Nitrogen deposition may enhance soil carbon storage via change of soil respiration dynamic during a spring freeze-thaw cycle period

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As crucial terrestrial ecosystems, temperate forests play an important role in global soil carbon dioxide flux, and this process can be sensitive to atmospheric nitrogen deposition. It is often reported that the nitrogen addition induces a change in soil carbon dioxide emission in growing season. However, the important effects of interactions between nitrogen deposition and the freeze-thaw-cycle have never been investigated. Here we show nitrogen deposition delays spikes of soil respiration and weakens soil respiration. We found the nitrogen addition, time and nitrogen addition × time exerted the negative impact on the soil respiration of spring freeze-thaw periods due to delay of spikes and inhibition of soil respiration (\(p < 0.001\)). The values of soil respiration were decreased by 6% (low-nitrogen), 39% (medium-nitrogen) and 36% (high-nitrogen) compared with the control. And the decrease values of soil respiration under medium- and high-nitrogen treatments during spring freeze-thaw-cycle period in temperate forest would be approximately equivalent to 1% of global annual C emissions. Therefore, we show interactions between nitrogen deposition and freeze-thaw-cycle in temperate forest ecosystems are important to predict global carbon emissions and sequestrations. We anticipate our finding to be a starting point for more sophisticated prediction of soil respiration in temperate forests ecosystems.

Carbon (C) cycles are increasingly paid attention under global climate change. Freeze-thaw-cycle (FTC) significantly affects soil C cycles as a crucial ecological process\textsuperscript{5,8} due to its more frequent appearance under global climate change\textsuperscript{4,6}. Therefore FTC is recognized as crucial ecological processes and has received increased attention. Studying on the impacts of FTC on soil C dynamic is beneficial to the further understanding of soil C cycle and their feedback to climate change.

Many previous studies have pointed out that the FTC-induced enhancement of carbon dioxide (CO\textsubscript{2}) emission was often observed\textsuperscript{7–18}. Wang et al.\textsuperscript{16} showed that the ephemeral burst of CO\textsubscript{2} occurred at the early stage of spring FTC period in a temperate forest. Song et al.\textsuperscript{15} found the high emission peaks of CO\textsubscript{2} during FTC period in a freshwater marsh. Wang et al.\textsuperscript{17} suggested that FTC play an important role in soil CO\textsubscript{2} emissions in a wet meadow. In addition, the CO\textsubscript{2} emission peaks during the FTC period were also detected in some laboratory incubations\textsuperscript{10,19}, which are consistent with most of the field studies. However, different conclusions have been also reported. For example, the FTC had no a significantly impact on CO\textsubscript{2} emission in broadleaf forests or it reduced the release of CO\textsubscript{2} in grassland\textsuperscript{20–21}. The emission of CO\textsubscript{2} from soil is one of major C exchanges between terrestrial ecosystems and the atmosphere\textsuperscript{22}. With global climate change, less snowfall and warming may lead to increasing the frequency and intensity of FTC, and then may cause the increase of CO\textsubscript{2} emission from soil to atmosphere. Sullivan et al.\textsuperscript{23} suggested that the pulses of CO\textsubscript{2} caused by FTC are jointly driven by biological and physical factors. Several potential mechanisms have been proposed to clarify the FTC-induced enhancement of CO\textsubscript{2} emissions: (1) burst of CO\textsubscript{2} during the FTC period largely resulted from the release of trapped CO\textsubscript{2} in the

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In addition, atmospheric nitrogen (N) deposition is another important factor to soil C cycle, because the cycles of soil C and N are closely coupled\(^{26-28}\). Some previous studies showed that simulated N addition has significantly increase release of CO\(_2\)\(^{29}\). Nevertheless, other studies found controversial affecting soil CO\(_2\) fluxes in terrestrial ecosystem\(^{30-31}\). The different responses of soil CO\(_2\) fluxes to N addition have been reported in the different ecosystem, including decreases\(^{32}\), increases\(^{33}\), and no significant difference\(^{33-34}\). Summary, most of the high concentration N deposition may limit CO\(_2\) release, and low concentration may promote or no changes. Several potential mechanisms have been proposed to clarify the N-induced change of CO\(_2\) emissions: (1) N inhibition of lignin degradation largely resulted from change of microbial composition\(^{35}\); (2) change of CO\(_2\) emissions may be due to ecological shifts in the soil microbiota under N deposition\(^{36}\); (3) the coupling of soil carbon and nitrogen was broken due to N deposition, which might lead to change of CO\(_2\) emission\(^{37}\). Although the effect of FTC on C cycles and the effect of atmospheric N deposition on C cycles have been investigated, respectively\(^{23,38,39}\), the effect of FTC together with atmospheric N deposition on C cycles has never been reported. We hypothesized that soil respiration (Rs) could have a special response pattern to N deposition due to the changes of soil physicochemical properties and microbial characteristic in the FTC period. The second objective of our study was to examine the impact of simulated N deposition together with spring FTC on soil CO\(_2\) fluxes in temperate forest.

