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Effect of carbon and nitrogen addition on nitrous oxide and carbon dioxide fluxes from thawing forest soils

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A b s t r a c t. Packed soil-core incubation experiments were done to study the effects of carbon (glucose, 6.4 g C m^{-2}) and nitrogen (NH_4Cl and KNO_3, 4.5 g N m^{-2}) addition on nitrous oxide (N_2O) and carbon dioxide (CO_2) fluxes during thawing of frozen soils under two forest stands (broadleaf and Korean pine mixed forest and white birch forest) with two moisture levels (55 and 80% water-filled pore space). With increasing soil moisture, the magnitude and longevity of the flush N_2O flux from forest soils was enhanced during the early period of thawing, which was accompanied by great NO_3^--N consumption. Without N addition, the glucose-induced cumulative CO_2 fluxes ranged from 9.61 to 13.49 g CO_2-C m^{-2}, which was larger than the dose of carbon added as glucose. The single addition of glucose increased microbial biomass carbon but slightly affected soil dissolved organic carbon pool. Thus, the extra carbon released upon addition of glucose can result from the decomposition of soil native organic carbon. The glucose-induced N_2O and CO_2 fluxes were both significantly correlated to the glucose-induced total N and dissolved organic carbon pools and influenced singly and interactively by soil moisture and KNO_3 addition. The interactive effects of glucose and nitrogen inputs on N_2O and CO_2 fluxes from forest soils after frost depended on N sources, soil moisture, and vegetation types.

K e y w o r d s: carbon dioxide, glucose, interactive effect, nitrous oxide, N sources, frozen soils

INTRODUCTION

In a high latitude and/or high altitude zone, long-term freezing during winter can cause death of microorganisms, breakdown of aggregates, and degradation of dead fine roots, which can induce an increase in nutrient availability in soil (Christensen and Christensen, 1991; Tierney *et al.*, 2001; Xu *et al.*, 2016). These released substrates can be utilized during nitrification, denitrification, and microbial respiration processes and thus may promote nitrous oxide (N_2O) and carbon dioxide (CO_2) fluxes from soils during spring thaw (Mørkved *et al.*, 2006; Goldberg *et al.*, 2008; Wu *et al.*, 2010). However, no pulse of CO_2 fluxes occurred during autumn and spring freeze-thaw periods in field experiments (Rong *et al.*, 2015) and the stimulus degree of freezing-thawing events on soil N_2O and CO_2 fluxes varies greatly among recent studies (Kim *et al.*, 2012). This can be contributed to the variations of freezing temperature, freezing-thawing cycles, freezing duration, soil moisture, the availability of carbon (C) and nitrogen (N) substrates and the properties of related microorganisms (Kim *et al.*, 2012; Tang *et al.*, 2016; Xu *et al.*, 2016).

During late autumn, due to the input of fresh litters, almost half of dissolved organic carbon (DOC) in water extracts of organic layers under temperate forest stands exists in the form of glucose-C (Wu *et al.*, 2016), which can be flushed into mineral soils during rainfall. The external active C input can normally give rise to strong short-term changes in the decomposition intensity of soil native organic C by enhancing microbial activities (Kuzyakov *et al.*, 2000), especially during spring thaw. During a freezing-thawing period, glucose addition in

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the field could increase CO_2 flux by 52 to 160% within 24 h (Brooks *et al.*, 2004) and an incubation experiment conducted by Sehy *et al.* (2004) showed that the availability of C substrates could play an important role in the soil N_2O flux. Besides the factor of the DOC input, soil moisture has a great spatial and temporal variability during spring thaw and increasing atmospheric N deposition imports different types of mineral N (NH_4^+-N and NO_3^--N) into the soil. More importantly, a laboratory experiment conducted by Xu *et al.* (2016) confirmed that N_2O and CO_2 fluxes from forest soils during thaw were correlated to the soil water content and the concentrations of soil inorganic nitrogen and dissolved organic matter. In addition, the addition of nitrate-N greatly promoted N_2O fluxes from soils with high water content during a freezing-thawing period and this promotion was stimulated by the addition of organic C (Cui *et al.*, 2016; Sehy *et al.*, 2004). Increased microbial respiration after the addition of organic C rapidly depleted O_2 supply, and the generated anoxic conditions promoted the soil N_2O flux especially under high soil moisture condition and the supply of nitrate-N (Nosalewicz *et al.*, 2013). Unfortunately, how the DOC input from forest organic layers and N deposition with different types interactively affected the N_2O and CO_2 fluxes from forest soils under different moisture conditions during thaw has been still not clear so far.

In Changbai Mountains area, northeastern China, continuous surface soil freezing normally lasted for two or three months each year and the following spring thaw period continued for about two weeks. The minimum air temperature during winter was low to nearly -30°C, which caused 1.0-1.5 m depth of frozen soil (Xu *et al.*, 2016). This severe winter freezing may affect the dynamics of soil nutrients and microbial activities at initial spring thaw. In this district, broadleaf and Korean pine mixed forest (BKPF) is the major component of forest ecosystems and lies in the climax community of forest succession (Supplementary Fig. S1). Surface soils under the BKPF stand possess greater soil organic matter content and lower bulk density than an adjacent secondary white birch forest (WBF) (Xu *et al.*, 2016). Due to the relatively lower vegetation coverage and phototaxis property, soil available nutrients, microbial properties, and hydrothermal conditions under the white birch forest stand are different from those under the mature mixed forest. The differences in soil properties under the two forest stands may influence the responses of soil N_2O and CO_2 fluxes to the addition of glucose and nitrogen as NH_4Cl or KNO_3. Furthermore, whether the increase in the DOC input from autumn freshly fallen leaves as glucose and in combination with increased N deposition can affect the N_2O and CO_2 fluxes from forest soils with different moisture levels during spring thawing periods has been unknown so far (Kim *et al.*, 2012). We hypothesized that the interactive effect of glucose and N addition on N_2O and CO_2 fluxes from thawing soils can be affected by N types and soil moisture. For this purpose, a series of laboratory incubation experiments were done to study (1) the single and interactive effects of soil moisture, C and N addition on N_2O and CO_2 fluxes from WBF and KBPF soils during thaw; (2) the main driving mechanisms of N_2O and CO_2 fluxes during soil thawing periods by considering the variations of soil properties such as labile C and N pools. The results improved our understanding of how soil moisture, DOC input from forest organic layers, and N deposition interactively affected soil N_2O and CO_2 fluxes under forest stands during the non-growing season.

