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Salt is a main factor shaping community composition of arbuscular mycorrhizal fungi along a vegetation successional series in the Yellow River Delta

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ABSTRACT

In coastal wetlands, salinity significantly influences the vegetation successional series. Arbuscular mycorrhizal fungal (AMF) are ubiquitous soil microorganisms which play important roles in plant establishment, diversifying plant communities and accelerating successional progress by improving the soil health. However, the structure and function of AMF communities in coastal wetlands are poorly understood. Here we investigated the AMF communities along a vegetation successional series (from intertidal to supratidal flats) and the sediment physicochemical factors affecting the variations. AMF community composition and diversity were analyzed by 18S rRNA genes using high-throughput sequencing technology. The results revealed that the maximum number of AMF sequences belonged to *Glomus* 74,660, 62.75%. The relative abundance of the top three genera differed significantly along the vegetation successional series. Redundancy analysis (RDA) results demonstrated that salinity was a major environmental factor structuring the AMF community in the sediment along the vegetation successional series. No significant differences of AMF diversity were observed between different sediment layers. Additionally, total nitrogen (TN) and total carbon (TC), important soil nutrients in young emergent wetland ecosystems, also contributed to AMF community structure. The results of this study provide a better understanding of the AMF community and the major factors impacting it along a coastal wetland successional series composed of different vegetation types.

1. Introduction

The Yellow River Delta, located at the mouth of the sediment-laden Yellow River, flows into the Bohai Sea, north of Shandong province, China. Approximately 1.05×10^7 tons of sediment per year is carried to the estuary, leading to an annual expansion of Yellow River Delta by 20–25 km^2 (Wang and Liang, 2000). The river's course is changed several times by the government over the history of the Yellow River Delta, and the current outlet at the north bank of the Qingshuigou channel is the result of a change in 1996 that redirected the southern course. As a result, the modern Yellow River Delta is characterized by newly formed wetlands. Driven by tidal actions and the sediment deposition, the wetlands generally consist of several hundred meters of land that follows a successional series from intertidal to supratidal flats, and includes changes in salinity, nutrient content, particle size distribution, and vegetation type and coverage (Peng et al., 2014; Liu et al., 2018). The different attributes of these newly formed coastal wetlands can influence the ecosystem services and functions of the coastal habitats.

As newly formed wetlands, soil microorganisms are recognized as the key factor in regulating ecological processes in the tidal flats (Böer et al., 2009; Lv et al., 2016). Soil microorganisms support the main biogeochemical processes such as primary production, remineralization of organic matter and supplying mineral nutrients to plants (Hu et al.,

Abbreviations: AMF, arbuscular mycorrhizal fungi; EC, electrical conductivity; AK, available potassium; AP, available phosphorus; TC, total carbon; TOC, total organic carbon; TN, total nitrogen; YRD, Yellow River Delta

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2014). However, abundance, diversity, and richness of soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) are possibly influenced by tidal water, soil salinity and also vegetation types (Ikenaga et al., 2010; Guo and Gong, 2014; Lv et al., 2016).

AMF are ubiquitous soil microorganisms which form symbiotic associations with the roots of over 80% of all land plants (Smith and Read, 2008; Guo et al., 2016). Even though AMF were sometimes considered unimportant in wetlands due to its aerobic characteristics (Peat and Fitter, 1993), many studies have found that the root-inhabiting fungi may affect plants by reducing negative effects of flood and salt stress, enhancing nutrient uptake, providing protection from pathogens and also may influence wetland plant community structure (Jumpponen 2001; Wang et al. 2004; Krishnamoorthy et al., 2014; Xu et al., 2016). The presence of AMF has been confirmed in soils under different environmental conditions, and they play an important role in plant establishment, diversifying plant communities and accelerating successional progress by improving the soil health (Daleo et al. 2008; Guo and Gong, 2014). Previous studies have discussed the diversity and identity of AMF in grasslands, forests and wetlands based on spore morphology, spore number and degree of root colonization (Wang et al., 2004; Kandalepas et al., 2010; Krishnamoorthy et al., 2014; Hu et al, 2015). However, due to the limited morphological differences between AMF species, it is hardly to uncover its diversity by using the culture-dependent approaches. High-throughput sequencing, a rapid and relatively inexpensive sequencing technology that produces hundreds of thousands of short DNA or RNA sequence reads, has revolutionized microbial ecology by allowing for the identification of microbial taxa based on genetic material (Lumini et al., 2010; Lin et al., 2012).

Effort has been made in the recent past to analyze the AMF diversity and distribution in saline soils, salt marshes and coastal areas (Lusite and Levinsh 2010; Guo and Gong, 2014; Krishnamoorthy et al., 2014). Soil salinity was shown to significantly reduce AMF coloninazion, spore count and glomalin related soil protein (Wang et al., 2004; Krishnamoorthy et al., 2014). However, much about AMF diversity in newly formed wetlands, particularly those with lower soil nutrient content (such as NH_4-N, NO_3-N or Available phosphorus) and high salinity (Yu et al., 2014, 2016), remains to be studied and will contribute to a better understanding of the nature and roles of AMF under such conditions.

