of AM fungi in the roots. Therefore, the maize, which worked well before [29], should be selected as the host for one-species cultures. The mycorrhizal structures of different fungi formed in the roots varied [30–33], so it could be preliminary inferred whether the dominant fungus successfully colonized in the three succession stages after comparing the colonized characteristics between three halophytes and maize. The results will lay a foundation for exploring the AM fungal species with excellent function, and provide support for the functional study of the fungus in the future.

In addition, AM fungal colonization and the formation of symbiosis are prerequisite for AM fungi to play functions, and AM fungal colonization will also be influenced by various abiotic factors [11,34,35]. However, there was a lack of research on the response of AM fungal colonization in saline-alkali habitats. To comprehensively clarify the correlation between AM fungal colonization and soil properties among succession stages of saline-alkali land and to illustrate the response relationship, the basic soil properties and AM fungal colonization characteristics were quantitatively determined in this experiment.

2 Materials and Methods

2.1 Sampling Sites and Sample Collection

The samples were collected in Zhaodong (125°52′54″–125°54′09″ E, 46°2′50.3″–46°2′58.1″ N), which is located in the middle of the Songnen plain. The climate pattern of the sampling sites is characterized by high temperature-rainy summers and dry-cold winters, and the annual average temperature is 3.6–4.4°C [36]. Five large quadrats (50 m × 50 m) separated by roads were set. Three small sites (15 m × 15 m), which possessed the three communities, were selected randomly within a quadrat. Ten clumps of *S. glauca*, *P. tenuiflora*, and *L. chinensis* with various statuses were selected respectively within each small site by "multi-point parallel sampling method" and "five-point sampling method" on May 24, 2021 [37]. After which the samples including rhizosphere soils (about 0.5 kg) and roots from depth 0–30 cm were collected at every small site. Three specimens of every succession stage harvested in three small sites within a large quadrat were mixed into a sample so that five samples were collected from each of the three succession stages and a total of 15 samples were obtained. After sifting through 0.85 mm mesh, the rhizosphere soils were labeled and stored at 4°C. The roots were cut into 1 cm-long segments and were soaked in FAA fixed solution (5 mL formalin, 5 mL glacial acetic acid, and 90 mL of 70% ethyl alcohol) till further use.

2.2 Determination of Soil Properties and Enzymatic Activities

Soil properties were determined in accordance with Bao [38] and the soil enzymatic activities were determined as the description in Guan [39]. The specific test methods were as follows: Soil pH of soil-water (1:5) saturated extract was measured using a PHS-3C pH meter. Soil salinity was represented by the conductivity (EC) which was measured by the DDS-11A conductivity meter. The carbonate content of soil-water immersion liquid (1:5) was estimated by the phenolphthalein-neutralization titration, and the OC and organic matter (OM) were measured by the potassium chromate volumetric analysis method. Total phosphorus content (Total P) was determined using the molybdenum antimony colorimetric method. Total N was estimated by Kjeldahl's semi-micro method after the soil samples were digested in the H_2SO_4 and accelerator at 410°C. Sucrase and cellulase activities were determined by the 3, 5-dinitrosalicylic acid colorimetry method. The NaClO-sodium phenolate colorimetry was used to measure urease activities. Catalase activity was determined by the potassium permanganate volumetric analysis. All the above-mentioned indices were measured three times.

2.3 AM Fungal Colonization

The determination of AM fungal colonization was performed in accordance with Philips et al. [40]. The steps were as follows: the root segments soaked in FAA solution were cleaned with distilled water and then were incubated in the 10% KOH solution (100°C, 90 min) to soften and transparentize, subsequently, the segments were neutralized with 2% hydrochloric acid for 5–10 min after the 20 min of treatment with 10% hydrogen peroxide, finally, staining them with 0.05% trypan-blue reagent for 20 min at 90°C. The colonization characteristics of 90 root segments from each sample were observed under the microscope (Zeiss Axio Scope. A1) after making mount. The AM fungal colonization was assessed and graded using the method of Trouvelot et al. [41]. The colonization intensity was divided into five levels: (1) 0%–1% as Level I, (2) 1%–10% as Level II, (3) 11%–50% as Level III, (4) 51%–90% as Level IV, and (5) 91%–100% as Level V. Similarly, the arbuscular abundance was divided into three grades: A1 indicated few arbuscles, at <5%, A2 indicated frequent arbuscles, at 5%–50%, and A3 indicated abundant arbuscles, at >50%. The classification method of vesicle abundance was consistent with that of arbuscular abundance, and it was recorded with V1, V2, and V3. Then the colonization rate (%), arbuscular abundance (%), vesicle abundance (%), and colonization intensity (%) were calculated using MYCOCALC software. The calculation formulas were as follows:

$$\text{Colonization rate (\%)} = \frac{\text{The number of colonized root segments}}{\text{The total number of root segments}} \times 100\% \quad (1)$$

$$\text{Colonization intensity} = \frac{95 \times N_V + 70 \times N_{IV} + 30 \times N_{III} + 5 \times N_{II} + N_I}{\text{Total number of root segments} \times 100} \times 100\% \quad (2)$$

N was the number of segments at the certain colonization level.

$$\text{Arbuscular abundance (\%)} = \frac{m \times A3 + 0.5 \times m \times A2 + 0.1 \times m \times A1}{100} \times 100\% \quad (3)$$

$$\text{Vesicle abundance (\%)} = \frac{m \times V3 + 0.5 \times m \times V2 + 0.1 \times m \times V1}{100} \times 100\% \quad (4)$$

$$m = \frac{\text{Colonization intensity (\%)} \times \text{The total number of root segments}}{\text{The number of colonized root segments}} \quad (5)$$

2.4 Isolation and Molecular Identification of Dominant Fungus

Twenty grams of rhizosphere soil was weighed from each sample to separate spores using the wet screening and sucrose density-gradient centrifugation method [42]. The spores were enumerated under a stereoscope (Leica MDG33) after they were cleaned in an ultrasonic washer, and the spore density was defined as the spore number in 20 g soil. Subsequently, the AM fungal spores in three succession stages were observed to record the characteristics under a digital optical microscope (Zeiss Axio Scope. A1). Finally, the species identification was performed based on the characteristics obtained in the above-mentioned operation and referred to "Chinese AM fungal resources and germplasm resources" [29], INVAM International website (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html), and Janusz Blaszkowski of Poland Agricultural University (http://www.zor.zut.edu.pl/Glomeromycota/Species%20descriptions%20of%20AMF.html). The isolation frequency, relative abundance, and importance value of the species were calculated referring to the method proposed by Yang et al. [43]. The extensively distributed fungus (dominant species) was determined according to the three values.

$$\text{Isolation frequency (F)} = \frac{\text{Occurrence frequency of individual species}}{\text{Total sample number}} \times 100\% \quad (6)$$

$$\text{Relative abundance (RA)} = \frac{\text{Spore number of single species}}{\text{Total quantity of AM fungal spores}} \times 100\% \quad (7)$$

$$\text{Importance value (IV)} = \frac{(F + RA)}{2} \times 100\% \quad (8)$$

After that, the high-frequency spores were transferred into a sterilized petri dish filled with ddH_2O. As the amplification template, a single spore (1 μL) was placed into a sterilized PCR tube, and then the spore was thoroughly crushed under a stereoscope (Leica MDG33). Subsequently, the Nested-PCR was performed to amplify the AML1/AML2 target of the 18S rDNA and the fragment was approximately 800 bp in length. GeoA2 (5'-CCAGTAGTCATATGCTTGTCTC-3') and Geo11 (5'-ACCTTGTTACGACTTTTACTTCC-3′) were used in the first round of amplification, and AML1 (5′-ATCAACTTTCGATGGTAGGATAGA-3') and AML2 (5'-GAACCCAAACACTTTGGTTTCC-3') were used for the second [44]. The reaction system and process were as follows (Table 1):

Table 1: The reaction system and process

Reaction system			Reaction process		
	First PCR	Second PCR		First PCR	Second PCR
Premix Taq™	10 μL	10 μL	Inital denaturation 94°C	4 min	4 min
DNA	1 μL	2 μL	Denaturation 94°C	30 sec	30 sec
10mmol/l F Pri	1 μL	1 μL	Annealing 55.2°C	30 sec	30 sec
10mmol/l R Pri	1 μL	1 μL	Extension 72°C	1 min	1 min
ddH_2O	7 μL	6 μL	Final extension 72°C	7 min	7 min
Total	20 μL	20 μL	Save at 4°C		

Note: F-Forward, R-Reverse, Pri-Primer, the denaturation, annealing and extension cycled 30 times.

