African Journal of Microbiology Research Vol. 6(22), pp. 4712-4721, 14 June, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1537
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Soil biochemical dynamics at three elevations during the soil thawing period, Eastern Tibetan Plateau: Nutrient availabilities, microbial properties and enzyme activities

Bo Tan, Fu-zhong Wu, Wan-qin Yang*, Ao Wang and Yu-lian Yang

Key Laboratory of Ecological Forestry Engineering, Institute of Ecological Forestry, Sichuan Agricultural University, Chengdu 611130, P. R. China.

Accepted 10 February, 2012

Studies during last decade have established that soil microbial biomass, enzyme activity and nutrient pools in some cold regions often keep relatively high level in winter and drop rapidly when soil temperatures approach and exceed 0°C during winter-spring transitional period. However, the inconsistent results were observed in different ecosystems due to various temperature dynamics. Based on previous observations on nutrient dynamics, soil microbial biomass, enzyme activity and soluble carbon and nitrogen were investigated in the highly humified soils of subalpine and alpine fir (*Abies faxoniana*) forests along an altitude gradient in the Eastern Qinghai–Tibet Plateau every 10 days from 5 March until 25 April, 2009, including the winter-spring transitional period. Soil temperature significantly changed during the soil thawing period with frequent soil temperature fluctuations around 0°C. Microbial biomass sharply increased as soil thawing proceeded until an obvious peak when soil temperatures rise to 0°C. Thereafter, microbial biomass declined during the thawing period when soil temperatures exceeded 0°C. Likewise, the rapid crashed in the soluble organic nutrient pools occurred shortly before or coincident with the microbial biomass dropped as soil temperature fluctuated close to 0°C. Meanwhile, the sudden decline in enzyme activity occurred just before the soluble organic nutrient pools collapsed. Additionally, the dynamic patterns of microbial biomass, enzyme activity and soluble organic substrate varied with altitudes due to different temperature fluctuations. The results indicate that the release of nutrients as microbial biomass decline might represent an important pulse of carbon and nutrients pools for vegetation growth in the early stage of growing season.

Key words: Subalpine and alpine forest, temperature dynamics, soil microbial biomass, soil enzyme activity, soluble organic substrate, soil thawing period.

INTRODUCTION

Soil thawing is assumed to be a critical period of dynamic transition between late winter and early growing season in the high altitude/latitude areas because of rapid snowmelt and high-amplitude fluctuations in temperature (Edwards et al., 2006; Buckeridge et al., 2010). Soil that was frozen and therefore relatively dry during winter is subjected to rapid large influxes of snowmelt water as well as repeated freezing temperatures associated with diurnal cycles at this time (Buckeridge et al., 2010; Buckeridge and Grogan, 2010). The sudden change in the soil environment during soil thawing is suggested to have an important influence on soil microbial organisms and the physical-chemical structure of soils (Lipson et al., 2002; Sjursen et al., 2005; Larsen et al., 2007; Buckeridge and Grogan, 2010), and thus, the ecosystem

*Corresponding author. E-mail: ywq918@yahoo.com. Tel: +86 28 86290957. Fax: +86 28 82652669.

processes. On the one hand, snowmelt and temperature fluctuations (freeze-thaw cycle) can accelerate nutrients releases from microbes and from litter and soil organic matter into soil (Koponen et al., 2006; Hentschel et al., 2008). This thawing pulse of nutrients release could be an essential pool for microbial activity and plant community during this period (Schmidt and Lipson, 2004). Alternatively, a portion or all of the nutrients released during soil thawing can be leached into the aquatic system as snowmelt (Balcarczyk et al., 2009; Buckeridge and Grogan, 2010), maintaining ecosystem-wide nutrients limitations on the growth of vegetation community (Vitousek et al., 1998; Buckeridge and Grogan, 2010).

Studies in alpine and high latitude ecosystems have demonstrated that soil microbial biomass, activity, and soil soluble nutrient pools keep relatively high level in winter and drop rapidly in late winter and early spring (Brookes et al., 1998; Edwards et al., 2006; Larsen et al., 2007; Buckeridge and Grogan, 2010). The sudden collapse in soluble organic nutrient pools may occur shortly before (Edwards et al., 2006) or after (Larsen et al., 2007) the soil microbial biomass crashes during late snow pack thaw (Buckeridge and Grogan, 2010). The potential competition for nutrients from roots and changes in the physical state of cold soils result in the drop of the microbial pools (Lipson and Monson, 1998; Edwards et al., 2006; Jefferies et al., 2010; Xu et al., 2011). However, the highly sensitive microbial response to environmental change and substrate fluctuations is difficult to capture in field studies (Schimel and Clein, 1996; Lipson et al., 2002; Koponen et al., 2006). As a result of it, the microbial-biogeochemical dynamics in late winter and early spring remains uncertain in many ecosystems, particularly in the forest ecosystem.

Freezing and freeze-thaw cycle are known to kill amounts of microbes and to destroy soil physical structure, subsequently influence microbial biomass and activity as well as nutrients availability in soils (Larsen et al., 2002; Campbell et al., 2005; Koponen et al., 2006). The surviving microbes are often in state of dormancy under frozen temperature unless the changes of substrate and water availability. Therefore, elevated soil temperature and moisture as soil thawing proceeded can favor microbial organisms growth and activity as the increase of substrate availability (Herrmann and Witter, 2002; Grogan et al., 2004; Schmidt and Lipson, 2004). Nevertheless, the freeze-thaw cycle during soil thawing period can kill massive surviving microbes and in turn decrease microbial biomass and activity, and that simultaneously release massive available nutrients (Skogland et al., 1988; Sjursen et al., 2005; Edwards et al., 2006). Moreover, snow pack and environment condition change rapidly with the temperature fluctuation can also lose amounts of available nutrients (Buckeridge et al., 2010; Jefferies et al., 2010). In addition, the rapid consumption of available substrate, and the competition for nutrients with awaken plants can significantly affect the dynamics the dynamics of microbial biomass and activity as well as nutrients availability in soils (Lipson and Monson, 1998; Lipson et al., 2000; Schimel and Mikan, 2005; Henry and Jefferies, 2003; Xu et al., 2011). These positive or negative effects play essential roles in the microbial-biogeochemical dynamics during soil thawing period (Schimel and Mikan, 2005; Edwards et al., 2006; Larsen et al., 2007). However, the detail processes have not been well examined since the fact in field could be more complicated than the speculations in different ecosystems.

