Long-term reindeer grazing limits warming-induced increases in CO$_2$ released by tundra heath soil: potential role of soil C quality

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Abstract

The current climate warming in the Arctic may increase the microbial degradation of vast pools of soil carbon (C); however, the temperature sensitivity of decomposition is often highly dependent on the quality of accumulated soil C. Grazing by reindeer (Rangifer tarandus L.) substantially affects the dominant vegetation and often increases graminoids in relation to dwarf shrubs in ecosystems, but the effect of this vegetation shift on the soil C quality has not been previously investigated. We analyzed the soil C quality and rate of microbially mediated CO$_2$ release at different temperatures in long-term laboratory incubations using soils from lightly grazed dwarf shrub-dominated and heavily grazed graminoid-dominated tundra ecosystem. The soil C quality was characterized by solid-state cross-polarization magic angle spinning (CPMAS 13C NMR spectroscopy), which showed a higher relative proportion of carbohydrate C under light grazing and higher relative proportion of aliphatic not-O-substituted C under heavy grazing. Initial measurements showed lower temperature sensitivity of the CO$_2$ release in soils under light grazing compared with soil under heavy grazing, but the overall CO$_2$ release rate and its temperature sensitivity increased under light grazing as the soil incubation progressed. At the end of incubation, significantly more carbohydrate C had been lost in soils under light grazing compared with heavy grazing. These findings indicate that there may be a link between the grazer-induced effects on soil C quality and the potential of soils to release CO$_2$ to atmosphere. We suggest that vegetation shifts induced by grazing could influence the proportion of accumulated soil C that is vulnerable to microbial degradation under warming climate.

Introduction

Because low temperatures limit decomposition, tundra ecosystems store substantial quantities of C in the form of old organic matter (OM). This accumulated soil C constitutes by far the largest C stock of tundra ecosystems, and overall, tundra soils store half of the global soil carbon (C) stocks [1]. The ongoing warming of the Arctic may enhance the decomposition of accumulated soil C, which would release vast amounts of CO$_2$ to the atmosphere and create a positive feedback loop with respect to climate change [2]. Investigations on the susceptibility of soil C stocks to increasing temperatures have recognized that the effects of increasing temperatures on soil C decomposition may depend on the chemical quality of the accumulated soil C. The temperature sensitivity of decomposition has been repeatedly demonstrated to increase with declining C quality (i.e. decomposability of accumulated C) in the soil and litter [3–7].

The chemical composition of the accumulated tundra soil C may largely be determined by the dominant vegetation composition, because the different plant species and growth forms vary in the chemical composition and the decomposability of litter produced [8–10]. In tundra, grazing by large ungulates exerts important effects on vegetation, often promoting the abundance of graminoids in relation to dwarf shrubs and mosses [11–16]. Although many tundra ecosystems are grazed by reindeer/caribou (Rangifer
effect of grazing on the C quality and the temperature
stocks to warming, a separate study investigating the
importance for the response of the total ecosystem C
susceptibility of soil C stocks to warming has a key
temperature respiration
ability, litter and soil decomposition rates and ecosys-
terandus L.
stutes an important means of land-use [17], it has not
been investigated how the grazer-induced vegetation
shifts influence on the quality of accumulated soil C.
Furthermore, it is unclear whether the complex chan-
ges in soils induced by grazing may influence the sensi-
tivity of soil C decomposition to increasing
temperatures. Grazer-induced shifts in the vegetation
from mosses and dwarf shrubs to graminoids is likely
to alter the soil C quality. Graminoid litter is generally
degraded more rapidly than dwarf shrub and moss litter [18, 19]; however, graminoids may also promote
soil C accumulation through the production of dense
mats of fibrous root biomass [9]. Dwarf shrub-domi-
nated vegetation produces phenol-rich and highly
aromatic litter that decomposes at a slow rate [9, 18],
which—according to kinetic theory—should increase
with temperature to a greater extent than the degrada-
tion of labile C [2, 20]. Along with soil C quality, graz-
ing causes substantial changes in the soil microclimate
and nutrient concentrations [21, 22], which could also
alter soil microbial responses to increasing tempera-
tures. The decomposition rates are regulated by com-
plicated interactions among soil C quality, substrate
diffusion, soil microbial temperature acclimation, and
nutrient stoichiometry [23–25]. Grazers in turn simul-
taneously alter several of these properties, making it
difficult to isolate mechanisms by which grazers influence
microbial activity as well as its temperature
sensitivity.

