WINTER FORAGING BY MIGRATORY UNGULATES MODIFIES THE COMPOSITION AND FUNCTION OF SOIL MICROBIAL COMMUNITIES

By

Anna Riba Hellzèn Peschel

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Faculty advisors:
Professor Donald R. Zak
Professor Bilal Butt

Abstract

Saprotrophic microbial communities in soil are commonly thought to be structured by bottom-up ecological forces that arise from resource availability (*i.e.,* plant detritus). However, it is plausible that ungulate foraging could indirectly modify resource availability by altering plant community composition, thereby exerting an indirect, top-down ecological force on soil microbial communities. To test this idea, we quantified microbial community taxonomic composition and functional potential inside and outside of four long-term winter foraging exclosures in sagebrush steppe in Wyoming, USA. Winter foraging exclusion significantly increased *Artemisia tridentata* biomass and reduced forb percent cover (-6 %). The change in plant composition caused significant changes in microbial taxonomic composition and functional potential by altering the composition of bacteria and fungi with lignocellulolytic function. Our observations provide evidence that winter foraging by ungulates in the sagebrush steppe exerts an indirect top-down ecological force that shapes the composition and function of soil microbial communities.

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Introduction

Soil food webs are thought to be structured by bottom-up ecological forces via the production and biochemistry of plant detritus, the limiting resource for saprotrophic metabolism in soil (Zak et al. 2011). However, evidence is also mounting that soil microbial communities can be influenced by top-down forces, such as the consumption of vegetation by grazing animals (Pastor et al. 1988). For example, herbivores could shape microbial communities by altering the biochemical composition of detritus (*e.g.,* dead leaves and roots) entering the soil food web (Hobbs 1996), as well as through the physiological responses in plants that are induced by grazing (e.g., plant production; Holland et al. 1992). These observations suggest foraging animals could modify bottom-up ecological forces that structure soil food webs, thereby linking their foraging behavior to the composition and function of soil microbial communities.

Plant species vary in the biochemical constituents of their leaves and roots, therefore, changes in plant community composition elicited by herbivory could alter the biochemistry of substrates that fuel microbial metabolism in soil. For example, plants allocate photosynthate to grow or defend from herbivory, thus plants rich in protein (*i.e.,* N) have low levels of metabolically costly plant defenses (*e.g.,* tannins and lignin; Herms & Mattson

1992). Herbivores can change plant community composition by preferentially consuming the most protein-rich plant material (*i.e.,* N; Augustine & McNaughton 1998; Hobbs 1996), which can lead to a dominance of unpalatable plants (Augustine & McNaughton 1998; Pastor et al. 1988). For example, moose (*Alces alces*) browsing changed forest composition on Isle Royal, MI, from protein-rich deciduous trees (*i.e., Populus tremuloides*) to spruce (*Picea glacua*), which has a high lignin and low protein concentration (Pastor et al. 1988). Conversely, intensive foraging can promote the occurrence of palatable plant species (McNaughton 1985). In the Serengeti grasslands of East Africa, for example, heavy grazing by ungulates shifted photosynthate allocation in graminoids from the roots to shoots, causing the rapid regrowth of protein-rich leaves after defoliation (McNaughton 1985).

Unlike these examples, ungulate populations in northern temperate montane ecosystems have a limited forage choice during winter, which has consequences for the subsequent composition of plant communities. To escape deep snow, North American elk (*Cervus elaphus*) and other migratory ungulates in the Rocky Mountains, journey from montane forests in autumn to lower elevation valleys to find winter forage (Irwin 2002). In winter, protein-rich herbs and grasses are dormant and hidden by snow; thus, the portion of browse (*i.e.,* protein-poor, lignin-rich shrubs) in

their diets increases (Irwin 2002). In addition, the energetic cost to search and dig for these plants beneath snow increases, making them less desirable (Parker et al. 1984). Therefore, winter foraging in these ecosystems can plausibly decrease shrub abundance and increase forb and grass abundance.

Plant communities can shape microbial community composition by altering the organic compounds that enter the soil from dead plant leaves and roots (Meier & Bowman 2008). Therefore, foraging-induced changes in plant community composition may feedback to alter the composition and function of soil microbial communities. Organic substrates metabolized by the saprotrophic microbial community are provided in different proportions depending on the plant composition (Swift et al. 1979). Generally, bacteria enzymatically harvest energy from organic acids and simple sugars, whereas some basidiomycete fungi have lignolytic physiologies that enable them to harvest energy by metabolizing polyphenolic compounds, such as lignin, that protect more energetically favorable cellulose and hemicellulose in the plant cell wall (Swift et al. 1979). Taken together, these observations suggest that winter foraging by native ungulates may alter the composition and function of soil microbial communities by modifying abundance of shrubs, forbs, and grasses.

