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*Full Length Research Paper*

# Changes in soil microbial biomass and bacterial diversity during the transition from winter to growing season in the subalpine/alpine forests

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**Changes in microbial community from winter to growing season are helpful to understand their self-adaptations in the high-frigid ecosystem. A field experiment was conducted to investigate soil microbial biomass and bacterial diversity by PCR-DGGE in the primary fir (*Abies faxoniana*) forest (PF), fir and birch (*Betula albosinensis*) mixed forest (MF) and secondary fir forest (SF) in western China. Soil samples were collected in March, April, May and August, 2009. The microbial biomass C (MBC) in soil organic layer (OL) increased from winter to growing season, but insignificant changes were observed in soil thawing period (April and May). In contrast, MBC in soil mineral layer (ML) displayed an obvious decrease at the end of soil thawing (May) and then increased. Rich DGGE bands indicating rich bacteria populations have been detected even under completely soil frozen condition. The richness of bacteria community significantly decreased during soil thawing period and then increased to growing season, except for which in OL of MF. The similarity of bacterial communities implied significant community changes during the transition, showing more sensitivity to temperature than forest type and soil layer. The results here are of ecological significance in explaining the adaptation of microorganisms in the high-frigid areas.**

**Key words:** Microbial biomass, bacterial diversity, soil thawing period, alpine forest, freeze-thaw events.

## INTRODUCTION

Soil microbial community is involved in numerous ecosystem functions, such as nutrient cycling and organic matter decomposition, and plays an essential role in the terrestrial C cycle (Schimel, 1995). As an active component in a terrestrial ecosystem, soil microbial community propagates and turns over more rapidly than other organisms in respond to external stress (Panikov, 1999; Sowerby et al., 2005). Seasonal freeze-thaw cycle is one of the most significant environmental changes in many high latitude/altitude areas such as in alpine/ subalpine regions (Poutou et al., 2004; Hentschel et al., 2008). Including others, the transition from winter to summer is a crucial stage which connects the winter to growing season (Brooks et al., 1998; Edwards et al., 2006). Soil freezing and thawing with temperature fluctuations around frozen point might greatly regulate soil microbial community during this transitive period. These changes of soil microbial community are crucial to understand the ecological linkages between wintertime and growing season.

It is well known that frozen temperature could change microbial population even kill amounts of micro-organisms, and decrease microbial biomass (Larsen et al., 2002; Koponen et al., 2006).

However, recent studies have demonstrated that soil

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microorganism does not exactly cease in winter (Koponen et al., 2006; Monson et al., 2006; Larsen et al., 2007). Relative higher soil microbial biomass has also observed under snowpack (Brooks et al., 1998; Jefferies et al., 2010). Initially, available C is sufficient to saturate microbial activity near frozen point under snowpack. But as temperature increased, soils become warm and metabolism increases, leading to soil C reserves are no longer sufficient to microorganism reproduction. The C limitation induced starvation and hence reduction of the microbial biomass (Schimel et al., 2004; Campbell et al., 2005). In contrast, increased levels of labile substrate in the soil and damage to microbes by freeze-thaw events could favor the growth of survived microorganisms as soil thawing proceeded (Herrmann and Witter, 2002; Grogan et al., 2004). These different effects including both positive and negative factors play essential roles in soil microbial community during the transition from wintertime to growing season (Larsen et al., 2002; Schmitt et al., 2008). Some studies have therefore reported that soil microbial biomass showed no response to freeze-thaw cycle (Lipson et al., 2000; Koponen et al., 2006), although differences of winter and summer microbial biomass are always demonstrated. Moreover, Schimel and Clein (1996) and our previous observations (Liu et al., 2010; Wang et al., 2010) suggested that a shift in species composition occurred during freeze-thaw season. The changed microbial biomass together with microbial composition from winter to summer is important in understanding ecological linkages between wintertime and growing period, but poorly understood.

Additionally, many evidences have implied that wintertime and high–latitude areas are more sensitive to the ongoing global climate change (Poutou et al., 2004; Joseph and Henry, 2008). The changed temperature fluctuations might influence soil microbial activity, and subsequently alter soil processes (Campbell et al., 2005). Including others, the subalpine/alpine forests at low-latitude displayed obvious frozen winter and growing season with temperature fluctuations, which might experience great and sensitive responses to global climate change. The transition between wintertime and growing season could be altered with the changes of soil thawing/freezing in the global warming scenarios, which might play important roles in soil processes, especially microbial community in subalpine/alpine forests. However, little attention has been given to microbial community in the subalpine/alpine forests at low–latitude during the transition. Thereby, the differences of soil temperature fluctuations along an elevational gradient provide a favorable research site to simulate the pattern of the ongoing global warming.