It was recently discovered that tundra grazing his-
tory may be an important determinant for the
response of ecosystem C balance to climate warming
[29]. Using a site where different sub-sections have
been subjected to drastically differing grazing intensi-
ties for the past 50 years, it was found that warming
decreased the ecosystem C sink under light grazing,
but had no effect under heavy grazing [29]. The long-
term differences in grazing intensity had induced a
vegetation shift from evergreen and deciduous dwarf
shrubs under light grazing toward graminoids under
heavy grazing [13], and increased soil nutrient avail-
bility, litter and soil decomposition rates and ecosys-
tem respiration [21, 26–29]. Given that the
susceptibility of soil C stocks to warming has a key
importance for the response of the total ecosystem C
stocks to warming, a separate study investigating the
effect of grazing on the C quality and the temperature
sensitivity of CO₂ release in the accumulated soil C was
warranted. We characterized litter and soil at different
long-term grazing intensities, and conducted labora-
tory incubations at different temperatures with litter
and soil. Soil and litter was analyzed using solid-state
cross-polarization magic angle spinning nuclear mag-
netic resonance (CPMAS 13C NMR) spectroscopy,
which is a powerful tool for characterizing the struc-
ture of soils and litters [30–32]. We based our hypo-
heses on the predictions of the kinetic theory. Because
vegetation is more lignin rich under light grazing than
heavy grazing [13, 28], we first hypothesized that (1)
litter and soil C under light grazing should be more
aromatic and recalcitrant to microbial decomposition
[2]; therefore, lower rates of CO₂ release should be
observed in soil incubations compared with those of
soils under heavy grazing. Because high aromaticity is
often linked with a higher temperature sensitivity of
decomposition [2, 3, 20], we hypothesized that (2) soil
C decomposition rates should show greater increases
with temperature in soils under light grazing than
those under heavy grazing.

Materials and methods

Study site

The study site was a mesic, nutrient-rich tundra heath
(Raisduoddar (69°31’N, 21°19’E), located in North-
ernmost Norway). The soil is classified as Inceptisol,
and has a coarse texture typical of mountain soils being
composed of sand and silt fractions with a consider-
able gravel component. The soils are freely draining
with a surface organic horizon of 0.5–11 cm thick,
while pH varies from 4.8 to 5.4 independent of grazing.
The vegetation community is characterized as an
Arctic Empetrum–Dicranum–Lichen type heath [33].
Because of a pasture rotation fence built in the 1960s,
one sub-section of lightly grazed tundra (LG) is briefly
used as a passage, whereas the other sub-section of
heavily grazed tundra (HG) is subjected to intensive
grazing during reindeer migration. The highest graz-
ing intensity is encountered in a 50 m wide and several
kilometers long zone on the HG side of the fence
during the first weeks of August, when reindeer gather
near the fence before migrating to the winter ranges
[28]. The abundances of evergreen and deciduous
dwarf shrubs are higher in the LG tundra, while the
abundances of graminoids, plant productivity, soil
nutrient availability and soil temperature are consider-
ably higher in the HG tundra [13, 21, 27, 28]. The
average soil temperatures for June–August 2010 mea-
sured at approximately 3 cm depth (n = 3, EasyLog
EL-USB, Lascar Electronics, Erie, Pennsylvania, USA)
were 7.9 ± 0.2 °C and 9.2 ± 0.2 °C (mean ± S.E.)
for the LG and HG tundra communities, respectively.