The major objective of this paper was to evaluate the change quantities of CO\(_2\) due to N deposition addition in FTC period in temperate forests which cover 9.7% of the earth's continental surface\(^{36}\). We hypothesized that N deposition would inhibit CO\(_2\) emissions via delay burst or decrease fluxes in spring FTC period in temperate forest. In addition, previous studies did not show an understandable mechanism regarding impact of FTC and N deposition on CO\(_2\) fluxes. The FTC and N deposition could affect the soil biological and physicochemical processes leading to C dynamic change. Therefore, we conducted a simulated N deposition experiment from May 2010 to present, and investigated the interactive effects of N deposition and spring FTC on soil C fluxes in a temperate forest and the potential mechanisms in 2015.

Materials and Methods

Site description. This study was conducted at the Fenligjiang Natural Reserve of Lesser Khingan Mountains in Heilongjiang province, Northeast China (48°02′–48°12′N, 128°58′–129°15′E). The climate is continental monsoon climate, with dry, cold winters, and humid, warm summers. The forests had a mean annual temperature of −0.5 °C from 1959 to 2013, with the lowest and highest monthly mean air temperature being −25.6 °C in January to 23.8 °C in July, respectively. The mean annual precipitation is 728 mm, of which approximately 75% falls between July and August. The snowpack lasted for 148 days with the snow depth ranging from 0 to 42 cm during the measurement years (Nov, 2014–Mar, 2015). The soil is classified as a dark brown forest soil\(^{41}\). The vegetation type is a cold-temperate spruce-fir Korean pine forest with the age of exceeded 200 years. The community is dominated by Picea koraiensis, Abies nephrolepis and Pinus koraiensis. The mean stand density is 972 ± 96 trees ha\(^{-1}\), the mean diameter at breast height is 13.7 ± 7.5 cm and the mean tree height is 16.7 ± 5.3 m. The major species in the canopy layer are Pinus koraiensis, Abies nephrolepis, Picea koraiensis, Picea jezoensis var. microsperma, Larix gmelini, Betula platyphylla, Acer mono, Fraxinus mandshurica and Betula costata.

Experimental design. To investigate changes in soil CO\(_2\) fluxes (Rs) following N application, we established three random blocks in May 2010, and each consisting of four research plots measuring 20 m × 20 m. The plots were separated by 10 m wide buffer strips to avoid horizontal movement of the soil N. The simulated N deposition was initiated at the onset of this experiment and included four treatments, control (no added N), low-N (5 g N·m\(^{-2}\)·yr\(^{-1}\)), medium-N (10 g N·m\(^{-2}\)·yr\(^{-1}\)) and high-N (15 g N·m\(^{-2}\)·yr\(^{-1}\)), with three replicates randomly distributed at each treatment. The N was applied as ammonium nitrate (NH\(_4\)NO\(_3\)) solution and was distributed across each plot to ensure an even distribution of the fertilizer. The control plots received 32 L water without N addition. The simulated N deposition was applied from May 2010 to present.

Soil CO\(_2\) fluxes measurements during spring FTC period. The spring FTC period soil CO\(_2\) fluxes (Rs) were measured every day from April 1\(^{st}\) to May 5\(^{th}\) 2015. For each of 12 plots, three polyvinyl Chloride (PVC) collars (20 cm inside diameter and 12 cm in height) were randomly inserted approximately 9 cm into the soil, with 3 cm left above the ground surface for Rs measurements, one week before N addition in 2010. A total of 36 soil collars were installed. The collars were left in the same place throughout the entire study period for exploring the change of the spring FTC period in Rs. The Rs was measured with a Li-8100 automated soil CO\(_2\) flux system (Li-Cor Inc, Lincoln, NE, USA) between 10:00–14:00 in spring FTC period. Each measurement was repeated 3 times for each collar to produce a collar’s mean Rs rate. Rs were calculated using exponential regression model with the Li-8100 file viewer application software (LI-8100/8150 Instruction Manual).

Soil physical and chemical properties and microbial characteristic measurements. The soil temperature at 5 cm depth (T\(_{5}\)) and soil volumetric water content at the 5 cm depth (W\(_{s}\), % v/v) were monitored simultaneously with the measurement of Rs by using a soil temperature probe (Omega Engineering Inc. USA) and soil moisture probes (Deltat Devices Ltd., Cambridge, England) connected to Li-8100. The continuous soil
temperature at 5 cm depth ($T_{5cm}$) was monitored hourly by Em-50 data logger (Decagon Devices, Inc. USA). The air temperature ($T_a$) was same measured hourly by Em-50 data logger (Decagon Devices, Inc. USA).

During the measurement of Rs, because of the difficulty in collecting soil samples from frozen soil, all soil samples were collected days nearby the soil collars from a depth of 0–10 cm using a specially designed auger (2.5 cm in diameter). Three soil cores were collected and pooled to one composite sample at each plot. All of the visible extraneous materials (such as roots, stones, etc.) were removed by hand, and then divided the composite sample into three sub-samples. One sub-sample was air-dried at ambient temperature, and then sieved (2 mm) and ground for the analysis of soil total C and total N by using an automated TOC/TN analyzer (multi N/C3100, Analytikjene AG, Germany). In addition, soil pH values were measured by a pH meter (SX7150, China) with soil:water ratio of 1:2.5. The second sub-sample was maintained original state, and taken back to laboratory. Thawed soils were mixed, whereas frozen soil was reduced to small pieces, with the pieces being homogenized to the extent possible. Immediately following, the inorganic N concentrations were determined by extracting fresh soil with $K_2SO_4$. The extractable NH$_4^+$-N concentrations were measured by using the indophenol blue method, followed by the colorimetric analysis. The NO$_3^-$-N content was determined by using the copper-cadmium reduction method. The third sub-sample was also maintained original states, and taken back to the laboratory immediately to assess microbial biomass C (MBC) and N (MBN). The MBC and MBN were measured by using a fumigation-extraction method. The extracts of N and C from fumigated and unfumigated samples were analysed by an automated TOC/TN analyzer (multi N/C3100, Analytikjene AG, Germany). The MBC and MBN were calculated from the difference between extractable N and C contents in the fumigated and the unfumigated samples using conversion factors (KEN and KEC) of 0.45 and 0.38, respectively. All extraction of NO$_3^-$-N, NH$_4^+$-N, MBC and MBN was done with $K_2SO_4$ of 0.5 mol l$^{-1}$ in 25 °C, and the duration of extraction was half an hour.

