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LETTER

Soil microbial communities and elk foraging intensity: implications for soil biogeochemical cycling in the sagebrush steppe

Lauren C. Cline,[1]* Donald R. Zak,[2,3] Rima A. Upchurch,[2] Zachary B. Freedman[4] and Anna R. Peschel[5]

Abstract

Foraging intensity of large herbivores may exert an indirect top-down ecological force on soil microbial communities via changes in plant litter inputs. We investigated the responses of the soil microbial community to elk (*Cervus elaphus*) winter range occupancy across a long-term foraging exclusion experiment in the sagebrush steppe of the North American Rocky Mountains, combining phylogenetic analysis of fungi and bacteria with shotgun metagenomics and extracellular enzyme assays. Winter foraging intensity was associated with reduced bacterial richness and increasingly distinct bacterial communities. Although fungal communities did not respond linearly to foraging intensity, a greater β-diversity response to winter foraging exclusion was observed. Furthermore, winter foraging exclusion increased soil cellulolytic and hemicellulolytic enzyme potential and higher foraging intensity reduced chitinolytic gene abundance. Thus, future changes in winter range occupancy may shape biogeochemical processes via shifts in microbial communities and subsequent changes to their physiological capacities to cycle soil C and N.

Keywords

Bacteria, community assembly, decomposition, extracellular enzyme, foraging, functional gene, fungi, herbivore, metagenome.

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INTRODUCTION

Large herbivores function as 'engineers' in a wide variety of ecosystems (Frank & Groffman 1998; Tanentzap & Coomes 2012) through the removal of plant biomass, which alters plant physiology and litter biochemistry (Bardgett *et al*. 1998; Olofsson *et al*. 2004). Saprotrophic microorganisms are limited by the energy they enzymatically harvest from plant detritus; therefore, the production of plant detritus and its biochemical composition function as selective forces that shape the composition of microbial communities in soil (e.g. Cline & Zak 2015). It stands to reason that foraging intensity of large herbivores may structure the composition and function of microbial communities in soil via changes in the abundance and biochemistry of plant litter substrates. Understanding this potential is of ecosystem-level significance, because soil microorganisms regulate key ecosystem functions, including soil C storage and plant nutrient availability (van der Heijden *et al*. 2008; Clemmensen *et al*. 2013).

Although evidence indicates that large herbivores can modify soil C and N cycling (e.g. Olofsson *et al*. 2004), understanding the mechanism by which migratory ungulates structure the composition and function of the soil microbial community remains largely unknown. Selective foraging by large mammalian herbivores can reduce soil microbial biomass and significantly modify microbial community composition (Pastor *et al*. 1993; Peschel *et al*. 2015). Changes in the size and membership of the soil community can, in turn, feedback to alter microbial metabolic potential for biogeochemical cycling (Yang *et al*. 2013), which has direct implications for the cycling and storage of soil C and N. For example, ungulate foraging on winter range decreased the abundance of microbial functional genes encoding enzymes involved in lignocellulose metabolism, as well as soil respiration and net N mineralisation (Peschel *et al*. 2015). In contrast, herbivore foraging can also increase the abundance of functional genes encoding enzymes mediating the decay of plant detritus (Yang *et al*. 2013). These mixed responses by soil microbial communities to ungulate foraging may be the result of differences in foraging intensities across regions (McSherry & Ritchie 2013), although this hypothesis remains to be tested.

North American elk (*Cervus elaphus*) are dominant ungulate herbivores in the sagebrush steppe and present a unique opportunity to investigate the microbial mechanism by which migratory ungulates induce belowground responses (Middleton *et al*. 2013; Jones *et al*. 2014; Cole *et al*. 2015). Inhabiting mountainous regions, North American elk migrate from high-elevation summer range and congregate in valley bottoms

[1]*Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, USA*
[2]*School of Natural Resources & Environment, University of Michigan, Ann Arbor, MI, USA*
[3]*Department of Ecology & Evolution, University of Michigan, Ann Arbor, MI, USA*
[4]*Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA*
[5]*Department of Conservation Biology, University of Minnesota, St. Paul, MN, USA*
**Correspondence: E-mail: clinela@umn.edu*

during winter, avoiding deep snow and accessing forage (Boyce 1991). In spring, these large ungulates follow the snowmelt to high elevations in which young plants emerge with protein-rich foliage (Sawyer & Kauffman 2011). Because winter range occupancy by elk and other ungulate herbivores can alter the membership of soil microbial communities (Peschel *et al*. 2015), winter range occupancy may have consequences for rates of biogeochemical cycling. Specifically, net N mineralisation was significantly greater in the absence of elk winter foraging across the sagebrush steppe, suggesting that soil N cycling rates may be reduced by the removal of plant inputs and subsequent changes to the soil microbial community (Peschel *et al*. 2015).

To elucidate the microbial mechanism by which foraging intensity in winter range may reduce rates of soil C and N cycling, we combined phylogenetic analyses of microbial communities with shotgun metagenomics and extracellular enzyme assays. We used these approaches across a range of foraging intensities in a long-term foraging exclusion experiment within the sagebrush steppe of the northern Rocky Mountains. In this region, ungulate foraging on winter range can dramatically reduce the occurrence of shrubs (e.g. *Artemisia tridentata*), which emerge above the snow, thereby increasing the abundance of grasses and forbs. These changes in plant community composition cascade to alter plant litter production and biochemistry, which in turn, may slow rates of soil nutrient cycling through changes in microbial community composition. We hypothesised that the magnitude of microbial compositional and functional responses will be governed by winter foraging intensity. Specifically, we expected that a high intensity of winter foraging will lead to larger reductions in fungal and bacterial richness, larger compositional changes in both communities, as well as reduced extracellular enzyme activity and lower abundance of genes mediating the cycling of C and N in soils.