Soil and litter sampling

Five blocks were established along the reindeer fence
that separates LG and HG sub-sections (distance
between blocks >20 m) in 2010. Within each block,
we selected plots with similar exposure and hydro-
logical status of approximately 1 × 1 m on both the
LG and HG sides of the reindeer fence (distance
between plots with differing grazing intensity <20 m).
Soil material was collected before the annual reindeer
migration (8 August 2010) by coring approximately
1 kg of fresh soil, which corresponded to 3–5 soil cores
(diameter 10 cm) of 5–10 cm depth in the soil organic
layer. In the laboratory, soils were sieved (2 mm mesh size) and pre-incubated for 2–3 months at 4 °C to deplete soils of the most labile C substances. Senescent leaves of bilberry (*Vaccinium myrtillus* L.), bog bilberry (*Vaccinium uliginosum* L.), dwarf birch (*Betula nana* L.) and mountain crowberry (*Empetrum nigrum* L. ssp. *hermaphroditum*, Hagerup) were collected from LG tundra and senescent stems and leaves of the dominant sedge species (*Carex bigelowii* L.) from HG tundra at the end of the growing season (GS) (17 September 2010). Numerous plant individuals were sampled from several locations within the study area. Unsorted root biomass from the LG and HG tundra and composite moss litter (*Dicranum* spp., *Polytrichum* spp., *Pleurozium schreberi*) from the LG site were collected in July 2011. Litter samples were pooled by species, whereas root biomass was pooled by grazing intensity. All of the samples were stored at 4 °C (2 weeks) before chemical analyses.

**Soil properties and C quality using solid-state 13C NMR**

The moisture (105 °C, 12 h) and OM content (loss on ignition at 475 °C, 4 h) of the fresh soil and litter samples were determined gravimetrically; the total C and nitrogen (N) concentrations were analyzed (EA 1110 CHNS-O) as the % dry weight, and these amounts were used to calculate the C:N ratios and soil C stock (kg m⁻²). To characterize the litter and soil C quality at different levels of grazing intensity, we used solid-state 13C CP/MAS NMR spectroscopy. An NMR analysis was conducted for fresh soils (*n* = 5) and litter (*n* = 1). Sub-samples of sieved soil and mixed litter were dried (two days, 60 °C), ground to a fine powder, and treated with 4 M HCl to increase the signal-to-noise ratio [34]. Comparisons between the NMR spectra for untreated and HCl-treated samples, which had high signal to noise ratios, showed that the HCl treatment did not affect the shape of the spectra. We acquired CP/MAS 13C NMR spectra for soil (initial, at 19 °C) and litter samples using a DSX200 spectrometer (Bruker, Coventry, UK) equipped with double-boring cylindrical probes (4 mm) for cross polarization and magic angle spinning (detailed description for data acquisition parameters and conditions see [35]). Bruker WinNMR software was used to measure the peak areas for the following chemical shift regions: 0–50 ppm, (aliphatic O-unsaturated), 50–60 ppm (methoxyls), 60–90 ppm (carbohydrates), 90–110 (carbohydrates and aliphatic lignin), 110–160 (aromatic lignin), and 160–210 (carboxyl). Areas of the chemical shift regions were expressed as percentages of the total area, and all of the NMR results are expressed as a % of the total C. The chemical shift regions were treated as functional classes of C. Carbohydrates and methoxyls are labile substrates easily degradable for many soil microorganisms, whereas aromatic lignin, aliphatic non-O-substituted and carboxyls are more resistant to microbial decomposition and contribute to the formation of soil OM [36]. Aromaticity and alkyl-to-O-alkyl ratios were calculated to describe the decomposability of litter and soil C.

