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Full Length Article

Response of Soil AMF Diversity to Nitrogen Deposition in a *Calamagrostis augustifolia* Wetland of Sanjiang Plain, China

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Abstract

Calamagrostis angustifolia Kom, a perennial plant of the family Gramineae, is the main species of the Sanjiang Wetland, China. Because of global climate change and the impact of human activities, the Sanjiang Plain Wetland has been seriously degraded, which has had a serious impact on stability of the ecosystem in Northeast China. Arbuscular mycorrhizal (AM) symbiosis can play a major role in enhancing stress resistance. In this study, soil diversity, physiochemical properties, and ITS rDNA responses of AM fungi with *C. augustifolia* under three nitrogen depositions at different loads were investigated. The results showed that both low-and high-level nitrogen deposition reduced the AM fungal diversity. In each of the three types of nitrogen-amended soils, the most predominant phylum was *Glomeromycota*. The abundance of *Glomeromycota* decreased with nitrogen deposition. A heatmap tree based on internal transcribed spacers (ITS) rDNA sequences established that different arbuscular mycorrhizal fungal communities respond differently to nitrogen deposition. Based on our results, we posit that changes in nitrogen deposition gradient in this study influenced the soil AM fungal community structure in Sanjiang Plain Wetland, especially those belonging to the phylum *Glomeromycota.*

Keywords: AMF diversity; *Calamagrostis augustifolia* wetland; ITS rDNA; Nitrogen deposition

Introduction

Atmospheric nitrogen deposition is increasing rapidly because of large-scale combustion of fossil fuels associated with technological advances and industrial and agricultural development (Tian *et al.*, 2019). According to the NITREX (Nitrogen Saturation Experiments) project in Europe, the critical point of forest nitrogen saturation is 25–60 kg hm^{-2} (Van Dobben and De Vries, 2017). The annual deposition of atmospheric nitrogen in North America has already increased to 40 kg hm^{-2} (Li *et al.*, 2013), which exceeds the maximum saturation concentration of nitrogen. From 1980 to 2010, the total nitrogen deposition in China increased at an average rate of 0.41 kg N hm^{-2} $year^{-1}$, resulting in nitrogen deposition in some areas (North China Plain) reaching 150 kg N $hm^{-2}a^{-1}$ (He *et al.*, 2010). The phenomenon of atmospheric nitrogen deposition has played a crucial role in variations in global ecosystems, aggravating the acidification of soil and reducing the variety and function of living life.

The arbuscular mycorrhizal network formed by the arbuscular mycorrhizal fungi (AMF) in the soil drives ecosystem nutrient cycling, improves plant nutrition, promotes biological material exchange and energy flow, enhances plant stress resistance, and repairs degradation and pollution (Javaid, 2009). Arbuscular mycorrhizal fungi play important roles in plant establishment, community succession, species diversity formation and stabilization of the ecosystem (Chu *et al.*, 2016). AMF are aerobic microorganisms, and their spores and mycelia require certain ventilation conditions for growth and development; therefore, the relatively low gas content in the soil environment in the field limits the symbiosis between AMF and plants to some extent. In the past, it was widely believed that AMF do not exist in wetlands or their role is very limited. In recent years, many studies have demonstrated the symbiosis and wide distribution of AMF in wetland plants, and research on the AMF structure of wetland plants has also received attention (Duck *et al.*, 2010). To date, no studies have investigated the Sanjiang Wetland ecosystem and AMF. Many researchers have found that an increased nitrogen deposition may have adverse impacts on AMF (Yang *et al.*, 2013), indicating that nitrogen deposition could change the compositions and functions of AMF communities. Hence, understanding the factors that cause variations in AMF diversity is of great importance to understanding the responses of ecosystems to nitrogen deposition.

