



Herbivory changes soil microbial communities and greenhouse gas fluxes in a high-latitude wetland

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Received: 8 December 2020 / Accepted: 9 March 2021

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Abstract

Herbivory can have strong impacts on greenhouse gas fluxes in high-latitude ecosystems. For example, in the Yukon-Kuskokwim (Y-K) Delta in western Alaska, migratory goose grazing affects the magnitude of soil carbon dioxide (CO₂) and methane (CH₄) fluxes. However, the underlying drivers of this relationship are unclear, as few studies systematically tease apart the processes by which herbivores influence soil biogeochemistry. To examine these mechanisms in detail, we conducted a laboratory incubation experiment to quantify changes in greenhouse gas fluxes in response to three parameters altered by herbivores in situ: temperature, soil moisture content, and nutrient inputs. These treatments were applied to soils collected in grazing lawns and nearby ungrazed habitat, allowing us to assess how variation in microbial community structure influenced observed responses. We found pronounced differences in both fungal and prokaryotic community composition between grazed and ungrazed areas. In the laboratory incubation experiment, CO₂ and CH₄ fluxes increased with temperature, soil moisture, and goose fecal addition, suggesting that grazing-related changes in the soil abiotic environment may enhance soil C losses. Yet, these abiotic drivers were insufficient to explain variation in fluxes between soils with and without prior grazing. Differences in trace gas fluxes between grazed and ungrazed areas may result both from herbivore-induced shifts in abiotic parameters and grazing-related alterations in microbial community structure. Our findings suggest that relationships among herbivores and soil microbial communities could mediate carbon-climate feedbacks in rapidly changing high-latitude ecosystems.

Keywords Carbon dioxide · Grazing · Methane · Migratory geese · Soil carbon cycling · Tundra · Yukon-Kuskokwim Delta

Introduction

High-latitude ecosystems contain approximately one third of the global terrestrial carbon (C) stock [1] and are experiencing unprecedented climate change, with poorly understood consequences for global C-climate feedbacks. Within these high-latitude ecosystems, climate-induced shifts in soil C cycling can be strongly mediated by herbivory; however, the direction

and magnitude of herbivore effects on greenhouse gas (GHG) fluxes from high-latitude soils vary widely. These uncertainties are especially large in high-latitude wetlands, which are responsible for a large fraction of global methane (CH₄) emissions [2] and which are vulnerable to both increasing warming and altered hydrological regimes [3]. We require a more generalizable framework for studying the effects of herbivores on GHG dynamics in high-latitude wetlands, which can only be achieved through a more mechanistic understanding of interactions between herbivores and soil biogeochemistry.

The mechanisms responsible for altered GHG fluxes under herbivory are likely multifactorial and therefore difficult to disentangle with purely observational studies. Grazing changes plant biomass and community composition [4], which in turn impacts soil moisture, temperature, and nutrient availability [5]. For example, reduced rates of evapotranspiration can occur in grazed wetland areas due to lower plant biomass, resulting in wetter soils [6]. Lower plant biomass in grazed areas can also increase soil temperatures by enhancing direct insolation [6, 7]. Grazing enhances nutrient availability via

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changes in plant tissue chemistry [4, 8], increased root exudation [9, 10], and herbivore fecal waste [11, 12]. Grazing in wetlands can also directly affect soil redox status, because the abovementioned changes in vegetation and soil microclimate, combined with trampling, may reduce soil oxygen levels [13] and thereby enhance the production of CH₄. Finally, each of the abovementioned mechanisms can influence the soil microbial communities that govern major aspects of nutrient cycling [14].

Whether the direction and magnitude of GHG shifts are dependent on soil microbial community structure is a central uncertainty in predicting herbivore effects on ecosystem C cycling. If changes in microbial community structure are unrelated to the dynamics of organic matter decomposition, then the impacts of herbivory on soil C cycling can be predicted based on herbivore-induced changes in abiotic parameters alone (e.g., soil moisture, temperature, and organic matter inputs). However, it is increasingly recognized that microbial communities can influence the shape of the relationship between environmental drivers (e.g., temperature) and biogeochemical processes [15, 16]. Microbial community shifts might amplify or dampen relationships between soil abiotic variables and GHG fluxes [17]. For example, thermal adaptation and compositional changes within microbial communities can attenuate the increase in CO₂ flux generally observed under warming [18].