We hypothesized that AMF community structure and function will differ spatially (horizontal and vertical) in the successional series of tidal flats from intertidal to supratidal flats, reflecting systematic changes in site conditions along the successional series. In this study, we applied the high-throughput sequencing technique to investigate AMF communities in four typical vegetation communities along the successional series from intertidal to supratidal flats. The objectives of the present study were (1) to characterize the molecular diversity and community composition of AMF along the successional series, and (2) to explore the possible relationship between AMF and sediment properties (salinity and nutrients) in the newly formed coastal wetland.

2. Materials and methods

2.1. Study sites description and sample collection

The study was conducted in the Yellow River Delta Natural Reserves (YRDNR), which established in 1992 to preserve habitat for birds and the unique coastal wetland ecosystem, at 37°35′–38°12′N, 118°33′–119°20′E between the Bohai Gulf and the Laizhou gulf in eastern China (Fig. 1). It is one of the most active regions of land-ocean interaction among the large river deltas in the world. The average annual temperature is 11.5 °C. The annual potential evaporation is 1900–2400 mm and annual precipitation is 530–630 mm, of which 70% is rainfall that occurs between June to August. *Suaeda salsa* (Linn.) Pall., *Tamarix chinensis* Lour., and *Phragmites australis* Trin. are the dominant plant communities in the YRDNR.

Tidal flats in the YRD have clear horizontal distribution vegetation zones from low tidal flats to high tidal flats. Four sampling sites (represent 4 typical successional communities along the tidal gradient) were established. The dominant plant species in sampling plots were *Suaeda salsa*, *Tamarix chinesis*, *Phragmites australis* and *Calamagrostis pseudophragmites* (Hall. f.) Koel., respectively. The plant species in different sampling sites are shown in Table S1 (Supplementary material).

We established three replicate plots (5 × 5 m^2) at each vegetation community site (the replicate plots were separated at least 100 m from each other). In each plot, four random sediment cores from three different depths (0–10, 10–20, 20–30 cm) were collected, homogenized, and the plant debris was removed to form a composite sample. The composite sediment cores for each plot were pooled in a sterile plastic bag and transported to the laboratory within 24 h. Samples were stored at −80 °C for total DNA extraction within 30 days, and a subset of the sediment was air-dried and sieved (2 mm mesh size) for chemical analysis.

2.2. Chemical analysis

NH_4^+-N, NO_3^--N in sediment samples were extracted from lyophilized sediments with 2 M KCl using a 1:10 sediment: extractant ratio, and then measured with Continuous Flow Analyzer (Seal AutoAnalyzer III, Germany) (Grasshoff, et al., 2007). TN and TC were determined by the elemental analyzer (Elementar Vario Macro, Germany). Sediment pH and EC values were measured with electricity conduction method (sediment/water = 1:5) (The Committee of Agro-chemistry of the Chinese Society of Soil Science, 1983). Available phosphorus (AP) was determined after extraction using sodium bicarbonate (Tiessen and Moir, 1993). Sediment available potassium (AK) contents were determined by atomic absorption spectrophotometry (AA-6800, Shimadzu, Japan).

2.3. DNA extraction and PCR amplification

Total DNA was extracted from 0.5 g of sediment using a Fast DNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA) according to the manufacturer's instructions. The extracted soil DNA was dissolved in 50 µl TE buffer. The DNA concentrations were determined by optical density at OD 260 nm using a Genesys 5 spectrophotometer (Spectronic instrument, Inc., New York, USA) and stored at −20 °C.

Two 18S rRNA specific primers AMV4.5NF (AAGCTCGTAGTTGAA TTTCG) + barcode and AMDGR (CCCAACTATCCCTATTAATCAT) were used to amplify 18S rRNA gene fragments by Polymerase Chain Reaction (PCR) (Sato et al., 2005; Lin et al., 2012). PCR was performed containing a high-performance Taq antibody and Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, Inc.). A sample of 5–10 ng total DNA was added to a PCR mixture containing 2 µM AMV4.5NF and AMDGR in a final reaction volume of 30 µl with the following components: 15 µl Phusion High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, United States), 1.5 µl (initial 6 µM each) of forward and reverse primers, 10 µl (1 ng/µl) of template containing approximately 10 ng of total genomic DNA and 2 µl of H_2O. The amplification program consisted of 1 min at 98 °C for initial denaturation followed by 30 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 30 s and a final 5 min extension at 72 °C. The PCR products were separated in 2% agarose gel and stained with ethidium bromide. The PCR products were then purified with Qiagen Gel Extraction Kit (Qiagen, Germany). The bar-coded PCR products from all samples were combined in equimolar amounts, then prepared for sequencing using the TruSeq™ DNA Sample Preparation Kit and sequenced using the HiSeq PE250 (Illumina) following the manufacturer's protocols.