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Sanjiang Plain, which is the largest freshwater wetland in China (Sui *et al.*, 2016), has a great influence on climate stability in Northeast China. The wetland had been seriously damaged by anthropogenic activities, which have changed the structure and function of the wetland system (Sun *et al.*, 2007). Our laboratory has investigated nitrogen deposition effects on the community composition of soil bacteria in a *Calamagrostis augustifolia* wetland, the effect on biomass in this type of wetland (Zhao *et al.*, 2012), or effects on bacterial species present in soils at 2016 (Sui *et al.*, 2016). It has also been reported that increased nitrogen did not have significant effects on the bacterial community but did show decreased abundance in a number of primary bacterial groups (Sui *et al.*, 2016). Nevertheless, the effects of nitrogen deposition on the arbuscular mycorrhizal fungal community are not well known. Therefore, this study was conducted to investigate the effects of simulated nitrogen deposition on the soil AMF diversity of a *C. augustifolia* wetland in Sanjiang Plain. Field investigation combined with morphological observations and molecular biology and other methods were used to investigate the diversity of AMF and analyze the changes in its community structure and composition to understand the differences in structure and function of AMF in Sanjiang Wetland and provide references to help reveal the function and mechanism of AMF in the wetland ecosystem. The results presented herein provide a scientific basis for studying the changes in wetland ecosystems in the Sanjiang Plain in the background of nitrogen deposition, as well as a theoretical reference for the protection and management of wetlands in Sanjiang Plain.

Materials and Methods

Overview of the Study Area

The research area is located in the Honghe National Nature Reserve, Sanjiang Plain Wetland (47°45′39″N, 133°37′04″E). The altitude of this area is 55–65 m, the average annual temperature is 1.9°C and the average annual precipitation is 585 mm, 50%–70% of which occurs in July–September and the average annual evaporation is 1,166 mm. This study was conducted at a *C. augustifolia* wetland that is typical of this region.

Sampling

The experimental set-up considered three nitrogen treatments, control or N1 (no added nitrogen), low nitrogen or N2 (4 g N hm^{-2} a^{-1}) and high nitrogen or N3 (8 g N hm^{-2} a^{-1}). Treatment plots were 20 m × 20 m. The control plots (N1) were treated with water, while the other plots were uniformly sprinkled with an aqueous solution of NH_4NO_3 at the specified dose from 2011 to 2014 in May. A total of 5–8 sampling sites within each plot were sampled at random by collecting surface soils (0–10 cm in depth), after which they were mixed to give samples representative of each plot. After the three soil samples were obtained, the plant and animal debris were removed. Prior to AMF community analysis of soil composition, one aliquot of each sample was stored at -80°C. The remaining soil was then air dried thoroughly. The sieved samples were used to determine the soil physical and chemical properties.

Measurements of Soil Physicochemical Properties

Soil pH was measured in suspension at a water-soil ratio of 2.5:1. The total organic carbon (TOC) of the soil was measured by a carbon and nitrogen analyzer (Jena-2100S, Germany). The total nitrogen (TN) was determined using the semi-micro Kjeldahl method, while the nitrate nitrogen (NO_3) was determined by phenolsulfonic acid colorimetry and the ammonia nitrogen (NH_4^+) was extracted with potassium chloride and then determined by the dophenol blue method. The total phosphorus (TP) was determined using 6.5 N molybdenum sulphate coloring solution, while available phosphorus (AP) was extracted with 0.5 mol L^{-1} sodium bicarbonate and determined by molybdenum rhodium coloration.

Isolation of Soil Total DNA

Total DNA was extracted from the soil using a Soil DNA Isolation Kit (PowerSoil®, Mobio, USA) according to the manufacturer's instructions. The extracted DNA was dissolved in 100 μL of deionized water, after which 2 μL of the synthetic solution was taken to determine the purity and concentration of the extracted DNA.

Sequencing of Soil AMF rDNA

ITS DNA was amplified using the 1737F and 2043R primers. The PCR reaction system included 2 μL of template DNA (10 ng), 1 μL of each primer (30 μmol L^{-1}), 12.5 μL of PCRMix (TAKARA, Dalian, China) and 9.5 μL of deionized water. The amplification reaction procedure was as follows: pre-denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, extension at 72°C for 30 s and then final extension at 72°C for 10 min. Each sample was repeated three times and the PCR products were combined and purified using an agarose gel DNA purification kit (TaKaRa, Dalian, China). The recovered product was assayed for concentration using a Qubit 2.0 DNA Detection Kit (Life Technologies, USA) and then subjected to MiSeq sequencing.

