Winter climate controls soil carbon dynamics during summer in boreal forests

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Abstract
Boreal forests, characterized by distinct winter seasons, store a large proportion of the global terrestrial carbon (C) pool. We studied summer soil C-dynamics in a boreal forest in northern Sweden using a seven-year experimental manipulation of soil frost. We found that winter soil climate conditions play a major role in controlling the dissolution/mineralization of soil organic-C in the following summer season. Intensified soil frost led to significantly higher concentrations of dissolved organic carbon (DOC). Intensified soil frost also led to higher rates of basal heterotrophic CO₂ production in surface soil samples. However, frost-induced decline in the in situ soil CO₂ concentrations in summer suggests a substantial decline in root and/or plant associated rhizosphere CO₂ production, which overrides the effects of increased heterotrophic CO₂ production. Thus, colder winter soils, as a result of reduced snow cover, can substantially alter C-dynamics in boreal forests by reducing summer soil CO₂ efflux, and increasing DOC losses.

Keywords: summer season, carbon dynamics, boreal forest, heterotrophic CO₂ production, dissolved organic carbon, soil frost, winter

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1. Introduction
Boreal forests cover one third of the world’s forested area [1] and store about 30% of the global terrestrial carbon pool [2]. The boreal region is characterized by distinct winter seasons and long periods of snow cover. Increasing air temperature induced by climate change has already occurred in the higher latitudes of the northern hemisphere including the boreal region [3]. This is predicted to be exaggerated in the future with the most dramatic changes occurring in winter [4]. Such changes may alter the timing of snow-pack formation, and reduce the duration of snow covered periods [5, 6]. As winter soil temperature largely depends on the insulating effects of snow cover, any reduction in snow cover may increase temperature variability and result in colder soils despite a warming climate in northern ecosystems [7, 8]. Furthermore, single cold extremes in air temperature are projected to persist even in a generally warmer future [9].

Soil organic carbon originates primarily from litter production, root exudates, and microbial biomass. This organic carbon is lost from the system through microbial mineralization to CO₂ and under low redox conditions also to CH₄ [10, 11], and via lateral transport of dissolved and particulate forms into rivers and streams [12]. The CO₂ lost from forest soils originate both from living
roots and their associated microorganisms (root and/or rhizosphere respiration) and from the activity of heterotrophic soil microorganisms decomposing dead organic matter. Production of both CO$_2$ and dissolved organic carbon (DOC) in soils are regulated by vegetation type [13], soil decomposer community [14], redox conditions, the amount and composition of soil organic matter [11, 15, 16], environmental conditions such as temperature and moisture [16, 17] and other soil properties [18].

The state of knowledge on C cycling in seasonally snow covered regions has recently been reviewed highlighting the importance of winter soil processes [19]. A key knowledge gap identified was the lack of understanding about how winter processes affect C-dynamics during the subsequent growing season. Considering anticipated climate changes in this region, filling this gap is a research priority. In a warmer climate, changes in soil conditions during winter have the potential to affect ecosystem processes during the growing season [20], but to what extent and in what way(s) are presently not known [19, 21].

Changes in the soil frost regime, resulting from alterations in snow-pack, have been shown to cause root injuries [22, 23], change the composition and activity of the soil decomposer community [24] and alter the concentrations of both CO$_2$ and DOC in soils [25, 26]. In a boreal forest of northern Sweden, we manipulated soil frost in the field since 2002, and previously showed that cold winter conditions and soil frost significantly increase the DOC concentration in soils during the following spring [25, 26] but also that winter climatic conditions influence the spring flood DOC concentrations in adjacent streams [26, 27]. Based on these previous findings, we hypothesized that (i) enhanced winter soil frost will increase the soil DOC concentration in the following summer, and (ii) these enhanced DOC concentrations are able to sustain higher rates of heterotrophic CO$_2$ production. In order to test these, we used (i) the summer season soil DOC concentration data collected in the soil frost manipulation experiment (summers 2004–2010), and (ii) a laboratory-generated estimate of heterotrophic soil CO$_2$ production based on basal respiration measurements on the soil samples collected from the same experimental plots as for DOC (August 2007). Our soil frost manipulation, in addition to altering the winter soil conditions, changed the soil conditions during the spring season [28]. Thus, we also evaluated the relative importance of winter versus pre-summer (the period between the end of winter and sampling in summer) soil conditions for both DOC and heterotrophic CO$_2$ production, using variance partitioning analysis. In addition, and using the same approach, we assessed the available data on soil CO$_2$ concentrations (summer 2004) in the soil frost manipulation experiment in order to identify to what extent changes in the heterotrophic CO$_2$ production may impact the total CO$_2$ concentrations in the soil.