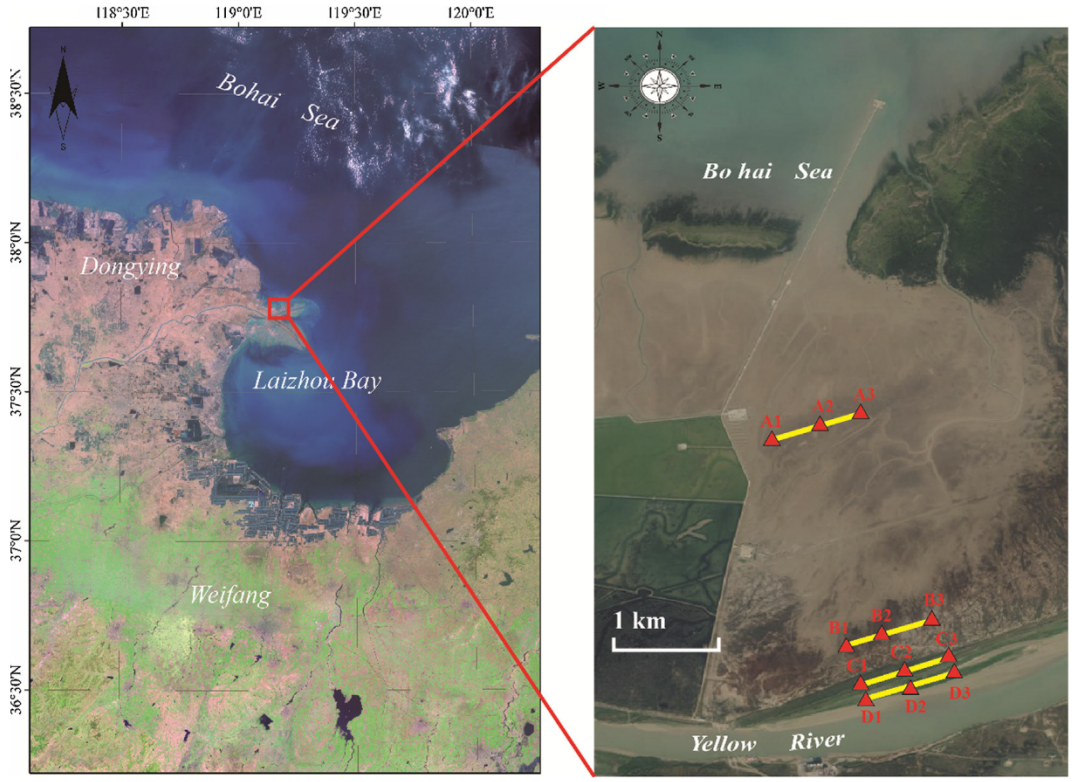

Fig. 1. Location of the Yellow River Delta and sampling site (obtained from Google Earth).

2.4. Illumina HiSeq sequencing and data processing

The library was constructed by TruSeq DNA PCR-Free Library Preparation kit (Illumina, USA), and it was sequenced on an Illumina HiSeq 250 platform and 250 bp paired-end reads were generated.

We used the Quantitative Insights Into Microbial Ecology (QIIME) pipeline to process the 18S molecular data. In brief, sequences with ambiguous nucleotides, a quality score below 30, shorter than 200 bp in length were excluded from further analysis. Sequences analysis was performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) (Edgar, 2013). Sequences with ≥97% similarity were assigned to the same OTUs. For each representative sequence, the Greengenes Database (DeSantis et al., 2006) was used with the RDP classifier (Wang et al., 2007) algorithm to annotate taxonomic information. OTUs abundance was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed based on the sequences and/or OTUs obtained from the Glomeromycota phylum. The sequences from this study have been deposited at GenBank with accession number SRP127525.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS 20.0 for Windows. All acquired data were represented by the average of three replicate measurements and standard deviation (S.D.). Significance was tested at the 5% level.

Alpha diversity was applied to analyze the complexity of species diversity, including Observed species, Chao1, Shannon, Simpson and Coverage. All these indices were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3) (http://www.r-project.org) (Caporaso et al., 2010). Beta diversity analysis was used to evaluate differences in AMF community structures, Principal Coordinate Analysis (PCoA) based on the unweighted UniFrac distance metric (Lozupone and Knight, 2005) was displayed by the WGCNA, stat and ggplot2 packages in the R program (R Development Core Team, 2010). In order to assess the correlation between AMF community structure and environmental parameters, redundancy analysis (RDA) was conducted using CANOCO 4.5 software. A Monte Carlo permutation test (999 random unrestricted permutations) was performed to test the statistical significance of the environmental variables.

3. Results

3.1. Sediment physicochemical properties

The sediment salt content significantly decreased along the successional series from intertidal to supratidal flat (Table 1). The highest EC was recorded in the *S. salsa* plant community, which was about 6 times higher than that in the *C. pseudophragmites* community (Table 1). No detectable difference in pH was observed between different plant communities or between different sediment layers, but changed dramatically under the interaction of vegetation and sediment layer (Table 1). Similarly, the total C and N remained stable along the successional series. The NO_3-N content decreased significantly from the sediment surface to the lower sediment layers (Table 1). The NH_4-N and NO_3-N content were significantly affected by the interaction of vegetation and sediment layers. AP and AK content changed significantly between different vegetation types. The highest AK content appeared in the *S. salsa* plant community, and the lowest was in the *C. pseudophragmites* community (Table 1).

