Utah State University

DigitalCommons@USU

All Graduate Theses and Dissertations | Graduate Studies

5-2020

Herbivory Changes Soil Microbial Communities and Greenhouse Gas Fluxes in High-Latitude Wetlands

Karen M. Foley
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd

 Part of the Biology Commons

Recommended Citation

Foley, Karen M., "Herbivory Changes Soil Microbial Communities and Greenhouse Gas Fluxes in High-Latitude Wetlands" (2020). *All Graduate Theses and Dissertations*. 7795.
https://digitalcommons.usu.edu/etd/7795

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

HERBIVORY CHANGES SOIL MICROBIAL COMMUNITIES AND GREENHOUSE GAS FLUXES IN HIGH-LATITUDE WETLANDS

by

Karen M. Foley

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

Approved:

_____	_____
Bonnie G. Waring, Ph.D. Major Professor	Karen H. Beard, Ph.D. Committee Member
_____	_____
Trisha B. Atwood, Ph.D. Committee Member	Richard S. Inouye, Ph.D. Vice Provost for Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2020

Copyright © Karen M. Foley 2020

All Rights Reserved

ABSTRACT

Herbivory Changes Soil Microbial Communities and Greenhouse Gas Fluxes in High-latitude Wetlands

by

Karen M. Foley, Master of Science

Utah State University, 2020

Major Professor: Dr. Bonnie G. Waring
Department: Biology

Although herbivory can have strong impacts on greenhouse gas (GHG) fluxes in high-latitude ecosystems, few studies quantify the microbial mechanisms that link herbivore-induced environmental shifts with changes in soil biogeochemistry. In the Yukon-Kuskokwim (Y-K) Delta in western Alaska, grazing by Pacific black brant affects the magnitude of soil carbon dioxide (CO_2) and methane (CH_4) fluxes, but the underlying drivers of this relationship are unclear. The existing variation in soil characteristics and microbial community structure across four different plant communities on the Y-K Delta was assessed with emphasis on grazed and un-grazed habitats in the wet sedge meadows. This allowed for quantification of the magnitude of herbivore effects on soil biogeochemistry against the backdrop of landscape-scale environmental variation. Potential drivers of herbivory-induced changes in GHG fluxes were evaluated by incubating grazed and un-grazed soils under controlled conditions, manipulating factors impacted by herbivores in the field: soil moisture, temperature, and nutrients. Results

showed soil characteristics and microbial community structure varied greatly across the four plant communities at the landscape scale, but the variation between adjacent grazed and un-grazed habitat was nearly as pronounced. In the laboratory incubation, GHG fluxes increased with temperature and nutrient additions, but not moisture. Moreover, these effects were magnified in previously grazed soils, in which CO_2 and CH_4 fluxes were increased by up to 50% and 500%, respectively. Temperature sensitivity of respiration was increased by up to 32% in grazed soils. This variation in GHG fluxes was associated with significant turnover in fungal and prokaryotic community composition between habitats with and without prior exposure to herbivores. These results suggest that relationships among herbivores, soil microbial communities, and belowground carbon cycling will mediate carbon-climate feedbacks in rapidly changing high-latitude ecosystems.

(52 pages)

PUBLIC ABSTRACT

Herbivory Changes Soil Microbial Communities and Greenhouse Gas Fluxes in High-latitude Wetlands

Karen M. Foley

Herbivory by migratory animals in high-latitude ecosystems is known to impact greenhouse gas emissions from soils. However, few studies quantify the relationships between changes herbivores make to plant communities and soil conditions, and the biological interactions soil organisms have with their environment that result in changes to greenhouse gas emissions. These relationships are important to understand because they capture important carbon-climate feedbacks that may have implications for climate change policy and land management decisions, especially since high-latitude systems are experiencing unprecedented changes in climate.

In the Yukon-Kuskokwim (Y-K) Delta in western Alaska, herbivory by migratory geese affects the magnitude of greenhouse gas emissions coming from soils, but the mechanisms driving these relationships are poorly understood. To determine these mechanisms, variation in soil environments between adjacent grazed and un-grazed sites were compared to variation in soil environments across a landscape-scale gradient of plant communities to better understand the magnitude of differences in soil environments created by grazing. Soil environment characteristics measured included soil pH, moisture, total organic carbon and nutrients, and microbial community structure and dynamics. We also performed an incubation experiment on soils from grazed and un-grazed sites to assess the mechanistic drivers of changes in greenhouse gas emissions by manipulating

soil environment characteristics that change with herbivory in the field: soil moisture, temperature, and nutrient content.

We found that soil environments between adjacent grazed and un-grazed sites had nearly as much variation as soil environments across the landscape, including in microbial communities. From the incubation experiment, greenhouse gas emissions increased with temperature and nutrient content, but there was no synergistic effect of moisture. Moreover, the effects of temperature and nutrients on greenhouse gases was increased in soils from grazed sites. The differences in the greenhouse gas emissions were not due to differences in absolute abundances of soil microbes. Instead, the results suggest that differences in relative abundances of soil microbial taxonomic groups with known differences in physiological traits or life-history strategies may account for the observed differences in greenhouse gas emissions.

These results have major implications for high-latitude ecosystems because these ecosystems are warming twice as fast as lower-latitude ecosystems, suggesting that greenhouse gas emissions will increase in grazed sites and contribute to positive feedbacks in climate. These results also suggest that relationships among herbivores, soil microbial communities, and belowground carbon cycling are important to capture ecological relationships that impact global climate.

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Bonnie Waring, who took a risk in taking me on as a student, showed endless patience, and provided excellent mentorship throughout every aspect of my program. I would like to thank my committee member, Dr. Karen Beard, for her valuable guidance in the fieldwork and writing process. I would also like to thank my committee member, Dr. Trisha Atwood, for her feedback and input on the statistical analysis of the data and the preparation of the manuscript. I would like to thank Ryan Choi for his advice on sampling site locations in the field and plant identification during digital image analysis of field photos. I would also like to thank Mark Lindberg and his crew for their logistical help during field collection of samples.

I would like to thank the Yukon-Kuskokwim National Wildlife Refuge for allowing us to conduct this research and collect soil samples. I would also like to thank Utah State University for funding this project through a Research Catalyst grant awarded to Dr. Karen Beard.

Finally, I would like to thank my family, friends, and colleagues for their constant support and encouragement throughout all aspects of my master's program.

Karen M. Foley

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

High-latitude ecosystems, which contain approximately one-third of the global terrestrial carbon (C) stock (Hugelius et al., 2014), are experiencing unprecedented changes in climate, with poorly understood consequences for global carbon-climate feedbacks (National Oceanic and Atmospheric Administration [NOAA], 2019). Within these high-latitude ecosystems, climate-induced shifts in soil C cycling are mediated by herbivory (e.g. Falk, Schmidt, Christensen, & Ström, 2015; Lara, Johnson, Andresen, Hollister, & Tweedie; 2017; Sjögersten, van der Wal, & Woodin, 2008), which can reduce net soil C uptake nearly three-fold (Cahoon, Sullivan, Post, & Welker, 2012). However, few studies quantify mechanisms that link herbivores with changes in soil biogeochemistry (Schmitz et al., 2018). Poor understanding of the ecological linkages among grazers, plants, and decomposer microorganisms precludes their representation in ecosystem models, challenging our ability to predict how high-latitude C stocks will respond to environmental change.

Herbivory in high-latitude ecosystems has been documented to impact vegetation community composition and plant biomass (Bråthen et al., 2007; Cahoon et al., 2012; Falk et al., 2015; Kelsey et al., 2016; Sjögersten et al., 2008; van der Wal & Brooker, 2004), alter patterns of nutrient cycling (Gao, Luo, Wu, Chen, & Wang, 2008; Leffler et al., 2019; McKendrigk, Batzli, Everett, & Swanson, 1980; Stark, Männistö, & Eskelinen, 2015) and affect soil characteristics, such as organic C and nitrogen (N) availability and erosion rates (Stark, Tuomi, Strömmer, & Helle, 2003; van der Wal et al., 2007; Zacheis,

Ruess, & Hupp, 2002). The simultaneous modification of plant and soil characteristics from the process of grazing makes predicting the impacts of herbivores on carbon dioxide (CO_2) and methane (CH_4) flux difficult. For example, Sjögersten et al. (2008) found that high intensity grazing reduced soil C sink strength by 99% in a wet meadow site, while having no effect on C sink or source strength in a mesic heath site. This highlights the need for a more generalizable framework for predicting the effects of herbivores on greenhouse gas (GHG) dynamics, which can only be achieved through a more mechanistic understanding of interactions between grazers and soil biogeochemical cycles across diverse high-latitude ecosystems.

The mechanisms responsible for altered GHG fluxes under herbivory are likely multifactorial, and therefore difficult to disentangle with the types of grazing manipulation studies that have been conducted to date. Grazing changes plant biomass and community composition in high-latitude ecosystems (Ruess, Uliassi, Mulder, & Person, 1997), which in turn impacts soil moisture, temperature, and nutrient availability (Kelsey et al., 2016). A reduced rate of evapotranspiration occurs in grazed areas due to lower plant biomass, resulting in higher soil moisture content where ambient temperatures are not high enough to dry soils (Asner, Elmore, Olander, Martin, & Harris, 2004). Lower plant biomass in grazed areas also increases soil temperatures by enhancing direct insolation of soils (Ruess et al., 1997; van der Wal and Brooker, 2004). Grazing can also enhance nutrient availability via increased root exudation (Bardgett, Wardle, & Yeates, 1998; Hamilton and Frank, 2001) and herbivore fecal waste (Hik & Jefferies, 1990; Ruess et al., 1997; Sjögersten et al., 2008). These herbivore-induced nutrient ‘hotspots’ can stimulate soil microbial metabolic processes, priming decomposition of

organic matter, and potentially enhancing rates of soil respiration (Bradford, Keiser, Davies, Mersmann, & Strickland; 2013; Goldfarb et al., 2011; McKendrigk et al., 1980). Finally, changes to soil moisture, temperature, and nutrient availability are known to cause shifts in microbial community composition, which can directly influence CO_2 and CH_4 fluxes from soils (Castro, Classen, Austin, Norby, & Schadt, 2010; Koyama, Wallenstein, Simpson, & Moore, 2014; Schimel & Schaeffer, 2012).

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 grazing 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 (Glassman et al., 2018; Schimel and Schaeffer, 2012). Microbial community shifts might amplify or dampen relationships between soil abiotic variables and GHG fluxes. For example, changes in the composition of bacterial and fungal communities can drive functional acclimation to long-term warming, reducing temperature-induced losses of soil C (Melillo et al., 2017). Another major knowledge gap concerns the temporal dynamics of microbial community shifts in response to grazing. Soil microbial community composition can resist environmental change for years (Hawkes, Waring, Rocca, & Kivlin, 2017), up to decades (Bond-Lamberty et al., 2016). Therefore, the structure and composition of microbial communities may reflect past grazing history, which often

remains untested.

