Soil Microbial Community and Enzyme Activity Responses to Herbaceous Plant Expansion in the Changbai Mountains Tundra, China

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Abstract: As one of the most sensitive regions to global climate change, alpine tundra in many places around the world has been undergoing dramatic changes in vegetation communities over the past few decades. Herbaceous plant species in the Changbai Mountains area have significantly expanded into tundra shrub communities over the past 30 yr. Soil microbial communities, enzyme activities, and soil nutrients are intertwined with this expansion process. In order to understand the responses of the soil microbial communities to such an expansion, we analyzed soil microbial community structures and enzyme activities in shrub tundra as well as areas with three different levels of herbaceous plant expansion. Our investigation was based on phospholipid fatty acid (PLFA) analysis and 96-well microtiter plates. The results showed that herbs have expanded greatly in the tundra, and they have become the dominant species in herbaceous plant expansion areas. There were differences for community composition and appearance among the shrub tundra and the mild expansion, moderate expansion, and severe expansion areas. Except for soil organic matter, soil nutrients were increased in herbaceous plant expansion areas, and the total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), and available phosphorus (AP) were greatest in moderate expansion areas (MOE), while soil organic matter levels were highest in the non-expanded areas (CK). The total soil PLFAs in the three levels of herbaceous plant expansion areas were significantly higher than those in the non-expanded areas, and total soil PLFAs were highest in the moderately expanded area and lowest in the severely expanded area (SEE). Bacteria increased significantly more than fungi and actinomycetes with herbaceous plant expansion. Soil hydrolase activities (β-1,4-glucosidase (βG) activity, β-1, 4-N-acetylglucosaminidase (NAG) activity, and acid phosphatase (aP) activity) were highest in MOE and lowest in the CK treatment. Soil oxidase activities (polyphenol oxidase (PPO) activities and peroxidase (PER) activities) were also highest in MOE, but they were lowest in the SEE treatment. The variations in total soil PLFAs with herbaceous plant expansion were mostly correlated with soil organic matter and available phosphorus concentrations, while soil enzyme activities were mostly correlated with the total soil nitrogen concentration. Our results suggest that herbaceous plant expansion increase the total soil PLFAs and soil enzyme activities and improved soil nutrients. However, soil microorganisms, enzyme activity, and nutrients responded differently to levels of herbaceous plant expansion. The soil conditions in mild and moderate expansion areas are more favorable than those in severe expansion areas.

Keywords: Changbai Mountains; tundra; herbaceous plant expansion; soil microorganism; soil enzyme activity; soil nutrients

1 Introduction

Vegetation composition is changing due to climate change (Tape et al., 2006; Devi et al., 2008), and this change can influence the physical, chemical, and biological properties of soil. In turn, these soil properties

can affect plant community composition, plant productivity, and ecosystem function (Wardle et al., 2004; Chu et al., 2011).

High-latitude and high-altitude areas are especially sensitive to climate change (Symon et al., 2005). Many species in low elevation areas have extended their ranges into high elevation areas of alpine and subalpine regions (Beckage et al., 2008; Parolo and Rossi, 2008). This upward expansion due to climatic warming is particularly obvious in herb species (Walther et al., 2005; Pauli et al., 2007; Thuiller et al., 2008). The changes in plant community composition and structure disrupt the long-term equilibrium between native plants and soil microorganisms in the alpine tundra. The abundance and composition of microorganisms can alter the quality and magnitude of soil enzyme activity as well as soil metabolic processes (Ushio et al., 2010). Meanwhile, changes in the physical and chemical properties of soil due to changes in climate and vegetation, such as soil temperature, water content, organic carbon, nutrient availability, cation exchange capacity, and soil pH, also influence the microbial community structure and function (Brockett et al., 2012; Sardans and Peñuelas, 2013). However, there is a paucity of information on the processes and mechanisms for herbaceous plant expansion into alpine tundra. For example, the responses of soil microorganisms, nutrients, and enzyme activities to herbaceous plant expansion remain to be determined.

As a result of global warming, there are substantial evidences indicating that distinct changes in plant community composition and structure have been occurred in circumpolar tundra and alpine tundra over the past three decades (Tape et al., 2006; Devi et al., 2008). The soil biogeochemical qualities as well as the structure and function of microbiological community have also varied substantially (Björk et al., 2007; Buckeridge et al., 2010; Chu et al., 2011). Several studies have found a strong interdependence between changes in plant community and in soil properties (Dias et al., 2011; Sardans and Peñuelas, 2013). Plant species with different litter and root exudates selectively stimulate soil microorganisms, thus affecting microbial community structure, function (soil enzyme activity), and diversity (Meier and Bowman, 2008; Dean et al., 2015). Distinct microbial communities and/or activities have been reported for low Arctic tundra ecosystems with different vegetation types (dry heath, birch hummock, tall birch, and wet sedge) (Chu et al., 2011). These patterns have even been reported in adjacent study sites with different tree species and in different succession stages within the same site (Grayston and Prescott, 2005; Bach et al., 2008; Mitchell et al., 2010).

As mediators of carbon decomposition, carbon stabilization, and nutrient cycling, soil microorganisms play a critical role in the processes that control ecosystem function (Coleman and Whitman, 2007; Brockett et al., 2012). Studies suggest that soil microbes (soil enzyme activity) exhibit a more significant response to shifts in vegetation when compared to the changes in soil nutrient levels (Mitchell et al., 2010; Lau and Lennon, 2012). The flourishing of expanded plants in new environments has been implicated in changes in the soil microbial community and function (Duda et al., 2003). This change can subsequently influence other soil properties. For example, if soil available nitrogen is increased, then herbaceous plants are able to take advantage of growing in soil with a high N concentration. However, native plants may not be able to adapt to the new environment (Dassonville et al., 2008).

Plant-soil interactions can affect plant community dynamics by influencing processes of coexistence or expansion as well as by maintaining alternate stable states. Negative effects ultimately result in a decline in the population growth rate of a plant species, while positive effects ultimately result in an increase in population growth rate (Bever et al., 1997, Bever, 2003). Studies have stressed the influence of the soil community on competitive interactions between plants via positive or negative effects on the growth of specific plants (van der Heijden et al., 2003; Revilla et al., 2013). The soil biotic component has a pronounced ability to facilitate and inhibit the growth of plants, and this can affect the rate and direction of succession (van de Voorde et al., 2011).

