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Full Length Article

Abundance and Diversity of Arbuscular Mycorrhizal Fungi in *Calamanrostis angustifolia* Wetlands of the Sanjiang Plain, China

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Abstract

Calamanrostis angustifolia is the major colonizing plant in the wetlands of the Sanjiang Plain in northeastern China. These wetlands have severely suffered from agricultural practices that resulted in lowered water tables. Hence, in this study, we compared the soil arbuscular mycorrhizal fungi (AMF) content from three types of *C. angustifolia* wetlands. Based on the water gradient, from high to low, the research site was categorized into marsh *C. angustifolia* wetland (W1), marsh meadow *C. angustifolia* wetland (W2) and meadow *C. angustifolia* wetland (W3). The fungal infection rate of *C. angustifolia* roots was investigated by microscopy. This showed presence of vesicles, arbuscules, infection sites and intra-and inter-cellular hyphae, from which we conclude this plant species is a typical arbuscular mycorrhizal host. Isolation of spores from the soil and morphological characterization revealed presence of at least 14 different AMF types, of which 10 could be identified at genus or species level. Total DNA was isolated from the three soil types and subjected to amplification and meta-genomic sequencing. This revealed the presence of fungal OTUs belonging to 2 orders, 7 families and 14 genera. Among the detected genera, *Redeckera*, *Pacispora*, *Racocetra*, *Cetraspora*, *Dentiscutata*, and *Rhizophagus* were detected for the first time in a wetland environment. The diversity index and an RDA analysis revealed that as the water content in the soil decreased, the soil nutrients and the pH increased. This resulted in a significant increase in the total amount and diversity of the AMF community in the intermediate W2 wetland. There was a significant difference in the community structure of W2 compared to W3 or W1. The reasons for this remain elusive but oxygen and nutrient levels may be partly responsible for the observations. This study provides scientific insights in the structural diversity and the spatial heterogeneity of the AMF community in various types of *C. angustifolia* wetlands.

Keywords: Arbuscular mycorrhizal fungi; *C. angustifolia*; Diversity; Wetland; China

Introduction

Wetland ecosystems account for 5–8% of the earth's surface area (Sui *et al.*, 2017), and with their characteristic mix of land and water, they display the richest biodiversity, highest productivity and highest ecological impact of all terrestrial ecosystems. Sanjiang Plain Wetland is the largest freshwater wetland in northeast China (Zheng *et al.*, 2013; Liu *et al.*, 2014; Taufik *et al.*, 2015). Due to agricultural development, submerged land coverage was reduced from 80% of the area in the early 1950s to only 20% in the 1980s (Zhang *et al.*, 2015). *Calamanrostis angustifolia* is the most abundant plant species in this wetland. Agricultural activity has locally lowered the water level which has resulted in three distinct ecosystems: meadow wetland, marsh meadow wetland and marsh wetland. As the area of the wetland decreased, the structure and function of these ecosystem changes accordingly (Ji *et al.*, 2006; Song *et al.*, 2006; Sun *et al.*, 2009).

The mycorrhiza networks resulting from the symbiosis of arbuscular mycorrhizal fungi (AMF) in the soil and plants has multiple functions, including promoting the nutrient cycle, improving plant nutrition, increasing the mass exchange and energy flow in the biosphere, enhancing the stress resistance of plants and recovering degraded and polluted ecosystems (Li *et al.*, 2013). These networks are also important for plant colonization, community competition and species succession, formation of species diversity and stabilization of ecosystems. AMF are aerobic microorganisms. However, the actual gas content in wetland soil is relatively low, so that access to oxygen to a certain extent limits the symbiosis of AMF and plants. Thus it was generally accepted that AMF played no or a limited role only in wetland ecosystems, however, in recent years a number of studies have demonstrated the importance of the symbiosis of AMF with plants in wetlands (Dunham *et al.*,

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2003; Carvalho *et al.*, 2004; Kai and Zhao, 2006; Ipsilantis and Sylvia, 2007). At present, there are no reports available about *C. angustifolia* colonized wetlands and a possible interaction with AMF in the wetland ecosystem of the Sanjiang Plain. Therefore, in this study, the occurrence of AMF was investigated in three ecosystem types: *C. angustifolia* meadow wetland, marsh meadow wetland and marsh wetland. By field investigations, morphological observations and the use of molecular biological techniques, we investigated the AMF diversity, analyzed its community structure and characterized changes in composition related with the various ecosystems. This work aimed to increase our understanding of the different characteristics of AMF populations and functions in the three *C. angustifolia* wetlands, and to provide reference to revealing the mechanisms of the function and impact of AMF in these wetland ecosystems.