The purpose of this study was to identify the mechanisms by which herbivory mediates CO_2 and CH_4 fluxes emitted from high-latitude wetland soils. To do so, two linked research aims were addressed. First, existing variation in soil characteristics and microbial community structure along gradients of plant community composition in high-latitude wetlands were quantified. The goal here was to assess the magnitude of herbivore effects on soil properties against the backdrop of landscape-scale environmental variation. Second, potential drivers of herbivory-induced changes in GHG fluxes were evaluated via a fully factorial manipulation of soil moisture, temperature, and nutrients, each of which are affected by herbivory in situ. These treatments were applied to soil from grazed and un-grazed habitats to assess whether herbivore-induced variation in soil microbial community composition affects the relationship between GHG fluxes and climate.

CHAPTER 2

METHODS

2.1 Study site description

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. The Y-K Delta is one of the largest deltaic systems in the world, with over 125,000 km^2 of coastal tundra and heterogenous salt marsh between the Yukon and Kuskokwim Rivers, which empty into the Bering Sea (Kincheloe and Stehn, 1991; MacDonald, 1977). Mean monthly temperatures range from -14°C in midwinter to 10°C in midsummer and the mean annual precipitation is approximately 43 cm (Tande & Jennings, 1986).

Coastal regions of the Y-K Delta are characterized by low elevation and relief across large spatial scales (e.g., less than 1 m of elevation rise within 7.5 km of the coast). Along the Tutakoke River, there are four parallel sets of sediment deposits moving inland that are approximately 2-5 km in width (Tande & Jennings, 1986), which I identify as 'terraces'. These terraces are defined by small elevational differences (about 50 cm), unique sedimentation rates, and plant communities that develop as a consequence of the frequency of tidal and storm-surge inundation (Jorgenson & Ely, 2001). Terrace one (T1), located nearest to the coast, is dominated by wet herb meadows (e.g. *Argentina egedii* and *Leymus mollis*) interspersed with mudflats, while terrace two (T2) is occupied by brackish wet sedge meadows (e.g. *Carex subspathacea*). Terrace three (T3) is dominated by a mixture of brackish wet sedge meadows and shrubland (e.g. *C. rariflora* and *Salix fuscescens*). Terrace four (T4) consists of lowland bog meadows and moist low scrub (e.g. *C. aquatilia*, *Betula nana*, *Cladina rangiferina*, and *Sphagnum* spp.) and

contains permafrost 10-20 cm below the surface (Jorgenson and Ely, 2001; Kincheloe and Stehn, 1991; Figure 1).

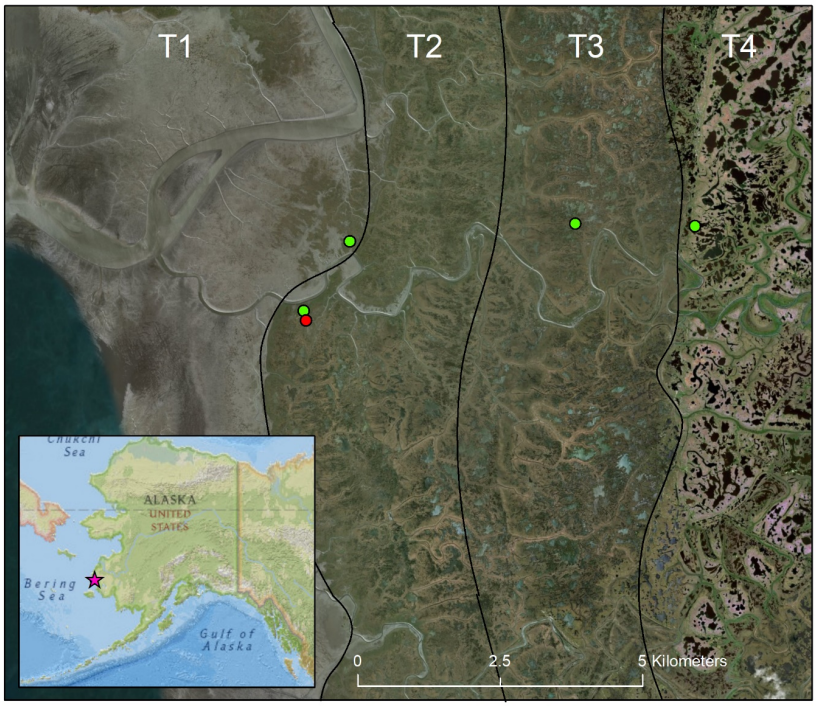

FIGURE 1 Satellite image map of the study site. The study site is located along the Tutakoke River within the Yukon-Kuskokwim Delta in western Alaska. Green points represent the location of the four 100 m transects within each terrace (i.e. T1 to T4). The red point represents the general location of sampled grazing lawns on Terrace two (T2). Data sources include USGS, National Geographic, Esri, and field-collected GPS data.

The Y-K Delta is an important breeding area for many migratory birds, including 50% of Pacific black brant (*Branta bernicla nigricans*), all emperor geese (*Chen canagica*), all cackling geese (*B. canadensis minima*), and a large majority of the Pacific flyway population of greater white-fronted geese (*Anser albifrons*). Geese breed and rear broods in the thousands within 15 km of the coast during the growing season of late May to late July, when preferred plant communities are most abundant (Amundson et al.,

2019; Babcock and Ely, 1994). Pacific black brant are the only colony breeders at the study site, and graze at such high intensity on T2 that they create mosaic patches of shorter, more nutritious *C. subspathacea*, described as 'grazing lawns' (Person, Babcock, & Ruess, 1998; Uher-Koch et al., 2019; Figure 2).

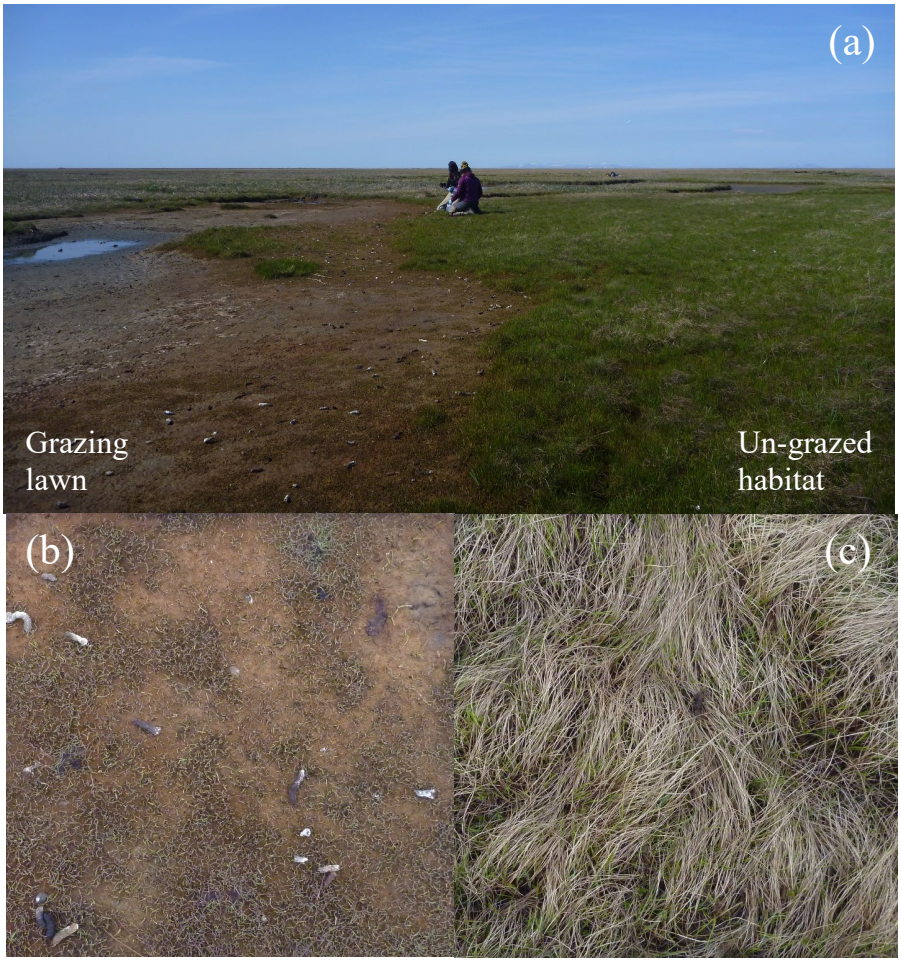

FIGURE 2 Images of (b) grazing lawns and (c) un-grazed habitat on Terrace two. Both grazing lawns and un-grazed habitat are made of mostly monospecific stands of *Carex subspathacea.* Grazing lawns are created by yearly grazing by Pacific black brant during breeding and brood-rearing months (i.e. June to early August) and (a) do not exhibit large patches of continuous habitat compared to adjacent un-grazed habitat. Grazing lawns also exhibit lower total plant biomass and senesced plant biomass and higher density of goose feces compared to adjacent un-grazed habitat.

2.2 Soil collection and determination of vegetation community composition

On 8-9 June 2018, 100 total soil samples were collected along 100 m transects in each of the four terraces: 20 samples each from T1, T3, and T4, and 40 samples in total from T2 (20 from the heavily grazed 'grazing lawns': T2G; and 20 from a transect in nearby 'un-grazed' meadows: T2UG). Every 20 m along each transect, four sampling points were selected: one in each cardinal direction and 50 cm from the transect line. The T2G points were sampled on a point-by-point basis, grouping four samples in each of five different grazing lawns, because grazing lawns are patchy and do not exhibit continuous habitat for 100 m. A 50 cm x 50 cm quadrat was placed over the center of each sampling point and photographed from a height of 1 m for subsequent digital analysis of live and dead plant biomass and bare ground. The number of individual goose feces was also counted within the quadrats. Within each quadrat, one 10 cm x 10 cm soil block was excavated to a 10 cm depth using a sterile knife. A representative subsample of each 1000 cm^3 block was immediately removed and stored on ice for microbial community analysis and quantification of gravimetric soil moisture, pH, total organic carbon (C), total nutrients [organic nitrogen (N), potassium (K), and phosphorus (P)], and microbial extracellular enzyme activities. For samples on T2, the remainder of each 1000 cm^3 soil block were stored on ice for a microcosm incubation experiment. Additional soil samples were harvested from grazing lawns and un-grazed habitat on T2 for determination of dry bulk density.

Vegetation community composition of each terrace was determined using vegetation community classifications from Kincheloe and Stehn (1991) and Jorgenson and Ely (2001). Vegetation communities 1a,b and 6a,b were used for T1, 6a-d were used

for T2, 8b was used for T3, and 10c was used for T4. Species in each community were categorized into functional groups of graminoid, forb, shrub, moss, or lichen. Percent cover for each plant functional group was determined by summing percent cover of all species within a plant functional group within a community, with species denoted by "+" rounded up to one percent cover. For T1 and T2, percent cover for a given plant functional group was averaged across all communities. Because percent cover often exceeded 100% due to ignoring the constancy of a species, the percent cover for a plant functional group was divided by the total percent cover of all plant functional groups combined. This approach allowed for calculation of the relative abundance of each plant functional group in each terrace. These results were compared to point estimates of percent cover of each plant functional group (Beard & Choi, n.d.) for accuracy. Percent live and dead plant biomass and bare ground for each quadrat for each sampling point was determined by a digital analysis of photos via SamplePoint (Booth, Cox, & Berryman, 2006).