3.2. Variation in AMF OTU number and richness

Overall, 118,978 sequences of *Glomeromycota* were obtained across the samples with the primer pair AMV4.5NF/AMDGR (Table S2). Of these, 15,935 sequences were found in *S. salsa* community, 15,611 sequences were found in *T. chinesis* community, 36,544 sequences in *P. australis* community and 40,888 in *C. pseudophragmites* community.

Based on 97% species similarity, a total of 127 operational

Table 1
The sediment physicochemical characteristics at the sample sites of different vegetation communities and sediment layers.

Sampling site	Main vegetation	Sediment layers (cm)	EC (ms/cm)	pH	TC (mg/g)	TN (mg/g)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	AP (mg/kg)	AK (mg/kg)
A	*S. salsa*	0–10	3.92 ± 0.42	8.02 ± 0.11	16.61 ± 0.98	0.26 ± 0.03	6.73 ± 3.33	9.78 ± 1.24	10.33 ± 1.87	699.85 ± 42.82
		10–20	2.65 ± 0.39	7.58 ± 0.07	14.52 ± 3.86	0.21 ± 0.12	9.28 ± 3.59	7.11 ± 0.97	11.64 ± 1.02	670.38 ± 55.80
		20–30	2.81 ± 0.40	7.86 ± 0.34	13.40 ± 0.36	0.16 ± 0.02	4.36 ± 2.22	4.38 ± 1.05	9.69 ± 1.50	560.79 ± 7.27
B	*T. chinensis*	0–10	2.15 ± 0.32	7.94 ± 0.11	15.28 ± 2.18	0.28 ± 0.11	13.96 ± 6.55	11.32 ± 0.04	10.09 ± 2.75	257.94 ± 11.24
		10–20	2.07 ± 0.54	8.04 ± 0.23	12.73 ± 0.84	0.16 ± 0.04	5.78 ± 1.79	5.23 ± 4.12	3.03 ± 0.17	217.71 ± 31.25
		20–30	2.25 ± 0.74	7.85 ± 0.28	15.38 ± 3.52	0.28 ± 0.16	5.22 ± 2.09	3.46 ± 2.74	3.15 ± 0.51	240.70 ± 31.44
C	*P. australis*	0–10	0.97 ± 0.21	7.73 ± 0.12	18.65 ± 4.47	0.45 ± 0.18	12.83 ± 3.72	22.09 ± 6.93	14.43 ± 5.46	409.60 ± 21.76
		10–20	1.24 ± 0.27	7.77 ± 0.48	13.36 ± 2.02	0.17 ± 0.06	15.69 ± 4.15	3.48 ± 1.68	11.23 ± 3.30	251.93 ± 53.40
		20–30	1.57 ± 0.07	8.09 ± 0.19	12.27 ± 1.62	0.14 ± 0.04	11.64 ± 2.33	6.63 ± 1.82	14.31 ± 5.01	413.42 ± 56.69
D	*C. pseudophragmites*	0–10	0.41 ± 0.18	8.20 ± 0.16	12.37 ± 0.99	0.16 ± 0.07	18.96 ± 0.45	7.72 ± 1.82	7.38 ± 3.24	253.03 ± 71.29
		10–20	0.54 ± 0.12	8.24 ± 0.09	12.78 ± 1.53	0.15 ± 0.03	6.90 ± 4.35	0.67 ± 0.26	2.25 ± 0.42	122.66 ± 27.41
		20–30	0.59 ± 0.21	8.18 ± 0.17	14.08 ± 1.08	0.21 ± 0.08	6.49 ± 4.09	2.01 ± 0.66	2.61 ± 1.10	108.27 ± 22.70
Analysis of variance										
Sediment layers			n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.
Vegetation			**	n.s.	n.s.	n.s.	n.s.	n.s.	**	***
Sediment layers × Vegetation			**	*	n.s.	*	*	**	n.s.	***

n.s., *, **and *** indicate non-significant or significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level.

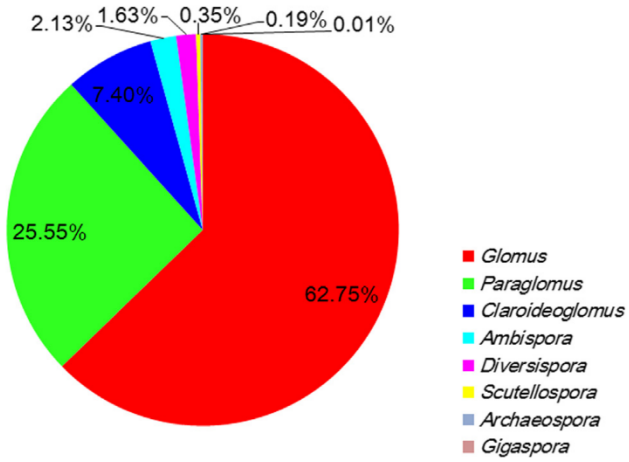

Fig. 2. The total genus distribution of the coastal wetland in the Yellow River Delta. Data are presented as the mean of three replications.

taxonomic units (OTUs) of *Glomeromycota* were detected for the entire range of successional sampling sites and depths (Table S2). The greatest number of OTUs obtained was 111 in *T. chinesis* community and the least number of OTUs obtained was 96 in *S. salsa* plant community. Among them, 21 OTUs (~16.7% of all OTUs) were discovered in common throughout the successional sampling sites and the entire depth range.