The subalpine and alpine forest of Western China located in the transitional area between the Qinghai–Tibet plateau and Sichuan basin (Yang et al., 2005; 2007). Our previous observations showed that there were significant temperature fluctuations during the transition between wintertime and growing season (Tan et al., 2010; Wu et al., 2010), which could lead to great effects on soil microbial community during the transition, but less information available on it.

Therefore, the dynamic of soil microbial biomass and community structure from wintertime to growing period was examined in the subalpine and alpine forests of Western China. The objectives were to 1) characterize the dynamics of soil microbial biomass and bacterial diversity from wintertime to growing period, and 2) analysis the effects of altitude–controlled temperature fluctuations with freezing and thawing events on the dynamic of soil microbial biomass and bacterial diversity.

## MATERIALS AND METHODS

### Site description

This study was conducted in the Bipenggou Nature Reserve (E102°53′–102°57′, N31°14′–31°19′, 2458–4619m a.s.l.), which is located in Li County, western China. The mean annual temperature ranges from 2 to 4°C with maximum and minimum temperatures of 23 and –18°C depending on altitude, respectively. Annual precipitation is about 850 mm. The thawing period starts in later March as the temperatures goes above 0°C. There are obvious frequently freeze-thaw cycles before soil completely thawed (Tan et al., 2010; Wu et al., 2010).

Three sites were selected covering a 600 m vertical transition zone at elevations around 3600, 3300 and 3000 m, respectively. The primary forest (PF) at 3600 m is dominated by fir and larch (*Larix mastersiana*) with a few azalea (*Rhododendron* spp.) and willow (*Salix paraplesia*) in shrub layer; the mixed forest (MF) with conifer and broad-leaf trees at 3300 m is dominated by spruce, fir and birch with dwarf bamboo (*Fargesia spathacea*) in the shrub layer; the secondary forest (SF) at 3000 m is dominated by spruce and fir with dense shrub including dwarf bamboo, *Lonicera* spp and *Rubus corchorifolius* (Tan et al., 2010). The characteristics of sample forests are described in Table 1.

### Soil sampling

Five 5 m × 5 m sampling plots were selected randomly with similar environmental factors in each forest. Soil samples were sampled in each sampling plots at the stage of soil completely frozen (March 5), soil thawing (April 5), soil completely thawed (May 20) and growing period (August 12), 2009. Based on the previous observation in soil characteristics (Yang et al., 2005), soil about 500g was sampled from soil organic layer (OL, 0-8 cm) and mineral soil layer (ML, 10-20 cm). All soil samples were stored in freezer boxes, and transported to laboratory within 24 h. Two buttony DS1923–F5 recorders (Maxim Com. USA) that recorded soil temperature every 2 h was buried in the soil to a depth of 5 and 10 cm in each of the three forests on March 1, 2009. Temperature fluctuations during the transition (March 1 to May 20) were given in Figure 1.

### Analysis

#### *Microbial biomass carbon*

Soil microbial biomass carbon (MBC) was determined according to the differences between unfumigated and fumigated samples by the dichromate oxidation–ferrous sulphate titration method following

**Table 1.** Characteristics of sampled forests.

| Sampled forests | Aspect/ Slope (°) | Layer | Bulk density (g soil · $cm^{-3}$) | Gravel content (%) | pH |
|---|---|---|---|---|---|
| Primary fir forest (PF) | NE45/34 | Organic Layer | 0.43±0.11 | 4-7 | 6.2±0.3 |
| | | Mineral Layer | 0.92±0.07 | 12-17 | 5.8±0.2 |
| Fir and birch mixed forest (MF) | NE42/31 | Organic Layer | 0.34±0.12 | 5-8 | 6.6±0.2 |
| | | Mineral Layer | 0.94±0.10 | 11-15 | 5.9±0.2 |
| Secondary fir forest (SF) | NE38/24 | Organic Layer | 0.38±0.09 | 7-12 | 6.5±0.3 |
| | | Mineral Layer | 0.96±0.06 | 15-19 | 5.9±0.3 |

