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Response of the soil fungal community to multi-factor environmental changes in a temperate forest

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ABSTRACT

Both environmental and climatic changes are known to influence soil microbial biomes in terrestrial ecosystems. However, there are limited data defining the interactive effects of multi-factor environmental disturbances, including N-deposition, precipitation, and air temperature, on soil fungal communities in temperate forests. A 3-year outdoor pot experiment was conducted to examine the temporal shifts of soil fungal communities in a temperate forest following N-addition, precipitation and air temperature changes. The shifts in the structure and composition of soil fungal communities were characterized by denaturing gradient gel electrophoresis and DNA sequencing. N-addition regimen induced significant alterations in the composition of soil fungal communities, and this effect was different at both higher and lower altitudes. The response of the soil fungal community to N-addition was much stronger in precipitation-reduced soils compared to soils experiencing enhanced precipitation. The combined treatment of N-addition and reduced precipitation caused more pronounced changes in the lower altitude versus those in the higher one. Certain fungal species in the subphylum Pezizomycotina and Saccharomycotina distinctively responded to N fertilization and soil water control at both altitudes. Redundancy discrimination analysis showed that changes in environmental factors and soil physicochemical properties explained 43.7% of the total variability in the soil fungal community at this forest ecosystem. Variations in the soil fungal community were significantly related to the altitude, soil temperature, total soil N content (TN) and pH value ($P < 0.05$). We present evidence for the interactive effects of N-addition, water manipulation and air temperature to reshape soil fungal communities in the temperate forest. Our data could provide new insights into predicting the response of soil micro-ecosystem to climatic changes.

1. Introduction

Atmospheric concentrations of CO_2 and other greenhouse gases are already rising and may lead to an increase in global air temperature ranging from 1.4 to 5.8 °C by the end of the 21st century (IPCC, 2007). It is expected that climatic warming may impact regional and global precipitation patterns (Dore, 2005; Groisman et al., 2005). In the past decades, atmospheric nitrogen (N) deposition has substantially increased in most industrialized areas, such as USA, Europe and Asia (Galloway et al., 1995; Howarth et al., 2002; Janssens et al., 2010; Lü and Tian, 2007), which would affect the biogeochemical cycling of C and N at local, regional and global scales (Galloway et al., 2004; Mark et al., 2004). The environmental changes could cause alterations in soil physicochemical properties including soil moisture, pH values, C/N ratio and soil temperature, and in turn impact the composition and function of soil microbial community (Myers et al., 2001; Treseder, 2004; Wakelin et al., 2007; Castro et al., 2010). Soil microbes play a key role in controlling consumption of greenhouse gases including CO_2, CH_4 and N_2O (Conrad, 1996), with consequent feedbacks to climatic changes. Therefore, a further understanding of microbial response to environmental changes is of critical significance in predicting terrestrial ecosystem feedbacks in a warmer climate (Margesin et al., 2009).

Atmospheric and climatic changes are happening in concert with one another, and the ecosystems are simultaneously experiencing higher input of atmospheric N, warming and changes in precipitation regimes (IPCC, 2007; Dore, 2005; Groisman et al., 2005; Galloway et al., 1995). Soil fungal community exists in a complex and dynamic environment controlled by multiple abiotic factors, including atmospheric N-deposition, elevated CO_2 concentration, precipitation and air temperature (Lilleskov et al., 2001, 2002; Treseder, 2004; Drenovsky et al., 2004; Kihara et al., 2012;

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Hirose et al., 2009). It has been indicated that atmospheric N-deposition could alter the diversity of ectomycorrhizal fungi in white spruce forests of Alaska (Lilleskov et al., 2001, 2002), and N-fertilization results in a reduction of 15% in mycorrhizal abundance in the field ecosystem (Treseder, 2004). Soil water deficit could reduce colonization of some fungal species (Drenovsky et al., 2004). Due to significant differences in soil moisture, the composition of the fungal communities in the semiarid and arid areas is apparently distinguished from those in humid regions (Kihara et al., 2012). The fungal diversity and richness decline with increasing altitudes in alpine ecosystems such as the Alps and Tibetan Plateau (Margesin et al., 2009; Hirose et al., 2009). Since these studies have linked the changes of soil fungal community with single climate factor, our knowledge concerning the interactive effects of multi-factor environmental changes such as atmospheric N-deposition, altered precipitation patterns and climatic warming on the fungal community remains very limited. Ectomycorrhizal abundance significantly increases in soils exposed to N fertilization and elevated CO_2, suggesting a potentially interactive effect of atmospheric N-deposition and CO_2 on fungal community (Garcia et al., 2008; Tingey et al., 1997; Lagomarsino et al., 2007). In addition, soil fungal response to N-deposition can also be influenced by alterations in soil moisture, increased ozone (O_3), and nutrient availability (Treseder, 2004; Lilleskov, 2005; Nicol et al., 2004; DeForest et al., 2004). Concurrent changes in atmospheric N deposition, precipitation regimes and climatic warming may have interactive effects on soil fungal community; however, little attention is paid to disclose how multiple climate change factors interact with each other to influence fungal community structure and composition in temperate forest ecosystem. Alterations in composition and taxonomic diversity of fungal communities could feed back into influencing soil and ecosystem processes (Schroter et al., 2005; Lilleskov et al., 2008). Elucidation of the interactive effect of multi-factor environmental variations on the communities would contribute to better understand the ecosystem processes.

