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Effects of *in situ* freeze-thaw cycles on winter soil respiration in mid-temperate plantation forests

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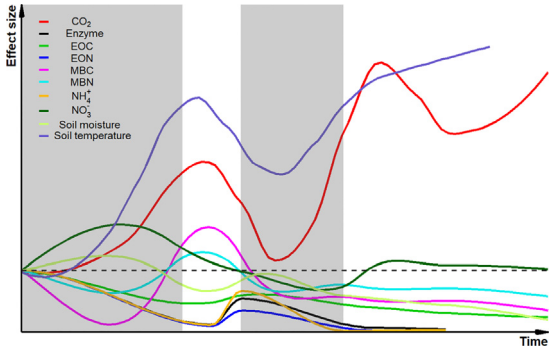
HIGHLIGHTS

- CO_2 flux exhibited a small response to FTC due to the exhaustion of resources.
- The intensity and frequency of FTC affected the effect of FTC on soil CO_2 flux.
- The burst of CO_2 flux during thawing period was mainly due to the increase in MBC.
- The thickness of litter layer affected the response of soil CO_2 flux to FTC.

GRAPHICAL ABSTRACT

The locally weighted polynomial regression analysis indicated that during the first FTC, the changing trends of MBC and MBN were consistent with that of soil CO_2 efflux, whereas the changing trends of EOC, EON, enzyme, and NH_4^+ were opposite to that of soil CO_2 efflux. Soil CO_2 efflux was significantly correlated with soil temperature during the entire FTC.



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ABSTRACT

As an important factor regulating soil carbon cycle, freeze-thaw cycle significantly affects winter soil respiration in temperate regions. However, few *in situ* studies have been carried out to evaluate the effect of freeze-thaw cycle on soil respiration. Here, a field experiment was conducted to explore the response of winter soil respiration to freeze-thaw cycle and the underlying mechanisms in larch and Chinese pine plantation forests in a mid-temperate region. These results indicated that CO_2 emissions during the freeze-thaw period accounted for 18.89–18.94% and 0.79–1.00% of the cumulative winter CO_2 emissions and the annual soil CO_2 emissions, respectively. Soil respiration rates during the thawing phase were 1.54–3.95 times higher than those during the freezing phase, which was mainly due to the increase of soil microbial biomass upon thawing. This effect declined during the second freeze-thaw cycle compared to the first freeze-thaw cycle due to the exhaustion of resources for microbes. The different responses of soil CO_2 flux to freeze-thaw cycle between the two types of forests were mainly because of the difference in the thickness of litter layer, which plays an important role in regulating soil temperature and enzyme activity. These results suggest the intensity and frequency of freeze-thaw cycle strongly affect soil carbon emissions during the freeze-thaw cycle period. Therefore, these factors should be considered in laboratory studies and model simulations under climate change scenarios.

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1. Introduction

Freeze-thaw cycle (FTC) events mainly occur at high latitudes, high altitudes, and some temperate regions (Grogan et al., 2004; Yu et al., 2011). Approximately 55% of the total land area in the northern hemisphere experiences seasonal soil freezing (Grogan et al., 2004; Kreyling et al., 2008; Brooks et al., 2011). FTC has a strong impact on soil physical structure, soil nutrients, microbial activities, all affecting the fluxes of CO_2 from soil (Sawicka et al., 2010; Pelster et al., 2013; Chai et al., 2014; Xiao et al., 2019). A burst of soil CO_2 after soil thawing has often been observed in many ecosystems (Phillips et al., 2012; Sullivan et al., 2012; Wang et al., 2014; Walz et al., 2017; Han et al., 2018). Global climate change has altered the patterns of FTC (Mellander et al., 2007), which may influence soil CO_2 emission and carbon cycling. However, previous studies on the response of soil CO_2 emission to changing FTC are mainly laboratory incubation studies (Song et al., 2017). The unrealistic timing of soil collection and unrealistic patterns of FTC in laboratory studies may significantly affect the response of soil respiration to FTC (Henry, 2007). Hence, more realistic simulation experiments are needed to better predict the response of carbon cycling to changing freeze-thaw regimes under the context of global changes (Korell et al., 2020). To date, there have been few studies conducted in the field on the effects of FTC on soil respiration (Song et al., 2017; Gao et al., 2018b).

Two main mechanisms have been explored to explain the freeze-thaw-induced enhancement of soil CO_2 emission (Teepe and Ludwig, 2004; Holst et al., 2008; Wang et al., 2013; Congreves et al., 2018). On the one hand, the disruption of soil aggregate subject to FTC promotes the release of soil inorganic and organic nutrients from soil lattice and colloid, beneficial for survived soil microbes and their respiration during the thawing period (Oztas and Fayetorbay, 2003; Six et al., 2004; Edwards, 2013). In addition, the dead soil microorganisms caused by FTC can also release nutrients into soil and reduce the immobilization of soil nutrients, stimulating soil enzymatic activities and further enhancing soil respiration during the thawing period (Grogan et al., 2004; Groffman et al., 2011). On the other hand, soil microorganisms could remain active when the soil is frozen (Robinson, 2001; Price and Sowers, 2004; Öquist et al., 2007; Peng et al., 2019), and the produced CO_2 gas during the freezing period is preserved in soils due to the physical barrier of the frozen layer (Koponen et al., 2004; Teepe and Ludwig, 2004; Yang et al., 2014). Consequently, the physical release of trapped CO_2 gas during freezing may also cause a sharp rise of CO_2 emission after thawing (Maljanen et al., 2007; Song et al., 2008).

At present, whether the above-mentioned mechanisms persist after the first FTC is still unclear because the nutrient release from disruption of soil aggregate and dead soil microorganisms may decrease or even no longer happen after the first FTC. In addition, during later FTC the adsorption of nutrients by soil aggregates, the immobilization of nutrients by soil microorganisms, or the leaching of dissolved organic carbon in the plots with FTC might be higher than those in the control plots without FTC, making soil CO_2 emission even lower than in the corresponding control plots (Song et al., 2017; Gao et al., 2018b). Some studies revealed that soil respiration showed a pulse increase of CO_2 during the initial FTC and no change or decrease in soil CO_2 emission during the subsequent FTC (Elberling and Brandt, 2003; Kurganova and Tipe, 2003; Feng et al., 2007; Wei et al., 2016). Contrarily, the increase of CO_2 emission has been discovered during the entire freeze-thaw period in some other studies, although the increment of CO_2 may have a reducing tendency with more FTCs (Wei et al., 2016; Walz et al., 2017). The contradiction may be attributed to the variation of nutrient supply during FTC period under different experimental methods. For instance, under field conditions, litter and dead roots can release nutrients during the FTC period, thus providing more nutrients to soil microorganisms continuously (Gaul et al., 2008; Song et al., 2017). In laboratory studies, however, soil nutrients are continuously consumed over time without additional sources of input (Gao et al., 2018b). Understanding the changes in soil nutrients, soil enzyme during each FTC could help better explain this discrepancy.

