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Original Articles

Effects of winter snowpack and nitrogen addition on the soil microbial community in a temperate forest in northeastern China

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ABSTRACT

Nitrogen (N) deposition and change of snowpack are two critical factors for soil microbial community in temperate forests. The responses of soil microbial community composition and diversity to snowpack changes and N deposition are rarely investigated in winter. Therefore, we conducted an experiment to detect the microbial responses to the different snowpack depths (0 cm and 40 cm) and N addition (0 g N m^{-2} yr^{-1} and 5 g N m^{-2} yr^{-1} for four years) in a cold temperate forest in northeastern China in winter. The results indicated that decreased snowpack and increased N caused the significant changes in the soil microbial community composition in winter. The N addition significantly decreased bacterial abundance and diversity, which may be caused by decrease of pH and increase of inorganic N concentration under N addition. The decreased temperature associated with the removal of snowpack was considered to be able to trigger the variability in the soil fungal and bacterial community composition and diversity. The reduction in snowpack also decreased the soil respiration rate and the microbial biomass carbon (C) and N. However, the inhibition effect of N addition on soil respiration only occurred in the no snowpack treatments during the winter in our study, which indicates that N addition might change the activity of cold-tolerant microorganisms. In general, increased N deposition and reduced snowpack might potentially affect ecosystem functioning and terrestrial ecosystem C cycling in temperate forests under global change scenarios.

1. Introduction

The thickness of snowpack influences the subsurface soil temperature and soil metabolic activity (Wang et al., 2013; Freppaz et al., 2014), and its insulating properties protect soil microorganisms (Hinkler et al., 2008). However, it has been predicted that snowfall will decrease under global warming (Kunkel et al., 2009; IPCC, 2013), leading to a thinner snowpack in winter (Kunkel et al., 2009; Kapnick and Delworth, 2013). The decrease in snowpack can affect soil microbial community composition and diversity, triggering changes in essential ecosystem functions (Campbell et al., 2005; Zhang, 2005). Allison et al. (2013) also suggested that characterizing soil microbial mechanisms was critical for understanding how ecosystem processes respond to changes in snowpack under global climate change. The responses of soil microbial community composition and diversity to snowpack changes are rarely investigated, although these responses are responsible for changes in soil carbon (C) cycles in winter.

The effects of snowpack on soil microbial community composition and diversity could interact with atmospheric nitrogen (N) deposition, which is also a critical factor affecting soil physicochemical processes and soil microbial communities (Zhang et al., 2011; Philippot et al., 2013). With respect to N deposition, many soil ecosystems receive large amounts of N from anthropogenic activities (Ramirez et al., 2012). The alteration of global N cycles is predicted to increase the global deposition of N from 100 Tg N yr^{-1} in 1995 to 200 Tg N yr^{-1} by 2050, with some areas of the world expected to experience N deposition rates exceeding 3–5 g N m^{-2} yr^{-1} (Galloway et al., 2008). The average annual bulk deposition of N in China increased from 13.2 kg N hm^{-2} in the 1990s to 21.1 kg N hm^{-2} in the 2000s (Liu et al., 2013). Bulk N deposition often results in the significant accumulation of soil organic matter in temperate forest ecosystems (Janssens et al., 2010; Frey et al., 2014). However, the accumulation of soil organic matter due to

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increased N deposition can have dramatic impacts on ecosystem processes and microbial communities, especially in temperate forests, which are often N-limited under natural conditions (Du et al., 2014; Frey et al., 2014). The responses of soil microbial communities to N addition have been extensively studied during the growing season (Janssens et al., 2010; Fierer et al., 2012; Zhou et al., 2014), but less in winter. With respect to snowpack, in temperate forests, snowpack usually lasts for half a year (Wang et al., 2013). The global mean temperature increased by approximately 0.85 °C between 1880 and 2012, and surface temperatures across the globe are predicted to rise an additional 0.3–4.8 °C by the end of this century (IPCC, 2013). Warming could affect the thickness and cover-time of snowpack, alter soil microbial communities. Under global climate change, snowpack and nitrogen deposition will be two important factors affecting soil microbial community composition and diversity in temperate forests in winter.

However, there are two unclear issues that need to be investigated. First, it is not clear how snowpack and N addition affect the soil microbial community in temperate forests, although previous studies have shown that changes in snowpack and N addition affect the soil carbon cycles (Bai et al., 2010; Fierer et al., 2011; Philippot et al., 2013; Liu et al., 2016). Second, the effects of N addition on microbial community composition and diversity have not been thoroughly examined in winter due to the difficulty in field sampling, although such effects have been extensively discussed in the growing season (Fierer et al., 2012; Ding et al., 2015; Zhong et al., 2015). We think that the microorganisms still active in winter. Therefore, we conducted an experiment to examine the effects of N addition and reduced snowpack on the microbial community in temperate forests. The phylogenetic structure of the soil microbial communities was determined by Illumina MiSeq sequencing, and the major soil parameters were also investigated. Our results could provide insight into how N addition and the thickness of snowpack affect microbial communities and cause changes in C cycles.