The amplified products were examined by 1.0% agarose gel electrophoresis, and the products with the correct length were sequenced in Comate Biotechnology Co., Ltd. (China). The accession number of the three sequences obtained in the study were ON150848, ON150849, and ON150850.

2.5 Colonization Verification of Dominant AM Fungus

The *Zea mays* Linn. 'Zhengdan 958' was seeded in the culture dish after it was disinfected with 0.1% $KMnO_4$. After 10 days, the seedlings were transplanted into the sterilized substrate (sand:vermiculite = 3:1, approximately 900 g). At the same time, the single AM fungal species, which existed extensively in three succession stages, was inoculated to the roots referring to the method proposed by Wang et al. [29], and 100 spores were eventually added to the substrate. Maintaining light 14 h, light intensity 5000lx, the temperature at 20–25°C, in addition, irrigating 100 mL Hoagland nutrient solution (phosphorus deficiency) every week. The colonization was observed 90 days after inoculation.

2.6 Statistical Analysis

MYCOCALC was used to calculate the AM fungal colonization rate, colonization intensity, arbuscular abundance, and vesicle abundance. SPSS 25 was used for the one-way ANOVA test of soil properties, enzymatic activities, and AM fungal colonization, and the significance of differences was marked according to the Waller Duncan's test results. A phylogenetic tree (Maximum-likelihood) was constructed using MEGA 6.0. The "Psych" package in R 4.1.1 was used to construct the correlation matrix between soil properties and colonization indices. The "Vegan" package was used to test the axis lengths and then the redundancy analysis (RDA) was constructed to illustrate the influence of soil properties on AM fungal colonization. Photoshop CC 2019 was used for image processing and editing.

3 Results

3.1 AM Fungal Colonization and Soil Properties of Three Succession Stages

One-way ANOVA test for different succession stages (Table 2) showed that the soil properties improved with the succession of saline-alkali grassland. Soil pH, EC, and carbonate content of rhizosphere soil, which were indicators of soil salinization level, decreased significantly with the succession ($P < 0.05$). There was no significant difference in some soil indices between *P. tenuiflora* and *S. glauca* ($P > 0.05$), while *L. chinensis* showed a significantly excellent level ($P < 0.05$), such as C/N. In addition, as the intermediate stage of succession, *P. tenuiflora* presented significantly high activity in cellulase activity ($P < 0.05$). Total P, sucrase activity, and catalase activity showed the highest value in *L. chinensis* stage and followed by *P. tenuiflora* and *S. glauca*, which was contrary to the succession sequence of Songnen saline-alkali grassland. The content of OC, OM, and Total N showed a similar tendency and they decreased gradually in turns of *L. chinensis*, *S. glauca*, and *P. tenuiflora*.

