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ARBUSCULAR MYCORRHIZAL FUNGI AND THEIR INFLUENCING FACTORS FOR *AEGICERAS CORNICULATUM* AND *ACANTHUS ILICIFOLIUS* IN SOUTHERN CHINA

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Abstract

Our study aimed to explore Arbuscular mycorrhizal fungi (AMF) colonization and spore density for *Aegiceras corniculatum* and *Acanthus ilicifolius* across five mangrove ecosystems in southern China, focusing mainly on the relationships between AMF and biotic/abiotic factors. Soil physicochemical properties and seawater salinity, as well as the numbers of culturable soil microbes (bacteria, fungi and actinmycetes) were measured to analyze their potential effects on AMF colonization. The results showed that AMF were very common for both plant species in the investigated mangrove ecosystems, and hyphae were the dominant structures for both species. Total AMF colonization rates (TC%) ranged from 0.33% to 36.50%, while the average TC% for *A. ilicifolius* (13.47%) was slightly higher than for *A. corniculatum* (9.47%). The average spore density for *A. corniculatum* was 49.0 spores per 25g air dried soil, and 51.7 for *A. ilicifolius*. Soil physicochemical analysis showed that soil in mangroves was with high moisture and organic matter content, slightly acidic pH, low levels of total and available P and high levels of N content. Microbial counting experiment recorded high microorganism numbers in mangroves. Data analysis revealed that soil available P content and seawater salinity may be important factors influencing AMF in mangroves. The two mangrove species showed different correlations with microbial numbers, which may illustrate that host plant is a key factor influencing AMF and other microbes.

Key words: Mangrove, Arbuscular mycorrhizal fungi, Colonization, Spore density, İnfluencing factors.

Introduction

Arbuscular mycorrhizal fungi (AMF) are well known for forming symbiotic relationships with most terrestrial plants, and their distribution dynamics and functional roles have been well documented (Li *et al*., 2008; Grace *et al.*, 2009). Since living organisms in wetland habitats are exposed to soil with low oxygen levels, extremely high or low mineral concentrations and constant flooding, many wetland plants were described as non-mycorrhizal in early studies (Anderson *et al.*, 1984). Also, it has been assumed that AMF have little significance in wetland ecosystems, especially in mangroves, because of the high concentrations of saline and heavy metal contamination (Juniper *et al.*, 2006; Wang *et al.*, 2013).

Recent studies show that AMF do exist in wetlands, forming symbiotic relationships with many hydrophytic plants (Turner *et al.*, 2000; Bohrer *et al.*, 2004). The dynamics and role of AM fungi in wetland ecosystems were also described (Wang *et al*., 2015). Yang *et al.* (2010) identified 48 taxa of AMF, which belong to 7 genera from 13 plant species in the Lhalu wetland in Tibet. Soil salinity, available P and N content in roots were related to AMF symbiosis or spore density (Kumar & Ghose, 2008; Likar *et al.*, 2009). Ipsilantis & Sylvia (2007) reported that AMF symbionts impact on wetland plants through complex interactions, which included AM fungi, plant species, water condition and P level.

In mangroves, AMF symbionts are also common. Many mangrove species, such as *Acanthus ilicifolius, Acrostichum aureum, Aegiceras corniculatum, Sesuvium portulacastrum, Borassus flabellifer*, form symbionts with AM fungi (Sengupta & Chaudhuri, 2002; Wang *et al.*, 2003). Arbuscular mycorrhizal fungi were also found in semi-mangrove plant communities, such as *Heritiera littoralis* Dryand., *Pongamia pinnata* L., *Cerbera manghas* L. and *Hibiscus tiliaceus* L. (Wang *et al*., 2014). D'Souza & Rodrigues (2013a) reported 28 AMF species of 5 genera in mangroves of West India and found that AMF colonization and spore density varied by both plant species and sites, and were co-affected by season and host(D'Souza & Rodrigues, 2013b). Our previous studies showed that there are plenty of AMF species in mangrove ecosystems in southern China, with abiotic factors such as hydrological conditions and flooding greatly affecting AMF diversity in mangroves (Wang *et al.*, 2010, 2011). These studies have indicated that AMF are an important component in mangrove ecosystems, yet no research has explored the AMF colonization, spore density, and the factors influencing different mangrove plant species in southern China.