The tundra is located in the upper part of the Changbai Mountains volcanic cone (altitude: 2000–2500 m) and represents a typical Asian tundra mountain area (Huang and Li, 1984). The local climate has been stable for hundreds of years, but the climate has undergone a significant change during the past 30 yr. Both annual temperatures and accumulated temperature during the growing season have increased, and the growing season has lengthened (Zong et al., 2013a). Herbaceous plants on the western slopes of the Changbai Mountains tundra

have increased since the 1990s, and they have successfully invaded this alpine landscape with a gradual trend that follows an altitudinal gradient. These herb species, which previously either occurred in the birch forest zone or were occasionally observed in tundra, are now forming patches and have often become common or even dominant species (Jin et al., 2016a). Currently, the expansion range has extended from relatively low elevations to higher elevations in the tundra. Herb patches at lower elevations are interconnected and form relatively large patches after years of expansion. At higher elevations in the tundra landscape, these herb patches are smaller and more scattered because they are in the initial stage of expansion.

Interactions between above- and below-ground biological communities are potential drivers of community and ecosystem dynamics (Wardle et al., 2004). To date, studies of changes in the tundra vegetation of the Changbai Mountains have focused on the herbaceous plant expansion pattern (Zong et al., 2013b, 2016; Jin et al., 2016b). Expansion of the herbaceous plant *Calamagrostis angustifolia* into tundra areas is thought to be related to climate change and vegetation succession (Xu and Zhang, 2010). Water erosion of volcanic ash, vegetation succession, as well as the physical and chemical soil properties can also impact herbaceous plant upward expansion from low elevation forest areas into high elevation tundra areas (Jin et al., 2013; 2014; 2016a). However, long-term observation of the below-ground processes for herb expansion phenomenon in the tundra of the Changbai Mountains is limited. We used the spatiotemporal substitution method to further understand the responses of soil microorganisms, changes in soil enzyme activity to herbaceous plant expansion, and the possible change process of tundra vegetation (Hollingsworth et al., 2010). This method divides the tundra vegetation into different levels of herbaceous plant expansion to reflect spatial patterns in different stages. We conducted the vegetation survey to evaluate the degree of herbaceous plant expansion. We analyzed the changes in the plant community after herbaceous plant encroachment. We hypothesized that soil microorganisms and soil enzyme activities would be significantly increased with intensified herbaceous plant expansion. We also hypothesized that soil pH and organic matter should largely be correlated with microorganisms and enzyme activities. To test the hypotheses, we determined soil microbes, enzyme activity, as well as physical and chemical properties at different degrees of herbaceous expansion. We then explored the relationships of these variables with key factors such as soil temperature, water content, pH, and nutrients. We aimed to determine whether these changes would facilitate further expansion of the herbaceous plant habitat.

2 Materials and Methods

2.1 Study area

Changbai Mountains (41°23'N–42°36'N, 126°55'E–129°00'E) are located in the southeast portion of Jilin Province in Northeast China, which extends along the border of China and North Korea (Fig. 1), reaching an elevation of 2744 m with the peak. Due to the high elevation, altitudinal zonation occurs with regard to climate, vegetation, and soils. From the mountain base to the summit, the vertical zones include mountain mixed broadleaf-coniferous forest, mountain coniferous forest, subalpine *Betula ermanii* forest, and alpine tundra.

Tundra on Changbai Mountains occurs above 2000 m in elevation and is well developed on the upper part of the volcanic cone at altitudes between 2000 and 2300 m. The tundra area has a cold climate with low temperatures (daily mean temperature is 5.9℃ from June to September). The mean annual precipitation is 1340 mm. This precipitation mostly occurs during June to September, which account for about 80% of the yearly total (The Tianchi Meteorological Station is located in the tundra zone, 42°01'N, 128°05'E, 2623 m) (Zong et al., 2016). The surface is mostly covered by weathered alkaline trachyte and small amounts of volcanic ash (local term: trass). The weathered material is rich in potassium. The landform is characterized by water-transformed volcanic slopes with thin tundra soil (common thickness ranging from 10–20 cm). The soil has high organic matter content (generally 20%–30%) but a low number of microorganisms, low enzyme activity, low base saturation, and little N and P (Chen and Zhang, 1983).

The tundra of the Changbai Mountains can be divided into two categories (Huang and Li, 1984). The main type is shrub tundra which occupies most of this area. Dwarf shrubs, such as *Rhododendron chrysanthum* and *Vaccinium uliginosum*, are the dominant plants within the shrub tundra category. There are also many other
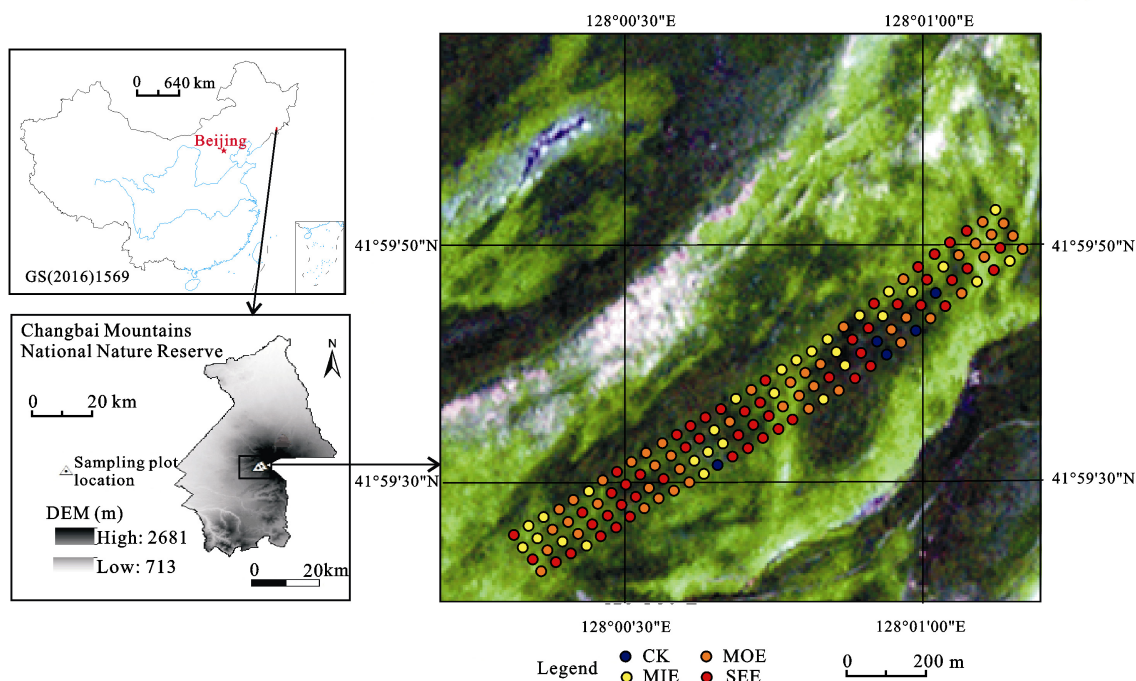
Fig. 1 Location of study area and sampling sites (The background image was acquired by TDI-CCD images sensor on July 13, 2013, with an accuracy of 0.5 m)