Table 2: The colonization indices and soil properties

	L. chinensis	*P. tenuiflora*	*S. glauca*
Colonization rate (%)	86.87 ± 10.56a	66.11 ± 9.51b	45.00 ± 8.24c
Colonization intensity (%)	38.24 ± 14.10a	7.28 ± 4.48b	2.68 ± 1.94b
Arbuscular abundance (%)	4.25 ± 4.61a	0.43 ± 0.33b	0.27 ± 1.94b
Vesicle abundance (%)	21.09 ± 12.63a	3.70 ± 2.60b	1.30 ± 1.74b
Spore density	1792 ± 159a	854 ± 504b	859 ± 510b
pH	9.292 ± 0.255c	10.136 ± 0.096b	10.2967 ± 0.058a
EC	0.641 ± 0.302c	1.796 ± 0.131b	2.763 ± 0.828a
Carbonate	1.463 ± 0.918c	5.885 ± 0.421b	8.039 ± 1.606a
OC	35.711 ± 6.962a	12.236 ± 3.897c	19.352 ± 2.958b
OM	61.565 ± 12.003a	21.095 ± 6.719c	33.362 ± 5.099b
Total N	1.44 ± 0.41a	0.28 ± 0.08c	0.52 ± 0.09b
Total P	0.337 ± 0.048a	0.255 ± 0.014b	0.219 ± 0.028c
C/N	25.681 ± 5.459b	43.518 ± 6.474a	38.047 ± 5.082a
Sucrase activity	3.981 ± 0.071a	2.371 ± 0.28b	1.875 ± 0.172c
Cellulase activity	4.146 ± 0.32b	6.423 ± 0.739a	4.24 ± 0.264b
Urease activity	5.523 ± 0.321a	5.015 ± 0.691a	3.231 ± 0.584b
Catalase activity	2.143 ± 0.006a	2.08 ± 0.062b	1.949 ± 0.046c

Note: Different letters in same row indicated significant differences among succession stages, and we marked the largest value with a, followed by b, c. Standard deviation (SD) was used for data statistics of 15 samples.

AM fungi successfully colonized the *S. glauca* (Figs. 1a–1c), *P. tenuiflora* (Figs. 1d–1f), and *L. chinensis* (Figs. 1g–1i) growing in Songnen saline-alkali grassland. AM fungi colonized the roots from the outside of the host and some of the hyphae expanded to be vesicles, in addition, some hyphae branched densely to shape arbuscules. The variation trend of AM fungal colonization was similar to that of soil properties (Table 2). The colonization rate significantly differed among the three succession stages ($P < 0.05$), and the values increased gradually with the succession processing. The colonization rate of *L. chinensis* ranged from 66.67% to 100% in five sampling sites, and that of *P. tenuiflora* ranged from 50% to 80%, while the *S. glauca* was only 35%–60%. There was no significant difference in colonization intensity, arbuscular abundance, vesicle abundance, and spore density in the adjacent succession stages (*P. tenuiflora* and *S. glauca*) ($P > 0.05$), while *L. chinensis* showed a significantly large value ($P < 0.05$).

3.2 Dominant AM Fungus in Three Succession Stages

A total of fifty-eight species which belonged to 17 genera were identified in three stages. There was an AM fungal species distributed in all the 15 soil samples collected from three succession stages, and its image was shown in Fig. 2. The species was identified as the dominant species in three succession stages with the isolation frequency, relative abundance, and importance value of 100%, 18.1%, and 59.1%, respectively, while the isolation frequency, relative abundance, and importance value of others only were 6.67%–86.67%, 0.1%–9.13%, and 3.38%–46.41%, respectively. The morphological characteristics of this species were as follows: the sporal color was orange-brown to red-brown, the shape was globose or subglobose and the diameter was 80 μm approximately, the appearance presented as protuberant blisters, hypha was occasionally seen, and no obvious color variation was recorded after the spore was stained by Melzer's reagent.