2.3 Soil biogeochemical analyses

Roots were removed from 100 soil subsamples before the following biogeochemical analyses. Gravimetric soil moisture was determined on all subsamples by weighing approximately 3 g of wet soil from each subsample, drying these at 60°C for 48 h, and measuring weight loss of the dried soil. Soil pH and total C, N, K, and P content were determined on groups of subsamples from each cluster of four sampling points along the 100 m transects (N = 5 per transect, 20 samples total). Soil pH was measured on a 1:5 soil to deionized water solution using a pH meter (Fisherbrand accumet AE150 Benchtop pH meter, Thermo Fisher Scientific, Waltham, MA, USA). For total C and

nutrient analyses, inorganic C was first removed from the soil using 5 mL of 6% sulfurous acid (H_2SO_3) at 80°C for 24 h. Approximately 14 mg of soil was used for all but T4 samples, and approximately 1.5 mg of soil was used for T4 sample groups (because T4 soils were composed mostly of organic material) for total C and N content. Total P and K were determined for ground, air-dried samples. Total organic C and N content were measured on an elemental analyzer (ECS 4010 Elemental Analyzer, Costech Analytical Technologies, Valencia, CA, USA) following acidification to remove inorganic C, and total P and K were measured via inductively coupled plasma mass spectrometry at the USU Analytical Lab. Dry bulk density was measured on soils from grazing lawns and un-grazed habitat. Total organic C and N and total K and P were multiplied by bulk density measurements to estimate differences in C and nutrient stocks between the habitats.

Activities of the extracellular enzymes acid phosphatase (AP), β-glucosidase (BG), leucine aminopeptidase (LAP), and β-N-acetylglucosaminidase (NAG) were assayed for each of the 100 soil subsamples using p-nitrophenol conjugated substrates following German et al. (2011). Absorbance of each microplate was read at 410 nm on a microplate spectrophotometer (Spectramax M2 microplate spectrophotometer, Molecular Devices, San Jose, CA, USA).

2.4 Microbial community analyses

A DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) was used to extract DNA from each of the 100 soil subsamples collected in the field. 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. The QIIME 2 (version 2018.11.0) sequence curation pipeline (Bolyen et al., 2019) was used to determine the bacterial, archaeal, and fungal community composition for each sample. The DADA2 algorithm (Callahan et al., 2016) 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 (DeSantis et al., 2006). For fungi, the Blast+ algorithm (Camacho et al., 2009) was used to assign taxonomy in reference to the UNITE (version 8.0) database (Nilsson et al., 2019). 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 sample, sequences were rarified to 20,000 bacterial sequences and 12,000 fungal sequences. The universal 16S primers used did not effectively amplify archaeal sequences, resulting in some samples with no sequences and others with over 2,000. For cursory analysis of archaeal diversity, a rarefaction threshold of 200 sequences was used to allow for the inclusion of most samples.

2.5 Microcosm incubation experiment

The experiment consisted of a fully factorial manipulation of soil moisture, soil temperature, and nutrient addition. To determine whether herbivory induces microbial community shifts that impact GHG fluxes, treatments were replicated in soils collected from T2 grazing lawns (G) and un-grazed habitat (UG), resulting in 16 unique treatment

combinations with six replicates each (N = 96).

Prior to starting the incubation experiment, G and UG soils were air-dried and sieved at 2 mm to remove large roots; 45 g of air-dried soil were added to each 250 mL soda-lime glass microcosm (Wide-Mouth Septa Jars, Thermo Fisher Scientific, Waltham, MA, USA). To establish soil moisture treatments, soil water content was adjusted to match mean soil moisture measured in grazing lawns (104 ± 4.52% [Standard error (SE)]) and un-grazed habitat (81 ± 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 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 treatments were determined using an early growing season temperature of 8°C (Kelsey et al., 2016; Leffler et al., 2019) and a 10-degree temperature difference to evaluate the Q_{10} temperature coefficient. While uncommon, soil temperatures can reach 18°C in the field (Kelsey et al., 2016). Finally, nutrient availability was manipulated in half of the microcosms by adding field-collected goose feces. Feces were dried in an oven at 60°C for 48 hours and then ground using a Wiley Mill. Mimicking the observed fecal density m^{-2} on the grazing lawns (Beard and Choi, 2017), 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 (Dual Program Illuminated Incubator 818, Precision Scientific, Chennai, Tamil Nadu, India) in the dark in their respective temperature

treatments for eight weeks (the length of the growing season), with headspace sampled once a week for GHG quantification. Microcosm locations within incubators were randomized at the start of the experiment and following each headspace sampling. If necessary, soil moisture treatment levels in each microcosm were maintained by adding deionized water. Headspace CO_2 and CH_4 concentrations 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 (Vance, Brookes, & Jenkinson, 1987), and extracts were analyzed for non-particulate organic C content (TOC-L, Shimadzu, Kyoto, Japan).

2.6 Statistical analyses

To quantify differences in soil characteristics across the four terraces, one-way analyses of variance (ANOVA) were conducted for all measured soil variables. Response variables were transformed as appropriate to meet assumptions of normality and homogeneity of variance. Spearman's correlations were conducted to explore relationships among all measured variables.

To analyze microbial community data, the R software package vegan (Oksanen et al., 2019) was used to calculate alpha- and beta-diversity metrics and to perform permutational ANOVAs across sample locations and soil characteristics. Percent bare ground was not included in the permutational ANOVAs because only grazing lawns exhibited high percentages of bare ground. Community data was visualized with non-metric multidimensional scaling (NMDS) plots. Redundancy analyses were performed to determine how much of the compositional variation within fungal and prokaryotic communities could be explained by differences in soil characteristics, plant

characteristics, and geographical distance between samples. To examine relationships between microbial community structure and ecological functions, a partial Mantel test was performed between distance/dissimilarity matrices for bacterial and fungal communities and soil enzyme activities.

Linear mixed effects models were conducted with the R package lme4 (Bates, Maechler, Bolker, & Walker, 2015) to determine the individual and interactive effects of each fixed treatment factor on CO_2 and CH_4 fluxes, with sampling timepoint as the random factor. P-values for each model term were calculated using Satterthwaite's approximation in the R software package lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017). Microbial biomass was analyzed with a four-way ANOVA with treatment factors as fixed effects.

CHAPTER 3

RESULTS

3.1 Soil biogeochemistry across environmental gradients on the Y-K Delta

Plant communities exhibited steep turnover among terraces (Table 1) and image analysis demonstrated that the proportion of living versus dead plant biomass also exhibited significant variation (Table 2). T2G exhibited 98% lower percent dead plant biomass than any other terrace, including compared to adjacent un-grazed habitat. Goose fecal density was also higher in the grazing lawns (34.8 ± 3.08 droppings m^{-2}) compared to all other habitats (< 3.5 droppings m^{-2} on average).

TABLE 1 Relative abundances of plant functional groups per terrace

Terrace	Graminoids	Forbs	Shrubs	Mosses	Lichens
T1	86.4 %	13.1 %	0.24 %	0 %	0.2 %
T2	83.2 %	13.6 %	2.57 %	0 %	0.6 %
T3	46.1 %	13.6 %	18.8 %	0.5 %	20.9 %
T4	12.4 %	9.8 %	27.5 %	22.9 %	27.5 %

Abbreviations: T1, Terrace one; T2, Terrace two; T3, Terrace three; T4, Terrace four.

TABLE 2 Summary statistics of digital image analysis results

Terrace	Live plant biomass (%)	Dead plant biomass (%)	Bare ground (%)
T1	64.6 ± 4.35	25.8 ± 2.78	9.65 ± 5.39
T2UG	52.2 ± 3.14	47.8 ± 3.14	0
T2G	52.0 ± 3.39	1.15 ± 0.519	46.9 ± 3.42
T3	51.7 ± 1.78	48.4 ± 1.78	0
T4	67.7 ± 2.25	32.4 ± 2.25	0

Notes. Summary statistics given are mean percent ± standard error. Abbreviations: T1, Terrace one; T2UG, Terrace two un-grazed habitat; T2G, Terrace two grazing lawns; T3, Terrace three; T4, Terrace four.

All measured soil properties differed among terraces (Table 3). Soil moisture increased nearly 6-fold from T1 to T4 ($F = 231.1$, $p < 0.001$; Figure 3a), and was also 22% greater in grazing lawns than un-grazed habitat in T2. Soil pH was 38% lower in T4 vs. T1 ($F = 252.6$, $p < 0.001$; Figure 3b), but did not differ between grazing lawns and un-grazed habitat. Dry bulk density was 16% greater in soils from grazing lawns (0.695 ± 5.09E-17 g $cm^{3\ -1}$ [SE]) versus un-grazed habitat (0.598 ± 2.55E-17 g $cm^{3\ -1}$ [SE]). Total organic C ($F = 701.1$, $p < 0.001$) and N ($F = 189.1$, $p < 0.001$) increased 25-fold and 9-fold from T1 to T4, respectively (Table 3, Figure 4a,b). Organic C and N stocks were 35% and 54% greater in un-grazed habitat (organic C: 2.31 ± 4.78E-18 g $cm^{3\ -1}$ [SE]; organic N: 0.179 ± 4.18E-19 g $cm^{3\ -1}$ [SE]) than grazing lawns (organic C: 1.71 ± 2.02E-18 g $cm^{3\ -1}$ [SE]; organic N: 0.117 ± 8.75E-20 g $cm^{3\ -1}$ [SE]), respectively. Total K and P were 48 to 68% and 28 to 181% higher in T4 than the other transects (Table 3, Figure 4c,d). Moreover, both nutrients' stocks were 106% and 100% higher in grazing lawns (K: 0.051 ± 1.17E-19 g $cm^{3\ -1}$ [SE]; P: 0.003 ± 5.59E-21 g $cm^{3\ -1}$ [SE]) than un-grazed habitat (K: 0.025 ± 1.66E-20 g $cm^{3\ -1}$ [SE]; P: 0.001 ± 1.68E-21 g $cm^{3\ -1}$ [SE]), respectively.

TABLE 3 Summary statistics of soil characteristics

Terrace	Soil moisture (%)	Soil pH	Total organic C (%)	Total organic N (%)	Total P (%)	Total K (%)	C:N enzyme ratio	C:P enzyme ratio	N:P enzyme ratio
T1	62.2 ± 2.31	7.11 ± 0.021	1.74 ± 0.036	0.140 ± 0.004	0.003 ± 9.82E-5	0.042 ± 0.001	0.961 ± 0.032	0.994 ± 0.018	1.05 ± 0.033
T2UG	81.3 ± 6.43	7.05 ± 0.014	3.86 ± 0.188	0.300 ± 0.016	0.002 ± 6.59E-5	0.041 ± 0.001	0.943 ± 0.014	1.01 ± 0.006	1.08 ± 0.022
T2G	104 ± 4.52	6.82 ± 0.021	2.47 ± 0.040	0.168 ± 0.002	0.004 ± 1.10E-4	0.073 ± 0.002	0.994 ± 0.017	0.996 ± 0.007	1.01 ± 0.016
T3	255 ± 12.2	6.60 ± 0.016	12.3 ± 0.259	0.872 ± 0.017	0.002 ± 9.06E-5	0.047 ± 0.001	1.03 ± 0.010	0.925 ± 0.013	0.899 ± 0.012
T4	414 ± 32.1	4.43 ± 0.052	44.5 ± 0.257	1.34 ± 0.040	0.005 ± 1.85E-4	0.070 ± 0.001	0.934 ± 0.019	0.867 ± 0.014	0.955 ± 0.030

Notes. Summary statistics are given as mean ± standard error. Abbreviations: T1, Terrace one; T2UG, Terrace two un-grazed habitat; T2G, Terrace two grazing lawns; T3, Terrace three; T4, Terrace four; C, carbon, N, nitrogen, P, phosphorus; K, potassium.