plant species, and approximately 80% of those are restricted to alpine habitats. The community has two synusiae: shrubs and lichen/moss. The height of the shrub layer was within the range 8–22 cm (Huang and Li, 1984). The second category is herb-shrub tundra which covers a smaller area and is distributed in moist habitats. This category is mainly dominated by herbaceous hygrophytes, such as *Sanguisorba stipulata* and *Sanguisorba parviflora* (Lang and Li, 2010). Seven dominant plants were identified in the tundra of Changbai Mountains. These were *Rhododendron chrysanthum*, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*, *Phyllodoce caerulea*, *Dryas octopetala* var. *asiatica*, *Rhododendron confertissimum*, and *Sanguisorba sitchensis* (Qian and Zhang, 1980).

2.2 Experimental design

2.2.1 Vegetation sampling

The vegetation survey was conducted in the tundra zone in order to assess the herbaceous plant expansion. The survey area was divided into four levels based on the coverage of herbaceous plants.

In August 2015, a 1600 m × 100 m vegetation plot was established in the tundra zone on the west slope of Changbai Mountain within the altitude range of

2050–2300 m. The 100 m-long sample transects were set at 50 m intervals from low to high elevation, with a 5 m-wide spacer on both sides. Four 1 m × 1 m quadrants were set at 30 m intervals. A total of 132 quadrats were generated (Fig. 1). Plant community characteristics (species, plant number, plant height, and coverage) were recorded for the systematic quadrat survey.

The quadrats were characterized according to the coverage of herbaceous plants. The relative coverage equals the coverage of the specific species divided by the overall coverage of all species. According to the relative coverage of herbs, the tundra was characterized as shrub tundra (herb coverage <10%), tundra with mild expansion (10%–30%), tundra with moderate expansion (herb coverage 30%–70%), and tundra with severe expansion (herb coverage >70%).

2.2.2 Soil sampling

According to the four level division in the vegetation survey, the four treatments consisted of 'shrub area' (non-expanded control area; CK), 'mild expansion area' (MIE), 'moderate expansion area' (MOE), and 'severe expansion area' (SEE). In order to reveal the response of soil microbes, soil enzyme activity, as well as soil physical and chemical properties to the different levels of herbaceous expansion, we selected new sample sites

within the large vegetation survey plot. Site selection aimed at minimizing the probability of differences existing prior to the herbaceous expansion event. To that end, herbaceous expansion and control plots within a site had similar topography and soil texture. Furthermore, the plots of the same treatment had almost the same coverage of herbaceous plants.

Sampling areas with *R. chrysanthum* as the dominant shrub were selected on gentle slopes (slope < 5°) at an altitude between 2280–2300 m in the large vegetation survey plot. These areas included 'shrub area' (with coverage of herbaceous plant as 0) and 'tundra with herbaceous plant expansion area'. Tundra with herbaceous plant expansion area was divided into three types: 'mild expansion area' (MIE) with coverage of herbaceous plant of 25%, 'moderate expansion area' (MOE) with coverage of herbaceous plant of 50%, and 'severe expansion area' (SEE) with coverage of herbaceous plant of 75%. Four replicate sample sites were set with an area of 2 m × 2 m each (16 quadrats total).

Sampling was conducted in early September 2015. Soil temperature and soil water content from the 0–10 cm layer below the soil surface were measured by EM50 (Decagon, US) in each quadrat. Soil samples were collected from the 0–10 cm soil layer and a 20 cm × 20 cm volume was analyzed in the laboratory for chemical characteristics. Within each sample quadrat, soil samples were collected from the 0–10 cm layer at five equidistant points in a zig-zag pattern. This was followed by even mixing and storage in a cooler. The remaining plant, root, and gravel material was removed manually and the soil was passed through a 2 mm sieve. The sample was stored at 4℃ prior to analysis of the soil enzyme activities and chemical properties. The sample was stored in at –20℃ prior to analysis of microbial community structure.

2.3 Measurement of microbial community, soil enzyme activities and soil chemical properties

2.3.1 Measurement of microbial community

Phospholipid fatty acid (PLFA) analysis was used to investigate the total soil PLFAs and the structure of soil microbial communities according to Bååth and Anderson (2003). PLFAs were extracted from the cellular membrane of microorganisms using the Bligh and Dyer method (1959). PLFA samples were identified with a gas chromatograph to obtain the PLFA levels for bacteria, fungi, and actinomycetes. The sum of all PLFAs was used to represent the viable microbial biomass.

The 13 PLFAs (i15 : 0, a15 : 0, 15 : 0, i16 : 0, 16 : 1ω7c, i17 : 0, cy17 : 0, cy19 : 0, 17 : 0, 18 : 1ω9c, 18 : 2ω6, 10Me16 : 0, and 10Me18 : 0) which were consistently present in the samples were used for data analysis. The fatty-acid signatures 15 : 0, 17 : 0, i15 : 0, a15 : 0, i16 : 0, i17 : 0, 16 : 1ω7c, cy17 : 0, and cy19 : 0 are considered to be of bacterial origin (Frostegård and Bååth, 1996), and these were used as biomarkers for the bacterial biomass. The fatty acid 18:1ω9c and the isomer 18:2ω6 were used as indicators for the fungal biomass (Frostegård and Bååth, 1996), while 10Me16 : 0 and 10Me18 : 0 were used as indicators for the actinomycetes biomass (Frostegård and Bååth, 1996). The fatty acids i15 : 0, a15 : 0, i16 : 0, and i17 : 0 were used to represent the Gram-positive (G^+) bacteria, while 16 : 1ω7c, cy17 : 0, and cy19 : 0 were used to represent the Gram-negative (G^-) bacteria (Djukic et al., 2010).