**Soil incubation**

To analyze the rates of CO₂ release and the temperature sensitivity of soil decomposition at different levels of grazing intensity, laboratory incubations at different temperatures were conducted using the pre-incubated soils. First, we conducted soil incubation experiments using constant temperatures (hereafter referred to as constant temperature incubation, *n* = 5). Soil (1 g OM with 60% water-holding capacity (WHC)) samples were incubated at 4 °C, 9 °C, 14 °C and 19 °C in 120 ml glass vials for six months (27 September 2010–31 March 2011), and CO₂ release was analyzed eight times. Air samples (250 μl) were collected in the head space of the incubation bottles and analyzed for CO₂ concentrations using a gas chromatograph (HP 6890 equipped with a TCD detector and micro-packed column) and reported as mg CO₂–C produced per g OM initially present per hour. The CO₂ production at different temperatures (4 °C, 9 °C, 14 °C and 19 °C) under constant temperature incubation was used to calculate the Q₁₀ value (describing the temperature sensitivity of decomposition) by plotting the natural logarithm of CO₂ release against temperature and using the slope (*k*) of the linear regression, *Q*₁₀ = *e^*^*k* *10*. The CO₂ release and *Q*₁₀ were averaged over the measurements. The moisture content was monitored and adjusted to 60% WHC when necessary. To describe the differences in CO₂ release and temperature sensitivity at the beginning and end of the incubations, the results of the first and last measurement are presented (6 October 2010 and 31 March 2011, respectively).

Second, we conducted long-term soil incubation experiments that simulated seasonal incubation cycles between ‘growing seasons’ and ‘winters’ that mimic the field conditions ([37]; hereafter referred to as seasonal incubation, *n* = 5). Each cycle consisted of a GS (eight weeks at 19 °C, 14 °C and 9 °C) and winter (6–7 weeks at −5 °C). The length of the ‘GS’ was based on a finding that the soil temperature of our study site is above 9 °C for approximately eight weeks [28]. The soil samples were weighed (20 g fresh weight) into 500 ml glass bottles, and the moisture content was adjusted to 60% WHC. During each ‘GS’, the CO₂ release was analyzed four times as described above. The ‘winter’ CO₂ release at −5 °C was measured once after the first GS and assumed to be the same during the subsequent winters. The duration of the soil incubation was three complete cycles, which resulted in 299 d of incubation (30 December 2010–24 October 2011). Time-integrated CO₂–C loss estimates for different temperatures and grazing intensities in the seasonal incubation were calculated. The seasonal average CO₂ fluxes were calculated and further used to
calculate the average seasonal Q10. To analyze changes in the soil C quality during the incubation, we also characterized the post-incubation soils, which had been incubated at 19°C, using NMR analyses and similar protocols used with fresh soils. For each soil functional C class, the absolute C change between fresh and post-incubation soils was calculated according to the calculated cumulative C losses and expressed as g C lost per g initial C.

**Statistical analyses**

The effects of grazing intensity and temperature on the GS CO2 release were analyzed with a mixed model that included grazing (G) and temperature (T) as fixed factors, block nested with grazing as a random factor, and GS as a repeated factor. Because of statistically significant interactions, the effects of T and GS were also analyzed separately for the LG and HG sites. The treatment effects on seasonal Q10 were analyzed without T. Soil C stock, soil quality and Q10 at the first and last measurement under constant temperature incubation were tested with grazing as a fixed factor and block nested with grazing as a random factor, whereas for the CO2 release rates, T was included as a fixed factor. A Bonferroni test was used as a post hoc test to detect differences in T and GS between LG and HG tundra. All of the statistical analyses were conducted with IBM SPSS Statistics 21 for Windows (IBM SPSS, Inc., Chicago, IL, USA).