The importance of herbivore-plant-microbe interactions for the strength of the high-latitude carbon sink is exemplified by the ecological impacts of migratory geese, which are among the most abundant herbivores in high-latitude wetlands. Multiple species of geese have been shown to have strong impacts on whole-ecosystem C balance. At some wetland sites, geese can reduce CO₂ uptake by reducing plant biomass and altering community structure, transforming ecosystems from C sinks to sources [12, 19]. High densities of geese have also been shown to alter the dynamics of microbial biomass growth and nitrogen cycling [20]. However, few studies have attempted to disentangle the interconnected biotic and abiotic mechanisms by which goose herbivory mediates CO₂ and CH₄ fluxes emitted from high-latitude wetland soils. To do so, we examined a coastal wetland ecosystem on the Y-K Delta, where grazing by Pacific black brant (*Branta bernicla nigricans*) affects CO₂ and CH₄ fluxes in situ [5, 21]. We first characterized soil microbial communities in grazing lawns and adjacent ungrazed habitat in Y-K Delta wetlands. Then, in a laboratory incubation experiment, we manipulated each of the parameters modified by Pacific black brant in the field. In comparison with nearby ungrazed habitat, grazing lawns are wetter, warmer, and receive higher nutrient inputs through fecal deposition [5]; therefore, we manipulated soil temperature, moisture content, and nutrient availability. We applied these treatments to soils sampled from both grazing lawns and ungrazed meadows, which permitted direct comparison of

GHG fluxes in soils maintained at the same temperature and moisture content but with different grazing regimes.

Methods

Study site

The study site is located near the Tutakoke River in the central coastal region of the Yukon-Kuskokwim (Y-K) Delta (61.24 N, −165.63 W) in western Alaska, USA. Mean monthly temperatures range from −14°C in midwinter to 10°C in midsummer, and the mean annual precipitation is approximately 43 cm [22]. The Y-K Delta is an important breeding area for many migratory birds, including Pacific black brant (*Branta bernicla nigricans*), which graze in meadows dominated by *Carex subspathacea*. By returning to the same sites year after year, Pacific black brant create mosaic patches of shorter, more nutritious *C. subspathacea*, described as ‘grazing lawns’ ([23, 24], **Supplementary Fig. S1**).

Soil collection and biogeochemical analysis

On June 8–9, 2018, soil samples were collected in ungrazed meadows and in grazing lawns. To collect soils from ungrazed habitat, we established five sampling points every 20 m along a 100-m transect. At each point, we collected four unique soil cores, located 50 cm from the transect line in one of the four cardinal directions. We could not precisely replicate this sampling strategy in the grazing lawns, because they occur in small patches randomly distributed across the landscape. Therefore, working as closely as possible to the ungrazed transect, we identified five distinct grazing lawns and took four unique soil cores within each. This nested sampling design resulted in a total of 20 soil cores from each habitat type (grazed vs. ungrazed).

Cores were 10 cm × 10 cm sections of soil excavated to a 10-cm depth using a sterile knife. The core was removed from the center of a 0.25 m² quadrat which was photographed for determination of goose fecal abundance and the proportion of living and senesced plant biomass. Using SamplePoint [25], 100 systematically gridded points were overlaid upon each photograph and classified as either ‘live’ or ‘senesced’ plant tissue. All soils were stored on ice during transport. Subsamples for microbial community analyses were shaved from the four corners of each excavated core and stored in a −80°C freezer upon return to the laboratory within 48 h of collection.

We determined soil pH (in distilled water using a Fisherbrand accumet AE150 Benchtop pH meter, Thermo Fisher Scientific, Waltham, MA, USA) and gravimetric soil moisture (via oven drying for 96 h at 65°C) for each of the 40 individual field samples. We combined samples by sampling

point ($N = 5$ in grazed and 5 in ungrazed habitat) prior to determination of total organic C, N, K, and P content. All samples subject to chemical analysis were air-dried, sieved to 2 mm to remove roots, and finely ground. Total organic C and N content were measured on an elemental analyzer (ECS 4010 Elemental Analyzer, Costech Analytical Technologies, Valencia, CA, USA) following acidification with H_2SO_3 (5 mL per 1 g ground soil) to remove inorganic C, and total P and K were measured via inductively coupled plasma mass spectrometry on a Thermo Electron iCAP ICP at the USU Analytical Lab.

Microbial community analyses

A DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) was used to extract DNA from each of the 40 unique soil samples following manufacturer protocols. At the USU Center for Integrated Biosystems, DNA extracts were pooled in equimolar concentrations and sequenced on the Illumina MiSeq platform. Primers targeting the V4 region of the 16S SSU rRNA gene (primers 515F–806R) and the internal transcribed spacer region of the fungal rRNA gene (primers ITS1f-ITS2) were used to amplify prokaryotic and fungal marker genes following Earth Microbiome Project protocols [26–28]. The QIIME 2 (version 2018.11.0) sequence curation pipeline [29] was used to determine the bacterial, archaeal, and fungal community composition for each sample. The DADA2 algorithm [30] was used to denoise sequences, producing putatively error-free amplicon sequence variants (ASVs) that lacked chimeras. To assign taxonomy to bacterial and archaeal sequences, a Naïve Bayesian classifier was trained on the Greengenes (version 13_8) training dataset [31]. For fungi, the Blast+ algorithm [32] was used to assign taxonomy in reference to the UNITE (version 8.0) database [33]. Following taxonomic classification, a taxonomy-based filtering procedure was used to remove non-bacterial, archaeal, or fungal ASVs from the appropriate datasets. Then, for each of the 40 samples, sequences were rarified to 20,000 bacterial sequences and 12,000 fungal sequences.