2. Materials and methods

2.1. Study site

The study location is a riparian forest along the stream Västrabäcken (C2), in the Krycklan catchment within the Svarterget Long-term Ecological Research Forest (Svarterget LTER; 64°14′N, 19°46′E), 60 km northwest of Umeå, Sweden [29]. The catchment is mainly covered by 100 year old Norway spruce (Picea abies) and an understory layer dominated by blueberries (Vaccinium myrtillus) along with a continuous moss layer dominated by Pleurozium schreberi and Hylocomium splendens. The fine roots at the study site occur mainly in the upper 20 cm of the soil profiles with an average density of 6.31 mg cm$^{-3}$ [30]. The average annual air temperature in the study area is +1.8°C (1980–2009); average air temperatures in January and July are −9.5°C and +14.6°C, respectively. Snow accounts for ~40% of the annual precipitation which is 623 mm on average. Average annual maximum snow and soil frost depths in the area were 76.5 cm (43–113 cm; 1980–2010) and 17.7 cm (2.5–79 cm; 1993–2007), respectively [26]. The meteorological measurements were carried out in the Svarterget field station located 1.2 km southwest of the experimental location.

2.2. Field-scale soil frost manipulation experiment

Soil frost manipulation began in 2002 and thus represents one of the longest on-going experiments of this kind. The experiment included triplicates of the following treatments: deep soil frost (snow removal), shallow soil frost (increased insulation), and control. During winter, each of the deep soil frost treatment plots was covered with a transparent roof above a wooden platform 2.5 m tall. Snow accumulated on top of the roofs, thereby preventing snow-pack formation and inducing deep soil frost (49 ± 6 cm (average ± standard deviation)). The wooden platforms did not significantly alter the light regime during the growing season [30]. The shallow soil frost plots were surrounded by ~40 cm wooden walls in which the ground was insulated with geotextile bags containing Styrofoam pellets (average soil frost depth = 4 ± 5 cm). The control plots were exposed to ambient conditions (average soil frost depth = 29 ± 3 cm). Each of the manipulated plots covered an area of 9 m$^2$. To ensure the hydrological balance between the treatment plots during snowmelt, the accumulated snow on the roofs was added to the ground at the end of each winter prior to snowmelt.

2.2.1. Soil DOC sampling and measurement. Soil measurement equipment was installed in the center of each frost treatment plot at a distance of ~3 m from the stream. Suction lysimeters (SKP 100, UMS GmbH, München, Germany) and temperature probes (T03R; TOJO Skogsteknik, Bygdeå, Sweden) were installed at the depths of 0, 10, 25, 40, 60 and 80 cm. At these same depths (except for 0 cm), volumetric water content was monitored in one plot of each treatment using time-domain reflectometry (TDR) (CS615; Campbell Scientific, Logan, UT, USA). Soil temperature and soil water content were automatically logged every 4 h on a Campbell Scientific data logger (CR10, Campbell Scientific). Soil water samples were collected for chemical analysis, such as DOC, using pre-evacuated bottles (~100 kPa). The sampling was conducted 2–5 times during
summer seasons 2004–2010. Start and end of the summer season was determined to be when the average daily air temperature was above and below +10°C respectively for at least five consecutive days [31]. In the laboratory, DOC was measured using a TOC-5000 Shimadzu before 2009 and a Shimadzu TOC-VCPH/CPN (Shimadzu, Kyoto, Japan) analyzer afterwards.

2.2.2. Soil CO$_2$ sampling and measurement. Soil CO$_2$ was sampled using gas sampling probes at 10, 25, 40 and 60 cm soil depth every week during March–October 2003–2004, and the CO$_2$ concentration was measured using a GC-FID (Varian 3400, Varian Inc., Walnut Creek, CA, USA) (for more details see [25]).

