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The effect of simulated warming on root dynamics and soil microbial community in an alpine meadow of the Qinghai-Tibet Plateau

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ABSTRACT

Previous studies have explored the effects of global change on above-ground vegetation in grassland ecosystems. Few, however, have investigated the responses of root dynamics and soil microbes to simulated warming in alpine meadows of the Qinghai-Tibet Plateau. This research used field open top chamber experiment to examine warming's impacts. Root production, mortality, and turnover were observed in situ using minirhizotron tubes, while soil microbial community composition and diversity were assessed by using phospholipid-derived fatty acids (PLFA) and Biolog-Eco plates. The results showed that 1) warming shifted the root standing crop distribution to deeper soil while increasing the accumulated root mortality and root production at 0–10 cm soil depth; 2) warming increased the abundance of fungal PLFAs while decreasing the abundance of other functional groups; 3) warming decreased the soil microbial functional diversity at the different soil layers; 4) bacterial and fungal PLFA contents were positively correlated with root mortality and turnover but were negatively correlated with root standing crop; 5) root dynamics significantly affect carbon utilization of the soil microbial community; and warming induced changes in root dynamics are associated with soil microbial community structure and function. Overall, these findings indicate that warming alters root production, mortality, and turnover and the relative abundance of different microorganisms in different soil layers. As a result, warming tended to shift microbial communities from bacteria toward fungi, leading to changes in the microbial community structure in different soil layers.

1. Introduction

The global mean temperature is predicted to increase 1.0–3.5 °C in the next 50–100 years, primarily because of greenhouse gas emissions. The effects of global warming on terrestrial ecosystem processes vary with space and time, with high altitude and high latitude systems particularly vulnerable (Beniston, 2000; Rustad, 2001). Experimental warming has been shown to directly change the photosynthetic rate and growth rate, shift plant phenology to extend growth phase for alpine species (Klanderud and Totland, 2005; Walther et al., 2005). Additionally, simulated warming may result in a shift of below-ground biomass distribution to deeper soil layers (Li et al., 2011a; Yu et al., 2015). Previous studies have shown that warming may also indirectly affect soil moisture level and nutrient utilization, leading to changes in the production and distribution of alpine vegetation biomass (Asseng et al., 1998) and vegetation succession and C cycle (Yu et al., 2015).

Below-ground biomass of plants, which is one of the major components of total primary production, plays an important role in productivity of plants and storage of vegetation C (Wu et al., 2011). The root system serves as a dynamic interface between soil and plants (Cai and Shen, 2002) and as an important structural and functional component of the ecosystem. It has been demonstrated that soil C turnover, water balance, and biogeochemical cycling of minerals are largely determined by root morphology, configuration, and distribution of the dominant plants (Schlesinger, 1997). Of the different abiotic factors that may affect root and whole-plant physiology, temperature often has profound impacts (Wang et al., 2016a,b). For example, increased temperature associated with

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climate warming can directly stimulate root growth or indirectly through enhancing belowground C allocation (Bai et al., 2010). Gill and Jackson (2000) indicate that elevated temperature increases root length growth and mortality for many terrestrial ecosystems, but their study highlights the paucity of data for alpine tundra ecosystems. Therefore, understanding about the respond of root dynamics to increasing temperature is critical for predicting ecosystem responses to future global warming.

Soil microbial community composition, structure and diversity are important indicators of soil quality, fertility, and crop productivity (Zelles, 1999). Microorganisms may be able to rapidly respond to changing environments, resulting in altered community structure and functioning (Bardgett et al., 2008; Wallenstein and Hall, 2012). Numerous studies have shown that climate change significantly influences microbial composition and biomass. For instance, Zhang et al. (2005) found that warming decreased microbial biomass and led to a community shift toward fungi in soils of an unclipped tall-grass prairie. Warming also leads to significant reductions in the evenness of bacterial communities, while the evenness of fungal communities increased significantly in the Arctic Deslippe et al. (2012). In addition, warming can stimulate microbial decomposition of litter and SOM (Luo and Zhou, 2006). Moreover, vegetation provides nutrition and energy for microorganisms in the soil, and soil microbial communities are also important suppliers of nutrients to plants that play important roles in plant growth. Changes in plant species composition and community structure are an important part of community responses to climatic change that can generally alter ecosystem stability, biomass production, and nutrient inputs (Kardol et al., 2010; Yang et al., 2011). Different types of plant litter and roots secrete chemicals with different physical and chemical properties that differ upon decomposition, thereby affecting the structure, function and diversity of the microbial community (Keiblinger et al., 2010; Shu et al., 2012). Accordingly, knowledge of warming's effects would help in understanding the microbial roles in terrestrial C cycling and provide data future climate modeling (Zhang et al., 2015).

The Qinghai-Tibet Plateau is the world's largest high and single geomorphic unit, and it is suffering an unprecedented warming trend, which is much greater than the global average over, at last, the last half-century (Wang et al., 2016b; Supplementary Figure 1). Alpine meadows are a widely distributed vegetation type on the plateau, accounting for 35% of its area, and play a critical role in uptake and storage of C (Wang et al., 2016b). Change of root biomass and it decomposition influence soil C sequestration, and soil organic C is decomposed by soil microbial community which are one of the main drivers of the global C cycle (Schindlbacher et al., 2011). Furthermore, about 90% of total root biomass in these systems occurs in the top 30 cm of soil (Yang et al., 2009). In additin, soil depth is an important spatial factor determining microbial community composition assembly (Yuan et al., 2014). Soil microbial biomass and C metabolic activity have traditionally been used as indicators of soil fertility, with decreases of the above indicators indicating a decline in soil quality (Zheng et al., 2005). In recent years, the development of biochemical, physiological and molecular biological approaches have overcome the shortcomings of traditional culture methods, making it possible to understand changes in microbial community composition and diversity under different situations (Feng and Simpson, 2009).

In this study, we used a minirhizotron technique to monitor root growth and production under experimentally warmed soils, Phospholipid-derived fatty acids (PLFAs) and Biolog-Eco plates were used to identify microbial community composition and diversity in experiments conducted in open top chambers (OTCs) in an alpine meadow. Specifically, we investigated the effect of simulated warming on 1) root growth, production, and turnover at different depths of alpine meadow soil (0–10 cm or 10–20 cm); and 2) the amount and composition of the microbial community at different depths in alpine meadow soil (0–10 cm or 10–20 cm); we determined the relationship of plant root production and the soil

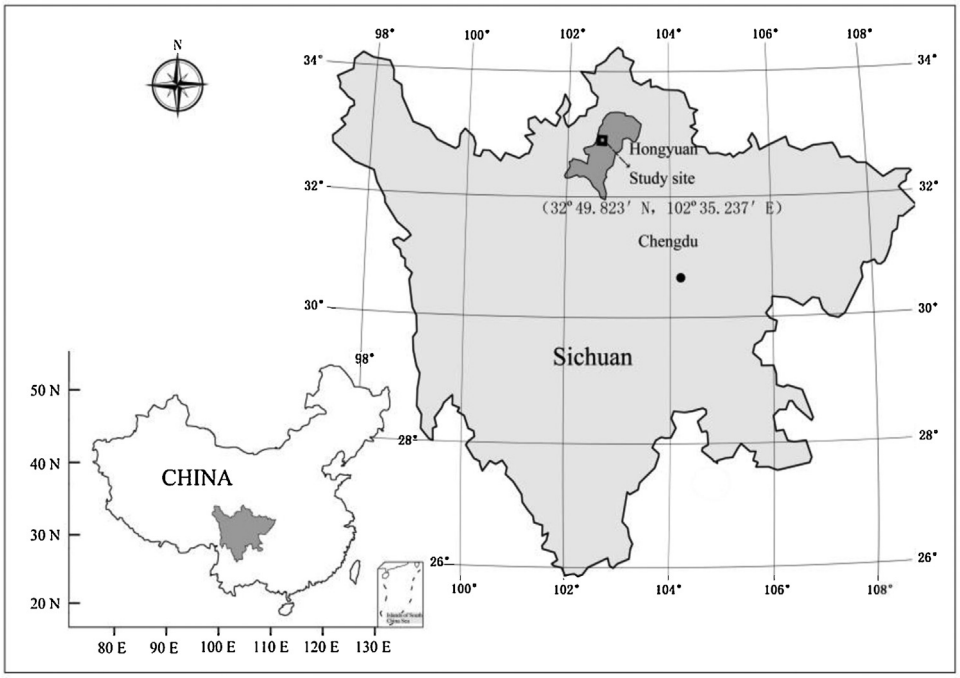

Fig. 1. The locations and long-term sampling map of the alpine meadow in the Northwestern Sichuan, China.

microbial community at different depths in alpine meadow soil (0–10 cm or 10–20 cm).