**Dividing the year into spring FTC period and Statistical analyses.** The spring FTC period was defined as the period that starts when soil surface snow is start to melt (the maximum $T_a$ is above 0 °C) and ends when daily minimum $T_{5cm}$ is above 0 °C. The spring FTC period lasted for 35 days (DOY 90–124 in 2015) in this study.

To assess the quantity of Rs under different N addition level in the FTC periods, Rs- $T_a$ models were constructed. Compared to the several commonly used models, such as the modified van’ Hoff’s model, the sigmoid-shaped Lloyd-Taylor and logistic models, the Gamma model performed either better or as good as the other models. In addition, Gamma model were tested across a wide $T_a$ range ($−18$–$35$ °C) and can also be expanded, using simple mathematics to help researchers analyse the Rs-$T_a$ relationship in the context of other environment factors, such as soil nutrients. The Gamma model was adopted based on $R^2$ and the Akaike Information Criterion (AIC). Therefore, Gamma model used to assess the impact of different quantities of N additions on Rs during the FTC period.

Gamma model was expressed as following:

$$Rs = (T)^a \times \exp (b + cT)$$

where $T$ is ($T_{5cm} + 40$), a, b and c are regression coefficients. $T_{5cm}$ is measured soil temperature under 5 cm below surface. 40 °C is added to $T_{5cm}$ because negative $T_{5cm}$ results in negative or imaginary Rs (or non-meaningful Rs), and 40 °C has been chosen as the lowest $T_{5cm}$ where Rs continues has been measured at $−39$ °C. The natural logarithm (Ln) transformed version of the Rs data was applied to alleviate the heteroscedasticity problem.

Two-ways analysis of variance was used to examine the influences of different quantities N deposition, spring FTC and their interactions on soil total C, total N, NH$_4^+$-N, NO$_3^-$-N, soil pH values, MBC, MBN. Fisher’s LSD followed the two-way analysis of variance between the N treatments. Tukey’s HSD tests were used to reveal the significant pairwise differences of the N addition. Pearson’s correlation analysis was used to determine the correlations between Rs and soil properties or microbial characteristics. Statistically significant differences were accepted at p < 0.05. All statistical analyses were performed using R 3.2.2 Version Software (R Development Core Team 2015).

**Results**

**Effects of spring FTC, N deposition and their interaction on Rs.** At the beginning of the spring FTC period, the daily maximum $T_a$ was above 0 °C, but all of $T_{5cm}$ were below 0 °C (Fig. 1(a)), and the snow was melting. However, the Rs remained at a low level (Fig. 1(b)), and the Rs under medium-N and high-N treatments was significantly lower than control and low-N treatments at the early stage of the spring FTC period (Fig. 1(b)). The significant differences in Rs were observed on next period of time, and temporal peaks of Rs occurred. The ephemeral burst of Rs observed from DOY 97 to DOY 102 under control treatments and lasted for 6 days, with the maximum Rs of 0.83 µmol m$^{-2}$ s$^{-1}$ (Fig. 1(b)). Simultaneously, we observed the high Rs occurred from DOY 98 to DOY 102 under low-N treatments and lasted for 5 days, with the maximum Rs of 0.76 µmol m$^{-2}$ s$^{-1}$ (Fig. 1(b)). During the period, the daily mean of air temperature and the mean of soil temperature in 5 cm depth increased continuously (Fig. 1(a)). The snowpack had melted completely. But, the ephemeral enhancement of Rs occurred at later stage of the spring FTC and lasted for 5 days (DOY 107–111) under medium-N and high-N treatments (Fig. 1(b)). The Rs pulse lasted for a short time period and after that the rate decreased to the normal status during the spring FTC period. During most of observation period, the Rs increased with temperature.

The effects of different quantity of N addition on Rs were highly variable during spring FTC period. During the measurement period, the mean of Rs was 0.58, 0.57, 0.47, 0.48 µmol m$^{-2}$ s$^{-1}$ for different quantity of N addition, i.e., control, low-N, medium-N, high-N treatments, respectively (Table 1). Our results found that the simulated N deposition had significantly impact on the Rs due to inhibiting CO$_2$ fluxes or delaying outburst event (Table 1; Fig. 1(b)). Likewise, the FTC also had a significantly impact on Rs (Table 2), which varied from 0.32 to 1.06 µmol m$^{-2}$ s$^{-1}$...
and showed the high fluctuations under natural status (control plots) (Fig. 1(b)). In addition, Rs was also significantly affected by the interaction of the simulated N deposition and spring FTC (p < 0.001) (Table 2). In general, the two-way ANOVA analysis showed that the simulated N deposition, the spring FTC and their interaction exhibited significant effects (p < 0.001) on the Rs during the whole measurement period (Table 2).