METHODS

Study sites

We investigated the effect of ungulate foraging intensity on soil microbial composition and function by sampling four sites in northwestern Wyoming, USA, in which winter foraging exclosures were constructed *c*. 60–80 years ago. Study sites are located on winter range in sagebrush steppe within the Bridger-Teton National Forest and the National Elk Refuge (NER) in Jackson Hole (see Fig. S1). These areas constitute winter range for large ungulate herbivores, including the North American elk (*Cervus elaphus*), moose (*Alces alces*), mule deer (*Odocoileus hemionus*), American bison (*Bison bison*), pronghorn (*Antilocapra americana*) and bighorn sheep (*Ovis canadensis*; Boyce 1991). Study sites include the Gros Ventre (GV), Lower Hoback (LH), Miller Butte (MB) and Upper Hoback (UH); detailed site information can be found in Peschel *et al*. (2015). These four sites were selected from nine grazing exclosures to minimise site-to-site variation in plant community composition, soil characteristics, as well as slope and aspect. During winter, these study sites are primarily occupied by elk, although bison occasionally graze MB located in the NER. Plant communities are dominated by bunch grasses and several varieties of *Artemisia tridentata*. In summer 2013, estimates of shrub, forb and graminoid cover were made within 10 1-m^2 sampling frames that were randomly located inside and outside each exclosure (Peschel *et al*. 2015).

Soil sampling and DNA extraction

We sampled soils inside and outside of the winter foraging exclosures at each study site in May 2015. Inside each exclosure, a 10 × 10-m plot was established at the centre. Similarly, we established a 10 × 10-m plot with matching slope and aspect, 10 m away from the outside fenced edge of each exclosure. Three composite soil samples were collected in each fenced and unfenced plot at each study site ($n = 4$). Each composite sample consisted of five soil cores, sampled to a depth of 10 cm (2.5 cm diameter); they were collected from random locations within each fenced and unfenced plot (hereafter foraged and unforaged). Composite soil samples were stored on ice in the field and immediately shipped (< 24 h) to the University of Michigan, where they were kept at −80 °C. Prior to extraction, soil samples were passed through a 2-mm sieve and roots were removed by hand. Six replicate extractions (3 g soil) were used to extract genomic DNA from each of the three composite soil samples collected in each foraged and unforaged plot using a PowerLyzer® DNA Isolation Kit (MO-BIO laboratories, Carlsbad, CA, USA).

Microbial community analysis

Targeted amplification of the fungal large ribosomal subunit (28S) and bacterial small ribosomal subunit (16S) was performed to characterise soil microbial community composition. Fungal α and β-diversity were estimated using primers LROR and LR3 (Vilgalys & Hester 1990). To quantify bacterial community composition, the 16S rRNA gene was targeted using primers 27f and 519r (Lane 1991). PCR protocol information can be found in Appendix S1. PCR products were purified using the Qiagen MinElute PCR kit and quantified using a Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, CA, USA). Sequencing was performed on the PacBio RS II system utilising circular consensus technology at the University of Michigan Sequencing Facility. Six barcoded samples, pooled in equimolar concentrations, were multiplexed on each SMRT chip for a total of eight chips.

Sequences were processed in Mothur using established pipeline procedures (Schloss *et al*. 2011). Briefly, sequences were sorted by barcode and trimmed to remove primers and barcodes, followed by alignment to SILVA 16S and RDP 28S reference alignments (Quast *et al*. 2013; Cole *et al*. 2014). DNA contaminants and chimeras, identified using uchime (Edgar *et al*. 2011), were removed prior to downstream analysis. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity for both fungal and bacterial sequences. Prior to the calculation of OTU richness, fungal and bacterial libraries were subsampled according to the library with the lowest sequence coverage (586 fungal and 1240 bacterial sequences). To calculate phylogenetic β-diversity, fungal and bacterial phylogenetic trees were constructed

using FastTree 2 (Price *et al*. 2010), followed by the calculation of weighted UniFrac distance between foraged and unforaged plots (Lozupone *et al*. 2006). Because subsampling can increase uncertainty in data (McMurdie & Holmes 2014), UniFrac distances were calculated from unrarefied sequence libraries with Hellinger transformations. Sequences were uploaded to the NCBI Sequence Read Archive under accession SRP079358.

Shotgun metagenomics

Eight libraries representing foraged ($n = 4$) and unforaged ($n = 4$) plots were multiplexed and sequenced on two lanes of the HiSeq 2500 Illumina instrument, with 150 bp single-end reads. All metagenome sequence data have been deposited and are publically available in MG-RAST (Meyer *et al*. 2008) under accession numbers 4670116.3–4670123.3. We annotated functional genes from each metagenome, which mediate soil carbon (C) and nitrogen (N) cycling processes, using DIAMOND (Buchfink *et al*. 2014) queries against curated databases (Fish *et al*. 2013; Table S1). The complete functional gene repository (FunGene) database for each gene was filtered, requiring sequences to have more than 50% coverage to the FunGene HMM and a score > 100. The abundance of genes was calculated following the assignment of metagenome sequences to functional gene databases using the 'BLASTX' function and default parameters in DIAMOND (v 0.7.9.58). Gene assignments were standardised to the number of sequences with predicted functions for each metagenome.