Materials and Methods

Description of the Research Site

The research site is located at the Sanjiang Plain Wetland Ecological Research Station, Institute of Natural Resources and Ecology, Heilongjiang Academy of Sciences, China (47˚45' – 47˚47'N, 133˚35'E) (Fig. 1). The altitude of the research station is 55–65 m, and the annual average temperature is 1.9ºC.The annual average precipitation is 550–600 mm. The frost-free season spans about 125 days. Based on the water gradient, from high to low, the research site was categorized into marsh *C. angustifolia* wetland (W1), marsh meadow *C. angustifolia* wetland (W2) and meadow *C. angustifolia* wetland (W3) as described before (Sui *et al.*, 2017). The main plant types found in these three wetlands are listed in Table 1.

Sample Collection

Sampling plots (5 m × 5 m) were set up in the marsh wetland W0, marsh meadow wetland W1 and typical meadow *C. angustifolia* wetland W2, where soil samples were collected in June 2016.In each type of wetland, five soil samples were collected from a depth of 0 – 20 cm using a soil drill of 10 cm in diameter. The roots of *C. angustifolia* were collected, washed with distilled water and put in glass bottles containing standard fixation solution (FAA), to determine the degree of root infection. After removing large plant parts, stones, fine roots and other debris, the weight of the samples was standardized to 20 g to minimize the influence of heterogeneity. Each soil sample was divided into three parts. One part was used for physico-chemical analysis; the second part was stored at -20ºC prior to characterization of AMF spores as a proxy for the AMF community structure; the third part was stored at -80ºC and sequenced AMF sequences by using high throughput sequence (Illuminate Miseq) at Bionova Company (Beijing, China).

Physico-chemical Characterization of the Soil Samples

Soil moisture was measured by drying method; pH value was determined by potentiometric method; soil total nitrogen, alkaline hydrolysis nitrogen, organic carbon mass fraction was measured by CN elemental analyzer; soil total phosphorus mass fraction was determined by molybdenum-antimony colorimetric method; the content of soil available phosphorus was determined by $NaHCO_3$ leaching colorimetric method. The content of total potassium and available potassium in soil was determined by flame photometry.

Determination of the Degree of Root Infection for AMF Spores

Collected *C. angustifolia* roots were cut into pieces of 1 cm long and treated in 10% KOH solution at 90ºC for 1 h, softened in alkaline H_2O_2 solution (10 mL 30% H_2O_2, 3 mL NH_4OH, add distilled water to 600 mL) for 20 min, acidified by addition of 1% HCl for 3 min, incubated at 0.05% Trypan Blue in lactic acid/glycerol (1:1, v:v) at 90ºC for 3h, and then de-colored in lactic acid/glycerol solution for 48 h. Subsequently, the degree of AMF infection in the root fragments was determined using by cross intersection.

AMF spore density in the soil samples was determined after spores were separated using wet sieving - sucrose centrifugation method from 10 g of dried soil (Bai, 2014). The collected spores were put in distilled water in a Petri dish and counted under the microscope.

Observation of Mycorrhizal Type and Infection

The morphology of mycorrhizae was observed under a microscope and counted the mycorrhizal infection. The rate of AMF infection was determined by the method of Phillips and Hayman (1970). Mycorrhizal infection grading standards: 1, the number of infected nutrition accounted for 0 to 5%; 2, the number of infected nutrition accounted for 6 to 25%; 3, the number of infected nutrition accounted for 26 ~ 50%; Grade 4, the number of infected nutrition accounted for 51 to 75%; 5, the number of infected nutrition accounted for 76 to 100%. Mycorrhizal infection intensity standard: "+" means weak: There are few fungi and vesicles in the roots and sporadic distribution; "++" means: there are more fungi, "++" means strong: root fungus branches and vesicles densely covered, connected into pieces.