Microcosm incubation experiment

The microcosm experiment consisted of a fully factorial manipulation of soil moisture, temperature, and nutrient addition, simulating three major factors affected by grazing geese in the field. Each microcosm consisted of 40 g soil (dry weight) in a 250 mL borosilicate glass jar with a septum in the lid to permit gas sampling. Prior to setting up the microcosms, soil samples were air-dried and sieved to 2 mm. We then combined soil cores by habitat type (grazing lawn vs. ungrazed), yielding two large, homogenized soil samples from which microcosms were created. This resulted in 16 unique treatment combinations with six replicates each ($N = 96$).

To establish soil moisture treatments, soil water content was adjusted to match mean soil moisture measured in grazing lawns ($104 \pm 4.52\%$ [SE]) and adjacent ungrazed habitat ($81 \pm 6.43\%$ [SE]). Soils were wet with brackish water with a mean salinity of 28 g L^{-1} total dissolved salts (Instant Ocean Sea Salt, Spectrum Brands, Blacksburg, VA, USA) to reach 104% soil moisture. Microcosms assigned to the lower soil moisture treatment level (81%) were allowed to air-dry until they reached the appropriate soil moisture; this approach avoided the confounding factor of differing total salts. The soil temperature manipulations consisted of a control treatment (early growing season temperature of 8°C [5, 21]) and a warmed treatment (18°C). While we lack high-resolution soil temperature data to quantify how much warmer grazing lawn soils are on average, soil temperatures can reach 18°C in the field [5]. Finally, nutrient availability was manipulated in half of the microcosms by adding field-collected goose feces once at the start of the experiment. Feces were dried in an oven at 60°C for 48 h and then ground using a Wiley Mill. Mimicking the observed fecal density m^{-2} on the grazing lawns [34], feces were applied to the surface of the microcosm soils at a rate of 27.94 g m^{-2} (0.08 g dry weight per microcosm). Feces were then mixed into the air-dried soil in each microcosm to mimic trampling effects prior to applying soil moisture treatments.

Microcosms were incubated for eight weeks (the length of the Y-K Delta growing season), with headspace gas sampled once a week for GHG quantification. Microcosm locations within incubators were randomized at the start of the experiment and following each headspace sampling. We weighed microcosms weekly to determine water loss; if necessary, soil moisture treatment levels in each microcosm were maintained by adding deionized water. Concentrations (μmol) of CO_2 and CH_4 accumulated in headspace over a 24-h window were analyzed using gas chromatography (GC-2010 Greenhouse Gas Analyzer, Shimadzu, Kyoto, Japan). At the conclusion of the incubation, microbial biomass in each microcosm was measured via fumigation-extraction techniques [35], and extracts were analyzed for non-particulate organic C content (TOC-L, Shimadzu, Kyoto, Japan).

Statistical analyses

Biogeochemical and microbial community analyses

Soil biogeochemical data collected in situ were analyzed with nested ANOVAs, with the point of sample collection nested within habitat type (grazing lawn vs. ungrazed habitat). Similar tests were conducted to analyze variation in bacterial and fungal ASV richness. Because soil samples were pooled by sampling point prior to analysis of total C, N, P, K, and pH, these data were analyzed with standard t tests. To analyze microbial community composition, the R software package *vegan* [36] was used to perform permutational ANOVAs to

quantify the effects of habitat type and soil characteristics on microbial community structure (i.e., counts of each ASV in each sample). The ANCOM function in QIIME2 was used to evaluate differential abundance of specific bacterial and fungal phyla across habitats [37]. We also used the *varpart* function in *vegan* to partition compositional variance associated with space (i.e., the coordinates of each sample point) vs. the abiotic environment (soil pH, moisture, grazing intensity [as indexed by goose fecal abundance], and stocks of C, N, P, and K). Community data was visualized with non-metric multidimensional scaling (NMDS) performed on Bray-Curtis dissimilarity matrices of ASV abundances.

Microcosm incubation experiment

Linear mixed effects models were conducted with the R package *lme4* [38] to determine the individual and interactive effects of each fixed treatment factor on CO₂ and CH₄ fluxes, with sampling time as the random factor. *P* values for each model term were calculated using Satterthwaite's approximation in the R software package *lmerTest* [39], and significant treatment interactions were evaluated through post hoc tests in the *multcomp* package in R [40]. Microbial biomass was analyzed with a four-way ANOVA with treatment factors as fixed effects. To explore treatment effects in more depth, we also calculated treatment response ratios (RRs) for CO₂ and CH₄ fluxes using the formula $RR = \frac{F1}{F2}$, where *F1* is the flux in the treatment condition (i.e., 18 °C, fecal addition, or 104% soil moisture, respectively) and *F2* is the flux in the control condition (8 °C, no nutrient inputs, or 81% soil moisture). RRs were calculated separately for soils from grazing lawn vs. ungrazed habitat. Finally, we calculated Q10 of respiration in grazed and ungrazed soil following the formula $Q10 = \frac{F1 - F2}{T1 - T2}$, where *F1* and *F2* are fluxes at temperature *T1* (18 °C) and *T2* (8 °C), respectively.