If winter foraging exerts an indirect top-down effect on soil microbial community composition and function, the exclusion of winter foraging should increase shrub abundance, which would favor fungal dominance and a greater abundance of genes with lignocellulolytic function. Alternatively, if the top-down force of foraging does not structure soil microbial communities, there should be no difference in composition and function of saprotrophic soil microbial communities in the presence or absence of winter foraging. We assessed the effect of winter foraging on soil microbial communities by quantifying their composition and function in the presence and absence of winter foraging in the sagebrush steppe in the northern Rocky Mountains of North America (Table 1).

Methods

Site description

During July 2013, soil and vegetation samples were collected in the Bridger-Teton National Forest and in the National Elk Refuge in Jackson Hole, Wyoming, USA (Table 1). The predominant ecosystem in the valley floor is sagebrush steppe, a dry-mesic environment with frequent fire, high levels of winter precipitation, and is dominated by bunch grasses and the shrub *Artemisia tridentata* (Knight 1994). Mean monthly precipitation is 27.5 mm (1930-2013), and mean monthly temperature is 2.5 °C (National Oceanic and Atmospheric Administration 2013). The main native

migratory ungulates are North American elk (*Cervus elaphus*), moose (*Alces alces*), mule deer (*Odocoileus hemionus*), and bighorn sheep (*Ovis canadensis*). Approximately 50-80 years ago, winter grazing exclosures were constructed throughout Jackson Hole to understand the influence of ungulate winter grazing on plant community composition; we sampled four of them, broadly distributed in Jackson Hole, to understand whether winter grazing could shape microbial communities in soil.

Vegetative community composition

Long-term effects of ungulate foraging on plant community composition were determined by inventorying plant species and aboveground biomass inside and outside of four winter-foraging exclosures. Inside each exclosure a 10-m x 10-m plot was established at the center. For adjacent winter-foraged sites, a 10-m x 10-m plot was created ten meters away from the fenced edge of each exclosure with matching slope and aspect. Percent cover of shrubs, forbs, graminoids, plant litter, organic horizon, and mineral soil was estimated within a 1-m^2 sampling frame at ten random locations within each plot.

Additionally, the aboveground biomass of shrubs, forbs, and graminoids was measured within each 1-m^2 sampling frame. Forbs and graminoids were clipped, and species-specific allometric equations (Table 2) were used to determine the aboveground

biomass of shrubs. Shrubs were clipped if they were < 5% of total plant cover. Clipped shrubs, forbs, and graminoids were bagged separately, dried at 45 ˚C, and weighed to determine their mass.

Soil properties and microbial communities

To determine the effect of winter foraging on soil microbial community composition and function, a 10-cm deep mineral soil sample (2.5 cm in diameter) was collected from the center of each 1-m^2 sampling frame. The ten soil core samples from each site were composited, homogenized, stored on ice, and shipped to the University of Michigan, where they were kept at -80 ˚C prior to DNA extraction. Homogenized soil samples were passed through a 2-mm sieve, from which genomic DNA was extracted using a MO-BIO PowerLyzer® PowerSoil® DNA Isolation Kit (MO BIO laboratories, Carlsbad, CA, USA), following manufacture's instructions. Triplicate extractions were performed on three subsamples from composited soil from each winter-foraged and unforaged site. Total soil N, total soil organic C, and soil pH, were measured to quantify edaphic properties. A slurry containing 10 g of soil and deionized water was used to measure pH. Total soil N was measured colorimetrically following digestion in concentrated H_2SO_4 (Lachet Instruments, Loveland, CO, USA). Total soil organic C was determined using a Leco CNS2000 Analyzer (LECO® St. Joseph, MI, USA).

Microbial activity

Microbial respiration, net N mineralization, and net nitrification were measured to determine the influence of winter foraging on microbial activity. Three 30-g soil samples from each foraged and unforaged site were brought to field capacity, and incubated at 25 °C in 1 L Mason jars equipped with septa for headspace gas sampling. Microbial respiration was estimated by measuring CO_2 accumulation in the headspace during a 14-d incubation using a Trace 200 Series gas chromatograph (Thermo Electron Corp., Austin, TX, USA). To calculate net N mineralization and net nitrification, the difference in NH_4^+ and NO_3^- production between the incubated soil samples and amounts present at the initiation of the incubation were measured using an AQ2 discrete analyzer (Seal Analytical, Mequon, WI, USA).

