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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 ^{13}C 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*

tarandus L.) especially in Northernmost Eurasia where the management of semi-domesticated reindeer constitutes 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 changes in soils induced by grazing may influence the sensitivity 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-dominated 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 degradation of labile C [2, 20]. Along with soil C quality, grazing causes substantial changes in the soil microclimate and nutrient concentrations [21, 22], which could also alter soil microbial responses to increasing temperatures. The decomposition rates are regulated by complex interactions among soil C quality, substrate diffusion, soil microbial temperature acclimation, and nutrient stoichiometry [23–25]. Grazers in turn simultaneously 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 history 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 intensities 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 availability, litter and soil decomposition rates and ecosystem 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 CO_2 release in the accumulated soil C was warranted. We characterized litter and soil at different long-term grazing intensities, and conducted laboratory incubations at different temperatures with litter and soil. Soil and litter was analyzed using solid-state cross-polarization magic angle spinning nuclear magnetic resonance (CPMAS ^{13}C NMR) spectroscopy, which is a powerful tool for characterizing the structure of soils and litters [30–32]. We based our hypotheses 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 CO_2 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 Northernmost 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 considerable 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 grazing 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 considerably higher in the HG tundra [13, 21, 27, 28]. The average soil temperatures for June–August 2010 measured 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 hydrological 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 ^{13}C 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 ^{13}C CPMAS 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 CPMAS ^{13}C NMR spectra for soil (initial, at 19 °C) and litter samples using a DSX200 spectrometer (Bruker, Coventry, UK) equipped with double-bearing 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 not-O-substituted*), 50–60 ppm (*methoxyls*), 60–90 ppm (*carbohydrates*), 90–110 (*carbohydrates and aliphatic lignin*), 110–160 (*aromatic lignin*), and 160–210 (*carboxyl/carbonyl*). 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 CO_2 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 CO_2 release was analyzed eight times. Air samples (250 μl) were collected in the head space of the incubation bottles and analyzed for CO_2 concentrations using a gas chromatograph (HP 6890 equipped with a TCD detector and micro-packed column) and reported as mg CO_2–C produced per g OM initially present per hour. The CO_2 production at different temperatures (4 °C, 9 °C, 14 °C and 19 °C) under constant temperature incubation was used to calculate the Q_{10} value (depicting the temperature sensitivity of decomposition) by plotting the natural logarithm of CO_2 release against temperature and using the slope (k) of the linear regression, $Q_{10} = e^{(10*k)}$. The CO_2 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 CO_2 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 CO_2 release was analyzed four times as described above. The 'winter' CO_2 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 CO_2–C loss estimates for different temperatures and grazing intensities in the seasonal incubation were calculated. The seasonal average CO_2 fluxes were calculated and further used to

calculate the average seasonal Q_{10}. 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 CO_2 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 Q_{10} were analyzed without T. Soil C stock, soil quality and Q_{10} 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 CO_2 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 LG 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 ($F_{1,\ 14} = 0.35$, $P = 0.56$; 2.7 ± 0.4 and 3.1 ± 0.5 kg m^{-2} for HG and LG, respectively). However, the C:N ratio was significantly lower ($F_{1,\ 8} = 25.97$, $P < 0.01$) in the HG soils (17.6 ± 1.3) compared with that of the LG (28.6 ± 1.7) soils.

CO_2 release and Q_{10} in constant temperature incubation

At the beginning of the constant temperature incubation, the CO_2 release from soils did not differ by grazing intensity (no G effect, $F_{1,\ 8} = 0.01$, $P = 0.94$; figure 1(a)); however, the Q_{10} value was higher in the HG than LG soils ($F_{1,\ 8} = 6.26$, $P = 0.04$; figure 1(a)). During the incubation, the CO_2 release and Q_{10} varied according to the grazing intensity, and at the end of incubation, Q_{10} had increased by 64% in LG soils but decreased by 35% in HG soils compared to the initial value. The CO_2 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 Q_{10} in LG soils was over two-fold higher than the Q_{10} in HG soils ($F_{1,\ 8} = 33.84$, $P < 0.01$; figure 1(b)).