MATERIALS AND METHODS

The study area is located near the National Research Station of Changbai Mountain Forestry Ecosystem (42°24′ N, 128°6′ E) in Jilin Province, northeastern China (Supplementary Fig. S1), characterized by a typical continental temperate climate. The average elevation of the area is 738 m with a flat topography. Based on regular meteorological measurements of the station during the period from 2001 to 2012, continuous surface soil freezing lasted for 52 to 89 d each year when air temperature persisted below 0°C and the average air temperature each year during the continuous surface soil freezing period ranged from -12.6 to -17.7°C. Snow depth in winter is normally within the range from 5 cm to 35 cm. The soil profile in winter can be frozen down to 1.0-1.5 m depth (Xu *et al.*, 2016). The dark brown forest soil originates from volcanic ash. A detailed description of the soil profile properties was reported by Xu *et al.* (2007). Mineral soil and organic layer samples were collected under the mature broadleaf and Korean pine mixed forest stand (BKPF) and the adjacent white birch forest stand (WBF), respectively. The former has a multi-layer structure with canopy density of 0.8 and the average age of dominant trees is about 200 years old; as a secondary forest, the latter has a more simple structure with canopy density of 0.6 and the average age of dominant trees is about 70 years. Due to the relatively lower vegetation coverage and phototaxis property, soil moisture under the white birch forest stand is smaller than that under the mature mixed forest over the year, and the former is characterized by relatively higher frequency of freezing and thawing cycles during the non-growth season.

To collect composite forest soil and organic layer samples, eighteen 1 × 1 m plots were selected in each forest stand in October 2012. Mineral soil samples (0-10 cm) in each plot were collected using an 8-cm diameter auger after removing the ground surface mulch, and organic layer samples including fresh and semi-decomposed litter were collected. All samples were kept separately in air-tight plastic bags and rapidly transported to the laboratory within 24 h. The soil samples from each forest stand were mixed

thoroughly, sieved (< 2 mm) to remove small stones and debris, and then stored in the dark at 4°C prior to incubation and analysis of soil properties. After drying at 60°C for 24 h, forest organic layer samples were finely milled to pass through a 0.5-mm screen mesh prior to chemical analysis.

Triplicate soils were dried at 105°C for 24 h to determine moisture content. Fresh soil pH (soil/water, 1/2.5, w/w) and pH values in water extracts of forest organic layers (sample/water, 1/10, w/w) were respectively measured with a portable pH meter (PB-10, Sartorius, Germany). Total C and N concentrations in soil samples were measured using an elemental analyzer (vario Macro cube, Elementar, Germany). Fresh forest soils (5.0 g) were extracted by shaking with 25 ml of deionized water for 30 min and dried organic layer samples (5.0 g) by shaking with 50 ml of deionized water for 24 h on an end-over-end shaker. The suspensions were centrifuged at 4 500 ***g*** for 5 min and then filtered into 50 ml plastic bottles *via* cellulose-acetate membrane filters (0.45 μm pore size). Concentrations of NH_4^+-N, NO_3^--N, total N (TN), and DOC in the soil water extracts and DOC in the organic layer water extracts were measured using a continuous flow analyzer (SAN^{++}, SKALAR, the Netherlands). Concentrations of soil dissolved organic N (DON) were calculated according to the differences between TN and mineral N (NH_4^+-N and NO_3^--N) concentrations in soil water extracts. Concentrations of soil microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation and extraction method, and were calculated by the differences of K_2SO_4-extractable DOC and TN pools between fumigated and non-fumigated soils divided by 0.45 (Jenkinson, 1988; Jenkinson *et al.*, 2004; Wu *et al.*, 1990), assuming that fumigation causes a release of microbial N in the same proportion as for microbial C. Both TN and DOC concentrations in the soil K_2SO_4 extracts were measured using a continuous flow analyzer as above. The glucose concentrations in water extracts of forest organic layers were determined by the anthrone-sulfuric acid colorimetric method (Deng *et al.*, 1994). The properties of mineral soils and water extracts of forest organic layers sampled under the two temperate forests were shown in Supplementary Table S1.

Packed soil cores were made according to the bulk densities of BKPF and WBF soils in the field (Supplementary Table S1). A factorial design with two forest types (BKPF and WBF), two soil moisture (55 and 80% water-filled pore space, WFPS), and the addition of nutrients (glucose, namely Glu 6.4 g C m^{-2}, NH_4Cl, 4.5 g N m^{-2}, KNO_3, 4.5 g N m^{-2}, Glu+NH_4Cl, and Glu+KNO_3) was established for the incubation experiment; no nutrient addition was considered as a control. The amounts of added N and C were approximately four-fold local annual wet N input and twice glucose concentration in water extracts of forest organic layers (Wu *et al.*, 2016) under the two study forest stands, respectively. All treatments were replicated three times, giving a total of 72 packed soil cores.

Homogenized fresh soils (85 g) were transferred into 100 ml stainless steel cylinders (diameter in 50.5 mm) as a soil core. For each core, appropriate nutrients were precisely sprayed with deionized water as solutions onto the homogenized soil before packing to reach the designed soil moisture; this operation was accomplished within 1 h. Then the 72 soil cores were frozen at -18°C for 50 days, which simulated a severe winter frost from late December to next February near the study area. After freezing, all the soil cores were immediately placed separately inside PVC cylinders and immediately incubated at 10°C in an incubator (LRH250, Yiheng Instruments, China) for 15 days to simulate the spring thaw process of soil. Three PVC cylinders without soil served as blank. The temperature of soil thaw was designed in accordance with maximum air temperature of the study area in late spring when soil freezing-thawing cycles intensively occur in the field. Deionized water was duly added for each soil core by weighting during the 15 day incubation to avoid evaporation. Gas sampling was performed from each soil core and the blank at 6, 12, 24, 37, 49, 75, 95, 119, 143, 167, 191, 215, 239, 263, 287, 311, 335, and 359 h after the incubation initiated, according to preliminary studies showing a linear increase in headspace N_2O and CO_2 concentrations within 24 h after closure. Headspace gas samples of 30 ml were collected using 50 ml polypropylene syringes equipped with 3-way stopcock. Each time when gas sampling finished, all PVC cylinders were immediately taken outdoor to be well ventilated for 20 min and then sealed to continue the incubation till the next sampling time. The concentrations of N_2O and CO_2 in headspace gas samples were quantified with a gas chromatograph (Agilent 7890A, Franklin, USA) equipped with an electron-capture detector and a flame ionization detector, respectively (Xu *et al.*, 2016). The detector responses were calibrated using a certified gas standard, which contains 2.02 ml l^{-1} CO_2 and 1.02 μl l^{-1} N_2O in air. The main properties of soil cores, including moisture, bulk density, pH, MBC, MBN, and concentrations of water-extractable NH_4^+-N, NO_3^--N, TN and DOC, were measured immediately when the last gas sampling finished (359 h), as mentioned above. Soil WFPS inside each core was calculated by soil bulk density and moisture (Franzluebbers, 1999).