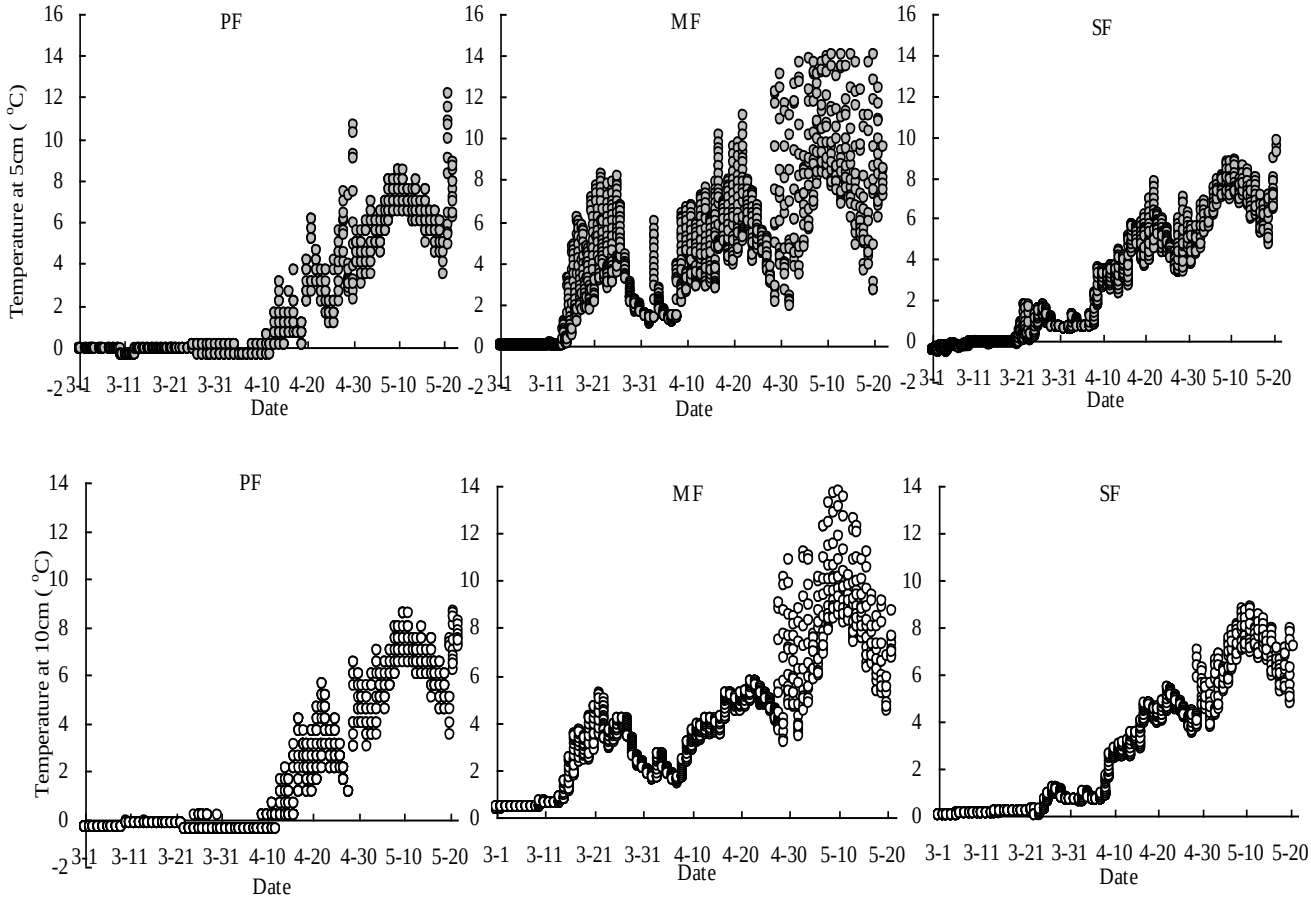


**Figure 1.** Soil temperature dynamics at 5 and 10 cm soil layer under the primary forest (PF), the mixed forest (MF) and the secondary forest (SF) from March 1 to May 22, 2009.

extraction with 0.5 mol $L^{-1}$ $K_2SO_4$ (Brookes et al., 1985; Vance et al., 1987). Efficiency factor (*Kc* = 0.38) was used to correct the incomplete extractability (Vance et al., 1987).

### *DNA extraction*

DNA of soil bacteria was extracted from the soil sample using the method of Griffiths et al. (2000). Briefly, soil samples were extracted with 0.5 g of glass beads (0.1 mm), 500 µl CTAB extraction buffer, and 500 µl phenol-chloroform isoamyl alcohol (25:24:1) (pH 8.0) by bead-beating at full speed for 30 s with Mini-bead beater (Biospec, Inc. USA). Separation of the phases and precipitation of the nucleic acids was performed. And then, the separation was treated by RNAase (Takara) according to the manufacturer's instructions.

### *PCR-DGGE*

The variable V3 region of 16S rRNA gene sequences was amplified by PCR by universal primers 341f and 534r and the touchdown protocol (Muyzer et al., 1993). The extracted DNA was amplified

with a PCR mixture (50 µl) containing 37.55 µl of sterilized MilliQ water, 5 µl of Mg-containing buffer, 4 µl of deoxynucleoside triphosphate mixture, 1 µl of each primer (20 µM), 1µl of the DNA solution, and 0.45 µl of Hotstart Version *ExTaq* DNA polymerase (Takara). PCR was performed with a Biorad iCycler thermocycler using the following protocol: 1 min at 94°C (denaturation), 1 min at 65°C (annealing), and 3 min at 72°C (elongation) with 1°C every second cycle to a "touchdown" annealing temperature for 20 cycles, followed by 10 cycles with an annealing temperature of 55°C and a final cycle consisting of 10 min at 72°C. After gel electrophoresis (1.5% [wt/vol] agarose gel) of 4 µl subsamples in the PCR product, the amount of amplified DNA was quantified by comparing band intensities to standard curves obtained with a low DNA Mass ladder (Takara). Band intensities were measured with Quantity-One analysis software (Bio-Rad Laboratories, Hercules, CA)