CO_2 release, Q_{10} and C losses during seasonal incubation

During seasonal incubation, the average growing season CO_2 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 CO_2 release rate increased with temperature (significant T effect, $F_{2,\ 60} = 199.08$, $P < 0.01$; figures 2(a) and (b)), although the effects of temperature varied temporally and according to the grazing intensity, with the temperature-induced increase in CO_2 release intensifying in LG soils during the soil incubation (significant GS × G × T interaction, $F_{4,40} = 3.99$, $P < 0.01$; figures 2(a) and (b)). Q_{10} 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 CO_2–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, carboxyl/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 $\pm$ SE, and the litter types are presented as the mean.

		Aliphatic not O-substituted 0–50 ppm	Methoxyl 50–60 ppm	Carbohydrate 60–90 ppm	Carbohydrate and aliphatic lignin 90–110 ppm	Aromatic lignin 110–160 ppm	Carboxyl/carbonyl 160–210 ppm	Aromaticity[a]	Alkyl-to-O-alkyl ratio[b]
LG, soil									
	Fresh soil	27.9 ± 0.6	5.1 ± 0.5	39.7 ± 1.4	10.4 ± 0.6	11.1 ± 0.4	5.7 ± 2.0	0.11 ± 0.00	0.51 ± 0.02
	Post-incubation soil	33.4 ± 1.3	6.6 ± 0.3	40.6 ± 1.3	9.9 ± 0.3	7.4 ± 0.4	2.1 ± 0.3	0.07 ± 0.00	0.59 ± 0.03
LG, litter									
	Mountain crowberry	42.3	4.1	32.0	7.2	11.3	3.1	0.11	0.98
	Dwarf birch	27.8	5.2	39.2	10.3	14.4	3.1	0.14	0.51
	Bog bilberry	24.2	5.1	43.4	10.1	14.1	3.0	0.14	0.41
	Bilberry	25.5	5.3	43.6	10.6	11.7	3.2	0.12	0.43
	Mosses	25.5	7.1	51.0	12.2	3.1	1.0	0.03	0.36
	Roots	20.4	7.1	51.0	12.2	9.2	0	0.09	0.29
HG, soil									
	Fresh soil	32.7 ± 1.9	6.0 ± 0.8	34.3 ± 1.1	9.2 ± 0.7	11.3 ± 0.6	6.6 ± 1.8	0.11 ± 0.01	0.66 ± 0.04
	Post-incubation soil	38.7 ± 1.6	7.1 ± 0.3	34.5 ± 0.9	8.4 ± 0.4	8.2 ± 0.7	3.2 ± 0.4	0.08 ± 0.01	0.78 ± 0.05
HG, litter									
	Sedge	13.4	7.2	56.7	12.4	8.2	2.1	0.08	0.18
	Roots	24.5	9.2	43.9	11.2	10.2	1.0	0.10	0.38

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	$F_{1,8} = 5.59^{*}$	$F_{1,8} = 0.75$	$F_{1,8} = 7.10^{*}$	$F_{1,8} = 2.41$	$F_{1,8} = 4.34$	$F_{1,8} = 0.05$		