Instant rates of N_2O and CO_2 fluxes from soils were calculated from the differences of headspace N_2O and CO_2 concentrations between each treatment and the blank divided by the sealing time before each gas sampling, and were expressed in μg N_2O-N m^{-2} h^{-1} and mg CO_2-C m^{-2} h^{-1}, respectively. The cumulative fluxes of N_2O and CO_2 during the 15 day incubation were calculated as the sum of N_2O and CO_2 fluxes for each sampling and were expressed in mg N_2O-N m^{-2} and g CO_2-C m^{-2}, respectively. The maximum rates of N_2O and CO_2 fluxes were determined by the maximum value of the instant rates during the 15 day incubation for each treatment, which were reasonable for the

relatively high gas sampling frequency. The Box-Lucas exponent model was used to simulate the relationships of cumulative fluxes of N_2O and CO_2 from each treatment against the incubation time according to the following equation:

$$Y = S_0 (1\text{-EXP}(-kt)),$$

where: Y is the cumulative N_2O flux (mg N_2O-N m^{-2}) or cumulative CO_2 flux (g CO_2-C m^{-2}); k is the rate constant of the model; t is the incubation time (h); S_0 is the upper-bound of the model; S_0 x k is the linearized ($R^2 > 0.95$) growth rate of the model during the early period, which represents the incipient rate of N_2O or CO_2 flux from each treatment and is expressed in mg N_2O-N m^{-2} h^{-1} or mg CO_2-C m^{-2} h^{-1}. The microbial metabolic quotient (qCO_2) values in all treatments during the 15 day incubation were calculated as the cumulative CO_2 flux divided by the soil MBC and the days of incubation, and were expressed in mg CO_2-C g^{-1} biomass C day^{-1}.

Glucose-induced stimulus effects were calculated by the differences of soil labile C and N pools and cumulative fluxes of N_2O and CO_2 in the presence and absence of glucose. Means and standard errors for three replicates were calculated. All measured variables were examined for normality (Shapiro-Wilk test) and homogeneity (Levene test) of variance and transformed where necessary. A three-factor analysis of variance (ANOVA) was used to assess the effect of soil moisture and addition of C and N on the incipient rates and maximum rates of N_2O and CO_2 fluxes, cumulative N_2O and CO_2 fluxes, qCO_2, and labile C and N pools of WBF and BKPF soils. Another three-factor ANOVA was used to study the influence of vegetation type, soil moisture, and N addition on the glucose-induced labile soil C and N pools and cumulative N_2O and CO_2 fluxes. Tukey honestly significant differences (HSD) were calculated at the p=5% level to assess the differences among treatments in instant rates of N_2O and CO_2 fluxes and cumulative N_2O and CO_2 fluxes at each sampling during the 15-day incubation. Stepwise regression analysis was performed to assess main soil properties, which can affect the cumulative fluxes of N_2O and CO_2 during the 15 day incubation. Significant effects were determined at the $p<0.05$ level using student *T*-test. All statistical analyses were conducted with the software SPSS for Windows (version 19.0, IBM Corp., USA).

RESULTS

The maximum rates of N_2O fluxes from WBF and BKPF soils with low moisture in all treatments were observed at the onset of thaw (Fig. 1a,b). Under high soil moisture condition, the N_2O flux peak was delayed by 24 h (Fig. 1c,d) and was significantly larger than that under low soil moisture condition ($p<0.001$) (Supplementary Table S2). At the end of the incubation, high soil moisture significantly increased cumulative N_2O fluxes from WBF and BKPF soils, respectively, by 7-462 and 18-91 times

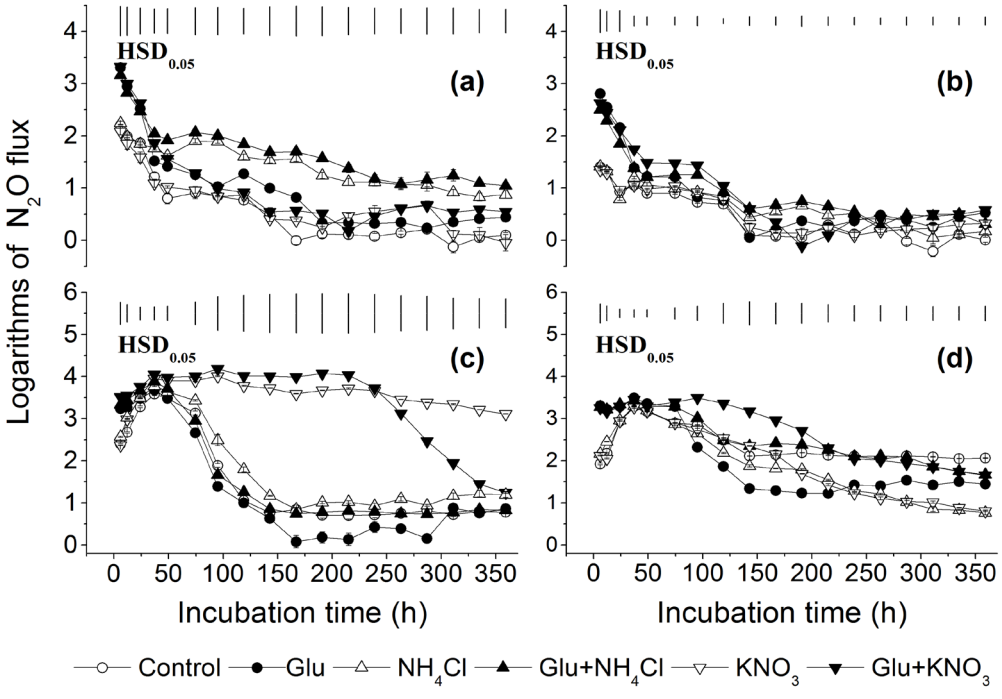

Fig. 1. Changes in the logarithms of N_2O flux during thawing of frozen WBF (a and c) and BKPF (b and d) soils treated with C and N under two soil moisture levels. (a) and (b) present low soil moisture; (c) and (d) present high soil moisture. Vertical bars show the Tukey honestly significant difference at the $p<0.05$ level ($HSD_{0.05}$) among all treatments at each sampling date. Error bars reflect the standard error of logarithms of N_2O flux between replicates (n = 3 for all treatments).