2.3.2 Measurement of soil enzyme activities

Potential activities of extra-cellular enzymes, which indicated the functional potential of the soil microbial community and soil enzyme activities, were measured according to Saiya-Cork et al. (2002). We measured the activities of three soil hydrolases and two oxidases which are significantly correlated with soil nutrient cycling (Xu, 2017). The three soil hydrolases were β-1,4-glucosidase (βG), β-1, 4-N-acetylglucosaminidase (NAG), and acid phosphatase (aP), with 4-MUB-β-D-glucoside, 4-MUB-N-acetyl-b-D-glucosaminide, and 4-MUB-phosphate as substrates, respectively. Activity of hydrolases in soil was measured using the fluorescence method with a microtiter plate. Acetate buffer was added to soil samples, and this was followed by mixing to obtain a soil suspension. The suspension was added to the microtiter plate using the eight-channel pipette, and the suspension was supplemented with respective substrate and MUB. The mixture was cultured in a 20℃ incubator for 4 h, and NaOH was then added to terminate the reaction. The microtiter plate was placed in a microplate reader to measure the fluorescence.

The two soil oxidases were polyphenol oxidase (PPO) and peroxidase (PER), with L-DOPA and EDTA (50 mM disodium ethylenediamine tetraacetic acid) as substrate, respectively. A spectrophotometric method was used to measure oxidases in soil. This was done by adding acetate buffer to soil samples and then mixing

the combination to obtain an even soil suspension. The suspension was added to a microtiter plate using an eight-channel pipette. This was followed by addition of the corresponding substrate and H_2O_2. The resulting mixture was then placed in an incubator to culture for 4h. This was followed by reading absorption values with a microplate reader.

We used the SynergyH4 full functional enzyme standard instrument (BioTek, US), eight-channel pipette (Eppendorf, Germany), incubator, analytical balance (METTLER TOLEDO, Switzerland), vortex oscillator, and high speed freezing centrifuge (centrifuge 5810 R) to complete the measurement of soil enzyme activities.

2.3.3 Soil chemical analyses

The pH of the soil in distilled water was measured at the ratio of 1 : 2.5. The soil organic matter (SOM) concentration was determined by the wet oxidation method (K_2CrO_4) (Lu, 2000). Soil total nitrogen (TN), total phosphorus (TP), and available phosphorus (AP) concentrations in the soil were examined by the continuous flow analyzer (SKALAR SAN++) after pretreatment. The soil was digested by H_2SO_4 to obtain TN with a mixture of Se, $CuSO_4$, and K_2SO_4 (1 : 10 : 100) acting as the catalysis (Lu, 2000). TP was determined via digestion by H_2SO_4 and $HClO_4$. The soil was extracted by $NaHCO_3$ to obtain AP (Huang et al., 2011). The available nitrogen (AN) concentration was determined with an alkali-diffusion method (Bao, 2000).

2.4 Data analysis

The importance value index, which describes the importance of a species within the studied area, was determined according to Mueller-Dombois & Ellenberg formulas (1974). One-way analysis of variance (ANOVA) and least significant difference (LSD) multiple range tests were used to determine the differences between soil PLFAs, soil enzyme activities, and soil physicochemical properties among the different levels of herbaceous expansion. The correlations between different groups of PLFAs, the enzyme activity, and soil physicochemical properties were calculated using Pearson correlation coefficients. Differences were considered significant at $P < 0.05$, with marginal significance set at $P < 0.01$. Data were analyzed by SPSS 18.0 statistical software (Statistical Graphics Crop, Princeton, USA).

3 Results

3.1 Degree of herbaceous plant expansion

Before 1984, shrubs occupied most of the tundra area on Changbai Mountains, and there were only two synusiae: shrubs and lichen/ moss (Huang and Li, 1984). Currently, quadrat investigation shows that shrub tundra accounts for 3.8% of the quadrats. The percentages of 'mild expansion', 'moderate expansion', and 'severe expansion' were 21.9%, 34.1%, and 40.2%, respectively. This indicated a high degree of herbaceous plant expansion in the tundra.

The community composition as well as the appearance of shrub tundra and tundra with different levels of herb expansion (MIE, MOE, and SEE) were significantly different. The dominant species within the different levels of herbaceous plant expansion were significantly different from those in the shrub tundra. Differences in the dominance degree (i.e., importance value index) of dominant species were also detected between the shrub tundra and the tundra with different levels of herbaceous plant expansion. The shrub *R. chrysanthum* was still the dominant species, while the occurrence of *V. uliginosum* dominance decreased dramatically in tundra with severe expansion (Table 1).

The height of shrubs was lower than the height of herbaceous plants in the tundra sites with three levels of herbaceous plant expansion. No significant difference in the height of shrubs was observed among mild expansion, moderate expansion, and shrub tundra ($P > 0.05$). However, a significant shrub height difference was detected between tundra with severe expansion and shrub tundra ($P < 0.05$). A significant difference in height of herbs was observed between the shrub tundra and the tundra sites with different levels of herbaceous plant expansion and ($P < 0.05$, Table 1). Height of herbs in tundra with mild expansion was significantly different between severe expansion and moderate expansion, while there was no significant difference between severe expansion and moderate expansion ($P > 0.05$, Table 1).