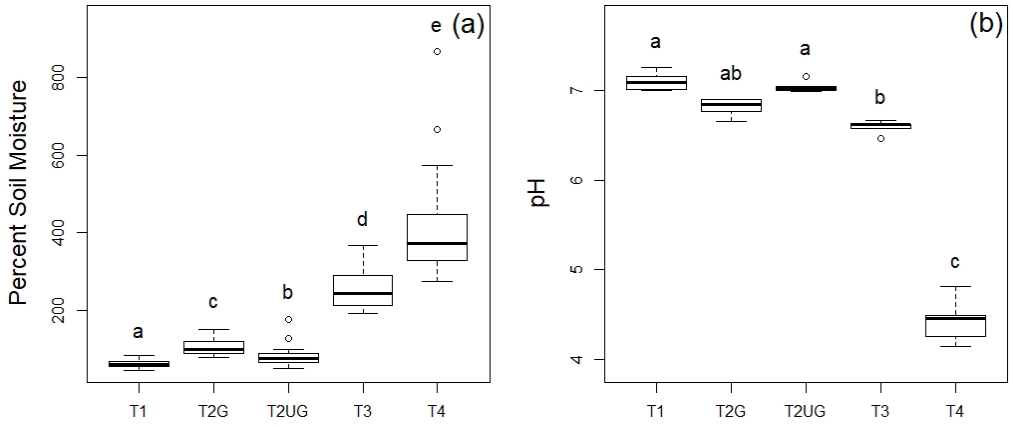

FIGURE 3 (a) Percent soil moisture and (b) soil pH across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Letters indicate significant differences based on Tukey's test.

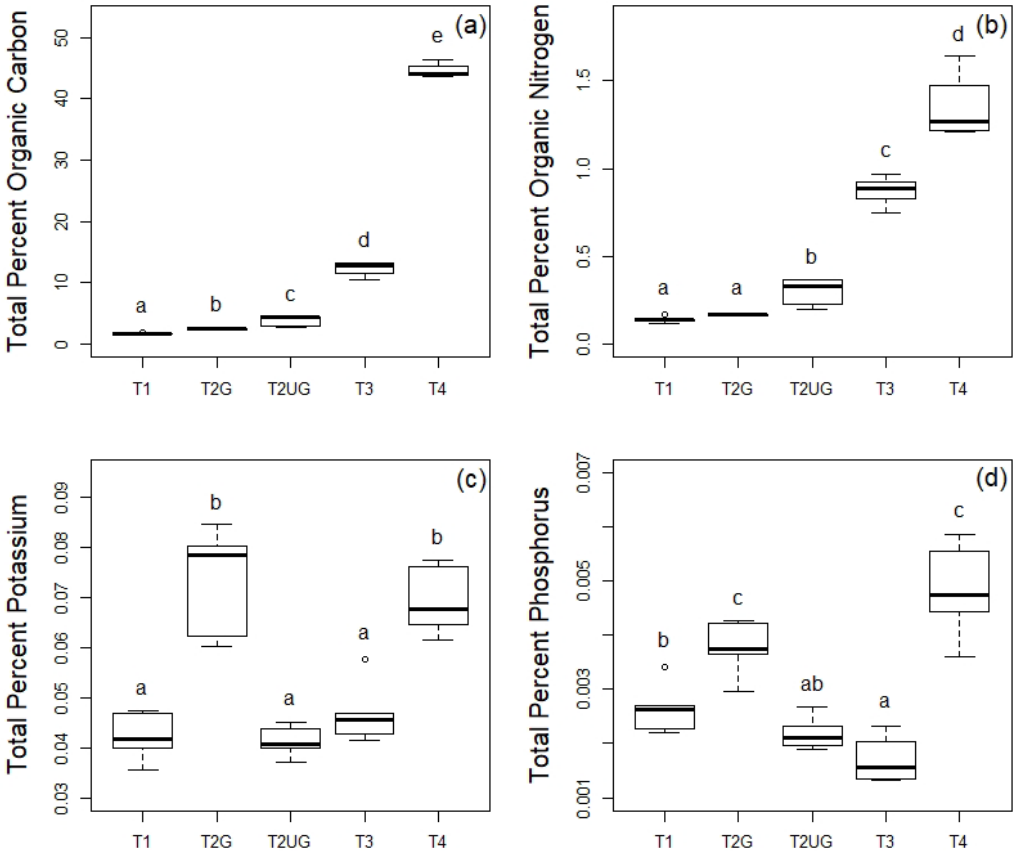

FIGURE 4 Total percent (a) organic carbon, (b) organic nitrogen, (c) potassium, and (d) phosphorus across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Letters indicate significant differences based on Tukey's test.

All extracellular enzyme activities differed among terraces (Figure 5). AP activity increased 1.4-fold from T1 to T4, whereas LAP activity showed the opposite pattern (Figure 5a,c). BG and NAG activities peaked in T2 (Figure 5b,d). As a result of these contrasting spatial patterns in enzyme activities, microbial allocation towards C, N, and P acquisition shifted across the landscape (Table 3). C:N enzyme activity ratios differed among terraces, but not in a consistent pattern (Figure 6a). N:P and C:P enzyme activity ratios were 6 to 17% and 6 to 14% lower in T3 and T4, respectively, compared to the other terraces (6b,c). Enzyme activities did not vary significantly between grazing lawns and un-grazed habitat.

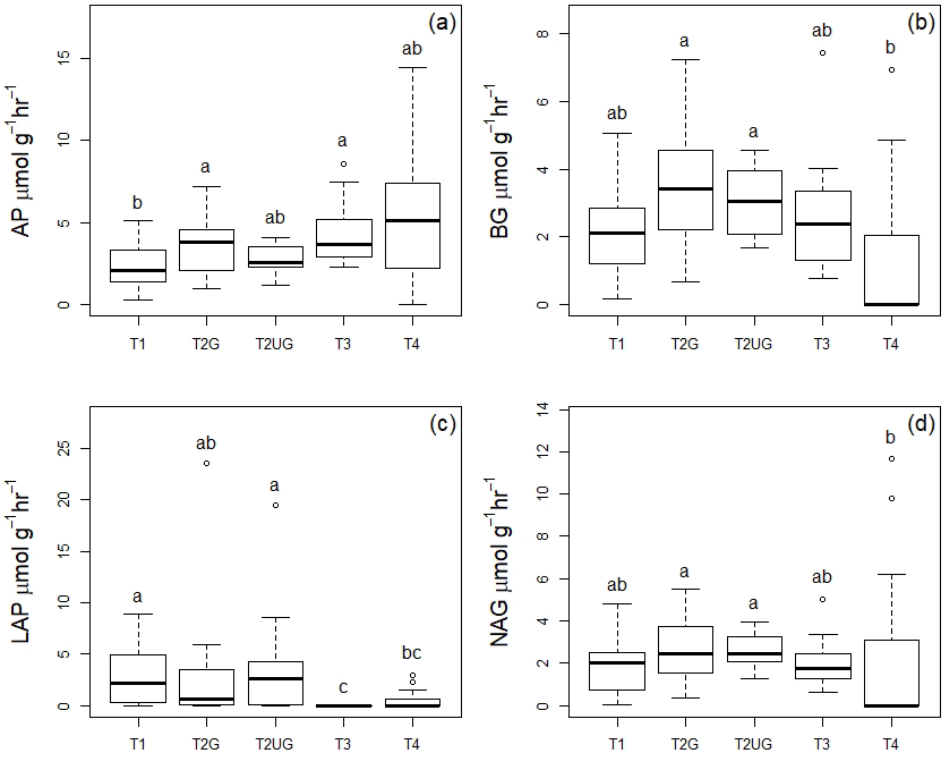

FIGURE 5 Soil microbial extracellular enzyme activities of (a) acid phosphatase (AP), (b) β-glucosidase (BG), (c) leucine aminopeptidase (LAP), and (d) β-N-acetylglucosaminidase (NAG) across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Letters indicate significant differences based on Tukey's test.

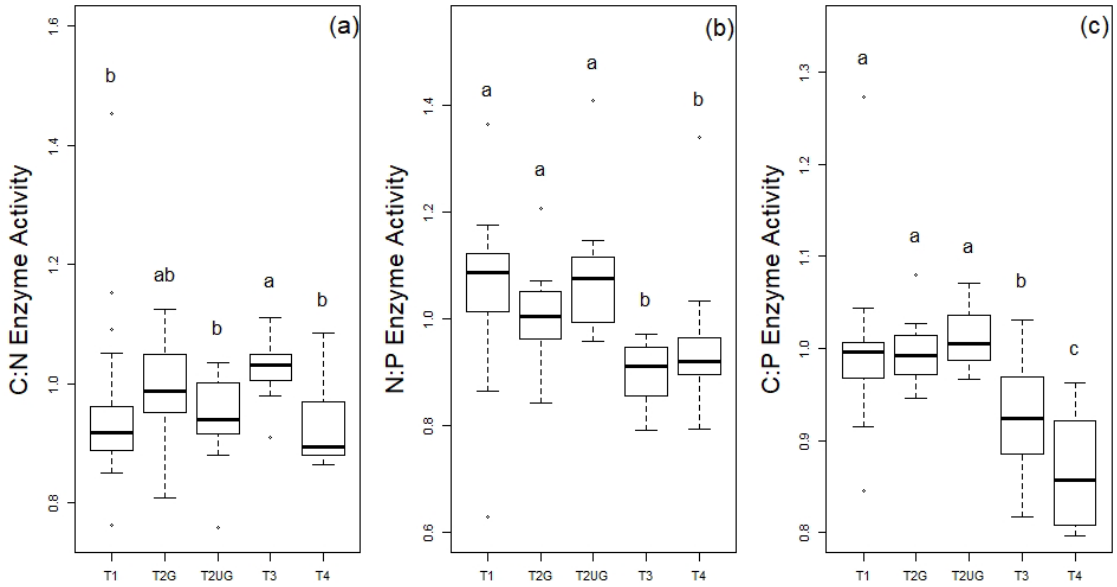

FIGURE 6 (a) Carbon to nitrogen (C:N), (b) nitrogen to phosphorus (N:P), and (c) carbon to phosphorus (C:P) soil microbial extracellular enzyme activity ratios across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Letters indicate significant differences based on Tukey's test.