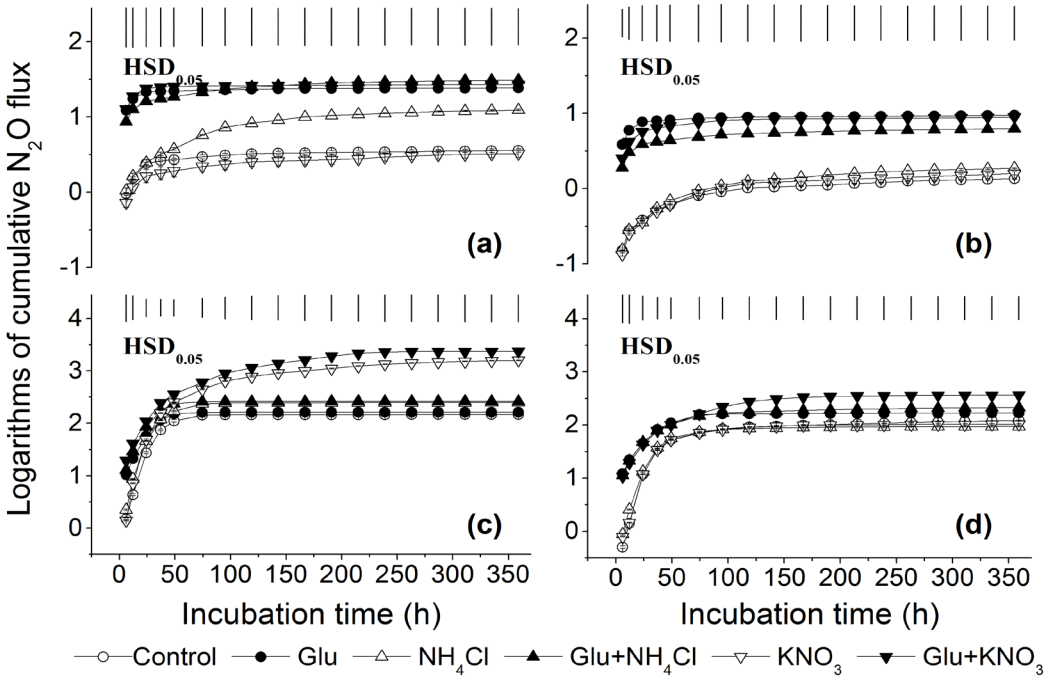

Fig. 2. Changes in the logarithms of cumulative N_2O flux during thawing of frozen WBF (a and c) and BKPF (b and d) soils treated with C and N under two soil moisture levels. (a) and (b) present low soil moisture; (c) and (d) present high soil moisture. Vertical bars show the Tukey honestly significant difference at the $p<0.05$ level ($HSD_{0.05}$) among all treatments at each sampling date. Error bars reflect the standard error of logarithms of cumulative N_2O flux between replicates ($n=3$ for all treatments).

with and without the addition of C and N, compared to those under low soil moisture condition ($p<0.001$) (Fig. 2 and Supplementary Table S2). Under low soil moisture condition, the addition of NH_4Cl alone significantly increased cumulative N_2O fluxes from WBF and BKPF soils ($p<0.05$), especially from WBF soil, but the addition of KNO_3 alone had no significant effect (Supplementary Table S2). Under high soil moisture condition, the addition of NH_4Cl and KNO_3 alone both significantly increased the cumulative N_2O flux from WBF soil ($p<0.05$), but the addition of N alone had no effect on the cumulative N_2O flux from BKPF soil (Supplementary Table S2). The increase in soil moisture greatly enhanced the stimulating effect of KNO_3 addition alone on the incipient rate of N_2O flux and cumulative N_2O flux from WBF soil ($p<0.001$), which was relatively larger than that from BKPF soil (Fig. 2 and Supplementary Table S2). The addition of glucose alone significantly increased the instant rate of N_2O flux from the two forest soils within 2 days after the incubation started ($p<0.05$) (Fig. 1). Under high moisture condition, the addition of glucose could significantly enhance the stimulating effect of KNO_3 addition on the incipient rate of N_2O flux and cumulative N_2O flux from the two forest soils, especially from WBF soil ($p<0.01$) (Fig. 2 and Supplementary Table S2). However, no interaction effect of glucose and NH_4Cl addition on the soil N_2O flux was observed under two soil moisture conditions (Supplementary Table S2). It is necessary to point out that the cumulative N_2O flux from WBF soil with high moisture in the Glu+KNO_3 treatment (2.34 g N_2O-N m^{-2}) accounted for 52% of the added N (4.5 g N m^{-2} as KNO_3). There were the interactive effects of glucose addition and soil moisture on the cumulative N_2O flux from the two forest soils, and the addition of glucose and soil moisture interactively affected the incipient rate of N_2O flux from WBF soil (Supplementary Table S2). The addition of N sources and glucose as well as soil moisture interactively affected the incipient rate of N_2O flux from WBF soil and cumulative N_2O flux from BKPF soil ($p<0.05$) (Supplementary Table S2).

In the absence of glucose, an increase in soil moisture could delay the appearance of CO_2 peak fluxes from the two forest soils (Fig. 3) and significantly decreased their maximum flux rates ($p<0.001$) (Supplementary Table S2). With increasing soil moisture, the cumulative CO_2 flux and qCO_2 decreased for the WBF soil in the control but both of them increased for the BKPF soil ($p<0.05$) (Supplementary Table S2). The addition of NH_4Cl and KNO_3 alone significantly decreased the incipient rate of CO_2 flux and cumulative CO_2 flux from WBF soil with low moisture ($p<0.05$) (Supplementary Table S2). The addition of KNO_3 alone significantly increased the cumulative CO_2 flux from BKPF soil with low moisture but decreased it under high soil moisture condition ($p<0.05$) (Supplementary Table S2). The addition of glucose substantially enhanced the CO_2