2. Materials and methods

2.1. Study region and site description

Field work was conducted in an alpine meadow ecosystem in Hongyuan County, Sichuan Province, on the eastern Qinghai-Tibetan Plateau (32°48′ N, 102°33′ E) (Fig. 1). The study site has an elevation of 3500 m a.s.l. (above sea level), an annual mean temperature of 0.9 °C and an annual mean precipitation of 690 mm. The dominant plant species in the alpine meadow include sedges (*Kobresia setchwanensis*,*Kobresia pygmaea*), grasses (*Agrostis clavata*, *Elymus nutans*) and forbs (*Anemone trullifolia*, *Potentilla anserine*, *Saussurea nigrescens*). The grass community typically grows in 1–2 layers, with a maximum height of 45–60 cm. Overall, grasses cover 60–95% of the ground. The soils at the study site are described as Cryosol according to the World Reference Base for Soil Resources (Li et al., 2011b). A detailed site description can be found in Li et al. (2011b).

2.2. Experimental design

In April 2009, we fenced a 100 × 100 m plot in a typical *Kobresia humilis* meadow with a fairly uniform mixture of vascular plant species, such that more than 90% of the plant species in the community could be found in any 50 × 50 cm square. Yak and Tibetan sheep grazing occurred before fencing, but was precluded thereafter. In May of 2009, twenty 2 × 2 × 2 m (height) open-top chambers (OTCs) (Supplementary Figure 2) were randomly deployed (at a minimum distance of 3 m between them) within the fenced area. The sides of the chambers were covered with polycarbonate screens with a transparency of over 90%. Each OTC was sunk 10 cm into the soil and firmly stabilized. OTCs are used in the International Tundra Experiment and are commonly employed to study the effects of climate warming on ecosystems (Walker et al., 2006). Moreover, OTCs are one of the most economic, simple and easy to do warming equipment, supporting cost is not high, can be used for there were no electricity and remote mountain area, but to simulate climate change may be inadequacy (Niu et al., 2007). To examine how well the OTCs simulated climate warming effects, we designated one 2 × 2 m plot near each OTC as the unwarmed control (CK), which was also fenced before the experiment. In the study, six OTCs and six CK plots were analyzed.

2.3. Soil temperature and moisture

A multi-point soil temperature and humidity meter (YM-01, Yiming Electronics of Handan, Hebei, China) was installed to record soil temperature and moisture at 10 min intervals at soil-layers of 0–10 cm, 10–20 cm, and 20–30 cm from November 2013 to January 2015. For every three months, batteries were changed and temperature and moisture data were downloaded. Data from January to December 2014 were further analyzed to determine the mean annual soil temperature and moisture. A multi-point air temperature and humidity meter (YM-17, Yiming Electronics, Handan, Hebei, China) was also installed to record air temperature and humidity for every 10 min at 30 cm above the ground. Data collected from January to December 2014 were used to determine the mean annual temperature and humidity.

The annual mean temperatures at soil depths of 0–10 cm, 10–20 cm, and 20–30 cm were 0.31, 0.57, and 0.30 °C higher in open top chambers (OTCs) than in CK, respectively. The annual mean soil moisture at the three different depths in the OTCs increased by −11.75, 5.13, and 37.25%, respectively, compared with in CK (Supplementary Table 1). At 30 cm above ground, the annual mean air temperature in the OTCs was 3.72 °C higher than that in the CK, while the annual mean humidity was 4.21% lower (Supplementary Table 2).

2.4. Plant sampling

Plant sampling was conducted during the peak of plant biomass in August from 2009–2014. Plant cover was measured annually in permanent quadrats (50 × 50 cm) from 2009 to 2014. One permanent quadrat (50 × 50 cm) was established in each OTC and CK in April 2009. During the measurements, a 50 × 50 cm frame with 100 equally distributed grids (5 × 5 cm) was placed above the canopy in each quadrat. The coverage of each species was visually estimated for all grids and summed to give the species coverage in the quadrat. The aboveground plant biomass was harvested and separated into four functional groups (grasses, sedges, legumes and forbs). Each species' height, frequency and coverage was recorded for each quadrat CK and OTC with six replicates, respectively.

The plant coverage, species richness and biomass were obtained to characterize the aboveground plant community. The important value (*IV*) of individual plant species was calculated by the following equation to determine the dominance of one species in the community:

$$IV = \frac{Cr + Hr + Fr}{3}$$

where, *Cr*, *Hr* and *Fr* are the relative coverage, relative height and relative frequency, respectively (Tan et al., 2008; Supplementary Table 3).

2.5. Minithizotron tube installation and date acquisition

On August 1, 2013, three experimental plots with warming chambers and three CK plots were selected at random. One polycarbonate minirhizotron tube each (100 cm long, external diameter of 7 cm, inner diameter of 6.4 cm) was buried in the middle under the target plant (dominant species, *Kobresia*) according to the method described by Majdi and Öhrvik (2004), giving a total six tubes. Briefly, a special Edelman drill that fits the CI-600 Root Scanning System (CID BioScience Inc., Camas, WA, USA) was used to drill a hole at 30° (Johnson et al., 2001) that was 60 cm deep and 6 cm in diameter. It should be noted that special care is needed when installing minirhizotron tubes. Specifically, a hole needs to be drilled in the soil, and the walls of the tube must tightly touch the soil as the sealed end of the tube is inserted into the hole. The partition of the tube that remains aboveground should be covered with an opaque black lid to block light exposure of the roots, which may interfere with growth. The space around the tube was then filled with soil, and care was taken to ensure the minirhizotron remained in close contact with the soil while minimizing disturbance around the tube. Finally, the tube was wrapped with a black plastic bag to reduce heat transfer when data are not being fetched (Johnson et al., 2001).

Data were read for the first time on April 27, 2014. Briefly, the black plastic bag wrapped around the minirhizotron tube was removed, after which the lid was opened and the tube wall was wiped with a cotton cloth to remove water condensation. The tube was then left open for 10 min to allow the temperature inside to equilibrate with the outside temperature, after which a calibrated CI-600 video camera was placed into the minirhizotron tube. Next, the location of the camera was marked to ensure subsequent readings were taken from the same spot, after which two sets of images were collected from two slope depths for each

minirhizotron tube, 0–20 cm and 20–60 cm, which correspond to vertical depths of 0–10 cm and 10–20 cm based on the installation angle. Following data collection, the tube was covered again as described above. Data were collected every two weeks until September 20, 2014, a total of 10 times for each tube at depths of 0–10 cm and 10–20 cm. After each data collection, data were imported to the WinRHIZO Tron MF root analysis software to analyze root morphology characteristics.

2.6. Processing of images from minithizotron tubes

The root temporal dynamic analysis software, WinRHIZO Tron MF (CID Biosciences Inc., Camas, WA, USA), was used to examine and analyze images scanned by the CI-600 camera. The roots captured in the images were traced manually using a mouse connected to a PC. The lengths of all roots were accurate to 0.01 cm. Live and dead roots were distinguished by colors and different root systems were numbered. For subsequent image data collected, the growing roots were traced based on previous roots, while roots that were no longer present were deleted. Among the images collected, brown and white roots were considered live, while black and disappeared roots were considered dead (Majdi and Öhrvik, 2004; Bai et al., 2010). All images of live and dead root systems collected were documented.

2.7. Estimates of root production, mortality and turnover

Root production and mortality were estimated as previously described (Majdi and Öhrvik, 2004; Bai et al., 2010). Root length production was defined as the total root length of all new roots at