Data Analysis

OTU-based Analysis

By extracting the total genomic DNA of the sample, PCR amplification was performed using universal primers targeting the ITS, and the microbial diversity in the sample was analyzed by sequencing. If the similarity of different rRNA sequences between sequences was greater than 98%,

they were defined as operational taxonomic units (OTUs), and each OTU corresponded to a different fungal species.

AMF Community Diversity and Rarefaction Curve

Alpha-diversity analysis is a single-sample method that can reflect the abundance and diversity of microbial communities. The α-diversity index of AMF communities was determined using a similarity level of 97%. The richness and evenness of soil microbial communities were indicated by the Chao and ACE indices, respectively. The diversity was represented by Shannon's index and Coverage was the sequencing depth index (Table 2).

Using a method of random sampling of optimized sequences, a rarefaction curve was constructed based on the number of sequences extracted and the number of OTUs they represented. A Rank-Abundance curve was used to simultaneously explain two aspects of sample diversity; namely, the richness and evenness of the species contained in the sample. The abundance of species was reflected by the length of the curve on the horizontal axis. A wider curve indicated greater species richness, while the uniformity of the species composition was reflected by the shape of the curve, with a flatter curve indicating higher uniformity of species composition.

Redundancy Analysis (RDA)

Redundancy analysis (RDA) were used to identify the abiotic factors that are most important to the AM fungal community composition and these results were used to construct the soil property matrix for variation partitioning analysis in R v3.4.3 using the vegan package.

Results

Soil Physiochemical Properties under Simulated Nitrogen Deposition Conditions

Table 1 shows the soil physical and chemical properties of the simulated nitrogen deposition in the test field. As expected, as nitrogen deposition increased, TN, NO_3^- and NH_4^+ showed an increasing trend, while AP showed a downward trend. Concentrations of TN, NO_3^-, NH_4^+ and AP all exhibited significant differences (P<0.05) among soils treated with different nitrogen concentrations, while soil pH, TOC and TP only produced significant differences for high levels of nitrogen deposition (N3) compared to the other two samples.

Distribution of Soil AMF Community under Different Nitrogen Deposition Conditions

The Venn diagram shows the number of common and unique OTUs and demonstrates the similarity and overlap of all samples. In total, 33,505 OTUs were detected, of which 3700

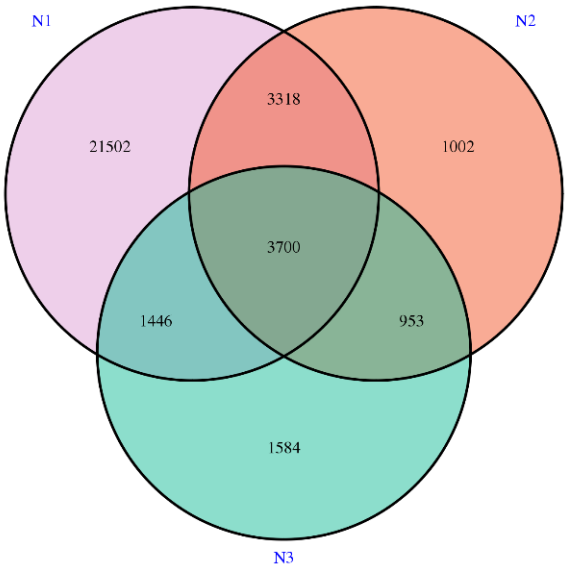

Fig. 1: Venn diagram showing the numbers of shared and exclusive OTUs of AMF identified from different soil samples

(11.04%) were shared between the N1, N2 and N3 treatments (Fig. 1). There were 1584 OTUs found only in N3, accounting for 4.73% of the total. N1 samples produced the highest number of specific OTUs (21 502, or 64.18%), while 1002 OTUs were specific for N2, accounting for 2.99%. A large proportion of OTUs were shared in N1 and N2 (9.90%), while 4.32% of OTUs were shared between N1 and N3 and 2.84% were isolated from both N2 and N3. Therefore, the distribution of OTUs from the N1 and N2 soil was most different among the three nitrogen treatments.