Profiles of the amplified 16S rRNA gene sequences were produced by DGGE using the CBS DGGE system (CBS Scientific, USA) (Muyzer et al., 1993). The PCR products were loaded onto a polyacrylamide gel (8% [wt/vol] acrylamide in 1×TAE buffer with a 45 to 65% denaturant gradient (100% denaturant was 7 M urea and 40% [vol/vol] deionized formamide). The wells were loaded with 25 µl of PCR product, and electrophoresis was carried out in TAE buffer at 100 V for 17 h at 60°C. The DNA fragments were stained by silver-stain as described by Radojkovic and Kušic (2000). The gel was destained in distilled water for 5 min. Images of the gels were obtained after destained by using Biorad GS-800 Calibrated Densitometer (Bio-Rad Laboratories, Hercules, CA). Band patterns were analyzed by using Quantity One software (Bio-Rad Laboratories, Hercules, CA). The phylogenetic trees were constructed by NYSYS 2.1 soft with matrix of sample similarity get from Quantity One software by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methods (Zhang and Jackson, 2008).

#### *Diversity indices calculation*

Each detected band was defined as an operational taxonomic unit (OTU), and the number of bands was defined as the genotypic richness of each sample (Bell et al., 2005). The pixel intensity for each band was detected by Quantity One software and was expressed as the relative abundance (*Pi*) (Reche et al., 2005). Richness index (Shannon-wiener index, *H'*), dominance index (Simpson index, *D*) and evenness index (Pielou index, $E_H$), the most widely used diversity indices (Luo et al., 2004; Trevors et al., 2010) were calculated using the richness and relative abundance data based on the following equation:

$$H = -\sum_{i=1}^{s} p_i \ln p_i \qquad D = \sum_{i=1}^{n} P_i^2$$

$$E_H = H / H_{\max} = H / \ln S$$

where $P_i=n_i/S$, $n_i$ is the abundance of the ith OTU and *S* is the total abundance of all OTUs in the sample.

#### Statistical analysis

All of the variables including measurements and calculations among sampled time and sampled forests were tested by analyses of variance (ANOVA). When significant differences were noted, LSD multiple range test was used to determine where differences existed. Differences were considered as significant at $P < 0.05$ levels. All of the statistical analyses were performed using the SPSS (Standard release version 11.5 for Windows, SPSS Inc., IL, USA) software package.

## RESULTS

### Microbial biomass

Microbial biomass carbon (MBC) was higher in OL compared with ML regardless of forests and sampling time (Figure 2). Insignificant differences of MBC were observed in OL among different forests. Although the MBC in ML also showed insignificant differences among sampled forests in May and August, which increased with the increase of altitude in March, decreased with the increase of altitude in April. The MBC in OL was continually increased from March to August under all three sampled forests, but insignificant changes were observed in April and May. In contrast, MBC in ML displayed the lowest values in May regardless of forest, the highest value in April for PF, August for MF and March for SF, respectively.

### DGGE bands

The PCR-DGGE analysis of the soil bacteria revealed that there were significant variations of DGGE bands among the soil samples from different soil layers under different forests (Figure 3). Although more DGGE bands were detected at growing period (August) compared with other stages, less DGGE bands were not detected at the stage of soil completely frozen (March), which was found at the stage of soil thawing (April) (Table 2). Among forests and soil layers, DGGE bands in OL of MF showed a continually increasing tendency from March to August, but which in other soil layer of other forests displayed an obviously decrease in April or May and then increase to August.

### Diversity indices

Similar to the changes of DGGE bands, the indices of *H* and $E_H$ showed a decrease in April or May depending on altitude and then increase to August, except for continually increase in OL of MF (Table 3). The lowest values were observed at the stage of soil thawing (April) except that the lowest *H* and $E_H$ in OL of SF were found in May. In contrast, the index of *D* showed the contrary tendency of *H* and $E_H$.