Isolation, PCR Amplification and Sequencing of DNA from the Soil

To extract total DNA from the soil microorganisms a Power Soil® DNA Isolation Kit (Qiagen, USA) was used, following the instructions of the product manual. The isolated DNA was dissolved in 100 μL of de-ionized water and 5 μL was analyzed by 1.0% agarosegel electrophoresis

(0.5 X TAE buffer), to examined the integrity of the DNA and its relative concentration. The DNA was then subjected to high-throughput sequencing. The sequencing primer was AMV 4.5NF/AMDGR (Sato *et al*., 2005). PCR amplification was performed by a two-step method with a reaction volume of 50 μL that included10 ng DNA template or 20 ng PCR product, 5 μL 10X PCR buffer, 0.5 μL dNTP (10 mmol/L each), 0.5 μLTaq DNA polymerase (5 U/μL), 0.5 μL of each primer (50 μmol/L each for forward and reverse primer), and double-distilled water to 50 μL. The amplification program included pre-denaturation at 95°C for 5 min, denaturation at 95°C for 15 s, annealing at 55°C for 15 s, elongation at 72°C for 30 s, 25 cycles, and a final elongation at 72°C for 10 min. The PCR products were isolated in 1.5% agarose gel electrophoresis, and recovered using the San Prep Column DNA Gel Extraction kit. The recovered product was quantified using Qubit2.0 DNA Detection Kit (Life Technologies, USA). After dilution at 1:1 ratio, all the samples were MiSeq sequenced (Liu *et al.,* 2016).

Analysis of Obtained DNA Sequences

The results of the MiSeq sequencing were paired-end data. In a first step, based on the overlap between the paired-end reads, paired reads were aligned into one sequence. For quality control on the quality of reads and alignment, effective sequences were obtained based on the barcode on the head and tail of the sequence and the primers. The sequence orientation was also corrected. During the quality control step, short sequences, reads containing incorrect barcodes or primer sequences, and any merged reads with a QC score <20 were removed. Sequence alignment and quality control was performed using Flask (v1.2.3) and Prinseq (v0.20.4) software, respectively. Taxonomic classification of the obtained sequences was determined based on the distance between the sequences, using the CROP method with Operational Taxonomic Units (OTU) defined at 97% similarity.

Statistical Analysis

Sequence coverage was used to obtain a sequence depth index. Community abundance was indicated using Ace and Chao indices. The richness and diversity of the AMF community species were represented by the Simpson and Shannon indices, respectively. All indices were analyzed by use of mother software. A principle component analysis was performed using vegan package R software and a redundancy analysis (RDA) was plotted in R software.

Results

The Physico-chemical Properties of the Soil

Sampled soil was characterized for the physico-chemical properties listed in Table 2. The water content of the soil from the three sampling plots varied considerably, illustrating the degree of dehydration of the wetlands W1 and W2. All three soils were acidic though the pH decreased as the water content increased. The amounts of various nutrients in the plots decreased as the water content increased.

AMF Infects the Roots of *C. angustifolia*

After the isolated roots of *C. angustifolia* were fragmented and stained by Acid Naphthol Red, the arbuscular mycorrhiza structure was examined by microscopy. The arbuscules, hyphae in roots and some vesicles were clearly visible, indicating that *C. angustifolia* is a host for AMF. The shape of the vesicles in the *C. angustifolia*roots varied from spherical too valor irregular. Generally, the vesicles were formed from the tips of inter-cellular hyphae in the root cortex. Vesicles were relatively rare and only found in a limited number of root pieces. The arbuscules were generally present at the tip of branches of the intra-cellular hyphae in the cortex cells (Fig. 2).

The degree of root infection was determined for all collected root fragments by quantization of mycorrhiza. This resulted in highly different mycorrhiza infection degrees for root fragments of different thickness. Roots with a thickness of 0.1–0.3 mm typically contained mycorrhiza infection in 29–31% of the fragments, while 0.3–0.6 mm thick roots were infected in 21–26% of the cases. Of these, the thinner root fragments were only lightly stained, while thicker fragments were stained darker. An example of both is shown in Fig. 3.

The spore density of AMF in the soil varied between the three wetland types, as shown in Table 3. All three soil types combined contained spores at a density varying between 80 – 120 spores per 50 g of dried soil. The average density was approximately 114 spores per 50 g of dried soil for marsh meadow *C. angustifolia* wetland (W2). This soil also harbored *C. angustifolia* roots with the highest degree of infection (36%, Table 3). The lowest spore density was detected in the soil of meadow wetland (W1) where roots of *C. angustifolia* also produced the lowest Infection rate of AMF (25%). The soil of W2 (meadow *C. angustifolia* wetland) reported values in between these extremes. The infection intensity was comparable among the three wetlands.