The litter layer in temperate forests plays an important role in insulation and patterns of FTC (Gao et al., 2018a). Under the same climatic conditions, different types of forests may have different thicknesses of the litter layer and thereby different patterns of FTC. The effect of the litter layer on soil respiration during the FTC period may be mainly attributed to three aspects. First, the litter layer as an insulating layer may weaken the intensity of freezing (Gao et al., 2018a). Second, the litter layer with a water-retaining effect may alter soil moisture. Lastly, the litter layer can be more decomposed due to the freezing and thawing effects, thus releasing more soluble substrates into soils (Gaul et al., 2008). These changes caused by the litter layer can significantly affect soil respiration in response to FTC. Additionally, differences in forest types also impact soil nutrient status and soil microbial characteristics (Gao et al., 2018a), which may result in a different effect of FTC on soil respiration. Until now, few studies have focused on the effects of forest types on the response of soil respiration to FTC (Shi et al., 2014).

Here, a field experiment was conducted to study the effect of FTC on soil respiration and to explore the underlying mechanisms in two types of mid-temperate plantation forests in Northeast China. The objectives of this study were: (1) to explore the response of soil respiration to FTC *in situ*; (2) to investigate whether the underlying mechanisms of the increase of soil CO_2 during the thawing period were different between different FTC from the perspectives of environmental factors, soil nutrient elements, and microbial characteristics; and (3) to compare the difference between the two types of plantation forests.

2. Materials and methods

2.1. Site description

The field experiment was carried out in two types of temperate plantation forests nearby Shenyang, Liaoning province, Northeast China (41°54′22″N, 123°35′48″E, 122 m a.s.l.). The region has a semi-humid temperate continental climate, characterized by the dry and cold winter season, and warm and humid summertime. The mean annual temperature is 8.3 °C, with the highest monthly mean temperature of 24.8 °C in July and the lowest monthly mean temperature of −10.5 °C in January. The mean annual precipitation is 726.2 mm, most of which falls between June and August, and the average winter precipitation is 30 mm. The soil in this area is classified as Aquic Brown soil by Chinese soil classification (equivalent to Typic Haplaqualf by USDA Soil Taxonomy) (Gong et al., 2003). The research forests are two types of plantation forests about 20 years old, mainly composed of Chinese pine (*Pinus tabuliformis*) and larch (*Larix gmelinii (Rupr.) Kuzen.*), respectively. During the freeze-thaw period, the thickness of litter layer in the Chinese pine plots (averaged 4.1 cm) was higher than that in the larch plots (averaged 2.1 cm). The basic physical and chemical properties of the studied soils (0–5 cm) were described by Gao et al. (2018a).

In situ observation of winter soil respiration during the freeze-thaw period was adopted in this experiment in larch forest and Chinese pine forest, respectively. There were five replicates for each type of forest (2.5 m × 2.5 m each replicate). Each plot contained one larch or Chinese pine tree. To minimize the interactions among plots, a buffer (approximately 50 cm) was applied to the edge of each plot.

2.2. Microclimate monitoring

The Thermochron iButton (iButton DS1923-F5, Maxim Com. USA) was used to continuously record air temperature at 2 m aboveground and soil temperature at 5 cm depth at 1-h intervals in each plot. Additionally, five portable thermometers were inserted into 1–2 cm of surface soil to monitor the soil temperature, determining whether the soil had started a freeze-thaw cycle. The intensity of FTC was represented by the minimum temperature during freezing. The number of

FTC was calculated based on soil temperature at 5 cm soil depth. One FTC was defined as follows: Soil temperature remained above 0 °C for at least 3 h and then dropped below 0 °C and remained for at least 3 h; or *vice versa* (Konestabo et al., 2007). In this study, the time of the beginning and end of the first freezing were determined when soil temperature at 5 cm depth started to increase and just reached 0 °C, respectively. The duration of the first thawing and the second freezing were the time when the soil temperature at 5 cm depth continued to be higher and lower than 0 °C, respectively. The time of the beginning and end of the second thawing were determined when soil temperature at 5 cm depth was above 0 °C and started to rise, respectively. Finally, in this experiment, the duration of the two freeze-thaw cycles was from February 29th to March 9th and March 10th to March 21st, respectively, and the total duration of FTC was 22 days.

2.3. Measurement of CO_2 flux and soil sampling

Soil CO_2 flux was measured using a Li-6400 soil CO_2 flux system (LI-COR INC., Lincon, NE, USA). During the snow-covered period, three polyvinyl chlorides (PVC) collars with a diameter of 10.5 cm were installed in the soil in each plot and stabilized one day before the measurement of soil CO_2 flux. To ensure a 3 cm height between the snow surface and the upper edge of the collar, different lengths of PVC collars were made and used according to the depth of snow. During the snow-free phase, three PVC collars were also inserted into the soil in each plot and made sure a 3 cm height was kept from the soil surface to the upper edge of the collar (Wang et al., 2010). Soil respiration was measured in the morning between 9:00 to 12:00 am, with a frequency of once every two or three days during the freeze-thaw period and once a week in other periods. The cumulative soil CO_2 fluxes were estimated by linearly interpolating between two measurements and integrating for the respective period (Gao et al., 2018a). Soil temperature measured by the portable thermometer inserted into 5 cm soil depth and weather forecast were used to determine the time of the FTC so that the time of soil sampling corresponded with the two FTCs well. The soil (0–5 cm) was randomly sampled at 3 points using a soil auger with a diameter of 5 cm for each plot. The three samples per plot were bulked homogeneously, stored in a freezer boxer, and then was immediately transported to the laboratory for further analysis. Fresh soils were passed through a 2 mm sieve and stored in the refrigerator at 4 °C for the analyses of microbial biomass, enzymatic activities, inorganic N, and extractable organic C and N.

2.4. Soil analysis

2.4.1. Soil NH_4^+, NO_3^-, and extractable organic C and N

To determine the concentrations of inorganic nitrogen (ammonium (NH_4^+) and nitrate (NO_3^-)), fresh soils were extracted with 2 M KCl solution (fresh soils: 2 M KCl solution = 1:5, shaken for 1 h). The concentrations of NH_4^+ and NO_3^- were determined using the indophenol-blue and the phenol disulphonic acid colorimetry, respectively (Lu, 1999). Extractable organic carbon (EOC) and total dissolved nitrogen (TDN) were extracted using 0.5 M K_2SO_4 solution and then were analyzed using a TOC/TN analyzer (MultiN/C3100, Analytic Jena, Germany). Thus, extractable organic nitrogen (EON) was equal to TDN minus the sum of soil NH_4^+ and NO_3^- (Ross et al., 2013).