3.2 Characteristics of soil physical and chemical properties response to herbaceous plant expansion

There were no significant differences in soil water content between the four treatments ($P > 0.05$). Soil

Table 1 Community characteristics of shrub tundra and tundra with herbaceous plant expansion

Community	Dominant species (importance value)	Average height of shrub (cm)	Average height of herb (cm)
Shrub tundra	*Rhododendron chrysanthum* (72.30) *Vaccinium uliginosum* (42.51) *Sanguisorba parviflora* (29.63) *Saussurea tomentosa* (22.09)	18.38a	17.67 c
Tundra with mild expansion	*Rhododendron chrysanthum* (70.14) *Sanguisorba parviflora* (39.96) *Vaccinium uliginosum* (31.37) *Saussurea tomentosa* (23.41) *Ligularia jamesii* (6.17) *Sanguisorba stipulata* (5.47) *Calamagrostis angustifolia* (4.70)	16.35a	23.67b
Tundra with moderate expansion	*Rhododendron chrysanthum* (68.18) *Sanguisorba parviflora* (44.38) *Saussurea tomentosa* (25.38) *Calamagrostis angustifolia* (19.38) *Ligularia jamesii* (11.06) *Sanguisorba stipulata* (10.73) *Vaccinium uliginosum* (9.57)	16.40a	29.78 a
Tundra with severe expansion	*Sanguisorba stipulata* (57.28) *Ligularia jamesii* (34.47) *Calamagrostis angustifolia* (30.11) *Sanguisorba parviflora* (14.39) *Rhododendron chrysanthum* (11.25) *Saussurea tomentosa* (7.67)	5.58b	31.74 a

Notes: The plots labeled with different letters (a, b, and c) indicate that the differences in average shrub height and average herb height among the different treatments are significant at the *P* < 0.05 level

temperature in the CK and MIE treatments were significantly higher than those of the MOE and SEE treatments (*P* < 0.05). Soil pH levels were significantly different between CK and SEE, with the lowest value in CK and the highest in SEE (*P* < 0.05). Soil pH in MIE was comparable with MOE and CK (*P* > 0.05), while a significant difference was observed in MOE and CK (*P* < 0.05, Table 2). The soil nutrient levels were greatest in MOE with the exception of soil organic matter. The variations in TN, TP, AN, and AP followed the pattern: MOE > SEE > MIE> CK. TN, TP, and AP levels in MOE were significantly higher than those in the SEE, MIE, and CK treatments (*P* < 0.05). However, AN levels in MOE were comparable with MIE and SEE

(*P* > 0.05). Soil organic matter levels in the CK treatment was significantly higher than those in the MIE, MOE, and SEE treatments (*P* < 0.05), and there was significant difference among the MIE, MOE, and SEE treatments (*P* < 0.05, Table 2).

3.3 Characteristics of soil microbial community response to herbaceous plant expansion

Total soil PLFAs were significantly higher in the 'tundra with herbaceous plant expansion' treatments than that in the CK treatment (*P* < 0.05), and they were the highest in the MOE treatment (Fig. 2). Total soil PLFAs in MOE were comparable to MIE, but levels were significantly higher in MOE than in SEE (*P* < 0.05; Fig. 2). Bacteria

Table 2 Soil physical and chemical properties (means±SE) for shrub tundra (CK), tundra with mild expansion (MIE), moderate expansion (MOE), and severe expansion (SEE) treatments

Treatments	Soil temperature (℃)	Soil water content (%)	pH	Soil organic matter (g/kg)	TN (mg/kg)	AN (mg/kg)	TP (mg/kg)	AP (mg/kg)
CK	27.4±0.09a	25.67±1.88	4.67±0.04c	312.58±5.77a	12065.62±104.42b	488.55±8.08b	369.95±3.49c	11.27±0.11d
MIE	27.1±0.06a	27.33±1.23	4.84±0.04bc	214.63±2.24c	12224.92±225.73b	497.54±28.07ab	418.5±2.08c	13.17±0.21c
MOE	26.5±0.21b	27.67±1.79	4.94±0.1b	236.34±2.17b	13241.08±260.34a	546.55±15.44a	466.95±8.1a	17.29±0.3a
SEE	26.4±0.17b	29.37±0.48	5.25±0.01a	188.39±2.47d	12441.99±57.29b	526.47±20.09a	443.16±7.51b	16.4±0.09b

Notes: The plots labeled with different letters (a, b, and c) indicate that the differences in soil physical and chemical properties among the different treatments are significant at the *P* < 0.05 level

and actinomycete PLFAs were comparable between MIE and MOE ($P > 0.05$), but they were significantly lower in SEE and CK ($P < 0.05$, Fig. 2). Soil fungi PLFAs in the MIE, MOE, and SEE treatments were significantly higher than in the CK treatment ($P < 0.05$), while no differences were observed among these three treatments ($P > 0.05$, Fig. 2).

The bacteria response to the herbaceous plant expansion was stronger than the response of fungi and actinomycetes. Herbaceous plant expansion increased the bacteria/fungi ratio compared with CK, and the ratios were relatively higher in MIE and MEE ($P < 0.05$, Fig. 3). The bacteria/actinomycetes ratio increased with the expansion of herbaceous plants, and it was highest in the SEE treatment ($P < 0.05$, Fig. 3). The G^+/G^- ratios in MOE and SEE treatments were significantly higher than in the CK treatment ($P < 0.05$). This indicates that the increase in G^+ bacteria was greater than the increase in G^- bacteria after the herbaceous plant expansion (Fig. 3).

3.4 Characteristics of soil enzyme activities response to herbaceous plant expansion

Soil hydrolase activities (βG activity, NAG activity, and aP activity) were highest in MOE and lowest in the CK treatment. Soil βG and NAG activities in MOE were comparable with MIE ($P > 0.05$), but these values were significantly higher than in SEE and CK ($P < 0.05$). Soil βG and NAG activities in SEE were comparable with MIE and CK ($P > 0.05$). Soil NAG activity in MIE was comparable with CK ($P > 0.05$), but soil βG activity in
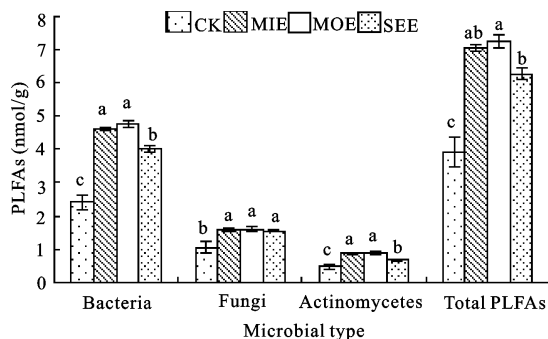

Fig. 2 The total soil phospholipid fatty acids (PLFAs) as well as bacteria, fungi, and actinomycetes PLFAs in shrub tundra (CK), tundra with mild expansion area (MIE), moderate expansion area (MOE), and severe expansion area (SEE) treatments. The plots labeled with different letters (a, b, and c) indicate that the differences in total soil, bacteria, fungi, and actinomycetes PLFAs between the different treatments are significant at the $P < 0.05$ level

MIE and CK exhibited a significant difference ($P < 0.05$, Fig. 4). Soil aP activity in MIE was comparable with MOE and SEE ($P > 0.05$), but the MIE levels were significantly higher than in CK ($P < 0.05$). Soil aP activity in the MIE, SEE, and CK treatments exhibited no significant differences ($P > 0.05$, Fig. 4). The variations in soil oxidase activities (PPO and PER) followed the pattern: MOE > MIE > CK, SEE ($P < 0.05$, Fig. 5).