Results

Soil biogeochemistry and herbivory on the Y-K Delta

Grazing lawns demonstrated dramatic biogeochemical signatures of herbivory. Even though all habitats were dominated by the same plant species, grazing had a significant impact on plant morphology. Grazing lawns exhibited 95 to 98% lower percent senesced plant cover than their ungrazed counterparts, and living biomass was much shorter in stature than in the ungrazed meadows (Supplementary Figure S1). Goose fecal density was also over 150 times higher in the grazing lawns than ungrazed habitat (Table 1, Supplementary Table S1). Both soil C and N stocks were 35 and 53% greater, respectively, in ungrazed habitat versus grazing lawns (Table 1),

although the difference in total C between habitats was marginally non-significant ($t = 2.38$, $P = 0.062$). However, K and P stocks were two-fold larger in grazing lawns (Table 1, Supplementary Table S1). Soil moisture was also 22% higher in grazing lawn, whereas pH was slightly lower (Table 1).

Soil microbial community responses to grazing

The richness of fungal ITS and prokaryotic 16S ASVs was 42% and 10% higher, respectively, in ungrazed meadow than in grazing lawns (Table 1, Supplementary Table S1). Moreover, the composition of both fungal and prokaryotic communities varied significantly between grazed and ungrazed habitats (Table 2, Fig. 1). All fungal phyla exhibited different patterns of abundance between these two habitats (Fig. 1, Supplementary Table S2). For prokaryotes, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Gemmatimonadetes*, *Parcubacteria* (OD1), and *Firmicutes* were differentially abundant between grazed and ungrazed meadow (Fig. 1). However, permutational ANOVAs showed that no individual soil parameter explained more than 4% of the compositional variation observed across fungal or bacterial communities (Table 2). Pure spatial effects explained only ~1% of variation in bacterial and fungal community dissimilarities, whereas the combination of spatial distance and measured environmental variables (fecal abundance and soil pH, moisture, and nutrient content) explained only 5% of variance in bacterial communities and 12% of the variance in fungal communities.

Greenhouse gas fluxes in grazed and ungrazed soils in a microcosm incubation experiment

Both GHG fluxes increased with temperature and fecal addition and were consistently higher from grazed soils versus ungrazed soils; there was a significant three-way interaction among these treatments for both CO₂ and CH₄ (Table 3, Fig. 2). These effects were consistent over time, although all trace gas fluxes declined over the course of the incubation. In planned comparison tests, while controlling for temperature and moisture, we found that adding goose feces to ungrazed soils did not increase CO₂ or CH₄ fluxes to the levels observed in unamended grazing lawns soils (Supplementary Figure S3). We also found that grazing regimes affected the temperature sensitivity of GHG production. Across the 10-degree temperature gradient imposed, CO₂ fluxes increased nearly 2-fold from grazing lawn soils ($Q_{10} = 1.93 \pm 0.10$), but only 63% from ungrazed soils ($Q_{10} = 1.63 \pm 0.09$) (Fig. 3). CH₄ fluxes were not different between the two temperature treatments in ungrazed soils ($Q_{10} = 1.79 \pm 0.48$), but increased 2-fold in grazing lawn soils ($Q_{10} = 2.36 \pm 0.29$) (Fig. 3). Additionally, whereas CO₂ and CH₄ fluxes were 4 and 74%, respectively, greater in wetter soils, the effects of the soil moisture treatment

Table 1 Summary statistics of soil characteristics

Habitat	Soil pH*	TOC	TN*	TP*	TK*	Fecal ab.*	Standing dead*	ITS*	16S*
Ungrazed	7.05 ± 0.03	2307 ± 225	179 ± 20	1.31 ± 0.08	24.7 ± 0.8	0.2 ± 0.2	47.8 ± 3.1	200 ± 10	1121 ± 36
Grazed	6.82 ± 0.04	1713 ± 55	117 ± 2	2.62 ± 0.15	50.8 ± 3.19	34.8 ± 3.1	1.15 ± 0.5	141 ± 7	1022 ± 33

Notes. Summary statistics are given as mean ± standard error. Asterisks following variable names indicate significant differences between grazed and ungrazed habitat in nested ANOVAs or *t* tests. Abbreviations: TOC, TN, TP, and TK indicate total C, N, P, and K, respectively (in units of g m⁻²). Fecal ab. refers to the abundance of goose feces in each quadrat (average number per m²), and standing dead refers to the coverage of senesced vegetation (%) in the sample quadrats. ITS and 16S indicate species richness of fungal and prokaryotic sequences in each soil core

were amplified (for CH₄) or slightly diminished (for CO₂) under warmer temperatures, regardless of past grazing regimes.