The subalpine and alpine forests of Western China locate in the Eastern Qinghai–Tibet Plateau (Yang et al., 2005; Tan et al., 2010), and it is well-known as the roof of world and the world 3rd polar, which is a typical forest ecosystem in mid-latitude region with high altitude. Frequent soil temperature fluctuations around 0°C were observed during early winter and later thawing ahead of spring in this region (Wu et al., 2010; Tan et al., 2011). Our recent research suggested that exchangeable inorganic nutrients keep relatively high level in late winter, and an abrupt decline in exchangeable inorganic nutrients was observed in these subalpine and alpine forests as soil thawing proceeded (Tan et al., 2011). The transitional period occurring before and during the final thaw is recognized as a critical time of year for understanding annual nutrient cycles and plant-nutrient acquisition in high altitude/latitude ecosystems (Edwards et al., 2006; Buckeridge et al., 2010). Therefore, a field soil sampling every 10 days was conducted to understand soil microbial biomass, activity, and nutrient dynamics during soil thawing period in the representative fir (*Abies faxoniana*) forests at different altitudes. The objectives were to (1) characterize the sequence of soil microbial and nutrient dynamics during soil thawing in temperate forest with lower latitude, (2) analysis the effects of altitude-controlled temperature fluctuations on the microbial-biogeochemical dynamics during soil thawing.

MATERIALS AND METHODS

Study sites

This study was conducted in the Bipenggou Nature Reserve (E102°53′ to 102°57′, N31°14′ to 31°19′, 2458 to 4619 m a.s.l.) in Li County. This region is a transitional area between the Qinghai–Tibetan Plateau and the Sichuan Basin. The forest vegetation is coniferous forest and mixed coniferous and broadleaf forests depending on elevations, which is mainly dominated by fir (*A. faxoniana*), spruce (*Picea purpurea*), and birch (*Betula albosinensis*) (Tan et al., 2010). The mean annual temperature ranges from 2 to 4°C with maximum and minimum temperatures of 23 and -18°C, respectively. The cold season starts in November as the soil temperatures goes down below 0°C after snow falls and the soil remains frozen for 5 to 6 months (Wu et al., 2010). Annual precipitation is about 850 mm. Soils are classified with Cambisols and Primosols (Gong et al., 2007). Three sites were selected covering a vertical 600 m transition zone at elevation with 3600 m (A1), 3300 m (A2) and 3000 m (A3), respectively. The forest at A1

Table 1. Basic soil properties in the sampling forests at different altitudes.

Altitude	Layer	Soil thickness (cm)	pH	TC (g·kg^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})	Aspect	Slope
A1	I	15 ± 2	6.2 ± 0.3	161.4 ± 20.3	9.5 ± 0.19	1.2 ± 0.2	NE45°	34°
	II	23 ± 3	5.8 ± 0.2	41.9 ± 15.8	2.8 ± 0.2	0.7 ± 0.2		
A2	I	12 ± 2	6.6 ± 0.2	174.0 ± 55.8	9.5 ± 2.1	1.5 ± 0.1	NE42°	31°
	II	24 ± 4	5.9 ± 0.2	53.7 ± 17.2	3.2 ± 0.2	1.2 ± 0.3		
A3	I	12 ± 2	6.5 ± 0.3	161.9 ± 31.1	8.1 ± 1.6	0.9 ± 0.1	NE38°	24°
	II	21 ± 3	5.9 ± 0.3	43.8 ± 10.8	2.0 ± 0.5	0.8 ± 0.1		

I, Soil rganic layer; II, mineral soil layer. A1, 3600; A2, 3300; A3, 3000.

was dominated by fir and larch (*Larix mastersiana*) in canopy, and that consisted of a few azalea (*Rhododendron* spp.) and willow (*Salix paraplesia*) in shrub layer; the forest at A2 was dominated by spruce, fir and birch in canopy, and the dwarf bamboo dominated the shrub layer; the forest at A3 was dominated by spruce and fir in canopy, and the dwarf bamboo, *Lonicera* spp and *Rubus corchorifolius* are consisted of shrub layer. The soil in all sites is a highly humified Cambisols (Gong et al., 2007). Basic soil properties in the sampling forests at different altitudes are shown in Table 1.

Temperature monitoring and soil sampling

Based on the field investigation and previous local data, soil thawing was known to begin in early March. The buttony DS1923–F5 recorder (Maxim Com. USA) that recorded soil temperature every 2 h was buried in the soil to a depth of 5 cm in each of the three forests on 1 March, 2009.

Five 5 m × 5 m sampling plots were selected randomly with similar environmental factors in each site. Soil samples were collected from each sampling plots on 5, 15 and 25 March, and 5, 15 and 25 April, 2009, as soil thawing proceeded. From the previous observations of the soil characteristics, about 500 g soils were sampled from the soil organic layer (I, 0 to 15 cm) and the mineral soil layer (II, 15 to 30 cm). All soil samples were stored in freezer boxes and transported to the laboratory within 24 h. A sub-sample of fresh soil was immediately sieved through a 2 mm mesh after excluding the visible fragments and debris of blocks, plants, roots and animals, and stored at 4°C. The samples were used to determine the microbial biomass, activity, and exchangeable inorganic nutrients as well as dissolve organic substrates. The remaining soil was allowed to air-dried for determination of pH, the concentrations of total organic C (TC), total organic N (TN) and total organic P (TP).