The taxonomic distributions over the currently recognized *Glomeromycota* genera are shown in Fig. 2. The greatest number of AMF sequences belonged to *Glomus* with 74,660, corresponding to 62.75% of the total sequences obtained. The second group was *Paraglomus* with 30,398 (25.55%), and the third was *Claroideoglomus* with 8802 (7.40%) (Fig. 2, Table S2).

3.3. Shifts in AMF diversity and community structure between vegetation successional series

Rarefaction curves indicated that our sequencing depth was sufficient to cover the majority of AMF species in all the samples (Fig. S1).

The Observed species, Chao1, Shannon, Simpson and Coverage indices were used to estimate and compare AMF alpha diversity and richness among different vegetation communities and sediment layers (Table 2). The results showed that the coverage values of all samples were more than 98%. Observed species increased from 70 in the *S. Salsa* community to 108 in the *C. pseudophragmites* community. Chao1 richness ranged from 87.38 to 132.28, Shannon's diversity ranged from 3.71 to 5.01, and Simpson's diversity varied between 0.81 and 0.94. We found that the highest values for observed species and Shannon's diversity appeared in the *C. pseudophragmites* community, the lowest values for observed species number and Shannon's diversity were in the *S. Salsa* community (Table 2). No significant differences were observed between vegetation types or sediment layers. However, the observed species and Shannon's diversity were significantly affected by the interaction of vegetation and sediment layer ($P < 0.05$).

All samples were clustered by principal coordinate analysis (PCoA) based on the UniFrac phylogenetic distance metric to detect differences between the different vegetation associated sediments. Our findings showed significant differences in the AMF community composition among different vegetation associated sediment communities (Fig. 3). There was a clear difference in AMF community structure between the *S. salsa* and *C. pseudophragmites* communities, whereas no significant difference in AMF community structure was observed between *T. chinesis* and *P. australis* dominated communities.

A total of 8 genera within *Glomeromycota* were identified in the sediment samples. The top three genera, *Glomus*, *Paraglomus* and *Claroideoglomus*, composed 95.70% of all sequences. *Glomus* was the

Table 2
Alpha diversity indices (at 97% sequence similarity) of AMF along different vegetation communities and sediment layers.

Sampling site	Sediment layers (cm)	Observed species	Chao1 richness	Shannon's diversity	Simpson's diversity	Coverage (%)
A	0–10	70	87.38	3.71	0.84	99.06
	10–20	83	103.59	4.09	0.88	98.72
	20–30	88	115.05	4.53	0.92	98.63
B	0–10	105	132.28	4.93	0.93	98.19
	10–20	97	125.20	4.57	0.92	98.33
	20–30	91	113.69	4.18	0.90	98.41
C	0–10	89	116.44	4.44	0.92	98.33
	10–20	96	124.46	4.66	0.94	98.15
	20–30	80	116.83	4.24	0.91	98.61
D	0–10	108	127.84	4.65	0.91	98.38
	10–20	94	120.11	4.00	0.81	98.68
	20–30	103	123.02	5.01	0.93	98.42
Analysis of variance						
Sediment layers		n.s.	n.s.	n.s.	n.s.	n.s.
Vegetation		n.s.	n.s.	n.s.	n.s.	n.s.
Sediment layers × Vegetation		*	n.s.	*	n.s.	n.s.

n.s. and * indicate non-significant or significant differences at $P < 0.05$ level.

dominant genus in each of the four vegetation communities we studied. The relative abundance of *Glomus* in *S. salsa* and *C. pseudophragmites* communities was 45.49% and 39.28%, which was lower than in that *T. chinesis* and *P. australis* communities (73.77% and 74.34%, respectively). The *Paraglomus* relative abundance was greatest in the *C. pseudophragmites* community, and significantly higher than other communities. The relative abundance of *Claroideoglomus*, with a range of 2.78–12.58%, had a similar trend to *Glomus* (Fig. 4).

3.4. Influences of environmental parameters on AMF diversity and community structure

Pearson correlation coefficients showed that EC had a strong negative correlation with observed species and Chao1 richness ($r = -0.459$, $p = 0.005$ and $r = -0.403$, $p = 0.015$) (Table 3). AK was negatively correlated with observed species, Chao1 richness and Shannon's diversity, suggesting it also played a significant role in reducing AMF alpha diversity.

Based on the result of the Mantel test, some sediment parameters significantly correlated with AMF community structure were selected for RDA analysis (Fig. 5). Variance inflation factors with 999 Monte-Carlo permutations showed that EC, TC and TN are significant factors that influence the AMF community structure in all samples (Fig. 5). The eigenvalues of the first and second axes were 42.3% and 7.9%, respectively. The cumulative percentage variance of species data showed that the first two RDA axes explain 50.2% of the structural variation.