Treatment response ratios (RRs) provide additional insight on how the multifactorial impacts of herbivory affect GHG fluxes (Fig. 4). For CO₂, the temperature treatment had the largest absolute impact on flux rate, with CO₂ fluxes approximately 1.5-fold greater at 18° vs. 8 ° C (Fig. 4). However, as noted above, both CO₂ and CH₄ fluxes in soils from grazing lawns responded more strongly to warming than those from ungrazed habitat. By contrast, whereas fecal addition and the high soil moisture treatment had little impact on CO₂ fluxes in grazing lawn soils, these treatments enhanced CO₂ by approximately 23 and 33%, respectively, in soils from ungrazed habitat. A similar pattern was observed for CH₄ fluxes, although the responses were more dramatic—in soils from ungrazed habitat, CH₄ flux increased over two-fold in response to fecal addition and 53% in response to elevated soil moisture.

Microbial biomass varied idiosyncratically among treatments, with a four-way interaction among grazing regime, temperature, moisture, and fecal addition (Table 3, Supplementary Fig. S4). Biomass was not consistently higher in the previously grazed soils, and there was no correlation between microbial biomass and mean CO₂ ($R^2 = 0.013$, $P = 0.139$) or CH₄ ($R^2 = 0.022$, $P = 0.079$) fluxes across all microcosms.

Table 2 Results of permutational analyses of variance (ANOVAs) of fungal (ITS) and prokaryotic (16S) community composition across grazed and ungrazed habitats. Significant predictors of microbial community structure are shown in bold

	ITS		16S	
	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²
Habitat identity (grazed vs. ungrazed)	<0.001	0.168	<0.001	0.074
Fecal abundance	0.163	0.027	0.065	0.028
Soil C	0.035	0.037	0.064	0.027
Soil N	0.130	0.029	0.218	0.025
Soil P	0.031	0.036	0.230	0.025
Soil K	0.032	0.036	0.012	0.031
pH	0.305	0.024	0.105	0.026
Soil moisture	0.444	0.021	0.402	0.024

Discussion

In this high-latitude wetland, herbivory has dramatic impacts on plant and soil properties, affecting soil microclimate and stocks of C, N, and base cations, which in turn shape bacterial and fungal communities. Our laboratory experiment results demonstrated that altering soil microclimate and nutrient status in ungrazed soils was not sufficient to replicate the higher GHG fluxes observed in grazing lawn soils. This suggests that higher CO₂ and CH₄ fluxes in grazing lawns are not only attributable to differences in soil moisture, temperature, and nutrients, but may also relate to the herbivore-induced changes in microbial community structure.

Soil biogeochemistry and microbial communities across the landscape

Both fungal and prokaryotic microbial communities exhibit dramatic differences between grazed and ungrazed habitat. These community differences may be explained, in part, by differences in soil temperature, moisture, and nutrient availability, which are impacted directly by herbivory. Grazing lawns exhibited lower organic C and N than adjacent ungrazed habitat, potentially due to lower aboveground biomass and litterfall on grazing lawns [41]. Additionally, soil K and P were highly enriched in grazing lawns, likely due to goose fecal deposition. However, measured soil variables (including nutrients, moisture, and pH at the time of sampling) explained only a small percentage of the observed variation in bacterial and fungal community structure. Herbivory can alter potentially important parameters which we did not quantify, including fine root productivity and turnover, rates of root exudation [10], temperature variability [5], and competition for nutrients between plants and soil microbes [42]. Such unmeasured factors likely contribute to the pronounced differences in bacterial and fungal communities that we observed.

Disentangling effects of temperature, soil moisture, and nutrient availability GHG fluxes

In the laboratory incubation experiment, CO₂ and CH₄ fluxes increased with temperature, soil moisture, and goose fecal addition, suggesting that grazing-related changes in the soil

