INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17F–003/2017/19–4–841–848
DOI: 10.17957/IJAB/15.0370
http://www.fspublishers.org

Full Length Article

Response of Soil Fungal Diversity to Nitrogen Deposition in a *Deyeuxia augustifolia* Wetland of Sanjiang Plain, Northeast, China

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Abstract

In order to understand the effects of nitrogen deposition on soil fungal diversity, a study was conducted with a *Deyeuxia augustifolia* wetland in the Sanjiang Plain, China, using an experimental setup simulating nitrogen deposition at different loads over a five-year period. Three conditions were investigated: N1 (no artificial deposits, control), N2 with low-level nitrogen deposition (4 g $N \cdot hm^{-2}$ a^{-1}) and N3 with high-level nitrogen deposition (8 g $N \cdot hm^{-2}$ a^{-1}). At the end of the experiment the soil physiochemical characteristics were determined and high-throughput sequencing was employed to monitor the soil fungal diversity. The addition of exogenous nitrogen to the soils changed the physicochemical properties of the soils and affected the fungal community composition and the relative abundance of species: low doses increased the diversity while high doses reduced the fungal diversity. In each of the three types of nitrogen-amended soils, the most predominant phyla were Ascomycota followed by Basidiomycota. The abundance of Basidiomycota decreased with nitrogen deposition, while Ascomycota changed in the opposite direction. A heatmap tree based on ITS rDNA sequences illustrated how different fungal communities responded differently to nitrogen deposition. The findings in the present study provide fundamental data and theoretical insights that can be employed to predict the effects of atmospheric nitrogen deposition on wetland soil fungi and wetland ecosystems in Sanjiang Plain.

Keywords: Fungal diversity; *Deyeuxia augustifolia* wetland; ITS rDNA; Nitrogen deposition

Introduction

Large-scale combustion of fossil fuels, resulting from technological advances and increased demands from industrial and agricultural development since the industrial revolution have resulted in a rapid increase of atmospheric nitrogen deposition. For instance, a European field-scale manipulation project pointed out that the annual nitrogen deposition in central European forests was 25～60 $kg \cdot hm^{-2}$ (Dobben and De Vries, 2017), well beyond the annual requisite amount in forests (Tatarinov *et al.*, 2011). Moreover, the annual deposition of atmospheric nitrogen in North America has already increased to 40 $kg \cdot hm^{-2}$ (Li *et al.*, 2013; Gryta *et al.*, 2014) and has exceeded the maximum saturation concentration of nitrogen. Researchers from China reported that the concentration (0.32×10^{-6} $mol \cdot L^{-1}$) of deposited atmospheric NO_3^- in China were close to the values reported for the U.S. and Japan. The concentration of NH_4^+ in China's precipitation was even higher, up to 3.77×10^{-6} $mol \cdot L^{-1}$, which was 4 times and 3.7 times the concentrations in the U.S. and Japan, respectively and the total mass of NH_4^+ in China's precipitation was 3 times and 1.8 times that in the U.S. and Japan, respectively (Djukic *et.al.*, 2010). The prolonged increased nitrogen deposition has shown to have profound impacts on ecosystems (Zhang *et al.*, 2013; Monteiro *et al.*, 2011; Li *et al.*, 2008). For instance, it has resulted in a decrease of biodiversity and ecosystem functions (Yu *et al.*, 2012; Malviya *et al.*, 2014).

Soil microbes are essential components of ecosystems (Li *et al.*, 2013). They play key roles in the cycling of soil elements (Djukic *et al.*, 2010; Gryta *et al.*, 2014), have essential roles in the sustainability of ecosystem functions (Zhang *et al.*, 2013), and are crucial for the stability of ecosystems (Li *et al.*, 2008). Therefore, understanding the factors that cause variation of soil microbial diversity is of great importance to understand the response of ecosystems to nitrogen deposition (Dong *et al.*, 2014). Many researchers have observed that an increased nitrogen deposition may

To cite this paper: Sui, X., R. Zhang, N. Xu, Y. Liu, H. Zhong, J. Wang, J. Lei, M. Li, X. Zhang and H. Ni, 2017. Response of soil fungal diversity to nitrogen deposition in a *Deyeuxia augustifolia* wetland of Sanjiang plain, Northeast, China. *Int. J. Agric. Biol*., 19: 841–848

have adverse impacts on soil microbes (Compton *et al.,* 2004; Deforest *et al.,* 2004), indicating that nitrogen deposition may change compositions and related functions of soil microbial communities. The variable soil microbial diversity is crucial to understand ecosystem functions under different environmental conditions (Yoshitake *et al.,* 2013).