Extracellular enzyme assays

To estimate lignocellulolytic activity of soil communities, extracellular enzyme assays were conducted in 96-well plates. To measure the activity of β-1,4-glucosidase, cellobiohydrolase and β-1,4-xylosidase, we used 200 μM methylumbelliferyl-linked substrates (Saiya-Cork *et al*. 2002). A 25 mM L-dihydroxyphenylalanine substrate was used to assay phenol oxidase. To obtain a soil slurry, one gram of soil was homogenised in 125 mL of 50 mM sodium acetate buffer (pH 5.0) for 1 min. Enzyme activity was measured in Synergy HT Multi-Mode Microplate Reader (Bio-Tek, Winooski, VT, USA) set at 360 nm excitation wavelength and 460 nm emission wavelength. Phenol oxidase assays were incubated for 24 h and rates were estimated spectrophotometrically (Saiya-Cork *et al*. 2002). Enzyme activities were expressed as $\mu mol\ g^{-1}\ h^{-1}$. To quantify overall variation in enzyme potential across sites and foraging treatments, a Euclidean distance matrix was calculated following square root transformation of enzyme activities.

Statistical analysis

Univariate and multivariate statistics were used to test whether winter foraging intensity significantly impacted microbial community composition and functional potential. The average difference in shrub cover (%) between the foraged and unforaged treatments at each site (Herrick *et al*. 2009) was calculated as a direct response to foraging intensity, representing the relative amount of plant material consumed by herbivores and microbial substrate availability. To quantify the effects of winter foraging exclusion on soil water and microbial relative abundance, we performed nested analysis of variance (ANOVA) with site, foraging treatment and their interaction as factors. Subplots were considered nested replicates within each treatment plot (Peschel *et al*. 2015). Using this nested statistical design, we investigated the effects of winter foraging exclusion, site and their interaction on OTU richness and extracellular enzyme activity. We included soil water content as a covariate in both analyses (analysis of covariance; ANCOVA) due to its known influence on microbial communities. To quantify phylogenetic β-diversity and the collective change in soil C and N cycling potential in response to winter foraging exclusion, we employed permutational multivariate analysis of variance with soil water as a covariate (PERMANOVA). To understand whether microbial responses were related to foraging intensity, we regressed the average site difference in shrub percent cover between foraged and unforaged treatments to differences in OTU richness, the weighted UniFrac distance, enzyme potential activity, as well as C and N cycling functional genes. By quantifying differences in plant and microbial characteristics between foraged and unforaged treatments at each site, site-to-site differences in microbial communities were normalised to pinpoint plant and microbial responses to winter foraging exclusion. Mantel correlations tested the hypothesis that changes in fungal and bacterial phylogenetic β-diversity across sites and foraging treatments resulted in concurrent changes to enzyme potential, and C and N cycling potential. Assumptions of linearity were verified prior to conducting linear regression, ANOVA and ANCOVA, followed by necessary log transformations. *Post hoc* analyses were conducted using Tukey's test. When applicable, *P*-values were corrected for multiple comparisons using the Benjamini and Hochberg false discovery rate correction (Benjamini & Hochberg 1995). All univariate analyses were performed in the R environment (http://www.R-project.org) using the stats package (Version 3.01; R Code Team 2016); multivariate analyses were conducted using the vegan package (Oksanen *et al*. 2015) and PERMANOVA statistics were executed in Primer (version 6, Primer-E Ltd., Plymouth, UK).

RESULTS

Winter foraging effects on plant communities and soil environment

Winter foraging had direct and indirect consequences on the plant and soil properties of sagebrush steppe, including a decrease in shrub percent cover (Fig. S2), subsequent increases in forb and graminoid cover, as well as a general decline in early spring soil water content (Fig. S3). The average reduction in shrub cover between foraged and unforaged treatments was largest at LH (68%), followed by UH (55%), MB (41%) and GV (14%; Fig. 1), indicating that our study sites spanned a range of winter foraging pressure. Ungulate winter foraging also exerted an indirect effect on the soil environment by modulating spring soil water content. Nested ANOVA revealed that site, foraging treatment and their interaction all significantly influenced soil water content (site: $F_{3,14} = 101.5$,

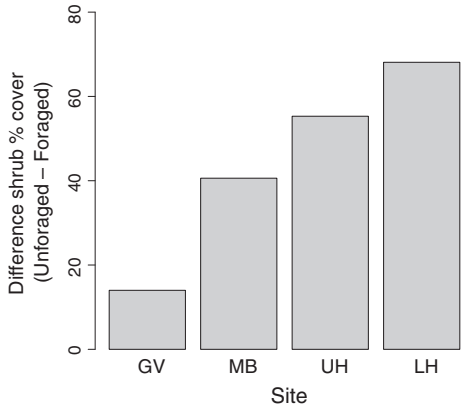

Figure 1 Experimental sites vary in ungulate winter foraging intensities, calculated as the average difference in shrub cover between winter foraging exclosure and foraged plots at each site. Sites are represented by GV (Gros Ventre), MB (Miller Butte), UH (Upper Hoback) and LH (Lower Hoback).