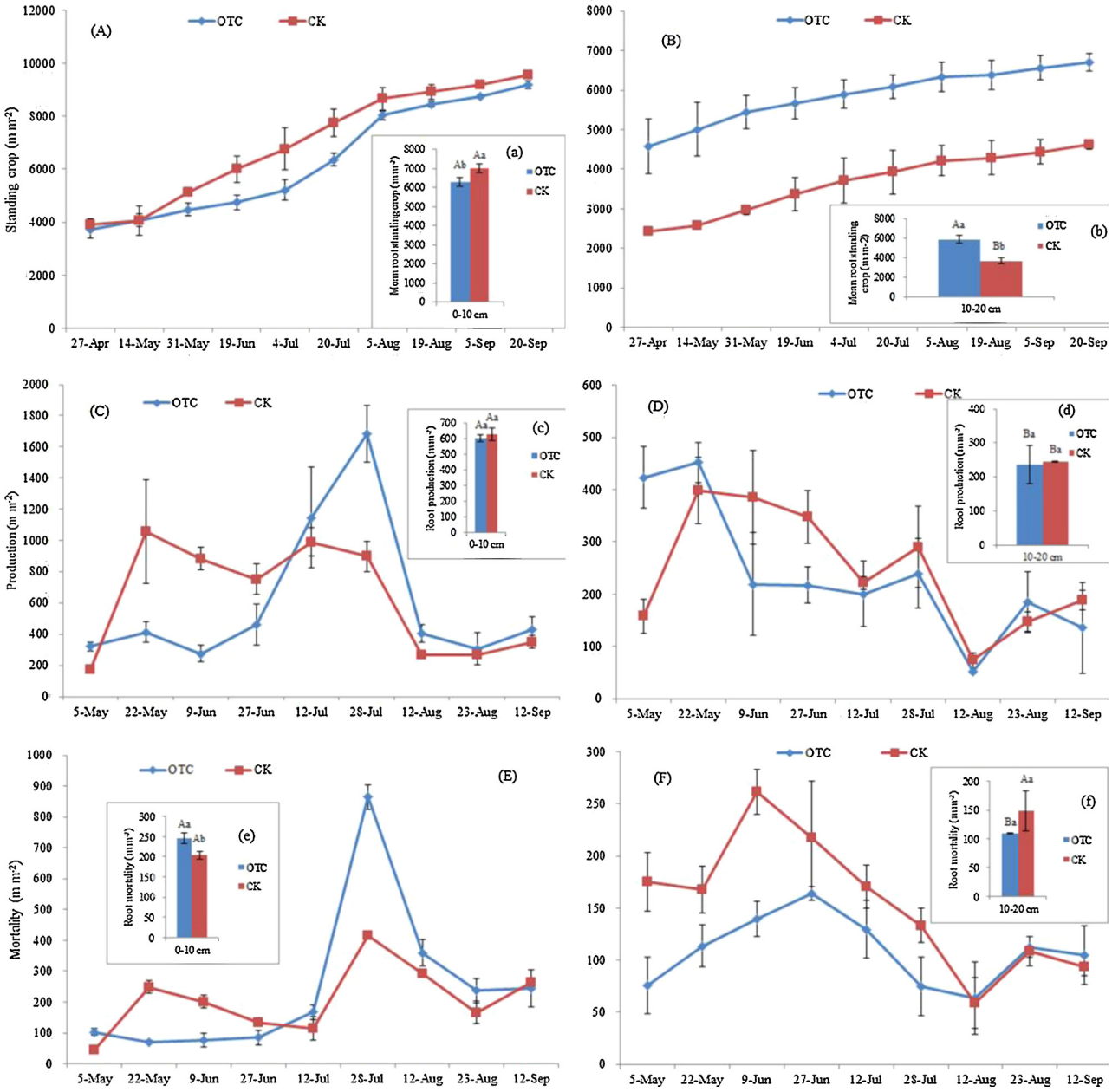

Fig. 2. Seasonal variation of standing crop (A, B), mean root standing crop (MSC a, b); root production (C, D), cumulative length production (CLP c, d); and mortality (E, F), cumulative length mortality (CLM e, f) ($m\,m^{-2}$; mean ± SE, $n = 3$) at the soil depths of 0–10 cm (left), 10–20 cm (right) in the open top chambers (OTCs) and control (CK). *Note*: Different lowercase letters indicate significantly different between different treatments in the same soil depths; Different capital letters indicate significant difference between different soil depths in the same treatments ($P < 0.05$).

time $t+1$ that were not present at time t, plus the growth increment of the roots present at time t. Mortality was measured as the total length of new and living roots at time t that had died by time $t+1$. Root production and mortality were expressed as root length per unit area of the tube wall measured ($m\,m^{-2}$). Changes in root standing crop are usually the differences between seasonal production and mortality (Norby et al., 2004). Annual root turnover is the ratio between the accumulative root length growth in each growing season and the mean length of live roots (Majdi and Andersson, 2005).

2.8. Soil sampling and PLFAs of the soil microorganisms

Soil samples (as close to the Minirhizotron tube as possible) were collected from OTCs and CK quadrats, respectively. For each quadrat, five soil cores (5 cm diameter) were collected and mixed to produce a single soil sample for that quadrat, after which soil samples were collected from areas from which the vegetation had recently been removed. These samples were split into 0–10 cm and 10–20 cm sections in August of 2014. Next, the samples were mixed by depth in each quadrat, stored in iceboxes, and transported to the molecular biology laboratory at Southwest University for Nationalities, China. After removing roots and stones by passing the samples through a 2 mm mesh sieve, samples were frozen at −70 °C until PLFA analysis (see Wang et al., 2015 for details), which was conducted using previously identified PLFA biomarkers for soil microbial biomass (Supplementary Table 4).

2.9. Microbial functional diversity

The functional diversity of the microbial community was measured using BIOLOG ECO plates (Biolog Inc., Hayward, CA, USA). The 96-well ECO plate comprised 31 C substrates in triplicate for each substrate and control well without any C substrate (detailed process description see Wang et al., 2016a). We analyzed the data from the ECO plates by averaging the three values for each individual substrate used within a plate.

2.10. Data analysis

Two-way analysis of variance (ANOVA)s with a randomized block design was used to evaluate the effects of warming and different soil layers (0–10, 10–20 cm) on root production, mortality, standing crop, and root turnover. Substrate utilization patterns (BIOLOG data) and PLFA profiles (three repeats) were analyzed by principal component analysis (PCA) to identify differences in the soil microbial community structure that were induced by warming. Soil microbial community functional diversity in different soil depth was tested using two-way ANOVAs with the least significant difference (LSD) tests at levels of $P=0.05$. Correlations between the soil PLFA content, substrate utilization and root production, standing crop and root turnover were determined using a linear Pearson's coefficient (r). PCA was performed using CANOCO for Windows, version 4.02 (ter Braak, 1998). All other analyses were conducted using SPSS 16.0 software (SPSS Inc., version 16.0).

3. Results

3.1. Effects of warming on root standing crop, production and mortality

At 0–10 cm soil layer, root standing crops increased during the growing season (April 27 to September 20) in both OTCs and CK, but after September, growth gradually stabilized (Fig. 2A). The maximum difference in root standing crop was observed on July 4, while the minimum was observed on May 14 (Fig. 2A). Standing crops were significantly decreased by OTCs (Fig. 2A). At 10–20 cm, the initial root standing crop in the OTCs was 2161.66 m m^{-2} higher than CK on April 27 (Fig. 2B). During the growing season, root standing crops were increasing gradually in the OTCs and CK (Fig. 2A, B).

Root production was decreased by OTCs treatment at 0–10 cm in CK (Fig. 2C). The maximum root production was observed on May 22 in the CK (Fig. 2C). Root production was high in July, reaching the maximum value on July 28 in the OTCs (Fig. 2C). At 10–20 cm, the maximum root production in the OTCs and CK was observed on May 22, after which it started decreasing (Fig. 2D).

Root mortality at 0–10 cm in the OTCs showed a single peak curve, with the maximum value being observed on July 28 (Fig. 2E). The maximum root mortality in the CK was also reached on July 28, but did not fluctuate greatly from month to month (Fig. 2E). At 10–20 cm, the maximum root mortality in the OTCs and CK was observed in June (Fig. 2F). Root mortality was lower in the OTCs than the CK (Fig. 2F).

3.2. Effects of warming on cumulative length production, cumulative length mortality and mean root standing crop

The mean root standing crop of the dominant plant species in the alpine meadow, *Kobresia*, is affected by warming, depth, and their interaction ($P<0.01$, Table 1). Warming inhibited the increase of mean root standing crop at 0–10 cm ($P<0.01$, Table 1 and Fig. 2a). Compared with the CK, warming lowered the root standing crop by 9.88% at 0–10 cm. At 10–20 cm, the root standing crop in the OTCs was 60.59% higher than the CK ($P<0.01$, Table 1 and Fig. 2b).

The cumulative root length production in the OTCs was less than the CK, but the difference was not significant ($P>0.05$, Table 1). The cumulative length production at 0–10 cm was significantly higher than at 10–20 cm ($P<0.01$, Table 1 and Fig. 2c, d).

The cumulative root length mortality was higher in the OTCs than the CK at 0–10 cm, while it was lower at 10–20 cm, but the differences were not significant. However, the cumulative root length mortality at 0–10 cm was significantly higher than at 10–20 cm in the OTCs ($P<0.01$, Table 1 and Fig. 2e, f).

Table 1
ANOVA for mean root standing crop (MSC), cumulative length production (CLP), cumulative length mortality (CLM) ($m\,m^{-2}$; mean ± SE, $n=3$), and root turnover ($year^{-1}$; mean ± SE, $n=3$) over the growing seasons of 2014 (April 27–September 20) at the soil depths of 0–10, 10–20 cm under warming manipulations in a *Kobresia tibetica* meadow.