3.2 Soil microbial communities across environmental gradients

Alpha-diversity (observed number of operational taxonomic units) of ITS sequences varied 1.5-fold among terraces ($F = 6.708$, $p < 0.0001$; Figure 7a) and was 41% higher in un-grazed habitat than grazing lawns ($p = 0.0032$). Alpha-diversity of 16S sequences varied 2-fold among terraces ($F = 31.15$, $p < 0.0001$; Figure 7b), and T4 had 45 to 52% lower prokaryotic diversity than all other terraces. There was not a significant difference in prokaryotic diversity between un-grazed habitat and grazing lawns.

The composition of fungal and prokaryotic communities also varied significantly among terraces (Table 4, Figure 8). However, only vegetation community composition explained more than 5% of variation in community structure; all other factors individually did not explain more than 1.5% of variation. In redundancy analyses, spatial

variation explained 5% of variation in fungal community structure and 4% of variation in prokaryotic community structure, while environmental drivers explained 14% of variation in fungal community structure and 8% of variation in prokaryotic community structure. Both fungal and prokaryotic community structure were correlated with ecological function (extracellular enzyme activity) (ITS data: r = 0.091, p = 0.006; 16S data: r = 0.159, p = 0.001).

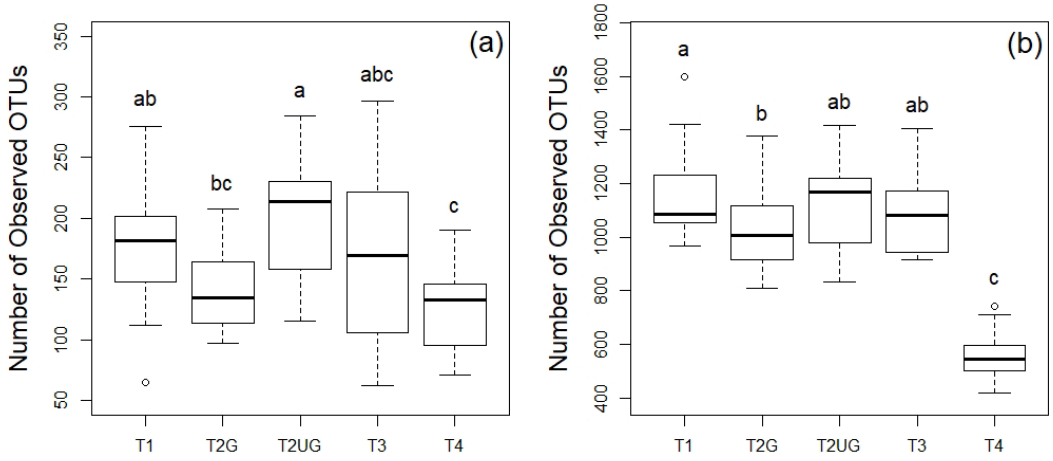

FIGURE 7 (a) Fungal and (b) prokaryotic alpha-diversity across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Alpha-diversity metric used was observed operational taxonomic units (OTUs) where amplicon sequence variants were considered as separate OTUs. Letters indicate significant differences based on Tukey's test.

TABLE 4 Results of permutational analyses of variance (ANOVAs) of fungal and prokaryotic communities' composition

Variable	ITS		16S	
	F-statistic	R^2	F-statistic	R^2
Transect Identity	5.4***	0.150	3.0***	0.089
Vegetation community composition	7.7***	0.071	5.2***	0.051
Live plant biomass (%)	1.5*	0.014	1.1	0.011
Dead plant biomass (%)	1.4*	0.013	1.2	0.011
Number of goose feces	1.1	0.010	1.2*	0.011
Soil moisture (%)	1.6**	0.015	1.2*	0.012
Soil pH	1.6**	0.014	1.1	0.011
Total organic carbon (%)	1.2	0.011	1.0	0.010
Total organic nitrogen (%)	0.8	0.007	0.9	0.009
Total potassium (%)	1.5*	0.014	1.1	0.011
Total phosphorus (%)	1.5*	0.014	1.1	0.011

Notes. Permutational ANOVAs performed on all soil samples across terraces and between grazing lawns and un-grazed habitat on Terrace two. Abbreviations: ITS, fungal sequence data; 16S, bacterial sequence data. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

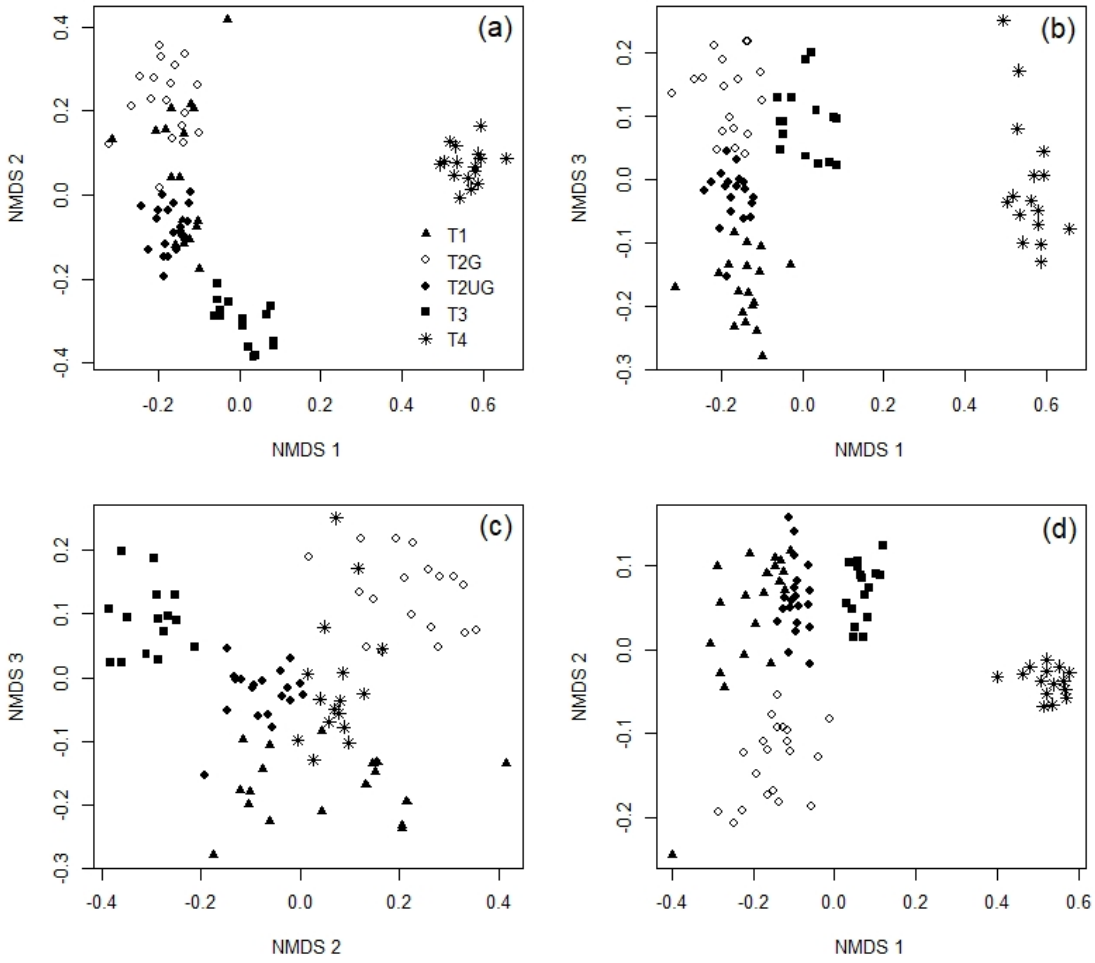

FIGURE 8 Non-metric multidemensional scaling (NMDS) plots of the Bray-Curtis diversity metric for (a-c) fungal and (b) prokaryotic communities by sample across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4). Fungal communities NMDS stress is 0.097 and prokaryotic communities NMDS stress is 0.072.

As the subsequent laboratory analyses focused on potential microbial legacies of herbivory, differences in the taxonomic composition of microbial communities in un-grazed habitat versus grazing lawns were quantified. All fungal phyla were differentially abundant between these two habitat types (Figure 9a). Additionally, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Gemmatimonadetes*, *Parcubacteria*

(OD1), and *Firmicutes* were differentially abundant between these two habitats (Figure 9b).

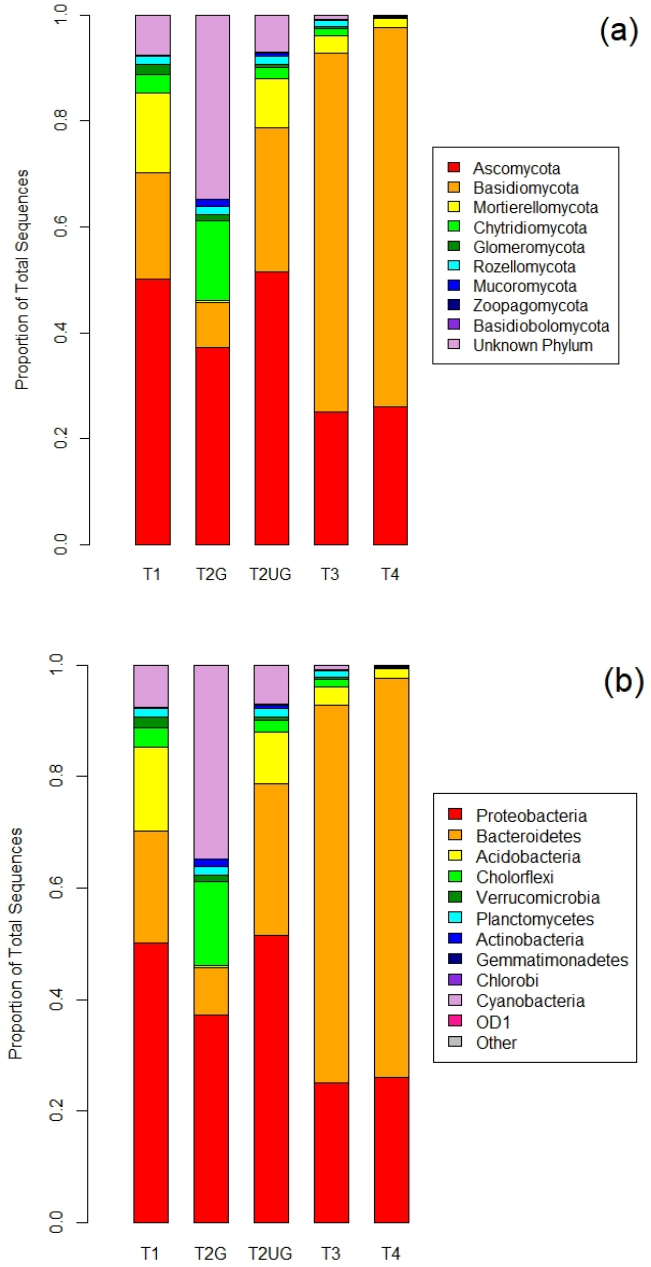

FIGURE 9 Proportions of the most abundant (a) fungal and (b) prokaryotic phyla across Terrace one (T1), Terrace two grazing lawns (T2G), Terrace two un-grazed habitat (T2UG), Terrace three (T3), and Terrace four (T4).