Quantitative polymerase chain reaction (qPCR) was performed to gain insight into the effect of winter foraging on the relative abundance of soil Bacteria and fungi. Bacterial abundance was estimated using universal primers Eub338F and Eub518R to amplify 180 bp of the V3 region of the 16S rRNA gene (Fierer et al. 2005). Fungal abundance was estimated using primers ITS1f (Gardes & Bruns 1993) and 5.8s (Vilgalys & Hester 1990) to amplify a ~300 bp region of the fungal internal transcribed spacer region (ITS). Both fungal and bacterial assays were performed in triplicate

on a Stratagene MX3000P real-time PCR (Agilent Technologies, Santa Clara, CA, USA).

GeoChip probe selection

Following DNA hybridization and data preprocessing (Lu et al. 2011), a subset of GeoChip 5.0 probes (n = 9,097) were analyzed to examine the effect of winter foraging on microbial taxonomic composition and functional potential. We included probes encompassing 18 bacterial and 15 fungal functional genes that encode enzymes which depolymerize the biochemical constituents of plant leaf litter, namely, starch, cutin, cellulose, hemicellulose, and lignin (Eisenlord et al. 2013). We analyzed bacterial and fungal genes separately (Table 3).

Statistical analysis

All statistical analyses were conducted in the software program RStudio (version 0.98.501; http://www.r-project.org/). Statistical significance was accepted at $\alpha = 0.05$. To determine the effect of winter foraging exclusion on plant composition, ordinations were created from principal coordinate analysis based on the percent cover of shrubs, forbs, graminoids, organic horizon, and mineral soil, for winter-foraged and unforaged treatments using the Bray-Curtis dissimilarity metric (Bray & Curtis 1957). Pearson's correlation coefficients were calculated to explore the relationship of each aforementioned cover variable with PCoA axis 1 or 2.

Permutational multivariate analysis of variance (perMANOVA; Anderson 2001) was used to determine the significance of plant compositional differences between treatments. Meter-squared plots were analyzed as replicates, with site and treatment as factors, with replicates nested in treatment. PerMANOVA allows variation to be partitioned according to the experimental design, and significance is determined by permutations (Anderson 2001). In addition, nested ANOVAs were used, to determine differences in shrub, forb, graminoid, organic horizon, and mineral soil percent cover between treatments.

Nested ANOVAs were also used to determine if ungulate foraging lead to differences in microbial respiration, net N mineralization, net nitrification, as well as fungal and bacterial abundance. Three replicates for each foraged and unforaged site were analyzed, with site and treatment as main factors, with replicates nested in treatment. Outliers, data points $\geq$ 2 SD from the mean, were removed from respiration, net N mineralization, and fungal and bacterial abundance data before statistical analysis. One-way ANOVAs were used to determine whether ungulate grazing lead to differences in shrub, forb, and graminoid biomass, as well as differences in edaphic properties between the four sites. Biomass and edaphic data were log transformed prior to analysis.

Differences in the functional composition of microbial assemblages between treatments were analyzed using multivariate statistics. The full GeoChip data matrix from microarray image analysis was normalized to relative abundance by dividing the signal intensity of each individual probe by the total signal intensity of all probes in each sample. The normalized data matrix was analyzed at the gene level (*i.e*., all genes associated with starch, cutin, cellulose, hemicellulose, and lignin), by summing the normalized signal intensity of all probes encoding a specific gene, and dividing by the number of probes representing a given gene, effectively accounting for different numbers of probes included on GeoChip for any given gene. Ordinations were created from principal coordinate analysis based on the normalized signal intensity of each gene of interest. The Bray-Curtis dissimilarity metric was used, and Pearson's correlation coefficients were calculated to explore the relationship of each functional gene with PCoA axis 1 or 2.

The effect of winter foraging on the taxonomic composition of fungal and bacterial functional assemblages was similarly analyzed using multivariate statistics. The GeoChip data matrix, which included probes associated with starch, cutin, cellulose, hemicellulose, and lignin, was converted into a presence-absence data matrix and reorganized by taxonomic order associated with

each probe. This matrix was normalized by summing the presence-absence values of probes associated with each order for a given sample, and dividing by the total number of probes associated with a given taxonomic order. Principal coordinate analysis was used to obtain ordinations based the normalized presence-absence GeoChip data matrix using the Bray-Curtis dissimilarity metric. Pearson's correlation coefficients were calculated to explore the relationship of each bacterial and fungal order with PCoA axis 1 or 2.