In this study, a 3-year manipulation experiment in outdoors potted with *Fraxinus mandschurica* was used to assess the interactive effects of the important environmental factors, atmospheric N-deposition, precipitation and/or air temperature on the soil fungal community in a temperate forest ecosystem using 18S rDNA-based fingerprinting and sequencing techniques. We applied redundancy discrimination analysis (RDA) to link with shifts of the fungal communities with these environmental variables and soil properties. We hypothesized that (1) N-addition, altered precipitation and altitude could interact to affect the soil fungal community in the temperate forest ecosystem; and (2) shifts in the soil fungal community under the complex environmental changes could be related to environmental factors and soil physicochemical properties.

2. Materials and methods

2.1. Study sites

This study was performed within the experimental sites of Changbai Mountain forest ecosystems research station (CBFERS), Chinese Academy of Sciences (42°24′09″N, 128°05′45″E) established in 1979 in northeastern China. The location is neither privately-owned nor protected in any way. The research area is situated in the temperate continental climatic zone. The study sites were selected at two altitudes, a lower site (740 m) and a higher (1200 m) at the north slope of Changbai Mountain. According to climate data collected from 1970 to 2008, the mean annual precipitation is 695 mm at the lower altitude area, 70–80% of which falls during the growing season from May to October (Zhang et al., 2005). The mean annual temperature is 2.1 °C, with average monthly temperatures of −15.6 °C in January and 19.7 °C in July. At the higher altitude region, the average annual precipitation is 755 mm, the mean annual temperature is 0.9 °C, and the average monthly temperature in January and July is −18.6 °C and 17.4 °C, respectively. In the area, the natural forest is a temperate broad-leaved Korean pine mixed forest. The main tree species include *Pinus koraiensis*, *F. mandschurica*, *Acer mono* and *Tilia amurensis* (Wang et al., 2012). The main shrub species are *Philadelphus schrenkii*, *Euonymus alatus*, *Lonicera japonica*, *Corylus mandshurica* and *Deutzia scabra*, and the main herbaceous species are *Anemone raddeana*, *Anemone cathayensis*, *Cyperus microiria*, *Funaria officinalis*, *Adonis vernalis*, *Brachybotrys paridiformis* and *Filipendula palmate*.

2.2. Experimental designs

We conducted the experiment in the two sites described above. A three water × two nitrogen full factorial experiment (i.e. six treatments) was designed at each altitude. Six treatments consisted of: (1) natural precipitation without N-addition (CK); (2) enhanced precipitation without N-addition (+W); (3) reduced precipitation without N-addition (−W); (4) natural precipitation with N-addition (+NCK); (5) enhanced precipitation with N-addition (+N+W); (6) reduced precipitation with N-addition (+N−W). Each treatment was replicated 30 pots at both altitudes. The position of each treatment was randomized. The experimental sites were fenced to prevent grazing. Water and N manipulations commenced on May 5th, 2007. Enhanced or reduced precipitation treatments were achieved by placing a hollow clear plastic ring (35 cm and 25 cm diameter for two edges) with certain angle onto the edge of the pots (Fig. S1), which could increase or decrease 30% areas of top pot. Three water levels, low (LW), medium (CK) and high (HW), were chosen to simulate the amount of precipitation during the growing season. As we did not account for possible leachate, the water levels should be regarded as relative rather than as absolute amounts.

Two N-fertilizer levels were achieved by using 0 g (soil without N-addition) or 10 g (soil with N-addition) of N m^{-2} $year^{-1}$ during the treatment period. The N supply dose roughly corresponds to the average annual N deposition in northeast China (Lü and Tian, 2007). To add N, NH_4NO_3 was diluted in deionized water (4.54 mM) and the solution was sprayed into the potted soil. The N-additions were performed with a sprayer on May 15th and July 15th, 2007, 2008 and 2009 as two equal applications (5 g N m^{-2}, i.e., 54.86 g NH_4NO_3) (Wang et al., 2012). Control pots without N-addition were simultaneously supplied with the same amount of deionized water.

The study was conducted at CBFERS using seedlings of *F. mandschurica*. Two-year-old seedlings of *F. mandschurica* were provided by the local tree nursery (Guangming seedling nursery Co., Erdao forest farm, Jilin Province). Whole plant dry mass, plant height and stem base diameter of the seedlings were determined to be 8.64 ± 0.49 g, 26.35 ± 0.80 cm and 8.33 ± 0.20 mm, respectively. Seedlings were planted in each pot (40 cm tall and 30 cm diameter; two seedlings per pot). The pots were incubated outdoors in the plots without shade. All pots placed in both altitudes were filled with dark-brown forest soil, which was uniformly collected from 0 to 20 cm under the floor of the temperate broad-leaved Korean pine (*P. koraiensis*) mixed forest at altitude 740 m. The soil, originating from volcanic ash, was classified as Eutric cambisol (FAO classification) with high organic matter content in the surface layer, a pH value of 5.8, an average of 156.6 g kg^{-1} organic carbon, and 7.17 g kg^{-1} total N. To reduce soil nutrient heterogeneity, the collected soil was passed through a 5 mm sieve. The experiment was started in May 2007 and soil samples were collected in August 2010.