OTUs Rarefaction Curves and Soil AM Fungal Diversity Indices under Simulated Nitrogen Deposition Conditions

The sequences were classified by distance between sequences, and OTUs were classified by 98% similarity using the CROP method. The obtained effective sequences were aligned in GenBank (National Center for Biotechnology Information), and each sequence was obtained for classification (Stach *et al.*, 2003). As shown in Fig. 2, the dilution curve, which can truly reflect the fungal community in the soil sample and represent the diversity of fungal communities, tended to be flat. The coverage rate reached more than 99%, indicating that the sampling was basically reasonable and the confidence of the fungal community structure in the actual environment was high.

The obtained diversity of soil fungal ITS rDNA under the three different nitrogen deposition conditions is outlined in Table 2. The Shannon indices for N1, N2 and N3 were 11.32, 9.79 and 9.64, respectively. The richness indices (Chao1) were 99,660, 11,534 and 9364 respectively. The control (N1) soil showed an increase in the diversity and richness of the soil AMF, while the soil exposed to nitrogen at low (N2) and high (N3) levels had diversity and richness

Table 1: Physical and chemical properties under different nitrogen concentration conditions

Treatment	pH	Organic carbon g kg^{-1}	Total Nitrogen g kg^{-1}	Ammonium Nitrogen mg kg^{-1}	Nitrate Nitrogen mg kg^{-1}	Total P g kg^{-1}	Available P mg kg^{-1}
Control (N1)	5.92±0.08b	35.05±1.76a	2.47±0.26a	16.37±0.56a	4.75±0.08a	1.12±0.12a	58.82±3.12c
Low nitrogen (N2)	5.87±0.05b	36.67±1.53ab	2.73±0.32b	19.45±0.68b	5.24±0.09b	1.15±0.11a	51.24±2.21b
High nitrogen (N3)	5.75±0.02a	38.23±1.28b	3.52±0.14c	24.31±0.43c	6.16±0.06c	1.21±0.15b	47.80±3.91a

Note: Different superscript letters in the same column indicate significant differences among different samples at $P < 0.05$

Table 2: AMF diversity indices of *Deyeuxia angustifolia* wetlands under simulated nitrogen deposition

Treatment	Ace	Chao1	Shannon	Simpson	Goods coverage
N1	92656	99660	11.32	0.999	0.982
N2	11359	11534	9.79	0.997	0.998
N3	9244	9364	9.64	0.998	0.998

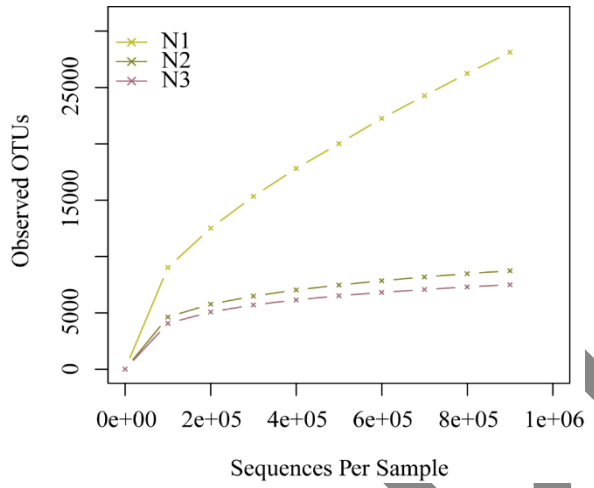

Fig. 2: Rarefaction curves of soil AM fungi OTUs in different nitrogen treatments