PerMANOVA was used to determine if ungulate foraging altered the taxonomic composition, as indicated by GeoChip, and functional composition of fungi and bacteria in the soil. Three replicates for each foraged and unforaged site were analyzed as replicates. PerMANOVA does not distinguish if dissimilarity between groups is the result of differences in location in multivariate space, differences in each group's relative dispersion, or both (Anderson 2001). Therefore, permutational analysis of multivariate dispersions (PERMDISP; Anderson 2006) was used to determine if significant differences between treatments was due to differences in the relative dispersion of each treatment from their mean. Similarity Percentage (SIMPER; Clarke & Warwick 2001), was used to ascertain the contribution of each functional gene or microbial order to average dissimilarity between treatments.

Results

Soil properties and plant biomass

Winter foraging did not have a significant influence on soil pH (ANOVA; $F_{1,6}$ = 0.43; P = 0.56), total soil N (ANOVA; $F_{1, 6}$ = 0.36; P = 0.57), or total soil organic C (ANOVA; $F_{1,6}$ = 0.16; P = 0.70). However, soil organic C content was ~9% greater in the unforaged treatment (2.39 mg C kg^{-1} in unforaged *vs.* 2.19 mg C kg^{-1} in winter-foraged), indicating the exclusion of winter foraging after ~60-80 yrs fostered accumulation of soil organic matter (Table 4).

Winter foraging exclusion increased total plant biomass ~12 fold (2199 g/m^2 vs. 179 g/m^2 in the unforaged and winter-foraged treatment, respectively). However, total plant biomass was marginally significant between treatments (ANOVA; $F_{1,6}$ = 4.4; P = 0.08). Additionally, winter foraging exclusion led to a ~25-fold increase in shrub biomass (2146 g/m^2 *vs.* 87 g/m^2 in unforaged and winter-foraged treatments respectively; Fig. 1). However, this difference also was marginally significant (ANOVA; $F_{1,6}$ = 4.7; P = 0.07). One site (Gros Ventre) was determined to have a particularly low shrub biomass in the unforaged treatment (316.7 g/m^2). When this site was removed from our analysis, shrub biomass (ANOVA; $F_{1,5}$ = 9.8; P = 0.026), and total plant biomass (ANOVA; $F_{1,5}$ = 9.3; P = 0.028) were significantly different between treatments.

Across all sites in the unforaged treatment, shrub biomass accounted for ~98% of total plant biomass. Specifically, winter foraging exclusion increased the biomass of *Aretemisia tridentata var. vaseyana* and *Artemisia tridentata var. tridentata*. Together these shrubs accounted for ~41% (874 g/m^2) of shrub biomass in the unforaged treatment. Forb biomass was similar between treatments (Fig. 1; ANOVA; $F_{1,6} = 3.2$; $P = 0.12$; 33 g/m^2 = unforaged *vs.* 65 g/m^2 = winter-foraged). Additionally, no difference was observed in graminoid biomass between treatments (Fig. 1; ANOVA; $F_{1,6} = 0.95$; $P = 0.51$; unforaged = 19.8 g/m^2 *vs.* winter-foraged = 26.5 g/m^2). Our observations reveal foraging exclusion increased shrub biomass, particularly *A. tridentata* biomass, which increased total plant biomass compared to the foraged treatment.

Plant community composition

Winter foraging exclusion significantly changed plant composition between treatments (perMANOVA; #$F_{1,79} = 32.4$; $P <$ 0.001). Plant composition shifted along principal coordinate analysis (PCoA) axes 1 and 2, which accounted for 43.2 and 13.6 percent variation, respectively (Fig. 2). Shrub percent cover was significantly and positively correlated to PCoA axis 1($r = 0.97$; $P = 0$) whereas, mineral soil ($r = -0.80$; $P < 0.0001$), forbs ($r = -0.50$; $P <$ 0.0001), and graminoids ($r = -0.24$; $P < 0.05$) were significantly and negatively correlated to PCoA axis 1. Graminoid ($r = 0.70$; $P < 0.001$),

organic horizon (r = 0.56; $P < 0.001$) and forb presence (r = 0.50; $P < 0.001$) were significantly and positively correlated with PCoA axis 2, but mineral soil (r = -0.55; $P < 0.001$) was negatively correlated with PCoA axis 2.