Sanjiang Plain is the largest freshwater wetland in China (Sui *et al.,* 2015), and is of great importance for climate stability in Northeast China. The nitrogen deposition concentrations of Sanjiang Plain increased severely due to the agricultural and industrial activities in recent years, and distributed mainly in the growing season and nitrogen form were NH_4^+ (Wang *et al.,* 2014). Hence, the nitrogen deposition characters were obviously affected by local agricultural activities and the structure and function of wetland systems were seriously affected. Because of the increasing deposition of atmospheric nitrogen, the structure and function of ecosystems in Sanjiang Plain have changed. Many studies have investigated the effects of nitrogen deposition on the wetland ecosystem in Sanjiang Plain. For instance, Sui *et al.* (2016) have investigated its effects on the community composition of soil bacteria in a *Deyeuxia augustifolia* wetland, the effect on biomass in this type of wetland (Zhao *et al.,* 2012), or effects on bacterial species present in soils (Sun *et al.,* 2010). It was reported that increased nitrogen did not show significant effects on the community of bacteria, but did show decreased abundance in a number of main bacterial groups (Sui *et al.,* 2016). However, little is known about the effects of nitrogen deposition on the community of soil fungi. Therefore, two questions were addressed here: (1) Are fungi likely to respond in a similar way as bacteria in response to nitrogen deposition? (2) Is the excess of nitrogen deposition likely to change the composition of soil nutrition and consequently change soil fungal communities?

Materials and Methods

Overview of the Study Area

The study station was set in the Sanjiang Plain Wetland Ecological Research Station, Institute of Natural Resources and Ecology of Heilongjiang Academy of Sciences, and was located in Honghe National Nature Reserve (Fig. 1). The altitude is 55~65 m. The local average annual temperature is 1.9°C, which was from −21.6°C in January to 21.5°C in July. The mean annual precipitation is 585 mm with 50%~70% of the precipitation occurring from July to September. The mean annual evaporation is 1,166 mm. This study was conducted at a *D. augustifolia* wetland that is typical in this region, for which physiochemical characters of the soils are outlined in Table 1.

Sampling

Nine experimental plots sized 20 m × 20 m each were set up in swamp meadows in the *D. augustifolia* wetland in May, 2010. Every three plots were grouped into a class, representing one of three levels of nitrogen treatment, i.e. control or N1 (no added nitrogen), low nitrogen or N2 (4 g $N \cdot hm^{-2}\ a^{-1}$) and high nitrogen or N3 (8 g $N \cdot hm^{-2}\ a^{-1}$). For five consecutive years (2010–2014) in May, the control plots were treated with water, while the other plots were uniformly sprinkled with an aqueous solution of NH_4NO_3 at the specified dose. In June of 2014, surface soils (0–10 cm in depth) in any given plot were collected by using an auger bore (4 cm in diameter) from randomly selected 5~8 sampling sites within the plot, and the collected soils were mixed to represent soil samples from that plot. For each level of nitrogen deposition, the soils from the corresponding plots were then combined into a bag of soil sample, and finally three bags of soil samples were obtained for the three levels of nitrogen disposition. After removal of debris of plants and animals, the soil samples were divided into four parts, sealed in plastic bags and quickly transported to the laboratory for further treatment. One aliquot of each sample was stored at -80ºC prior to analysis of community composition of soil fungi. Another aliquot was air dried, thoroughly grinded and passed through two sieves, (1.00 mm and subsequently 0.15 mm pore diameter). The sieved samples were used to determine the soil physicochemical properties.

Measurements of Soil Physicochemical Properties

Soil pH was measured in suspension with a water-to-soil ratio of 2.5:1. Soil total organic carbon (TOC) was measured on a carbon and nitrogen analyzer (Jena-2100S, Germany). Total nitrogen (TN) was determined by use of the semi micro Kjeldahl method. Nitrogen in the form of nitrate (NO_3^-) was determined with the phenol disulfonic acid colorimetric method. Ammonium-nitrogen (NH_4^+) was extracted with potassium chloride and was determined with the dophenol blue method. Total phosphorus (TP) was determined using a 6.5 N sulfuric acid-molybdenum-antimony coloration liquid. Available phosphorus (AP) was extracted with 0.5 mol/L sodium bicarbonate and was determined by the molybdenum-antimony coloration method.

Isolation of Soil Total DNA

The isolation of total DNA from the soil was conducted with a soil DNA isolation kit (PowerSoil®, Mobio, USA) following the protocols in the manual. The extracted DNA was dissolved in 100 µL deionized water, and 2 µL of the resultant solution was used to determine the purity and concentration of the extracted DNA.