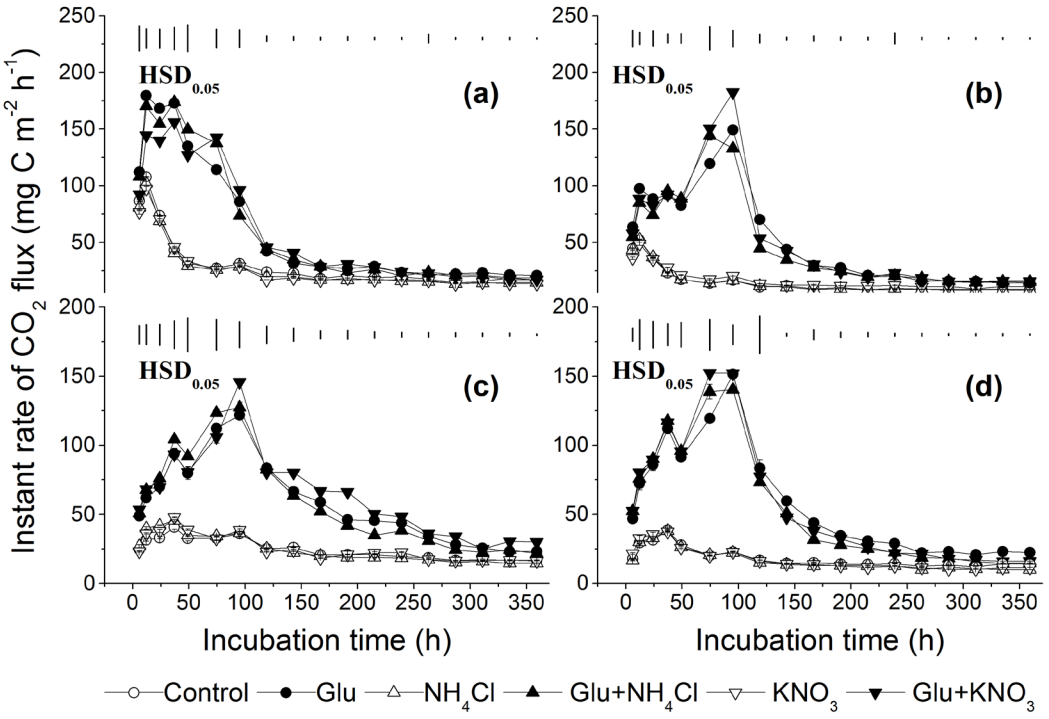

Fig. 3. Changes in the instant rate of CO_2 flux during thawing of frozen WBF (a and c) and BKPF (b and d) soils treated with C and N under two soil moisture levels. (a) and (b) present low soil moisture; (c) and (d) present high soil moisture. Vertical bars show the Tukey's honestly significant difference at the $p<0.05$ level ($HSD_{0.05}$) among all treatments at each sampling date. Error bars reflect the standard error of the instant rate of CO_2 flux between replicates (n = 3 for all treatments).

pulse fluxes from WBF and BKPF soils during thaw (Fig. 3) and their cumulative CO_2 fluxes were increased by 2.1-2.6 and 3.2-3.6 times, respectively, at the end of the incubation (Fig. 4 and Supplementary Table S2). According to the results of repeated measures ANOVA, it can be concluded that the addition of KNO_3 and glucose interactively affected cumulative CO_2 fluxes from the two forest soils and that the addition of N sources and glucose as well as soil moisture interactively affected the cumulative CO_2 flux from BKPF soil ($p<0.05$) (Supplementary Table S2).

The labile C and N concentrations of WBF and BKPF soils at the end of the incubation are shown in Supplementary Table S3. With increasing soil moisture, NO_3^--N concentrations of the two forest soils in the control significantly decreased ($p<0.001$). With increasing soil moisture, the concentrations of DOC and MBC and MBN of WBF forest soil in the control significantly increased, whereas MBC and MBN concentrations of BKPF soil in the control significantly decreased ($p<0.05$). The MBC:MBN ratios of WBF and BKPF soils in the control significantly decreased with increasing soil moisture ($p<0.05$). The addition of glucose alone significantly decreased the concentrations of NO_3^--N and NH_4^+-N and increased the MBC concentration in the two forest soils with two moisture levels ($p<0.05$). The addition of KNO_3 alone significantly increased NH_4^+-N concentrations in the two forest soils with two moisture levels ($p<0.001$). The addition of N sources and soil moisture interactively affected the DON concentration in the two forest soils ($p<0.05$). The addition of KNO_3 and glucose addition could significantly affect the MBC:MBN ratios singularly and interactively ($p<0.05$).

Without N addition, the decrease in labile TN induced by glucose addition in BKPF soil was significantly larger than that in WBF soil ($p<0.001$) (Supplementary Table S4). Glucose-induced cumulative CO_2 fluxes from the two forest soils were significantly larger than the dose of the C added (6.4 g glucose-C m^{-2}) ($p<0.001$) (Supplementary Table S4). Without N addition, the glucose-induced cumulative CO_2 fluxes from WBF and BKPF soils increased with increasing soil moisture, with a relatively larger flux from BKPF soil ($p<0.001$) (Supplementary Table S4). The addition of KNO_3 significantly increased the glucose-induced MBC and cumulative CO_2 flux from WBF soil with two moisture levels ($p<0.05$) (Supplementary Table S4). Under high moisture condition, KNO_3 addition significantly increased the glucose-induced cumulative N_2O flux from the two forests soils, especially from WBF soil ($p<0.001$) (Supplementary Table S4). According to the results of repeated measures ANOVA, it could be concluded that vegetation type and soil moisture interactively affected the glucose-induced cumulative N_2O flux from forest soils ($p<0.05$) and that the addition of N sources and vegetation types interactively affected the glucose-induced soil labile TN and DOC pools as well as the glucose-induced soil cumulative CO_2 flux (Supplementary Table S4). The glucose-induced cumulative N_2O and CO_2 fluxes from