Winter foraging exclusion increased shrub cover (48% and 16% cover in unforaged and winter-foraged treatment respectively; ANOVA; $F_{1,73} = 94.3$; $P < 0.0001$). Conversely, winter foraging exclusion decreased forb (11% and 17% cover in unforaged and winter-foraged respectively; ANOVA; $F_{1,73} = 44.8$; $P < 0.0001$), graminoid (9% and 19% cover in unforaged and winter foraged respectively; ANOVA; $F_{1,73} = 14.2$; $P < 0.01$), and mineral soil (20% and 41% cover in unforaged and winter foraged respectively; ANOVA; $F_{1,73} = 40.5$; $P < 0.0001$) cover. However, winter foraging exclusion did not change the percent cover of the organic horizon (9% and 8% in unforaged and winter foraged respectively; ANOVA; $F_{1,73} = 0.29$; $P = 0.59$). Therefore, the presence of shrubs in the unforaged treatment, as well as forbs, graminoids, and mineral soil in the winter-foraged treatment, drove significant differences in plant composition between treatments.

Microbial activity

Microbial respiration was ~1.5-fold greater in the unforaged treatment compared to the winter foraged treatment (935 and 616 mg CO_2 g soil^{-1} respectively; ANOVA; $F_{1,16} = 5.7$; $P = 0.03$). Similarly,

net N mineralization was greater in the unforaged treatment (7.01 μg N g^{-1} soil) than in the winter-foraged treatment (4.10 μg N g^{-1} soil; ANOVA; $F_{1,16} = 23.7$; $P < 0.001$). However, net nitrification was similar between treatments (ANOVA; $F_{1,16} = 3.1$; $P = 0.098$; 1.33 *vs.* 1.66 μg N g^{-1} soil in unforaged and winter foraged treatment respectively). The significantly greater rates of soil respiration and net N mineralization in the unforaged treatment provide evidence that winter foraging exclusion accelerated soil C and N cycling rates.

Microbial community abundance

In contrast to our expectation, the relative abundance of fungi and bacteria did not change with winter foraging exclusion. Bacterial 16S rDNA gene copy number did not differ between treatments (unforaged = 8.3×10^5 copies g $soil^{-1}$; winter-foraged = 6.6×10^5 copies g $soil^{-1}$; ANOVA; $F_{1,11} = 1.8$; $P = 0.21$). Additionally, there was no effect of winter foraging exclusion on the relative abundance of the fungal ITS gene copy number (unforaged = 2.7×10^5 copies g $soil^{-1}$; winter-foraged = 2.3×10^5 copies g $soil^{-1}$; ANOVA; $F_{1,9} = 0.78$; $P = 0.40$). These results indicate that the relative abundance of both fungi and bacteria was unchanged by winter foraging exclusion.

Microbial taxonomic and functional composition

GeoChip data were interpreted as i) taxonomic composition (*i.e.,* fungal and bacterial orders based on the presence-absence

GeoChip data matrix reorganized by taxonomic order associated with each probe) and ii) functional composition (*i.e.,* the normalized signal intensity of all genes associated with starch, cutin, cellulose, hemicellulose, and lignin depolymerization). Winter foraging exclusion changed the taxonomic composition of the soil bacterial community as indicated by GeoChip (perMANOVA; #$F_{1, 23}$ = 2.9; P = 0.041; Fig. 3A). Bacterial taxonomic assemblages shifted along PCoA 1 and 2, which accounted for 42 and 11 percent of the variation between treatments, respectively (Fig. 3A). Fifty-five out of 73 bacterial orders present in our sites were significantly and negatively correlated with PCoA axis 1 (Pearson's correlation coefficients from -0.93 to -0.41; Table 5). The order Actinomycetales correlated most significantly and negatively with PCoA axis 1 (Table 5; r = -0.93 and $P < 0.0001$). Furthermore, SIMPER analysis indicated bacterial taxonomic composition exhibited a 7% average dissimilarity between treatments, with the order Synergistales driving the majority of this difference (3.7%). Winter foraging exclusion did not change bacterial taxonomic heterogeneity between treatments (PERMDISP; $F_{1,23}$ = 2.11; P = 0.16).