Spring FTC period contribution of Rs to the winter and annual budget and assessing the future C dynamic in temperate forest. Applying the empirical Rs-T models assessed the quantities of Rs under different N addition levels (i.e., control, low-N, medium-N, high-N) during the spring FTC period. The ordinary least squares was used to calculate the coefficients (i.e., a, b, c), similar to what was performed in the Khomik 2009 Gamma model paper (Table 3). The predicated spring FTC period Rs was 17.53 ± 0.43 g C m⁻² yr⁻¹ in this temperate forest without N addition (Table 1). Low-N treatment exerted negative effects on spring FTC

Figure 1. (a) Mean daily variation of soil temperature at the 5 cm depth (T₅cm) during the spring Freeze-thaw cycle periods in 2015. (b) Daily variation of soil respiration at different added N level plots in spring Freeze-thaw cycle periods in 2015. Control refers the control treatment plots; Low-N refers the Low-N treatment plots; Medium-N represents the Medium-N treatment plots; High-N represents the High-N treatment plots.

| Specified Treatments | Rs (umol CO₂ m⁻² s⁻¹) | Cumulative Rs (g C m⁻²) | Contribution to winter Rs (%) | Contribution to annual Rs (%) |
|----------------------|------------------------|-------------------------|-------------------------------|-------------------------------|
| Control-N            | 0.58 ± 0.02            | 17.53 ± 0.43            | 37.49 ± 0.89                  | 1.80 ± 0.02                   |
| Low-N                | 0.57 ± 0.01            | 16.44 ± 0.58            | 46.88 ± 1.32                  | 1.69 ± 0.01                   |
| Medium-N             | 0.47 ± 0.02            | 10.67 ± 0.75            | 25.50 ± 1.47                  | 1.10 ± 0.01                   |
| High-N               | 0.48 ± 0.03            | 11.24 ± 0.69            | 18.03 ± 0.85                  | 1.15 ± 0.01                   |

Table 1. Spring FTC periods soil CO₂ flux and contribution of Rs to the winter and annual budget at the different quantities of nitrogen additions treatments.

| Effect             | Rs       | MBC      | MBN      | NO₃⁻-N  | NH₄⁺-N  |
|--------------------|----------|----------|----------|---------|---------|
| Treatment          | <0.001   | <0.001   | <0.001   | <0.001  | <0.001  |
| Date               | <0.001   | <0.001   | <0.001   | <0.001  | <0.001  |
| Treatment × Date   | <0.001   | <0.001   | <0.001   | <0.001  | <0.001  |

Table 2. ANOVA P-Values for impact of Treatment and FTC (Substitute Date for FTC) on soil respiration (Rs), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), ammonium nitrogen concentrations (NH₄⁺-N), nitrate nitrogen concentrations (NO₃⁻-N). P-Values < 0.001 denote very significance.
and 36% (high-N) compared with the control. The predicted annual 
decreased exponentially with the soil NO$_3^-$ concentrations, and determination coefficient ($R^2$) and Akaike Information Criterion (AIC) are given. 

**Table 3. Regression models of $R_s$ against soil temperature at the 5 cm depth ($T_s$) for the FTC period.** The regression models are of the form: $R_s = T_s^a \times \exp(b + c T_s)$, where $T$ is $(T_s + 40)$, a, b and c are regression coefficients, and determination coefficient($R^2$) and Akaike Information Criterion (AIC) are given.

| Treatments | a   | b    | c    | $R^2$ | AIC |
|------------|-----|------|------|-------|-----|
| Control    | 220.01 | 585.95 | 5.62 | 0.61 | 64  |
| Low-N      | -480.62 | 1285.47 | 12.17 | 0.43 | 48  |
| Medium-N   | 424.54   | -1166.35 | -10.02 | 0.80 | 79  |
| High-N     | 366.40   | 1009.17   | -8.59 | 0.81 | 84  |

**Figure 2.** Model-based contributions of spring Freeze-thaw cycle soil CO$_2$ flux to winter (a) and annual total (b) at the different quantities of N addition (control, Low-N, Medium-N, High-N). The winter and annual soil CO$_2$ efflux quote from Liu et al. 48.

$Rs$, and its value was 16.44 ± 0.58 g C m$^{-2}$ yr$^{-1}$ (Table 1). The cumulative $Rs$ during the spring FTC period were 10.67 ± 0.75 g C m$^{-2}$ yr$^{-1}$ in medium-N plots and 11.24 ± 0.69 g C m$^{-2}$ yr$^{-1}$ in high-N plots (Table 1). In general, the N addition exerted a negative impact on spring FTC $Rs$ and decreased it by 6% (low-N), 39% (medium-N) and 36% (high-N) compared with the control. The predicted annual $Rs$ was 974.3 ± 67.1 g C m$^{-2}$ yr$^{-1}$ without N addition treatment; the values of $Rs$ in winter were 46.8 g C m$^{-2}$ yr$^{-1}$ (control), 35.7 g C m$^{-2}$ yr$^{-1}$ (low-N), 41.89 g C m$^{-2}$ yr$^{-1}$ (medium-N) and 62.35 g C m$^{-2}$ yr$^{-1}$ (high-N) 48. Under different quantities of N addition, the cumulative $Rs$ during spring FTC period contributed 37.49% (control), 46.88% (low-N), 25.50% (medium-N) and 18.03% (high-N), respectively, to the winter $Rs$ and contributed 1.80% (control), 1.69% (low-N), 1.10% (medium-N) and 1.15% (high-N), respectively, to the annual $Rs$ (Fig. 2).