2.2.3. Soil heterotrophic CO$_2$ production. In the beginning of August 2007, we sampled the soil at five different horizons: (1) 0–5 cm, (2) 5–15 cm, (3) 20–30 cm, (4) 35–45 cm and (5) 55–65 cm in the nine plots of the soil frost manipulation experimental design. Samples were taken from 10 locations in each plot and lumped together. While sampling, a margin of 30 cm was left to the outside treatment perimeter to avoid an edge effect. The samples were transported from the field in zip-lock plastic bags and inside cooling boxes. In the laboratory, roots, needles and other large organic and inorganic material were removed and the soil samples were sieved and homogenized. Loss on ignition (LOI) was determined on three sub-samples (4 h at 500°C). Water content in the soil samples were adjusted to 60% water holding capacity (WHC) prior to respiration measurements. WHC was measured on soil samples placed in plastic cylinders (height: 20 mm, diameter: 10 mm, n = 3) using fine mesh bottoms, soaked in water for 12 h and drained for 1 h followed by 12 h drying at 105°C [32]. While measuring the LOI and WHC, the samples were stored at +4°C for three days. Thereafter, basal respiration was measured on the soil samples (n = 2) in a respirometer (Respicond IV, Nordgren Innovations AB, Djäkneboda, Sweden). Since the basal respiration measurements were entirely laboratory based and carried out on soils isolated from the natural field condition (and therefore no contribution from root and/or plant associated rhizosphere respiration), the basal respiration was used as a measure of soil heterotrophic CO$_2$ production. The measurements were done on equivalents of 1 g soil (d.w.) for the two surface layers (0–5 cm and 5–15 cm) and equivalents of 2 g soil (d.w.) for the three deeper layers. The soil samples were weighed into 250 ml polypropylene jars and the CO$_2$ production rate was recorded every hour at 20°C. In the respirometer, the evolved CO$_2$ was captured in KOH solution and the measurements were based on changes in the KOH conductivity. The reported CO$_2$ production (basal respiration) rates are based on the average of 40 hourly measurements after stabilizing [33, 34]. Thereafter, substrate-induced respiration (SIR) was achieved by adding equivalents of 0.2 g glucose per g dry organic material together with nutrients (nitrogen and phosphorous) with final molar ratio of 181:13:1 for C:N:P, to the soil samples which simulated the initial basal respiration. SIR was used as an indicator for the size of the living and active soil microbial population [35].

2.3. Data assessment

2.3.1. Soil frost treatment effect. At each soil horizon, the effects of frost manipulation treatments on the summer season’s DOC concentrations in the riparian soil horizons (2–5 sampling occasions in summers 2004–10), as well as on the heterotrophic CO$_2$ production (measured as basal respiration rate) and SIR (laboratory-based analysis, one occasion in August 2007) in the soil samples were assessed by analysis of variance (repeated measures ANOVA for the entire dataset) based on linear models in conjunction with Tukey HSD post hoc comparisons (significance level = 5%), after ensuring the normal distribution of data using one-sample Kolmogorov–Smirnov test (PASW Statistics 18, SPSS Inc., Chicago, IL, USA, 2009).

2.3.2. Importance of winter versus pre-summer soil conditions. As indicated by temperature and TDR-data, the soil frost manipulation treatments changed the soil conditions not only in the winter season, but also during the pre-summer period. Winter was defined as the period in which the average air temperature was below 0°C. Pre-summer was defined as the period between the end of winter (when the average air temperature rose above 0°C) and the sampling date in summer.

First, a variance partitioning based on linear regression and redundancy analysis ordination (RDA) [36] was used to differentiate between the percentages of variance in summer DOC concentrations and heterotrophic CO$_2$ production rates that were explained by two sets of winter and pre-summer variables jointly and individually. Hence, the seven-year data on soil DOC concentrations (summers 2004–2010) and the one-occasion laboratory-based heterotrophic CO$_2$ production measurements (August 2007) were evaluated in response to the two explanatory categories of variables: (1) winter variables included soil frost duration, soil minimum temperature, and winter soil and air temperature sums (daily resolution). For the heterotrophic CO$_2$ production, winter air temperature sum was not included in the analysis, since there was only a single data set available (table 1); and (2) Pre-summer variables included precipitation sum, air and soil temperature sums between end of winter and sampling date for the DOC analysis, soil water content at sampling as well as soil temperature sum between end of winter and sampling date (average daily resolution) for the heterotrophic CO$_2$ production. In the second step, the variance partitioning analysis was run using the winter variables only in order to investigate their joint and individual explanatory power (table 1; variables with regression $p < 0.1$). Both of the variance partitioning analyses were performed for the soil layers which experienced significant soil frost manipulation treatment effect (10 cm for DOC and 0–5 cm for basal respiration). For each explanatory variable, the significance of the optimal relation (i.e. quadratic, square root, log transformation) to the dependent variable was assessed.
beforehand by univariate linear least-squares regression analysis (significance was assessed via $F$-statistic). Only those variables with a significant relation were included in the final variance partitioning model using the function `varpart` from the package vegan version 1.17-11 for the R statistics system.