Soil analysis

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined by the chloroform fumigation extraction method followed by 0.5 M K_2SO_4 extraction method of both unfumigated and fumigated samples (Brookes et al., 1985; Vance et al., 1987). Microbial biomass P (MBP) also was determined by the chloroform fumigation extraction method followed by 0.5 M $NaHCO_3$ extraction method of both unfumigated and fumigated samples (Morel et al., 1996). Fumigations were carried out for a period of 48 h in vacuum desiccators with alcohol-free chloroform. Dissolved organic C (DOC), dissolved organic N (DON) and inorganic P in fumigated and unfumigated extracts were, respectively measured by the dichromate oxidation-ferrous sulphate titration method, semi-micro Kjeldahl method, and phosphorus molybdenum–blue colorimetry method. MBC, MBN and MBP were calculated by dividing the difference of total extract between fumigated and unfumigated samples with a conversion factor of 0.45 for MBC (Sparling and West, 1990), 0.54 for MBN (Brookes et al., 1985) and 0.40 for MBP (Kouno et al., 1995), respectively. Moreover, due to some of the inorganic P in solution that can be fixed by the soils during soil extraction, the amount of absorbed inorganic P during soil extraction was estimated by measuring the recovery of a known amount of P standard solution (2.5 mg P L^{-1}) added to the soil. The percentage recovery of added P ranged from 84 to 90% in the samples.

Soil invertase activity was assayed as described by Wang et al. (2008). In brief, 1 g of soil was incubated for 24 h at 37°C with 15 ml 8% sucrose and 5 ml phosphate buffer at pH 5.5 and 0.1 ml toluene. The glucose released by invertase reacted with 3, 5-dinitrosalicylic acid and 3-aminonitrosalicylic acid, and then was measured at 508 nm. Results were expressed as mg released glucose by 1 g soil per day. For soil urease activity, we used the Kandeler and Gerber (1988) method "1 g of soil was incubated for 24 h at 37°C with 10 ml 10% urea and 20 ml citrate buffer at pH 6.7 and 0.1 ml toluene" Released ammonium by urease was determined by Indophenol-blue colorimetry. Results were expressed as mg released ammonium by 1 g soil per day. Acid phosphatase activity was measured following Tabatabai and Dick (2002). "1 g of soil was incubated for 2 h at 37°C with 20 ml 0.5% disodium phenyl phosphate and acetate buffer at pH 5 and 0.1 ml toluene" Released phenol acid phosphatase was determined by 4-aminoantipyrine colorimetry. The incubated temperature was chosen in order to standardize conditions with other studies, although it may have led to denaturation of cold-adapted enzymes.

Exchangeable inorganic was extracted with a 2 M KCl extracting water solution. Ammonium (NH_4-N) and nitrate (NO_3-N) in extract was measured by Indophenol–blue colorimetry and phenol disulphonic acid colorimetry, respectively (Lu, 1999). Exchangeable inorganic P (P_2O_5) was extracted with a 0.5 M $NaHCO_3$ extracting water solution, and then determined by phosphorus molybdenum-blue colorimetry (Lu, 1999). DOC and total dissolved N (TDN) were extracted by the method of Jones and Willett (2006). In brief, 20 g of fresh soil was extracted with 100 ml ultra-pure water in a centrifuge tube by shaking the mixture for 1 h on a reciprocal shaker, and then centrifuging it at 13,000 rpm for 30 min at 4°C. The supernatant was filtered through a 0.45 mm glass fiber filter. The C and N in the extracts were also measured using a C/N analyzer (TOC-VcPH+TNM-1, Shimazu Inc., Kyoto, Japan). DON was calculated as DON=TDN − (ammonium + nitrate).

Air-dried soils were passed through a 0.25 mm sieve for determination of the TC, TN and TP as described by Lu (1999). TC concentration was determined by the dichromate oxidation-ferrous