Sequencing of its of Soil Fungal rRNA

The fungal community was analyzed using the

1737F/2043R primers to amplify the ITS region of the fungal ribosomal DNA. This region is usually subjected to pyrosequencing for environmental samples. Briefly, an aliquot of the extracted DNA from each sample was used as a template for amplification. The ITS regions were amplified with primers 1737F and 2043R containing the A and B sequencing adaptors. PCR reactions were performed in a 25 μL mixture containing 2 μL of template DNA (10 ng), 1 μL of of each primer at 30 μmol L^{-1}, and 12.5 μL of PCRMix (TAKARA, Dalian, China). The amplification reaction systems following the programs: 95ºC for 2 min, 30 cycles of 95□C for 60 s, 52□C for 30 s, 72ºC for 30 s, and an extension step at 72□C for 10 min. Each sample was amplified in triplicate, and the PCR products were pooled and purified using an Agarose Gel DNA purification kit (TaKaRa, Dalian, China). The Miseq High-throughput sequence were performed at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

Data Analysis

Preprocessing of pyrosequencing data: Pair-end sequencing was implemented in this study. In order to obtain high quality and accurate bioinformatics data, quality control was undertaken in preprocessing the raw sequencing reads. Pair-end sequences were implemented using the Quantitative Insights into Microbial Ecology (QIIME) toolkit. Firstly, Sequences that had lengths shorter than 150 bp, showed ambiguous base data, or contained more than two base mismatches from the primer were excluded from analysis. The resultant optimized sequences were subjected to statistical analysis for recovering the sequence to use in bioinformatics analysis.

OTU-based analysis: All sequences were identified to operational taxonomic units (OTU) for bioinformatic statistical analysis. Optimized sequences with lengths greater than 350 bp were selected, compared with the Unites database and then clustered. Clustering analysis was performed using the software packages mothur and chopseq (http://www.mothur.org/ wiki/Main_Page).

Fungal Community Diversity and Rarefaction Curve

Species richness and diversity of the fungal community were characterized by Chao1 and the Shannon index, and the sequencing depth index was expressed as Coverage. Alpha-diversity of the fungal community was measured at significance levels of 97% (0.03). The estimates were calculated by employing the tools Aligner, Complete Linkage Clustering, and Rarefaction of the RDP pyrosequencing pipeline.

Variation Partitioning Analysis (VPA)

BioEnv and canonical correspondence analysis (CCA) were also used to identify the abiotic factors that are most important to fungal community composition, and these results were used to construct the soil property matrix for variation partitioning analysis in R v2.8.1 using the vegan package.

Results

Soil Physiochemical Properties under Simulated Nitrogen Deposition Conditions

The soil physiochemical properties of the experimental plots subjected to simulated nitrogen deposition are listed in Table 1. The main soil type was meadow soil for all experimental plots. 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 compared to the other two samples. As expected, TN, NO_3^- and NH_4^+ showed increasing trends with nitrogen deposition, but AP showed a decreasing trend with nitrogen deposition.

Sequencing Data Analysis

Soil DNA was isolated and fungal DNA was amplified by ITS-rDNA PCR. After high-throughput sequencing and optimization, a total of 88,428 sequences were obtained from the amplicons. As outlined in Table 2, a total of 28, 943,067 bp was obtained, with a mean read length of 327.36 bp, and reads of 201–300 bp comprised 98.77% of the total sequences obtained.

Random sampling of the post-processed sequences was conducted. The number of operational taxonomic unit (OTU) related to the sampled sequences was used to generate rarefaction curves. For the three levels of nitrogen treatment, the obtained rarefaction curves all plateaued at higher numbers of sequences, indicating that the sequencing data was close to saturation and the current sequencing depth was reasonable (Fig. 2). Thus, it could be expected that the addition of more sequences would not result in detection of new OTUs.

The Distribution of Soil Fungal Community in Different Nitrogen Deposition Conditions

A Venn diagram illustrates the number of common and unique OTUs in the samples, as shown in Fig. 3. This simple numeric visualization shows the similarity and overlap of all samples. In total, 803 OTUs were detected, of which 513 (63.89%) were shared between the N1, N2 and N3 treatments (Fig. 3). There were 48 OTUs only found in N3, accounting for 6.98% of the total. N1 samples produced the highest number of specific OTUs (58, or 7.22%), and the OTUs specific for N2 was 31, accounting for 3.86%. A large proportion of OTUs were shared in N1 and N3 (8.97%), while 4.98% of OTUs were shared between N1 and N2 and 6.10% were isolated from both N2 and N3. Thus, of the three nitrogen treatments, the distribution of OTUs from the N1 and N3 soil was most different.

Table 1: The 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±0.08^{b}	35.05±1.76^{a}	2.47±0.26^{a}	16.37±0.56^{a}	4.75±0.08^{a}	1.12±0.12^{a}	58.82±3.12^{c}
Low nitrogen(N2)	5.87±0.05^{b}	36.67±1.53^{ab}	2.73±0.32^{b}	19.45±0.68^{b}	5.24±0.09^{b}	1.15±0.11^{a}	51.24±2.21^{b}
High nitrogen (N3)	5.75±0.02^{a}	38.23±1.28^{b}	3.52±0.14^{c}	24.31±0.43^{c}	6.16±0.06^{c}	1.21±0.15^{b}	47.80±3.91^{a}

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

Table 2: The number and distribution of valid sequences of ITS rDNA

Length in bp	Number of sequences	Percent (%)
1—200	21	0.02
201—300	87342	98.77
301—400	985	1.11
401—500	80	0.09

Table 3: Fungal diversity indices of *Deyeuxia angustifolia* wetlands under simulation nitrogen deposition