In response to changes in winter climate, the contribution of soil heterotrophic CO$_2$ production to the total summer soil respiration was evaluated at the top 60 cm soil depth: the relative importance of the winter variables for both heterotrophic CO$_2$ production and the total soil CO$_2$ concentration was assessed through univariate linear least-squares regression analyses as mentioned above, followed by variance partitioning analyses. Laboratory-based basal respiration measurements in the one occasion in August 2007 and CO$_2$ concentration data for summer 2004 (due to lack of data on winter variables, the data for year 2003 was not included here) were used as dependent variables indicating the heterotrophic CO$_2$ production and total soil CO$_2$ concentration, respectively. The explanatory winter variables for both analyses included soil frost duration, soil minimum temperature, and soil winter temperature sum (average daily resolution) (table 1). Both the response and explanatory variables were depth weighted over 60 cm soil depth (for the calculations of the depth-weighted values, see supplementary equations S1 and S2 available at stacks.iop.org/ERL/8/024017/mmedia).

### Table 1. Dependent variables and explaining winter variables as well as direction of relation, regression equation, adjusted $R^2$ and $p$-value as assessed by univariate least-squares regression analyses (for average values of the explanatory variables see supplementary table S1 available at stacks.iop.org/ERL/8/024017/mmedia).

| Dependent variable (y) | Explaining winter variable (x) | Direction of relation | Regression equation | Adj. $R^2$ | p-value |
|------------------------|--------------------------------|-----------------------|---------------------|-------------|---------|
| DOC concentration (10 cm depth) (mg l$^{-1}$) | Minimum soil temperature ($^\circ$C) | Negative | $\log(y) = 4.05 - 0.08x^2$ | 0.07 | 0.085 |
| | Winter soil temperature sum ($^\circ$C) | Negative | $\log(y) = 4.29 - 0.0003x$ | 0.17 | 0.012 |
| | Duration of soil frost (days) | Positive | $\log(y) = 3.90 + 0.005x$ | 0.23 | 0.004 |
| | Air winter temperature sum ($^\circ$C) | — | $\log(y) = 5.73 + 0.001x$ | 0.03 | 0.171 |
| Heterotrophic CO$_2$ production (0–5 cm depth) ($\mu$g h$^{-1}$ g$^{-1}$) | Minimum soil temperature ($^\circ$C) | Negative | $\log(y) = 0.59 - 0.05 \log(13 + x)$ | 0.63 | 0.019 |
| | Winter soil temperature sum ($^\circ$C) | Negative | $\log(y) = 0.60 - 0.02 \log(316 + x)$ | 0.59 | 0.027 |
| | Duration of soil frost (days) | Positive | $\log(y) = 0.48 + 4.7e - 52 \exp(x)$ | 0.55 | 0.034 |
| Depth-weighted-heterotrophic CO$_2$ production (top 60 cm depth) ($\mu$g h$^{-1}$ g$^{-1}$) | Minimum soil temperature ($^\circ$C) | Negative | $\log(y + 1) = 0.17 - 0.02 \log(4 + x)$ | 0.71 | 0.006 |
| | Winter soil temperature sum ($^\circ$C) | Negative | $\log(y + 1) = 0.38 - 0.04 \log(x)$ | 0.52 | 0.040 |
| | Duration of soil frost (days) | Positive | $\log(y + 1) = 0.15 + 1.6e - 34 \exp(x)$ | 0.61 | 0.013 |
| Depth-weighted-soil CO$_2$ concentration (top 60 cm depth) (mg l$^{-1}$) | Minimum soil temperature ($^\circ$C) | Positive | $y = 8.13 + 5.29 \log(5 + x)$ | 0.33 | 0.048 |
| | Winter soil temperature sum ($^\circ$C) | Positive | $y = 4.01 + 2.28x$ | 0.31 | 0.088 |
| | Duration of soil frost (days) | — | $y = 17.08 - 0.06x$ | 0.13 | 0.199 |