**Results**

**Chemical quality of litter C**

We characterized litter quality using one composite sample per litter type, and therefore, these analyses are considered qualitative. The proportion of aromatic lignin and aromaticity were higher in dwarf shrub litter than in graminoid litter (table 1). Of the analyzed litter types, moss litter (collected only from LG) showed the lowest proportion of aromatic lignin and aromaticity (table 1). Dwarf shrub litter and moss litter showed a higher proportion of aliphatic not-O-substituted C and higher ratio of alkyl to O-alkyl than graminoid litter. In the root biomass, the aromaticity did not differ by grazing intensity; however, the roots under HG showed a higher proportion of aliphatic not-O-substituted C and higher ratio of alkyl to O-alkyl than did the roots under LG, and the roots under HG showed a higher proportion of carbohydrates (table 1). The proportion of carbohydrates was high in the graminoid and moss litter (table 1).

**Chemical quality of soil C**

Similar to litter, soil carbohydrates and aliphatic not-O-substituted C constituted the most abundant functional C classes (table 1). The patterns in aromaticity observed in litters were not found in the underlying soil because the average aromaticity did not differ between LG and HG soils (tables 1 and 2). In contrast, the proportion of carbohydrates was higher under LG, whereas the proportion of aliphatic not-O-substituted C and ratio of alkyl to O-alkyl were higher under HG (tables 1 and 2). There was no significant difference in soil C stock between grazing intensities (F1, 14 = 0.35, P = 0.56; 2.7 ± 0.4 and 3.1 ± 0.5 kg m–2 for HG and LG, respectively). However, the CN ratio was significantly lower (F1, 8 = 25.97, P < 0.01) in the HG soils (17.6 ± 1.3) compared with that of the LG (28.6 ± 1.7) soils.

**CO2 release and Q10 in constant temperature incubation**

At the beginning of the constant temperature incubation, the CO2 release from soils did not differ by grazing intensity (no G effect, F1, 8 = 0.01, P = 0.94; figure 1(a)); however, the Q10 value was higher in the HG than LG soils (F2, 24 = 6.26, P = 0.04; figure 1(a)). During the incubation, the CO2 release and Q10 varied according to the grazing intensity, and at the end of incubation, Q10 had increased by 64% in LG soils but decreased by 35% in HG soils compared to the initial value. The CO2 release was significantly higher in LG soils compared with that of HG soils at all temperatures except at 4°C (significant G × T interaction, $F_{3, 24} = 36.18$, P < 0.01; figure 1(b)), and the Q10 in LG soils was over two-fold higher than the Q10 in HG soils ($F_{1, 8} = 33.84$, P < 0.01; figure 1(b)).

**CO2 release, Q10 and C losses during seasonal incubation**

During seasonal incubation, the average growing season CO2 release over all temperatures was 75% higher in the LG soils relative to that of the HG soils (significant G effect, $F_{1, 9} = 8.87$, P = 0.02). The CO2 release rate increased with temperature (significant T effect, $F_{4, 60} = 199.08$, P < 0.01; figures 2(a) and (b))), although the effects of temperature varied temporally and according to the grazing intensity, with the temperature-induced increase in CO2 release intensifying in LG soils during the soil incubation (significant GS × G × T interaction, $F_{6, 40} = 3.99$, P < 0.01; figures 2(a) and (b)). Q10 was higher in LG soils relative to HG soils throughout the incubation (significant G effect, $F_{1, 17} = 11.12$, P < 0.01; figures 2(a) and (b)). The cumulative CO2-C loss during incubation was higher in LG soils relative to HG soils. Changes in soil functional C classes during incubation at 19°C were relatively similar in soils under both grazing intensities. Whereas the proportions of aromatic lignin, carbonyl/carbonyl C and aromaticity decreased; proportions of aliphatic not-O-substituted C and alkyl-to-O-alkyl ratios increased; and proportions of methoxyl C, carbohydrates and aliphatic lignin remained unchanged (table 1). The cumulative losses of carbohydrates and aliphatic not-O-substituted C, however, were significantly higher in LG soils relative to HG soils (figure 3, table 2).
Table 1. The relative proportions of functional C classes expressed as a % of the total C for fresh and post-incubation soils incubated at 19 °C and the dominant litter types for the lightly grazed (LG) and heavily grazed (HG) tundra. The soil values are presented as the mean ± SE, and the litter types are presented as the mean.