$P < 0.001$; foraging: $F_{1,14} = 32.7$, $P < 0.001$; site x foraging interaction: $F_{3,14} = 11.9$, $P < 0.001$). *Post hoc* analysis revealed significantly lower soil water in the foraged treatment relative to the unforaged treatment, with the exception MB, in which soil water between foraging treatments was not significantly different (Fig. S2).

Microbial community response to ungulate foraging

In total, 14 045 unique 28S rRNA gene sequences were obtained (51 264 total sequences), and were assigned to the phyla Ascomycota (53%), Basidiomycota (20%), Blastocladiomycota (1.3%), Chytridiomycota (1.7%), fungi *incertae sedis* (1.3%), Glomeromycota (0.5%) and unclassified (22%). A total of 41 477 unique bacterial 16S sequences also were obtained (68 286 total sequences). The most abundant (> 5% relative abundance) bacterial phyla included Actinobacteria (36%), Proteobacteria (21%), Acidobacteria (12%), unclassified (7%), Chloroflexi (6.7%) and Bacteroidetes (6.7%). Of the 26 fungal classes identified, Dothideomycetes (+7.00% increase from unforaged treatment; $P_{adjust} = 0.003$), Sordariomycetes (+3.33%; $P_{adjust} = 0.029$) and Tremellomycetes (+1.66%; $P_{adjust} = 0.029$) had significantly higher relative abundances in the foraged treatment, relative to the unforaged treatment (Fig. S4). Conversely, the fungal class Eurotiomycetes (-4.46%; $P_{adjust} = 0.025$) was significantly less abundant in the foraged treatment. Winter foraging also altered the abundance of bacterial classes; for example, the relative abundance of Thermomicrobia significantly increased in the foraged treatment (+1.21%; $P_{adjust} = 0.001$). Negative responses to winter foraging were observed for the bacterial classes Gemmatimonadetes (−0.80%; $P_{adjust} = 0.049$), Deltaproteobacteria (−0.63%; $P_{adjust} = 0.049$) and Holophagae (−0.26%; $P_{adjust} = 0.049$).

Foraging treatment and site were significant factors accounting for fungal OTU richness, with a significant treatment by site interaction (Two-way ANCOVA; Fig. 2a; Table S2). Given the range of grazing intensity across study sites (Fig. 1), the significant interaction between site and foraging treatment could indicate that the degree of foraging intensity had site-specific effects on fungal richness. Despite these site-to-site differences, no linear relationship was observed between changes in fungal OTU richness and foraging intensity (Fig. 2c). Furthermore, *post hoc* analyses indicated that fungal richness was significantly lower in the MB foraged treatment, relative to the MB unforaged treatment ($P < 0.001$), although no other site comparisons were significant.

Winter foraging also decreased soil bacterial richness, with a significant site by treatment interaction, suggesting that differences in foraging intensity may lead to site-specific responses; soil water was a significant covariate in this analysis (Fig. 2b; Table S2). Bacterial richness was lower in foraged treatment at UH ($P = 0.003$) and marginally lower in LH foraged treatment ($P = 0.081$), relative to the unforaged treatment at those sites. Bacterial OTU richness between foraged and unforaged treatments at each site were marginally correlated with shrub cover differences ($r^2 = 0.89$; $P = 0.056$; Fig. 2d), indicating that sites with strong foraging pressure had larger reductions in bacterial OTU richness.

Phylogenetic composition was significantly different between foraging treatments and sites for both fungal and bacterial communities; soil water also was a significant covariate in this analysis (Fig. 3a and b; Table S2). A significant interaction between site and treatment for the bacterial β-diversity model indicated bacterial responses to foraging depended on sites, which experienced a range of foraging intensity. All site pairwise comparisons between foraging treatments were significantly different when considering fungal-weighted UniFrac distance ($P < 0.042$). Although bacterial phylogenetic composition was significantly different between foraging treatments at UH ($P = 0.037$), marginally different at GV and LH ($P = 0.052$–0.087), but not significantly different at MB. Furthermore, bacterial UniFrac distances between foraging treatments across sites were marginally correlated with average change in shrub percent cover ($r^2 = 0.87$, $P = 0.069$; Fig. 3c); whereas, no linear relationship was observed for fungal UniFrac distances ($P = 0.73$; Fig. 3d).

Microbial functional response to winter foraging

To test the hypothesis that ungulate winter foraging intensity altered the functional capacity of the microbial community, we quantified the relative abundance and composition of genes involved in the microbial metabolism of lignocellulose, as well as the processing of organic nitrogen (Table S1). The composition of genes mediating the decay of lignocellulose was neither altered by winter foraging exclusion (PERMANOVA; $P = 0.75$) nor was the composition of genes mediating soil N cycling processes ($P = 0.80$). However, the difference in chitobiase (*chb*) abundance, a gene encoding an enzyme mediating chitin depolymerisation, was positively correlated with differences in shrub abundance (Fig. 4; $r^2 = 0.99$; $P_{adjust} = 0.057$). Although no other genes involved in lignocellulolytic decay or soil N cycling processes were significantly related to site difference in shrub cover, this response to foraging intensity was correlated ($r^2 > 0.75$) with the relative abundance of nine of 20 investigated functional genes (Fig. S5 & S6).