Treatment	OTU	Ace index	Chao1 index	Shannon index	Simpson index
N1	260	213	270	3.49	0.0782
N2	339	307	347	3.83	0.0559
N3	243	270	253	2.92	0.134

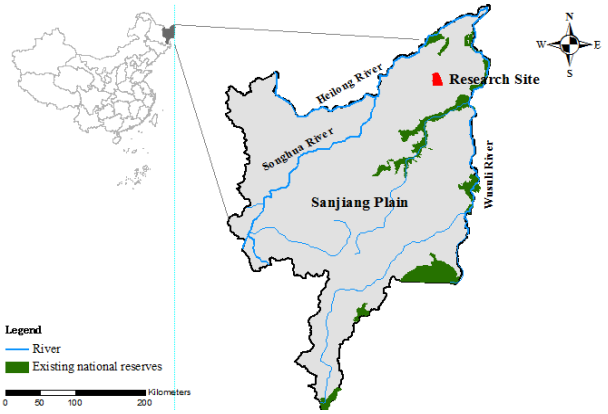

Fig. 1: Location of sampling sites in the Sanjiang Plain-Honghe national nature reserve

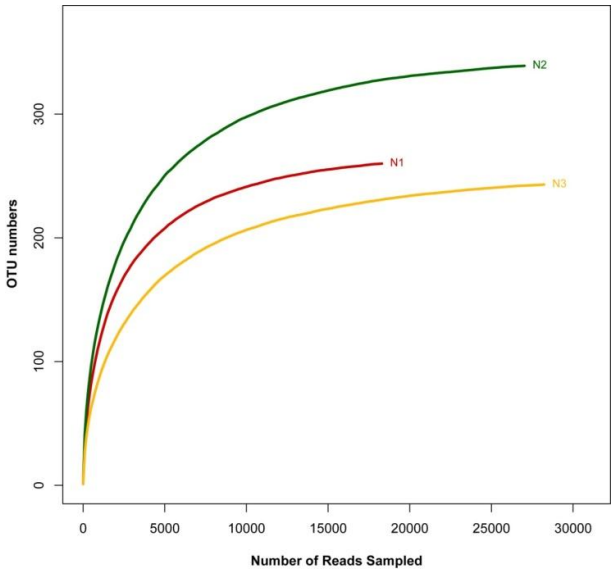

Fig. 2: OTUs rarefaction curves of soil fungi in the different nitrogen treatments

Soil Fungal Diversity Indices in Simulated Nitrogen Deposition Conditions

The obtained fungal ITS rDNA sequences were compared, and sequences with similarity of 98% or more were grouped into the same OUT (Stach *et al*., 2003). The obtained diversity of soil fungal ITS rDNA in the three different nitrogen deposition conditions is outlined in Table 3. The Shannon indices for N1, N2 and N3 were 3.49, 3.83 and 2.92, respectively, and the richness indices (Chao1) were 270, 347 and 253, respectively. Soils treated with low-level nitrogen (N2) showed increasing diversity and richness of soil fungi, while exposure to high-level nitrogen (N3) decreased the diversity and richness to levels below the control samples. Thus the soil fungal diversity and richness of *D. angustifolia* wetlands were significantly different under the tested nitrogen deposition concentrations. Moreover, the fungal community compositions in the soils were significantly different following prolonged exposure to enhanced nitrogen application.

Sequencing Analysis of ITS rDNA in Simulated Nitrogen Deposition

After comparing the obtained sequences of ITS rDNA in the UNITE ITS database, the soil fungal community compositions from different nitrogen treatments were outlined (Table 4). The richness of the dominant fungal species changed as a result of the treatment (Table 4). Nitrogen-amended soils showed an increase of richness of Sac fungi (Ascomyceta) by 24.31% and 27.61% for N2 and N3, respectively. However, nitrogen deposition decreased the richness of Basidiomycete by 21.16% and 26.65% for N2 and N3, respectively. Moreover, unclassified fungi decreased in richness with nitrogen deposition. Zygomycota decreased in low-level nitrogen, while they increased in high-level nitrogen. Chytridiomycota did not show any change due to nitrogen deposition. These findings indicate that the change of the wetland environment resulting from different amounts of nitrogen deposits had major effects on the soil fungal communities.

Table 4: Sequence analysis of ITS rDNA under different nitrogen concentration conditions

	Control (N1)		Low nitrogen (N2)		High nitrogen (N3)	
Phylum	Sequencenumber	Percent (%)	Sequencenumber	Percent (%)	Sequencenumber	Percent (%)
Ascomycota	10349	56.56	21856	80.87	23757	84.17
Basidiomycota	6338	34.64	3643	13.48	2255	7.99
Chytridiomycota	0	0.00	0	0.00	4	0.01
Zygomycota	938	5.13	529	1.96	1784	6.32
Fungi_unclassified	674	3.68	990	3.66	426	1.51
Total	18299	1	27025	1	28226	1