Differences in fungal taxonomic composition (as indicated by GeoChip) between winter-foraged and unforaged treatments were marginally significant (perMANOVA; #$F_{1,23}$ = 2.5; P = 0.076; Fig. 3B). Fungal taxonomic assemblages shifted along PCoA axis 1 and 2,

which accounted for 34 and 9 percent variation between treatments, respectively (Fig. 3B). Fourteen out of 29 fungal orders present in our sites were significantly ($P < 0.05$) and negatively correlated to PCoA axis 1 (Table 6). The fungal order Agaricales most strongly and negatively correlated with PCoA axis 1 (r = -0.90, $P < 0.0001$). SIMPER analysis indicated fungal richness exhibited 8% average dissimilarity between treatments, and the order Microascales contributed most to this difference (10%). Foraging exclusion did not change fungal taxonomic heterogeneity between treatments (PERMDISP; $F_{1,22} < 0.001$; $P = 0.99$). These changes in microbial composition occurred in parallel with differences in shrub cover and biomass, suggesting changes in plant composition from foraging exclusion altered microbial taxonomic composition between treatments.

Microbial community functional composition

Winter foraging exclusion significantly altered bacterial functional composition (perMANOVA; #$F_{1, 23} = 6.1$; $P = 0.015$). Bacterial functional assemblages shifted along PCoA axis 1 and 2, which accounted for 61 and 8 percent of the variation, respectively (Fig. 4A). Genes encoding for enzymes that depolymerize starch, cellulose, and hemicellulose (alpha-amylase, cellobiase and alpha-L-arabinofuranosidase respectively) had the strongest negative correlation with PCoA axis 1 (Table 7). In addition, 12 out of 18

lignocellulolytic genes present in our samples correlated with the unforaged treatment (Table 7). SIMPER analysis revealed bacterial functional assemblages exhibited a mean dissimilarity of ~4%, and the gene AmyX (which encodes for the enzyme isoamylase that hydrolyzes starch) contributed most to dissimilarity between treatments. In addition, there was no difference in assemblage heterogeneity between treatments (PERMDISP; $F_{1,22}$ = 0.92; P = 0.35), indicating the change in composition between treatments was not driven by a difference in relative dispersion, but a difference in functional composition.

Winter foraging exclusion did not change fungal functional composition (perMANOVA; #$F_{1,23}$ = 2.05; P = 0.153). However, fungal functional assemblages shifted along PCoA axis 1 and 2, which accounted for 57 and 7 percent variation between treatments, respectively (Fig. 4B). In addition, all fungal lignocellulolytic genes present in our samples were significantly and negatively correlated to PCoA axis 1(Fig. 4B; Table 8). Together these results suggest foraging exclusion promoted the presence of bacterial and fungal lignocellulolytic functional genes, which occurred simultaneously with an increase in *A. tridentata* abundance.

Discussion

Saprotrophic microbial communities in soil are thought to be structured by bottom-up ecological forces governing resource

availability; however, our observations provide evidence this relationship can be modified by foraging animals. For example, winter consumption of shrubs led to a reduced total plant biomass, which plauibly arose because *A. tridentata*, the most prevalent shrub in our study, is not tolerant to repeated browsing (Bilbrough & Richards 1993). Winter foraging further lead to an increase in forb cover, likely because forbs emerge following snowmelt, the time when many ungulates had already migrated to higher elevations, thereby avoiding consumption (Irwin 2002; Caldwell 1985). Taken together, the seasonal migration of ungulates in our study area from high elevations in summer to lower elevations in winter, reduced shrub cover and biomass, thereby changing plant composition, which modified microbial taxonomic composition and functional potential in soil. Our findings suggest that, in this system, the indirect top-down ecological force of winter foraging by migratory ungulates modifies the taxonomic composition and functional potential of saprotrophic soil microorganisms.

Soil organic C was greater in the absence of winter foraging, which may be driven by an increased litter input to soil by a more abundant shrub community. Shrubs play an important role in nutrient regulation in semi-arid ecosystems because their litter fall increases soil organic matter beneath them, relative to adjacent areas outside of the shrub canopy (Charley & West 1977).

Therefore, the greater shrub cover and lower bare soil cover may account for ~9% increase in soil organic C in the unforaged compared to the winter-foraged treatment. Additionally, *A. tridentata* presumably contributed to the increase in soil organic C we documented because its tissues have a high lignin:N ratio as compared to co-occurring graminoids and forbs in sagebrush steppe (Hooker et al. 2008; Shaw & Harte 2001). The initial lignin content in plant litter is inversely related to the rate of litter decay (Prescott, 2005), as most saprotrophic microorganisms lack the physiological capacity to oxidize this plant compound (Paustian et al. 1997); therefore, lignin from plant detritus enters the resistant pool of soil organic matter which increases soil organic C (Paustian et al. 1997). Thus, the difference in soil organic C between treatments can possibly be explained as a result of winter foraging by ungulates, which reduced abundance of *A. tridentata,* which may in-turn have decreased lignin inputs to soil, thereby reducing soil organic C.