| Litter Type | Fresh soil | Post-incubation soil |
|-------------|------------|----------------------|
| Aliphatic not O-substituted 0–50 ppm | 32.7 ± 1.9 | 38.7 ± 1.6 |
| Methoxyl 50–60 ppm | 6.0 ± 0.8 | 7.1 ± 0.3 |
| Carbohydrate 60–90 ppm | 34.3 ± 1.1 | 34.5 ± 0.9 |
| Carbohydrate and aliphatic lignin 90–110 ppm | 9.2 ± 0.7 | 8.4 ± 0.4 |
| Aromatic lignin 110–160 ppm | 11.3 ± 0.6 | 8.2 ± 0.7 |
| Carboxyl/carbonyl 160–210 ppm | 6.6 ± 1.8 | 3.2 ± 0.4 |
| Aromaticity ^a | 0.11 ± 0.01 | 0.08 ± 0.01 |
| Alkyl-to-O-alkyl ratio ^b | 0.66 ± 0.04 | 0.78 ± 0.05 |

LG soil

| Litter Type | Fresh soil | Post-incubation soil |
|-------------|------------|----------------------|
| Fresh soil | 27.9 ± 0.6 | 33.4 ± 1.3 |
| Post-incubation soil | 5.1 ± 0.5 | 6.6 ± 0.3 |
| Carbohydrate 60–90 ppm | 39.7 ± 1.4 | 40.6 ± 1.3 |
| Carbohydrate and aliphatic lignin 90–110 ppm | 10.4 ± 0.6 | 9.9 ± 0.3 |
| Aromatic lignin 110–160 ppm | 11.1 ± 0.4 | 7.4 ± 0.4 |
| Carboxyl/carbonyl 160–210 ppm | 5.7 ± 2.0 | 2.1 ± 0.3 |
| Aromaticity ^a | 0.11 ± 0.00 | 0.07 ± 0.00 |
| Alkyl-to-O-alkyl ratio ^b | 0.51 ± 0.02 | 0.59 ± 0.03 |

HG soil

| Litter Type | Fresh soil | Post-incubation soil |
|-------------|------------|----------------------|
| Fresh soil | 22.5 ± 0.3 | 28.4 ± 0.3 |
| Post-incubation soil | 4.5 ± 0.2 | 6.0 ± 0.2 |
| Carbohydrate 60–90 ppm | 33.0 ± 1.2 | 34.0 ± 1.2 |
| Carbohydrate and aliphatic lignin 90–110 ppm | 7.7 ± 0.3 | 9.3 ± 0.4 |
| Aromatic lignin 110–160 ppm | 11.3 ± 0.6 | 7.7 ± 0.5 |
| Carboxyl/carbonyl 160–210 ppm | 5.6 ± 0.7 | 3.0 ± 0.4 |
| Aromaticity ^a | 0.11 ± 0.01 | 0.08 ± 0.01 |
| Alkyl-to-O-alkyl ratio ^b | 0.66 ± 0.04 | 0.78 ± 0.05 |

HG litter

| Litter Type | Fresh soil | Post-incubation soil |
|-------------|------------|----------------------|
| Sedge | 13.4 | 14.5 |
| Roots | 24.5 | 34.5 |

For different litter types, the values are single determinations of composite samples.

^a Calculated as aromatic lignin/total signal from all compounds.