The Fenglin Natural Reserve (our study site) covered an area of 18165 hm$^2$. We hypothesized that whole Reserve was used to simulate the impact of N addition on Rs. The Rs in the study area was reduced by 1.97 × 10$^{-4}$ Tg C yr$^{-1}$ (low-N), 1.25 × 10$^{-3}$ Tg C yr$^{-1}$ (medium-N) and 1.14 × 10$^{-3}$ Tg C yr$^{-1}$ (high-N) during the spring FTC period. The temperate forest covers 9.7% of the earth's continental surface 46. The temperate forest covered an area of 14.5 million km$^2$. The Rs in whole temperate forest would be reduced by 15.81 Tg C yr$^{-1}$ (low-N), 99.47 Tg C yr$^{-1}$ (medium-N) and 91.21 Tg C yr$^{-1}$ (high-N) during the spring FTC period. Global total CO$_2$ emission (excluding Land-use Change and Forestry) cumulative value was 33843.05 Mt (about 9229.92 Tg C) in 2012 46. The decrease values of Rs under medium- and high-N treatments during spring FTC period in temperate forest would be approximately equivalent to 1% of global annual C emissions.

**Relationships between spring FTC Rs and soil biochemical property.** The mean values of soil biochemical property were summarized in Table 4. The correlation analyses between Rs and soil biochemical property were performed to attempt to explain the observed changes in Rs during spring FTC period. But we only found the Rs and soil NH$_4^+$-N, the soil NO$_3^-$-N, the soil MBC and the soil MBN are related. The Rs was positively correlated with the soil MBC and MBN during the spring FTC period (Fig. 3a,b). But, the Rs was positively correlated with the lower concentrations of the soil NH$_4^+$-N and the soil NO$_3^-$-N, and negatively correlated with the higher concentrations of the soil NH$_4^+$-N and the soil NO$_3^-$-N during spring FTC period (Fig. 3c,d).

We made the best fitting equation of the Rs and the soil biochemical property. The Rs increased linearly with the soil MBC ($y = 0.47x - 0.21$, $R^2 = 0.75$, $p < 0.01$, $y$ was defined as Rs values, $x$ was defined as MBC values) and the soil MBN ($y = 4.07x - 1.83$, $R^2 = 0.74$, $p < 0.01$, $y$ was defined as Rs values, $x$ was defined as MBN values). The Rs decreased exponentially with the soil NO$_3^-$-N concentrations ($y = 0.70e^{-0.03x}$, $R^2 = 0.63$, $p < 0.01$, $y$ was defined as Rs values, $x$ was defined as soil NO$_3^-$-N concentrations). The Rs changed irregularly with the soil NH$_4^+$-N concentrations ($y = 139.55x^2 - 139.89x + 64.82$, $R^2 = 0.33$, $p < 0.05$, $y$ was defined as Rs values, $x$ was defined as soil NH$_4^+$-N concentrations).
Discussion

We found that the ephemeral spikes of Rs occurred in control plots (without N addition) at the early stage of the spring FTC period, which was consistent with some previous observation. At the beginning of the outburst, the snow was completely melted. And, the soil microbes can recover rapidly from disturbance resulting from

|                          | Control-N            | Low-N              | Medium-N            | High-N              |
|--------------------------|----------------------|--------------------|---------------------|---------------------|
| Soil NH\textsubscript{4}\textsuperscript{+}-N (mg/kg) | 5.74 ± 4.64d        | 10.29 ± 5.55c      | 18.86 ± 12.19b     | 22.24 ± 16.27a     |
| Soil NO\textsubscript{3}\textsuperscript{-}-N (mg/kg)  | 32.48 ± 11.02c      | 32.47 ± 11.99d     | 38.31 ± 15.04b     | 39.12 ± 14.88a     |
| Soil Microbial C (mg/g)  | 1.64 ± 0.46a         | 1.63 ± 0.47b       | 1.49 ± 0.51c       | 1.47 ± 0.51d       |
| Soil Microbial N (mg/g)  | 0.20 ± 0.05a         | 0.18 ± 0.05b       | 0.16 ± 0.06c       | 0.15 ± 0.05d       |
| Soil Total C (mg/g)      | 103.10 ± 5.13b       | 169.32 ± 6.45a     | 171.85 ± 4.82a     | 166.63 ± 7.21a     |
| Soil Total N (mg/g)      | 13.17 ± 3.28b        | 15.43 ± 5.86b      | 16.19 ± 7.46a      | 19.29 ± 6.82a      |
| Soil pH                  | 5.10 ± 0.48a         | 5.00 ± 0.57a       | 4.87 ± 0.61b       | 4.72 ± 0.75b       |

Table 4. The mean values of Soil NH\textsubscript{4}\textsuperscript{+}-N, Soil NO\textsubscript{3}\textsuperscript{-}-N, Soil Microbial C, Soil Microbial N, Soil Total C, Soil Total N and soil pH during the spring FTC period. Value within the same column with the same letters (a, b, c and d) are not significantly different at \( p < 0.05 \). Data are shown as means with standard errors.