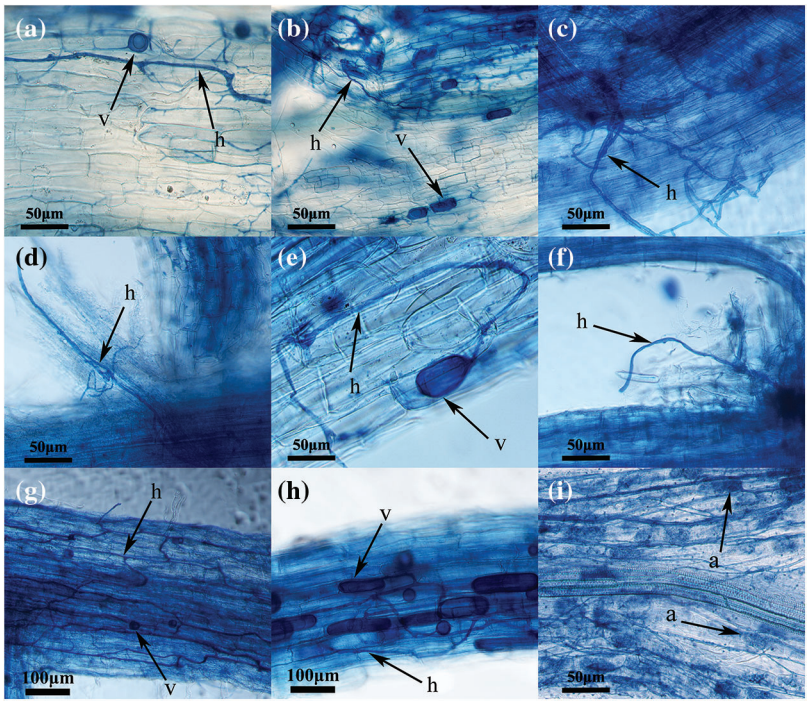

Figure 1: AM fungal colonization in *S. glauca*, *P. tenuiflora* and *L. chinensis*. a–c: The roots of *S. glauca*, d–f: *P. tenuiflora*, g–i: *L. chinensis*. h-hypha, v-vesicule, a-arbuscule

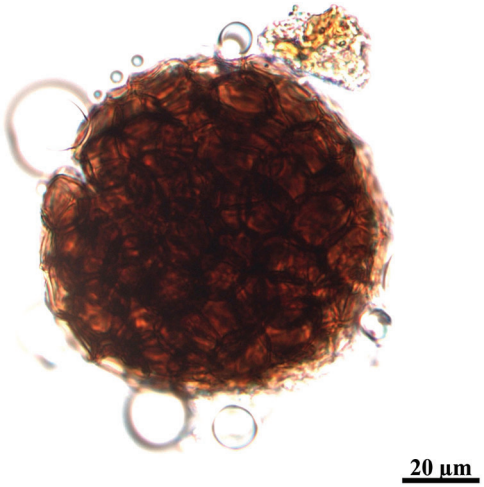

Figure 2: The morphological characteristics of dominant species

A total of three valid sequences (No.001, No.002, No.003) were extracted from the dominant fungus which existed extensively in three succession stages. The AML1/AML2 targets of the three sequences were 772 bp, 745 bp, and 747 bp in length, respectively. The 10 sequences with high homology of more than 98% to the three sequences were selected from the NCBI database. Subsequently, the alignment and construction of the phylogenetic tree were performed with MEGA. The Maximum-likelihood Phylogenetic tree was shown in Fig. 3. The results showed that the three sequences clustered with the MK592776.1 Glomeraceae sp., indicating that the dominant fungus was an uncultured species in

Glomeraceae and no artificial cultures have been obtained. Therefore, we temporarily named it Glomeraceae sp1 in this study.