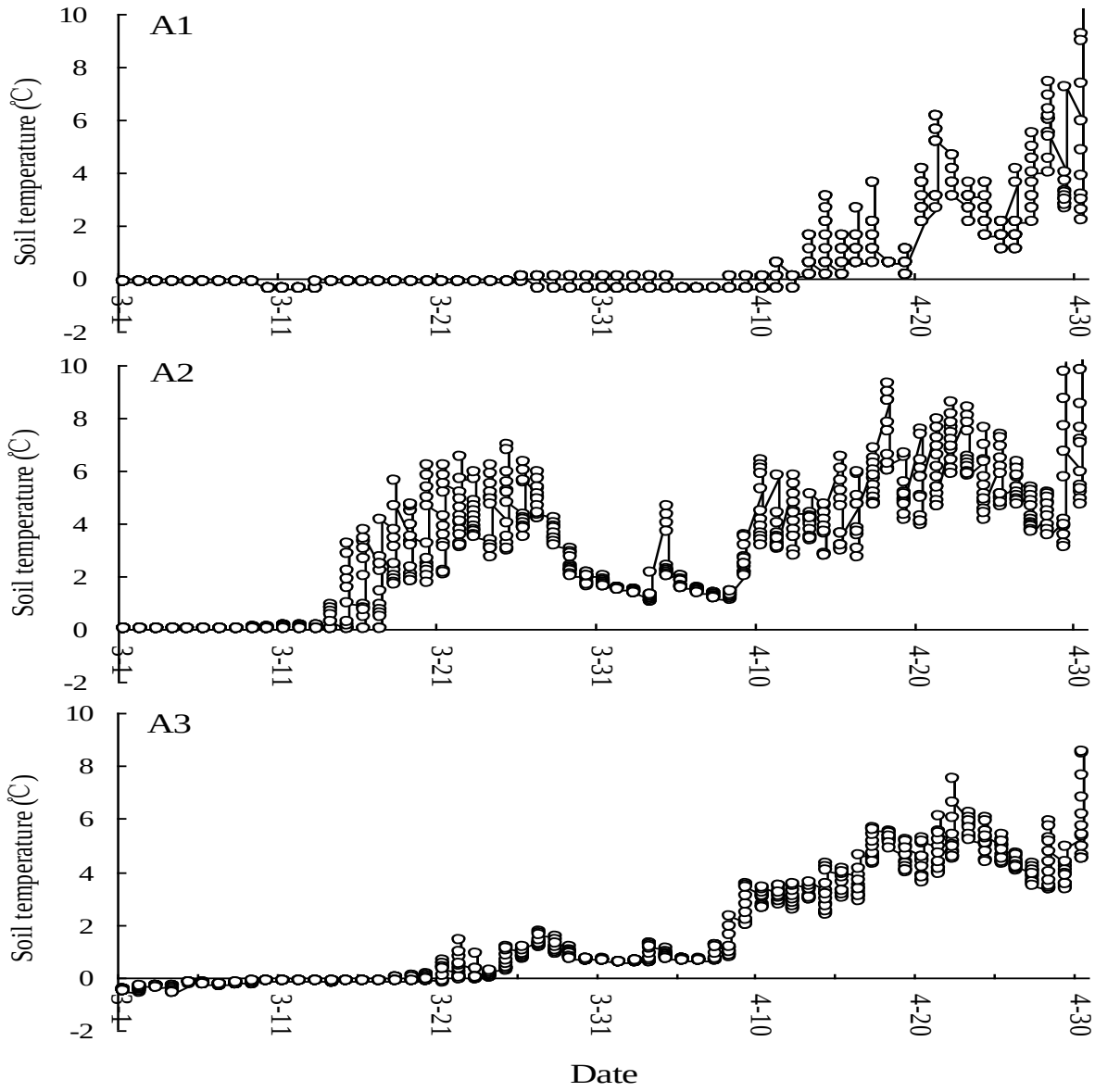

Figure 1. Daily soil temperature changes at depths of 5 cm at different altitudes during soil thawing period. A1, 3600; A2, 3300; A3, 3000.

sulphate titration method. After digestion with 8 ml H_2SO_4 (ρ = 1.84 g cm^{-3}) and 3 ml H_2O_2 solution, semi-micro Kjeldahl and phosphorus molybdenum-blue colorimetry were used to determine the concentrations of TN and TP, respectively.

Statistical analysis

Repeated measures analysis of variance (ANOVA) were used to test the main effects of altitude, sampling data, soil layer and their interactions on variables. Where significant main effects of altitude or sampling date were observed, Tukey's HSD post-hoc tests were applied. Before analysis, all dates were tested for the assumptions of ANOVA. If data were heterogeneous, they were in-transformed before analysis. The statistical tests were considered significant at the p=0.05 level. All of the statistical analyses were performed using SPSS software package (Standard released version 11.5 for Windows, SPSS Inc., IL. USA).

RESULTS

Soil temperature

Soil temperature significantly changed during the soil thawing period with frequent temperature fluctuations occurring at 0°C (Figure 1). The soil temperature above 0°C were first recorded on 26, 9 and 18 March, and that remained consistently above 0°C from 13 April, 18 March and 24 March in the A1, A2 and A3, respectively. There were 18 days (26 March to 12 April), 9 days (9 to 17 March), and 6 days (18 to 23 March) that soil temperature

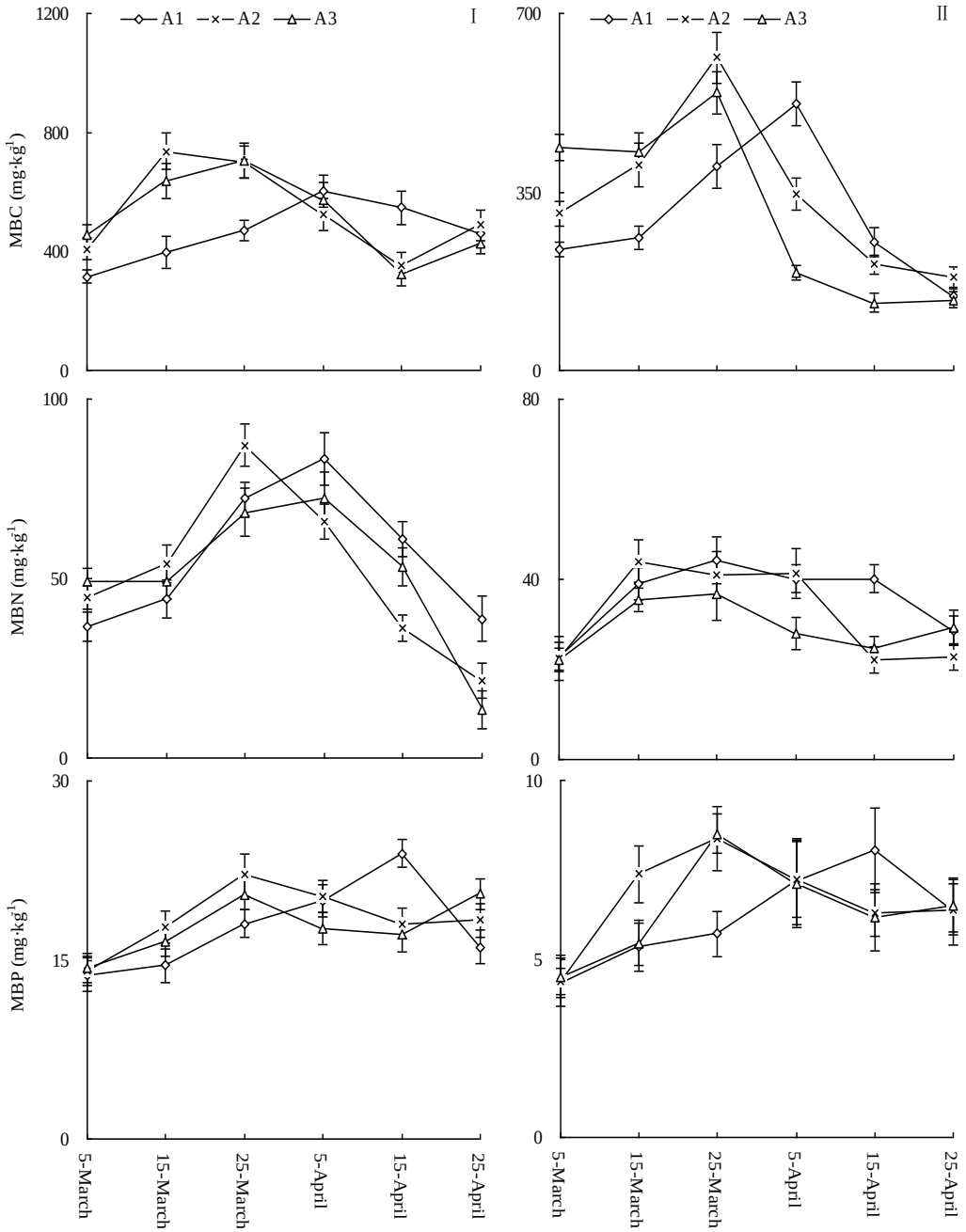

Figure 2. Dynamics of MBC, MBN and MBP in the sampled forests at different altitudes during soil thawing period. Means and standard errors are shown with the sample size n = 5. I, Soil organic layer; II, mineral soil layer. A1, 3600; A2, 3300; A3, 3000.

fluctuated at 0°C in the A1, A2 and A3, respectively.