Morphological Characterization of AMF Spores Isolated from the Soil

A total of 14 morphologically distinct spore types were identified from the three soil types combined. Examples of these spores are shown in Fig. 4. Their characteristics are summarized in Table 4. A number of the spores could be speciated based on their morphological characteristics. Four belonged to the Glomus species, of which *Glomus*

Table 1: General characteristic of the study areas

Wetland type	Main plant types
Marsh wetland (W1)	*D. angustifolia*, *Anemone rivularis*, *Stachys baicalensis*, *Sanguis orbatenuifolia*, *Carex lehmanii*, *Lathyrus quinquenervius*
Marshmeadow wetland (W2)	*D. angustifolia*, *Carex lehmanii*, *Caltha palustris*, *Sanguisorba tenuifolia*, *Geranium carolinianum*
Meadow wetland (W3)	*D. angustifolia*, *Phragmites communis*, *Carex miyabei* var. maopengensis, *Stachys baicalensis Fisch*, *Glyceria lithuanica* (*Gorski*)

Table 2: Soil physical and chemical properties

Wetland type	pH	Water content (%)	Organic carbon Oc (g/kg)	Total nitrogen TN (g/kg)	Available nitrogen AN (mg/kg)	Total phosphorus TP (g/kg)	Available phosphorus AP (mg/kg)	Total potassium TK (g/kg)	Available potassium AK (mg/kg)
W1	5.81	71	58.57	3.16	213.56	8.13	20.23	20.65	200.49
W2	5.65	90	56.43	2.62	116.25	6.36	17.34	14.27	150.35
W3	5.48	191	47.96	2.45	100.77	4.60	15.12	13.68	134.67

Marsh wetland (W1, Marsh meadow wetland (W2, Meadow wetland (W3)

Table 3: AMF mycorrhizal infection of *Deyeuxia angustifolia* roots in the different wetlands

Site	Spore density (spores/50 g soil)	Infection rate of *D. angustifolia* roots (%)	Infection grade	Infection Strength
W1	82.33 ±1.76 [a]	25%	2	+
W2	113.67 ±4.05 [c]	36%	3	+
W3	95.67 ±2.60 [b]	30%	3	+

Marsh wetland (W1, Marsh meadow wetland (W2, Meadow wetland (W3)

Table 4: Morphological characteristics of isolated AMF spores

Species	Color	Shape	Size (μm)	Description of spore walls (in case of two layers, L1 is the outer layer)
Acaulospora colossica, (panel 4A)	orange brown	oval	150-160× 120-140	L1 brown, 4-5 μm.; L2 brown, 4-5 μm
Glomus etunicatum (panel 4B)	Red-brown	spheric	50-90	L1 red- brown, layered, 1-2 μm; L2 light yellow-brown to red-brown, 4 μm, not closed, straight Cyprinosporium
Species unknown *(panel 4C)*	light gray		150-220	No distinct spore walls visible
Scutellopora spp.*(panel 4D)*	light gray		230-340	L1 transparent, membrane-like, 4-5 μm; L2 transparent or light yellow, layered
Acaulospora spinosa (panel 4E)	light yellow to yellow brown		150-230	L1 only residuals left; L2light yellow to light brown, 5-8 μm.
G. geosporum (panel 4F)	light brown to red brown		150-175	L1 yellow brown, 2-3 μm; L2 layered, yellow brown
G. aggregatum (panel 4G)	light brown	nearly spheric, oval, or irregular	41-64× 55-65	Single spore wall of 3-3.5 μm, not closed, Cyprinosporium straight or curved
G. claroideum (panel 4H)	light yellow	spheric	80-100	L1 transparent, < l μm; L2 light yellow, layered, 4-5 μm, wrinkled when the spore popped up.
Aaculosopora spp.*(panel 4I)*	Yellow brown	spheric	100-120	No distinct spore walls visible, rough surface, easy to rupture
Species unknown *(panel 4J)*	transparent (individual spores)	spheric	150-210	No distinct spore walls visible, easy to rupture, wrinkled when the spore popped up.
Species unknown *(panel 4K)*	yellow brown (individual spores)	oval	170-200×210-330	No distinct spore walls visible, easy to rupture
Aaculosopora spp.*(panel 4L)*	light yellow	close to spheric	80-90	Single spore wall of 2-3 μm, brown, layered
Species unknown *(panel 4M)*	white	oval	210-270× 320-350	

aggregatum (panel 4G) were typically clustered in the soil. Four types were members *of Acaulospora* of which two could be speculated. One spore type was recognized as a *Scutellopora* species and four types remained unidentified.