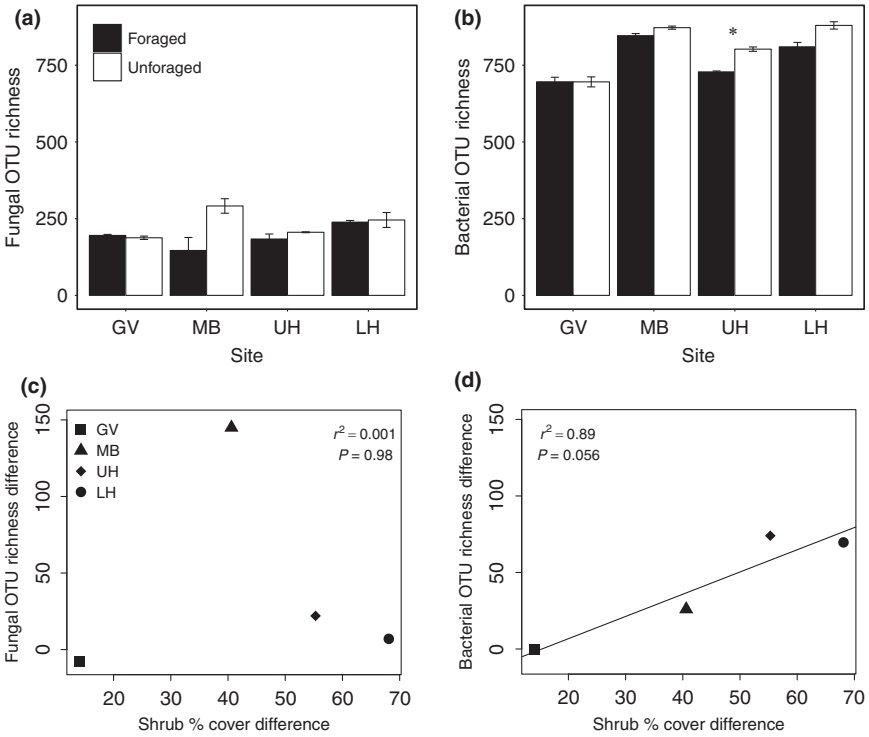

Figure 2 Operational taxonomic unit (OTU) richness of fungal (a) and bacterial (b) communities, as well as the relationship between site foraging intensity, calculated as the difference in shrub percent cover between winter foraging exclosures and foraged treatments, and site differences in fungal OTU richness (c), and site differences in bacterial OTU richness (d) across the four sites (GV = Gros Ventre, MB = Miller Butte, UH = Upper Hoback, LH = Lower Hoback). Fungal and bacterial OTUs were clustered at 97% sequence similarity. Error bars denote standard error and an asterisk indicates significant differences between treatments within a site at $\alpha < 0.05$. *P*-values indicate the linear relationship between x and y variables.

Extracellular enzyme assays

Two-way nested ANCOVAS revealed that foraging treatment, site and soil water influenced the activity of soil enzymes (Table S2). Soil water was a significant covariate in models of all enzyme activities. Foraging treatment significantly accounted for variation in cellobiohydrolase and β-1,4-xylosidase activity (Fig. 5), indicating that the influence of foraging treatment on these cellulolytic and hemicellulolytic enzymes was independent of corresponding treatment effects on soil water content. Site and the interaction of site with foraging treatment were significant in explaining variation in phenol oxidase activity, as foraging exclusion led to lower enzyme activities in the MB and UH sites; this enzymatic treatment response was directly opposite to the response observed at sites LH and GV. No site differences in enzyme activities between foraging treatments were significantly correlated with site reduction in shrub cover ($P > 0.30$).

Links between microbial community composition and metabolic potential

Mantel correlations tested the hypothesis that differences in the phylogenetic composition of the microbial community in response to ungulate winter foraging exclusion resulted in concurrent changes to microbial genetic and enzymatic potential across sampling locations. Supporting our hypothesis, fungal and bacterial-weighted UniFrac distance matrices were significantly correlated with Euclidean pairwise differences in C and N cycling gene abundance, and extracellular enzyme activity (Table 1).

DISCUSSION

Winter foraging by migratory ungulates in the sagebrush steppe exerts an indirect, top-down ecological force that shapes the microbial communities and potential rates of nutrient cycling (Peschel *et al.* 2015). Here, we provide evidence that winter foraging intensity modulates the magnitude of herbivore-driven change in microbial community composition and functional potential. For example, the highest level of winter foraging suppressed soil bacterial richness and increased bacterial phylogenetic β-diversity. Although there was no linear relationship between fungal community response and foraging intensity, winter foraging exclusion had a greater overall effect on the fungal phylogenetic community composition, such that foraged treatments contributed to more distinct fungal communities relative to bacteria. Furthermore, foraging-associated responses in microbial phylogenetic composition and richness had consequences for the microbial functional potential, including reduced cellulolytic and hemicellulolytic enzyme activity as well as decreased abundance of chitinolytic functional genes with increasing foraging intensity. Together, our results demonstrate that a microbial mechanism