3.3 Greenhouse gas fluxes from grazed and un-grazed soils in a microcosm incubation experiment

Both GHG fluxes increased with temperature and fecal addition and were consistently higher in grazing lawn versus un-grazed soils (Table 5). These treatments had synergistic effects on GHG fluxes, which were maximized in grazing lawn soils under fecal addition and at higher temperatures (Figure 10). Grazing also affected the temperature sensitivity of GHG production. Across the 10-degree temperature gradient imposed, CO_2 fluxes increased nearly 2-fold from grazing lawn soils ($Q_{10} = 1.93 \pm 0.10$), but only 63% from un-grazed soils ($Q_{10} = 1.63 \pm 0.09$). CH_4 fluxes were not different between the two temperature treatments in un-grazed soils ($Q_{10} = 1.79 \pm 0.48$) but increased 2-fold in grazing lawn soils ($Q_{10} = 2.36 \pm 0.29$). Additionally, whereas CO_2 and CH_4 fluxes were 4 and 74% greater in wetter soils, respectively, the effects of the soil moisture treatment were amplified (for CH_4) or slightly diminished (for CO_2) under warmer temperatures, regardless of the soils' origin.

Microbial biomass varied idiosyncratically among treatments, with a four-way interaction among grazing history, temperature, moisture, and fecal addition (Table 5, Figure 11). Biomass was not consistently higher in the previously grazed soils, and there was no correlation between microbial biomass and mean CO_2 ($R^2 = 0.013$, $p = 0.139$) or CH_4 ($R^2 = 0.022$, $p = 0.079$) fluxes across all microcosms.

TABLE 5 Mixed model results on greenhouse gas fluxes and microbial biomass following the incubation experiment

Treatment	CO_2	CH_4	Microbial biomass
		F-statistic	
Grazing	383.4***	135.7***	19.0***
Temperature	1064.1***	17.4***	0.1
Moisture	5.5*	14.5***	12.3***
Fecal Addition	112.2***	34.1***	12.1***
Grazing x Temperature	26.2***	8.8**	2.9
Grazing x Moisture	2.2	0.1	6.3*
Temperature x Moisture	13.5***	4.4*	3.1
Grazing x Fecal Addition	11.3***	0.7	1.5
Temperature x Fecal Addition	0.6	2.6	0.02
Moisture x Fecal Addition	3.2	6.1*	5.0*
Grazing x Temperature x Moisture	2.1	2.4	0.4
Grazing x Temperature x Fecal Addition	9.7**	4.8*	23.3***
Grazing x Moisture x Fecal Addition	0.5	1.2	14.4***
Temperature x Moisture x Fecal Addition	0.02	0.3	4.7*
Grazing x Temperature x Moisture x Fecal Addition	0.01	3.5	6.4*

Notes. Abbreviations: CO_2, carbon dioxide; CH_4, methane. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

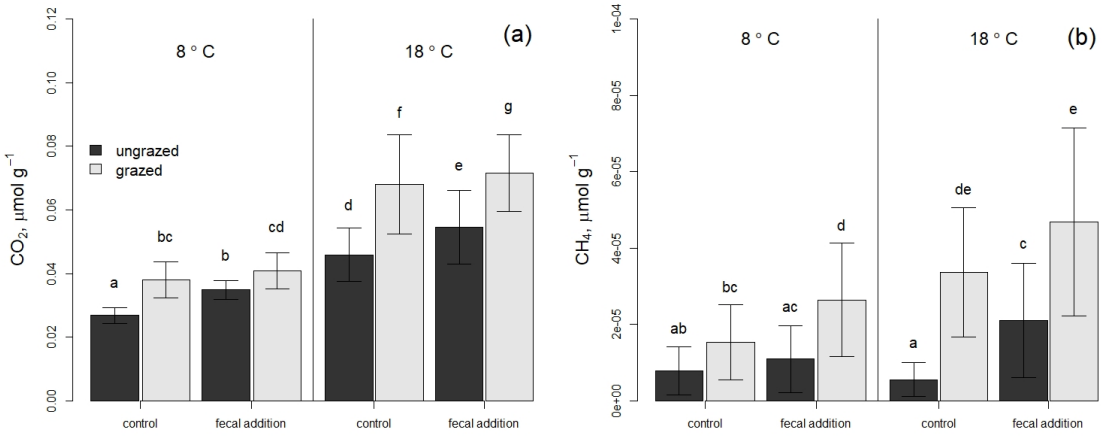

FIGURE 10 Mean (a) carbon dioxide (CO_2) and (b) methane (CH_4) fluxes (± standard error) during the incubation experiment for control and fecal addition treatments in low and high temperature treatments on soils from grazing lawns and un-grazed habitat. Data was averaged across all moisture treatments and timepoints. Letters indicate significant differences based on Tukey's test.

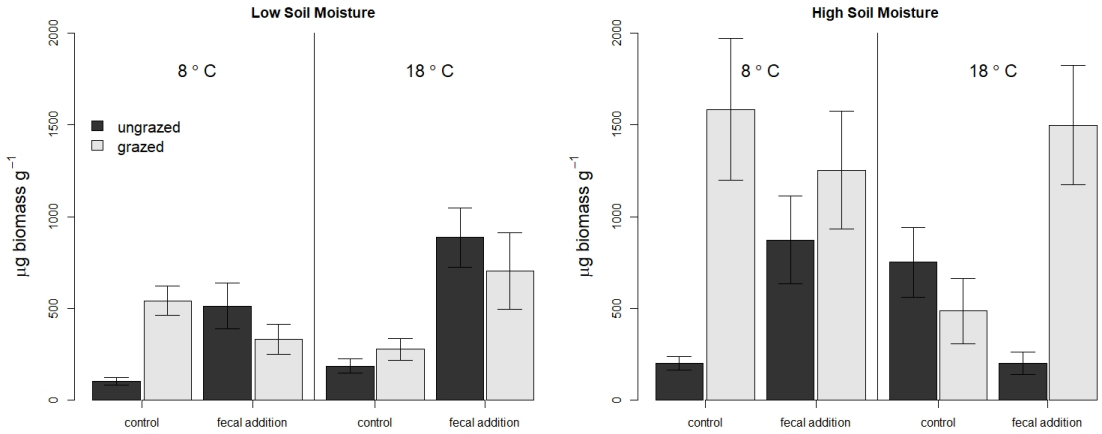

FIGURE 11 Mean soil microbial biomass (± standard error) following the incubation experiment for control and goose fecal addition treatments in low and high temperature treatments on soils from grazed and un-grazed habitat, separated into results from (left) low soil moisture treatments and (right) high soil moisture treatments.

CHAPTER 4

DISCUSSION

In this high-latitude wetland, soil chemistry and microbial community structure exhibit steep turnover across the landscape. Much of this variance is driven by distance from the Bering Sea, which influences the frequency of flooding, sedimentation rates and turnover in plant community structure (Jorgenson and Ely, 2001; Person and Ruess, 2003). However, the impacts of herbivory on soil chemistry and microbial community structure within a terrace are nearly as dramatic. Soils from grazed habitat are wetter with lower C and N content, but are enriched in K and P. The results suggest that higher CO_2 and CH_4 fluxes in grazing lawns are not attributable solely to herbivore-induced shifts in soil moisture, temperature, and nutrients, but also to differences in microbial community structure that reflect previous herbivory. The results highlight processes that control complex relationships among grazers, soil microbes, and belowground C cycling: the removal of plant biomass by herbivores that warms soils, the deposition of nutrients from herbivore waste, and the interaction of these with soil microbial community structure. The results suggest that incorporating the ecological relationships between herbivores, plants, and soil microbes into ecosystem models may help capture important carbon-climate feedbacks.

4.1 Soil biogeochemistry and microbial communities across the landscape

Most soil properties vary with distance from the Bering Sea (i.e. across terraces), and spatial turnover in plant community structure is associated with predictable gradients in soil moisture, pH, and total C and N. From terraces near the coast to inland (i.e., T1 to

T4), plant communities shift from salt-tolerant sedges and grasses to salt-intolerant woody plants, lichen and mosses (Jorgenson and Ely, 2001; Kincheloe and Stehn, 1991; Person and Ruess, 2003). Along this gradient, soil moisture and total C and N increase while pH decreases, reflecting increasing organic matter content of soils. Intense grazing within a terrace also has major impacts on soil biogeochemistry. For example, soil K and P are highly enriched on grazing lawns on T2, likely due to goose fecal deposition. Differences in soil moisture and total C and N between grazed and un-grazed habitats reflect at least in part lower aboveground biomass (Choi et al., 2019) and litterfall on grazing lawns due to consistent grazing by brant throughout the growing season and across years (Lohman et al., 2019).

Similar to patterns observed within plant communities, both fungal and prokaryotic microbial communities exhibit strong turnover among terraces. These patterns may reflect common microbial responses found in other ecosystems to soil abiotic properties that also vary with distance from the Bering Sea, such as pH (Lauber, Hamady, Knight, & Fierer, 2009), soil moisture (Strickland and Rousk, 2010), and organic C availability (Drenovsky, Vo, Graham, & Scow, 2004). The relative availability of C and nutrients may also vary among terraces, which is reflected by the differences in microbial extracellular enzyme activities. Enzyme activity ratios suggest that C and N are the limiting nutrients in the more mineral soils of T1 and T2, while P is the limiting element in the more organic-rich soils of T3 and T4. However, spatial and measured environmental factors only account for a minority of the observed variation in microbial community structure among terraces, even when accounting for different plant communities in each terrace. Unquantified factors, such as the flooding regime, dynamics

of the freeze-thaw cycles, and the chemistry of plant C inputs, may also be important.

Notably, differences in microbial community structure between grazed and un-grazed soils are similar in magnitude to differences across terraces. Both fungal and prokaryotic communities are responsive to soil properties that are impacted directly by herbivory, including increased soil moisture, nutrient content, and the relative abundance of living versus dead plant biomass. Herbivory can also alter rates of root exudation (Hamilton and Frank, 2001) and the temporal variability of temperature (Kelsey et al., 2016), which may also account for the large turnover in bacterial and fungal community structure between immediately adjacent grazing lawns versus un-grazed habitat.

4.2 Identifying ecological mechanisms of herbivore effects on greenhouse gas fluxes

In the incubation study, soils from grazed habitat had consistently higher CO_2 and CH_4 fluxes than soils from un-grazed habitat, agreeing with results from in situ herbivory manipulations (Kelsey et al., 2016; Leffler et al., 2019). CO_2 and CH_4 fluxes increase with temperature, soil moisture, and goose fecal addition, suggesting that grazing-related changes in the soil abiotic environment may enhance soil C losses. Yet while herbivore-induced changes in the soil microclimate and nutrient content impact GHG fluxes, these abiotic shifts are insufficient to explain differences in GHG fluxes across habitats. Differences in GHG fluxes from un-grazed versus grazed soils persist even when they are incubated under identical soil temperature and moisture conditions. Moreover, grazing history impacted not only the absolute magnitude of GHG fluxes, but also their relationship to soil climate: the temperature sensitivity (Q_{10}) of CO_2 and CH_4 flux is 18 and 32% greater, respectively, in previously grazed versus un-grazed soils. 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 (Kelsey et al., 2016), and because the Q_{10} of organic matter decomposition is higher.