^b Calculated as aliphatic not-O-substituted/(methoxyl + carbohydrate + carbohydrate and aliphatic lignin).
Table 2. The effect of grazing on relative proportions of soil functional C classes, indexes for fresh soils, and the absolute functional C-class changes during incubation. *F*-values and their corresponding df-values are presented.

|                  | Aliphatic not-O-substituted | Methoxyl     | Carbohydrate | Carbohydrate aliphatic lignin | Aromatic lignin | Carboxyl/carbonyl | Aromaticity | Alkyl-to-O-alkyl ratio |
|------------------|-----------------------------|--------------|--------------|-------------------------------|-----------------|-------------------|-------------|------------------------|
| Fresh soil       | $F_{1,8} = 5.60^*$          | $F_{1,8} = 0.83$ | $F_{1,8} = 9.26^*$ | $F_{1,8} = 1.61$            | $F_{1,8} = 0.05$ | $F_{1,8} = 0.10$  | $F_{1,8} = 0.05$ | $F_{1,8} = 11.37^{**}$ |
| C change         | $F_{1,8} = 5.59^*$          | $F_{1,8} = 0.75$ | $F_{1,8} = 7.10^*$ | $F_{1,8} = 2.41$            | $F_{1,8} = 4.34$ | $F_{1,8} = 0.05$  |             |                        |

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p < 0.001$. 
Discussion

Because the LG tundra is dominated by woody dwarf shrubs and the HG tundra is dominated by graminoids, we predicted higher soil C aromaticity, lower CO$_2$ release rates from soils, and higher temperature sensitivity under light grazing than heavy grazing [2, 20]. Opposite to our hypothesis, soil aromaticity did not differ according to grazing intensity despite the drastically higher aromaticity of the dominant litter types in LG areas. Furthermore, the effects of temperature on CO$_2$ release rates showed complex interactions...
between the grazing intensities that varied for the different phases of soil incubation. Initially, the CO₂ release rates did not differ between the grazing intensities and the temperature sensitivity of decomposition was lower under light grazing than heavy grazing (figure 1(a)). However, as the duration of incubation increased, the CO₂ release rate and temperature sensitivity increased in soils under light grazing compared with soils under heavy grazing (figures 1(b) and 2). The microbial responses to temperature under short-term soil incubations may depict the initial responses of microbial activities to temperature, whereas microbial responses to temperature under long-term soil incubation may reflect the responses of soil microbial activities under sustained higher temperatures (e.g., [38]). Soils incubated at higher temperatures may be depleted of labile C substrates at a faster rate compared with soils incubated at low temperatures [25, 39, 40]. Post-incubation temperature sensitivities and shifts in Q₁₀ during the course of incubation could thus reflect differing post-incubation C quality rather than temperature sensitivity alone. Our findings indicate that under prolonged warming, a long history of high grazing intensity might dampen the effects of increasing temperatures on the decomposition of accumulated soil C.

Instead of differing by soil C aromaticity, long-term grazing intensity altered the proportions of carbohydrate and aliphatic C, with the proportion of carbohydrate C higher in soils under light grazing and proportion of aliphatic not-O-substituted C higher in soils under heavy grazing. Similar soil C aromaticity at both levels of grazing intensity despite a drastic difference in the dominant vegetation aromaticity could result from a higher capacity of the soil microbial community for lignin degradation under light grazing. The higher carbohydrate abundance in soils under light grazing may be caused by higher moss abundance in the vegetation [29]. Moss biomass is largely composed of carbohydrates and considered to contribute significantly to soil C accumulation in the tundra [10]. A higher proportion of aliphatic not-O-substituted C in soils under heavy grazing may result from the dense fibrous mats of root biomass produced by the graminoid-dominant vegetation [9] as graminoid roots contain high concentrations of decomposition-resistant aliphatic compounds that often accumulate in soils [41, 42]. Higher alkyl-to-O-alkyl ratios in soils under heavy grazing may also reflect a more advanced state of decomposition [30, 43]; this would be consistent with observations that soil microbial activity is generally higher in soils under heavy than light grazing [21, 27, 28].