Items	*df*	MSC		CLP		CLM		Root turnover	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	1, 12	19.076	=0.002	0.57	=0.472	0.001	=0.972	5.843	=0.042
Depth	1, 12	117.157	<0.001	321.328	<0.001	73.602	<0.001	76.945	<0.001
Treatment × Depth	1, 12	69.418	<0.001	0.086	=0.776	13.108	=0.007	12.014	=0.008

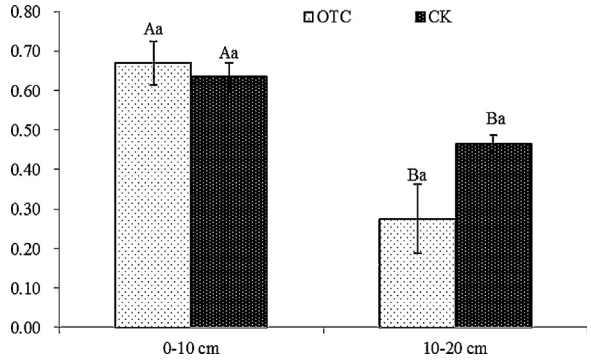

Fig. 3. Annual root turnovers (year^{-1}; mean ± SE, $n = 3$) over the growing seasons of 2014 (April 27–September 20) at the soil depths of 0–10, 10–20 cm under warming manipulations in a *Kobresia tibetica* meadow. Multiple comparisons of group means among treatments were carried out with LSD after two-way ANOVA indicated significant effect of treatment. *Note*: Different lowercase letters indicate significantly different between different treatments in the same soil depths; Different capital letters indicate significant difference between different soil depths in the same treatments ($P < 0.05$).

3.3. Effect of warming on root turnover

Root turnover is significantly affected by warming, soil depths and their interaction ($P < 0.01$, Table 1 and Fig. 3). At 0–10 cm, experimental warming promoted root turnover of the dominant species ($P < 0.01$, Table 1 and Fig. 3), with root turnover being 0.03/year higher than the CK. At 10–20 cm, warm temperature simulation was unfavorable for root turnover ($P < 0.01$, Table 1 and Fig. 3), resulting in turnover being 0.19/year lower than the CK.

3.4. Effect of warming on PLFA content and functional diversity of microorganisms

Warming had a significant impact on total microbial PLFAs, Gram-positive bacteria PLFAs (G^+ PLFAs), Gram-negative bacteria PLFAs (G^- PLFAs), and fungal PLFAs (F PLFA) at different soil depths ($P < 0.05$). At 0–10 cm, total PLFAs, G^+ PLFAs, and G^- PLFAs were significantly lower (by 12.21%, 18.94%, and 49.97%, respectively), whereas F PLFAs were significantly higher (by 49.52%) in the OTCs compared to CK. At 10–20 cm, total PLFAs and G^- PLFAs were significantly lower (by 16.44% and 21.67%, respectively), but F PLFAs were significantly higher (by 20.61%) when compared to CK. Total PLFAs and PLFAs of various microorganisms were significantly reduced as soil depth increased, regardless of the treatment (Fig. 4).

The soil microbial structure determined through PCA of the PLFAs data showed the distribution on the PC axis was significantly different (Fig. 5). Different soil depths of the warming and CK groups (OTC 0–10 cm, OTC 10–20 cm; CK 0–10 cm, CK 10–20 cm) were in different quadrants of the PCA plot, indicating that different soil microbial PLFAs responded differently to warming.

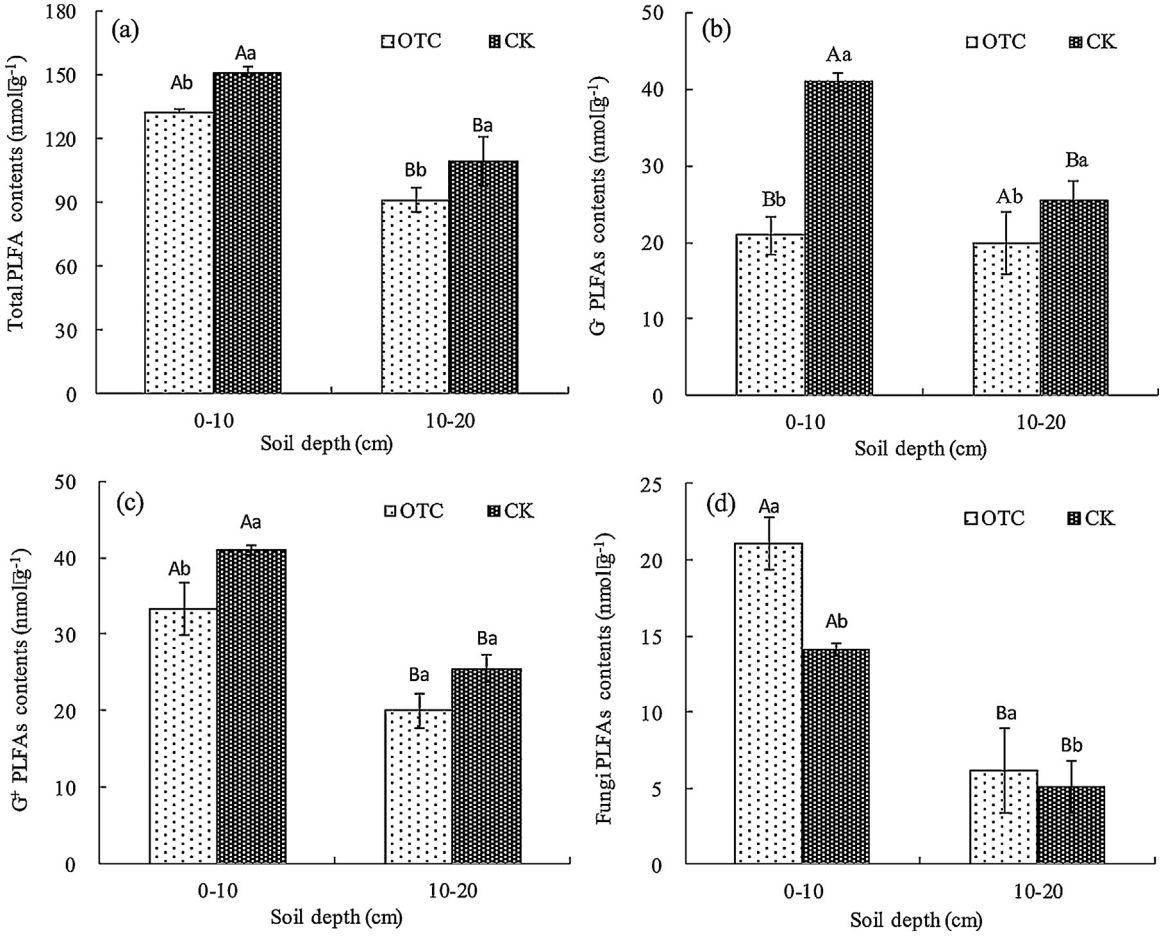

Fig. 4. Effects of warming on different soil microbial PLFAs groups at the soil depths of 0–10 cm, 10–20 cm in the open top chambers (OTCs) and control (CK). Multiple comparisons of group means among treatments were carried out with LSD after two-way ANOVA indicated significant effect of treatment (means ± SE, $n = 3$). *Note*: Different lowercase letters indicate significantly different between different treatments in the same soil depths; Different capital letters indicate significant difference between different soil depths in the same treatments ($P < 0.05$). B PLFAs: bacterial PLFAs; F PLFAs: fungal PLFAs; G^+ PLFAs: Gram-positive bacterial PLFAs; G^- PLFAs: Gram-negative bacterial PLFAs.

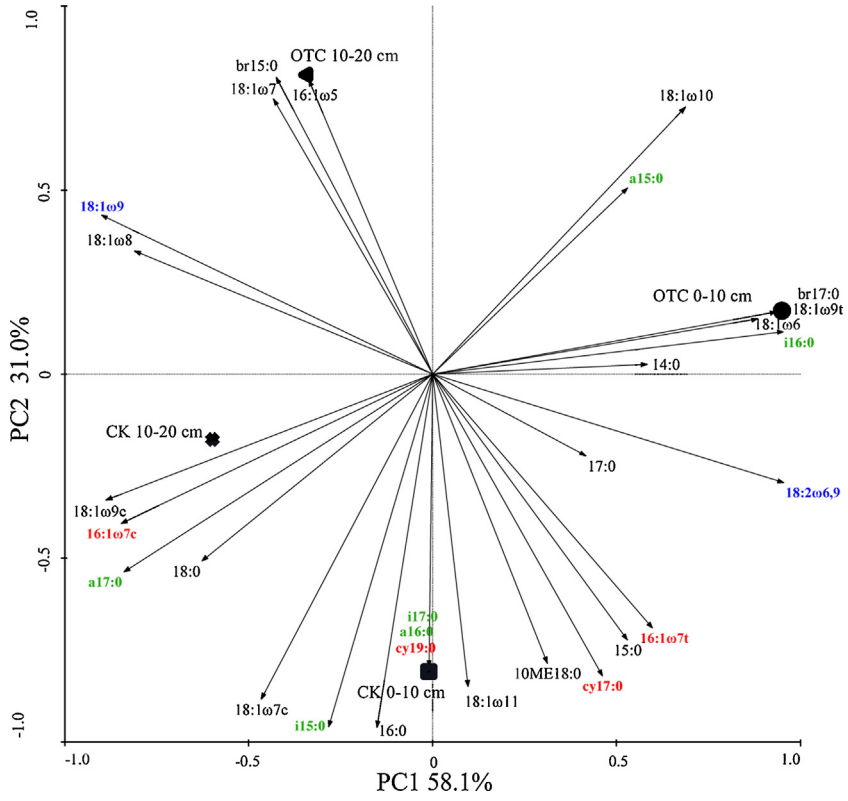

Fig. 5. Principal component analysis (PCA) plots for all phospholipid fatty acid (PLFA) signatures detected in the 0–10 cm and 10–20 cm soil layers in the warmed open top chambers (OTCs) and the control (CK). Principal components 1 (PC1) and 2 (PC2) explain 58.1% and 31.0% of the variation at the 0–10 cm and 10–20 cm soil layer in PLFAs in the alpine meadow, respectively.