Nitrogen mineralization and respiration rates were greater in the unforaged treatment, which may also be driven by an increased litter input to soil from a more abundant shrub community. In semi-arid ecosystems, shrubs increase rates of net N mineralization (Charley & West 1977) and microbial activity (Bolton et al. 1993), because of a localization of litterfall and the subsequent increase in SOM beneath them. Additionally, organic matter has been found to

increase the potential for N mineralization and soil respiration, as well as regulate N mineralization in soil from beneath *A. tridentata* (Burke et al. 1989; Burke 1989). Therefore, it appears in the absence of foraging, the greater *A. tridentata* biomass likely increased the amount of lignified plant material entering the soil food web, thereby increasing soil organic C which in turn may have driven the observed increase in N mineralization and respiration rates.

Our results differ from others who found ungulate grazing increased microbial respiration and net N mineralization (Frank et al. 2000; Frank & Groffman 1998). However, this response was driven by added ammonium and urea, a plant accessible mineral form of N from ungulate urine and fecal pellets (Frank et al. 2000; Frank & Groffman 1998; Tracy & Frank 1998; Hobbs 1996). In our study sites, ungulate excreta did not impact C and N cycling, presumably because it was not rapidly incorporated into the soil. Elk excreta was deposited in winter when there was snow accumulation, and likely leached with spring melt. In addition, elk pellets can persist for decades, as evidenced by elk pellets in the Miller Butte exclosure that have remained since its origin (Eric Cole, personal communication). Therefore, ungulate excreta did not increase C and N rates in the foraged treatment, and the greater rates observed in the absence of foraging could be the result of

increased soil organic C, presumably from a greater *A. tridentata* abundance.

The exclusion of winter foraging drove bacterial and fungal assemblages to favor taxa implicated in the degradation of lignified plant litter. Bacterial genes encoding for enzymes that depolymerize starch, cellulose, hemicellulose, and lignin, compounds found in high proportions in *A. tridentata* (Kinney & Sugihara 1943), were highly correlated with the unforaged treatment (Table 3). Among Bacteria, winter foraging exclusion promoted Actinomycetales, which have lignolytic physiological capacities (Kluepfel & Ishaque 1982), and Synergistales, which can ferment starch (Maune & Tanner 2012). Among fungi, winter foraging exclusion promoted Agaricales, which can oxidize lignin, and Microascales, which are saprotrophic fungi involved in plant litter decay (Sakayaroj et al. 2011). The increase in *A. tridentata* biomass with the absence of foraging likely increased litter inputs to soil, which could have stimulated lignolytic and saprotrophic metabolism by providing additional substrate for lignocellulolytic enzymes (Osono 2007; Högberg & Ekblad 1996; Raich & Schlesinger 1992). Therefore, the increase in *A. tridentata* with foraging exclusion could have promoted the observed taxonomic and functional shift within the soil microbial community.

Bacterial taxonomic and functional assemblages were compositionally distinct between treatments (Fig. 3A and 4A), but

were similarly heterogeneous. Fungal taxonomic assemblages were marginally different between treatments (#$F_{1,23}$ = 2.5; P = 0.076; Fig. 3B), yet fungal functional assemblages were similar between treatments (#$F_{1,23}$ = 2.05; P = 0.153), which suggests fungal communities were functionally equivalent but compositionally distinct. Similar to bacteria, both fungal taxonomic and functional composition was similarly heterogeneous across treatments, demonstrating that foraging did not reduce the relative dispersion, but changed the overall composition of the fungal and bacterial community. These results suggest the difference in plant composition between treatments constrained the microbial community, because soil microorganisms are limited by litter inputs from plant detritus. Therefore, winter foraging by native ungulates in this system, presumably altered microbial community taxonomic composition and functional potential via changes in plant composition.

Our research provides evidence that soil microbial community composition and function can be structured by indirect top-down effects of ungulate foraging. The sagebrush steppe we studied is highly affected by ungulate winter foraging, which, in our study, altered plant composition, and in turn, modified soil microbial community taxonomic composition and functional potential. In the absence of foraging, the abundance of forbs decreased and shrubs

increased, specifically *A. tridentata*. The increase in shrub biomass occurred concomitantly with changes in microbial community composition and functional potential, with organisms possessing genes with lignocellulolytic function accounting for much of the compositional differences. Together, our results provide evidence that winter foraging by native migratory ungulates in the sagebrush steppe exerts an indirect top-down ecological force that shapes soil microbial communities, reveling the potential for links between foraging animals and soil microbial communities in other grazing systems.