3.5 Relationship between soil properties and soil microorganisms and enzyme activity

Total soil PLFAs and the different groups of PLFAs were significantly and positively correlated with soil TN, AN, and AP concentrations. However, they were
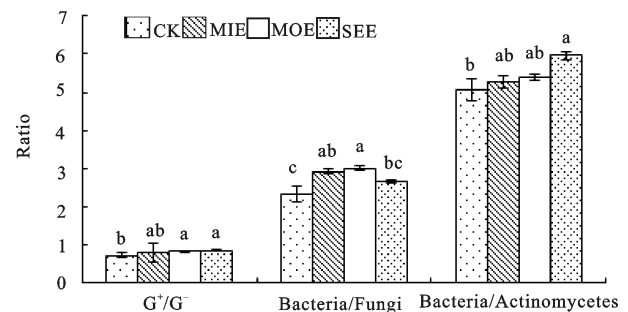

Fig. 3 The ratio of Gram-positive (G^+)/Gram-negative (G^-) bacteria phospholipid fatty acids (PLFAs), bacteria/fungi PLFAs, and bacteria/actinomycetes PLFAs in shrub tundra (CK), tundra with mild expansion (MIE), moderate expansion (MOE), and severe expansion (SEE) treatments. The plots labeled with different letters (a, b, and c) indicate that the differences between the ratio of G^+/G^- bacteria PLFAs, bacteria/fungi PLFAs, and bacteria/actinomycetes PLFAs among the different treatments are significant at the $P < 0.05$ level
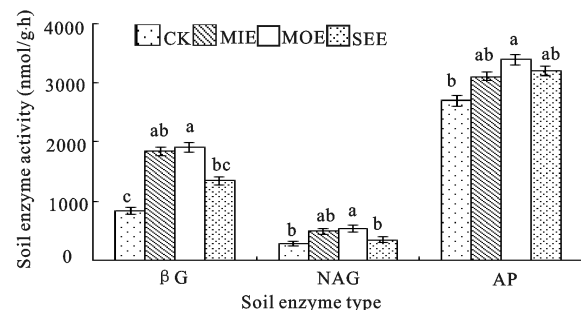

Fig. 4 The β-1,4-glucosidase (βG), β-1, 4-N-acetylglucosaminidase (NAG), and acid phosphatase (aP) activity in shrub tundra (CK), tundra with mild expansion (MIE), moderate expansion (MOE), and severe expansion (SEE) treatments. The plots labeled with different letters (a, b, and c) indicate significant differences in the βG activity, NAG activity, and aP activity at the $P < 0.05$ level
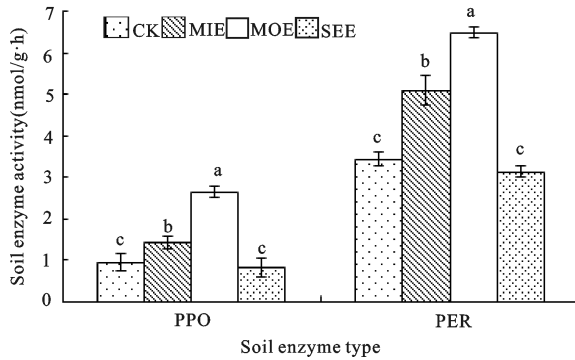
Fig. 5 The polyphenol oxidase (PPO) and peroxidase (PER) activity shrub tundra (CK), tundra with mild expansion (MIE), moderate expansion (MOE), and severe expansion (SEE) treatments. The plots labeled with different letters (a, b, and c) indicate that the differences in the PPO and PER activity among the different treatments are significant at the $P < 0.05$ level

4 Discussion

4.1 Plant change with herb expansion

Alpine tundra habitat is extremely fragile, vulnerable to outside interference, and ecologically sensitive (Wei et al., 2007; Seo et al., 2015). Tundra can be significantly affected by climate change (Mavris et al., 2015). Observed changes in plant communities in both Arctic and alpine environments have been associated with climate warming (Grabherr et al. 2010). Studies have suggested that alpine plants have different response patterns to recent trend of climate change. Tree lines displayed an upward shift in some alpine areas, which reduced the tundra scope, e.g., in Polar Urals (the Rai-Iz massif and Mounts Tchernaya and Malaya Tchernaya) (Shiyatov et al. 2007), in the Swedish of the Scandinavian mountain range (Kullman, 2001). Species in alpine areas also have shifted towards higher altitudes. For example, low altitude species invade the tundra so that a general trend to increased species richness with an accelerated rate had been observed. At the same time, most existing species increased in abundance and colonized new areas of the tundra, which put many alpine tundra species at the risk of the fragmentation and loss of habitat, or even extinction, e.g., in Central Altai, the European Alps, the Sierra Nevada and White Mountain ranges of California in the western U.S. (Butz et al. 2008; Holzinger et al., 2008; Wipf et al., 2013; Artemov, 2018). There were still differences in changes of species in tundra. One result was that the certain species of herbs decreased and certain shrub species increased. Another result was opposing trend which certain species of shrub decreased and certain herbs species increased (Mark et al., 2006; Pickering and Green, 2009). The results of this study show that herbs exhibit an upward shift to tundra, which is consistent with some reported studies (Mark, 2006; Walker et al., 2006; Pickering and Green, 2009; Danby et al., 2011). However, previous studies of the vegetation changes in tundra focused on species richness and diversity rather than community structure due to the absence of long-term monitoring data for vegetation (Danby et al., 2011). Our results suggest that there is a high degree of herbaceous plant expansion in the tundra zone of the Changbai Mountains at present. This suggests a trend towards significant changes in community composition and structure, but the dominant shrub species of the tundra were not entirely replaced or elimi-