2.4.2. Soil microbial biomass

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform fumigation extraction (Brookes et al., 1985; Vance et al., 1987). Briefly, 20 g fresh soils were extracted with 0.5 M K_2SO_4 solution (fresh soil: solution = 1:4, shaken for 1 h). Another 20 g fresh soils were fumigated with chloroform for 24 h, then were extracted with 0.5 M K_2SO_4 solution (fresh soil: solution = 1:4, shaken for 1 h). TOC/TN analyzer (MultiN/C3100, Analytik Jena, Germany) was used to analyze extractable C and N in both unfumigated and fumigated extracts. MBC was calculated by the difference in extractable C concentration between fumigated and unfumigated soils divided by 0.45 (Vance et al., 1987). MBN was calculated using the difference in extractable N concentration between fumigated and non-fumigated samples divided by 0.54 (Brookes et al., 1985).

2.4.3. Soil extracellular enzymatic activities

Four soil extracellular enzymatic activities involved in carbon cycling were determined using the fluorogenic substrates (Saiya-Cork et al., 2002). Soil samples at 0–5 cm depth were assayed for α-D-glucosidase (EC 3.2.1.20), β-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), and xylosidase (EC 3.2.1.37) using 4-methylumbelliferyl (MUB) -α-D-glucosidase, 4-MUB-β-D-glucosidase, 4-MUB-β-D-cellobioside, and 4-MUB-β-D-xylopyranoside as substrates, respectively. Briefly, 2 g fresh soils were extracted with 100 mL sodium acetate butter solution (pH = 5) and then stirred for 1 min using a magnetic stir plate. Next, different mixed solutions were added to a 96-well microplate. For example, 50 μL MUB and 200 μL sodium acetate butter, 50 μL substrates and 200 μL soil suspensions, and 50 μL sodium acetate butter and 200 μL soil suspensions were standards, samples, and blanks, respectively. Besides, 50 μL MUB and 200 μL soil suspensions were added into one well on the 96-well microplate to correct the error caused by the adsorption of fluorescent substances in the soils. Then, the 96-well microplate was incubated in an opaque oven for 4 h at 25 °C. After that, 10 μL 0.5 mol L^{-1} NaOH was immediately added into all wells on the microplate to terminate the reaction. Finally, a fluorimetric microplate reader (Synergy H1, BioTek, Winooski, VT) was used to analyze the intensity of fluorescence. The activities of these soil enzymes were calculated according to the method described by German et al. (2011).

2.5. Statistical analysis

The magnitude of changes in soil variables during the FTC period was calculated as the value of the samples determined at a given time point divided by the value at the initial time point (February 23, 2017). Data were analyzed by fitting linear mixed-effects models with maximum likelihood (using the lme function in the nlme package, R software version 3.5.3 (R Development Core Team, 2018)). During the entire freeze-thaw period, forest type (larch *vs.* Chinese pine) and time were used as fixed effects and the individual plot was included as random effects. The corAR1 function was used to account for repeated measurements with a first-order autoregressive covariate structure. *t*-tests were carried out to test whether there were statistically significant increases or decreases in soil respiration, environmental variables (soil temperature and soil moisture), soil nutrients (soil NH_4^+, NO_3^-, EOC, and EON) or microbial parameters (MBC, MBN, α-glucosidase, β-glucosidase, cellobiohydrolase, and xylosidase activities) during each FTC and the differences among treatments at single time-points. Subsequently, Pearson's correlations between soil respiration and environmental variables, soil nutrients, and microbial parameters were performed. All statistical analyses and figures were performed using R software version 3.5.3. The reported values in this study were presented as mean ± 95% confidence intervals.

Structure equation modeling (SEM) was further used to explore the impacts of freeze-thaw cycle-induced alterations in environmental variables, including soil temperature, soil moisture, NH_4^+, NO_3^-, EOC, and EON, MBC, MBN, and enzyme, on soil CO_2 flux. At first, a conceptual model of hypothetical correlations was built according to prior and theoretical knowledge. In the SEM analysis, model fit statistics were used to compare the observed variance-covariance matrix with the model-implied variance-covariance matrix. The chi-square test (χ^2) was used to evaluate the overall goodness of fit for SEM. When the χ^2 is small and the *p*-value is larger than 0.05, the SEM can be accepted (Schermellehengel et al., 2003). The SEM analysis was performed using AMOS 20.0 (Amos Development Company, Crawfordville, Florida, USA).

3. Results

3.1. Air and soil temperature, soil moisture, and CO_2 flux

Soil temperature (5 cm depth) fluctuated with air temperature during the entire experimental period (Fig. 1a, b), and two soil FTCs were found based on soil temperature during the study period. Soil temperature at 5 cm depth varied between −5 and 1 °C during the two FTCs in both forests. A higher freezing temperature was found in the larch plots (−5 °C) than in the Chinese pine plots (−3 °C) ($p = 0.002$). There was a decreasing tendency in soil moisture in the larch plots (from 78.91 to 24.31%) in the entire study period. By contrast, soil moisture remained rather constant in the Chinese pine plots ranging between 15.11 and 29.74% (Fig. 1c). In addition, there was a significant difference in soil moisture between the two forest types with higher soil moisture in the larch than in the Chinese pine plots during the two freezing periods ($p < 0.001$).

During the thawing period of both FTCs, an increase in soil CO_2 flux was observed in both forests (Fig. 1d). The peaks of soil CO_2 fluxes were significantly higher by 2.25 times and 1.54 times during the first thawing period (March 7, 2017) than those during the freezing period (March 4, 2017) in the larch ($p = 0.005$) and the Chinese pine forests ($p = 0.01$), respectively; and were significantly higher by 3.95 times and 1.87 times during the second thawing period (March 18, 2017) than those in the previous freezing period (March 14, 2017) in the larch ($p < 0.001$) and the Chinese pine forests ($p = 0.02$), respectively. Additionally, soil CO_2 flux was higher at the second thawing peak than that at the first thawing peak, but the difference was not significant. The cumulative CO_2 fluxes during the freeze-thaw period, the rest wintertime, and the entire year were 3.71, 19.65, and 450.46 g C m^{-2} for the larch forest, respectively, and 5.69, 30.04, and 541.10 g C m^{-2} for the Chinese pine forest, respectively. The cumulative C emissions during the freeze-thaw period accounted for 18.89 and 18.94% of the cumulative winter C emissions in the larch and the Chinese pine forests, respectively, and accounted for 0.79 and 1.00% of the annual C emissions in the larch and the Chinese pine forests, respectively (Fig. 2).

3.2. Soil inorganic N and extractable organic C and N

Soil NH_4^+, EOC, and EON in both forests had similar trends with a maximum value in the first freezing period, a sharp slump in the first thawing period, and then a quick rise in the second freezing period, a fast drop in the following thawing period, whereas soil NO_3^- maintained rather stable (Fig. 3). Mean soil NH_4^+, EOC, and EON in the first thawing phase were all significantly lower than the mean values in the first freezing period ($p < 0.05$).