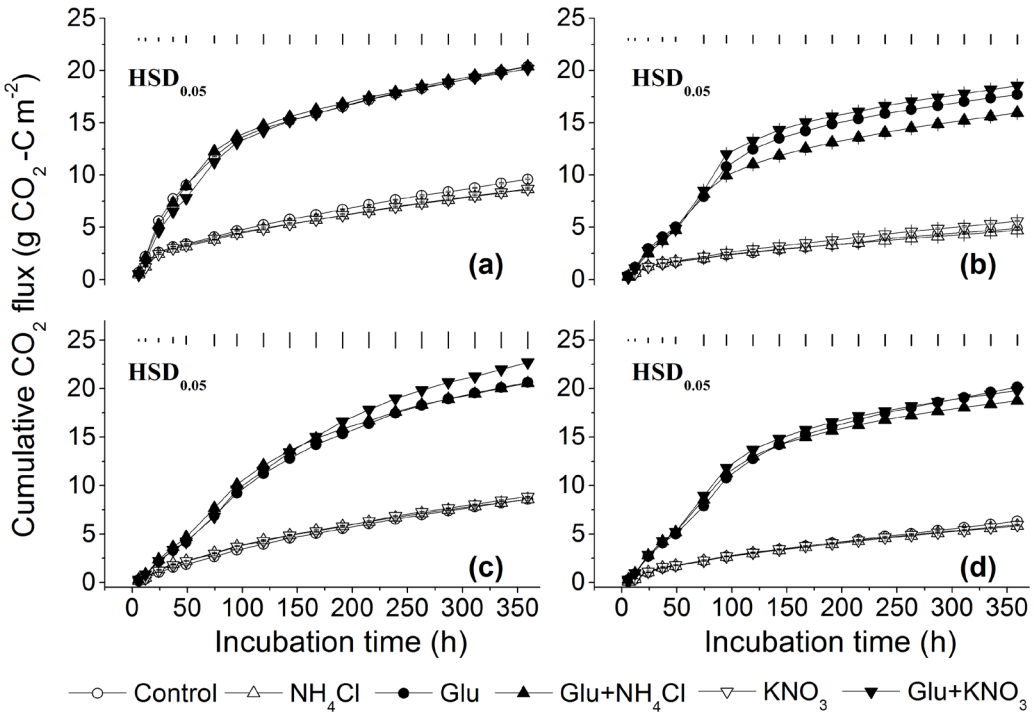

Fig. 4. Changes in the cumulative CO_2 flux during thawing of frozen WBF (a and c) and BKPF (b and d) soils treated with C and N under two soil moisture levels. (a) and (b) present low soil moisture; (c) and (d) present high soil moisture. Vertical bars show the Tukey honestly significant difference at the $p<0.05$ level ($HSD_{0.05}$) among all treatments at each sampling date. Error bars reflect the standard error of cumulative CO_2 flux between replicates (n = 3 for all treatments).

forest soils were both positively correlated to the glucose-induced DOC concentration but negatively correlated to the glucose-induced TN concentration (Fig. 5). Except for the N_2O flux from WBF soil with high moisture, cumulative N_2O and CO_2 fluxes were both positively correlated to the qCO_2 values of forest soils ($p<0.001$) (Fig. 6). With an increase in one unit of qCO_2, the increases in N_2O and CO_2 fluxes were higher from WBF soil than those from BKPF soil, and the increase in N_2O flux was much larger from the two forest soils with high moisture compared to that under low soil moisture condition (Fig. 6). According to the results of stepwise regression analysis, 68% of the variability in the N_2O fluxes from thawing forest soils could be attributed to the soil pH, NH_4^+-N, DON, and DOC, with the predominant influence of soil DOC (Supplementary Table S5). Meanwhile, 68% of the variability in the CO_2 fluxes from thawing forest soils could be explained by the soil NO_3^--N, NH_4^+-N, DOC, and MBC and were mostly affected by the MBC (Supplementary Table S5).

DISCUSSION

The duration and magnitude of N_2O and CO_2 flush fluxes following soil thawing are important indicators reflecting the impact of a freezing-thawing event on the fluxes of greenhouse gases from soils (Kim *et al.*, 2012). Similar to this study, Xu *et al.* (2016) reported that the peak of N_2O and CO_2 fluxes from forest soils occurred at the first or the second day after soil thaw started and the peak values of N_2O (0.01-1.8 mg N m^{-2} h^{-1}) and CO_2 (40-250 mg C m^{-2} h^{-1}) fluxes were similar to those reported in this study (Supplementary Table S2). In this study, an increase in soil moisture could delay the appearance of maximum N_2O and CO_2 fluxes by 24 to 48 h after soil thaw started (Figs 1 and 3). The peak of N_2O flux was reported to occur at 72 h when peatland soil was thawed with 100% water holding capacity (Cui *et al.*, 2016). However, the peak value of N_2O flux (0.02 mg N m^{-2} h^{-1}) reported by Cui *et al.* (2016) was lower than that in this study (Supplementary Table S2), which was probably attributed to the reduction of N_2O to N_2 in the soil with an extreme high water content. According to previous field experiments (Goldberg *et al.*, 2010; Wu *et al.*, 2010), the peak of N_2O flux from spruce forest soils during spring thaw period ranged from 0.04 to 0.5 mg N m^{-2} h^{-1}, which was relatively lower than that from BKPF and WBF soils with 80% WFPS, especially from WBF soil in this study (2.1-3.9 mg N m^{-2} h^{-1}). This could be partly attributed to the relatively high gas sampling frequency, which is hard to operate in the field, and the relative increase in DOC and DON pools released into the WBF soil at high soil moisture after a long-term freezing treatment (Supplementary Table S3). Compared to the N_2O flux, the pulse of CO_2 flux from soils during thaw became much weaker and no obvious flush CO_2 flux was even reported by Rong *et al.* (2015) in cropland and grassland. The maximum rates of soil CO_2 flux during thaw in this study (Supplementary Table S2) was within the reported values of 10-151 mg C m^{-2} h^{-1} in