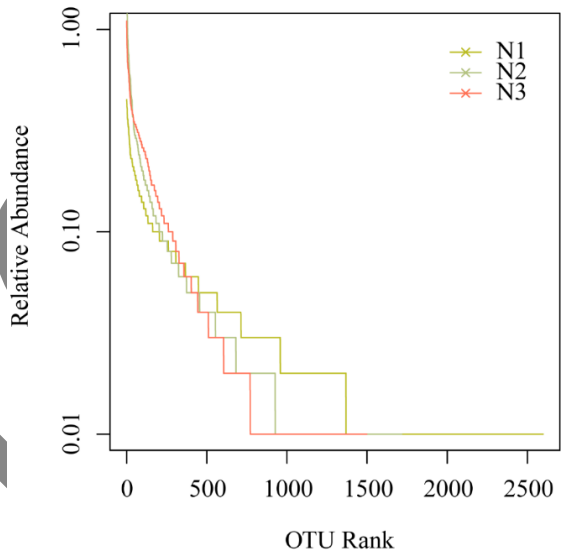

Fig. 3: Rank-abundance distribution curve of OTUs from soil samples

values that were lower than those of the control sample. Thus, under nitrogen concentration tested settling, the richness and diversity of soil fungi in *Deyeuxia augustifolia* wetlands showed significant differences. In addition, long-term exposure to enhanced nitrogen deposition causes the soil fungi community to differ significantly.

Analysis of AM Fungal Community Structure

The graded abundance curves of OTUs in different nitrogen treatments showed that, in the soil AM fungal communities at different altitudes, a small proportion of relatively high abundance OTUs were dominant (Fig. 3).

Based on the relative abundance of OTUs in the soil sample AMF, the first 30 OTUs with higher relative abundance were clustered and heatmaps were generated. The heatmap tree showed the differences among AM fungal communities in the different genera directly.

As shown in Fig. 4, four clusters were produced based on the ITS rDNA in the soil AM fungal communities. The OTUs of relatively dense AMF in Cluster 1 were mainly distributed in N3, of which OTU_111, OTU_30, OTU_449, and OTU_104 were dominant, while the relative abundance of OTUs of the AMF of the other two sample controls was very low or absent. The OTUs of AMF with relatively high abundance in Cluster 2 were mainly distributed in low-level nitrogen (N2), among which OTU_210, OTU_170 and OTU_7945 were the dominant clusters. The relative abundances of various AMF in this plot were averaged compared to the other two controls, while the relative abundances of the OTUs of the same plot in the other two controls were very low. OTU_661, OTU_120, OTU_8 and OTU_1069 of AMF in N2 and N3 in Cluster 3 were dominant groups, and the relative abundance of OTUs of AMF in these two sample sites was similar. In Cluster 4, OTU_577 was the dominant group in N2.

According to the latest AMF classification system (Wang *et al.*, 2013), a total of one class, two orders, seven families and 14 genera of AMF were obtained from the sequencing (Fig. 5). Species of three genera, *Funneliformis*, *Claroideoglomus* and *Diversispora*, were dominant in the control (N1), with relative abundances of 11.10%, 10.87%, and 10.21%, respectively. In low-level nitrogen (N2), the genera *Funneliformis*, *Claroideoglomus*, *Diversispora* and *Gigaspora* were the dominant groups, with relative