3.3. Soil microbial biomass C and N

Soil MBC and MBN in both types of forests had a consistent trend with a sudden increase in the first thawing period, and then a rapid decrease in the following period (Fig. 4). Soil MBC (663.46 and 666.70 mg kg^{-1} in the larch and the Chinese pine forests, respectively) and MBN (29.40 and 30.28 mg kg^{-1} in the larch forest and the Chinese pine forests, respectively) reached peak values in the first thawing period. There were significant differences in soil MBC and MBN between the first freezing period and the first thawing period ($p < 0.05$) in the Chinese pine forest, but not in the larch forest. Compared with the first thawing period, during the second freezing period soil MBC and MBN significantly decreased by 56.65 and 48.50% in the larch forest, respectively, and by 48.41 and 29.27% in the Chinese pine forest, respectively ($p < 0.05$).

3.4. Soil extracellular enzymatic activities

Soil cellobiohydrolase and xylosidase activities in two types of forests had a consistent trend with a rapid decline during the first thawing period and then remained at a low level (Fig. 5). The maximum values of four soil enzymatic activities in both forests appeared during the first freezing stage (2.24 and 4.55 nmol g^{-1} h^{-1} for α-glucosidase activity in the larch and the Chinese pine forests, respectively; 60.40 and 89.35 nmol g^{-1} h^{-1} for β-glucosidase activity in the larch and Chinese pine forests, respectively; 2.07 and 4.70 nmol g^{-1} h^{-1} for cellobiohydrolase activity in the larch and Chinese pine forests,

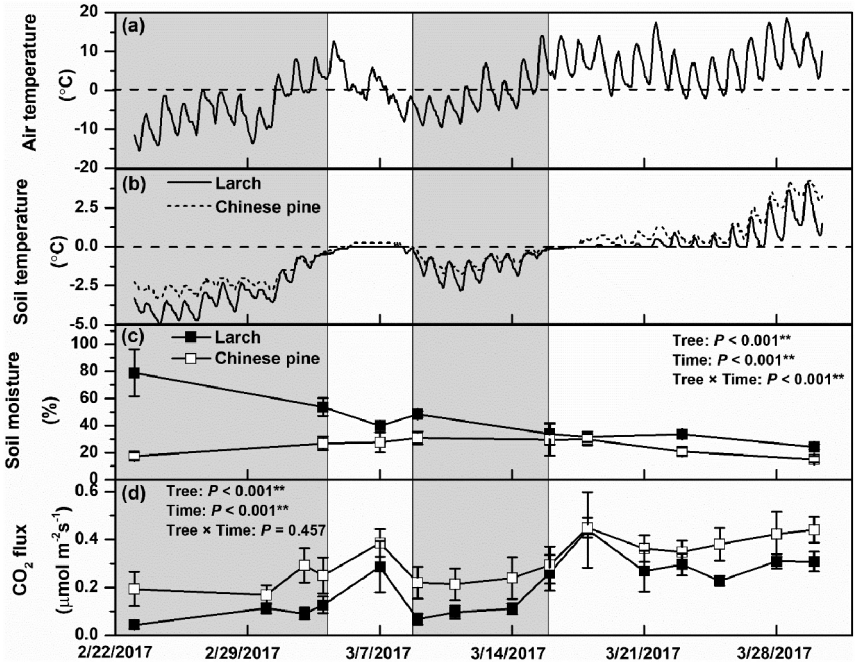

Fig. 1. Temporal dynamics of air temperature (a), soil temperature at 5 cm depth (b), soil gravimetric water content at 5 cm depth (c), and soil CO_2 flux (d) in the two types of plantation forests. Grey and white areas indicate the freezing and thawing period, respectively. The vertical bars stand for 95% confidence intervals ($n = 5$).

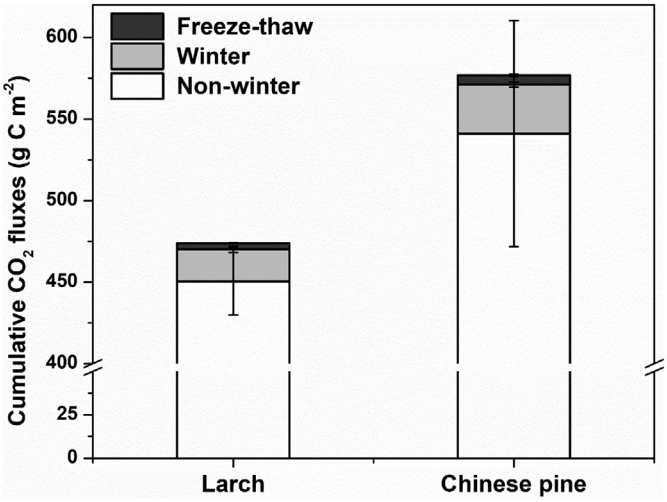

Fig. 2. Cumulative soil CO_2 fluxes during the freeze-thaw period, winter, and the rest time of the year (non-winter) in the studied larch and Chinese pine forests. The vertical bars stand for 95% confidence intervals (n = 5).

respectively; 6.11 and 6.07 nmol g^{-1} h^{-1} for xylosidase activity in the larch forest and Chinese pine forests, respectively).

3.5. Relationships between CO_2 flux and environmental variables

Pearson's correlation analysis indicated that soil CO_2 flux in both forests had significantly positive correlations with soil temperature ($p < 0.001$), MBC ($p = 0.02$), and MBN ($p = 0.04$), and significantly negative correlations with soil NH_4^+ ($p < 0.001$), EOC ($p = 0.03$), EON ($p = 0.003$), α-glucosidase ($p = 0.03$), β-glucosidase ($p = 0.01$), cellobiohydrolase ($p = 0.03$), and xylosidase ($p = 0.03$) during the first FTC. Soil CO_2 flux in both forests was only significantly positively correlated with soil temperature during the second FTC ($p = 0.01$) (Table 1). The locally weighted polynomial regression analysis also indicated that during the first FTC, the changing trends of MBC and MBN were consistent with that of soil CO_2 efflux, whereas the changing trends of EOC, EON, enzyme, and NH_4^+ were opposite to that of soil CO_2 efflux (Fig. 6). Soil CO_2 efflux was significantly correlated with soil temperature during the entire FTC ($p < 0.001$). Structural equation modeling further showed that soil CO_2 efflux was significantly positively correlated with MBC ($p < 0.001$) and significantly negatively correlated with NH_4^+ ($p = 0.005$) in the larch plots while soil CO_2 efflux was only significantly positively correlated with MBC ($p < 0.001$) in the Chinese pine plots (Fig. 7a, b). Interestingly, during the second freeze-thaw cycle, all of the above relationships disappeared and soil CO_2 efflux was only correlated with soil temperature ($p < 0.001$). Structural equation modeling also indicated that soil CO_2 flux in the two types of forests was only correlated with soil enzyme activity during the first thawing period ($p = 0.01$). The increasing thickness of litter layer significantly increased soil temperature ($p < 0.001$) and decreased soil moisture ($p < 0.001$) and soil temperature significantly enhanced soil enzyme activity ($p < 0.001$) and reduced EOC ($p = 0.049$) (Fig. 7c). Additionally, linear correlation analysis showed that the difference of CO_2 flux between the freezing and thawing periods was significantly negatively correlated with freezing temperature ($p < 0.001$), but significantly positively correlated with the differences of NH_4^+ ($p = 0.001$), EOC ($p < 0.001$), and EON ($p < 0.001$) between the freezing and thawing periods (Fig. 8).