negatively correlated with SOC concentration ($P < 0.05$, Fig. 6). Total soil PLFAs and bacteria PLFAs were significantly and positively correlated with the level of TP. Total soil PLFAs and the different groups of PLFAs were positively related with soil pH and soil water content, and they were negatively with soil temperature. However, these correlations were not significant. Soil hydrolase activities and oxidase activities all were significantly and positively correlated with soil TN concentration. Soil PPO activity was significantly and positively correlated with soil AN concentration ($P < 0.05$, Fig. 6). Soil βG, aP, PPO, and PER enzyme activities were significantly positively correlated with AP concentration.
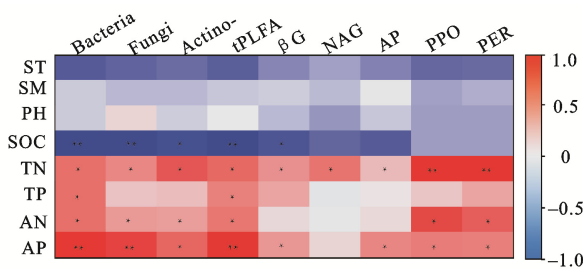

Fig. 6 Correlation analysis of soil microorganisms, soil enzyme activity, as well as soil physical and chemical properties. [*]indicates that the correlations are significant at $P < 0.05$. [**]indicates that the correlations are significant at $P < 0.01$, respectively. Bacteria, Bacteria phospholipid fatty acids (PLFAs); Fungi, Fungi PLFAs; Actino-, Actinomycetes PLFAs; tPLFAs, total soil PLFAs; βG, β-1,4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; aP, acid phosphatase; PPO, polyphenol oxidase; PER, peroxidase; ST, soil temperature; SM, soil water content; TN, total nitrogen; TP, total phosphorus; AN, available nitrogen; AP, available phosphorus

nated. In the three levels of herbaceous plant expansion, the number and importance value of dominant shrub species gradually decreased. Herbs gradually became dominant, and herbs synusiae appeared. The average height of herbs was higher than that of shrubs. In particular, there was only one dominant shrub species (i.e., *Rhododendron chrysanthum*) and importance value of *R. chrysanthum* decreased significantly. The dominant shrub species *Vaccinium uliginosum* disappeared, and the average height of shrubs was far below that of herbs in severe expansion areas. Compared with reported studies (Qian and Zhang, 1980; Huang and Li, 1984; Qian, 1990), our results indicate that shrub tundra of the Changbai Mountains is currently undergoing the process of transforming to herb-shrub tundra, the tundra landscape has gradually changed.

4.2   Responses of soil properties to herb expansion

Our results show that herbaceous plant expansion has significantly altered plant communities as well as the quantity and quality of plant litter. These changes subsequently affected soil physical and chemical properties in the tundra zone of the Changbai Mountains. These findings are consistent with earlier studies (Djukic et al, 2010; Xu et al, 2015). Soil pH increased with herbaceous plant expansion. Soil pH in moderate and severe expansion areas were significantly higher than in the non-expanded areas. This is in agreement with previous studies that reported significantly different soil pH under different plant communities as well as reports of higher soil pH in grassland when compared to acidophilic shrubland sites (Djukic et al, 2010; Li et al, 2017).

Plant residue is a main source of soil organic matter. Wei et al. (2007) suggested that the quantity and quality of plant litter was significantly different for shrubs and herbs in the Changbai Mountains tundra. The biomass of the shrub vegetation type was significantly higher than that of the herb type (Wei et al., 2007). Meanwhile, shrub plant litter, with a high C/N ratio, decomposed at a slower rate than herb plant litter which had a low C/N ratio (Silver, 2001). Our results also show that soil organic matter in the shrub tundra area was significantly higher than SOM levels in herbaceous plant expansion areas.

In the nutrient poor soils of the shrub tundra, low pH results in slow nitrification and mineralization (Williams et al., 1999). This reduces the availability of nitrogen

and limits productivity. Inputs of labile carbon substrates (herb litter) to the soil can significantly stimulate soil organic matter (SOM) decomposition (Xu et al., 2015). This process is termed the priming effect, and it can alter nitrogen cycling in soils and thereby increase soil nutrients (Kuzyakov et al., 2000). Our results show that the herbaceous plant expansion in the Changbai Mountains tundra accelerated the decomposition of soil organic matter and enhanced the content of TN, TP, AN, and AP. The change in species composition has been proved to affect decomposition rates, ecosystem carbon and nitrogen status in the circumpolar Arctic and north temperate alpine regions (Arft et al., 1999; Mclaren et al., 2107), which also was supported in our study.

Dense cushions of small perennial shrubs in this region insulate the ground and create a microclimate with create slightly warmer soil temperatures than areas with herbaceous plant cover (Huang and Li, 1984). The herbaceous plants grow taller and denser than tundra shrub, leading to the decrease of soil temperature and the increase soil moisture.

4.3   Responses of soil microbes and enzyme activity to herb expansion

Previous studies showed that despite the increase in microbial abundance of fungi and bacteria due to climate warming (Väisänen et al., 2019), there was no change in the fungal to bacterial ratio, suggesting that warming did not favor growth of either of the microbial groups (Kandeler et al., 1998; Bardgett et al., 1999). However, climate warming caused the transformation of vegetation type, legacy effects of plant change influences on biotic and abiotic soil properties (Van de Voorde et al., 2011). Our results show that the total soil PLFAs, bacterial/fungal ratio, bacterial/actinomycetes ratio, and soil enzyme activity significantly increased in herbaceous plant expansion areas compared to shrub areas. Our results support the view that plant change may influence the soil microbial community (Van de Voorde et al., 2011; Thakur et al., 2015), and may amplify the effects of climate warming on soil microbial community (Thakur et al., 2015).

In alpine environment, vegetation change may alter the quantity and quality of plant litter. This provides different substrates, which in turn may affect the size of the microbial community and their activity (Djukic et al. 2010). Mixed shrub and herb litter is formed in mild and

moderate herbaceous plant expansion area, and solely herbaceous litter is formed in severe herbaceous plant expansion areas in the Changbai Mountains tundra. Studies have suggested that a mixed litter of shrubs and herbs is more easily decomposed and more conducive to microbial growth than litter with a single component (Dirks et al., 2010; De Marco et al., 2011). We similarly concluded that the total soil PLFAs for mild expansion and moderate expansion areas were higher than those of shrub tundra and severe expansion areas. Meanwhile, our results also show that the total soil PLFAs in shrub tundra areas were lower than those of severe expansion areas.