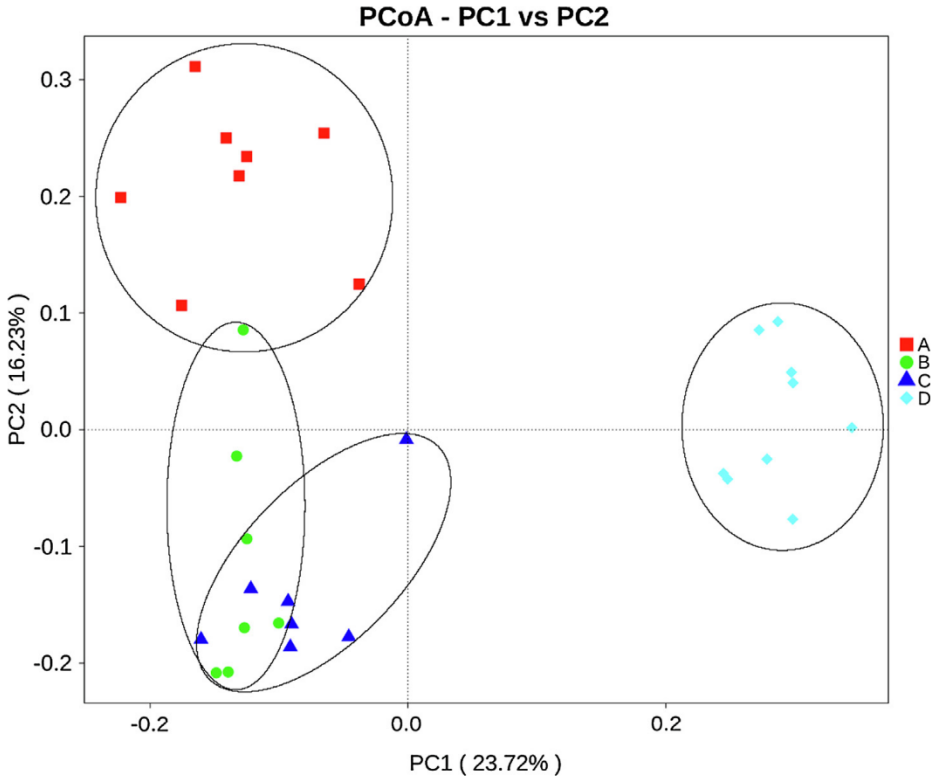

Fig. 3. The principal co-ordinates analysis (PCoA) of unweighted UniFrac distances of AMF community in sediments of different vegetation types and sediment layers. The contribution rates of the first and second principal component are 23.72% and 16.23%.