### Similarity of soil bacterial community

According to UPGMA, significant variations (similarity 0.48-0.92) among the bacterial communities were observed in different forests and soil layers from cluster tree (Figure 4). At the same stage, relative lower similarities were detected between OL and ML under the same forest. In contrast, relative higher similarities were found between the same soil layers even between the different soil layers under different forests. Compared

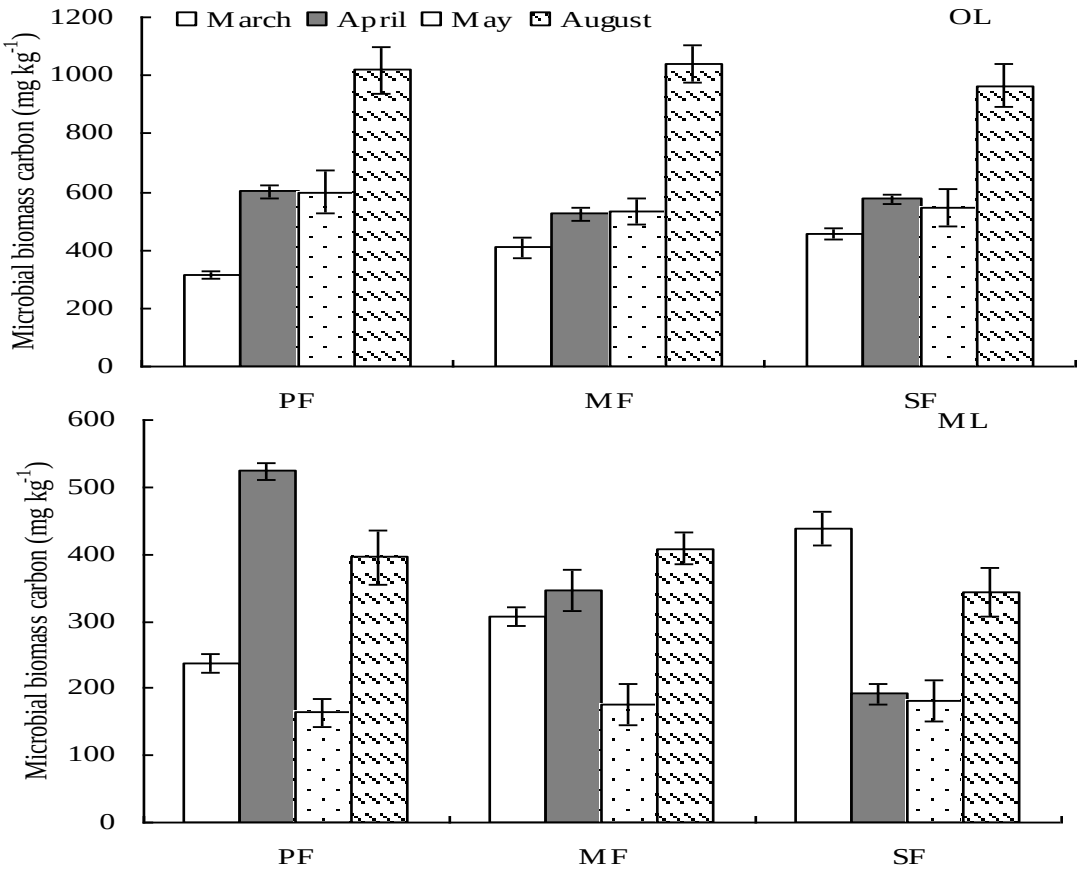


**Figure 2.** Microbial biomass carbon of soil bacterial community in soil organic layer (OL) and mineral soil layer (ML) under the primary fir forest (PF), fir and birch mixed forest (MF) and secondary fir forest (SF) from winter to growing season. Bars indicate SD, n=5.

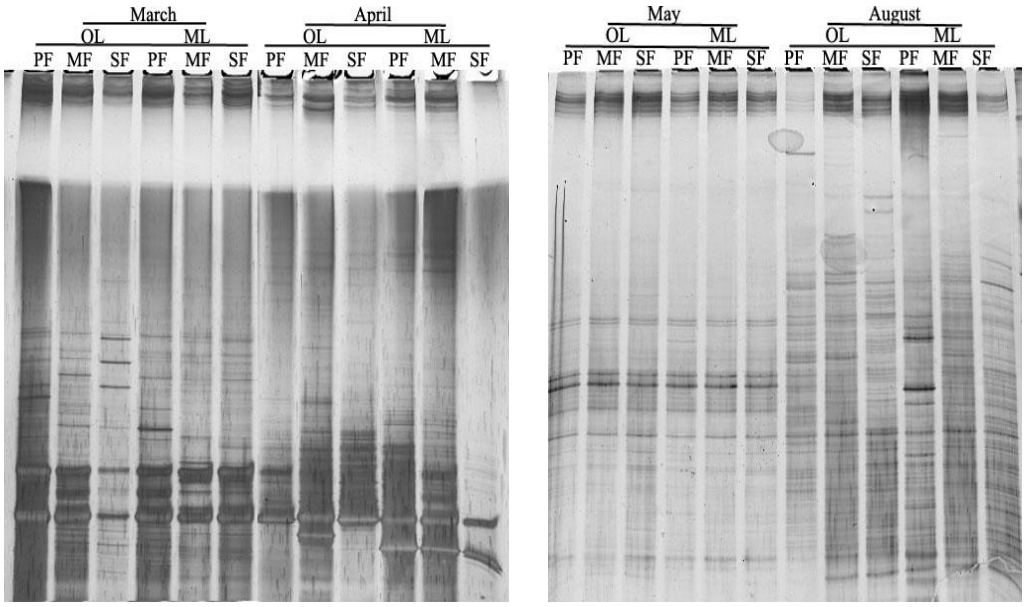


**Figure 3.** DGGE profiles of bacterial 16S rDNA showing the diversity of bacterial community in soil organic layer (OL) and mineral soil layer (ML) under the primary fir forest (PF), fir and birch mixed forest (MF) and secondary fir forest (SF) from winter to growing season.