3. Results

3.1. Soil frost treatment effect

3.1.1. Soil DOC. Summer DOC concentrations in the soil water (at 10 cm depth) were significantly different between the treatments in the soil frost manipulation experiment (ANOVA: $F = 10.5, p < 0.001$). At 10 cm depth, DOC concentration in the deep soil frost plots was on average ($\pm$ standard deviation (SD)) 106 $\pm$ 44 mg l$^{-1}$ ($n = 16$), while average concentrations ($\pm$SD) for control and shallow soil frost treatments were 64 $\pm$ 23 mg l$^{-1}$ ($n = 32$) and 62 $\pm$ 34 mg l$^{-1}$ ($n = 18$), respectively. The overall DOC concentrations in summer were significantly higher in the deep soil frost treatment than both control (Tukey HSD: $p < 0.001$) and shallow soil frost treatments ($p = 0.001$) at 10 cm depth, but no significant difference was found between the control and shallow soil frost treatments ($p = 0.97$) (figure 1(a)). The soil frost treatment effect on DOC concentration was not significant at deeper soil layers. In addition, we did not find a particular trend in DOC variations over the year (ANOVA: $p > 0.05$).

3.1.2. Soil heterotrophic CO$_2$ production. The soil frost manipulation treatment significantly affected the heterotrophic CO$_2$ production (basal respiration rate) in the surface soil (0–5 cm depth) in summer (ANOVA: $F = 5.59, p = 0.015$), while the treatment effect was not significant in deeper soil layers. In the soil samples collected at 0–5 cm depth in summer, heterotrophic CO$_2$ production (basal respiration rate) was highest in the deep soil frost treatment plots with an average ($\pm$SD) rate of 74 $\pm$ 7 $\mu$g CO$_2$ h$^{-1}$ g$^{-1}$ dry soil ($n = 6$). The control and shallow soil frost plots had average ($\pm$ SD) basal respiration rates of 60 $\pm$ 12 $\mu$g CO$_2$ h$^{-1}$ g$^{-1}$ dry soil ($n = 6$) and 59 $\pm$ 5 $\mu$g CO$_2$ h$^{-1}$ g$^{-1}$ dry soil ($n = 6$), respectively (figure 1(b)). Heterotrophic CO$_2$ production in the deep soil frost plots was
Figure 1. (a) Soil solution DOC concentration during the summer seasons 2004–2010 at 10 cm depth (n = 18, 32 and 16 for shallow soil frost, control and deep soil frost treatments, respectively) and (b) heterotrophic CO$_2$ production rate in samples taken in August 2007 at 0–5 cm depth (n = 6 for each treatment) in the soil frost manipulation plots. Each error bar covers the range between the minimum and maximum values. Different letters indicate significant difference as assessed by ANOVA in conjunction with Tukey HSD post hoc comparisons (significance level = 5%).

significantly higher than both control (Tukey HSD; $p = 0.032$) and shallow soil frost ($p = 0.025$) treatments. No significant difference was observed in heterotrophic CO$_2$ production rates between the control and the shallow soil frost treatments ($p = 0.99$). In addition, soil frost treatment did not result in significant changes in SIR ($p < 0.05$).

3.2. Importance of winter versus pre-summer conditions

3.2.1. Soil DOC. Winter and pre-summer variables together explained 50% of the variance in the summer DOC concentration at 10 cm soil depth. However, most of the variance (45%) was explained by winter variables with only 9% of the variance jointly explained by both winter and pre-summer variables (see supplementary figure S1(a) available at stacks.iop.org/ERL/8/024017/mmedia).

The variance partitioning on the winter variables revealed that the variation in summer DOC concentrations was not controlled by a single winter factor, rather the three variables: frost duration, soil winter temperature sum and soil minimum temperature (table 1) jointly explained the variation (37% of the total explained variation) (figure 2(a)). The summer DOC concentration was positively related to the duration of soil frost in the preceding winter, while it was negatively related to the soil minimum temperature as well as soil winter temperature sum (table 1).