Figure 3. Relationships between (a) the soil CO\textsubscript{2} efflux and microbial biomass carbon (MBC, \( R^2 = 0.75 \)), (b) the soil CO\textsubscript{2} efflux and microbial biomass nitrogen (MBN, \( R^2 = 0.74 \)), (c) the soil CO\textsubscript{2} efflux and nitrate nitrogen concentrations (NH\textsubscript{4}\textsuperscript{+}-N, \( R^2 = 0.63 \)), (d) the soil CO\textsubscript{2} efflux and ammonium nitrogen concentrations (NO\textsubscript{3}\textsuperscript{-}-N, \( R^2 = 0.33 \)) at the different quantities of N addition (CK refers the control treatment plots; TL refers the Low-N treatment plots; TM represents the Medium-N treatment plots; TH represents the High-N treatment plots) during the spring Freeze-thaw cycle periods. Pictures made by Lattice package (R 3.2.2 Version).
and wet growing season in the N addition plots\textsuperscript{62–64}, and not occurred in winter among treatment\textsuperscript{63}. In addition, the rate of N deposition during the spring FTC periods. In the previous studies, the decrease in Rs did not occur in this period. After this period of FTC, the pulse of N addition, we hypothesized N and salt in high concentrations inhibited microbial activity and biomass during the spring FTC period. Therefore, more attention should be paid to the impact of N deposition on soil respiration in the spring FTC period.

Conclusions

The simulated N addition delayed the outburst of Rs compared with control (no N addition). The soil spring FTC increased Rs during the spring FTC period to the annual Rs were 1.80%, 1.69%, 1.10% and 1.15% for control, low-N, medium-N and high-N treatment, respectively (Table 1, Fig. 3). Our results suggested that response of Rs to simulated N deposition in temperate forests is a decline, and it may vary depending on the level of N deposition during the spring FTC periods. In the previous studies, the decrease in Rs occurred in the warm and wet growing season in the N addition plots\textsuperscript{62–64}, and not occurred in winter among treatment\textsuperscript{65}. In addition, our results also suggested that contribution of Rs during the spring FTC period to the annual Rs will vary when the global N deposition are greatly altered with the atmospheric N levels rise\textsuperscript{64}. We suspected that the decline of Rs due to N addition may be an improvement in the C use efficiency of the soil microbial community, and might impact on the global C cycle. However, N deposition may enhance soil carbon storage via decrease of Rs during spring FTC period. Therefore, more attention should be paid to the impact of N deposition on soil respiration in the spring FTC period.