Mangroves only exist on tropical and subtropical coastlines. Special habitats and distribution pattern make mangroves unique to other wetland ecosystems, but also restrict research on AMF in mangroves. Little has been known about the distribution of AMF and culturable microorganisms in different mangrove ecosystems until now. The objectives of present study was i) understand the AMF status for both mangrove species; ii) study the differences for AM colonization and spore density across mangrove ecosystems; iii) identify the factors influencing the distribution of AMF in mangrove ecosystems.

Materials and methods

Study sites and sample collection: Five sampling areas (Fig. 1) were selected for this study: Zhangjiang Estuary National Nature Reserve (ZJ) in Fujian Province (23°54′N, 177°26′E), Su'ai Bay Mangrove Nature Reserve (SA) in Guangdong Province (23°18′N, 116°43′E), Qi'ao Mangrove Nature Reserve (QA) in Guangdong Province (22°26′N, 113°38′E), Gaoqiao Mangrove nature reserve (GQ) in Guangdong Province (21°34′N, 109°45′E) and Dongzhai Harbor Mangrove Nature Reserve (DZ) in Hainan Province (19°57′N, 110°33′E).

Two mangrove species, viz., *Aegiceras corniculatum* (L.) Blanco and *Acanthus ilicifolius* L., common to five selected study sites, were chosen and studied. Intertidal levels, along with hydrological and pH information at each particular sampling site are shown in Table 1. At each point, root and soil samples (10–20 cm beneath the soil surface) were collected from three individuals of each plant species, from November to December in 2011.

For root samples, only the young nutritive roots attached to the plant were collected. Soil that remained attached to the root after gentle shaking was also collected. Each soil sample was divided into three parts: one part was air dried, and used for physical and chemical properties analysis, one part was stored in a refrigerator at -80 for determining soil microbial number, and the other part was used for AMF spore counting. Seawater salinity at each sample point was also recorded using a salinometer.

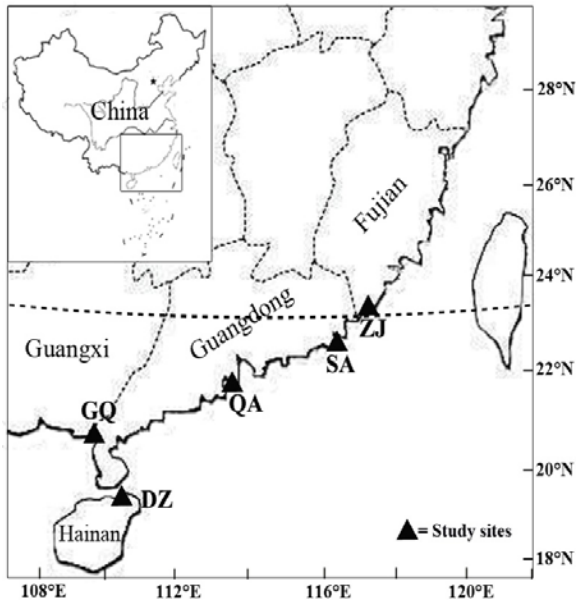

Fig. 1. Map showing locations of study sites. The dashed line across the map indicates the Tropic of Cancer. ZJ: Zhangjiang Estuary National Nature Reserve; SA: Su'ai Bay Mangrove Nature Reserve; QA: Qi'ao Mangrove nature reserve; GQ: Gaoqiao Mangrove nature reserve; DZ: Dongzhai Harbor Mangrove Nature Reserve.

Soil analyses: Soil moisture content was determined immediately after the samples arrived in our laboratory in Guangzhou. Organic matter content, pH, electrical conductivity (EC), total N and P and available N and P were analyzed using the methods described by Page *et al.* (1982). Soil pH was measured using a Rex PHS-3C (Shanghai) in a soil:water (1:2.5) suspension, and EC was measured in a 1:5 soil:water paste using a DDS-11A Conductivity meter (Chengdu).

Assessment of culturable microbial numbers: Dilution plating was used to determine soil microbial numbers. As established by our pre-experiment results, soil dilution ratios were set at 10^{-2} – 10^{-5} for bacteria and 10^{-1} – 10^{-4} for fungi and actinomycetes. Nutrient agar medium was used for determining the numbers of bacteria, modified Martin medium was used for determining the numbers of fungi and modified Gause's synthetic ager was used for determining the numbers of actinomycetes (all three types of medium were purchased from Guangzhou Huankai Microbial Sci. & Tech. Co., Ltd). Before using, each liter of modified Martin medium had 3.3mL Bangladesh Red solution ($10gL^{-1}$) and 3mL streptomycin solution ($10gL^{-1}$) added to it. 1mL $30gL^{-1}$ Potassium dichromate solution was added to every 300mL of modified Gause's synthetic ager. Due to the consistently high salinity in mangrove habitats, 10g NaCl was added to every liter of medium.