3.2.2. Soil heterotrophic CO$_2$ production. Variance in soil heterotrophic CO$_2$ production in soil samples (0–5 cm depth), was to a large extent (98%) explained by winter and pre-summer variables. While winter variables accounted for the whole variance, 58% of the total variance was jointly explained by both the winter and pre-summer variables (indicated as overlapping bars, figure S1(b) available at stacks.iop.org/ERL/8/024017/mmedia).

Based on variance partitioning analysis of the winter variables, no dominant individual explanatory variable was recognized for heterotrophic CO$_2$ production in the 0–5 cm soil depth (figure 2(b)). However, the three significant winter variables, namely soil frost duration, minimum soil temperature and soil winter temperature sum (table 1), together explained 77% of the variation in basal respiration rate at this surface soil layer (figure 2(b)). Soil heterotrophic CO$_2$ production during summer responded positively to the frost duration and negatively to both soil minimum temperature and soil winter temperature sum at 0–5 cm depth (table 1).

3.3. Total and heterotrophic CO$_2$ production in response to cold winter soils

In the top 60 cm of the soil, depth-weighted basal respiration rates (heterotrophic CO$_2$ production) in summer increased in
response to longer frost duration as well as colder winter soils (measured as minimum soil temperature and winter soil temperature sum) in the antecedent winter (table 1). There was, however, a strong autocorrelation among the winter variables which together explained 86% of the depth-weighted CO₂ production jointly (figure 3(a)). In contrast to rates of CO₂ production, winter temperature sum and minimum soil temperature were the significant explanatory winter variables which were both positively correlated to the soil CO₂ concentrations during the summer (table 1) and together explained 44% of the total variance in CO₂ concentration (figure 3(b)). Decreasing minimum soil temperature by 1 °C rendered a 4% decrease in the summer CO₂ concentration in the top 60 cm of the soil (table 1).

4. Discussion

The overall finding of this study is that winter climate and soil frost have substantial effects on summer season's soil carbon dynamics, through the production of DOC and heterotrophic CO₂ Reduction in snow cover, allowing deeper soil frost and colder soils in the preceding winter [37, 38], resulted in a significant increase in soil DOC concentrations in the late-summer. A significant increase in the summer soil heterotrophic CO₂ production (soil basal respiration rate) was also observed as a result of deeper soil frost and colder winter soil conditions. Therefore, these results confirmed our first and second hypotheses. However, based on the available data we cannot mechanistically link the increase in DOC concentrations to the increase in heterotrophic CO₂ production rates, although both processes were significantly susceptible to the variations in soil frost regime induced by the manipulation. Winter climatic conditions, compared to the pre-summer conditions, played a larger role in controlling the DOC concentrations and the heterotrophic CO₂ production rates in the following summer season. Total soil CO₂ concentrations in summer declined suggesting that the observed enhancement of heterotrophic CO₂ production (measured as basal respiration rate) did not drive the response of bulk soil CO₂ production to deep soil frost. The significant changes in both DOC and heterotrophic CO₂ production were observed in the upper soil horizons where both control and deep soil frost (and in some years also the shallow soil frost) treatment plots experienced soil frost. We name the different field treatments based on the differences induced in the soil frost depth. However, alterations in soil temperature and timing of soil freezing/thawing (frost duration) could be driving factors rather than the frost depth itself, and this should be considered in the interpretation of the data. The deep soil frost treatment over the study period led to ∼7 °C lower minimum soil temperature (∼8.6 °C), a doubling of soil frost duration (118 days) and 26 days delay in the spring thaw, in comparison with the control plots (10 cm soil depth).

Winter soil frost led to a significant increase in soil solution DOC concentrations at 10 cm depth in the following summer season, which was similar to what previously has been observed for the spring snowmelt period [26]. Soil frost has been suggested to increase DOC concentrations through several different mechanisms including fine root injuries [22], soil physical disturbance [39], microbial cell lysis [40] and freeze-out processes [41]. Based on our investigation it was not possible to identify the exact mechanism of the increase in summer season's DOC concentration caused by colder winter soils, but the likely effect of higher DOC was a stimulation of the soil heterotrophic respiration, especially since there was no observed changes in microbial biomass (as quantified by the SIR measurements). In a laboratory-based soil incubation experiment mimicking a range of different winter soil temperatures and winter conditions representative for boreal regions, no clear effect on the soil microbial biomass (as assessed by total phospholipid fatty acid (PLFA) content) was detected, while an increase in the soil basal respiration rate was attributed to the enhancement of DOC as induced by cold temperatures [24].