2. Materials and methods

2.1. Field site and experimental design

This study was conducted at Fenglin Natural Reserve in the Lesser Khingan Mountains in Heilongjiang Province, China (48°02′–48°12′ N, 128°58′–129°15′ E). This region is characterized by a continental monsoon climate. The mean annual air temperature is −0.5 °C, the mean altitude is 450 m and the mean annual precipitation is 720 mm. The snowpack lasted for 149 days in state the year. The soil is classified as a dark brown forest soil. The forest is dominated by spruce (*Picea koraiensis*), fir (*Abies nephrolepis*) and Korean pine (*Pinus koraiensis*).

The N addition field experiment began in May 2010. Two levels of N treatments were included in this study: no N addition and N addition at 5 g N m^{-2} yr^{-1} for four years (Zhang et al., 2008). This design was replicated in three random experimental blocks, each consisting of two plots measuring 20 m × 20 m each, and the plots were separated by 10 m wide buffer strips. A dilute ammonium nitrate (NH_4NO_3) solution was applied to the forest floor every two weeks during the growing season (May to October, total 10 times a year) from 15th May, 2010. In each plot where N was added at 5 g N m^{-2} yr^{-1}, the NH_4NO_3 was mixed with 32 L of water (17.86 g NH_4NO_3 L^{-1}) and was then applied using a backpack sprayer below the canopy. The no N addition plots received 32 L water without the addition of N. During the winter, each plot was randomly separated into two subplots of the same size and assigned to two snowpack treatments (natural snowpack treatment and no snowpack treatment). For the no snowpack treatment, eighteen snow fences (2 m height, 3 m width and 3 m length in each plot) were installed in the six subplots (three snow fences installed in each no snowpack subplot) on October 8th, 2013, and a nylon mesh was placed on top of the snow fences to prevent snow accumulation. Within each block, we sampled subplots with (I) natural snowpack and no N addition as a control (S40), (II) no snowpack and no N addition (S0), (III) no snowpack and N addition at 5 g N m^{-2} yr^{-1} (NS0) and (IV) natural snowpack and nitrogen N addition at 5 g N m^{-2} yr^{-1} (NS40).

2.2. Measurement of soil respiration and soil sample collection

In each of the 12 subplots, we randomly inserted 3 polyvinyl chloride collars (20 cm inside diameter and 8 cm in height) for the winter measurements on 8th October, 2013. The soil collars were inserted 5 cm into the soil, with 3 cm left above the ground surface (Heinemeyer and McNamara, 2011). The collars were maintained in the same locations throughout the study. Based on previous studies (Wang et al., 2013), the snowpack collars were directly inserted into the soil to allow the snowpack to naturally form in the collars. The heights of the collars were adjusted whenever a snowfall event was forecasted until the measurements took place (Wang et al., 2013). The soil respiration was measured with an LI-8100 Automated Soil CO_2 Flux System (LI-COR Inc., Lincoln, NE, USA) on 24th December, 2013. Each measurement was repeated 3 times for each polyvinyl chloride collar to calculate the mean soil respiration rate. Under the natural snowpack, the rates of soil respiration were revised due to changes in the total system volume (LI-8100 Instruction Manual). The soil temperature (T_{5cm}) and the soil moisture (W_{5cm}, %) at the 5 cm depth were monitored simultaneously with the measurement of soil respiration using a soil temperature probe (Omega Engineering Inc., USA) and soil liquid water probes (determination of the content of liquid water remaining in the soil) (Delta-T Devices Ltd., Cambridge, England) connected to the LI-8100.

After the measurement of soil respiration on December 24th, 2013, all soil samples were taken from a depth of 0–10 cm, which is the topsoil corresponding to the organic horizon, and inside of the soil collars use a handheld auger (2.5 cm in diameter). Three soil cores were collected in each subplot and pooled into one composite sample. All of the visible extraneous materials (such as roots, stones, etc.) were removed by hand. Each soil sample was partitioned into three sub-samples: the first was air-dried and then sieved (2 mm) for the analysis of basic soil properties; the second was maintained in its original state and taken back to laboratory for the measurement of soil microbial biomass; the third was immediately flash-frozen in liquid N_2 and stored in cooling boxes for transportation to the laboratory and then stored at −80 °C until it was processed for nucleic acid extraction.

2.3. Soil physicochemical measurements

The soil pH was measured in a 1:2.5 (soil:water) suspension using a pH meter (SX7150, China). The soil total C (TC) and N (TN) were measured using an automated TC/TN analyzer (multi N/C 3100, Analytik Jena AG, Germany). The soil ammonium nitrogen (NH_4^+-N) was measured using the indophenol blue method followed by colorimetric analysis. The soil nitrate nitrogen (NO_3^--N) was measured using the copper-cadmium reduction method. The soil microbial biomass C (MBC) and nitrogen (MBN) were estimated using the chloroform-fumigation extraction methods reported by Brookes et al. (1985) and Wu et al. (1990). The conversion factors (kEN and kEC) of 0.45 and 0.38 were used for calculating the MBC and MBN values, respectively (Brookes et al., 1985). All these soil physiochemical measurements were performed in triplicate.

2.4. DNA extraction, PCR amplification and sequencing

The soil microbial DNA was extracted from 0.5 g of freeze-dried soil samples using an E.Z.N.A.® Soil DNA Kit for soil. The bacterial 16S ribosomal RNA genes were amplified with the primers 338F and 806R. The fungal 18S ribosomal RNA genes were amplified with the primers ITS1F and 2043R. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. The purified amplicons were