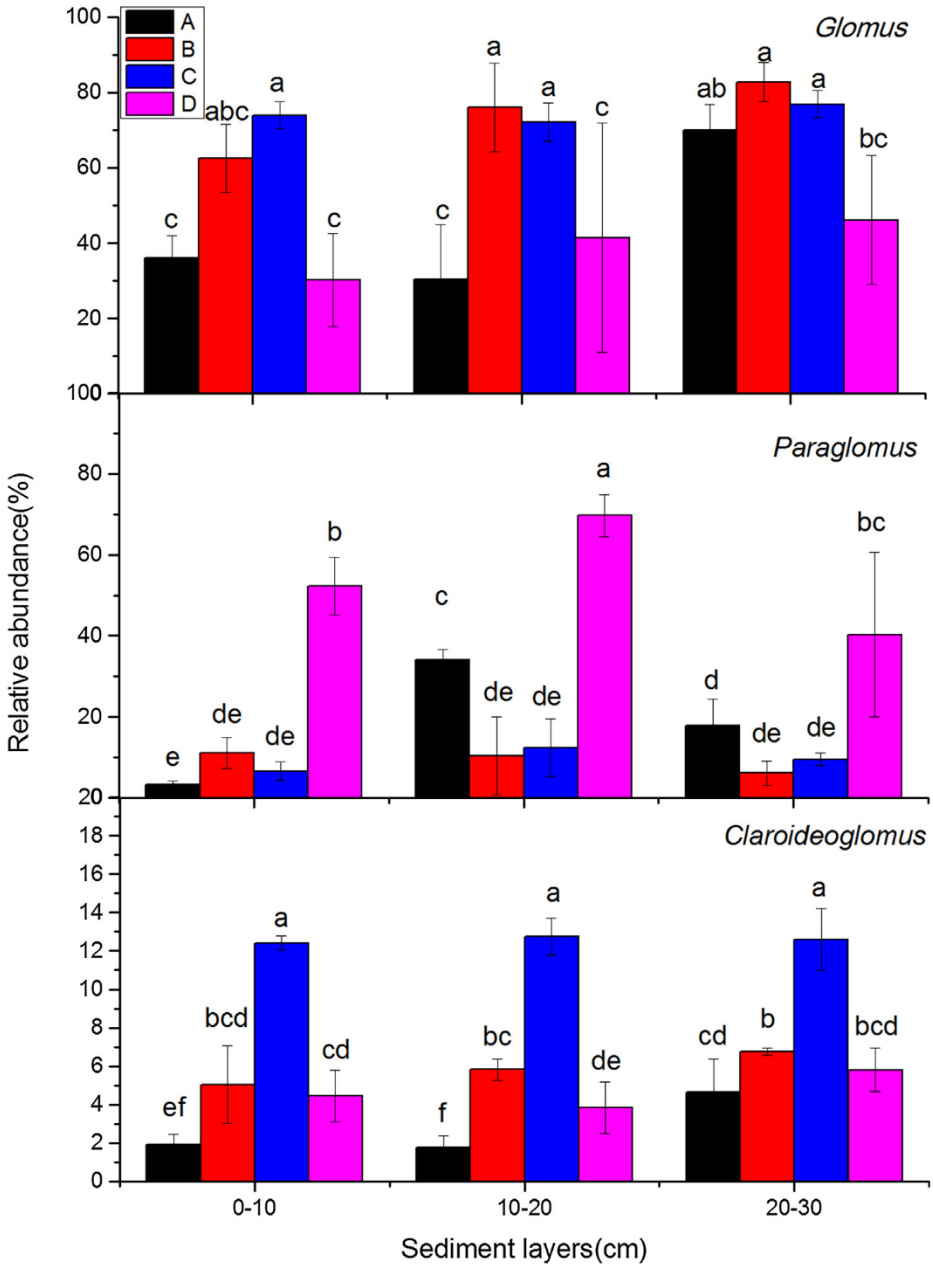

Fig. 4. The top three genera relative abundance of phylum *Glomeromycota* (*Glomus, Paraglomus* and *Claroideoglomus*) in sediments of different vegetation types and sediment layers.

Table 3
Coefficients of Pearson's correlation between AMF alpha diversity and the sediment physicochemical characteristics.

	Observed Species	Chao 1 richness	Shannon's diversity	Simpson's diversity
EC	−0.459**	−0.403*	−0.276	−0.098
pH	0.182	0.196	0.043	−0.133
NO_3-N	−0.128	−0.100	−0.033	0.096
NH_4-N	0.316	0.285	0.312	0.308
AK	−0.618***	−0.514**	−0.380*	−0.126
AP	−0.269	−0.091	−0.106	0.104
TC	−0.185	−0.188	−0.007	0.064
TN	−0.036	−0.063	0.097	0.138
C/N ratio	−0.049	0.042	−0.176	−0.157

*, **, *** indicate significant differences at 0.05, 0.01 and 0.001 level.

4. Discussion

Tidal currents are a dominant factor controlling coastal wetland ecosystem evolution (Mariotti and Fagherazzi, 2013). The distribution of plants or sediment microorganisms in tidal flats depends on flooding and salt tolerance. In our study sites, as the EC decreased from intertidal to supratidal flat, the plant community changed from the pioneer halophyte plant (*S. salsa*) to a non-halophyte (*C. pseudophragmites*). The tidal action also significantly increased the sediment surface EC of the *S. salsa* community when compared with lower sediment layers. However, opposite trend was found in the *P. australis* and *C. pseudophragmites* communities, attributable to the frequent scour of the Yellow River.

In the present study, 118,978 sequences of *Glomeromycota* forming 127 OTUs were detected with the primer set AMV4.5NF/AMDGR, which was previously shown to give good coverage of *Glomeromycota* taxa (Sato et al., 2005; Lumini et al., 2010). The AMF richness in the

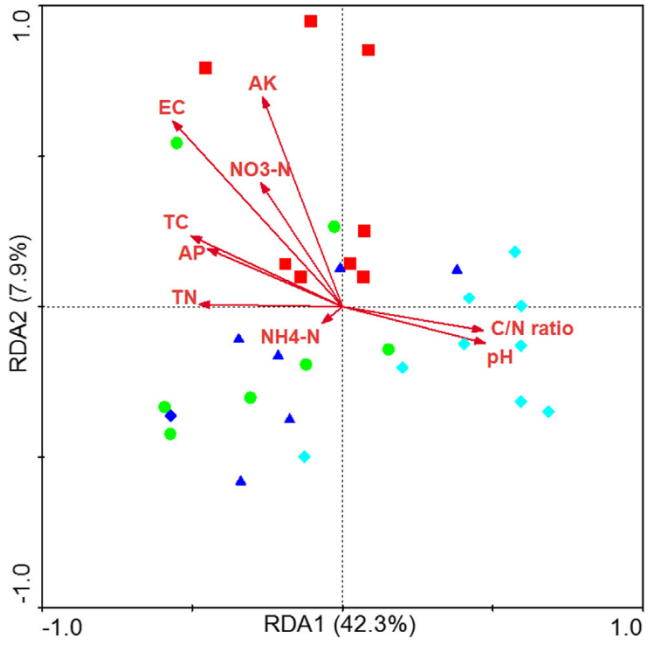

Fig. 5. Redundancy analysis (RDA) compares the AMF community structure in sediment samples and environmental factors (red arrows), including pH, salinity, TC, TN, C/N ratio, NH_4-N, NO_3-N, AK, and AP. Red squares, green circles, blue triangles and cyan diamonds indicate the samples of *S. salsa*, *T. chinesis*, *P. australis* and *C. pseudophragmites*, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sediment of the coastal wetland ecosystem of the YRD was unexpected higher than other ecosystems, such as farmland (Lin et al., 2012) (e.g. 15 soil samples from a maize field with different nutrient treatments) or grassland (Lumini et al., 2010) (e.g. 25 soil samples from 5 different locations), suggesting that the YRD wetland sediments contain abundant AMF DNA compared to other ecosystems.