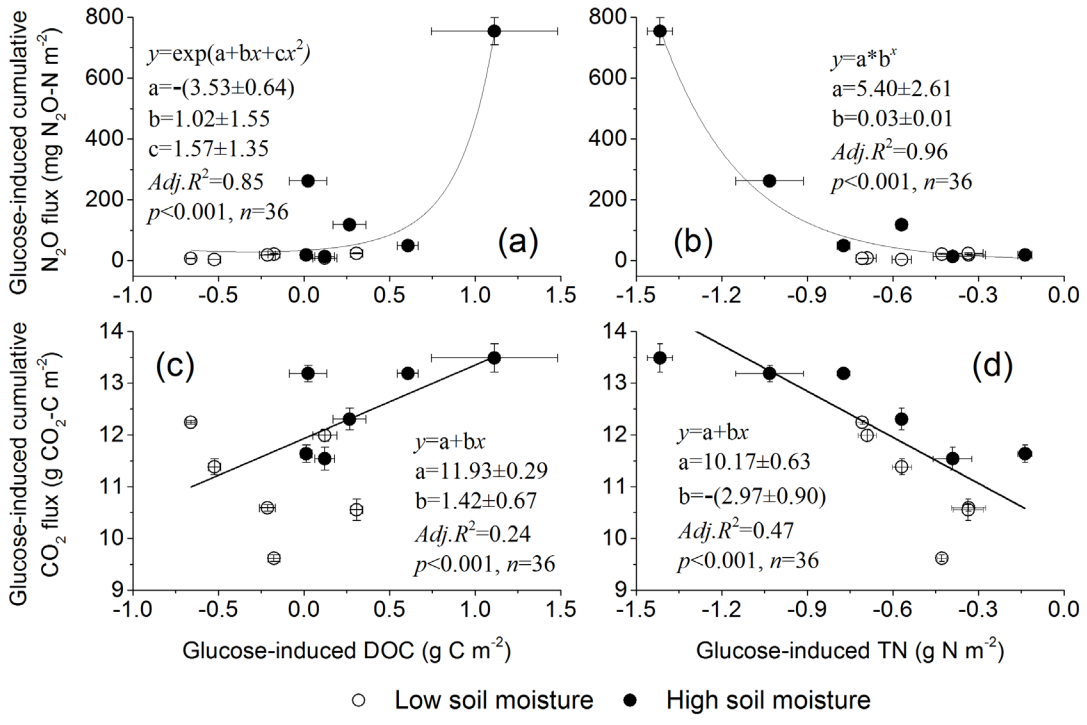

Fig. 5. Relationships between the glucose-induced fluxes of CO_2 and N_2O against the glucose-induced DOC and TN concentrations of forest soils. Error bars reflect the standard error of glucose-induced N_2O flux, CO_2 flux, DOC and TN between replicates, respectively (n = 3 for all treatments).

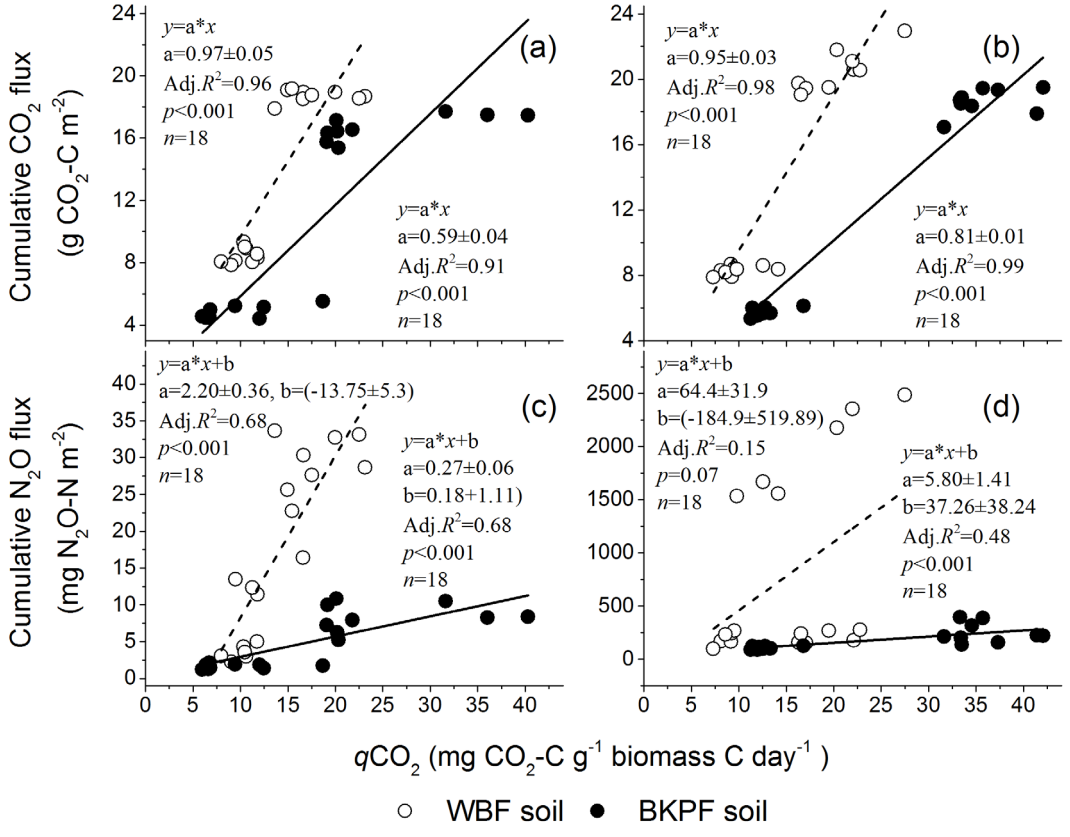

Fig. 6. Relationships between N_2O and CO_2 fluxes against the qCO_2 value of forest soils during thaw. (a) and (c) represent low soil moisture; (b) and (d) represent high soil moisture.

spruce and Korean pine forest lands (Wu *et al.*, 2010; Yan *et al.*, 2016). Considering the absence of peak flux in the field observation, the incipient rates of N_2O and CO_2 fluxes calculated in this study (Figs 2 and 4) would be appropriate to represent the magnitude of N_2O and CO_2 flush fluxes following spring soil thawing. From the results of this present study and previous studies, it can be reasonably concluded that an increase in soil moisture can delay the appearance of N_2O and CO_2 peak fluxes from thawing soils and their maximum fluxes vary with different soil types, which depends on soil moisture, freeze-thaw conditions, and the substrates supplied (Goldberg *et al.*, 2008; Kim *et al.*, 2012; Sehy *et al.*, 2004; Vilain *et al.*, 2010).