Differences in GHG fluxes between grazed and un-grazed soils are likely attributable to three interrelated mechanisms: variation in soil nutrient content across habitats, differences in the size of the microbial biomass, and variation in fungal and prokaryotic community structure. Despite lower soil C and N stocks, soils from grazed habitat have higher K and P content and tend to have a larger microbial biomass, factors which are associated with higher decomposition rates. Yet differences in GHG flux between habitats persist even under the fecal addition treatment, suggesting that nutrient limitation on microbial biomass cannot fully account for differences in gas fluxes between the soil types. Moreover, across all microcosms, CO_2 and CH_4 fluxes were unrelated to the size of the microbial biomass. Therefore, larger fluxes in the soils from grazing lawns are not attributable to differences in absolute microbial abundance between grazed and un-grazed soils. Instead, variation within fungal and prokaryotic community compositions likely drives patterns of CO_2 and CH_4 flux under herbivory. For example, *Acidobacteria* is proportionally three times more abundant in un-grazed soils. Because this phylum contains many oligotrophic bacteria (Fierer, Bradford, & Jackson, 2007), which grow slowly and thus have slower respiration rates, their higher relative abundance may partially account for the observed lower rates of CO_2 flux. Although not measured in in this study, herbivory-induced shifts in bacterial or fungal community structure may also impact important community-level physiological parameters, such as C-use efficiency (the partitioning of C uptake between biomass growth and respiration; Allison, Wallenstein, & Bradford, 2010).

4.3 Implications for dynamic high-latitude ecosystems

Conditions at high latitudes are expected to change rapidly over time, as Arctic ecosystems are warming twice as fast as the rest of the globe, experiencing shifting precipitation regimes, and losing snow and ice cover (NOAA, 2019). The results imply that C cycling in grazed and un-grazed habitats will respond to the diverse effects climate change in different ways due to microbial legacies of herbivory that arise from the maintenance of the grazing lawns from year-to-year which keeps the soil environment different between the two habitats. However, it is not known how long these legacies persist. Microbial communities can change within short periods of time, such as a growing season (Shade, Caporaso, Handelsman, Knight, & Fierer, 2013). By contrast, other studies have noted a persistence in functional composition over several years, even following major landscape change (Bond-Lamberty et al., 2016; Hawkes et al., 2017). Furthermore, herbivory regimes are also shifting with climate change due to phenological mismatches between animals and their historical food sources (Beard, Kelsey, Leffler, & Welker, 2019; Cohen, Lajeunesse, & Rohr, 2018), of which migratory animals are particularly vulnerable (Robinson et al., 2009), and changes in population sizes of migratory animals. Brant populations in the Y-K Delta have declined by more than 4% annually over the past two decades (Fischer et al., 2018; Sedinger et al., 2016), while increasing on the Arctic Coastal Plain of Alaska (Flint, Meixell, & Mallek, 2014; Tape, Flint, Meixell, & Gaglioti, 2013). Conversely, emperor geese, cackling geese, and greater white-fronted geese populations in the Y-K Delta have increased by 25%, 300%, and 500%, respectively, during this time (Fischer et al., 2018). The degree of microbial community resistance to environmental change will shape the trajectory of C-climate

feedbacks as the Y-K Delta undergoes warming and changes in grazing regimes (Lohman et al., 2019; Uher-Koch et al., 2019).

The results have implications for understanding of soil biogeochemistry both within the Y-K Delta and across other high-latitude wetland ecosystems. The impacts of grazing on ecosystem-scale C cycling on the Y-K Delta will depend on the extent of grazing lawns, which vary in extent from year to year (Uher-Koch et al., 2019). However, the results suggest the differences in GHG fluxes between grazed and un-grazed habitat may be large enough to affect the strength of the terrestrial C sink in these high-latitude wetlands. Extrapolating these results to other high-latitude ecosystems is more complex because grazing has variable impacts on GHG fluxes from soils in different systems (Falk et al., 2015; Lara et al., 2017; Schmitz et al., 2018) as well as between habitats in the same systems (Sjögersten et al., 2008). For example, herbivore exclusion caused GHG fluxes to change, though in opposite directions, via interactions with plant productivity and soil abiotic characteristics (Falk et al., 2015; Lara et al., 2017). This study demonstrates that herbivore-mediated changes in plant communities are likely to cascade through belowground ecosystems, altering soil microbial community structure and GHG fluxes.

Although microbial community shifts clearly play an important role in mediating belowground responses to herbivory, terrestrial ecosystem models do not explicitly represent microbial controls over soil C cycling (Campbell and Paustian, 2015; Schmitz et al., 2018). Therefore, most predictive models would be unable to capture the interactions among herbivory, climate, and soil biogeochemistry observed on the Y-K Delta and elsewhere. Microbial community-by-environment interactions and the impacts

of herbivores are increasingly recognized as a major driver of regulating ecosystem process rates (Glassman et al., 2018; Schmitz et al., 2018). Incorporating microbial community dynamics into ecosystem models may be necessary to capture ecological feedbacks on environmental change (Crowther et al., 2019; Wieder, Bonan, & Allison, 2013).

REFERENCES

Allison, S. D., Wallenstein, M. D., & Bradford, M. A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, *3*(5), 336–340. https://doi.org/10.1038/ngeo846

Amundson, C., Flint, P., Stehn, R., Platte, R., Wilson, H., Larned, W., & Fischer, J. (2019). Spatiotemporal population change of Arctic-breeding waterbirds on the Arctic Coastal Plain of Alaska. *Avian Conservation and Ecology*, *14*(1). https://doi.org/10.5751/ACE-01383-140118

Asner, G. P., Elmore, A. J., Olander, L. P., Martin, R. E., & Harris, A. T. (2004). Grazing Systems, Ecosystem Responses, and Global Change. *Annual Review of Environment and Resources*, *29*(1), 261–299. https://doi.org/10.1146/annurev.energy.29.062403.102142

Babcock, C. A., & Ely, C. R. (1994). Classification of vegetation communities in which geese rear broods on the Yukon – Kuskokwim delta, Alaska. *Canadian Journal of Botany*, *72*(9), 1294–1301. https://doi.org/10.1139/b94-158

Bardgett, R. D., Wardle, D. A., & Yeates, G. W. (1998). Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry*, *30*(14), 1867–1878. https://doi.org/10.1016/S0038-0717(98)00069-8

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1-48. https://doi.org/10.18637/jss.v067.i01

Beard, K. H., & Choi, R. T. (2017). Asynchrony in the timing of goose-vegetation interactions: implications for biogeochemical cycling in wet sedge tundra Tutakoke River, Yukon Delta NWR, Alaska, 2014-2016. Arctic Data Center. https://doi.org/10.18739/A22274

Beard, K. H., & Choi, R. T. (n.d.) [Estimates of percent cover of each plant functional group on Terraces 1-3]. Unpublished raw data.

Beard, K. H., Kelsey, K. C., Leffler, A. J., & Welker, J. M. (2019). The Missing Angle: Ecosystem Consequences of Phenological Mismatch. *Trends in Ecology and Evolution*, *34*(10), 885–888. https://doi.org/10.1016/j.tree.2019.07.019

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bond-Lamberty, B., Bolton, H., Fansler, S., Heredia-Langner, A., Liu, C., McCue, L. A., … Bailey, V. (2016). Soil Respiration and Bacterial Structure and Function after 17 Years of a Reciprocal Soil Transplant Experiment. *PLoS ONE*, *11*(3), e0150599. https://doi.org/10.1371/journal.pone.0150599

Booth, D. T., Cox, S. E., & Berryman, R. D. (2006). Point Sampling Digital Imagery with 'Samplepoint.' *Environmental Monitoring and Assessment*, *123*(1–3), 97–108. https://doi.org/10.1007/s10661-005-9164-7

Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., & Strickland, M. S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. *Biogeochemistry*, *113*(1), 271–281. https://doi.org/10.1007/s10533-012-9822-0

Bråthen, K. A., Ims, R. A., Yoccoz, N. G., Fauchald, P., Tveraa, T., & Hausner, V. H. (2007). Induced Shift in Ecosystem Productivity? Extensive Scale Effects of Abundant Large Herbivores. *Ecosystems*, *10*(5), 773–789. https://doi.org/10.1007/s10021-007-9058-3

Cahoon, S. M. P., Sullivan, P. F., Post, E., & Welker, J. M. (2012). Large herbivores limit CO2 uptake and suppress carbon cycle responses to warming in West Greenland. *Global Change Biology*, *18*(2), 469–479. https://doi.org/10.1111/j.1365-2486.2011.02528.x

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*(1), 421. https://doi.org/10.1186/1471-2105-10-421

Campbell, E. E., & Paustian, K. (2015). Current developments in soil organic matter modeling and the expansion of model applications: A review. *Environmental Research Letters*, *10*(12), 123004. https://doi.org/10.1088/1748-9326/10/12/123004

Castro, H. F., Classen, A. T., Austin, E. E., Norby, R. J., & Schadt, C. W. (2010). Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology*, *76*(4), 999–1007. https://doi.org/10.1128/AEM.02874-09

Choi, R. T., Beard, K. H., Leffler, A. J., Kelsey, K. C., Schmutz, J. A., & Welker, J. M. (2019). Phenological mismatch between season advancement and migration

timing alters Arctic plant traits. *Journal of Ecology*, *107*(5), 2503–2518. https://doi.org/10.1111/1365-2745.13191

Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, *8*(3), 224–228. https://doi.org/10.1038/s41558-018-0067-3

Crowther, T. W., van den Hoogen, J., Wan, J., Mayes, M. A., Keiser, A. D., Mo, L., … Maynard, D. S. (2019). The global soil community and its influence on biogeochemistry. *Science*, *365*(6455), eaav0550. https://doi.org/10.1126/science.aav0550

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., … Andersen, G. L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied and Environmental Microbiology*, *72*(7), 5069–5072. https://doi.org/10.1128/AEM.03006-05

Drenovsky, R. E., Vo, D., Graham, K. J., & Scow, K. M. (2004). Soil Water Content and Organic Carbon Availability Are Major Determinants of Soil Microbial Community Composition. *Microbial Ecology*, *48*(3), 424–430.

Falk, J. M., Schmidt, N. M., Christensen, T. R., & Ström, L. (2015). Large herbivore grazing affects the vegetation structure and greenhouse gas balance in a high arctic mire. *Environmental Research Letters*, *10*(4), 045001. https://doi.org/10.1088/1748-9326/10/4/045001

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an Ecological Classification of Soil Bacteria. *Ecology*, *88*(6), 1354–1364.