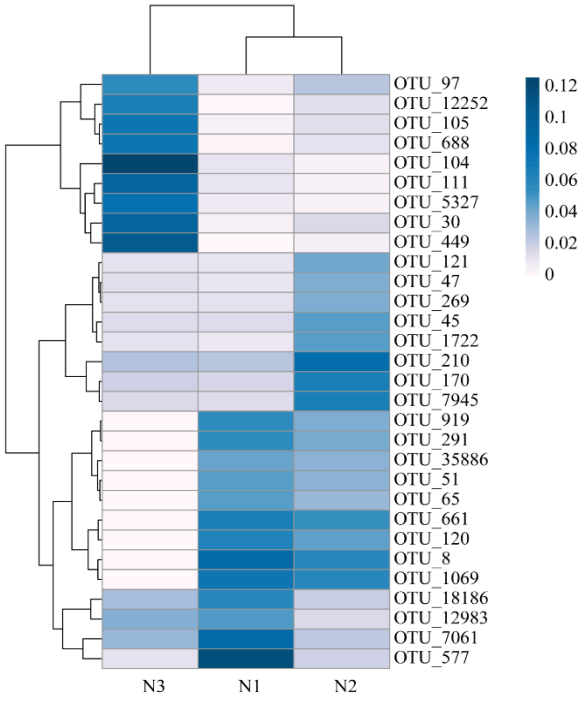

Fig. 4: Heatmap tree based on the AM fungal ITS rDNA sequences

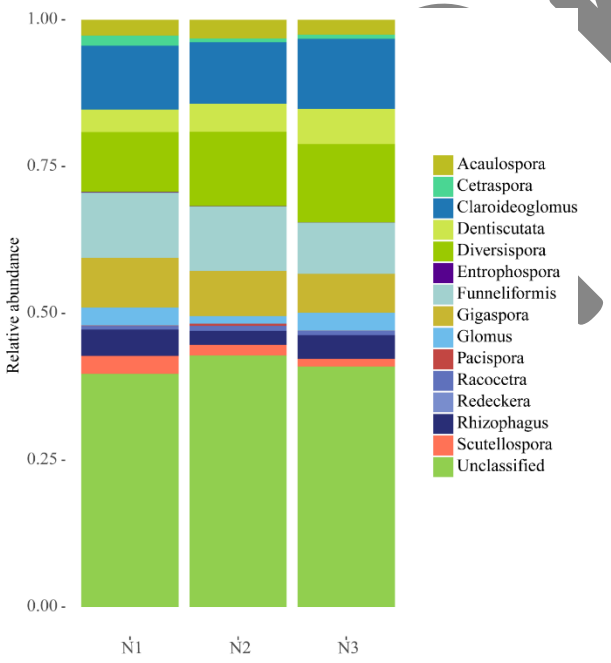

Fig. 5: Genera found in AM fungal communities derived from different elevations

abundances of 10.97%, 10.41%, 12.67%, and 7.70%, respectively. The high-level nitrogen (N3) group was dominated by the genera *Claroideoglomus*, *Diversispora*, *Funneliformis* and *Gigaspora*, which were present with relative abundances of 11.95%, 13.34%, 8.72% and 6.63%, respectively.

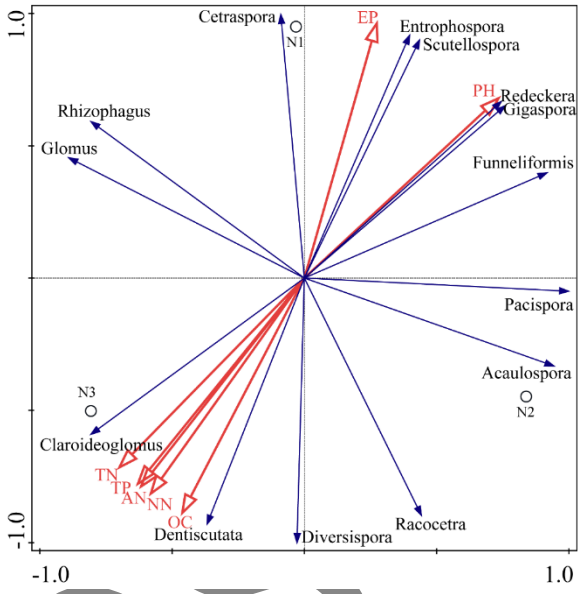

Fig. 6: RDA based on soil AMF community at the genus level and environmental factors of the soil

Correlation between Soil AM Fungal Diversity and Environmental Factors

Redundancy analysis (RDA) was employed to quantitatively analyze the contributions of the factors to the soil AM fungal communities (Fig. 6). N1 is located in the first and second quadrant, N3 in the third quadrant and N2 in the fourth quadrant, which indicates that the three plots are different due to differences in their nitrogen gradients. The soil pH was positively correlated with the first axis, indicating that the soil nutrients decreased with increasing soil pH in the three plots. Among all AMF groups, the genera *Claroideoglomus* and *Dentiscutata* were positively correlated with soil nutrients and negatively correlated with soil pH, while *Redeckera* and *Gigaspora* were negatively correlated with soil nutrients and positively correlated with soil pH.

Discussion

Biological diversity indices are very useful for analyzing microbial communities in different soils. While higher values of such indices indicate a higher diversity of microbial communities, the indices consist of two components: richness and evenness (Harcha *et al.*, 1997; Fontúrbel *et al.*, 2012). The findings in this study indicated that the diversity of AMF had changed significantly because of the different nitrogen deposition conditions, and the Shannon indices decreased in the order of N3>N2>N1 (Table 2). This implied that nitrogen deposition decreased the wetland soil diversity. Many studies have included nitrogen application experiments that showed increases in N availability significantly changed the richness and structure of plants and arbuscular mycorrhizal fungi (Van Diepen *et al.*, 2010).