**Table 2.** The abundance (DGGE bands) of soil bacteria community in soil organic layer (OL) and mineral soil layer (ML) under the primary fir forest (PF), fir and birch mixed forest (MF) and secondary fir forest (SF) from winter to growing season.

| Sampled forests | | March | April | May | August |
|---|---|---|---|---|---|
| PF | OL | 19 | 10 | 14 | 24 |
| | ML | 27 | 10 | 14 | 19 |
| MF | OL | 11 | 14 | 16 | 23 |
| | ML | 24 | 12 | 18 | 21 |
| SF | OL | 23 | 18 | 11 | 24 |
| | ML | 22 | 6 | 14 | 28 |

**Table 3.** Richness index (Shannon-wiener index, *H'*), evenness index (Pielou index, $E_H$) and dominance index (Simpson index, *D*) of soil bacterial community in soil organic layer (OL) and mineral soil layer (ML) under the primary fir forest (PF), fir and birch mixed forest (MF) and secondary fir forest (SF) from winter to growing season.

| Sampled forests | | Biodiversity indices | March | April | May | August |
|---|---|---|---|---|---|---|
| PF | OL | *H'* | 2.908 | 2.247 | 2.537 | 3.168 |
| | | $E_H$ | 0.696 | 0.538 | 0.607 | 0.758 |
| | | *D* | 0.056 | 0.111 | 0.087 | 0.042 |
| | ML | *H'* | 3.251 | 2.254 | 2.561 | 2.927 |
| | | $E_H$ | 0.778 | 0.539 | 0.613 | 0.701 |
| | | *D* | 0.041 | 0.109 | 0.083 | 0.054 |
| MF | OL | *H'* | 2.343 | 2.565 | 2.738 | 3.461 |
| | | $E_H$ | 0.561 | 0.614 | 0.655 | 0.829 |
| | | *D* | 0.101 | 0.081 | 0.067 | 0.032 |
| | ML | *H'* | 3.058 | 2.425 | 2.821 | 3.048 |
| | | $E_H$ | 0.732 | 0.581 | 0.675 | 0.731 |
| | | *D* | 0.051 | 0.093 | 0.064 | 0.049 |
| SF | OL | *H'* | 3.034 | 2.839 | 2.357 | 3.159 |
| | | $E_H$ | 0.726 | 0.681 | 0.564 | 0.756 |
| | | *D* | 0.051 | 0.061 | 0.098 | 0.043 |
| | ML | *H'* | 3.091 | 1.673 | 2.581 | 3.309 |
| | | $E_H$ | 0.741 | 0.401 | 0.618 | 0.792 |
| | | *D* | 0.045 | 0.215 | 0.081 | 0.037 |

with March and August, the similarity of soil bacterial communities in April and May showed higher similarity among different soil layers and forests.

## DISCUSSION

Microbial biomass and microbial diversity from DGGE is considered to be a measure of the viable microbial community because dead cells from microorganism are decomposed quickly in soil. It is not surprising that microorganisms in a cold ecosystem can tolerate below frozen temperature and moderate freeze-thaw events. The results here indicated that MBC in OL did a continually increase tendency from winter to growing period, and showed few responses to freeze-thaw events during the transition. However, freeze-thaw events did a significant negative effect on MBC in ML, displaying an obvious decrease in April or May depending on altitude. Moreover, the results from PCR-DGGE showed that the richness of bacteria community significantly decreased in April or May as temperature increased except for which in OL of MF. The similarity among the bacterial communities according to UPGMA implied that there might be a significant species shift as temperature increased during the transition from winter to growing season. This is consistent with the observations of Ludwig et al. (2004) and Smith et al. (2010), which could be helpful to understand the ecological linkages of wintertime and growing season in high frigid forest.