$^{*}p \leqslant 0.05, ^{**}p \leqslant 0.01, ^{***}p < 0.001.$
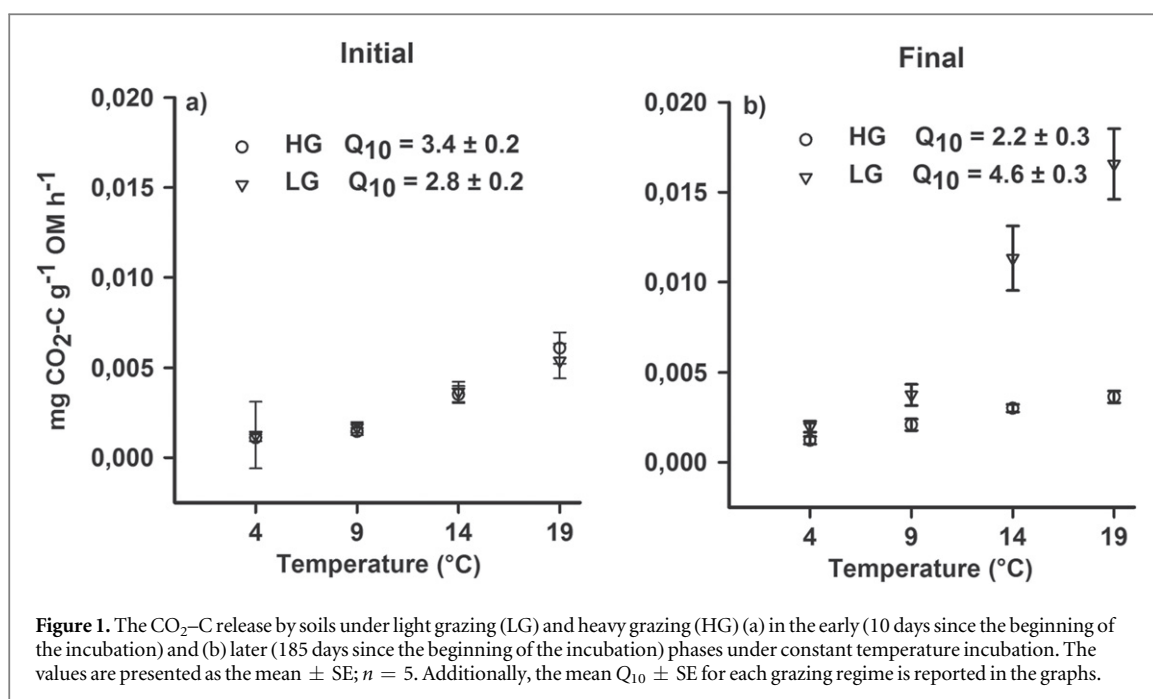
Figure 1. The CO_2–C release by soils under light grazing (LG) and heavy grazing (HG) (a) in the early (10 days since the beginning of the incubation) and (b) later (185 days since the beginning of the incubation) phases under constant temperature incubation. The values are presented as the mean $\pm$ SE; $n = 5$. Additionally, the mean $Q_{10} \pm$ SE for each grazing regime is reported in the graphs.
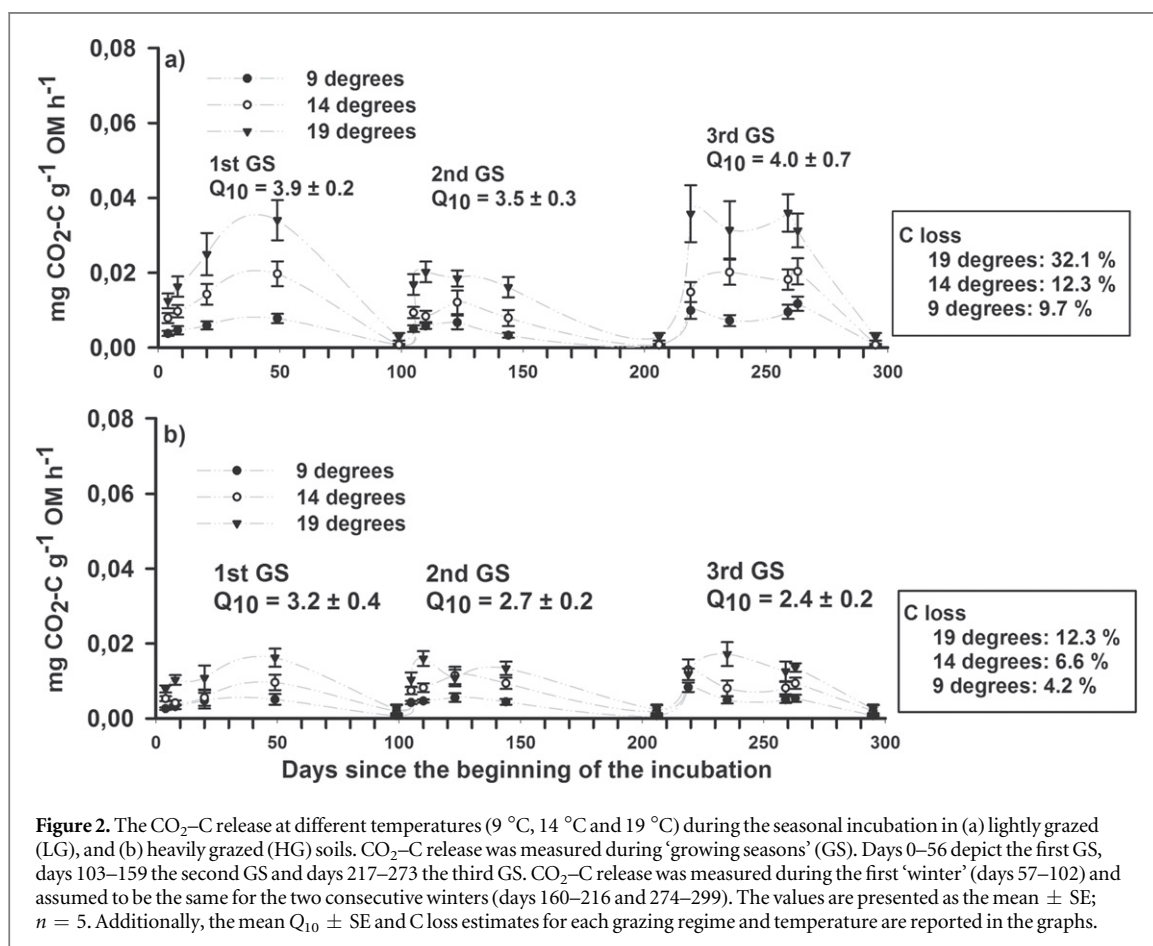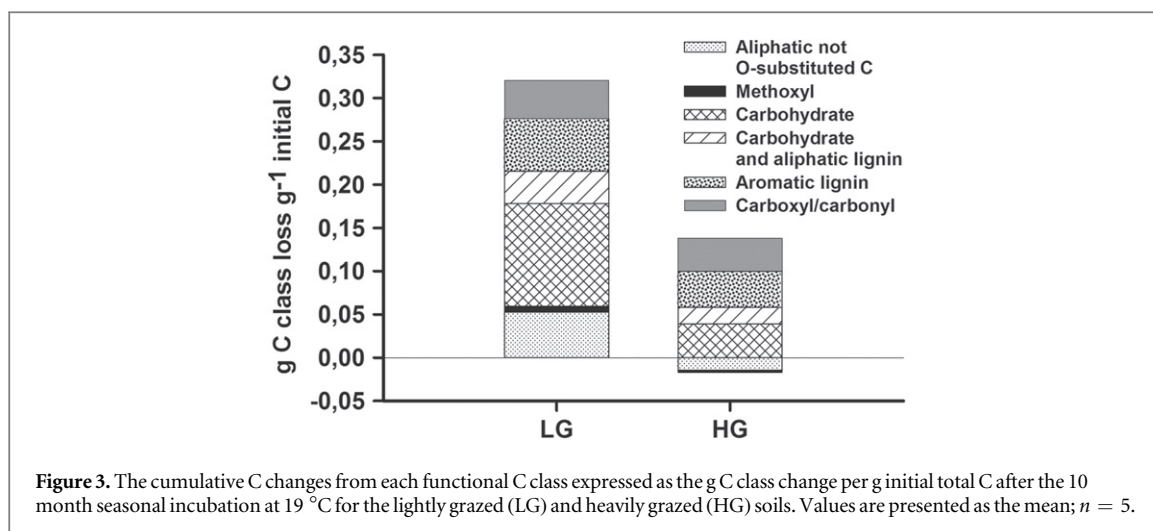