Assessment of AMF colonization and spore density: Rinsed fine root samples were cleared with 10% KOH at 90°C for 40 – 60 min, then washed with 2% HCl and stained with 0.05% trypan blue (Phillips & Hayman, 1970). The AMF colonization rate was calculated by scoring 200 spots on 40 root segments under a microscope (Carl Zeiss Germany). The total colonization rate (TC%) was calculated by summing the number of occurrences of vesicles, arbuscules or hyphae; when the structures occurred together, they were counted as a single colony. The colonization rate of hyphae (HC%), colonization rate of vesicles (VC%) and colonization rate of arbuscules (AC%) were all counted, using the method described by McGonigle *et al*. (1990). Arbuscular mycorrhizal fungi spores were extracted from air dried soil samples (25g) using the wet sieving and decanting method (An *et al*., 1990), and then counted on a grid-patterned dish under a binocular stereo microscope.

Data analysis: The Pearson correlation coefficient was used to determine the relationships between arbuscular mycorrhizal colonization, spore density and soil parameters. A parametric One-Way Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) at the 5% confidence level, were used to determine differences in colonization rates among different mangrove species at each site. All statistical analyses were performed using SPSS Base 17.0 (SPSS Inc., USA).

Table 1. Intertidal level, soil moisture content and soil pH at each sampling site.

Species	Study sites	Intertidal level	Moisture content	Soil pH
A. c	Zhangjiang	low	0.54 ± 0.01	5.88 ± 0.07
	Su'ai Bay	middle	0.49 ± 0.06	5.57 ± 0.19
	Qi'ao	low	0.51 ± 0.03	6.44 ± 0.20
	Gaoqiao	middle	0.43 ± 0.13	5.78 ± 0.12
	Dongzhai	low	0.55 ± 0.12	5.75 ± 0.19
A. i	Zhangjiang	low	0.57 ± 0.01	6.26 ± 0.37
	Su'ai Bay	low	0.61 ± 0.03	6.28 ± 0.36
	Qi'ao	low	0.48 ± 0.03	6.21 ± 0.09
	Gaoqiao	high	0.26 ± 0.01	5.93 ± 0.58
	Dongzhai	low	0.60 ± 0.10	5.27 ± 0.38

Note: Intertidal level was estimated using flooding time and moisture content in the rhizoshperic soil recorded when taking samples. Low: flooding time more than 7h a day according to our observation; middle: flooding time from 2h to 4h; high: daily flooding time less than 1h. Values are represented shown as mean ± SE. A.c: *Aegiceras corniculatum* and A.i: *Acanthus ilicifolius.*

Table 2. Seawater salinity, EC, OM, soil total P, available P, total N and available N content at each sample site.

Species	Study sites	Salinity (‰)	EC (mS/cm)	OM (%)	Total P (g/Kg)	Available P (mg/Kg)	Total N (g/Kg)	Available N (mg/Kg)
A. c	Zhangjiang	14.8 ± 0.7	4.38 ± 0.25	5.9 ± 0.0	0.62 ± 0.01	9.3 ± 0.3	2.24 ± 0.13	180.4 ± 32.1
	Su'ai Bay	13.8 ± 0.1	6.99 ± 0.63	5.3 ± 0.6	0.40 ± 0.01	1.7 ± 0.2	2.14 ± 0.48	218.6 ± 65.4
	Qi'ao	8.7 ± 0.4	2.65 ± 0.28	5.6 ± 0.5	1.03 ± 0.02	4.8 ± 0.6	2.57 ± 0.42	212.6 ± 47.2
	Gaoqiao	28.0 ± 1.6	4.27 ± 0.85	4.8 ± 1.7	0.58 ± 0.16	6.7 ± 1.0	2.51 ± 0.96	198.1 ± 73.2
	Dongzhai	25.0 ± 5.1	5.51 ± 1.52	4.7 ± 1.2	2.81 ± 0.98	26.2 ± 8.0	3.15 ± 1.17	376.4 ± 55.3
A. i	Zhangjiang	14.8 ± 0.7	4.71 ± 0.17	5.9 ± 0.2	0.64 ± 0.01	9.6 ± 0.6	2.18 ± 0.09	193.2 ± 22.8
	Su'ai Bay	13.8 ± 0.1	6.76 ± 0.57	5.6 ± 0.4	0.43 ± 0.05	2.2 ± 0.2	2.00 ± 0.12	301.5 ± 11.2
	Qi'ao	7.3 ± 0.5	2.76 ± 0.32	4.9 ± 0.5	1.00 ± 0.03	2.5 ± 0.7	1.93 ± 0.15	177.8 ± 5.6
	Gaoqiao	27.1 ± 0.0	2.44 ± 0.07	2.0 ± 0.2	0.41 ± 0.06	10.8 ± 0.8	0.79 ± 0.09	72.8 ± 38.9
	Dongzhai	21.0 ± 5.8	5.57 ± 0.29	5.9 ± 0.9	1.45 ± 0.60	18.3 ± 4.6	3.57 ± 0.81	363.5 ± 59.2