Microbial biomass

Microbial biomass based on estimates of MBC showed significant changes (Figure 2). MBC increased steadily to an obvious peak when soil temperatures started to exceed 0°C as soil thawing proceeded, although the tendency was inconsistent at different altitudes. Thereafter, there was a continual decreased in MBC, particularly between 15 and 25 April, followed by a crash. MBN and MBP in soils (Figure 2) also showed significant peaks between 25 March and 15 April, and these approximately coincident with maximum values of MBC. Subsequently, MBN and MBP also declined steadily. MBC, MBN and MBP were significantly influenced by sampling date, and the interactive effects of altitude and sampling date were significant on MBC and MBN (Table 2)

Table 2. Results of repeated measures ANOVA showing the *p* values for response of MBC, MBN, MBP, DOC, DON, invertase, urease and acid phosphatase to altitude (A) and sampling date (D).

Factor	MBC	MBN	MBP	DOC	DON	Invertase	Urease	Acid phosphatase
Altitude	0.009*	0.024*	0.699	0.001*	0.001*	0.021*	0.011*	0.009*
Date	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
A × D	0.001*	0.001*	0.227	0.006*	0.006*	0.055	0.074	0.658

*, $p < 0.05$.

Enzyme activity

Activities of invertase, urease and acid phosphatase, which were measured from early March until late April, showed a similar pattern in March (Figure 3). Activity sharply reached the peak value when soil temperatures approached 0°C (March 15), but the hysteresis was observed for peak values of invertase and acid phosphatase in the soil organic layer in the A2. There was a crash decreased in activity which lasted 10 days after the peak value occurred. Meanwhile, the invertase activity declined continually in the organic layer in April; conversely, the acid phosphatase activity increased steadily (Figure 3). Activities of invertase, urease and acid phosphatase were significantly influenced by altitude and sampling date, but their interactive effects showed marginal significance on activities of invertase, urease and acid phosphatase (Table 2).

Soluble organic substrate

DOC and DON in soil solutions of these alpine and subalpine forests steadily increased to a peak value from early March until the end of the month, although the tendency was inconsistent at different altitudes (Figure 4). Thereafter, there was a steep and significant decline in DOC and DON from 25 March until 15 April. Amounts of DOC and DON were significantly influenced by altitude, sampling date and their interactive effects (Table 2).

DISCUSSION

Previous studies have established that microbial activity continues during the winter often resulting in high microbial biomass at some alpine meadow and arctic tundra sites, and microbial biomass usually reaches a peak in late winter and then declines during the period when soil temperatures rise to 0°C (Broo ks et al., 1998; Edwards et al., 2006; Larsen et al., 2007). In agreement with these results, the MBC and MBN here was not the lowest at the frozen soil (soil temperature below 0°C) in the subalpine and alpine forests during the study period. MBC, MBN and MBP sharply increased, and an obvious peak was detected when the soil temperature was around 0°C, as the soil temperature increased. Because the soil was frozen and relatively dry during winter, it was subjected to continual influxes of snowmelt during the thawing period (Buckeridge et al., 2010). The melt water streamed down channels in frozen soil, and that rapidly raised soil moisture (Jefferies et al., 2010). Meanwhile, massive soil available inorganic nutrient, which is presumed to come from the lysis of cells of plant and soil organisms (microbes or animals) necromass, was suggested to flood into frozen soil with the melting snow water (Herrmann and Witter, 2002; Grogan et al. 2004; Hentschel et al., 2008; Tan et al., 2011). Therefore, nutrients released from the senescing cell can be utilized by surviving microbes, contributing to the rapid reproduction of microbes and high microbial biomass (Larsen et al., 2002; Lipson et al., 2002; Edwards et al., 2006). However, the changes in soil physical state and freeze-thaw cycle could reduce and even kill a portion of microbes due to soil water osmolarity alteration when soil temperature above 0°C (Jefferies et al., 2010). Furthermore, the exhaustion of available substrates by abrupt reproduction might limit the growth of microbes (Lipson et al., 2000; Schimel and Mikan, 2005; Edwards et al., 2006). Additionally, the snowmelt water rapidly leaches available nutrients and might also terminate the sharp growth of microbes (Balcarczyk et al., 2009; Buckeridge and Grogan, 2010). Therefore, the sudden crash in microbial biomass was observed after the bloom of microbial biomass lasting 10 days. The dynamics in microbial biomass in these subalpine and alpine forests support the view that soil microbial biomass was resistant to repeated fluctuations around 0°C, but the winter microbial community was sensitive to soil temperatures and moisture that remained above the freezing point (Lipson et al., 2000; Jefferies et al., 2010).

Similarly, concentrations of soluble organic substrates (DOC and DON) in the soil remained increased until the crash which occurred at soil temperature around 0°C. In addition, invertase, urease and acid phosphatase activities were maintained until 15 or 25 March when a steep decline occurred just prior the soil temperature rise to 0°C. The results indicated that dramatic changes occurred in both soluble organic substrate and enzyme activity over a period as little as 10 days in March, just prior to the sustained rise in soil temperatures to above the freezing point in these subalpine and alpine forests.