Shrub litter with high C/N ratio provides a poor substrate which is low in nutrients and rich in recalcitrant compounds for microbial growth. This may lead to low microbial population size (Swift et al., 1979). Herb litter is more likely to be decomposed by microorganisms than shrub litter and is more conducive to microbial growth (Kazakou et al., 2009).

The composition of the soil microbial community was mainly controlled by the pH and C/N ratio of the substrate. The increase of bacterial dominance in soils with high pH and a low C/N ratio is already well documented (Alexander, 1977; Sterner and Elsner, 2002). Among the three types of microorganisms, bacteria have high fecundity, nutrient competition ability, and adaptability to environmental stress. Bacteria can more readily decompose organic matter containing low molecular weight components (Kazakou et al., 2006). Our result is consistent with this view. The herbaceous plant expansion directly inhibits the growth of shrubs, therefore herb litter increased and shrub litter decreased. Compared to the CK, the mixed litters of shrub and herbs as well as the homogenous herb litter exhibited increased pH. This can result in the increase of soil bacteria PLFAs. The fast-growing herb plant species, especially those with highly branched fine root systems, supply large quantities of exudates (Personeni and Loiseau, 2005). These exudates are favored by bacteria, and this pattern is in agreement with our results. Meanwhile, fungi are primarily responsible for decomposing complex compounds with higher C/N ratios (Fierer et al., 2009). Therefore, fungi are more suitable for shrub environments with litter containing higher C/N ratios and nitrogen deficiencies. Actinomycetes mainly decompose recalcitrant substances. The amount of recalcitrant shrub litter is reduced with herbaceous expansion, and this is a likely cause for the increase in the ratio of bacteria.

Soil enzyme activity is significantly positively correlated with soil microorganisms (Groffman, 2001), and root exudates are an important source of soil enzymes (Gramss, 1999). Herb litter increased the total soil PLFAs, and this promoted soil enzyme activity (Kourtev, 2000). The large quantities of root exudates produced by herbaceous plants with highly branched fine root and fibrous roots systems would also increase enzyme activity (Personini and Loiseau, 2005). Therefore, we suggest that expansion of herbaceous plants to the tundra of Changbai Mountains increased soil enzyme activity.

4.4   Implications for plant change in the tundra

Total soil PLFAs and soil enzyme activities were significantly correlated with soil nutrients, but they were not correlated with soil pH. In contrast to the positive correlation between the levels of microorganisms and the content of soil organic matter seen in most regions (Franklin and Mills, 2009; Brockett et al., 2012), a negative association was observed between microorganism levels and soil organic matter concentration in the Changbai Mountains tundra. The soil of shrub tundra has high organic matter concentration, but it has a low number of microorganisms and low enzyme activity (Chen and Zhang, 1983). Our results show that herbaceous plant expansion leads to low organic matter concentration with a high number of microorganisms and high enzyme activity. This is mainly because the sources of soil organic matter is lacking due to the lower quantity of plant litter in the herbaceous plant expansion areas compared to shrub tundra areas. Furthermore, the quality of soil organic matter in the herbaceous plant expansion areas is higher and provides substrates which are more likely to be decomposed by microorganisms. This decreases soil organic matter concentration which results in enhanced soil N and P concentration, increased total soil PLFAs, and higher enzyme activities. Anderson (2011) believed that soil biota exhibit a strong response to vegetation change and that these shifts in soil biota control nutrient availability. They considered the mechanisms by which changes to disturbance regimes might result in changes in productivity and shifts in plant community composition at a range of spatial scales. Increasing available N and P enhanced the com-

petitiveness of herbaceous plants, and it had a direct adverse effect on shrubs which was reflected by gradual replacement of shrubs by herbs (Aerts et al., 1990; Alons et al., 2001). Jin et al. (2016a) also found that increased available nitrogen significantly promoted nitrophilous herbs such as *C. angustifolia*. This increased the competitive ability of these herbs, and these herbs became the new dominant species by gradually replacing shrubs such as *R. chrysanthum* and *V. uliginosum*.

Tundra vegetation in many alpine and subalpine areas has changed over the last few decades, and the upward expansion of herbaceous plants is particularly obvious. Many studies focused on results of vegetation changes, however, the lack of information makes it difficult for us to predict that how will increasing herbs affect decomposition, microbial community structure, function and C and N cycling in Arctic and alpine tundra (Myers-Smith et al. 2011). We present new insights into the process of herbaceous plant expansion into tundra according to our results. Herbaceous plant species in this system create a soil nutrient environment conducive to their own growth or the growth of other herbaceous plants. A positive effect develops between mild and moderate herbs expansion, increased soil microbial biomass, enzyme activity, and soil nutrients. This promotes herb expansion and increases herb dominance. However, severe expansion of herbs represented by one or two dominant herb species results in the replacement of complex mixed litter with solely herbaceous litter. This situation can reduce levels of soil microorganisms and lead to a decrease in soil enzyme activity and soil nutrients. Ecological conditions of severe expansion are less favorable than that of mild and moderate expansion. In turn, inhibition of herbaceous plant growth, the rate of expansion, and expansion degree of herb plants may result from this negative effect.

5   Conclusions

In conclusion, herbs have expanded in the tundra zone in the Changbai Mountains. They have become dominant species in herbaceous plant expansion areas. There were differences in the community composition and appearance of shrub tundra, mild expansion, moderate expansion, and severe expansion areas. The tundra on Changbai Mountains was originally dominated by slow-growing low shrubs. The soils had relatively low total PLFAs and low enzyme activity. The soil was enriched with organic matter but decomposition was slow. This formed an oligotrophic system sensitive to alterations of plant community composition and structure. Expansion of herb plants into the tundra significantly increased soil microbial levels and enzyme activity. This expansion also significantly decreased soil organic matter. Increased available nitrogen and phosphorus in soil favored the growth of herbs. However, soil microorganisms, soil enzyme activity, and soil nutrients responded differently to the various levels of herbaceous plant expansion. The soil conditions of mild and moderate expansion are more favorable than that of severe expansion, and these modifications of soil environment will therefore favor or inhibit the process of herbaceous plant expansion.

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