4. Discussion

4.1. Response of soil respiration to in situ freeze-thaw cycles

In this study, the contributions of cumulative CO_2 fluxes during the freeze-thaw period to cumulative winter CO_2 fluxes and annual soil CO_2 fluxes were 18.89–18.94% and 0.79–1.00%, respectively, in two types of mid-temperate plantation forests (Fig. 2), which is similar with the results reported by previous field studies (Wang et al., 2013; Shi et al., 2014; Yan et al., 2016; Walz et al., 2017). Although soil respiration during the studied freeze-thaw period (22 days) accounts for a low proportion of annual C emissions in the mid-latitude regions, it cannot be ignored because of the increasing intensity and duration of FTC caused by global climate warming. Only two short-term FTC events were found in this *in-situ* study, and soil CO_2 fluxes continuously

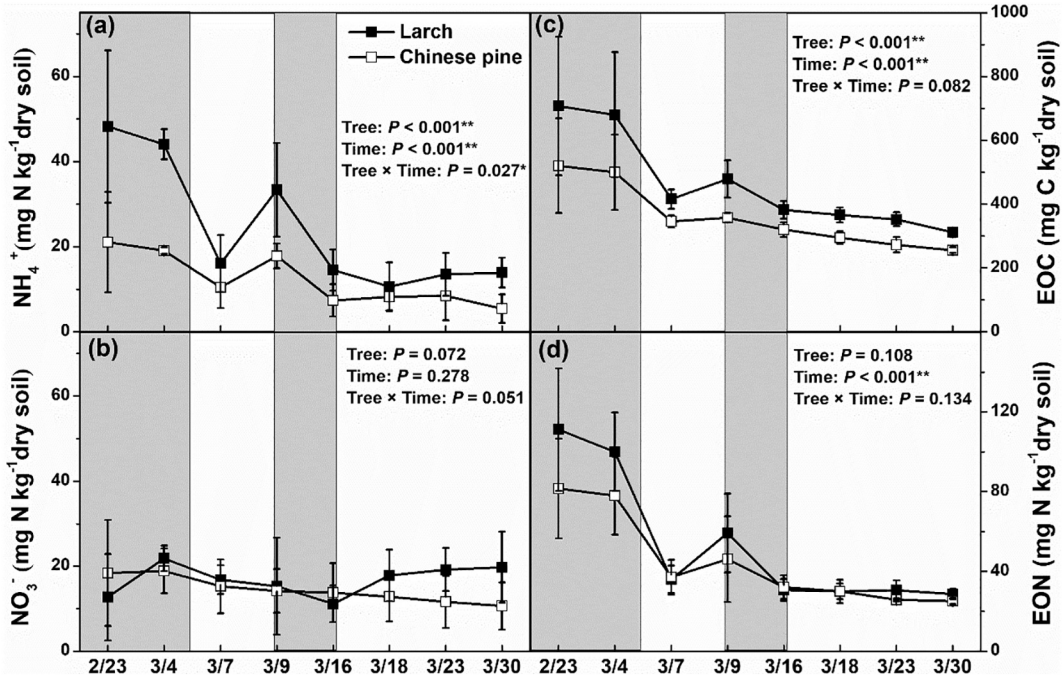

Fig. 3. Temporal dynamics of soil NH_4^+ (a), soil NO_3^- (b), extractable organic carbon (EOC) (c) and extractable organic nitrogen (EON) (d) in the two types of forests (black symbol: larch forest, white symbols: Chinese pine forest). Grey and white areas indicate the freezing and thawing period, respectively. The vertical bars stand for 95% confidence intervals (n = 5).

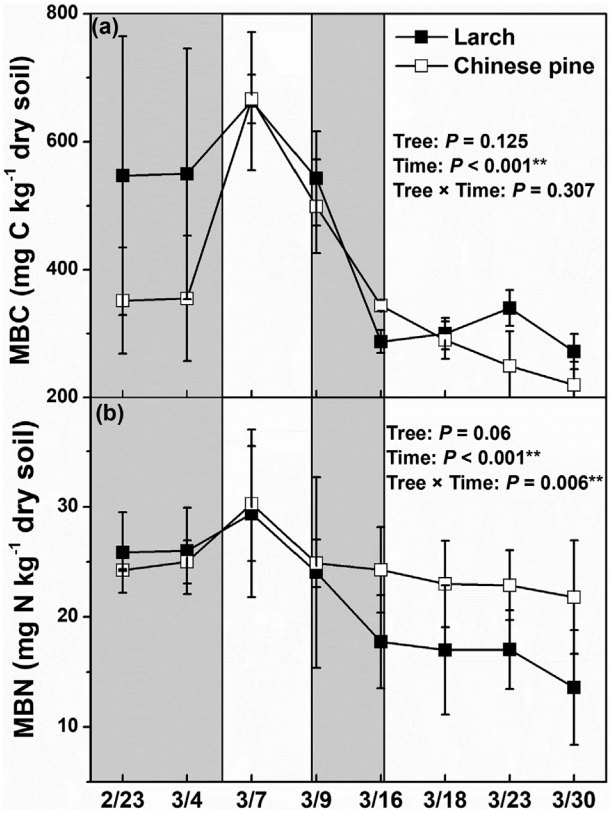

Fig. 4. Temporal dynamics of soil microbial biomass carbon (MBC) (a) and soil microbial biomass nitrogen (MBN) (b) in the two types of forests (black symbol: larch forest, white symbols: Chinese pine forest). Grey and white areas indicate the freezing and thawing period, respectively. The vertical bars stand for 95% confidence intervals (n = 5).

increased with increasing number of the FTC. Previous studies also found that the C-flush can still be observed with increasing number of FTCs under *in situ* conditions (Wei et al., 2016; Walz et al., 2017; Han et al., 2018). Conversely, some laboratory incubation experiments showed that soil respiration maintained at a relatively stable level and even decreased with the increasing number of FTCs (Elberling and Brandt, 2003; Kurganova and Tipe, 2003; Feng et al., 2007; Wang et al., 2014; Wei et al., 2016). Soil temperature in the thawing period increased with time under *in situ* conditions in this study but often remains constant in lab incubation studies, which could be the major reason why this study found increasing soil respiration with more cycles of freezing-thawing. Besides, the increment of CO_2 emissions induced by FTC was found to decline only when the number of FTCs exceeded a certain number (Wang et al., 2014), while there were only two cycles of freezing-thawing in this study. The limitation of the *in situ* approach adopted in this study is that it is cannot accurately control the duration,

Table 1
Pearson's correlation coefficients between CO_2 flux and soil temperature, soil moisture, NH_4^+, NO_3^-, extractable organic carbon (EOC), extractable organic nitrogen (EON), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), α-glucosidase, β-glucosidase, cellobiohydrolase, and xylosidase activities during the first and second freeze-thaw cycle (FTC) ($n = 10$). Numbers in bold indicate statistically significant results.