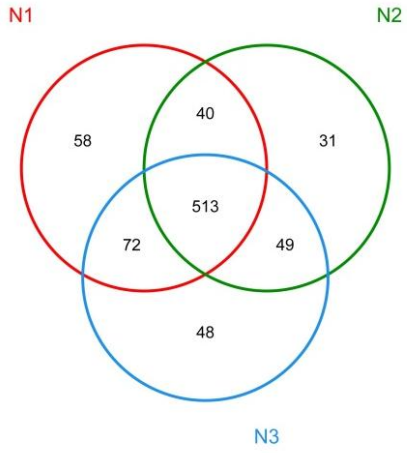

Fig. 3: Venn diagram showing the numbers of shared and exclusive OTUs of fungus identified from the different soil sample

Analysis of Fungal Community Structure

A heatmap tree was constructed to provide a graphical representation of the data, where fungal genera were first clustered based on genus classification and then plotted against the relative sequence abundance values within each OTU. In this manner, the heatmap tree directly showed the differences among fungal communities in the different genera. As shown in Fig. 4, 6 Clusters were produced based on the ITS rDNA in the soil fungal communities. The relative fungal abundance changed in samples subject to different nitrogen deposition conditions, with some noticeable trends briefly summarized here.

Cluster 1 contained *Miceosphaerosis, Mortierella, Penicilium, Pseudogymnoescus, Trichoderma,* and *Arthrinium* species. In this cluster, the relative fungal abundance in N3 samples was high, while it was lower in soil from N1 and N2.

Cluster 2 consisted of 18 OTUs, amongst others containing members of *Preussia, Pezizales, Chloridium, Rhizoscyphus, Oidiodendron, Microdochium, Phacellium, Plectosphaerellaceae,* and *Talaromyces.* As shown in Fig. 4, N3 produced higher fungal abundance for this clusterthan N1 and N2, which both produced relatively low fungal abundances. The overall trend was thus similar to that in Cluster 1 though the members of Cluster 2 were of lower abundance than those of Cluster 1.

Cluster 3 contained 16 OTUs, mainly comprising of *Stagonospora, Agaricomycetes, Leptosphaeriaceae, Basidiomycetes, Ascomycota, Schizothecium, Lachnum, Paraconiothyrium* and *Chaetosphaeriates* species. Particularly, the *Basidomicota* and *Ascomycota* were highly abundant in N1 and N2. As a general trend, in this cluster N3 showed lower fungal abundance than N1 and N2, which was in contrast to the trend observed for Clusters 1 and 2. Overall, the abundance of fungal OTUs was highest for this cluster.

Cluster 4 (15 OTUs) mainly contained *Filosporella, Xylodon, Sordariales, Archaeorhizomyces, Curvularia, Sebacinales, Dokmaia, Guehomyces, Sporobolomyces, Sporobolomyces* and *Canidida* species, while the fungal abundance was higher in N1 than in N2 and N3. Thus, this cluster showed a different trend compared to Clusters 1 to 3.

Cluster 5 was the largest cluster obtained and contained 27 OTUs. It contained, amongst others, *Aureobasidium, Galerina, Gibberella, Herpotrichiellaceae* and *Capnobotryella* species. The fungal abundance for this cluster was highest in N2 and lowest in N1, a trend resembling that of Cluster 3.

The cluster represented at the bottom of the figure (Cluster 6, with 17 OTUs), mainly contained low abundance OTUs in N3 and N1, with moderate abundance in N2 for species of *Phaeococcomyces, Devriesia, Sporidiobolales, Erythrobasidium, Articulospora, Agaricales, Varicosporium, Rachicladosporium, Eudarluca, Periconia, Rhodotorula, Hypocreales, Aspergillus, Phialocephala, Arnium,* and *Kurtzmahomyces*.

On the whole, the control (N1) and high-level nitrogen (N3) resembled each other more than they resembled N2, in terms of fungal community composition and fungal abundance. This is represented by the tree at the top of the figure. The observations indicate that the compositions of soil fungal communities showed evident changes, as a result of nitrogen deposition.

Correlation between Soil Fungal Diversity and Environmental Factors

Variation Partitioning Analysis (VPA) was employed to quantitatively analyze the contributions of the