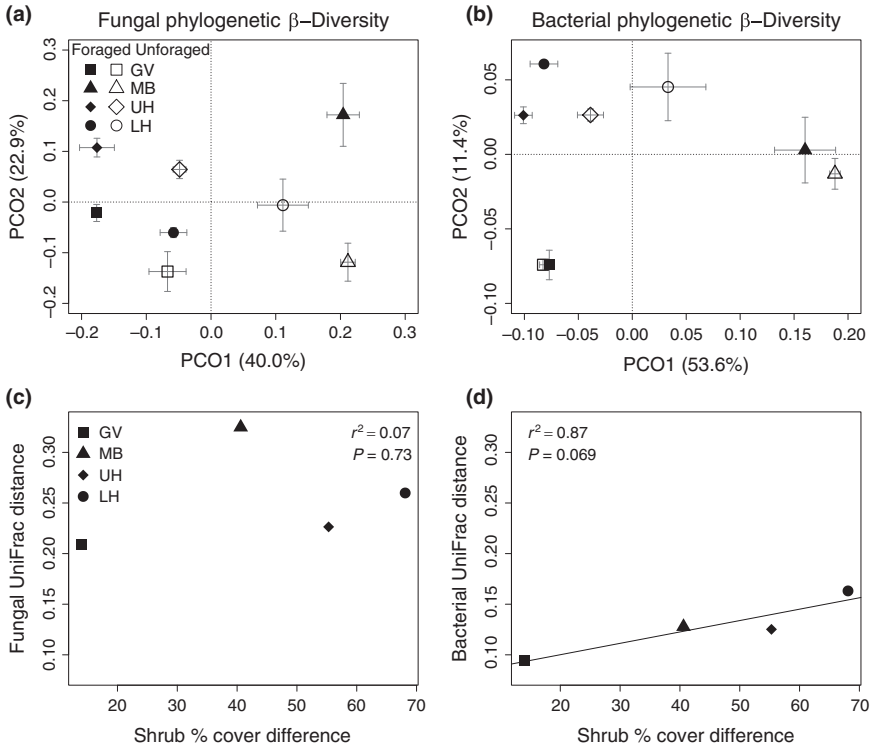

Figure 3 Principal coordinate (PCo) analysis of fungal (a) and bacterial (b) phylogenetic composition of unforaged and foraged treatments across four sites, as well as the relationship between site foraging intensity, calculated as the difference in shrub percent cover between winter foraging exclosures and foraged treatments and site fungal phylogenetic β-diversity (c) as well as site bacterial phylogenetic β-diversity (d) between foraging treatments. Phylogenetic distances between were calculated by the weighted UniFrac distance metric. Error bars denote standard error. Sites are represented by GV (Gros Ventre), LH (Lower Hoback), MB (Miller Butte), and UH (Upper Hoback). *P*-values indicate the linear relationship between x and y variables.

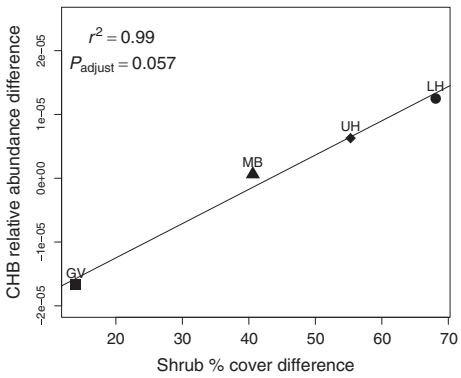

Figure 4 Site differences in chitobiase (*chb*) abundance between winter foraging exclosures and foraged treatments were positively correlated with increases in foraging intensity across sites, calculated as the difference in shrub percent cover, between winter foraging exclosures and foraged treatments at each site.

underlies previously observed reductions in rates of soil C and N cycling in the presence of elk winter foraging.

Ungulate winter foraging is a top-down force shaping microbial communities across the sagebrush steppe, generally resulting in phylogenetically distinct fungal and bacterial communities (Fig. 3). Limited resource availability in the foraged treatment likely decreased microbial richness, because reduced plant detrital inputs to soil constrained the number of microbial taxa able to meet their minimum resource requirements (Waldrop *et al.* 2006). Furthermore, reductions in shrub abundance resulted in phylogenetically distinct microbial communities, supporting the assertion that microorganisms have varied physiological capacities to degrade the biochemical components of plant detritus (McGuire *et al.* 2010; Martiny *et al.* 2013). For example, with a significantly higher proportion of lignin-rich detritus from shrubs (Thines *et al.* 2008; Perryman *et al.* 2011), the unforaged treatment had an increased proportion of Agaricomycetes, the fungal class in which the ancestral lineage of white rot fungi originated (i.e. the capacity to completely degrade lignin to CO_2; Floudas *et al.* 2012), as well as the filamentous Actinobacteria, which also play a role in lignin decomposition (Kirby 2006).

The extent of community response to winter foraging was greater in soil fungi relative to bacteria. For example, larger UniFrac distances were observed in fungal communities between foraging treatments relative to bacteria (Fig. 3c and d); moreover, fungal communities were phylogenetically distinct between foraging treatments at all four sites. Given the limited resource availability for microbial growth in the foraged treatments, the larger response observed in soil fungi is consistent with evidence that fungi serve as predominant degraders of lignin and cellulose in plant litter (Schneider *et al.* 2012) and have a relatively higher sensitivity to changes in plant functional group composition via changes in plant