Note: EC: electrical conductivity; OM: organic matter content; values are shown as mean ± SE. A.c: *Aegiceras corniculatum* and A.i: *Acanthus ilicifolius*

Results

Mycorrhizal colonization and spore density: Both mangrove species showed associations with AMF at all the investigated sites (Fig. 2). TC% ranged from 0.33 ± 0.33% (*A. corniculatum*, DZ) to 36.50 ± 16.63% (*A. ilicifolius*, SA). The average TC% for *A. ilicifolius* (13.47%) was slightly higher than for *A. corniculatum* (9.47%). Hyphae were the dominant structures, while the highest and lowest observed HC% measurements were the same as TC%. Vesicles were also found in most of the sites for both species, while arbuscules were rare or absent, except for *A. ilicifolius* at SA with an AC% of 14.83 ± 7.17%. The average spore density was 50.4 spores per 25g soil (51.7 for *A. ilicifolius*, 49.0 for *A. corniculatum*), with the lowest spore density 13.5 ± 3.1 spores per 25g soil (*A. ilicifolius*, DZ), regarded as a low AMF spore density (Fig. 3).

Soil properties and seawater salinity: Average soil moisture content ranged from 0.26 to 0.61, while pH ranged from 5.27 to 6.44 (Table 1). EC ranged from 2.44 to 6.99 mS/cm (Table 2). Organic matter content was greater than 4.5%, except for *A. ilicifolius* at GQ (2.0%). Available P content was below 30 mg/Kg while all available N content measured was greater than 120 mg/Kg except for *A. ilicifolius* at GQ (72.8 mg/Kg). Overall, soil from all sample points showed some concordant trends, with high moisture and organic matter content, slightly acidic pH, low levels of total and available P and high levels of N content.

Numbers of microbes in soil: The number of culturable microorganisms in our five sample sites revealed that bacteria formed the most abundant microorganism group (Fig. 4), ranging from 7.35×10^5 $CFUg^{-1}$ (*A. corniculatum*, SA) to 8.05×10^6 $CFUg^{-1}$ (*A. ilicifolius*, QA). The number of fungi ranged from 4.3×10^3 $CFUg^{-1}$ (*A. corniculatum*, SA) to 1.62×10^5 $CFUg^{-1}$ (*A. corniculatum*, QA), meaning that fungi were the least abundant microorganism group detected. For actinomycetes, the maximum colony count was for *A. ilicifolius,* SA (9.14×10^5 $CFUg^{-1}$), while the minimum colony count was 4.1×10^4 $CFUg^{-1}$ (*A. corniculatum*, ZJ).