Data from the present study showed that the AMF community of the investigated sediments (including different sediment layers) was characterized by the dominance of *Glomus*. Many previous studies have also reported the dominance of *Glomus* spp. from widespread soils including from agro-ecosystems (Hijri et al., 2006; Lin et al., 2012), forest ecosystems (Wang et al., 2015), grasslands (Lumini et al., 2010; Guo et al., 2016), reclamation land (Krishnamoorthy et al., 2014), and coastal wetlands (Guo and Gong, 2014). It is well established that the *Glomeraceae*, the largest family of AMF, can adapt to diverse ecosystems. In this study, the *S. salsa* and *C. pseudophragmites* communities were affected frequently by seawater and Yellow River water, respectively. The inundation situation (ether caused by seawater or river water) could be the reason why *Glomus*, an aerobic microorganism, had a significantly lower relative abundance in *S. salsa* and *C. pseudophragmites* communities (45.49% and 39.28%) than that in *T. chinesis* and *P. australis* communities (73.77% and 74.34%).

The second most abundant group detected in this study, *Paraglomus*, had a relative abundance of 40–52% in the sediment of *C. pseudophragmites* community, which was significantly higher than other communities. In contrast, previous studies have reported that *Gigasporaceae*, *Diversispora* or *Acaulosporaceae* are the most frequently detected families after *Glomeraceae* in coastal wetlands such as mangrove swamps (Wang et al., 2011), while other researchers observed that *Paraglomus* could be dominant in a grassland ecosystem (Hempel et al., 2007). In this study, highest numbers of OTUs were detected in the sediment of the *C. pseudophragmites* community, which is found not far from fresh water (Yellow River). This finding is in line with a recent study that *Paraglomus* species prefer well-watered environments (Symanczik et al., 2015).

Sediment salinity is not only a key factor structuring the natural vegetation distribution in coastal wetlands (He et al., 2007), but also influences AMF abundance, such as percentage of root colonization, spore count, and AMF community structure (Guo and Gong, 2014; Krishnamoorthy et al., 2014). In this study, we found that AMF diversity and community structure changed significantly along the successional series from intertidal to supratidal flat, which was represented as a salinity gradient. On the one hand, high soil salinity could inhibit AMF hyphal growth and colonization, and also postpone AMF spore germination (Hajiboland et al., 2010; Hammer and Rillig, 2011; Krishnamoorthy et al., 2014). On the other hand, soil salinity may also affect AMF communities through influence on plant species composition (Guo and Gong, 2014). The present study did not differentiate the effects of plant and salinity on AMF, but strongly supports that soil salinity is a major influencing factor on AMF communities.

It is widely accepted that AMF has a positive effect on the host plant growth and nutrient uptake, and could return nutrients to their plant hosts in exchange for carbon (Daleo et al., 2008; Delavaux et al., 2017). Conversely, AMF-derived carbon in itself could also be a significant component of soil organic matter (Rillig, 2004). As a recently emerged coastal wetland, nutrient contents (such as TN or TOC) in the sediments in the YRD were proved lower than in other coastal wetland sediments (Yu et al., 2016). Along with soil salinity, TN and TOC are limiting factors affecting distribution patterns of plant communities in this area (Bai et al., 2012; Liu et al., 2018). In the present study, although no significant effects of TN and TC on AMF alpha diversity were detected, TN and TC were found to be important factors affecting AMF community structure based on Monte-Carlo permuted variance inflation factors.

In conclusion, AMF diversity and structure along a vegetation successional series of newly formed coastal wetlands in the Yellow River Delta were obtained from high-throughput sequencing of 18 s variable region amplicons from soil DNA. The results demonstrated that *Glomus* was the dominant AMF genus in the coastal wetlands of the YRD. RDA indicated that salinity had a considerable impact on AMF community structure and abundance in the sediment along the successional series. In addition, as important soil nutrient elements, TN and TC also played important role in AMF community structure. The relative abundance of *Glomus* in *S. salsa* and *C. pseudophragmites* communities were lower likely due to the tidal activity and frequent flooding from the Yellow River. However, we speculate that, besides the influence of water, the increased relative abundance of *Glomus* in the sediments of *T. chinesis* and *P. australis* communities could also be related to the change in vegetation type, which should be investigated in future studies. These observations thus provide new insights about AMF in the newly formed wetlands of the YRD, specifically their relationship to soil nutrients and salinity. The YRD wetlands are a unique ecosystem influenced by both fresh water and seawater, and offer an ideal case study for understanding the impact of environmental changes on AMF structure and function in coastal ecosystems.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2019.104318.

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