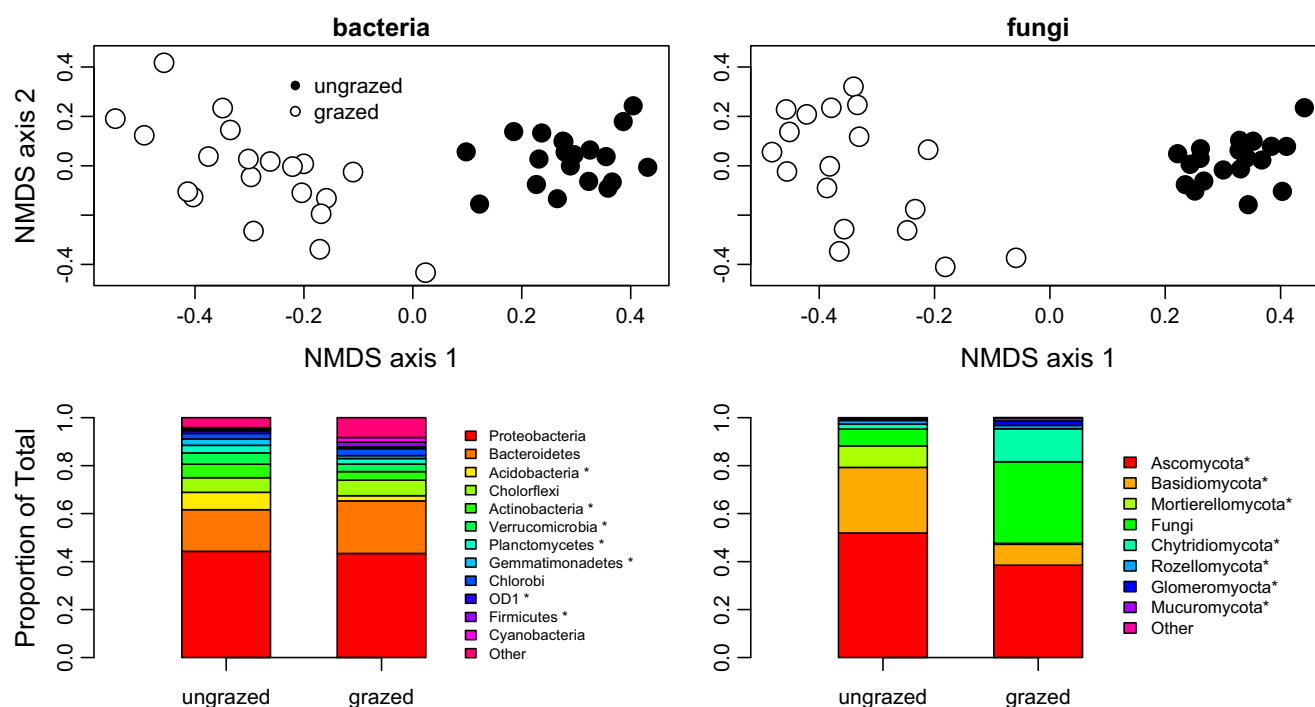


Fig. 1 NMDS plots showing variation in bacterial (left panel; stress = 0.085) and fungal (right panel; stress = 0.164) communities in soil cores collected in Pacific black brant grazing lawns or adjacent, ungrazed habitat. Bar plots show the relative abundance of microbial phyla; those highlighted with an asterisk show significant differences in their

abundance between habitats. In both panels, the category ‘Other’ refers to all phyla whose relative abundance was <1%; the label ‘Fungi’ designates sequences that were confirmed as fungal in origin, but could not be assigned a phylum

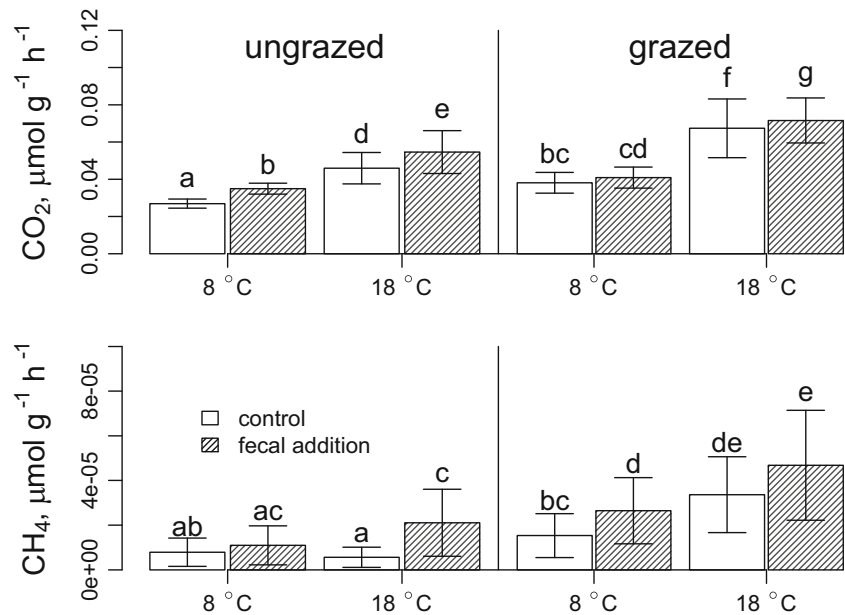
abiotic environment may enhance soil C losses. Many studies have shown that warming enhances CO₂ and CH₄ losses from high-latitude soils [3, 43]; thus, to the extent that herbivores increase soil temperature via modifying plant cover and soil microclimate, we can expect GHG fluxes to increase in grazed habitat. Fecal addition also enhanced CO₂ and CH₄ losses,

likely by relieving microbial nutrient limitation. Notably, fecal fertilization had a much larger effect in soils from ungrazed habitat, which have significantly lower stocks of P and K. This suggests that these nutrients—which also tend to be elevated in fecal matter [44]—may enhance microbial activity in the wetland soils we studied. Finally, higher soil water content

Table 3 *F* statistics from a mixed model analyzing greenhouse gas fluxes and microbial biomass in the microcosm incubation experiment. Significant results are highlighted in bold