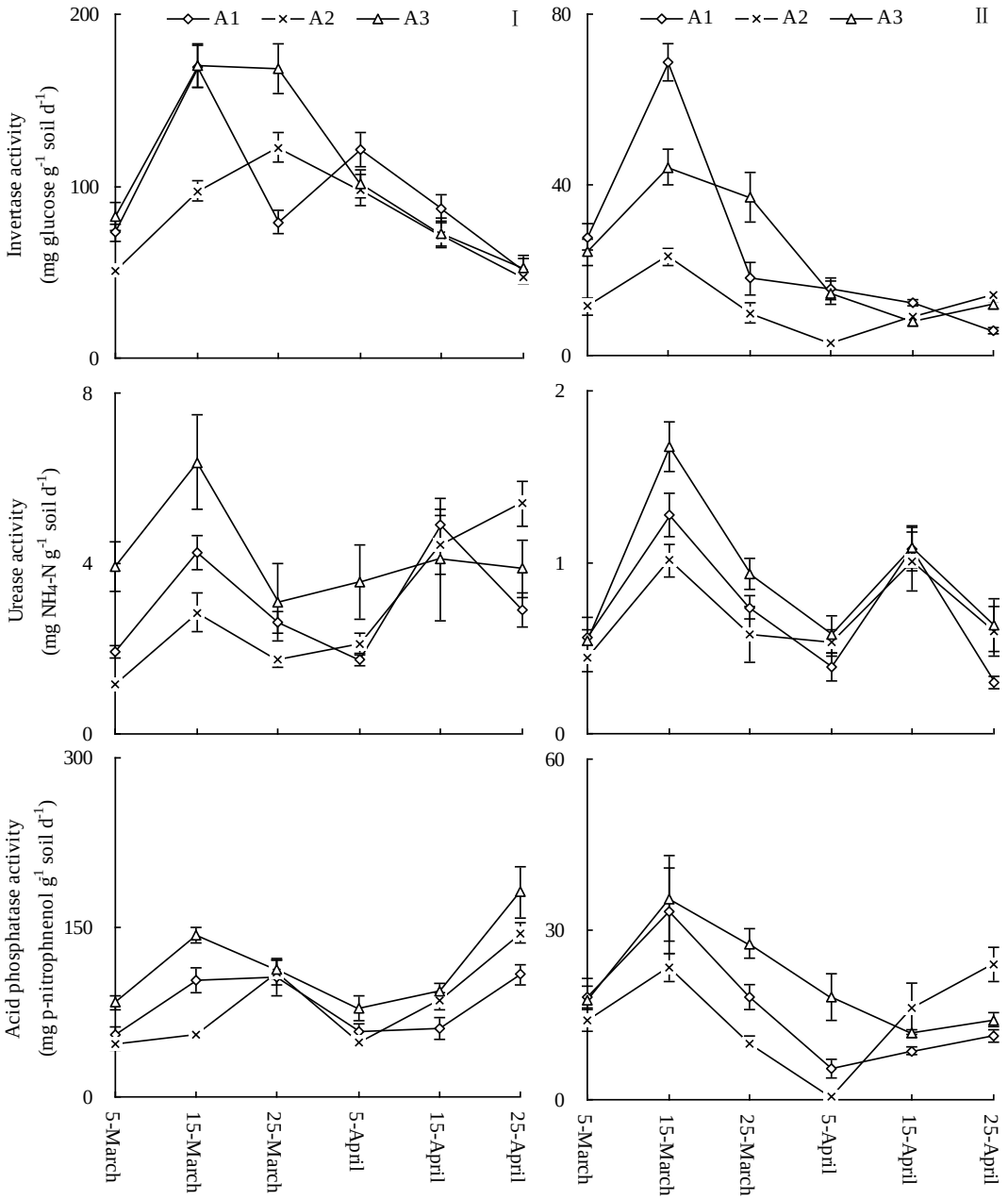

Figure 3. Invertase, urease and acid phosphatase activities (measured at 37°C) in the sampled forests at different altitudes during soil thawing period. Means and standard errors are shown with the sample size n = 5. I, Soil organic layer; II, mineral soil layer. A1, 3600; A2, 3300; A3, 3000.

These changes were shortly before or coincident with the soil microbial biomass dropped. However, in spite of the decline in microbial biomass and soluble organic substrates during the final stages of soil thawing, there was no subsequent flush of inorganic nutrients (Tan et al., 2011). Hence, the decline in microbial biomass and in soluble organic substrates as well as in enzyme activity precedes the sustained temperature rise above the freezing point and the results strongly suggest that the lack of resource availability contributes to the crash of microbial biomass (Lipson et al., 2000; Schmidt and Lipson, 2004; Edwards et al., 2006). The sequence of nutrient availability over time, therefore, is initially a decline in exchangeable inorganic nutrient from early March (Tan et al., 2011), followed by a very large drop in soluble organic substrates and enzyme activities from mid-March until late March that precedes a steep decline in microbial biomass. Nevertheless, the inconsistent decline of microbial biomass implies that other drivers are forcing the changes. The most likely causes of the