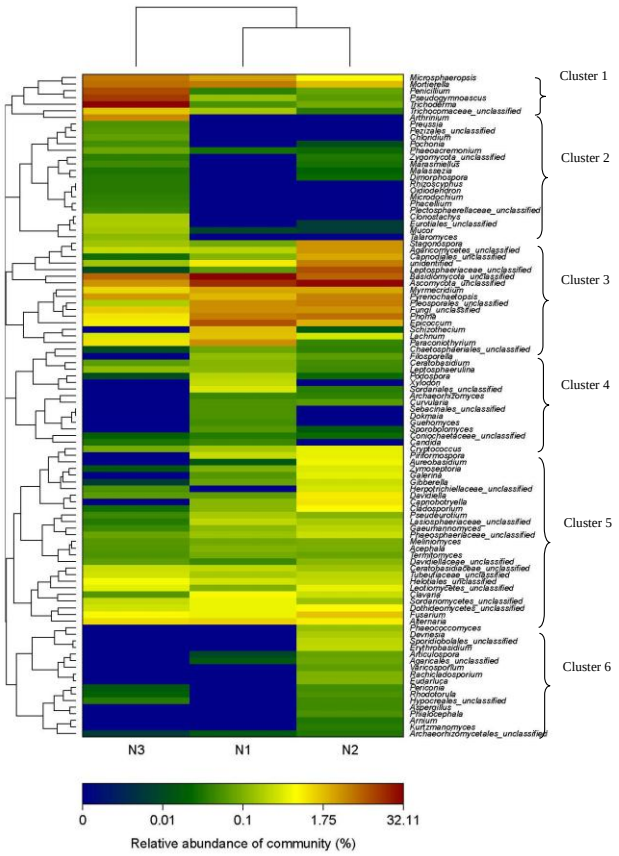

Fig. 4: Heatmap tree based on the fungal ITS rDNA sequences

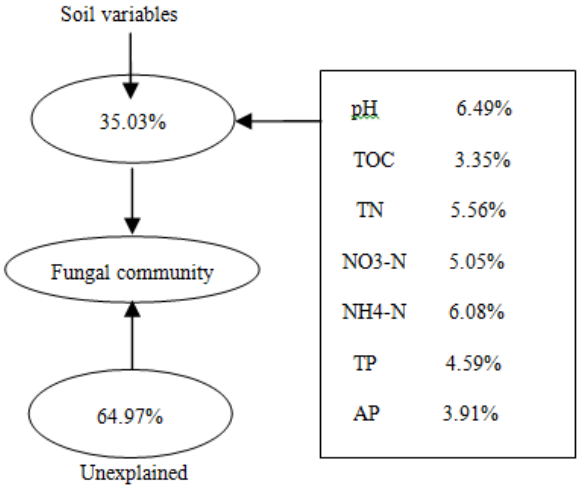

Fig. 5: Variation partitioning analysis of the effects of soil variables on the phylogenetic structure of fungal communities

environmental factors to the soil fungal communities. The environmental factors accounted for 35.03% of the total variation in the soil fungal community, where soil pH, soil TOC, nitrogen in the form of NH_4^+ and NO_3^-, TP and AP accounted for 6.49%, 3.35%, 5.56%, 5.05%, 6.08%, 4.59% and 3.91% of the total variation, respectively. This indicates that all environmental factors influenced the soil fungal community observed in this study and in combination they attributed to over one third of the observed variation.

Discussion

Soil pH is an important factor affecting soil microbial diversity. The variation of pH plays essential roles in the decomposition and formation of soil organic litter, and in the synthesis and transformation of N, P and K-containing nutrients (Ndour *et al.*, 2008). In different nitrogen deposition conditions, the N1 and N2 samples had significantly higher pH than N3, although the difference in pH between N1 and N2 was not statistically significant. An increase of nitrogen deposition would affect soil nutrition. As compared with N1, the two soil samples of N2 and N3 showed increased content of TOC, in addition with the expected increase in nitrogen following the treatment (Table 1). This may be because the introduction of nitrogen would facilitate carbon sequestration and thus would increase soil TOC content, which is in line with the viewpoints of Conant *et al*. (2001). These findings indicated that the introduction of exogenous nitrogen to the wetland increased the available nitrogen in the wetland ecosystems, and thus produced significant effects on the soil TOC content in the wetland. On the other hand, the introduction of exogenous nitrogen in the form of NO_3^- and NH_4^+ raised the mineralization rate of soil nitrogen, which resulted in an increase of mineralized nitrogen and TN in soils. This was in agreement with the findings by the majority of other researchers, who reported that soil NO_3^- and NH_4^+ tend to increase with nitrogen deposition until soil nitrogen saturation is reached (Fan *et al*., 2007). Wang *et al.* (2008) investigated the early-stage response of soil available nitrogen to a simulated nitrogen deposition in tropical forests of South Asia, and found that atmospheric nitrogen deposition would lead to an increase of soil available nitrogen, and a higher level of nitrogen deposition would produce a higher increase of soil available nitrogen.

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 (Harch *et al*., 1997; Fontúrbel *et al*., 2012). The findings in this study indicated that the compositions of microbial fungal communities had undergone significant as a result of the different nitrogen deposition conditions, and Shannon indices decreased in the order of N2>N1>N3 (Table 3). In other words, low-level nitrogen deposition increased the diversity while high-level nitrogen suppressed the fungal diversity. This implied that

the dependence of fungal diversity on nitrogen deposition was related to a threshold value of nitrogen concentration, beyond which higher nitrogen concentration would suppress fungal diversity. 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 Liu *et al*. (2016). Exceeding the threshold concentration, high-level nitrogen would change the availability of soil nutrients, facilitate the growth of certain microbial species (e.g., those preferentially utilizing plant debris) while suppressing other microbial species, and thus make the microbial community diversity decrease. Thus, nitrogen deposition plays a complex role in the composition change 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 (Zhou and Yan, 2001; Zheng *et al.*, 2014; He *et al.*, 2016).