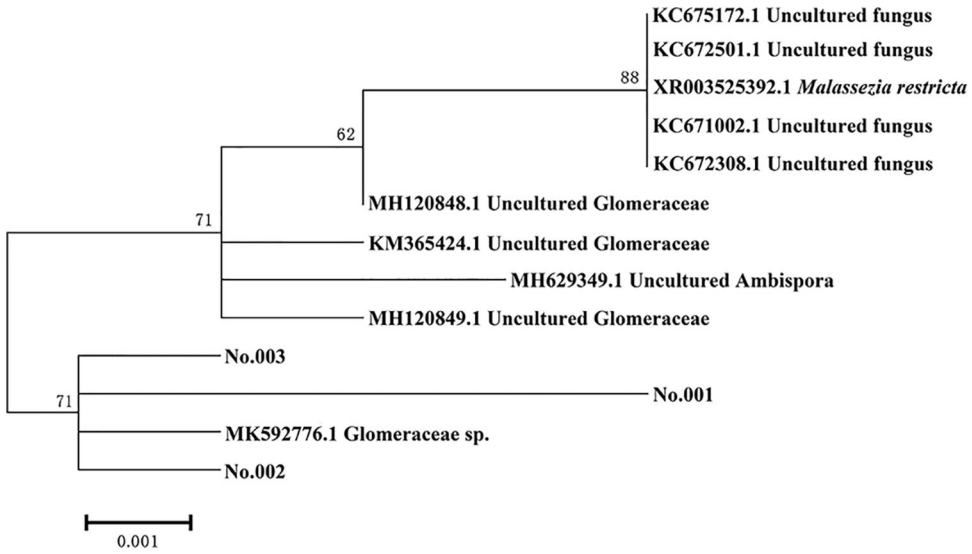

Figure 3: Phylogenetic tree of dominant AM fungi species

3.3 Colonization Verification of Glomeraceae sp1

The results showed that Glomeraceae sp1 could successfully colonize the roots of maize with the colonization rate ranged from 80% to 87%, and the spore number in substrate increased (2–3/g). The colonization characteristics of Glomeraceae sp1 in maize were shown in Figs. 4d–4f. In some root segments, dense mycelia were observed, while there was no arbuscule was detected. In most cases, abundant vesicles with only a few mycelia were observed in the root segments. It was worthwhile to note that the similar colonization characteristics, which numerous vesicles with almost no hypha, were detected in the roots of *P. tenuiflora* (Fig. 4b), *S. glauca* (Fig. 4a), and *L. chinensis* (Fig. 4c) after comparing the images. Based on the fact that Glomeraceae sp1 widely distributed in saline-alkali habitats, and the mycorrhizal structure of the single fungus was found in the roots of field samples, it could be preliminary speculated that Glomeraceae sp1 colonized the *P. tenuiflora*, *S. glauca*, and *L. chinensis* growing in Songnen saline-alkali grassland.

3.4 Effects of Soil Properties on AM Fungal Colonization

The correlation matrix (Fig. 5a) was constructed to realize the relationship between AM fungal colonization and soil properties. The results showed that pH, EC, carbonate content, and C/N were significantly negatively correlated with multiple colonization indices. Among which the effect of pH was the most obvious, the negative correlation between it and AM fungal colonization was extremely significant at the level of 0.01, and the pH related to the colonization intensity and vesicle abundance with the $R = -0.96$ and -0.93, respectively. Among the soil properties which positively correlated with AM fungal colonization, total P correlated with the colonization indices the best, and the $R = 0.65$–0.93 with the $P < 0.01$. No significant correlation was observed between cellulase activity and colonization indices ($P > 0.05$), although the other enzyme activities showed a significant positive correlation with AM fungal colonization ($P < 0.05$). Besides, the analysis also indicated that colonization rate, colonization intensity, and vesicle abundance were closely related to environmental factors the most, and they related significantly to all the soil properties except cellulase activity.

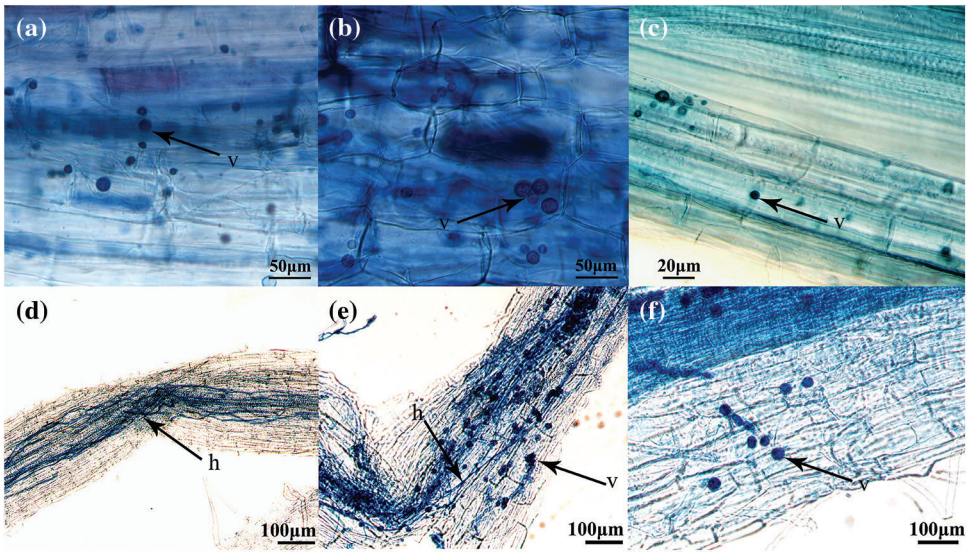

Figure 4: The colonization of Glomeraceae sp1. a: The roots of *S. glauca*, b: *P. tenuiflora*, c: *L. chinensis*, d–f: Maize. h-hypha, v-vesicule, a-arbuscule