Treatment	CO ₂	CH ₄	Microbial biomass
Grazing	383.4	135.7	16.8
Temperature	1064.1	17.4	0.3
Moisture	5.5	14.5	10.8
Fecal addition	112.2	34.1	10.9
Grazing x temperature	26.2	8.8	3.6
Grazing x moisture	2.2	0.1	5.4
Temperature x moisture	13.5	4.4	3.8
Grazing x fecal addition	11.3	0.7	2.0
Temperature x fecal addition	0.6	2.6	0.00
Moisture x fecal addition	3.2	6.1	6.1
Grazing x temperature x moisture	2.1	2.4	0.1
Grazing x temperature x fecal addition	9.7	4.8	21.6
Grazing x moisture x fecal addition	0.5	1.2	13.4
Temperature x moisture x fecal addition	0.02	0.3	5.3
Grazing x temperature x moisture x fecal addition	0.01	3.5	5.7

Fig. 2 Mean ($\pm$ SE) fluxes of CO_2 (top) and CH_4 (bottom), averaged over all timepoints and moisture treatments, in the microcosm incubation experiment. Letters indicate treatments that were significantly different in post hoc tests



tended to boost both CO_2 and CH_4 fluxes, although there were no significant interactions between the moisture treatment and soil grazing regimes.

Although we carefully controlled soil abiotic conditions in the context of our laboratory microcosm experiment, soils from grazed vs. ungrazed habitat may differ in other ways that affect patterns of GHG flux. Micro-topographical differences between grazing lawns (which tend to occur around localized depressions) and ungrazed habitat can affect abiotic properties not measured here, such as soil texture or salinity. These

factors may have influenced GHG fluxes and their responses to treatments, either directly (e.g., by influencing water infiltration into the soil) or indirectly (via their contributions to variation in microbial community structure), which we discuss in more detail below.

Identifying microbial mechanisms of herbivore effects on GHG fluxes

Although enhancing soil temperature, moisture, and nutrient availability increased CO_2 and CH_4 losses, these abiotic drivers were insufficient to explain variation in GHG fluxes

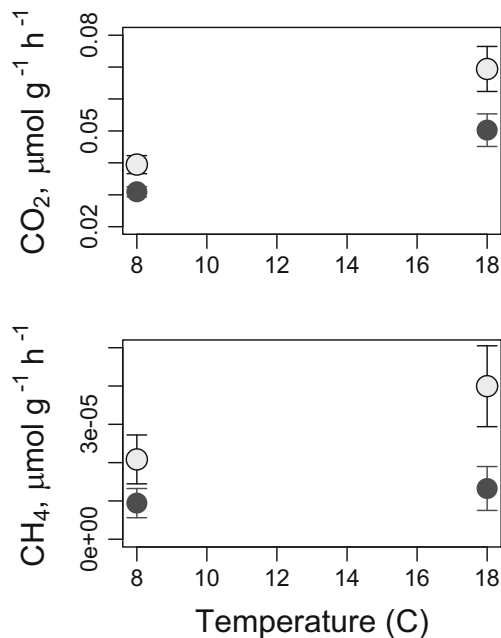


Fig. 3 Mean fluxes of CO_2 (top panel) and CH_4 (bottom panel), averaged over all moisture and nutrient treatments, in soils from grazing lawn (light gray symbols) and ungrazed habitat (dark gray symbols)

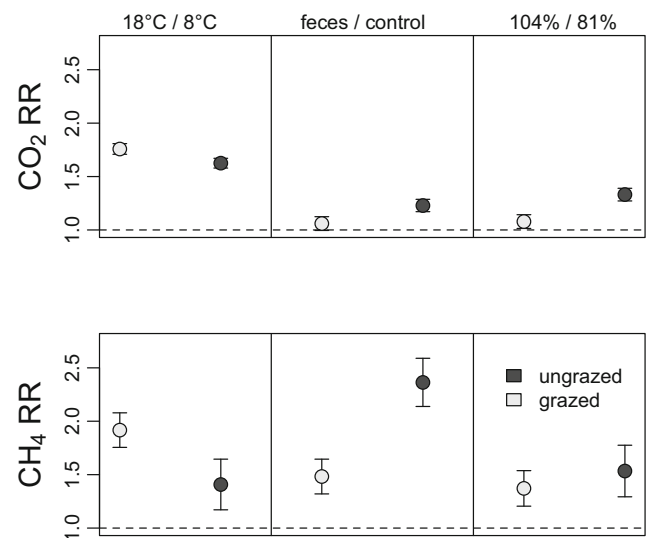


Fig. 4 Response ratios (RR) and their 95% confidence intervals. RRs were calculated as the ratio of GHG fluxes in 'treatment' conditions (18°C, fecal addition, and 104% soil moisture) relative to 'control' conditions (8°C, no nutrient addition, and 81% soil moisture)

between soils with and without prior grazing. Trace gas fluxes were higher in soils from grazing lawns vs. ungrazed habitat, even when incubated under identical soil temperature and moisture conditions. Higher GHG fluxes in previously grazed soils could be attributable to three interrelated mechanisms: variation in soil chemical composition across habitats, differences in the size of the microbial biomass, and variation in fungal and prokaryotic community structure. However, CO₂ and CH₄ emissions in nutrient-amended ungrazed soils from wet sedge meadows were lower than those observed in unamended grazing lawn soils, suggesting that higher fluxes in the latter are not driven entirely by greater nutrient availability. Moreover, across all microcosms, CO₂ and CH₄ fluxes were unrelated to the size of the microbial biomass. Instead, we suggest that the dramatic, herbivore-induced variation in fungal and prokaryotic community structure contributes to the observed patterns of CO₂ and CH₄ flux.