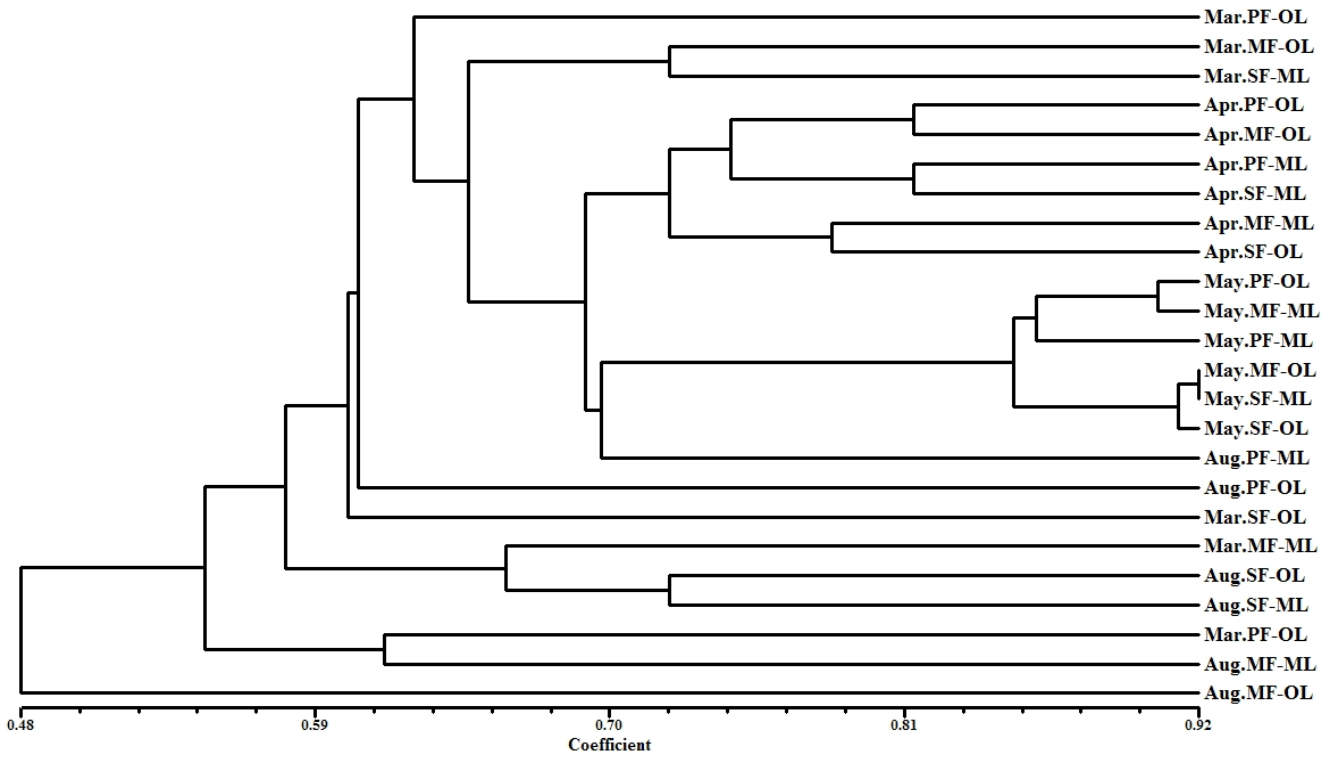


**Figure 4.** Dendrogram from UPGMA showing the similarity of DGGE bands of soil bacterial community in soil organic layer (OL) and mineral soil layer (ML) under the primary fir forest (PF), fir and birch mixed forest (MF) and secondary fir forest (SF) from winter to growing season.

A couple of laboratory experiments conducted in microcosms reported no change in the microbial biomass with the progress of single freeze-thaw in soil (Lipson et al., 2000; Nielsen et al., 2001; Grogen et al., 2004; Sharma et al., 2006). Larsen et al. (2002) reported that microbial biomass C in subarctic heath decreased after 18 FTCs. In the present study, MBC in OL showed few variations as soil thawing proceeded (April and May), which is in good agreement with the results of laboratory microcosms experiments (Lipson et al., 2000; Sharma et al., 2006). In contrast, microbial biomass C in ML displayed a significant decrease tendency during soil thawing stage (April or May depending on temperature fluctuation in different forests). This could be attributed to the soil thawing time and substrate availability. Substrate and nutrients often abrupt released from frozen soil as soil thawing proceeded with the contributing factors of leaching and physical destruction (Lipson et al., 2000; Wu et al., 2010 ), which could lead to a rapid increase of microorganisms, although only a part of microorganisms could be adapted to the extreme environment. With temperature continually increasing, substrate exhausted and competition with awaken other creatures (plants and soil faunas) could induce rapid decrease of microorganisms (Lipson et al., 2000; Edwards et al., 2006). The forest with lower altitude (SF) showed earlier thawing (Figure 1), and displayed earlier increase (March) of microbial biomass and also earlier decrease (April). In addition, the up/down movement of microorganisms and the variation of substrate could explain the different dynamics pattern of microbial biomass between OL and ML.