The sequencing analysis of ITS rDNA showed that Ascomycota was the predominant fungal phylum in each of the soil samples of N1, N2 and N3, accounting for 56.56%, 80.87% and 84.17% of the fungal population, respectively. Many studies have indicated that microbial communities in wetland soils are primarily comprised of *Ascomycota* and *Basidiomycota*. For instance, Chen (2013) reported *Ascomycota* as the predominant fungal phylum in wetlands of Sanjiang Plain, a finding in agreement with that in this study. Nitrogen deposition changed the relative abundance of *Ascomycota*, which decreased with low-level and increased with high-level nitrogen deposition. Conversely, *Basidiomycota* reduced in abundance with both tested nitrogen deposition levels, which agreed with the finding by Xue *et al.* (2004) who found a negative correlation between ectomycorrhizal fungi and soil nitrogen content. Our study found that at high-level nitrogen the soil *Basidiomycota* abundance decreased, which could be because the soil nitrogen was over-saturated. Over-saturation of nitrogen in soils would suppress the supply of soil nutrition to fungi and thus decrease fungal abundance.

Conclusion

An enhanced nitrogen deposition led to a dramatic increase of soil mineral nitrogen and made the soils slightly more acidic. The relatively high abundance of *Ascomycota* in the three types of soil samples (N1–N3) was likely attributed to this more acidic soil environment, as a soil pH slightly below 7 facilitates the growth of some species of *Ascomycota*. Soil microbes in wetlands play very important roles in the recycling of organic matter and energy-carriers throughout the soils. However, relatively few studies have been conducted for wetlands, and the studies on nitrogen deposition in wetlands are rare. Such environments are typically seasonally or annually waterlogged, in contrast to forests or grasslands, which could potentially produce discrepancy in research results obtained from wetlands depending on the season and height of the water table. For this reason we took all samples in the same calendar month. Nevertheless, research about soil microbes of wetlands is complex and requires a sophisticated and systematic approach, with multiple disciplines involved to broaden and deepen our understanding of the subject. Nitrogen deposition increases on a global scale, and the region of Northeast China is no exception. Therefore, understanding the responses of fungal community compositions and their structures to nitrogen deposition in wetland soils produces valuable insights into effects of climate and atmospheric changes in the future on diversity and functions stability in wetland ecosystems.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NO.31470019, 31400429), Fundamental Research Funds for the Central Non-profit Research Institution of CAF (CAFYBB2014ZD001). Fund of Heilongjiang academy of sciences (STJB16-01, ZNJZ2017ZR01).

References

Chen, H.H., 2013. Diversity Analysis of Soil Microbial Community Structure in Sanjiang Wetlands. *Master Degree Dissertation*, Northeast Agricultural University, Harbin, China

Compton, J.E., L.S. Watrud and L.A. Porteous, 2004. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *Forest Ecol. Manage.*, 196: 143–158

Conant, R.T., K. Paustian and E.T. Elliot, 2001. Grassland management and conversion into grassland: Effects on soil carbon. *Ecol. Appl.*, 11: 343–355

Deforest, J.L., D.R. Zak and K.S. Pregitzer, 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. *Soil Biol. Biochem.*, 36: 965–971

Djukic, I., F. Zehetner and A. Mentler, 2010. Microbial community composition and activity in different Alpine vegetation zones. *Soil Biol. Biochem.,* 42: 155–161

Dong, D., D.P. Zhang and W.C. Liu, 2014. Diversity analysis of bacterial community from permafrost soil of Mo-he in China. *Ind. J. Microbiol.*, 54: 111–113

Fan, H.B., W.F. Liu, Y.Y. Li, Y.C. Liao, Y.H. Yuan and L. Xu, 2007. Tree growth and soil nutrients in response to nitrogen deposition in a subtropical chinese fir plantation. *Acta Ecol. Sin.*, 27: 4630–4642

Gryta, A., M. Frąc and K. Oszust, 2014. The application of the BiologEcoPlate approach in ecotoxicological evaluation of dairy sewage sludge. *Appl. Biochem. Biotechnol.,* 174: 1434–1443

Harch, B.D., W. Meech, C.E. Pankhurst and R.L. Correll, 1997. Using the Gini coefficient with BIOLOG substrate utilization data to provide an alternative quantitative measure for comparing bacterial soil communities. *J. Microbiol. Meth.*, 30: 91–101

He, D., X. Xiang and J.S. He, 2016. Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan plateau. *Biol. Fert. Soils*, 52: 1059–1072

Li, F.L., M. Liu and Z.P. Li, 2013. Changes in soil microbial biomass and functional diversity with a nitrogen gradient in soil columns. *Appl. Soil Ecol.,* 64: 1–6

Li, T., P. Wang and P.X. Wang, 2008. Microbial diversity in surface sediments of the Xisha trough the South China sea. *Acta Ecol. Sin.*, 28: 1166–1173