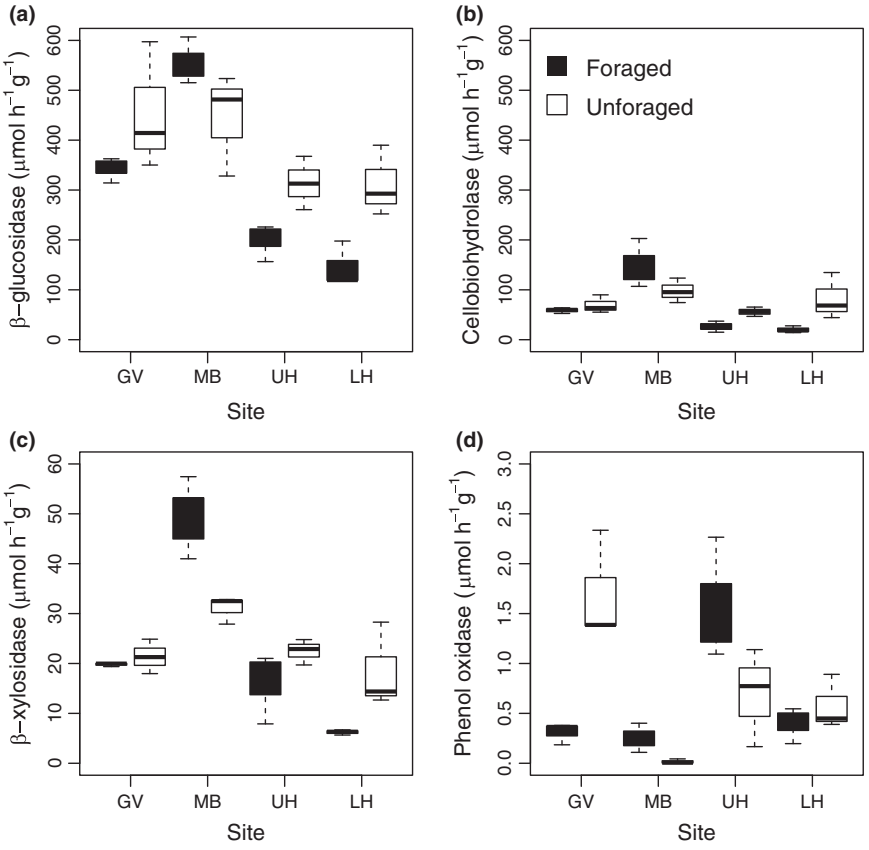

Figure 5 Soil microbial enzyme potential for β-glucosidase (a), cellobiohydrolase (b), β-xylosidase (c), phenol oxidase (d). Sites are represented by GV (Gros Ventre), LH (Lower Hoback), MB (Miller Butte) and UH (Upper Hoback).

Table 1 Mantel correlations tested the relationship between variation in fungal and bacterial community composition and differences in microbial genetic potential across the experiment. Fungal and bacterial community distance matrices were calculated using the weighted UniFrac distance metric. Euclidean distance matrices were calculated for extracellular enzyme potential, as well as the composition of genes classified to C and N cycles from the functional gene repository.

Distance matrix	*R*	Adjusted P
Correlation with Fungal UniFrac		
Enzyme potential	0.32	0.0020
C genes	0.80	0.0018
N genes	0.40	0.0170
Correlation with Bacterial UniFrac		
Enzyme potential	0.46	0.0005
C genes	0.96	0.0018
N genes	0.51	0.0034

litter biochemistry (Cline & Zak 2015). Although few studies have compared compositional changes in soil fungi and bacteria in response to ungulate herbivory, fungi appear to respond to changes in plant litter biochemistry associated with selective foraging (Davinic *et al*. 2013), whereas bacteria appear more sensitive to foraging effects on physical soil properties (Hodel *et al*. 2014).

In our study, bacterial richness and composition were influenced by foraging intensity (Fig. 2c and d; Fig. 3c and d), indicating that the bacterial community responded to the magnitude of foraging-associated changes in plant community composition and soil micro-environment. However, this relationship was not observed in soil fungi; largest deviations in fungal richness and phylogenetic composition were observed at MB, a site located within the National Elk Refuge (NER). While MB does not appear to receive high amounts of winter foraging (Fig. 1), proximity to supplemental feeding grounds in the winter months (Cole *et al*. 2015) likely increased the number of elk passing through the site. In turn, elevated ungulate traffic may increase urine and faecal pellet deposition, plausibly representing an important N source for fungal communities in foraged treatments and accounting for compositional differences observed between foraging treatments (van Wal *et al*. 2004). Alternatively, supplemental feeding may encourage ungulate foraging at MB later in the winter season relative to other sites (Jones *et al*. 2014), due to access to snow-free forage at low elevations. Thus, fungal β-diversity between foraging treatments at this site may be the result of temporal variation in plant belowground C allocation or the activity of particular fungal decomposers (Kaiser *et al*. 2011) relative to other sites. Together, these observations indicate that current reductions in winter foraging, as a result of environmental and anthropogenic influences, may lead to a predictable response in soil bacterial composition and richness (Bardgett *et al*. 2001). Gaining a firm grasp of the fungal community response will require understanding the interaction

between foraging intensity and management practices (Sawyer & Kauffman 2011; Middleton *et al*. 2013).