Figure 2. The CO_2–C release at different temperatures (9 °C, 14 °C and 19 °C) during the seasonal incubation in (a) lightly grazed (LG), and (b) heavily grazed (HG) soils. CO_2–C release was measured during 'growing seasons' (GS). Days 0–56 depict the first GS, days 103–159 the second GS and days 217–273 the third GS. CO_2–C release was measured during the first 'winter' (days 57–102) and assumed to be the same for the two consecutive winters (days 160–216 and 274–299). The values are presented as the mean $\pm$ SE; $n = 5$. Additionally, the mean $Q_{10} \pm$ SE and C loss estimates for each grazing regime and temperature are reported in the graphs.

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

Figure 3. The cumulative C changes from each functional C class expressed as the g C class change per g initial total C after the 10 month seasonal incubation at 19 °C for the lightly grazed (LG) and heavily grazed (HG) soils. Values are presented as the mean; $n = 5$.

between the grazing intensities that varied for the different phases of soil incubation. Initially, the CO_2 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 CO_2 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 Q_{10} 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 CO_2 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]. CO_2 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 CO_2 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 alter 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 CO_2 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 CO_2 (so-called overflow metabolism; [53]).

Our findings of higher temperature sensitivity of CO_2 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_e; 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_e similarly at both levels of grazing intensity [29]. Increased R_e 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_e, 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_e 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.

Acknowledgments

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