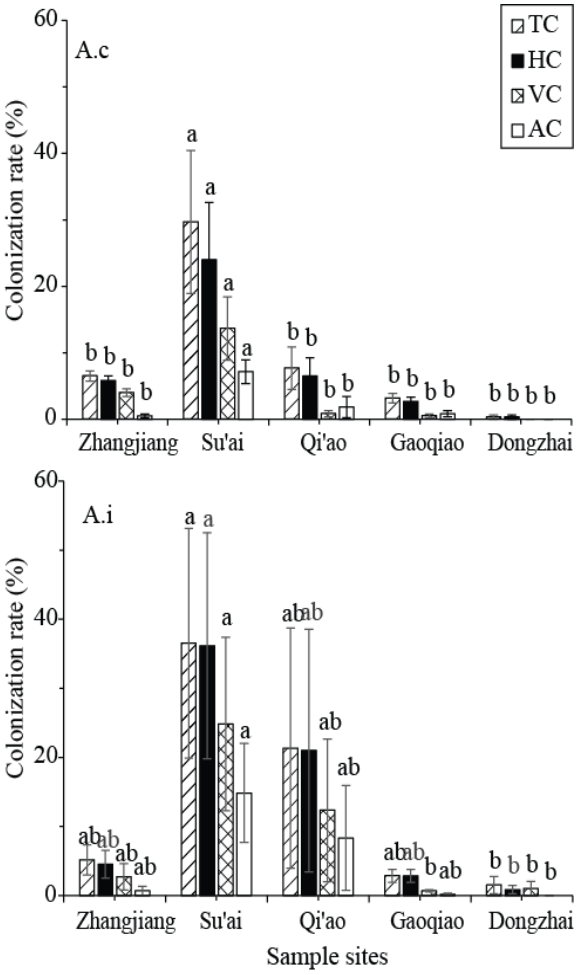

Fig. 2. Arbuscular mycorrhizal fungi colonization rate at different sites. Letters above columns indicate significant difference across different sites at 5% confidence level; mean ± SE. A.c: *Aegiceras corniculatum* and A.i: *Acanthus ilicifolius.*

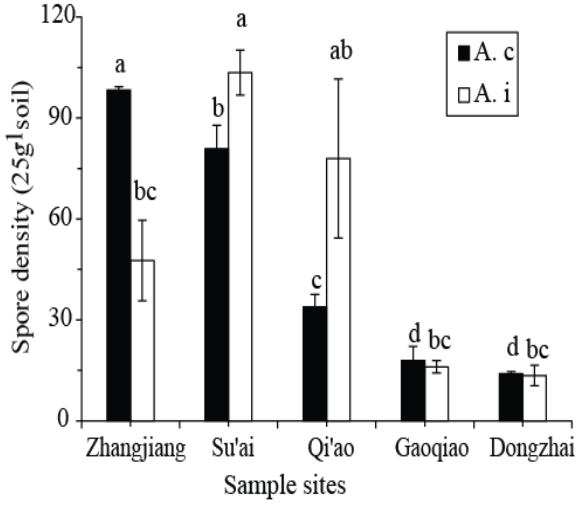

Fig. 3. Arbuscular mycorrhizal fungi spore density at different sites. Letters above columns indicate significant difference across different sites at 5% confidence level; mean ± SE. A.c: *Aegiceras corniculatum,* A.i: *Acanthus ilicifolius.*

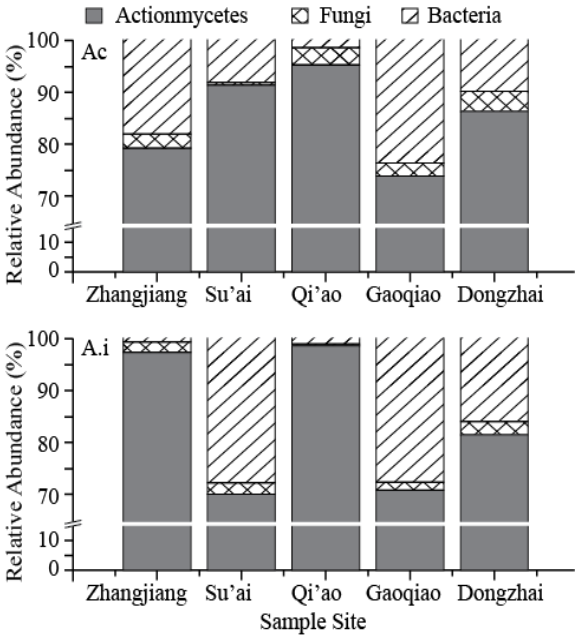

Fig. 4. Relative abundance of three groups of microorganisms. A.c: *Aegiceras corniculatum,* A.i: *Acanthus ilicifolius.*

Discussion

Presence and influencing factors on AMF colonization rates and spore density: Arbuscular mycorrhizal fungi colonization rates agreed with our previous study (Wang *et al.*, 2010), which reported a 20% total colonization rate for *A. ilicifolius* at low tide level in the Qi'ao Nature Reserve, while in this study, the rate was 21.3% (Fig. 2). The recorded AMF spore density ranged from 13.5 to 98.3 spores per 25g soil, representing a low spore density, something that is common in mangrove ecosystems (Kothamasi *et al.*, 2006). These results revealed that the AMF colonization rate and spore density were lower in mangrove plants than in most terrestrial ecosystems, indicating the specificity of AMF in mangroves.