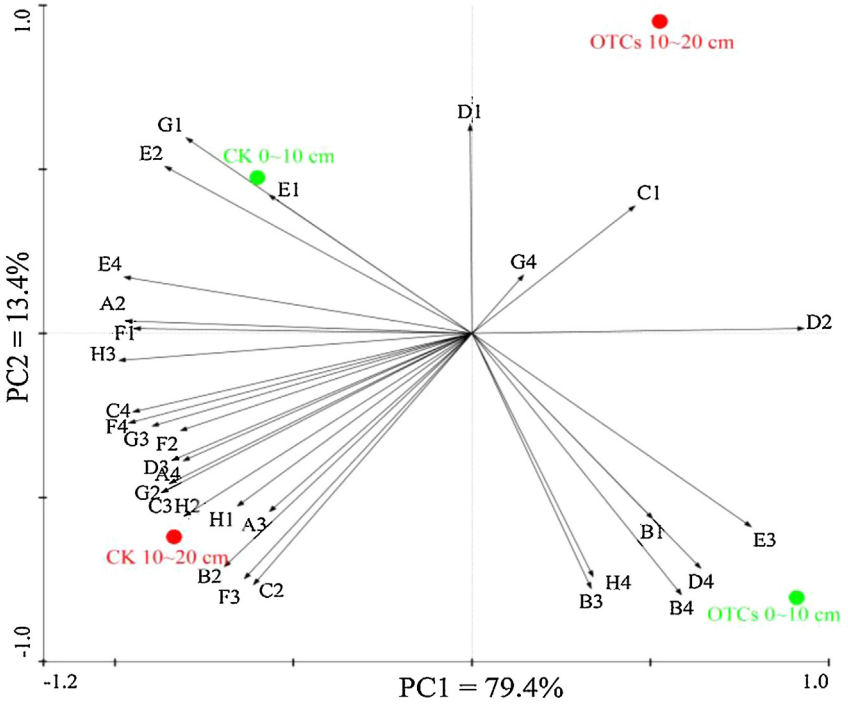

Fig. 6. Principal component analysis (PCA) for BIOLOG data in the 0–10 cm and 10–20 cm soil layers in the warmed open top chambers (OTCs) and the control (CK). Principal components 1 (PC1) and 2 (PC2) explain 79.4% and 13.4% of the variation at the 0–10 cm and 10–20 cm soil layer in substrate utilization pattern in the alpine meadow, respectively. A 2: β-methyl-D-glucoside; A 3: D-galactonic acid lactone; A 4: L-arginine; B1: pyruvic acid methyl ester; B 2: D-xylose; B 3: D-galacturonic acid; B 4: L-asparagine; C 1: Tween 40; C 2: i-erythritol; C 3: 2-hydroxybenzoic acid; C 4: L-phenylalanine; D 1: Tween 80; D 2: D-mannitol; D 3: 4-hydroxybenzoic acid; D 4: L-serine; E 1: α-cyclodextrin; E 2: N-acetyl-D-glucosamine; E 3: γ-hydroxybutyric acid; E 4: L-threonine; F 1: glycogen; F 2: D-glucosaminic acid; F 3: itaconic acid; F 4: glycyl-L-glutamic acid; G 1: D-cellobiose; G 2: α-D-glucose-1-phosphate; G 3: α-ketobutyric acid; G 4: phenylethylamine; H 1:α-D-lactose; H 2: D,L-α-glycerol phosphate; H 3: D-malic acid; H 4: putrescine.

More PLFAs i15:0, a16:0, i17:0, and cy19:0, cy17:0, 16:1ω7t indicative of gram-positive and gram-negative bacteria (G^+ and G^-) were located near CK 0–10 cm than OTC 0–10 cm, indicating that warming decreased the proportion of G^+ and G^-. This could also be concluded by comparing CK 10–20 cm and OTC 10–20 cm. Conversely, the fungal PLFAs 18:2ω6, 9 and 18:1ω9 located near OTC 0–10 cm and OTC 10–20 cm showed changes in warmed plots compared with CK plots (Fig. 5).

Warming significantly changed average well color development (AWCD) value at the different soil layer. At 0–10 cm soil layer, AWCD was increased by warming at 24–168 h, but at 172 h AWCD values were less in the OTC than the CK. At the 10–20 cm, after 72 h, AWCD value was significantly increased by warming. In addition, AWCD value was lower with soil depth increased except 172 h (Supplementary Figure 3). Warming decreased significantly microbial diversity indices (*H* and *J*) compared with the CK, but soil microbial diversity indices no significantly changed (Supplementary Figure 4). Principal Components Analysis of the Biolog data indicated differentiation of microbial community diversity between warming and CK soil (Fig. 6). The PC1 explained 79.4% of total variance in the data, and PC2 explained 13.4%. The locations of the OTCs and CK were distinct, thus, the soil microbial communities at the OTCs and CK exhibited different levels of metabolism of the substrates from varying sources (Fig. 6). Furthermore, C substrate utilization (metabolic activity) was different between warming and CK soil. There were less C sources (e.g. carbohydrates, carboxylic acids, amino acids, polymers, and amines) used at 0–10 cm, 10–20 cm soil depth in warming than in CK (Fig. 6).

3.5. Relationship between plant root and soil microbial community

At 0–10 cm, F PLFA content was positively correlated with root mortality ($P < 0.01$) and root turnover ($P < 0.05$). Total PLFA content was positively correlated with root production ($P < 0.05$) (Table 2).

At 10–20 cm, B PLFA content was negatively correlated with root standing crop ($P < 0.01$), but positively correlated with root mortality ($P < 0.05$) and root turnover ($P < 0.01$). F PLFA content ($P < 0.05$) was negatively correlated with root standing crop and positively correlated with root mortality ($P < 0.05$) and root turnover ($P < 0.01$). Total PLFA was negatively correlated with root standing crop ($P < 0.05$) and positively correlated with root turnover ($P < 0.05$) (Table 2).

At 0–10 cm, carbohydrates were positively correlated with root standing crop ($P < 0.05$), but negatively correlated with root mortality ($P < 0.01$); carboxylic acids were positively correlated with root standing crop ($P < 0.05$), negatively correlated with root mortality ($P < 0.05$); Polymers were positively correlated with root production ($P < 0.05$) (Table 3).

At 10–20 cm, carboxylic acids were negatively correlated with root standing crop ($P < 0.01$), positively correlated with root turnover ($P < 0.05$); phenolic acids were negatively correlated with root standing crop ($P < 0.05$) (Table 3).

4. Discussion

4.1. Effect of warming on root dynamics

In the arctic alpine ecosystem, the high rate of biomass distribution to roots and the low tissue turnover rate are closely correlated with low temperature and poor nutrients (Shaver and Chapin, 1991). In cold alpine meadows, the underground part accounts for more than 80% of the total biomass (Yang et al., 2009). Therefore, examining the response of underground plant parts to temperature increase is essential to understanding the effects of climate warming on alpine ecosystems. We found that root standing crop, root production and turnover in different soil depth responded differently to warming, leading to changes in the underground root distribution in alpine meadows. When the root standing crop and root production in topsoil decreased, root mortality increased, promoting root turnover of the dominant species, *Kobresia*, in alpine meadows. However, at 10–20 cm, root standing crop and root production increased, and root mortality decreased, which slowed down the root turnover of the dominant species. Overall, warming shifted the root biomass distribution toward deeper soil and increased root turnover at low soil layer. These results are consistent with those of Wu et al. (2013), suggesting that fine root biomass, production and turnover varied with grazing intensity (Gao et al., 2008), vegetation types (Schoettle and Fahey, 1994), warming time (Bai et al., 2010), and different methods (Wu et al., 2013).

Warming affected root dynamics through inducing changes in soil moisture and temperature.

Changes in temperature and humidity in the OTCs due to warming may partially be responsible for these results of root dynamics. For example, the soil temperature increased (Liu et al., 2010), and the topsoil moisture decreased (De Valpine and Harte, 2001), belowground biomass decreased by 23% due to the reduction of soil moisture induced by warming (De Boeck et al., 2007). Our study showed that, as the temperature in the OTCs increased, air humidity decreased (Supplementary Table 2). Warming also led to decreased soil moisture so that plants faced drought stress. Warming-driven changes in moisture (e.g., decreased soil moisture at 0–10 cm), plant species composition and their importance may also have intermediate effects on litter quality and quantity, which affect both superficial roots respiration and nutrient absorption. These conditions limited the

Table 2
Pearson correlation between root standing crop, production, mortality, and turnover and microbial PLFAs contents in the 0–10 and 10–20 cm soil layers with warming manipulations in the alpine meadow ($m\,m^{-2}$; mean ± SE, $n = 3$).