Knops *et al.* (2002) found that, after application of nitrogen, the structure of AMF species changed, and the number of spores decreased. The AMF species richness also showed a downward trend.

Soil microbes, which are the most active part of soil living organisms, are involved in the decomposition of organic matter, synthesis of humus, nutrient transfer, and the formation and development of soil. After nitrogen fertilizer is applied, it needs to be decomposed into small molecules in the soil, which will locally form a higher concentration of substances, thereby influencing soil microorganisms. Nitrogen affects soil microbial communities as well as cycling of carbon and other nutrient elements in forest soils. In field control experiments, nitrogen addition reduced microbial biomass by 15% (Treseder, 2008) and in some experiments, nitrogen deposition altered soil microbial community structure (Compton *et al*., 2004; Frey *et al*., 2004).

Nitrogen has important biogeochemical effects in the biosphere and increasing levels of anthropogenic nitrogen deposition have caused unpredictable and devastating damage to terrestrial ecosystems, including acidification and eutrophication, which in turn alters vegetation. Mycorrhizal fungi and rotted organisms are particularly susceptible to nitrogen deposition in many ecosystems (Treseder, 2004). Changes in these microbial communities not only impact biodiversity, but also affect the biogeochemical cycles of plant functions, nutrients and carbon. After two years of nitrogen application, the total microbial biomass of soil microorganisms decreased by 49%, which is consistent with the results of Diepen *et al.* (2010), who found that the total biomass of soil microorganisms decreased by 24% in a continuous nitrogen addition experiment. In this experiment, nitrogen application reduced the total biomass of microorganisms and the fungi: bacteria ratio. Wallenstein *et al.* (2006) also found that, in forest ecosystems, long-term nitrogen application reduced soil total microbial biomass and fungal:bacterial ratio. The short-term application of nitrogen fertilizer in the boreal forest system has no effect on microbial biomass and fungal: bacterial ratio. Meta-analysis by Treseder (2008) indicated that the microbial biomass showed a decreasing trend with increasing nitrogen addition and the extension of nitrogen retention time.

This might be because nitrogen deposition would increase soil nutrients, facilitating fungal growth, but only at concentrations within a certain range, which is in agreement with results of a study conducted by Liu *et al.* (2012). Exceeding the threshold concentration, high-level nitrogen would change the availability of soil nutrients and facilitate the growth of certain microbial species (*e.g.*, those preferentially utilizing plant debris) while suppressing other microbial species, and thus make the AMF community diversity decrease. Thus, nitrogen deposition plays a complex role in changes in the composition of soil fungal communities. If the observed trends can be generalized to other ecosystems, high nitrogen deposition in some regions of China is likely to affect local soil fungal communities (Zheng *et al.*, 2014; He *et al.*, 2016).

Analysis of soil AMF diversity revealed that pH is an important factor affecting their diversity. Changes in pH plays an important role in the decomposition and formation of soil organic litter and the synthesis and transformation of nutrients containing N, P and K (Harcha *et al.*, 1997). The pH of the N1 and N2 samples was higher than N3 under different nitrogen deposition conditions. Two soil samples, N2 and N3, showed an increase in TOC content and an expected increase in nitrogen after treatment compared to N1. This may have been because the introduction of nitrogen promotes carbon sequestration, thereby increasing soil TOC content, which is consistent with the results reported by Fontúrbel *et al.* (2012). These findings suggest that the introduction of exogenous nitrogen into the wetland increases the available nitrogen in the wetland ecosystem and has a significant impact on the TOC content of the wetland soil. However, the introduction of exogenous nitrogen in the form of NO_3^- and NH_4^+ increases the mineralization rate of soil nitrogen, resulting in an increase in mineralized nitrogen and TN in the soil. These findings are consistent with the results of most other researchers, who have reported that soil NO_3^- and NH_4^+ tend to increase with nitrogen deposition until soil nitrogen saturation occurs (Ling *et al.*, 2015). Wang *et al.* (2013) studied the early response of soil available nitrogen to simulated nitrogen deposition in southern subtropical forests. The results revealed that atmospheric nitrogen deposition leads to an increase in soil available nitrogen and that higher levels of nitrogen deposition result in more soil available nitrogen. Evaluation of the early response of soil available nitrogen to simulated nitrogen deposition in southern subtropical forests revealed that atmospheric nitrogen deposition caused an increase in soil available nitrogen, and that higher levels of nitrogen deposition resulted in more soil available nitrogen.