Sequencing Results of the Soil Samples

Total DNA isolated from the soil samples was subjected to PCR to amplify the 28S and the amplicons were sequenced to identify Operational Taxonomic Units (OTUs) of the fungi that were present. A total of 30,006 OTUs was identified in soil from W2 but only 7264 from W3 and 5159 from W1. Thus, the samples with the lowest and highest water content had far fewer fungal OTUs than marsh meadow wetland soil. A Venn diagram is shown in Fig. 5 to illustrate the shared OTUs between the wetland types. As expected, the number of wetland-type specific OTUs was highest for W2 (24513) while the largest shared fraction was between W2 and W3 (4090). Of these, 1322 OTUs were isolated from all the three soil types. These results suggest more similarity in fungal communities between W2 and W3 than between either of these wetland types and W1.

The sequence data were used to calculate a coverage index as a measure for the coverage rate of the various sample libraries. Higher index values indicate a lower

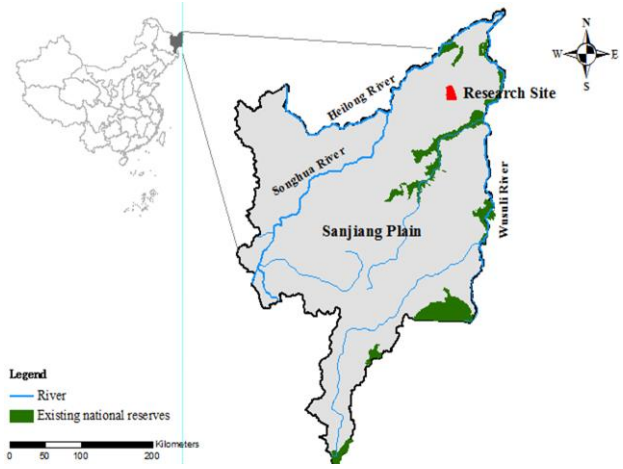

Fig. 1: Location of the sampling site (red) in the Sanjiang Plain-Honghe national nature reserve

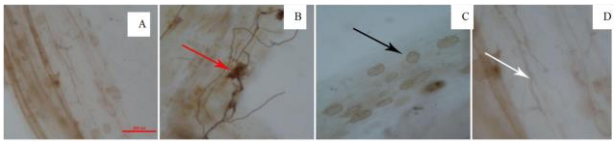

Fig. 2: Arbuscular mycorrhizal fungi on the roots of *C. angustifolia*. The red arrow (panel B) indicates the arbuscule structure (200×), the black arrow (C) indicates presence of a vesicle (100×) and the white arrow (D) points to intra radical hyphae (400×)

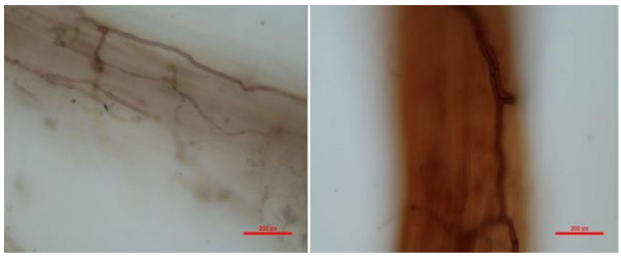

Fig. 3: Stained arbuscular mycorrhizal fungi in thin (0.1-0.3 mm, left) and thick (0.3-0.6 mm, right) roots of *C. angustifolia*

probability that a sequence is missed in the sample due to incomplete coverage. As given in Table 5, the coverage indices of all three soil samples was >0.98, with a slightly lower coverage for W2 (0.9818) than for W3 and W1. These values suggest that the sequencing results quite accurately reflect the real presence of fungi in the sampled soils.

The Ace and Chao indices estimate the species total numbers and the fungal species richness. As shown in Table 5, these two indices were much higher for W2 than for the other two wetland soil types, corresponding with the higher total numbers of OTUs in the marsh meadow wetland, which has the greatest

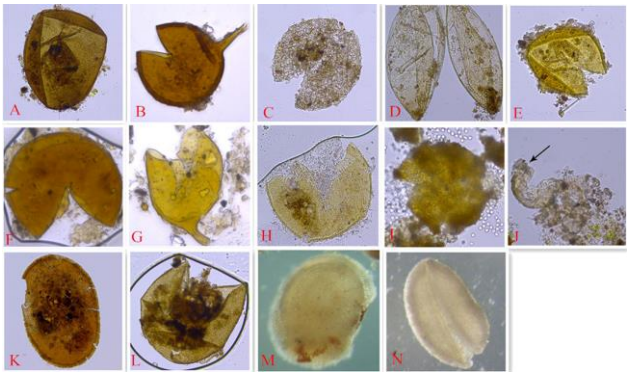

Fig. 4: AMF spores with different morphologies isolated from the soils of *C. angustifolia* wetlands. See Table 4 for a description of the panels