Depth	Items	Root standing crop		Root production		Root mortality		Root turnover	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
0–10 cm	B PLFAs	0.714	0.111	0.771	0.072	−0.714	0.111	−0.086	0.872
	G^+ PLFAs	0.543	0.266	0.429	0.397	−0.600	0.208	−0.086	0.872
	G^- PLFAs	0.771	0.072	0.143	0.787	−0.771	0.072	−0.714	0.111
	F PLFAs	−0.771	0.072	−0.029	0.957	**0.943****	**0.005**	**0.829***	**0.042**
	Total PLFAs	0.771	0.072	**0.829***	**0.042**	−0.600	0.208	−0.029	0.957
10–20 cm	B PLFAs	**−0.986****	**0.001**	0.371	0.468	**0.829***	**0.042**	**0.943****	**0.005**
	G^+ PLFAs	−0.371	0.468	0.314	0.544	0.086	0.872	0.429	0.397
	G^- PLFAs	−0.657	0.156	0.371	0.468	0.429	0.397	0.771	0.072
	F PLFAs	**−0.829***	**0.042**	0.200	0.704	**0.829***	**0.042**	**0.943****	**0.005**
	Total PLFAs	**−0.829***	**0.042**	0.543	0.266	0.543	0.266	**0.886***	**0.019**

* Significant at $P < 0.05$; $N = 6$.
** Significant at $P < 0.01$.

Table 3
Pearson correlation between root standing crop, production, mortality, and turnover and substrate utilization in the 0–10 and 10–20 cm soil layers with warming manipulations in the alpine meadow ($m\,m^{-2}$; mean ± SE, $n = 3$).

Depth	Items	Root standing crop		Root production		Root mortality		Root turnover	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
0–10 cm	CAR	0.807	0.052	0.161	0.76	**−0.946****	**0.004**	−0.566	0.242
	AMA	0.784	0.065	0.269	0.606	−0.425	0.401	−0.445	0.377
	CAA	**0.875***	**0.022**	0.064	0.905	**−0.830***	**0.041**	−0.737	0.095
	POL	0.493	0.320	**0.876***	**0.022**	−0.168	0.751	0.409	0.421
	PHA	0.385	0.451	0.290	0.577	−0.373	0.467	−0.077	0.885
	AMI	−0.511	0.300	−0.272	0.602	0.170	0.747	0.200	0.704
10–20 cm	CAR	−0.688	0.131	−0.162	0.759	0.675	0.141	0.515	0.296
	AMA	−0.716	0.109	−0.021	0.968	0.508	0.304	0.594	0.214
	CAA	**−0.987****	**0.000**	0.231	0.686	0.802	0.055	**0.909***	**0.012**
	POL	−0.083	0.876	−0.358	0.487	−0.069	0.897	−0.019	0.972
	PHA	**−0.913***	**0.011**	−0.068	0.899	0.749	0.087	0.760	0.079
	AMI	0.031	0.954	−0.161	0.760	−0.386	0.450	−0.089	0.867

CAR: carbohydrates; AMA: amino acids; CAA: carboxylic acids; POL: polymers; PHA: phenolic acids; AMI: amines.
* Significant at $P < 0.05$; $N = 6$.
** Significant at $P < 0.01$.

photosynthesis capability of some plants (Shen et al., 2006), and reduce the plant's net primary productivity. As a result, the underground biomass, especially of superficial roots, was reduced. Short term warming revealed that the biomass distribution in the alpine meadow had a tendency to shift to deeper soil layers (Li et al., 2011a). Our study confirmed this tendency by indicating that warming led to a shift of the underground root standing crop to deeper soil. Conversely, warming weakened the dominance of shallow root plants such as *K. humilis*, *Deschampsia caespitosa*, and *Festuca rubra*, but enhanced the dominance of plants with axial roots and taproots such as *Saussurea*, *Gentiana*, *Polygonum sibiricum* Laxm., *Polygonum viviparum* or *Potentilla fruticosa* (Zhou et al., 2000; Supplementary Table 1).

Temporal and spatial changing in soil environmental factors along with soil depth may be the main cause of the different production, mortality and turnover of roots distributed in different soil layers. Numerous studies have shown that soil temperature, nutrient availability, and moisture status control the timing and duration of root growth (Brassard et al., 2009). Majdi and Andersson (2005) report that fine root turnover and production increased with higher nitrogen availability in Norway spruce. In addition, herbaceous roots have been shown to be very sensitive to dry conditions (Wu et al., 2013). Under lower water availability, substantial grass roots mortality was detected in savannah bunchgrasses (West et al., 2004), and this involved both perennial and annual grasses (Peek et al., 2005). Therefore, warming-driven changes in moisture may directly influence roots distribution in the surface soil of alpine meadows. It is likely that the comprehensive effects of related factors influences the distribution of root dynamics across the soil profile, including temperature, moisture, and available nitrogen.

Experimental warming has been reported to have negative effects on root production, mortality and root standing crop of the semi-arid temperate grassland in northern China (Bai et al., 2010). Differences in the amount of root production and mortality led to decreases in root standing crop (Wan et al., 2004). There is also evidence that warming had no effect on fine root production, mortality, and root standing crop in forest ecosystems (Johnson et al., 2006). Warming had different direct or indirect effects on different ecosystems processes (Wan et al., 2007). If the plant water use efficiency in an ecosystem was relatively high, the direct effects of warming would be higher than the indirect effects, thereby promoting root production and mortality (Majdi and Ohrvik, 2004; Sullivan and Welker, 2005). Warming can directly promote plant growth (Zhang et al., 2005) and indirectly affect other underground process, such as underground C distribution and utilization (Bai et al., 2010), transfer of nutrients toward plants, nitrogen utilization, and competition for soil nutrients between plants and the soil microorganisms, resulting in an impact of nutrients on the soil microbial community (Bardgett et al., 2008).

4.2. Effect of warming on microbial communities

Changes in soil temperature directly affect microbial growth, mineralization rate, enzyme activity, and microbial community composition, especially in topsoil (Zogg et al., 1997). PLFA analysis revealed that warming led to a reduction of microbial PLFAs species and abundance, especially among soil surface microorganisms, but that this effect decreased with increasing soil, consistent with the results of a previous study (Fierer et al., 2003). The decrease in biomass of total PLFAs, G^+ PLFAs, and G^- PLFAs with significant increase in fungal biomass observed in response to warming in the present study was consistent with the results of previous studies (Zhang et al., 2005). Moreover, previous investigations indicated that microbial communities may be in a specialized environment that is clearly different from the surface soil after warming (Blume et al., 2002). In this case, the function of soil microbial community at 10–20 cm may be different from that of topsoil. Specifically, their metabolic characteristics and ability to use soil nutrients (substrate) may vary. Changes in environmental conditions such as soil temperature and soil moisture can affect soil microbial community composition (Zhang et al., 2014). Differences in soil microbial community PLFAs composition and amount may be a result of changes in plant root production, mortality and turnover (Table 2). Likewise, there exists a clear difference between the soil microbial community of warming and CK soil in using carbohydrate, carboxylic acids, phenolic acids, amines, amino acids, and polymers C-substrate (Fig. 6). It suggests that microbial metabolic diversity is different in the warming and CK soil (Supplementary Figure 4). The high oxidation of C sources supplied (high AWCD) reflects an increase of bacterial density (Haack et al., 1995). Catabolic diversity and the number of substrates used reflect the diversity of carbon-oxidation pathways and therefore functional diversity (Insam et al., 1996).