Considering parallel findings of higher soil C quality and decomposition temperature sensitivity, it is possible that the higher proportion of carbohydrate C under light grazing explains the increased CO₂ release rates under prolonged warming. This hypothesis is supported by the finding that the total carbohydrate-C loss during the incubation was higher under light than heavy grazing (figure 3). Mid- to long-term temperature sensitivities of microbial respiration are primarily driven by the availability of readily decomposable C [39, 44]. Tundra soils harbor large portions of bioavailable and potentially degradable C, which is one of the primary causes of the high vulnerability of accumulated tundra soil C to increasing temperatures [10, 36, 37, 45–47]. CO₂ release rates in tundra correlate also positively with the proportion of polysaccharides in the accumulated soil C [48]. Because soil carbohydrates are often stored in ligno–cellulose complexes and protected by lignin [31, 49], they would be degraded at later stages of soil incubation, thus explaining why CO₂ release rates were higher under light grazing only as the soil incubation progressed. Soils under light grazing could thus harbor substantially greater amounts of potentially mineralizable C than soils under heavy grazing and release larger quantities of C under warmer climate.

In addition to soil C quality, the effect of grazing on microbial temperature adaptation or soil nutrient...
availability could underlie the differing effects of temperatures on microbial activity. Soil temperatures are higher under heavy than light grazing [28]. In another laboratory study at this site, we found that extracellular enzymes under light grazing catalyzed OM degradation more efficiently at low temperatures than that under heavy grazing, indicating differing capacity for temperature adaptation depending on grazing intensity [22]. Increasing temperatures also increased the microbial community composition and induce functional adaptations to higher temperatures [50–52], and this temperature acclimation during the incubation could be stronger under light grazing with initially more cold-adapted microbial community. It is also important to note that soil nutrient availability is drastically higher under heavy relative to light grazing [21]. There are a multitude of mechanisms by which high soil nutrient availability may either intensify or weaken the effects of temperature on microbial CO2 release [25]. It has also been suggested that if nutrients limit microbial growth, a larger proportion of C may be respired to the atmosphere as CO2 (so-called overflow metabolism; [33]).

Our findings of higher temperature sensitivity of CO2 release from accumulated soil C under light grazing contrast with previous studies at the same study site showing higher microbial respiration in fresh soils [21] as well as ecosystem respiration (\(R_{ec}\); the sum of plant and soil faunal respiration, and microbial decomposition of fresh plant litter, plant root exudates and accumulated soil C) under heavy grazing. Warming implemented using open-top chambers also increased \(R_{ec}\) similarly at both levels of grazing intensity [29]. Increased \(R_{ec}\) by warming resulted in negligible effect on the C sink under heavy grazing due to higher gross ecosystem production (GEP) [29]. Field observations reflect the balance between GEP and \(R_{ec}\), whereas the data from the present investigation depict the response of accumulated soil C to increasing temperatures. The divergent findings of field and laboratory studies suggest that plant respiration is probably more important source for increased \(R_{ec}\) under warming but that prolonged warming may trigger stronger response in the decomposition of accumulated soil C pool under light grazing. Given that soil C constitutes the largest ecosystem C stock in tundra and arctic tundra stores half of the global soil C [1], this is an important finding. Grazing by domestic and wild ungulates is the most widespread land use worldwide [54], and large grazers induce vegetation shifts across biomes and climatic vegetation zones [55]. Grazers have been demonstrated to influence soil C stability and the temperature sensitivity of decomposition in temperate grasslands [56, 58]. Our investigation in tundra demonstrates for the first time that the effects of grazing on the temperature sensitivity of decomposition may result from differences in the quality of accumulated soil C. In our study site, a reduction in the soil C quality in response to grazing coincided with a weaker response of decomposition to increasing temperature. These findings indicate that grazers have a potential to limit warming-induced climate feedback from enhanced soil C decomposition.

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