	CO_2 flux			
	Larch		Chinese pine	
	First freeze-thaw cycle	Second freeze-thaw cycle	First freeze-thaw cycle	Second freeze-thaw cycle
Soil temperature	**0.947****	**0.762***	**0.894****	**0.903****
Soil moisture	−0.564	−0.368	−0.253	−0.111
NH_4^+	**−0.891****	−0.081	**−0.645***	0.163
NO_3^-	−0.479	0.537	−0.436	−0.036
EOC	**−0.696***	−0.328	**−0.632***	−0.513
EON	**−0.793****	0.038	**−0.692***	−0.351
MBC	**0.739***	0.507	**0.883****	−0.632
MBN	**0.646***	−0.469	**0.815****	−0.288
α-glucosidase	**−0.725***	−0.448	**−0.791****	0.067
β-glucosidase	**−0.773***	−0.379	**−0.701***	0.199
cellobiohydrolase	**−0.721***	−0.135	**−0.721***	0.555
xylosidase	**−0.709***	−0.153	−0.622	0.301

** Highly significant ($p < 0.01$).
* significant ($p < 0.05$).

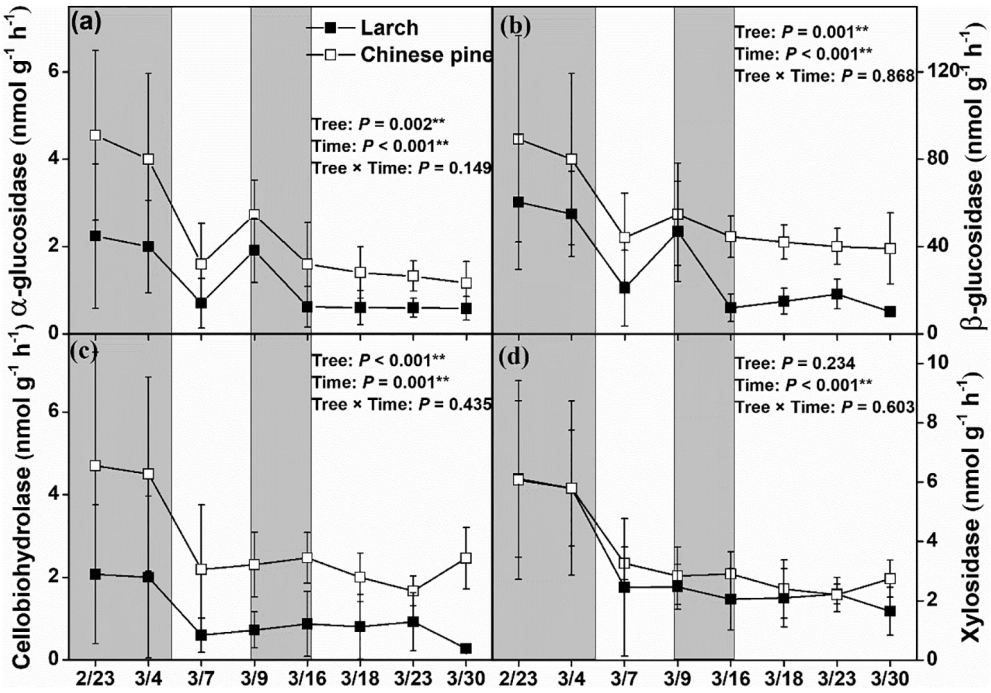

Fig. 5. Temporal dynamics of soil α-glucosidase (a), β-glucosidase (b), cellobiohydrolase (c), and xylosidase (d) activities in the two types of forests (black symbol: larch forest, white symbols: Chinese pine forest). Grey and white areas indicate the freezing and thawing period, respectively. The vertical bars stand for 95% confidence intervals (n = 5).

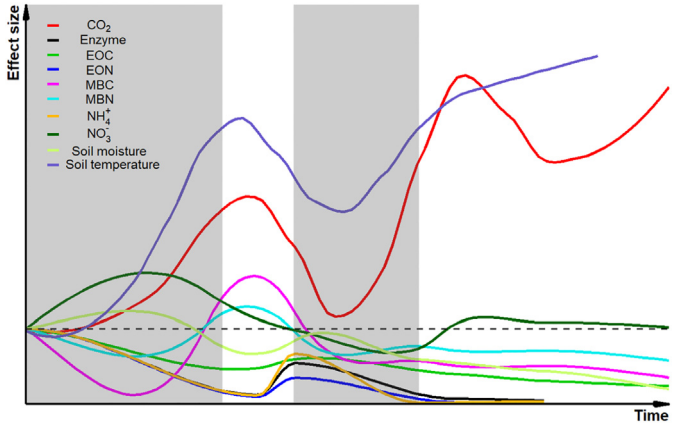

Fig. 6. Changes of the effect sizes of soil CO_2 fluxes, EOC (extractable organic carbon), EON (extractable organic nitrogen), MBC (microbial biomass carbon), MBN (microbial biomass nitrogen), NH_4^+, NO_3^-, and soil moisture with time during the freeze-thaw cycle (FTC). The effect size was calculated as the value of the samples determined at a given time point divided by the value at the initial time point (February 23, 2017). Solid lines represented best fit using locally weighted regression ("loess"). The dashed line indicates that the effect size is 1, below which the effect is negative and above which the effect is positive. Grey and white areas indicate the freezing and thawing period, respectively.

frequency, and intensity of FTC compared to laboratory research, thus making it difficult to explore the effect of higher frequency of FTC on soil C emission. However, in mid-latitude areas, the number of FTCs is generally not high and the mean temperature of each cycle generally increases with time, different from the unrealistic simulations in lab incubation studies. An alternative explanation for this discrepancy may be that the enhanced nutrient release from the litter layer and dead roots during the FTC under field conditions could sustain the supply of substrates for soil microorganisms while the labile substrates were quickly consumed in laboratory studies (Hirano, 2005; Walz et al., 2017).