Plant litter on the ground, roots and root exudates are major sources of soil organic matter (Fierer et al., 2003) that impact the quality and quantity of soil organic matter (Saleska et al., 2002), which could affect the soil microbial community and structure (Zhang et al., 2005). Campbell et al. (1997) showed that BIOLOG C sources reported as constituents of root exudates discriminate bacterial communities from different sites. Each plant species produced characteristic C source utilization patterns (Fang et al.,

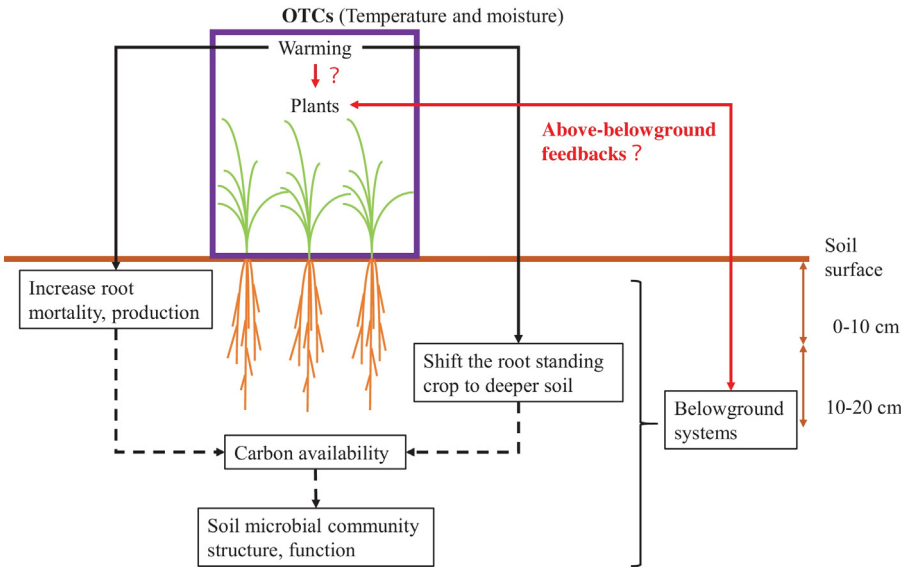

Fig. 7. Conceptual model of direct and indirect effects of warming on belowground [illegible]luding potential feedbacks based on results from this study. → Warming impacts root dynamics directly through changes in temperature and moisture, and ➡ indirectly impacts soil microbial community through changes in carbon availability. Combined, these cause changes in the belowground systems (e.g. roots, soil microoganisms), may be lead to change aboveground systems (e.g. plants) in red colors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2001). Therefore, we may conclude that, in our experiment, both microbial functional diversity and substrate utilization pattern decrease with decreasing plant diversity (Supplementary Table 3). On the other hand, differences in soil resource availability and environmental stress (such as warming) may be the main factors leading to changes in microbial community composition and content in different soil layers.

4.3. Plant roots and soil microbial community relationships

Increased carbon input and decreased nitrogen utilization may help enrich fungi in the soil microbial community (Smith et al., 2003), and changes in plant species composition caused by warming may facilitate growth of fungi in soil (Zhang et al., 2005). The C resources that support soil microbial communities are primarily derived from plants (Broughton and Gross, 2000), so it is likely that the soil microbial community should respond to changes in plant productivity including plant roots. The results of this study showed that, in the 0–10 cm soil layer, root mortality and root turnover levels increased (Fig. 3) and the F PLFAs content was positively correlated with root mortality (Table 2), substrate utilization pattern correlated significantly with root dynamics (Table 3). This was likely because the soil resource available to soil microorganisms is limited by the chemical composition of dead leaves and roots (Smith and Paul, 1990), and plants differ in chemical composition, which influences the composition and function of the microbial community. For example, slow-growing plants (e.g., the alpine herb *Acomastylis rossi*) may produce large amounts of phenolic-rich litter that may control nitrogen-immobilization by microorganisms (mostly fungi) and exacerbate the low-nutrient status upon entry into the soil (Wilson and Agnew, 1992). Conversely, other fast-growing plants (e.g., *Deschampsia caespitose*) show a high rate of fine roots turnover and produce large amounts of high quality (rich in N) litter, promoting a food web dominated by bacteria and improving the nutrient status of the habitat (Bardgett et al., 2005).

Therefore, above plant communities change, differences in the chemistry and quantity of litter and root biomass and exudates have been shown to influence belowground microbial composition and activity (De Graaff et al., 2010). Our data reveal that significant relationships between plant root properties and individual microbial group PLFAs. Spatial variability in soil resources and microenvironmental conditions may affect the soil microbial community composition.

Climate change will have direct and indirect impacts on terrestrial ecosystems, both above- and belowground (Fig. 7). Plants can provide nutrients to soil microorganisms through root exudation, plant litter, root production, root mortality and root turnover, which results in co-evolution of plants and microbes and promotes soil microbial diversity.

5. Conclusions

Our data show that six consecutive years of simulated warming changed the spatial distribution and structure of the underground root system in alpine meadows. The warming reduced topsoil root production and promoted root mortality and turnover. Additionally, warming promoted root production and reduced root mortality and turnover at 10–20 cm, and may lead to a loss of soil organic carbon at 0–10 cm, and enhance soil organic carbon at 10–20 cm. Overall, warming changed the distribution of root production toward deeper soil in an alpine meadow. Warming also changed the type and abundance of different microorganisms based on PLFAs profiling and microbial functional diversity, especially in topsoil. These results also indicate that the interaction between the underground root system and soil microorganisms is important for maintaining the stability of the whole ecosystem.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.03.005.

References

Asseng, S., Ritchie, J.T., Smucker, A.J.M., Robertson, M.J., 1998. Root growth and water uptake during water deficit and recovering in wheat. Plant Soil 201, 265–273.

Bai, W.M., Wan, S.Q., Niu, S.L., Liu, W.X., Chen, Q.S., Wang, Q.B., Zhang, W.H., Han, X. G., Li, L.H., 2010. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. Global Change Biol. 6, 1306–1316.

Bardgett, R.D., Bowman, W.D., Kaufmann, B., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. Trends Ecol. Evol. 20, 634–641.

Bardgett, R.D., Freeman, C., Ostle, N.J., 2008. Microbial contributions to climate change through carbon cycle feedbacks. ISME J. 2, 805–814.

Beniston, M., 2000. Environmental Change in Mountains and Uplands. Arnold/ Hodder and Stoughton/Chapman and Hall Publishers, London, United Kingdom, pp. 200.

Blume, E., Bischoff, M., Reichert, J., Moorman, T., Konopka, A., Turco, R., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Appl. Soil Ecol. 592, 1–11.

Brassard, B.W., Chen, H.Y.H., Bergeron, Y., 2009. Influence of environmental variability on root dynamics in northern forests. Crit. Rev. Plant Sci. 28, 179–197.

Broughton, L.C., Gross, K.L., 2000. Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. Oecologia 125, 420–427.

Cai, K.Z., Shen, H., 2002. Root: dynamic interface of plant and soil – introduce for the sixth international roots research conference. Acta Ecol. Sin. 22, 139–140 (in Chinese).

Campbell, C.D., Grayston, S.J., Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J. Microbiol. Meth. 30, 33–41.

De Boeck, H.J., Lemmens, C.M.H.M., Gielen, B., Bossuyt, H., Malchair, S., Carnol, M., Merckx, R., Ceulemans, R., Nijs, I., 2007. Combined effects of climate warming and plant diversity loss on above- and below-ground grassland productivity. Environ. Exp. Bot. 60, 95–104.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytol. 188, 1055–1064.

Deslippe, J.R., Hartmann, M., Simard, S.W., Mohn, W.W., 2012. Long-term warming alters the composition of Arctic soil microbial communities. FEMS Microbiol. Ecol. 82, 303–315.

De Valpine, P., Harte, J., 2001. Plant responses to experimental warming in a montane meadow. Ecology 82, 637–648.

Fang, C., Radosevich, M., Fuhrmann, J.J., 2001. Characterisation of rhizosphere microbial community structure in five similar grass species using FAME and BIOLOG analyses. Soil Biol. Biochem. 33, 679–682.

Feng, X.J., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biol. Biochem. 41, 804–812.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

Gao, Y.Z., Giese, M., Lin, S., Sattelmacher, B., Zhao, Y., Brueck, H., 2008. Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. Plant Soil 307, 41–50.

Gill, R.A., Jackson, R.B., 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147, 13–31.

Haack, S.K., Garchow, H., Klug, M.J., Forney, L.J., 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. Appl. Environ. Microbiol. 61, 1458–1468.

Insam, H., Amor, K., Renner, M., Crepaz, C., 1996. Changes in functional abilities of the microbial community during composting of manure. Microbial. Ecol. 31, 77–87.

Johnson, M.G., Rygiewicz, P.T., Tingey, D.T., Phillips, D.L., 2006. Elevated CO_2 and elevated temperature have no effect on Douglas-fir fine-root dynamics in nitrogen-poor soil. New Phytol. 170, 345–356.

Johnson, M.G., Tingey, D.T., Phillips, D.L., Storm, M.J., 2001. Review: advancing fine root research with minirhizotrons. Environ. Exp. Bot. 45, 263–289.

Kardol, P., Campany, C.E., Souza, L., Norby, R.J., Weltzin, J.F., Classen, A.T., 2010. Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. Global Change Biol. 16, 2676–2687.

Keiblinger, K.M., Hall, E.K., Wanek, W., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiol. Ecol. 73, 430–440.

Klanderud, K., Totland, O., 2005. Simulated climate change altered dominance hierarchies and diversity of an alpine biodiversity hotspot. Ecology 86, 2047–2054.

Li, G.Y., Liu, Y.Z., Frelich, L.E., Sun, S.C., 2011b. Experimental warming induces degradation of a Tibetan alpine meadow through trophic interactions. J. Appl. Ecol. 48, 659–667.

Li, N., Wang, G.X., Yan, Y., Gao, Y.H., Liu, L.A., Liu, G.S., 2011a. Short-term effects of temperature enhancement on community structure and biomass of alpine meadow in the Qinhai-Tibet Plateau. Acta Ecol. Sin. 31, 895–905 (in Chinese).