The driving effects of soil properties on AM fungal colonization were verified by RDA (the axis length 0.53 < 4), and the cumulative percentage of the explanatory variance of the first two axis reached 90.57%, $P = 0.024$ (Fig. 5b). Consistent with the results of correlation matrix analysis, all the soil properties except cellulase activity drove the variation of AM fungal colonization ($P < 0.05$, $r^2 > 0.41$). Among these, soil pH and Total P performed the most significant driving effects, and the r^2 were 0.90 and 0.88 at the significance level of 0.001, respectively. Among the soil enzymes, sucrase activity showed the strongest driving effect on AM fungal colonization ($P = 0.001$, $r^2 = 0.75$). Besides, OC, OM, carbonate, and Total N showed similar driving strength with the $r^2 = 0.77$–0.78 at the level of 0.001.

4 Discussion

The land in Songnen saline-alkali grassland has further degraded in recent years, and the pH and salt content increased greatly [42,45]. The degraded saline-alkali ecosystem was in urgent need of restoration. The soil properties and enzyme activities gradually improved with the succession, indicating that the selection of the three communities was appropriate. The results showed that AM fungal colonization was influenced by soil properties and that was proved in previous investigations [11,46]. The pH was negatively related to the colonization at the level of 0.01, which was consistent with the research results of Gai et al. [47]. This was likely because the soil pH affected the relative abundance and sporulation of AM fungi, and neutral to slightly acidic salinity was more conducive to AM fungal colonization, while high pH inhibited the formation of AM fungal mycelia [48]. The previous investigation reported that the colonization rate, colonization intensity, and arbuscular abundance reached the maximum when the total salt content was in the range of 0.21%–0.25% [11]. However, the total salt content in our sampling sites was well beyond the value, presenting a negative correlation, which was consistent with the research results of Ren et al. [49]. Carbonate content, which was another indicator of soil salinization level, was similar to the effects of pH and EC. OC, OM, Total N, and Total P were positively correlated with AM fungal colonization, which was consistent with the reported results [50]. Joner et al. declared that the high content of soil organic matter played a positive effect on the formation and development of mycelia of *Glomus diuphauam* and could promote the occurrence of arbuscular [51]. In addition, the conclusion, that which relative abundance of AM fungal spores was positively correlated with organic matter content in

saline-alkaline soil of Egypt, was presented by Agwa et al. [52]. However, some studies showed that the colonization rate and arbuscular abundance decreased with the increase of organic matter content [53] and the augmented phosphorus content inhibited the elongation and thickening of mycelia [54]. These might be due to the excessive accumulation of nutrients and elements in the soil. Sucrase activity was index related to soil nutrition and urease activity was associated with nitrogen supply [39] and thus these showed similar results with OC, OM, and element content. Catalase activity was positively correlated with AM fungal colonization, it was speculated that the harmful substances were removed timely in sites with high activity so that avoiding the impact on AM fungal colonization [39]. The above reasons could also explain why soil properties and enzyme activities drove the variation of AM fungal colonization and spore production.