In our study, soil heterotrophic CO₂ production (measured as basal respiration rate) in surface soil samples collected in summer were higher in the deep soil frost treatment plots. As reported previously from the same site [25], summer CO₂ concentrations in the upper 60 cm of the soil, and subsequent CO₂ emission from the soil surface both decreased in the deep soil frost treatment plots. Thus, even if the deep soil frost treatment enhanced heterotrophic CO₂ production, the net declining effect on soil CO₂ concentrations and emissions [25] suggested that the total soil respiration at the site was less sensitive to alterations in the heterotrophic CO₂ production compared to the root and/or rhizosphere respiration (figure 4). An increase in the heterotrophic respiration, particularly in the in situ measurements, might be driven by the availability of labile organic matter released in the form of root exudates or resulted from the decomposition of fine roots [42]. A 50% decrease in the fine root biomass, including both tree and understory roots, was previously reported in the snow removal (deep soil frost) plots as compared to reference plots [30]. In a tree girdling experiment in a forest stand located only one
km away from our experiment, root associated respiration accounted for about 50% of soil respiration [43]. Thus, assuming a similar partitioning of respiration in our study site, it is not surprising that a 50% decrease in fine root biomass can have a significant effect on soil respiration. Frost damage to tree roots has also been reported from other soil frost manipulation experiments [22, 44] and root injuries have been proposed to be mainly driven by direct cellular damage [23]. Although the tree roots are injured by frost, they have a short turnover time which makes it difficult to detect the changes during mid- and late-summer. In addition, trees can acquire water and nutrients from both inside and outside the plots. Therefore, the fine root biomass reduction in the experimental plots was attributed to the decline in the understory plant cover mainly [30]. The reduction in understory root abundance could have contributed to a decline in CO₂ production through root respiration. This concurs with the conclusion that understory vegetation is an important driver of the soil environment in boreal forests of northern Sweden, despite providing a relatively small proportion of the total plant biomass [45].

Our results contradict those from a study on Norway spruce forest soils in Germany [46] in which a significant decrease in summer soil respiration was found in the frost treated soil profiles. Yet, as indicated by radiocarbon signature of CO₂ (Δ¹³C), the decrease was mainly driven by a considerable reduction in heterotrophic respiration and was not compensated by increasing root respiration. However, the decrease in heterotrophic respiration was not only attributed to the winter soil frost and damage to the heterotrophic community, but also to drought conditions during sampling which inhibited the recovery of the heterotrophic community [46].

In our study site, the deep soil frost treatment significantly affected both the concentrations of DOC and CO₂ in soil during spring snowmelt [26] and summer [25]. There is, however, a need for complementary data such as soil physical properties and water status to be able to more precisely estimate the total amount of soil carbon loss either through respiration or leaching in dissolved form. Although it is challenging to translate our concentration-based data into quantitative amounts of carbon, the observed alterations in carbon concentrations indicate that a larger partitioning of carbon into dissolved organic forms rather than the inorganic form occurs during the summer season after winters with extensive soil frost conditions (figure 4).

It has recently been suggested that manipulation experiments often overestimate the impacts of climate change on ecological processes due to abrupt, short-term manipulations [47]. Although a rise in the air temperature is not expected to offset the increase in soil frost depth caused by less snow insulation, the predicted climate change scenarios may not be as severe as achieved in our study [5, 38]. Our results, however, show a strong and consistent effect of the winter manipulations on DOC concentrations in summer over almost a decade. This indicates that the susceptible C-pool is either very large or refilled before each summer season.

How soil carbon will respond to changes in climate will ultimately depend on a number of interdependent processes and their interactions that include physical, biological, chemical and hydrological aspects. Our results emphasize the importance of winter conditions for the dynamics and partitioning of dissolution and mineralization of the soil organic pool. Our findings indicate that colder and deeper soil frost during winter has significant effects on summer soil carbon dynamics through the production of soil DOC and CO₂. This, in particular, is important for the northern regions predicted to experience colder winter soils, due to less insulating snow cover, in a warmer future climate. Although our results are specific in terms of study location, number of samples and different time-scales, the implication of these results are that winter conditions have much larger influence on soil carbon dynamics than has previously been understood.

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