The sequencing results of ITS rDNA showed that *Glomeromycota* was the main component of AM fungi, accounting for 60.29%, 57.17% and 59.05% of the AM fungal population in the three nitrogen deposition plots, respectively. Nitrogen deposition changes the relative abundance of *Glomeromycota* and decreases with the deepening of nitrogen deposition. Our study found that the soil *Glomeromycota* abundance in low-level nitrogen decreased more than high-level nitrogen, which may be the reason for soil nitrogen saturation in high-concentration nitrogen.

RDA analysis revealed that soil pH was negatively correlated with soil nutrients in most of the three treatments. Most AMF, such as *Redeckera* and *Gigaspora*, were positively correlated with soil pH, indicating that the three treatments increased with increasing of nitrogen deposition. Many studies have concluded that the primary factor affecting the composition of AMF is the available phosphorus (Ling *et al.*, 2015; Chen *et al.*, 2017), while some studies have found that fast-acting nitrogen also has a greater

impact on AMF (Van Diepen *et al.*, 2011; Yang *et al.*, 2013). In this study, AMF of *Claroideoglomus* and *Dentiscutata* were found to be positively correlated with soil nutrients, while most other AMF were negatively correlated with soil nutrients, which was related to the soil organic matter and nitrogen fertilizers. Phosphorus and other factors that can significantly affect the results of AMF diversity were similar.

The arbuscular mycorrhizal fungal community is considered to be a sensitive indicator of N enrichment. In a low-forest in southern California, two years of nitrogen fertilizer addition experiments revealed that N enrichment reduced the diversity of arbuscular mycorrhizal fungi and changed the mycorrhizal community structure. The species of arbuscular mycorrhizal fungi change with nutrient uptake and metastasis, which also affects plant symbiosis. Many factors lead to the reduction of arbuscular mycorrhizal symbiosis under nitrogen-rich conditions. One possible reason for this is that nitrogen addition reduces the host plant's carbon supply to AMF for growth, while another is that mycorrhiza is directly affected by the soil: high nitrogen content. Wallander (1995) argues that the reduction in mycorrhizal growth is not due to a decrease in C delivered to AMF; rather, this occurs because increasing N input increases C use by mycorrhizal fungi as a cost for the absorption assimilation of N rather than for its own growth. Zheng *et al.* (2014) suggested that soil available phosphorus, pH and nitrate nitrogen content were important factors affecting the AMF community structure. EgertonWarburton *et al.* (2007) concluded that the AMF community structure is significantly related to soil pH and N availability. Changes in cations and anions under nitrogen addition potentially influence soil pH and soil inorganic nitrogen availability. Moreover, the effects of nitrogen application on the spore density and community structure of arbuscular mycorrhizal fungi are not direct and instead mainly occur through soil regulation, followed by plants. Some scholars have pointed out that N input can influence AMF community structure through indirect plant regulation pathways. Moreover, the N-soil-AMF pathway found in severe P-restricted areas has been shown to be more potent than the N-soil-AMF pathway (Liu *et al.*, 2013). Nitrogen addition may weaken the inter-linkage between plants and microorganisms and is achieved by the inhibitory effects of soil nutrient availability.

Conclusion

Simulation experiments show that nitrogen deposition has a significant impact on the physical and chemical properties of the soil in Sanjiang Plain wetland and AMF community structure. As nitrogen deposition levels increased, the soil pH decreased significantly and the soluble carbon nutrient content increased significantly. The diversity, richness, and evenness index of soil AMF varied significantly and the composition of AMF communities decreased with increasing nitrogen deposition. The relatively high *Glomeromycota* abundance in the three soil samples (N1, N2, N3) may be because the soil pH is slightly below 7 and more conducive to the growth of certain species of *Glomeromycota*.

Acknowledgements

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