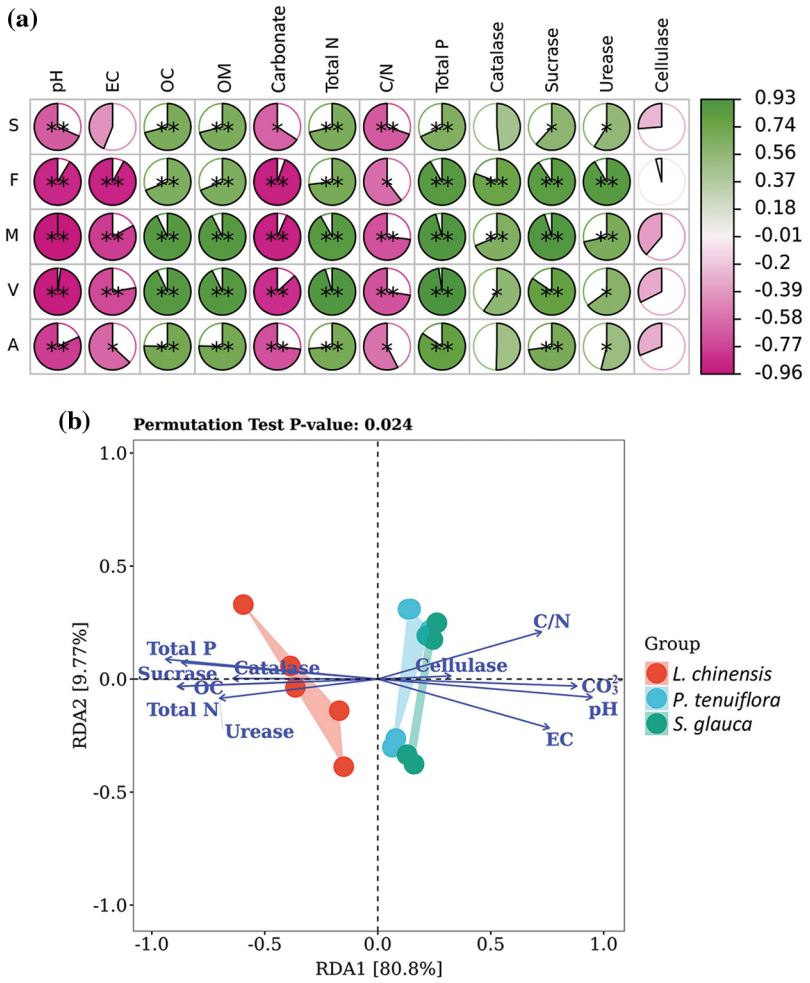

Figure 5: The correlation matrix and RDA between AM fungal colonization and soil properties
Note: a: The correlation matrix. S-spore density, F-colonization rate, M-colonization intensity, V-vesicle abundance, A-arbuscular abundance, ** indicated that the correlation was significant at 0.01 level; * indicated that the correlation was significant at 0.05 level. b: The RDA between AM fungal colonization and soil properties.

Glomeraceae sp1 distributed in all the 15 soil samples collected from the three succession stages. Previous investigations on AM fungal resources in saline-alkali land reported that the fungus presented in the rhizosphere of *Inula japonica* and *P. tenuiflora* [24,42], and they named it *Glomus convolutum* based

on the morphology, the above results proved that Glomeraceae sp1 widely distributed in saline-alkali land. However, sequencing results of AML1/AML2 showed that the fungus was an uncultured species in Glomeraceae, indicating that it was not objective to identify AM fungal species only relying on morphological information [55]. Furthermore, Glomeraceae sp1 has not been reported in habitats except saline-alkali land, which indicated it was highly possible to play excellent functions in halophytic environment. Differing from many AM fungi species that cannot colonize in one-species cultures, such as *Scutellospora calospora*, *Glomus corymbiforme*, and *Acaulospora thomii*, etc. (http://www.zor.zut.edu.pl/Glomeromycota/Species%20descriptions%20of%20AMF.html), Glomeraceae sp1 colonized well in this research with the colonization rate ranged from 80% to 87%, and the spore number increased. Besides, based on the colonization characteristics, it was preliminary inferred that Glomeraceae sp1 successfully colonized the halophytes in the wild environment. The above information indicated that it not only had strong salt-alkali adaptation but also was likely to play an important role in the salt-alkali tolerance of hosts. Further studies should be performed to explore its functional characteristics.

5 Conclusion

The soil properties improved with the succession of saline-alkali land. The plants' communities of the three stages could be colonized by AM fungi, in addition, AM fungal colonization indices were closely related to soil conditions and the changes were driven by soil properties. Glomeraceae sp1 was identified as the dominant species, besides, the maize was successfully colonized by it in one-species cultures and similar mycorrhizal characteristics were detected in the roots of the three halophytes, indicating that Glomeraceae sp1 colonized the samples in the field and was likely to play an important role in salt-alkali tolerance of hosts.

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