Liu, C., Y. Dong and Q. Sun, 2016. Soil bacterial community response to short-term manipulation of the nitrogen deposition form and dose in a Chinese fir plantation in Southern China. *Water Air Soil Pollut*., 227: 447

Fontúrbel, M.T., A. Barreiro and J.A. Vega, 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. *Geoderma*, 191: 51–60

Malviya, N., M.S. Yandigeri and A.K. Yadav, 2014. Isolation and characterization of novel alkali-halophilic actinomycetes from the Chilika brackish water lake, India. *Ann. Microbiol.*, 64: 1829–1838

Monteiro, J.M., R.E. Vollú, M.R.R. Coelho, A. Fonseca, S.C. Gomes Neto and L. Seldin, 2011. Bacterial communities within the rhizosphere and roots of vetiver (*Chrysopogon zizanioides* L. Roberty) sampled at different growth stages. *Eur. J. Soil Biol.*, 47: 236–242

Ndour, N.Y.B., W. Achouak, R. Christen, T. Heulin, A. Brauman and J.L. Chotte, 2008. Characteristics of microbial habitats in a tropical soil subject to different fallow management. *Appl. Soil Ecol.,* 38: 51–61

Stach, J.E.M., L.A. Maldonado and D.G. Masson, 2003. Statistical Approaches for Estimating Actinobacterial Diversity in Marine Sediments. *Appl. Environ. Microbiol.,* 69: 6189

Sui, X., R.T. Zhang and L.B. Yang, 2015. Effects of Long-term Elevated CO 2 Fumigation on Microbial Communities in a Wetland Soil. *J. Residuals Sci. Technol.*, 12: S93–S96

Sui, X., R.T. Zhang and L.B. Yang, 2016. Effect of simulation nitrogen depositions on bacterial diversity of *Deyeuxia angustifolia* in wetland of Sanjiang Plain. *Pratacultural Sci.*, 33: 589–598

Sun, Z.G., J.S. Liu and X.J. Mou, 2010. Seasonal Change Characteristics of Nitrate Nitrogen and Ammonium Nitrogen in Typical *Calamagrostis angustifolia* Wetland Soils of Sanjiang Plain. *System Sciences and Comprehensive Studies in Agriculture*, 26: 277–282

Tatarinov, F.A., E. Cienciala and P. Vopenka, 2011. Effect of climate change and nitrogen deposition on central-European forests: Regional-scale simulation for South Bohemia. *Forest Ecol. Manage.,* 262: 1919–1927

Van Dobben, H.F. and W. De Vries, 2017. The contribution of nitrogen deposition to the eutrophication signal in understorey plant communities of European forests. *Ecol. Evol.*, 7: 214–227

Wang, H., J.M. Mo, X.K. Lu, J.H. Xue, J. Li and Y.T. Fang, 2008. Effects of elevated nitrogen deposition on soil microbial biomass carbon in the main subtropical forests of southern china. *Acta Ecol. Sin.*, 2: 470–478

Wang, Y., J. Liu and L. He, 2014. Responses of carbon dynamics to nitrogen deposition in typical freshwater wetland of Sanjiang *Plain. J. Chem. New York*, 2014: 1–9

Xue, J.H., J.M. Mo and J. Li, 2004. Effects of nitrogen deposition on ectomycorrhizal fungi. *Acta Ecol. Sin.*, 24: 1785–1792

Yoshitake, S., M. Fujiyoshi and K. Watanabe, 2013. Successional changes in the soil microbial community along a vegetation development sequence in a subalpine volcanic desert on Mount Fuji, Japan. *Plant Soil*, 364: 261–272

Yu, Y., H. Wang, J. Liu, Q. Wang, T. Shen, W. Guo and R. Wang, 2012. Shifts in microbial community function and structure along the successional gradient of coastal wetlands in Yellow River Estuary. *Eur. J. Soil Biol.*, 49: 12–21

Zhang, H.F., G. Li and X.L. Song, 2013. Changes in soil microbial functional diversity under different vegetation restoration patterns for Hulunbeier Sandy Land. *Acta Ecol. Sin.*, 33: 38–44

Zhao, G.Y., J.X. Dou and D.M. Hao, 2012. Effects of Nitrogen Decomposition on Biomass and Allocation of *Deyeuxia angustifolia* in Freshwater Marsh in Sanjiang Plain. *Geography Geo-Information Sci.*, 28: 103–106

Zheng, Y., Y. Kim and X. Tian, 2014. Differential responses of arbuscular mycorrhizal fungi to nitrogen addition in a near pristine Tibetan alpine meadow. *FEMS. Microbiol. Ecol.,* 89: 594–605

Zhou, G.Y. and J.H. Yan, 2001. The influences of regional atmospheric precipitation characteristics and its element inputs on the existence and development of Dinghushan forest ecosystems. *Acta Ecol. Sin*., 21: 2002–2012

(Received 24 March 2017; Accepted 07 April 2017)