This study found that the peak of CO_2 flux during the thawing period was significantly 1.54–3.95 times higher than that during the freezing period, while previous researchers reported higher differences between the thawing and freezing periods (Priemé and Christensen, 2001; Koponen et al., 2006; Wang et al., 2014; Gao et al., 2015; Kurganova and de Gerenyu, 2015; Walz et al., 2017; Wu et al., 2020). This was probably because of the lower range (−5–1 °C) and lower rate (2 °C d^{-1}) of temperature change in this study (Fig. 1b) while the range and rate were usually large (generally range from −20–−10 °C to 5–20 °C) and fast (general range from 10–30 °C d^{-1}) in laboratory studies (Henry, 2007; Matzner and Borken, 2008). When the range of temperature change is the same, soil microorganisms show higher sensitivity to the faster rates of freezing than the slower rates of freezing (Lipson et al., 2000). Additionally, this study found significant positive relationships between CO_2 flux and freezing intensity, NH_4^+, EOC, and EON during the two FTCs periods (Fig. 8). Soils exposed to high intensity and rapid rates of FTC can release more amounts of nutrients than soils exposed to low intensity and slow rates of FTC (Elliott and Henry, 2009; Urakawa et al., 2014; Pelster et al., 2019; Kreyling et al., 2020), resulting in a higher response of soil CO_2 flux to FTC. Thus, the FTC effects on soil CO_2 effluxes may be overestimated in laboratory studies and more *in situ* studies are needed to better predict the response of C cycling to the changing climate. Although this experiment was carried out under field conditions, the results obtained from this study are only based on the results of one ecosystem and one year. Therefore, different ecosystems and long-term FTC effects should be considered in future research.

4.2. Controlling factors of soil respiration responses to the freeze-thaw cycles

First, the response of soil respiration to the FTC was mainly controlled by soil temperature and MBC, both of which showed positive relationships with CO_2 (Table 1). The nutrients released from dead microbes, soil aggregate disruption, and root fracture during the freezing period were beneficial for microbial growth during the thawing period and therefore CO_2 emission. However, this effect was only present in the first FTC but disappeared during the second FTC (Table 1), indicating this mechanism is unsustainable. Overall, both enzymes and nutrients showed a declining pattern during the whole FTC period (Figs. 3, 4), suggesting that the rate of soil carbon emissions may decline with increasing duration of FTC. This further suggests that CO_2 emission may not increase linearly with the increasing number of FTCs under the context of global warming.

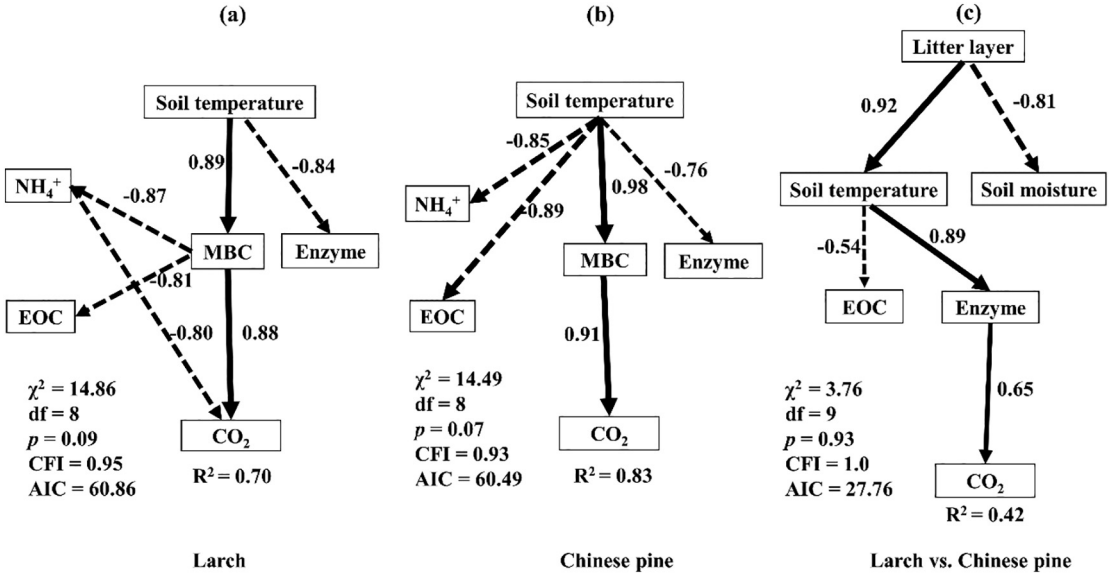

Fig. 7. Structural equation models (SEM) indicating the effects of soil temperature, NH_4^+, EOC (extractable organic carbon), MBC (microbial biomass carbon), enzyme on soil CO_2 flux in the larch (a) and the Chinese pine forests (b) during the first freeze-thaw cycle period and the effects of litter layer, soil temperature, soil moisture, EOC, and enzyme on soil CO_2 flux in the two types of forests during the first thawing period (c). The thickness of the arrows stands for the magnitude of the standardized path coefficients. The dashed and solid lines represent significantly negative and positive relationships, respectively. R^2 denotes the proportion of soil CO_2 explained by these drivers. χ^2, Chi-square; df, degrees of freedom; *p*, probability level; CFI, comparative fit index; AIC, akaike information criteria.