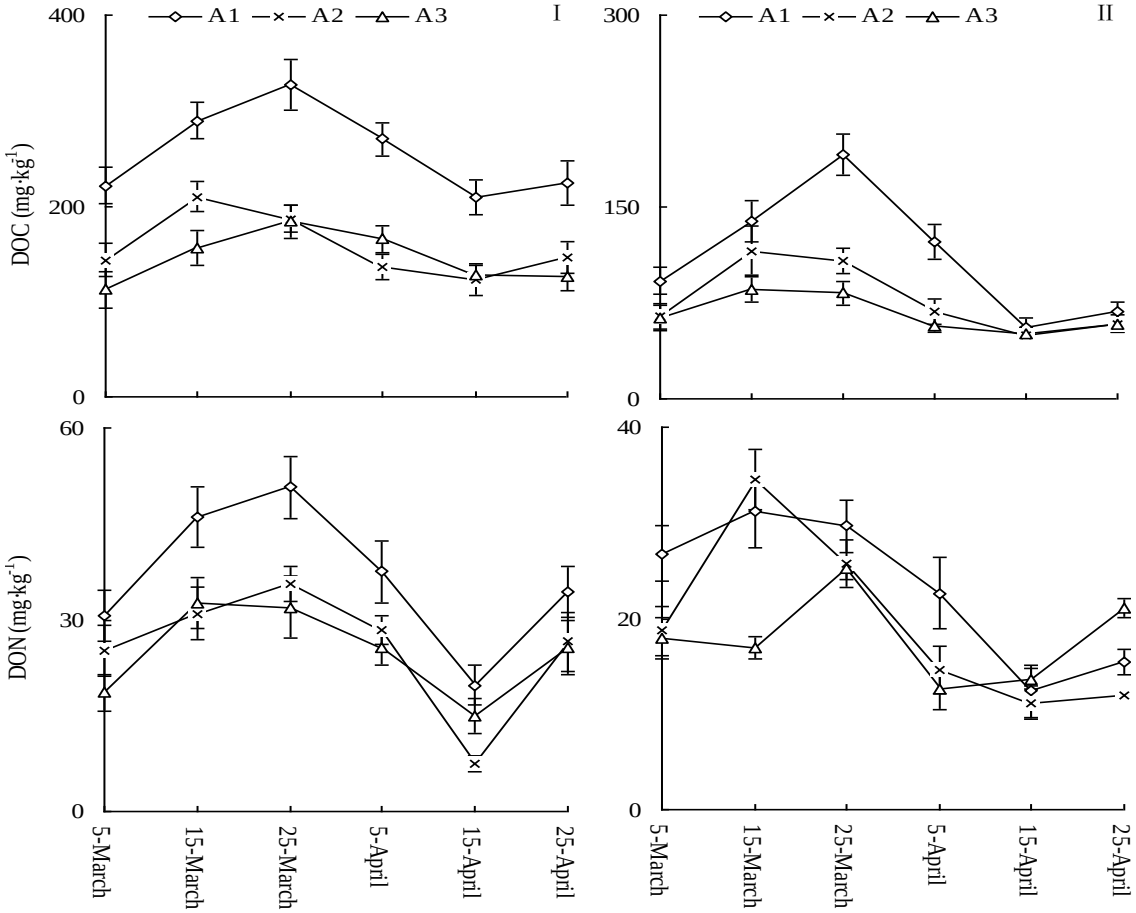

Figure 4. Amounts of DOC and DON in the sampled forests at different altitudes during soil thawing period. Means and standard errors are shown with the sample size n = 5. I, Soil organic layer; II, mineral soil layer. A1,3600; A2, 3300; A3, 3000.

declines are: (1) the lysis of microbial cells in response to the changes in soil physical state during transient temperatures above 0°C (Jefferies et al., 2010), (2) the depletion of soil C, N and P substrates (Lipson et al., 2000; Schimel and Mikan, 2005), (3) the difference of thawing time (Figure 1) and vegetation community, and (4) competition for organic C and N from plant roots, which are known to take up amino acids directly from soil at low temperatures in arctic systems (Lipson and Monson, 1998; Henry and Jefferies, 2003; Xu et al., 2011). Additionally, Schmidt and Lipson (2004) have pointed out that the microbial community acts first as a sink for nutrients in late winter when snow cover prevails and then as a source of nutrients in early spring at the time of snow melt. The release of nutrients from lysis microbes as microbial biomass decline might represents the immediate single input of available nutrients into these forest soils and is mostly absorbed by plants for early spring growth (Lipson and Monson, 1998; Xu et al., 2011), but it appears that this source is rapidly depleted in both the studies of Edwards et al. (2006) and our study. The global warming scenarios suggested that freezing characteristics in winter, such as the frequency, intensity and length of freezing and thawing, might experience significant increase in the future warmer condition as the disappearance of insulating snow pack (Campbell et al., 2005). The present study also showed that the dynamics of microbial biomass, activity and nutrients were inconsistent at different altitudes due to different temperature fluctuations. Since the A1 had longer length of the temperature fluctuations as compared with the A2 and A3, the sequence of microbial biomass, activity and nutrients in the A1 showed a more distinct dynamics following soil thawing proceeded. This indicated that the dynamics of microbial biomass between wintertime and springtime would have an important role in controlling the annual carbon cycle in coniferous forest ecosystems in the context of global climatic change. Meanwhile, although similar dynamics were observed in both the soil organic layer and mineral soil layer, more significant dynamics were detected in the soil organic layer and a hysteresis phenomenon in the mineral soil layer. The closely explanation is that the soil organic layer had relative higher availability of substrate (Tan et al., 2011),

and the soil organic layer directly faced to the environment changes such as temperature fluctuations (Figure 1). Furthermore, due to the different characteristics of N and P during soil thawing (Tan et al., 2011), there were apparent differences between the changes of MBN and MBP. More significant changes in MBN indicated that N might be a more limiting element compared with P in the subalpine/alpine forests during soil thawing period. However, although this study tried to analyze the effects of altitude-controlled temperature fluctuations on the microbial-biogeochemical dynamics during soil thawing change in these forests, the responses of soil microbial biomass, enzyme activity, and nutrients to various temperature fluctuations were confused since many other factors have not been examined. This warrants further study.

Conclusion

Soil microbial biomass increased as soil thawing proceeded until an obvious peak when soil temperatures exceed 0°C in these subalpine/alpine forests at different altitudes. Thereafter, soil microbial biomass did not increase with the increase of temperature, which continually declined until the moment that may be the end of soil thawing. Likewise, the rapidly crash in the soluble organic nutrient pools occurred shortly before or coincident with the microbial biomass dropped as soil temperature around 0°C. Meanwhile, the sudden decline in enzyme activity occurred just before the soluble organic nutrient pools collapsed. Additionally, the dynamic patterns of microbial biomass, enzyme activity and soluble organic substrate varied with altitudes due to different temperature fluctuations. The results show similarities to trends observed in alpine meadow and arctic tundra soils, which indicate that the release of nutrients as microbial biomass decline represent important pulse of carbon and nutrients pools for the growth of vegetation in the early growing season.

ACKNOWLEDGEMENTS

This project was financially supported by National Natural Science Foundation of China (No. 31000213, 31170423), Program for New Century Excellent Talents in University (No. NCET-07-0592), National Key Technologies R & D in China (2011BAC09B05), the Provincial Key Science and Technology R & G Program of Sichuan (No. 2010NZ0051) and Program of Sichuan Youth Sci-tech Foundation (No. 2012JQ0008). This research was carried out in compliance with the laws of People's Republic of China.

REFERENCES

Balcarczyk KL, Jones JB, Jaffé R, Maie N (2009). Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. Biogeochem., 94: 255–270.

Brookes PC, Landman A, Pruden G, Jinkenson DS (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem., 17(6): 837–842.