Soil freezing-thawing events have been suggested to destroy microbial cells (Christensen and Tiedje, 1990), but more and more studies have declared that amounts of microorganisms in the cold region could survive under soil frozen condition (Monson et al., 2006; Larsen et al., 2007; Liu et al., 2010; Wang et al., 2010), and play important roles in continuing soil ecological processes during winter. In agreement with them, rich DGGE bands indicating rich bacterial community have also detected at the stage with temperature completely below 0°C in this study. Shortly after snow melts in the spring as temperature increased, an initial kill of cells occurs with abrupt temperature change, followed by population growth of adapted microorganisms (Cookson et al., 2002; Smith et al., 2010).

Koponen et al. (2006) have stated that the anaerobic and/or facultative anaerobic bacteria might benefit especially from soil freezing and thawing and cited extra substrates released during temperature stress as a contributing factor, but parts of microbes have died. As a result of it, there might be a transition stage of composition changes of bacteria community, showing lower

richness at thawing stage (April) in this study. The results of the diversity indices here also reflect the same phenomenon, showing increased dominance index (*D*) but decreased richness index (*H'*) and evenness index ($E_H$) in April.

Including composition changes with temperature fluctuations, the follow reasons could also attribute to the detected tendency of soil bacterial community from winter to growing season. First, snow cover could provide relative stable environment condition to the microorganisms in winter (Brooks et al., 1993; 1998), showing relative higher richness of bacterial community. Few changes of temperature are also the evidence in the stage with soil completely frozen (Figure 1). Second, although easily decomposable material becomes available after frozen and freeze-thaw cycle as soil thawing proceeded with the increase of temperature (Herrmann and Witter, 2002), soils have to receive significant environment changes with frequently temperature fluctuations and loss of snow-pack protection (Lipson et al., 2000).

This might be related to the lower richness of bacteria community in the thawing stage. Because a little earlier thawing was occurred in the OL of MF (Figure 1) and relative higher soil fertility (Tan et al., 2011) could provide a possible buffer to resist environment change, the richness of bacterial community increased with the increase of temperature. Third, after a frozen winter and a transitive period, the released available substrate from frozen condition (Wu et al., 2010), the rapid turnover of microbial biomass (Cookson et al., 2002) and favorable living condition with increased temperature and moisture could beneficial to the growth of microorganisms, showing higher richness but lower dominance of bacterial community in the growing season (August). There results also indicated that soil bacterial community could be a sensitive indicator to environment changes, and the changes of soil bacterial community are essential to understand the ecological linkages between winter and growing season.

Similarity cluster tree according to UPGMA is the direct evidence for the shift of bacterial community from winter to growing season. Our results indicated that the similarity of soil bacteria community was relative lower among different sampling stages from same soil layer under same forests, but higher similarity was observed among the different soil samples at the same stage especially in April and May. This might be related to the movement of microorganisms and the extreme living conditions. On the one hand, soil surface as OL layer often faced much stronger environment changes such as temperature fluctuations compare with ML (Figure 1). The living microorganisms could avoid the harm from environment changes and move to soil sub-layer, only parts of cold-tolerant populations reserved in the surface layer. This is in agreement with our previous observations (Liu et al., 2010; Wang et al., 2010), implying the self-adaptation mechanisms of soil microorganisms in respond to frigid environment. In fact, relative more bacteria populations were also observed in ML than OL in March and May under the forests (PF and MF) with higher altitude (Table 1). On the other hand, only a few microorganisms could survive in the extreme environment with freeze-thaw cycles in the processes of soil thawing, resulting higher similarity of soil bacterial community at soil thawing stage. Smith et al. (2010) have also observed the similar phenomenon in an agriculture system. More sharply decrease of bacterial populations in ML than OL was observed as soil thawing proceeded from March to April, which indicated that OL could provide more substrate for microorganisms although faced much stronger environment change, and also implied that the extreme environment changes could have deadly effects on many bacteria populations. This is in line with the results of Ludwig et al. (2004), suggesting an anaerobic environment has formed by soil thawing in ML. As the result of it, relative higher similarity was detected between the same soil-layers under different forests in April compared with other stages. Just after soil completely thawing (May), similar temperature condition and rich substrate could lead to higher similarity among soil samples from different soil layers under different forests.

In conclusion, it is clear from our study that there are significant changes in the microbial biomass and community composition from winter to growing season in these alpine/subalpine forests. The soil thawing processes have few effects on MBC in OL, but have an obvious negative effect on MBC in ML. The richness of soil bacterial community decreased during the transition of winter to growing season, but the dominance increased, and species shifts occurred. Due to the variations of temperature fluctuations, substrate availability and microorganism movement, differences in dynamics pattern of soil bacterial community and microbial biomass were observed among different soil layers and forests. We know the molecular approach used here may have its flaws as many others mentioned (Farrelly et al., 1995; Lopez et al., 2003; Sharma et al., 2006), but it enables the comparison of the spatial and temporal differences between bacterial communities, and estimations of the bacterial diversity. The observed results could be beneficial to understand the self-adaptation of soil microorganisms to extreme environment change in cold region.

## ACKNOWLEDGEMENTS

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