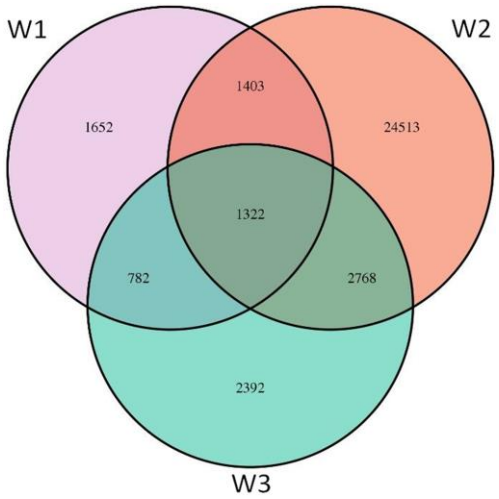

Fig. 5: Venn diagram of fungal OTUs reported from soil obtained from marsh wetland (W1), marsh meadow wetland (W2) and meadow wetland (W3)

AMF total amount and species richness.

The Shannon and Simpson Index were used to estimate the alpha diversity of the AFM in the sampling soils. Both indices were highest in W2, lower in W3 and lowest in W1, suggesting fungal communities of W2 had the highest complexity and was the least even.

Structure, Composition and Analysis of AMF Communities

According to the relative abundance of the AMF OTUs in the different soil samples, the 30 OTUs with the highest relative abundance were selected and a heatmap was generated of their abundance, to demonstrate the structural difference of the communities in the different soil types at the genus level. As shown in Fig. 6, these abundant OTUs formed 2 relatively close clusters (Cluster 1 and 2) and one more diverse cluster (Cluster 3).

Table 5: Diversity index of each soil sample at similarity 97%

Site	Coverage index	Ace index	Chao1 index	Shannon index	Simpson index
W1	0.9988	6524	6331	8.3195	0.9918
W2	0.9818	92574	97883	11.3243	0.9991
W3	0.9984	8780	8622	9.4273	0.9964

Marsh wetland (W1, Marsh meadow wetland (W2, Meadow wetland (W3)

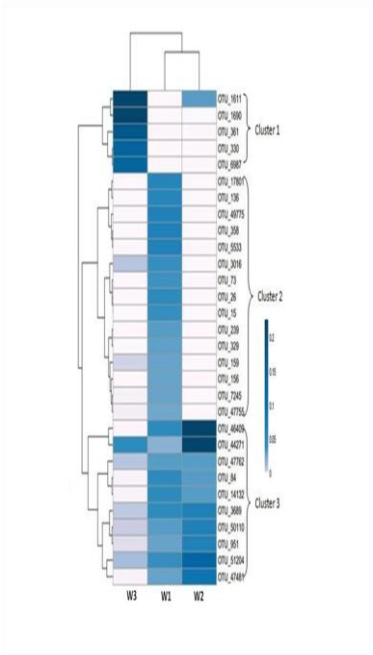

Fig. 6: Heat map plot based on the ITS sequences of the 30 most abundant OTUs in the three plots. The color scale indicates relative abundance in 1%

These three clusters segregated for the three wetland types from which the soils were sampled. Cluster 1 was mainly distributed in soil from W2, with members OTU_1611, OTU_1690, OTU_361, OTU_330 and OTU_6987 being dominant with an abundance of 6.81%, 6.60%, 6.20%, 5.67% and 5.27%, respectively. Compared to the soils in other two plots, the relative abundance of the AMF in W1 was relatively even, and the abundant OTUs found here were only present in very low numbers in the other two plots. In contrast, OTUs found in Cluster 3, typical for W2, were also found at medium abundance in W1 and at variable abundance in W3. The dominant populations in the AMF of W1 were OTU_46409, OTU_44271, OTU_51204, OTU_47481, OTU_3689, OTU_50110 and OTU_951, with relative abundances of 22.28%, 22.58%, 12.90%, 9.68%, 6.45%, 6.45% and 6.45%, respectively.