Brooks PD, Williams MW, Schmidt SK (1998). Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry, 43(1): 1–15.

Buckeridge KM, Cen YP, Layzell DB, Grogan P (2010). Soil biogeochemistry during the early spring in low arctic mesic tundra and the impacts of deepened snow and enhanced nitrogen availability. Biogeochemistry, 99(1-3): 127–141.

Buckeridge KM, Grogan P (2010). Deepened snow increases late thaw biogeochemical pulses in mesic low arctic tundra. Biogeochemistry, 101(1-3): 105–121.

Campbell JL, Mitchell MJ, Groffman PM, Chrisenson LM, Hardy JP (2005). Winter in northeastern North America: a critical period for ecological processes. Front. Ecol. Environ., 3(6): 314–322.

Edwards KA, McCulloch J, Kershaw GP, Jefferies RL (2006). Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. Soil Biol. Biochem., 38(9): 2843–2851.

Gong ZT, Zhang GL, Chen ZC (2007). Pedogenesis and soil taxonomy. Science Press, Beijing, pp. 307–329.

Grogan P, Michelsen A, Ambus P, Jonasson S (2004). Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. Soil Biol. Biochem., 36(4): 641–654.

Henry HAL, Jefferies RL (2003). Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed Arctic salt marsh. J. Ecol., 91(4): 627–636.

Hentschel K, Borken W, Matzner E (2008). Repeated freeze-thaw events affect leaching losses of nitrogen and dissolved organic matter in a forest soil. J. Plant Nutr. Soil Sci., 171(5): 699–706.

Herrmann A, Witter E (2002). Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. Soil Biol. Biochem., 34(10): 1495–1505.

Jefferies RL, Walker NA, Edwards KA, Dainty J (2010). Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils? Soil Biol. Biochem., 42(2): 129–135.

Jones DL, Willett VB (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem., 38(5): 991–999.

Kandeler E, Gerber H (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils, 6(1): 68–72.

Koponen HT, Jaakkolaa T, Keinanen-Toivola MM, Kaipainena S, Tuomainena J, Servomaac K, Martikainen PJ (2006). Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles. Soil Biol. Biochem., 38(7): 1861–1871.

Kouno K, Tuchiya Y, Ando T (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol. Biochem., 27(10): 1353–1357.

Larsen KS, Grogan P, Jonasson S, Michelsen A (2007). Dynamics and microbial dynamics in two subarctic ecosystems during winter and spring thaw: effects of increased snow depth. Arct. Antarct. Alp. Res., 39(2): 268–276.

Larsen KS, Jonasson S, Michelsen A (2002). Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. Appl. Soil Ecol., 21(3): 187–195.

Lipson DA, Monson RK (1998). Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. Oecologia, 113(3): 406–414.

Lipson DA, Schadt CW, Schmidt SK (2002). Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microb. Ecol., 43(3): 307–314.

Lipson DA, Schmidt SK, Monson RK (2000). Carbon availability and temperature control the post-snowmelt baseline in alpine soil microbial biomass. Soil Biol. Biochem., 32(4): 441–448.

Lu RK (1999). Soil and Agro–chemical Analytical Methods. China Agricultural Science and Technology Press, Beijing, pp. 227–448.

Morel C, Tiessen H, Stewart JWB (1996). Correction for P-sorption in the measurement of soil microbial biomass P by $CHCl_3$ fumigation. Soil Biol. Biochem., 28(12): 1699–1706.

Schimel JP, Clein JS (1996). Microbial response to freeze-thaw cycles in tundra and taiga soils. Soil Biol. Biochem., 28(8): 1061–1066.
Schimel JP, Mikan C (2005). Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. Soil Biol. Biochem., 37(8): 1411–1418.
Schmidt SK, Lipson DA (2004). Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil, 259(1-2): 1–7.
Sjursen H, Michelsen A, Holmstrup M (2005). Effects of freeze-thaw cycles on microarthropods and nutrient availability in a sub-Arctic soil. Appl. Soil Ecol., 28(1): 79–93.
Skogland T, Lomeland S, Goksoyr J (1988). Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. Soil Biol. Biochem., 20(6): 851–856.
Sparling GP, West AW (1990). A comparison of gas chromatography and differential respirometer methods to measure soil respiration and to estimate the soil microbial biomass. Pedobiologia, 34(2): 103–112.
Tabatabai MA, Dick WA (2002). Enzymes in soil. In: Burns, RG, Dick RP (Eds.) Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker, New York, pp. 567–596.
Tan B, Wu FZ, Yang WQ, Liu L, Yu S (2010). Characteristics of soil animal community in the subalpine/alpine forests of western Sichuan at the early stage of freeze-thaw season. Acta Ecol. Sin., 30(2): 93–99.
Tan B, Wu FZ, Yang WQ, Yu S, Liu L, Wang A (2011). The dynamics pattern of soil carbon and nutrient as soil thawing proceeded in the alpine/subalpine forest. Acta Agric. Scand. B–S. P., 61(7): 670-679.
Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem., 19(6): 703–707.
Vitousek PM, Hedin LO, Matson PA (1998). Within-system element cycles, input-output budgets, and nutrient limitations. In: Pace ML, Groffman PM et al (Eds) Successes, limitations and frontiers in ecosystem science. Springer–Verlag, New York, pp. 432–451.
Wang Q, Wang S, Liu Y (2008). Responses to N and P fertilization in a young Eucalyptus dunnii plantation: Microbial properties, enzyme activities and dissolved organic matter. Appl. Soil Ecol., 40(3): 484–490.
Wu FZ, Yang WQ, Zhang J, Deng RJ (2010). Litter decomposition in two subalpine forests during the freeze–thaw season. Acta Oecol., 36(1): 135–140.
Xu XL, Ouyang H, Richter A, Wanek W, Cao GM, Kuzyakov Y (2011). Spatio-temporal variations determine plant-microbe competition for inorganic nitrogen in an alpine meadow. J. Ecol., 99(2): 563–571.
Yang WQ, Wang KY, Kellomäki S, Gong HD (2005). Litter dynamics of three subalpine forests in Western Sichuan. Pedosphere, 15(5): 653–659.