The main abiotic factors affecting AMF colonization rate have been well described, such as hydrological conditions (Miller & Sharitz, 2000; Wang *et al.*, 2010), phosphorus levels (Chen *et al.*, 2008), organic matter content (Albertsen *et al.*, 2006), nitrogen levels (Eom *et al.*, 1999). In our study, EC was significantly (r= 0.54, *p*= 0.038) correlated with VC% for *A. corniculatum*, while other soil parameters didn't show correlations with AMF colonization rates for both species. Seawater salinity (r= -0.63, *p*= 0.012) and available P content (r= -0.73, *p*= 0.002) were both negatively correlated with spore density. When analyzing data for both species together, soil available P content showed significantly negative correlations with both AMF colonization rate and spore density, which was agree with the results of Kumar and Ghose (2008). Both total N and available N content do not show any correlation with AMF colonization or spore density. As soil P content was very low in this study, it may be the limiting factor for AMF colonization, while abundant soil N content did not seem to affect AMF colonization rates and spore density. The result that seawater salinity was negatively correlated

with spore density agreed with results of previous studies of mangrove (Sengupta & Chaudhuri, 2002) and coastal ecosystems (Guo & Gong, 2014), indicating that seawater salinity may be an important abiotic factor influencing AMF colonization in mangrove ecosystems.

The correlation between AMF and soil microbial numbers: Arbuscular mycorrhizal fungi are among the most important and influential soil microbes, as they constantly interact with a wide range of soil microorganisms (Miransari, 2011), significantly affecting them (Dumbrell *et al.*, 2010). Mycorrhiza helper bacteria can promote the activity of AMF (Klett *et al.*, 2007), and pathogenic fungi may induce plant resistance to AMF (Kloepper *et al.*, 2004). Zhang *et al.* (2010) also suggested that the bacterial and AMF diversity in *H. rhamnoides* and *C. microphylla* rhizosphere were positively correlated.

In this study, bacteria number was significantly negatively (r= -0.054, *p*= 0.039) correlated with AC%, while fungi number was negatively correlated with both VC% (r= -0.54, *p*= 0.040) and AC% (r= -0.61, *p*= 0.016) for *A. corniculatum*. However, no significant correlation was found between the numbers of any type of microbes and AMF colonization rates or spore density for *A. ilicifolius*. The soil microbial community can positively or adversely affect the development of AMF colonies. The interactions between AMF and soil bacteria are influenced by many factors, including AMF species and bacterial strains, plant species, rhizosphere and climate properties (Sanon *et al.*, 2009). Previous work has also demonstrated that soil properties such as pH, OM and nutrient availability are strong determinants of microbial community structure in mangrove ecosystems (Colares & Melo, 2013). The different correlations for the two mangrove species with microbial numbers illustrate that host plant is a key factor influencing AMF and other microbes.

Arbuscular mycorrhizal fungi distribution across different mangroves: When analyzing AMF colonization rate and spore density data (Figs. 2 and 3), it seems that AMF colonization rate for both plant species distributed a trend to first increase and then decrease from north to south, spore density for *A. ilicifolius* also shows the trend. Variance analysis revealed that for *A. corniculatum*, all types of AMF colonization rates were significantly different across five sample sites, but for *A. ilicifolius*, no type of AMF colonization rates were not different. Spore density was extremely significantly different across different sites for both *A. corniculatum* (*p*= 0.000) and *A. ilicifolius* (*p*= 0.002). Due to the restricted sample size and study scale, we can't say for sure whether there existed a distribution trend for AMF in mangrove ecosystems. But we think that this is an interesting topic for studying AMF in mangrove ecosystems, as mangrove species diversity decreases and become less well-developed with increasing latitude (Li & Lee, 1997). How greatly the distribution of mangrove plant species can affect AMF below ground? What are the factors influencing AMF distribution in different mangrove ecosystems? To answer these questions, we still need further and deeper researches.

Conclusion

The study showed that AMF were common for both *A. corniculatum* and *A. ilicifolius* across the five sample sites. Although the recorded AMF colonization rate and spore density were lower in mangrove plants than in most terrestrial ecosystems, the extensive existence showed the importance of AMF in mangrove ecosystems. Soil available P content and seawater salinity may be important factors influencing AMF in mangroves, while the different correlations for the two mangrove species with microbial numbers illustrate that host plant is a key factor influencing AMF and other microbes. Until now, our understanding of AMF colonization and spore density in mangroves is still in its infancy. Studying the characterization of AMF colonization and spore density in mangroves may provide some help in understanding the factors that influence AMF distribution, as well as the application of AMF in the bioremediation of mangroves.

Acknowledgements

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