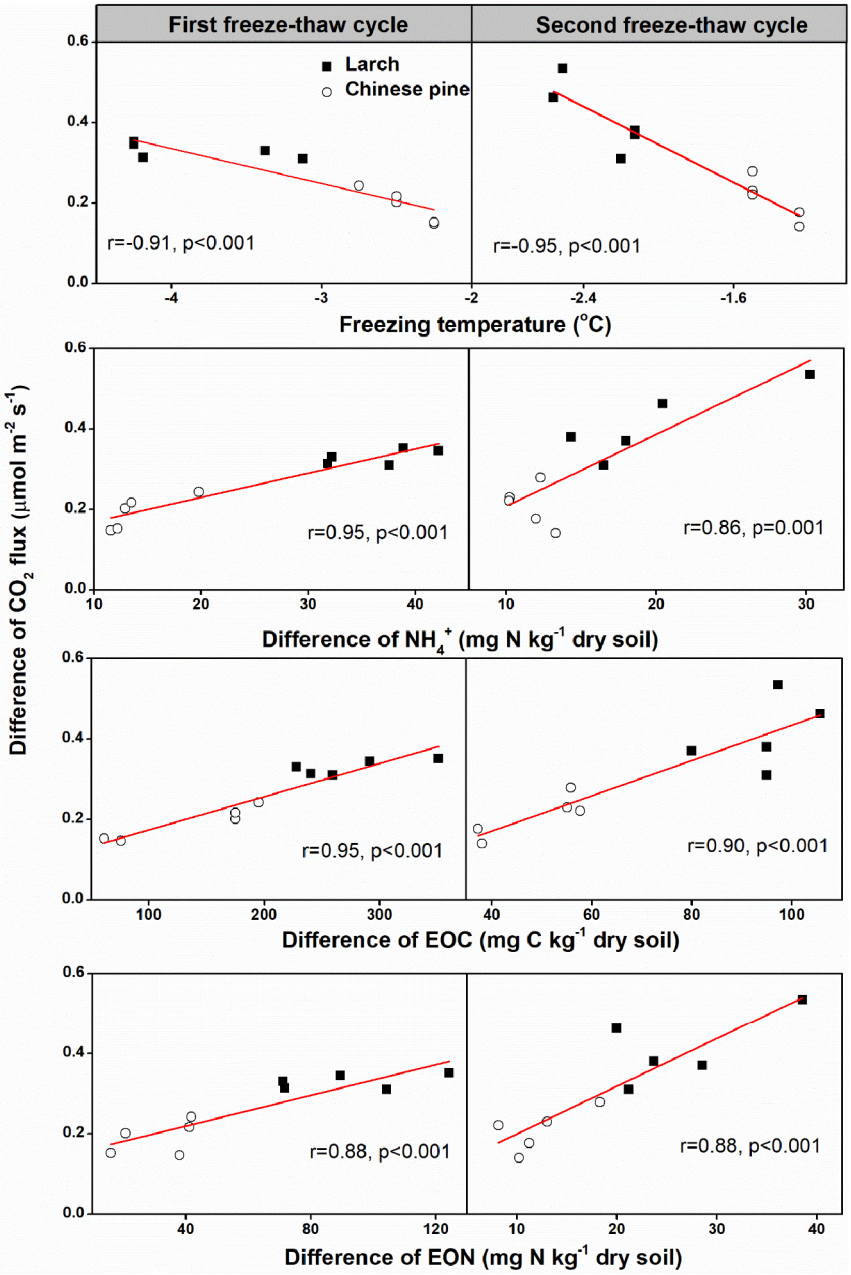

Fig. 8. Relationships between the difference of CO_2 flux between the freezing and thawing periods and freezing temperature, the differences of NH_4^+, EOC, and EON between the freezing and thawing periods in the two types of forests (black symbol: larch forest, white symbols: Chinese pine forest).

Second, the bursts of CO_2 fluxes during the thawing period were because that some microbes remained active during the thawing period. This study found higher enzymatic activities in the freezing period than in the thawing period (Fig. 5), suggesting the potential activity of soil microbes during freezing (Wallenstein et al., 2009; Nikrad et al., 2016; Gavazov et al., 2017). The low range and low rate of temperature change may not be able to kill soil microorganisms, resulting in a high capacity of soil microorganisms to produce enzymes during the freezing period. Also, there was probably a lag response of enzymes since the increase of MBC in the thawing period. The time of enzyme secretion by soil microorganisms may lag by 24 h or even more after microbial changes (Blagodatskaya and Kuzyakov, 2013). The higher enzymatic activities in the freezing period than in the thawing period could also be due to the release of endoenzyme during some cell disruption upon frozen, the same mechanism as NH_4^+ and DON responses (Fig. 3). This was why negative relationships between soil enzymes and soil microbial biomass or CO_2 fluxes were observed during the first FTC (Table 1), contrary to the common phenomena of positive relationships between enzymes and CO_2 emissions (Tao et al., 2018). Therefore, changes in enzymes and nutrients were dominated by the output processes (*i.e.* consumptions by microbes or inhibition by minerals, *etc.*) during the FTC, unlike during other periods, which should be paid attention to and further studied.

4.3. Different responses between the two types of forests

Under the same climatic conditions, different types of forests basically showed similar underlying mechanisms of the response of soil respiration to FTC. However, they experienced different intensity of the freeze-thaw effect and thereby different temporal dynamics of soil respiration (Fig. 1d), mainly due to the insulation effects of the litter layer because the larch forest had a thinner litter layer (averaged 2.1 cm) than

the Chinese pine forest (averaged 4.1 cm). Although the variances in soil nutrients and enzymatic activities between the two types of forests may cause the change in soil respiration, these results showed that soil respiration had a significant positive correlation with soil temperature during the two freeze-thaw cycles and had no significant relationships with soil nutrients and enzymatic activities during the second freeze-thaw cycle (Table 1; Fig. 7), suggesting that soil respiration during the freeze-thaw cycle is mainly controlled by soil temperature. This result was also supported by previous research which showed that there was a close correlation between soil respiration and soil temperature in the freeze-thaw system (Ouyang et al., 2015). Because the litter layer, which plays an insulation effect, can delay or reduce soil freezing at cold winter temperatures. Increased soil temperature due to increasing thickness of the litter layer during the FTC period can stimulate soil enzyme activity, thereby enhancing the flux of soil CO_2 (Fig. 7c). Therefore, during the freeze-thaw cycle, we speculate that the difference in litter thickness may be the main reason for the difference in soil respiration between the two types of forests. Furthermore, almost all examined parameters fluctuated more in the larch forest than in the Chinese pine forest (Fig. 8), resulting in higher changes in soil respiration in the larch forest than in the Chinese pine forest too. This is consistent with previous studies which found higher intensity of freezing increased the effect of FTC on CO_2 fluxes (Goldberg et al., 2008) and our above statement that higher intensity and frequency of the FTC treatments in laboratory studies could overestimate the effects of FTC in the field. Thus, forest types and thickness of the litter layer should be considered in process-based models to better simulate the response of soil carbon emissions to changing regimes of the FTC.

5. Conclusion

This study suggested that frequency plays an important role in the effects of FTC on soil respiration and with higher FTC intensity and frequency under climate change in the future, soil respiration is expected to increase, causing a positive feedback to climate change. The higher rate of soil respiration during the thawing phase than during the freezing phase was dominantly driven by biological processes because higher microbial biomass in the thawing period than in the freezing period was observed. This effect became weak during the second FTC because of the consumption of soil nutrients, resulting in a non-linear increase in CO_2 emissions with the increased number of FTCs. Due to the important control of temperature on CO_2 emission, the different responses of soil CO_2 flux to FTC between the two types of forests were mainly caused by the difference in the thickness of litter layer. Future experiments on FTC effects should focus more on realistic field studies to explore the underlying biotic mechanisms due to the maintenance of activity of soil microbes during mild freezing.

CRediT authorship contribution statement

All authors contributed intellectual input and assistance to this study and manuscript preparation. Decai Gao and Edith Bai developed the original idea. Decai Gao and Edith Bai conducted data analysis. Decai Gao and Edith Bai interpreted results and wrote the paper. Decai Gao, Edith Bai, and Ziping Liu revised the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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