References

1. Cooley, K. R. Effects of CO\textsubscript{2}-induced climatic changes on snowpack and streamflow. Hydrolog Sci J. 35, 511–522 (1990).
2. Kunkel, K. E. et al. Trends in Twentieth-Century US Extreme Snowfall Seasons. J Climate 22(23), 6204–6216 (2009).
3. IPCC. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (The Stocker, T. E., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA (2013).
4. Freppaz, M., Williams, B. L., Edwards, A. C., Scalenghe, R. & Zanini, E. Simulating soil freeze/thaw cycles typical of winter alpine conditions: Implications for N and P availability. Appl Soil Ecol. 35, 247–255 (2007).
5. Schimel, J. P. & Mikan, C. Changing microbial substrate use in Arctic tundra soils through a freeze–thaw cycle. Soil Biol Biochem. 37, 1411–1418 (2005).
6. Sjursen, H. S., Michelsen, A. & Holmstrup, M. Effects of freeze–thaw cycles on microarthropods and nutrient availability in a sub-Arctic soil. Appl Soil Ecol. 28, 79–93 (2005).
7. Elberling, B. & Brandt, K. K. Uncoupling of microbial CO\textsubscript{2} production and release in frozen soil and its implications for field studies of Arctic C cycling. Soil Biol Biochem. 35, 263–272 (2003).
8. Henry, H. A. L. Soil freeze–thaw cycle experiments: trends, methodological weaknesses and suggested improvements. Soil Biol Biochem. 39, 977–986 (2007).
9. Holst, J. et al. Fluxes of nitrous oxide, methane and carbon dioxide during freezing-thawing cycles in an Inner Mongolian steppe. Plant Soil. 308, 105–117 (2008).
10. Goldberg, S. D., Muhr, J., Borken, W. & Gebauer, G. Fluxes of climate relevant trace gases between a Norway spruce forest soil and atmosphere during repeated freeze-thaw cycles in mesocosms. J Plant Nutr Soil Science 171, 729–739 (2008).
11. Ludwig, B., Teepe, R., de Gerenyu, V. L. & Flessa, H. CO\textsubscript{2} and N\textsubscript{2}O emissions from gleicy soils in the Russian tundra and a German forest during freeze-thaw periods – a microcosm study. Soil Biol Biochem. 38, 3516–3519 (2006).
12. Monson, R. K. et al. The contribution of beneath-snow soil respiration to total ecosystem respiration in a high-elevation, subalpine forest. Global Biogeochem Cy. 20, 13 (2006).
13. Neilsen, C. B. et al. Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. Soil Sci Soc Am J. 65, 1723–1730 (2001).
14. Sharma, S., Saele, Z., Schilling, R., Munch, J. C. & Schloter, M. Influence of freeze–thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Appl Environ Microbiol. 72, 2148–2154 (2006).
15. Song, C. C., Wang, Y. S., Wang, Y. Y. & Zhao, Z. C. Emission of CO₂, CH₄, and N₂O from freshwater marsh during freeze–thaw period in Northeast of China. *Atmos Environ.* **40**, 6879–6885 (2006).
16. Wang, C. *et al.* Seasonality of soil CO₂ efflux in a temperate forest: Biophysical effects of snowpack and spring freeze–thaw cycles. *Agr Forest Meteorol.* **177**, 83–92 (2013).
17. Wang, J. & Wu, Q. Annual soil CO₂ efflux in a wet meadow during active layer freeze–thaw changes on the Qinghai–Tibet Plateau. *Environ Earth Sci.* **69**, 855–862 (2013).
18. Wu, X. *et al.* Effects of soil moisture and temperature on CO₂, CH₄, soil-atmosphere exchange of various land use/cover types in a semi-arid grassland in Inner Mongolia, China. *Soil Biol Biochem.* **42**, 773–787 (2010).
19. Wu, X. *et al.* Environmental controls over soil-atmosphere exchange of N₂O, NO and CO₂ in a temperate Norway spruce forest. *Global Biogeochem Cy.* **24**, GB2012 (2010).
20. Feng, X., Nielsen, L. L. & Simpson, J. Response of soil organic matter and microorganisms to freeze/thaw cycles. *Soil Biol Biochem.* **39**, 2027–2037 (2007).
21. Groffman, P. M., Hardy, J. D., Driscoll, C. T. & Fahey, T. J. Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. *Global Change Biol.* **12**, 1748–1760 (2006).
22. Schlesinger, W. H. & Andrews, J. A. Soil respiration and the global carbon cycle. *Biogeochemistry* **48**, 7–20 (2000).
23. Sullivan, B. W., Dove, S., Montes-Helu, M. C., Kolb, T. E. & Hart, S. C. Pulse emissions of carbon dioxide during snowmelt at a high-elevation site in northern Arizona, USA. *AuricAntarct Alp Res.* **44**, 247–254 (2012).
24. Hirano, T. Seasonal and diurnal variations in topsoil and subsoil respiration under snowpack in a temperate deciduous forest. *Global Biogeochem Cy.* **19** (2005).
25. Kim, D. G., Vargas, R., Bond-Lamberty, B. & Turetsky, M. R. Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research. *Biogeochemistry* **93**, 2459–2483 (2012).
26. Wang, X. *et al.* Contrasting effects of ammonium and nitrate inputs on soil CO₂ emission in a subtropical coniferous plantation of southern China. *Biol Fert Soils* **51**, 815–825 (2015).
27. Thornton, P. E. & Rosenzloom, N. A. Ecosystem model spin-up: estimating steady state conditions in a coupled terrestrial carbon and nitrogen cycle model. *Ecol Model.* **189**, 25–48 (2005).
28. Chapin, F. S. Principles of terrestrial ecosystem ecology Vol. 2 (eds Matso, P. A. *et al.*) (Springer, New York, 2011).
29. Graham, S. L. *et al.* Effects of Soil Warming and Nitrogen Addition on Soil Respiration in a New Zealand Tussock Grassland. *PLoS ONE* **9**(3), e91204 (2014).
30. Gong, Y. M. *et al.* Response of carbon dioxide emissions to sheep grazing and N application in an alpine grassland—Part 2: Effect of N application. *Biogeochemistry* **11**, 1751–1757 (2014).
31. Wei, D., Xu-Ri., Liu, Y., Wang, Y. & Wang, Y. Three-year study of CO₂ efflux and CH₄/N₂O fluxes at an alpine steppe site on the central Tibetan Plateau and their responses to simulated N deposition. *Geoderma.* **232–234**, 85–96 (2014).
32. Jiang, C., Yu, G., Fang, H., Cao, G. & Li, Y. Short-term effect of increasing nitrogen deposition on CO₂, CH₄, and N₂O fluxes in an alpine meadow on the Qinghai–Tibetan Plateau, China. *Atmos Environ.* **44**, 2920–2926 (2010).