Without C and N addition, the concentration of NO_3^--N in BKPF soil with high moisture remained at 0.77 g N m^{-2} at the end of incubation (Supplementary Table S3). In addition, besides the possible dissimilatory nitrate reduction to ammonium (Wu *et al.*, 2016), the addition of nitrate (4.5 g KNO_3-N m^{-2}) alone was mainly reserved in BKPF soil with high moisture along with no significant change in cumulative N_2O flux compared to the control (Supplementary Tables S2 and S3). This indicated a weak denitrification potential in the BKPF soil with high moisture. However, the addition of KNO_3 alone stimulated N_2O flux from WBF soil with high moisture during the whole incubation along with a large consumption of the added NO_3^--N (Fig. 1c and Supplementary Table S3). Compared to the WBF soil, the relatively weaker denitrificaiton potential in the BKPF soil with high moisture could be related to a smaller soil DOC concentration and lower carbon availability in the forest organic layer (Supplementary Table S1). The extraneous DOC addition in the form of glucose promoted NO_3^--N consumption, N_2O and CO_2 fluxes, and microbial biomass C concentration in the two forest soils during thaw (Supplementary Tables S2 and S3). A decrease in the soil NO_3^--N concentration and an increase in the microbial biomass C upon addition of crop residues was reported by Pelster *et al.* (2013), who attributed that to N immobilization by soil microbes during the freezing-thawing period. The extraneous C stimulated soil microbial respiration and consumed O_2, which can benefit the denitrification process and promotes the soil N_2O flux (Sehy *et al.*, 2004). This glucose-induced N_2O flux from forest soils could be further enhanced under high soil moisture condition upon the addition of NO_3^--N (Supplementary Table S4) (Sehy *et al.*, 2004), which was accompanied by the largest glucose-induced CO_2 flux especially from the WBF soil (Supplementary Table S4). Mørkved *et al.* (2006) reported that, during the freezing-thawing period, the plant extract (0.34 mg C g^{-1} dry soil and 28 μg N g^{-1} dry soil) induced CO_2 flux from arable soil with gravimetric water content of 68% decreased with a decreasing soil O_2 concentration but increased with the increase in the amount of the added plant extract. Probably, the significant increase in the glucose-induced CO_2 flux under 80% WFPS condition in this study was related to the glucose-induced DOC and dissolved total N concentrations in the soil rather than the limitation of headspace O_2 (Fig. 5 and Supplementary Table S4). An incubation experiment conducted by Cayuela *et al.* (2010) showed that a single addition of NH_4NO_3 (15 g N m^{-2}) or glucose (150 g C m^{-2}) had no significant effect on N_2O flux from arable soil with 80% WFPS, but the flux was strongly increased by their combinative addition. The synergistic effect of C and N addition on the soil N_2O flux might be different in the soil with an extreme high moisture level. As reported by Sanchez-Martin *et al.* (2008), compared to the single addition of $(NH_4)_2SO_4$ (5 g N m^{-2}), the addition of $(NH_4)_2SO_4$ together with glucose (1%, *w*/*w*) significantly reduced cumulative N_2O fluxes from arable soil with 90% WFPS by 94%, which resulted from the completed denitrification to N_2 in saturated soil with high availability of labile C. Thus, the increase in soil C availability and N input can synergistically affect the N_2O and CO_2 fluxes from forest soils during thaw, which varies with soil moisture, soil types, and the types of the added N.

The microbial metabolic quotient, qCO_2, was regarded as a bio-indicator for disturbances and ecosystem maturity and represented the substrate utilization efficiency of soil microorganisms (Wardle and Ghani, 1995). Following the addition of glucose alone, qCO_2 increased in the two forest soils, along with increased fluxes of N_2O and CO_2 (Supplementary Table S3). These responses probably varied with the sources of extraneous C. Bamminger *et al.* (2014) reported that the addition of biochar, recalcitrant C, was reported to reduce qCO_2 and the fluxes of N_2O and CO_2 from an arable soil. Despite this, the consistent responses of the two gas fluxes and qCO_2 to extraneous C addition could partly support the positive relationship of N_2O and CO_2 fluxes against the qCO_2 in forest soils (Fig. 6). The qCO_2 was reported to decline with ecosystem development (Bastida *et al.*, 2008), which was consistent with the relatively smaller qCO_2 value of BKPF soil in the control compared to the WBF soil under low moisture condition. As shown in Fig. 6, an increase in one unit of qCO_2 can induce a larger increase in N_2O and CO_2 fluxes from WBF soil compared to those from BKPF soil, especially for N_2O flux under high moisture condition. This implied that N_2O and CO_2 fluxes from forest soils after addition of labile C and N during thaw was related to the substrate utilization efficiency of soil microorganisms, which varied with soil moisture and vegetation types.

Nitrification was rather low in the forest soil with 80% WFPS after further O_2 consumption with glucose addition. The increase in the MBC:MBN ratio after glucose addition (Supplementary Table S3) indicated an increase

in the relative abundance of fungi compared to that of bacteria (Dilly, 2004). For these reasons and no significant changes in the soil MBN concentration (Supplementary Table S3), the decrease in the glucose-induced NH_4^+-N of forest soils with a high moisture level (Supplementary Table S3) might be attributed to fungi dominating the codenitrification process instead of bacteria dominating anaerobic ammonium oxidation (Hayatsu *et al.*, 2008). Codenitrification controlled by denitrifying fungi produced N_2 or N_2O by combined nitrogen atoms from nitrate and other N compounds, such as organic N (Spott *et al.*, 2011). However, the DON concentration in forest soils slightly changed or even increased after the addition of glucose (Supplementary Table S3). This could be attributed to rapid inherent organic matter turnover after the addition of glucose, which could be reflected by the larger glucose-induced CO_2 flux from forest soils (Supplementary Table S4) than the dose of the added C in the form of glucose (6.4 g C m^{-2}), namely, a priming effect (Kuzyakov *et al.*, 2000; Wu *et al.*, 2015). Thus, the glucose addition could result in rapid turnover of C and N in soil, which was well followed by the increase in the soil glucose-induced N_2O and CO_2 fluxes (Fig. 5). The freezing-thawing period has been known to promote the release of DOM and inorganic N, especially NH_4^+-N, into the soil (Kim *et al.*, 2012). In addition, the spring snow thaw can increase soil moisture and probably results in a large input of available C from the forest organic layer (Supplementary Table S1) into mineral soils. The anaerobic environment and increased dissolved organic matter and NH_4^+-N concentrations in the soil during spring thaw period can promote the codenification process, which has been still unclear in forest soils so far (Xi *et al.*, 2016). Field experiments should be done in the future to study the effect of C and N addition on N_2O and CO_2 fluxes from soils with different water contents during the spring thaw period and their microbial mechanisms.

CONCLUSIONS

1. The flush fluxes of N_2O and CO_2 were observed from forest soils following thaw and their peak fluxes were both delayed with increasing soil moisture.

2. The glucose-induced cumulative CO_2 fluxes from the two forest soils ranged from 9.61 to 13.49 g CO_2-C m^{-2}, which was larger than the dose of carbon added as glucose. The addition of glucose alone increased microbial biomass C but slightly affected the dissolved organic carbon concentration in the two soils. Thus, the extra carbon released upon the addition of glucose could result from the microbial decomposition of soil native organic carbon.

3. The glucose-induced N_2O and CO_2 fluxes from the two forest soils during thaw were both significantly correlated to the glucose-induced soil total N and dissolved organic carbon concentrations and influenced by the soil moisture and KNO_3 addition singly and interactively.

4. Both cumulative N_2O and CO_2 fluxes were both positively correlated to the qCO_2 values of the two forest soils during thaw.

5. The interactive effects of glucose and N inputs on N_2O and CO_2 fluxes from forest soils during the thawing period depended on the types of the added N, soil moisture, and vegetation types.

Conflict of interest: The Authors do not declare conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// doi: 10.1515/intag-2016-0065

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