Liu, W., Wang, C.T., Zhao, J.Z., Xu, Q.M., Zhou, L., 2010. Responses of quantity characteristics of plant community to simulating warming in alpine *Kobresia humilis* meadow ecosystem. Acta Boreali-Occidentalia Sin. 30, 995–1003 (in Chinese).

Luo, Y., Zhou, X., 2006. Soil Respiration and the Environment. Elsevier, San Diego.

Majdi, H., Andersson, P., 2005. Fine root production and turnover in a Norway Spruce stand in Northern Sweden: effects of nitrogen and water manipulation. Ecosystems 8, 191–199.

Majdi, H., Öhrvik, J., 2004. Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a norway spruce stand in northern Sweden. Global Change Biol. 10, 182–188.

Niu, S.L., Han, X.G., Ma, K.P., 2007. Field facilities in global warming and terrestrial ecosystem research. J. Plant Ecol. 31, 262–271.

Norby, R.J., Ledford, J., Reilly, C.D., Miller, N.E., O'Neill, E.G., 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. Proc. Natl. Acad. Sci. U.S.A. 101, 9689–9693.

Peek, M.S., Leffler, A.J., Ivans, C.Y., Ryel, R.J., Caldwell, M.M., 2005. Fine root distribution and persistence under field conditions of three co-occurring Great Basin species of different life form. New Phytol. 165, 171–180.

Saleska, S.R., Shaw, M.B., Fischer, M.L., 2002. Plant community composition mediates both large transient decline and predicted long-term recovery of soil carbon under climate warming. Global Biogeochem. Cycl. 16, 1055.

Schindlbacher, A., Rodler, A., Kuffner, M., 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. Soil Biol. Biochem. 43, 1417–1425.

Schlesinger, W.H., 1997. Biogeochemistry: An Analysis of Global Change. Academic Press.

Schoettle, A.W., Fahey, T.J., 1994. Foliage and fine root longevity of pines. Ecol. Bull. 43, 136–153.

Shaver, G.R., Chapin, I.I.I.F.S., 1991. Production: biomass relationships and element cycling in contrasting arctic vegetation types. Ecol. Monogr. 6, 1–32.

Shen, H., Klein, J.A., Zhao, X., Tang, Y., 2006. Leaf photosynthesis and simulated carbon budget of *Gentiana straminea* from a decade-long warming experiment. J. Plant Ecol. 2, 207–216.

Shu, W., Jiang, Q., Wang, Z., He, J., 2012. Research advances about the effects of vegetation recovery on soil microorganism. J. Agric. Sci. 33, 73–79 (in Chinese).

Smith, R.S., Shiel, R.S., Bardgett, R.D., Millward, D., Corkhill, P., Rolph, G., Hobbs, P.J., Peacock, S., 2003. Soil microbial community, fertility, vegetation and diversity as targets in the restoration management of a meadow grassland. J. Appl. Ecol. 40, 51–64.

Sullivan, P.F., Welker, J.M., 2005. Warming chambers stimulate early season growth of an arctic sedge: results of a minirhizotron field study. Oecologia 142, 616–626.

Tan, Y., Pan, B.R., Duan, S.M., Zhang, Q., Zhang, Y.Z., 2008. Characteristic of *Calligonum* L. community and species diversity in China. Acta Bot. Boreali-Occidential Sin. 28, 1049–1055.

ter Braak, C.J.F.T., 1998. CANOCO Reference Manual and User's Guide to CANOCO for Windows: Software for Canonical Community Ordination (Version 4.02). Microcomputer Power, Ithaca, NY.

Walker, M.D., Wahren, C.H., Hollister, R.D., Henry, G.H.R., Ahlquist, L.E., Alatalo, J.M., Bret-Harte, M.S., Calef, M.P., Callaghan, T.V., Carroll, A.B., Epstein, H.E., Jonsdottir, I.S., Klein, J.A., Magnusson, B., Molau, U., Oberbauer, S.F., Rewa, S.P., Robinson, C. H., Shaver, G.R., Suding, K.N., Thompson, C.C., Tolvanen, A., Totland, O., Turner, P. L., Tweedie, C.E., Webber, P.J., Wookey, P.A., 2006. Plant community responses to experimental warming across the tundra biome. Proc. Natl. Acad. Sci. U.S.A. 103, 1342–1346.

Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109, 35–47.

Walther, G.R., Beissner, S., Burga, C.A., 2005. Trends in the upward shift of alpine plants. J. Veg. Sci. 16, 541–548.

Wan, S., Norby, R.J., Ledford, J., Weltzin, J.F., 2007. Responses of soil respiration to elevated CO_2, air warming, and changing soil water availability in a model old-field grassland. Global Change Biol. 13, 2411–2424.

Wan, S., Norby, R.J., Pregitzer, K.S., Ledford, J., O'Neill, E.G., 2004. CO_2 enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. New Phytol. 162, 437–446.

Wang, C.T., Wang, G.X., Wang, Y., Rashid, R., Ma, L., Hu, L., Luo, Y.Q., 2015. Urea addition and litter manipulation alter plant community and soil microbial community composition in a *Kobresia humilis* meadow. Eur. J. Soil Biol. 70, 7–14.

Wang, C.T., Wang, G.X., Wang, Y., Wang, Y., Rafique, R., Ma, L., Hu, L., Luo, Y.Q., 2016a. Fire alters vegetation and soil microbial community in alpine meadow. Land Degrad. Dev. 27, 1379–1390.

Wang, Z., Ding, L., Wang, J., Zuo, X., Yao, S., Feng, J., 2016b. Effects of root diameter, branch order, root depth, season and warming on root longevity in an alpine meadow. Ecol. Res. 31 (5), 1–9.

West, J.B., Espeleta, J.F., Donovan, L.A., 2004. Fine root production and turnover across a complex edaphic gradient of a *Pinus palustris–Aristida stricta* savanna ecosystem. For. Ecol. Manag. 19, 397–406.

Wilson, J.B., Agnew, A.D.Q., 1992. Positive-feedback switches in plant communities. Adv. Ecol. Res. 23, 263–336.
Wu, Z.T., Dijkstra, P., Koch, G.W., Peñuelas, J., Hungate, B.A., 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biol. 17, 927–942.
Wu, Y.B., Deng, Y.C., Zhang, J., Wu, J., Tang, Y.H., Cao, G.M., Zhang, F.W., Cui, X.Y., 2013. Root size and soil environments determine root lifespan: evidence from an alpine meadow on the Tibetan Plateau. Ecol. Res. 28, 493–501.
Yang, Y., Fang, J., Ji, C., Han, W., 2009. Above- and belowground biomass allocation in tibetan grasslands. J. Veg. Sci. 20, 177–184.
Yang, H.J., Wu, M.Y., Liu, W.X., Zhang, Z., Zhang, N.L., Wan, S.Q., 2011. Community structure and composition in response to climate change in a temperate steppe. Global Change Biol. 17, 452–465.
Yu, X.C., Yao, B.Q., Zhou, H.K., Jin, Y.X., Yang, Y.J., Wang, W.Y., Dong, S.K., Zhao, X.Q., 2015. Variable responses to long-term simulated warming of underground biomass and carbon allocations of two alpine meadows on the Qinghai-Tibet Plateau. Chin. Sci. Bull. 60, 379–388 (in Chinese).
Yuan, Y.L., Si, G.C., Jian, W., Luo, T.X., Zhang, G.X., 2014. Bacterial community in alpine grasslands along an altitudinal gradient on the Tibetan Plateau. FEMS Microbiol. Ecol. 87, 121–132.
Zhang, W., Parker, K.M., Luo, Y.Q., Wan, S.Q., Wallace, L.L., Hu, S., 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biol. 11, 266–277.
Zhang, B., Chen, S.Y., He, X.Y., Liu, W.J., Zhao, Q., Zhao, L., Tian, C.J., 2014. Responses of soil microbial communities to experimental warming in alpine grasslands on the Qinghai-Tibet plateau. PLOS ONE 9, e103859.
Zhang, B., Chen, S.Y., Zhang, J.F., He, X.Y., Liu, W.J., Zhao, Q., Tian, C.J., 2015. Depth-related responses of soil microbial communities toexperimental warming in an alpine meadow on the Qinghai-Tibet plateau. Eur. J. Soil Sci. 66 (3), 496–504.
Zheng, H., Ouyang, Z.Y., Wang, X.K., Fang, Z.G., Zhao, T.Q., Miao, H., 2005. Effects of regenerating forest cover on soil microbial communities: a case study in hilly red soil region, southern China. For. Ecol. Manag. 217, 244–254.
Zhou, H.K., Zhou, X.M., Zhao, X.Q., 2000. A preliminary study of the influence of simulated greenhouse effect on a *Kobresia humilis* meadow. Chin. Plant Ecol. 24, 547–553 (in Chinese).
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biol. Fert. Soils 29, 111–129.
Zogg, G.P., Zak, D.R., Ringelberg, D.B., Macdonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Sci. Soc. Am. J. 61, 475–481.