Fischer, J. B., Stehn, R. A., Bowman, T. D., Platte, R. M., Eldridge, W. D., Hodges, J. I., & Butler, W. I. (2018). Coordinated aerial and ground surveys document long-term recovery of geese and eiders on the Yukon–Kuskokwim Delta, Alaska, 1985–2014. In W. D. Shuford, R. E. Gill, & C. M. Handel (Eds.), *Trends and Traditions: Avifaunal Change in Western North America* (pp. 148–160). Camarillo, CA: Western Field Ornithologists. https://doi.org/10.21199/SWB3.7

Flint, P. L., Meixell, B. W., & Mallek, E. J. (2014). High fidelity does not preclude colonization: Range expansion of molting Black Brant on the Arctic coast of Alaska. *Journal of Field Ornithology*, *85*(1), 75–83. https://doi.org/10.1111/jofo.12051

Gao, Y. h, Luo, P., Wu, N., Chen, H., & Wang, G. (2008). Impacts of Grazing Intensity on Nitrogen Pools and Nitrogen Cycle in an Alpine Meadow on the Eastern Tibetan Plateau. *Applied Ecology and Environmental Research*, *6*, 69–79. https://doi.org/10.15666/aeer/0603_069079

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, *43*(7), 1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

Glassman, S. I., Weihe, C., Li, J., Albright, M. B. N., Looby, C. I., Martiny, A. C., … Martiny, J. B. H. (2018). Decomposition responses to climate depend on microbial community composition. *Proceedings of the National Academy of Sciences*, *115*(47), 11994–11999. https://doi.org/10.1073/pnas.1811269115

Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., … Brodie, E. L. (2011). Differential Growth Responses of Soil Bacterial Taxa to Carbon Substrates of Varying Chemical Recalcitrance. *Frontiers in Microbiology*, *2,* 94. https://doi.org/10.3389/fmicb.2011.00094

Hamilton, E. W., & Frank, D. A. (2001). Can Plants Stimulate Soil Microbes and Their Own Nutrient Supply? Evidence from a Grazing Tolerant Grass. *Ecology*, *82*(9), 2397–2402. https://doi.org/10.2307/2679923

Hawkes, C. V., Waring, B. G., Rocca, J. D., & Kivlin, S. N. (2017). Historical climate controls soil respiration responses to current soil moisture. *Proceedings of the National Academy of Sciences*, *114*(24), 6322–6327. https://doi.org/10.1073/pnas.1620811114

Hik, D. S., & Jefferies, R. L. (1990). Increases in the Net Above-Ground Primary Production of a Salt-Marsh Forage Grass: A Test of the Predictions of the Herbivore-Optimization Model. *Journal of Ecology*, *78*(1), 180–195. https://doi.org/10.2307/2261044

Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J. W., Schuur, E. a. G., Ping, C. L., … Kuhry, P. (2014). Estimated stocks of circumpolar permafrost carbon with quantified uncertainty ranges and identified data gaps. *Biogeosciences (Online)*, *11*(23). https://doi.org/10.5194/bg-11-6573-2014

Jorgenson, M. T. (2000). Hierarchical Organization of Ecosystems at Multiple Spatial Scales on the Yukon-Kuskokwim Delta, Alaska, U.S.A. *Arctic, Antarctic, and Alpine Research*, *32*(3), 221–239. https://doi.org/10.1080/15230430.2000.12003360

Jorgenson, T., & Ely, C. (2001). Topography and Flooding of Coastal Ecosystems on the Yukon-Kuskokwim Delta, Alaska: Implications for Sea-Level Rise. *Journal of Coastal Research*, *17*(1), 124–136.

Kelsey, K. C., Leffler, A. J., Beard, K. H., Schmutz, J. A., Choi, R. T., & Welker, J. M.

(2016). Interactions among vegetation, climate, and herbivory control greenhouse gas fluxes in a subarctic coastal wetland. *Journal of Geophysical Research: Biogeosciences*, *121*(12), 2960–2975. https://doi.org/10.1002/2016JG003546

Kincheloe, K. L., & Stehn, R. A. (1991). Vegetation patterns and environmental gradients in coastal meadows on the Yukon–Kuskokwim delta, Alaska. *Canadian Journal of Botany*, *69*(7), 1616–1627. https://doi.org/10.1139/b91-205

Koyama, A., Wallenstein, M. D., Simpson, R. T., & Moore, J. C. (2014). Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Frontiers in Microbiology*, *5,* 516. https://doi.org/10.3389/fmicb.2014.00516

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 1-26. http://doi.org/10.18637/jss.v082.i13

Lara, M. J., Johnson, D. R., Andresen, C., Hollister, R. D., & Tweedie, C. E. (2017). Peak season carbon exchange shifts from a sink to a source following 50+ years of herbivore exclusion in an Arctic tundra ecosystem. *Journal of Ecology*, *105*(1), 122–131. https://doi.org/10.1111/1365-2745.12654

Lauber, C. L., Hamady, M., Knight, R., & Fierer, N. (2009). Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. *Applied and Environmental Microbiology*, *75*(15), 5111–5120. https://doi.org/10.1128/AEM.00335-09

Leffler, A. J., Beard, K. H., Kelsey, K. C., Choi, R. T., Schmutz, J. A., & Welker, J. M. (2019). Delayed herbivory by migratory geese increases summer-long CO2 uptake in coastal western Alaska. *Global Change Biology*, *25*(1), 277–289. https://doi.org/10.1111/gcb.14473

Lohman, M. G., Riecke, T. V., Acevedo, C. R., Person, B. T., Schmutz, J. A., Uher-Koch, B. D., & Sedinger, J. S. (2019). Changes in behavior are unable to disrupt a trophic cascade involving a specialist herbivore and its food plant. *Ecology and Evolution*, *9*(9), 5281–5291. https://doi.org/10.1002/ece3.5118

MacDonald, K. B. (1977). Plant and animal communities of Pacific North America salt marshes. In V. J. Chapman (Eds.), *Wet coastal ecosystems* (pp. 167-191).

McKendrigk, J. D., Batzli, G. O., Everett, K. R., & Swanson, J. C. (1980). Some Effects of Mammalian Herbivores and Fertilization on Tundra Soils and Vegetation. *Arctic and Alpine Research*, *12*(4), 565–578. https://doi.org/10.1080/00040851.1980.12004213

Melillo, J. M., Frey, S. D., DeAngelis, K. M., Werner, W. J., Bernard, M. J., Bowles, F. P., … Grandy, A. S. (2017). Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science*, *358*(6359), 101–105. https://doi.org/10.1126/science.aan2874

Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., … Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Oksanen, J. F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5-4 [Data code package]. Retrieved from https://CRAN.Rproject.org/package=vegan

Person, B. T., & Ruess, R. W. (2003). Stability of a subarctic saltmarsh: Plant community resistance to tidal inundation. *Écoscience*, *10*(3), 351–360. https://doi.org/10.1080/11956860.2003.11682784

Person, B. T., Babcock, C. A., & Ruess, R. W. (1998). Forage variation in brood-rearing areas used by pacific black brant geese on the Yukon-Kuskokwim delta, Alaska. *Journal of Ecology*, *86*(2), 243–259. https://doi.org/10.1046/j.1365-2745.1998.00249.x

National Oceanic and Atmospheric Administration. (2019). Arctic Report Card 2019. Retrieved from https://www.arctic.noaa.gov/Portals/7/ArcticReportCard/Documents/ArcticReportCard_full_report2019.pdf

Robinson, R., Crick, H., Learmonth, J., Maclean, I., Thomas, C., Bairlein, F., … Visser, M. (2009). Travelling through a warming world: Climate change and migratory species. *Endangered Species Research*, *7*, 87–99. https://doi.org/10.3354/esr00095

Ruess, R. W., Uliassi, D. D., Mulder, C. P. H., & Person, B. T. (1997). Growth responses of Carex ramenskii to defoliation, salinity, and nitrogen availability: Implications for geese-ecosystem dynamics in western Alaska. *Écoscience*, *4*(2), 170–178. https://doi.org/10.1080/11956860.1997.11682392

Schimel, J., & Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in Microbiology*, *3,* 348. https://doi.org/10.3389/fmicb.2012.00348

Schmitz, O. J., Wilmers, C. C., Leroux, S. J., Doughty, C. E., Atwood, T. B., Galetti, M.,

… Goetz, S. J. (2018). Animals and the zoogeochemistry of the carbon cycle. *Science*, *362*(6419). https://doi.org/10.1126/science.aar3213

Sedinger, J. S., Nicolai, C. A., VanDellen, A. W., Leach, A. G., Wilson, H. M., & Anthony, R. M. (2016). Predation and reduced grazing interact to reduce recruitment and population growth in Black Brant. *The Condor*, *118*(2), 433–444. https://doi.org/10.1650/CONDOR-15-109.1

Shade, A., Caporaso, G. J., Handelsman, J., Knight, R., & Fierer, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *The ISME Journal*, *7*(8), 1493–1506. https://doi.org/10.1038/ismej.2013.54

Sjögersten, S., Wal, R. van der, & Woodin, S. J. (2008). Habitat Type Determines Herbivory Controls Over CO2 Fluxes in a Warmer Arctic. *Ecology*, *89*(8), 2103–2116. https://doi.org/10.1890/07-1601.1

Stark, S., Männistö, M. K., & Eskelinen, A. (2015). When do grazers accelerate or decelerate soil carbon and nitrogen cycling in tundra? A test of theory on grazing effects in fertile and infertile habitats. *Oikos*, *124*(5), 593–602. https://doi.org/10.1111/oik.01355

Stark, S., Tuomi, J., Strömmer, R., & Helle, T. (2003). Non-parallel changes in soil microbial carbon and nitrogen dynamics due to reindeer grazing in northern boreal forests. *Ecography*, *26*(1), 51–59. https://doi.org/10.1034/j.1600-0587.2003.03336.x

Strickland, M. S., & Rousk, J. (2010). Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Tande, G. F., & Jennings, T. W. (1986). Classification and mapping of tundra near Hazen Bay, Yukon Delta National Wildlife Refuge, Alaska. Available from the Alaska Investigations Field Office, U.S. Fish and Wildlife Service, Anchorage.

Tape, K. D., Flint, P. L., Meixell, B. W., & Gaglioti, B. V. (2013). Inundation, sedimentation, and subsidence creates goose habitat along the Arctic coast of Alaska. *Environmental Research Letters*, *8*(4), 045031. https://doi.org/10.1088/1748-9326/8/4/045031

Uher-Koch, B. D., Schmutz, J. A., Wilson, H. M., Anthony, R. M., Day, T. L., Fondell, T. F., … Sedinger, J. S. (2019). Ecosystem-scale loss of grazing habitat impacted by abundance of dominant herbivores. *Ecosphere*, *10*(6), e02767. https://doi.org/10.1002/ecs2.2767

van der Wal, R., & Brooker, R. W. (2004). Mosses mediate grazer impacts on grass

abundance in arctic ecosystems. *Functional Ecology*, *18*(1), 77–86. https://doi.org/10.1111/j.1365-2435.2004.00820.x

van der Wal, R., Sjögersten, S., Woodin, S. J., Cooper, E. J., Jónsdóttir, I. S., … Huiskes, A. D. (2007). Spring feeding by pink-footed geese reduces carbon stocks and sink strength in tundra ecosystems. *Global Change Biology*, *13*(2), 539–545. https://doi.org/10.1111/j.1365-2486.2006.01310.x

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wieder, W. R., Bonan, G. B., & Allison, S. D. (2013). Global soil carbon projections are improved by modelling microbial processes. *Nature Climate Change*, *3*(10), 909–912. https://doi.org/10.1038/nclimate1951

Zacheis, A., Ruess, R. W., & Hupp, J. W. (2002). Nitrogen dynamics in an Alaskan salt marsh following spring use by geese. *Oecologia*, *130*(4), 600-608. https://doi.org/10.1007/s00442-001-0837-9