Winter foraging elicited changes in microbial community composition that cascaded to influence the genetic and enzymatic potential of the soil microbial community, although this effect appeared to attenuate from community to functional levels of investigation (Patra 2005). Consistent changes in microbial community composition and functional potential (Table 1) indicated that relatively large phylogenetic shifts in microbial composition led to correspondingly large differences in enzymatic and genetic potential. More importantly, microbial community response to winter foraging may have implications for soil biogeochemical cycling via changes in the genetic and enzymatic capacity of fungi and bacteria (Yang *et al*. 2013; Peschel *et al*. 2015). For example, we previously documented that winter foraging significantly reduced net N mineralisation (Peschel *et al*. 2015). However, because winter foraging exclusion did not significantly alter the composition of microbial genes mediating the decay of lignocellulose or the processing of soil nitrogen, some degree of functional equivalency may exist between phylogenetically distinct microbial communities across foraging treatments (Talbot *et al*. 2014). The observed lack of statistical significance may also be the result of our lower metagenomic sampling effort, relative to microbial community characterisation via rDNA. A clear understanding of the relative importance of winter foraging effects on microbial composition and function will require greater replication of soil metagenomes. Nonetheless, winter foraging appears to exert an indirect influence on the genetic and enzymatic potential of soil microbial communities, evidenced by suppressed soil cellulolytic and hemicellulolytic enzyme potential in foraged treatments.

Although functional gene assemblages were not significantly different in composition between foraging treatments, the direction and magnitude of individual genetic responses depended on degree of winter foraging (Figure S5; Yang *et al*. 2013). For example, microbial potential for chitin and hemicellulose depolymerisation (i.e. *chb, nag3* and *xylA* gene abundances) was lower in sites in which foraging intensity was high; whereas, high cellulolytic potential (i.e. *cbh1*) corresponded to high foraging intensity. Reduced fungal biomass under high intensity foraging (Bardgett *et al*. 2001) may explain the observed decline in chitinolytic genetic potential, as a result of reduced substrate availability; moreover, it is also plausible that such a response is implicated in differences in net N mineralisation between foraging treatments (Peschel *et al*. 2015). Similarly, via changes in plant litter biochemistry associated with grass and forb dominance (Thines *et al*. 2008; Perryman *et al*. 2011), high intensity foraging may increase the amount of cellulose relative to lignin available for microbial metabolism in soil. Thus, the magnitude of microbial community response to winter foraging may have important functional implications for the microbial capacity to degrade plant detritus (Stark *et al*. 2015).

Winter foraging intensity appears to have additional consequences for the cycling of C and N in soil via an interaction with the soil environment. For example, winter foraging significantly altered extracellular enzyme activity, although the overall decline in potential activity in the presence of winter foraging was predominantly driven by declines in soil water content (Table S2) and not directly by foraging intensity. This relationship was observed in all sites except MB, in which enzyme activity and soil water content were elevated in the foraged treatment (Fig. 5; Fig. S3). This general pattern of increased soil water content in unforaged treatments may be the result of hydraulic lift by sagebrush, an adaptation for drought tolerance by which roots re-distribute water from deep soil horizons to the surface (Ryel *et al*. 2004). The primary role of soil water indicates that physical factors govern extracellular enzyme activity, independent of substrate availability and microbial genetic capacity (Burns *et al*. 2013). Furthermore, observed declines in microbial respiration and nitrification under winter foraging (Peschel *et al*. 2015) indicate enzyme activity may be tied to soil C and N cycles via microbial activity.

We fully recognise that our study is not without limitations. Despite careful selection of study sites, differences in plant community composition, soil characteristics and microclimates may influence elk herbivory and microbial community composition. To minimise potential site-to-site variation, we calculated the difference in microbial composition and function between foraging treatments within a site. This approach normalised site differences to focus analysis on the microbial response to foraging-associated changes in plant communities. Additionally, there was a temporal disconnect between plant sampling (2013) and soil sampling (2015). However, due to slow plant growth in this arid climate, it is unlikely that the plant community would change substantially, and any changes should occur concomitantly across the landscape.

CONCLUSION

The observed changes in microbial community composition and functional potential with elk winter foraging suggests that a microbial mechanism may underlie the influence that herbivores have on soil C turnover in the Greater Rocky Mountain Ecosystem (Frank *et al*. 2011). Furthermore, the relationship between foraging intensity and phylogenetic β-diversity, as well richness and genetic potential to degrade plant litter, suggests that current declines in the number and distance of ungulate migrations across many regions of the Earth (e.g. Middleton *et al*. 2013) may have important consequences to soil biogeochemical processes. Our results further imply that the extent of this response will be contingent on the degree to which changes in migratory behaviour impact foraging intensity. Evidence for this comes from the consistent interaction between site and foraging treatment, which plausibly arose from the range of foraging intensities across our study sites. In the future, lower winter occupancy by foraging ungulates in sagebrush steppe may increase microbial richness and enzyme activity; whereas, reduction in microbial richness and enzyme activity may be observed in areas frequented by growing resident populations. Our results also raise questions about the influence that summer foraging may have on soil microbial communities, given that available forage, plant activity levels and ungulate foraging behaviour vary between

seasons. Importantly, significant interactions between foraging intensity and the soil microclimate indicate that global changes in temperature and precipitation are also important determinants of soil C and N cycling in the foraging system we studied. Human interventions that alter the timing and duration of migration, including supplemental feeding, will further impact soil biogeochemical processes via the indirect top-down ecological effects we document here.

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AUTHOR CONTRIBUTIONS

DRZ and LCC designed the experiment. DRZ collected samples. RAU and ZBF processed and analysed metagenome and extracellular enzyme data. ARP processed and analysed plant community data. LCC performed all other analyses and wrote first draft of paper. DRZ, RAU, ZBF and ARP contributed substantially to manuscript revisions.

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