According to the latest classification of AMF (Wang *et al*., 2013), the detected OTUs could be classified into 1 class, 2 orders, 7 families and 14 genera. At the genus level

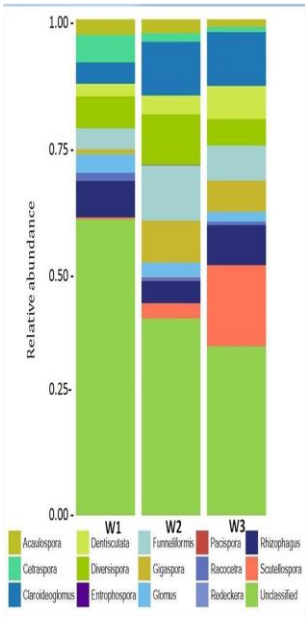

Fig. 7: Composition of the fungal community at genus level

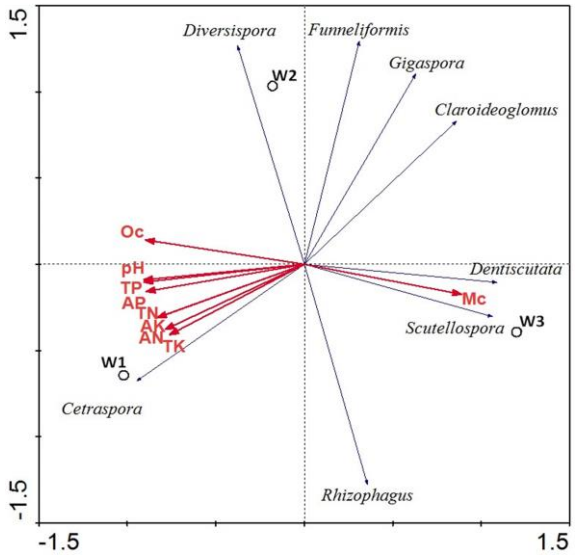

Fig. 8: RDA plot based on soil AMF communities at genus level and physico-chemical characteristics of the soil

(graphically shown in Fig. 7), *Rhizophagus*, *Diversispora* and *Cetraspora* were the major dominant population in W1, with a relative abundance of 7.34, 6.42 and 5.53%, respectively. In W2, *Funneliformis* (11.1%), *Claroideoglomus* (10.87%), *Diversispora* (10.21%) and *Gigaspora* (8.47%) were dominant, while the dominant genera in W3 were *Claroideoglomus* (10.87%), *Rhizophagus* (8.07%), *Funneliformis* (7.10%), *Dentiscutata*

(6.65%), *Scutellospora* (6.36%), *Gigaspora* (6.26%), and *Diversispora* (5.33%) (all members of Glomeromycetes). The relative abundance of members of the genus *Redeckera* was relatively low (0.01%) in all three plots. Members of the genus *Pacispora* were not detected in W1.

The Impact of the Physico-chemical Properties of the Soil on the AMF

A principle component analysis was performed to assess the impact of the soil's physico-chemical properties on the AMF community and a redundancy analysis (RDA) plot was constructed (Fig. 8).The three wetlands W1, W2 and W3 were positioned in three different quadrants, suggesting significant differences in underlying factors of the three wetland types resulting in different fungal communities that correlated to their different water tables. Indeed, the water content in the soils correlated positively with the x-axis, while the content of nutrients in the soils correlated negatively with the x-axis and thus with the water content. Table 1 already showed that the higher the water content in the soil was, the fewer nutrients were found in the soil. Among the different AMF communities, members of the genus *Cetraspora* (which were mostly found in W1, see Fig. 7) had a positive relationship with presence of soil nutrients and a negative relationship with the water content. In contrast, the genera *Dentiscutata* and *Scutellospora* (mostly found in W3 marshland) correlated negatively with soil nutrients and positively with water content. Additionally, in terms of the distribution pattern of the AMF communities, the dominant genus/genera in the three plots were, respectively, *Cetraspora* in W1, *Diversispora* in W2, *Dentiscutata*, *Scutellospora* and *Rhizophagus* in W3.

Discussion

The symbiosis of AMF with plants is a common phenomenon in terrestrial ecosystems, and the wetlands of Sanjiang Plain are no exception (Shi *et al.*, 2017). AMF are important members of the soil microbiota with the largest biomass. A number of studies have investigated the structure of the AMF population in various wetlands and their findings suggest that AMF are present in wetland, though significantly fewer types are found compared to dry terrestrial ecosystems (Li *et al.*, 2013). In wetlands, the most common AMF types belong to the genus *Glomus[21-23]*, although other genera such as *Paraglomus*, *Archaeospora*, *Geosiphon*, *Acaulospora*, *Entrophospora*, *Scutellospora*, *Diversispora*, and *Gigaspora* can also be found (Bohrer *et al.*, 2004; Wang and Zhao, 2006; Wang *et al.*, 2008; Choudhury *et al.*, 2010). In this study, we characterized the spores isolated from soil sampled in three wetland types of the Sanjiang Plain at different levels of dewatering. By microscopic examination of the spores morphology we identified members representing at least 3 genera, including 4 members of the genus *Glomus*, 4 *Acaulospora* spp. and 1 *Scutellospora* spp. Four AMF remained unidentified based on morphological characteristics of the spores. By amplification and sequencing of the ITS of the ribosomal gene locus, DNA could be identified that belonged to AMF from 1 class, 2 orders 7 families, that included 8 previously described wetland-associated genera (*Claroideoglomus, Funneliformis, Diversispora, Scutellospora, Gigaspora, Glomus, Acaulospora* and *Entrophospora*) as well as 6 genera that have, to the best of our knowledge, not been observed in wetlands before (*Racocetra, Pacispora, Rhizophagus, Dentiscutata, Cetraspora* and *Redeckera*). A comparison of the results obtained by the two methods showed that identification by morphology of isolated spores did not necessarily detect the most abundant species and left a number of spores unidentified.