33. Li, K. *et al.* Responses of CH₄, CO₂ and N₂O fluxes to increasing nitrogen deposition in alpine grassland of the Tianshan Mountains. *Chemosphere* **88**, 140–143 (2012).
34. Krause, K., Niklaus, P. A. & Schleppi, P. Soil-atmosphere fluxes of the greenhouse gases CO₂, CH₄, and N₂O in a mountain spruce forest subjected to long-term N addition and to tree girdling. *Agr Forest Meteorol.* **181**, 61–68 (2013).
35. Berg, B. & Matzner, E. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environmental Reviews* **5**, 1–25 (1997).
36. Fierer, N. *et al.* Toward an ecological classification of soil bacteria. *Ecology* **88**, 1354–1364 (2007).
37. Manzoni, S. *et al.* Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* **196**, 79–91 (2012).
38. Sun, Z. Z. *et al.* The effect of nitrogen addition on soil respiration from a nitrogen-limited forest soil. *Agr Forest Meteorol.* **197**, 103–110 (2014).
39. Joseph, G. & Henry, H. A. L. Soil nitrogen leaching losses in response to freeze–thaw cycles and pulsed warming in a temperate old field. *Soil Biol Biochem.* **40**, 1947–1953 (2008).
40. Schulz, J. *The Ecosystems of the World.* (Springer Berlin, 1995).
41. Soil classification research group of Nanjing Soil Institute of Chinese Academy of Sciences & China Soil Classification Research The Retrieval System for China Soil Classification Vol 3 (eds). (University of Science & Technology China, 2001) (in Chinese).
42. Schimel, J. P., Bilbrough, C. & Welker, J. M. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biol Biochem.* **36**, 217–227 (2004).
43. Brookes, P. C., Landman, A., Prud, N. G. & Je NkiNson, D. S. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem.* **17**, 837–842 (1985).
44. Yan I.Hoff, J. H. *Etudes de dynamique chimique.* (Frederick Muller, Amsterdam, 1884).
45. Lloyd, J. & Taylor, J. On the temperature dependence of soil respiration. *Aquat Ecol.* **8**, 315–323 (1994).
46. Richards, F. J. A flexible growth function for empirical use. *J Exp Bot.* **10**, 290–300 (1959).
47. Khomik, M., Arain, M. A., Liaw, K.-L. & McCaughey, J. H. Decoupled dynamic model for simulating soil respiration–soil temperature relationship: Gamma model. *J Geophys Res.* **114**, doi: 10.1029/2008GC000851 (2009).
48. Liu, B. *et al.* Annual soil CO₂ efflux in a cold temperate forest in northeastern China: effects of winter snowpack and artificial nitrogen deposition. *Sci Rep-UK.* **6**, 18957, doi: 10.1038/srep18957 (2016).
49. CAFE 2.0 Climate explorer. World Resource Institute, Washington. http://cafe2.wri.org/WRI/Country%20GHG%20Emissions/indicator=Total%20GHG%20Emissions%20Excluding%20LUC&indicator=Total%20GHG%20Emissions%20Including%20LUC&fyear=2010&sortID=6&sortDir=d&chartType= &Cited FAO 2014, FAOSTAT Emission Database (2015).
50. Aanderud, Z. T., Jones, S. E., Schoolmaster, D., Fierer, N. & Lennon, J. T. Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biol Biochem.* **57**, 217–227 (2013).
51. Fries, A. & Christensen, S. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soil. *Environ Biogeochemistry* **33**, 2083–2091 (2001).
52. Yanai, T., Toyota, K. & Okazaki, M. Effects of Successive Soil Freeze–Thaw Cycles on Soil Microbial Biomass and Organic Matter Decomposition Potential of Soils. *Soil Sci Plant Nutr.* **50**(6), 821–829 (2004).
53. Schimel, J. P. & Klein, J. S. Microbial response to freeze–thaw cycles in tundra and taiga soils. *Soil Biol Biochem.* **28**, 1061–1066 (1996).
54. Haei, M. *et al.* Effects of soil frost and growth, composition and respiration of the soil microbial decomposer community. *Soil Biol Biochem.* **43**, 2069–2077 (2011).
55. Frey, S. D., Knorr, M., Parrent, J. L. & Simpson, R. T. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecol Manag.* **196**(1), 159–171 (2004).
56. Gilleam, F. S., Cook, A. & Lyter, S. Effects of experimental freezing on soil nitrogen (N) dynamics in soils of a net nitrification gradient in an N-saturated hardwood forest ecosystem. *Can J Forest Res.* **34**, 805–814 (2004).
57. Grogan, P., Michelsen, A., Ambus, P. & Jonasson, S. Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biol Biochem.* **36**, 641–654 (2004).
58. Zhao, H. T. et al. Effect of freezing on soil nitrogen mineralization under different plant communities in a semi-arid area during a non-growing season. *Appl Soil Ecol.* **45**, 187–192 (2010).
59. Judd, K. E., Likens, G. E. & Groffman, P. M. High nitrate retention during winter in soils of the hubbard brook experimental forest. *Ecosystems* **10**, 217–225 (2007).
60. Schmidt, S. K. & Lipson, D. A. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant Soil* **259**, 1–7 (2004).
61. Tierney, G. L. et al. Soil freezing alters Wne root dynamics in a northern hardwood forest. *Biogeochemistry* **56**, 175–190 (2001).
62. Mo, J. et al. Response of soil respiration to simulated N deposition in a disturbed and a rehabilitated tropical forest in southern China. *Plant soil* **296**(1), 125–135 (2007).
63. Mo, J. et al. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. *Global Chang Biol.* **14**, 403–412 (2007).
64. Janssens, I. A. et al. Reduction of forest soil respiration in response to nitrogen deposition. *Nat Geosci.* **3**, 315–322 (2010).

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**Author Contributions**

G.Y., Y.X. and L.X. contributed the same for the whole manuscript preparation and design. Q.W., S.H., G.Y., Y.X. and L.X. contributed the whole manuscript preparation and design. Q.W., S.H., G.Y., Y.X. and L.X. wrote the main manuscript text. Q.W., G.Y., Y.X. and L.X. prepared all figures. J.W., W.M., Z.Z., Z.W., S.J., S.H., J.Y. and B.L. collected literatures and prepared Tables 1–4. All authors reviewed the manuscript.

**Additional Information**

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