Because individual soil cores were combined within treatment group (grazed vs. ungrazed habitat) prior to creating the microcosms, and because we did not have the resources to resequence microcosms at the conclusion of the experiment, we do not have the statistical power to link GHG dynamics to the presence or absence of individual taxa. We do know, however, that the initial microbial communities in the experimental microcosms likely reflected some systematic differences related to soil habitat of origin. Microbial communities from grazing lawns were characterized by much higher abundance of *Bacteroidetes*, *Cyanobacteria*, and *Chytridiomycota*, but significantly lower abundance of *Acidobacteria*, *Actinobacteria*, *Ascomycota*, and *Basidiomycota*. Of course, there is significant ecological variation within these extremely broad taxonomic groups, but relative abundances of some of these phyla have been linked with variation in CO₂ flux rates [45]. Soils from grazed and ungrazed habitat likely also differ in the abundance and community structure of soil fauna, which could also contribute to the biogeochemical patterns we observed.

Finally, we observed that grazing regimes impacted not only the absolute magnitude of GHG fluxes but also their relationship to soil climate: the temperature sensitivities (Q₁₀) of CO₂ and CH₄ fluxes were 18% and 32% greater, respectively, in previously grazed versus ungrazed soils from the same vegetation community. Therefore, warming-induced losses of soil C may be accentuated in grazing lawns, both because the soils are typically 1 to 4 °C warmer during the growing season [5], and because the temperature sensitivity of organic matter decomposition is higher in those soils, as demonstrated in this experiment. Our results are consistent with in situ observations of trace gas fluxes on the Y-K Delta: CH₄ fluxes were more sensitive to temperature in grazing lawns vs. ungrazed habitat [5]. This pattern may reflect the differences in microbial community composition discussed above, variation in the chemical composition of organic matter within the

soils, or both. The Q₁₀ of respiration is hypothesized to be higher when the microbial biomass is consuming chemically recalcitrant organic matter, as the decomposition of complex biomolecules is intrinsically more sensitive to temperature [46]. At the same time, reorganization of microbial community structure and/or thermal adaptation within microbial populations can influence the types of substrates that are preferentially metabolized and thereby amplify or diminish the temperature sensitivity of decomposition [17, 47].

Conclusions

Conditions in high latitude wetlands are expected to change rapidly over time, as Arctic ecosystems are warming twice as fast as the rest of the globe, experiencing altered precipitation regimes, and losing snow and ice cover [48]. Herbivory regimes are also changing: Pacific black brant populations in the Y-K Delta have declined by approximately 50% since 2000 [49] while increasing on the Arctic Coastal Plain of Alaska [50, 51], whereas populations of many other geese are increasing [52]. Our results imply that C cycle responses to climate change on the Y-K Delta will be mediated by the multifactorial impacts of herbivory. We provide evidence that geese may alter soil biogeochemistry via three non-exclusive pathways: through direct modification of soil microclimate and nutrient availability, via changes in microbial community structure, and through the interaction of microbes with their abiotic environment. Yet, terrestrial ecosystem models do not explicitly represent herbivory effects on soil biogeochemistry or microbial controls over soil C cycling [53]. Therefore, most predictive models would be unable to capture the interactions among herbivory, climate, and soil biogeochemistry observed in the Y-K Delta and elsewhere. To accurately predict ecosystem GHG losses in a rapidly changing Arctic environment, future studies must carefully evaluate how herbivory-driven shifts in microbial communities influence fluxes of carbon among plants, soils, and the atmosphere.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00248-021-01733-8>.

Acknowledgements We thank M. Lindberg and his crew for logistical support during field collection of samples, R. Choi for advice on study site locations and plant identification, and the Yukon-Kuskokwim Delta National Wildlife Refuge for allowing us to collect soil samples.

Availability of data and materials Upon acceptance of the manuscript for publication, all data will be archived in Dryad digital repository (www.datadryad.org).

Author contributions All authors designed the study; KF, BW, and KB collected samples and data in the field; KF performed sample processing

and laboratory analyses; KF, BW, and TA performed statistical analyses of output data and all authors reviewed analyses for accuracy; KB arranged funding for the research; KF wrote the initial draft of the manuscript, and all authors contributed substantially to revisions.

Funding The work was funded by the National Science Foundation awards ARC-1304523 and ARCCS-1932889, a Utah State University (USU) Research Catalyst grant, a USU Research and Graduate Studies Dissertation Enhancement, and the Utah Agricultural Experiment Station, approved as journal paper 9298.

Declarations

Ethics approval and consent to participate Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflicts of interest None to declare

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