There are multiple factors affecting the diversity of the AMF population, including the plant hosts, oxygen and water levels, season, pH and availability of soil nutrients (Yan *et al.*, 2008). In this work, the ranking of both Ace and Chao indices suggested that the difference in the water levels of the three *C. angustifolia* wetland types related to distinct AMF total amounts and community abundance. An RDA analysis showed that in the three sampled wetlands, the soil water content correlated negatively with the soil nutrients, and most of the AMF belonging to *Dentiscutata* and *Scutellospora* species had a positive correlation with the soil water content. This suggests that in the three wetland types, as the soil water content decreased, the pH value increased, soil nutrients became more abundant, and the AMF abundance decreased. It was demonstrated by numerous studies that the AMF infection rate of plant roots is inhibited by water, so that soil with lower water content resulted in an increase in AMF diversity (Mentzer *et al.*, 2006; Ipsilantis and Sylvia, 2010). That is not exactly what we found, as the richest and most diverse AMF population was found in the soil type that was only partly dewatered. This is consistent with the findings of Carvalho *et al.* (2006), who sampled wetland along a water gradient. They found that 9 AMF species were recovered in total but the composition and the structure of the population were different in the plots (Carvalho *et al.*, 2001; Bohrer *et al.*, 2004; Ipsilantis and Sylvia, 2010). It was previously reported that the primary factor affecting the AMF composition was readily available phosphorus (Lin *et al.*, 2012; Likar *et al.*, 2013; Xiang *et al.*, 2014), while some other studies claimed that readily available nitrogen influenced the AMF equally (Hao *et al.*, 2011; Lin *et al.*, 2012). In our study, AMF of the *Cetraspora* related positively with soil nutrients, but most of the other AMF types had a negative correlation. This is similar to findings of Li *et al.* (2013) from the Qinghai-Tibet Plateau and Bohrer *et al.* (2004) who studied a forest near the Atlantic Ocean. In those studies, organic matter, nitrogen and phosphorus in the soil affected the AMF diversity significantly. Landwehr *et al.* (2002) showed that there was a large number of *Glomus geosporum* in alkaline soil.

Others have argued that the pH value not only impacts the AMF composition of the wetland plants, but also the type of plants, which again affects the associated AMF (Yan *et al*., 2008). These results were consistent with our findings that the diversity of AMF correlated positively with pH.

Among the three *C. angustifolia* wetlands compared here, the driest meadows wetland (W1) was no longer submerged by water while the marsh wetland (W3) was completely submerged. Our findings indicate that the largest and most diverse AMF population can be found in the intermediate marsh meadow wetland, which is occasionally flooded. These results are consistent with the aerobic growth of AMF, as the high water content of marsh wetland would result in poor aeration in the soil, which may have limited the growth and development of AMF (Yan *et al.*, 2008). In contrast, the high nutritional value of the soil in dry meadow wetland (W1) may also have limited AMF abundance. Although, some researchers have argued that AMF may obtain oxygen by forming special structures through the aeration tissues of the plant host (Bohrer *et al.*, 2004) in our study, the *C. angustifolia* floated or was positioned at the water-air interface, which may have improved the aeration and may have led to a large number of AMF. Different wetlands are populated by different plant species, and the characteristic difference of the root systems of plants may further affect the community structure of the AMF that live in symbiosis with plants.

Conclusion

At present, the knowledge on AMF abundance and diversity in wetlands is still limited, but comparative studied such as the one presented here are a good start. These data need to be completed with more research studies, more detailed morphologic characterization, and growing databases to improve our knowledge on the presence of AMF populations in wetlands.

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