Figures and tables

Table 1. Physiographic and edaphic characteristics of winter foraging exclosures in northwestern Wyoming.

Site	Location	Elevation m	Soil Type	Year Established	Area m^2
Gros Ventre	43°34’10”N 110°18’40”W	2263	fine sandy loam	1958	3,721
Lower Hoback	43°17’45”N 110°39’27”W	1894	gravelly loam	1938	961
Miller Butte	43°30’52”N 110°42’42”W	1938	loam/silt loam	1958	10,920
Upper Hoback	43°18’0”N 110°39’38”W	1951	gravelly loam	1938	961

Table 2. Allometric equations used to estimate shrub biomass.

Shrub species	Variable	Allometric equations	Source
Artemisia tripartite	X= percent cover	y=(A+B)*x1*N*%	Riccardi et al. 2007
Symphoricarpos oreophilus	X=VOL 2	$y=a(x^b)$	Reiner et al. 2010
Chrysothamnus vicsidiflorus	X=VOL 2	$y=a(x^b)$	Reiner et al. 2010
Artemisia tridentata var.tridentata	X=percent cover	y=(A+B)*x1*N*%	Riccardi et al. 2007
Purshia tridentata	X= percent cover	y=(A+B)*x1*N*%	Riccardi et al. 2007
Artemisia tridentata var. vaseyana	X=VOL2	$y=a(x^b)$	Reiner et al. 2010
Artemisia arbuscula	X=VOL 2	$y=a(b^e)$	Reiner et al. 2010
Amelanchier alnifolia	X=basal diameter cm	$Y=a(x^b)$	Smith & Brand 1983

Table 3. List of fungal and bacterial genes chosen for analysis. Number of gene variants is the number of probes representing this gene on the microarray. Substrate category as predefined on the microarray. EC number retrieved from BRENDA (http://www.brenda-enzymes.info).

Fungi				Bacteria			
Enzyme (gene) name	**EC number**	**# Variants**	**Substrate category**	**Enzyme (gene) name**	**EC number**	**# Variants**	**Substrate category**
acetylxylan esterase (axe)	3.1.1.72	41	Cellulose	cellobiase	3.2.1.21	224	Cellulose
cellobiase	3.2.1.21	80	Cellulose	endoglucanase	3.2.1.4	148	Cellulose
endoglucanse	3.2.1.4	63	Cellulose	exoglucanase	3.2.1.91	16	Cellulose
exoglucanse	3.2.1.91	81	Cellulose	cutinase	3.1.1.74	124	Cutin
cutinase	3.1.1.74	51	Cutin	alpha-L-arabinofuranosidase (ara)	3.2.1.55	285	Hemicellulose
alpha-L-arabinofuranosidase (ara)	3.2.1.55	68	Hemicellulose	mannanase	3.2.1.25	119	Hemicellulose
mannanase	3.2.1.25	23	Hemicellulose	Xylose isomerase (xyla)	3.5.1.5	211	Hemicellulose
xylanase	3.2.1.8	47	Hemicellulose	xylanase	3.2.1.8	279	Hemicellulose
glyoxal oxidase (glx)	1.4.3.11	52	Lignin	glyoxal oxidase (glx)	1.4.3.11	16	Lignin
ligninase	1.11.1.14	10	Lignin	phenol oxidase	1.10.32	110	Lignin
phenol oxidase	1.10.32	154	Lignin	vanillate demethylase (vana)	1.2.3.12	161	Vanillin/Lignin
vanillin dehydrogenase (vdh)	1.2.1.67	3	Vanillin/Lignin	vanillin dehydrogenase (vdh)	1.2.1.67	44	Vanillin/Lignin
Manganese peroxidase	1.11.1.13	32	Lignin	alpha-amylase (AmyA)	3.2.1.1	2363	Starch
alpha-amylase (AmyA)	3.2.1.1	51	Starch	isoamylase (AmyX)	3.2.1.68	1	Starch
glucoamylase	3.2.1.3	48	Starch	cytidine deaminase (cda)	3.5.4.5	166	Starch
				glucoamylase	3.2.1.3	67	Starch
				neopullulanase (npIT)	3.2.1.135	40	Starch
				pullulanase(pula)	3.2.1.41	153	Starch

Table 4. Soil chemical properties of winter foraging exclosures and adjacent winter-foraged plots. Means with the same letter are not significantly different at $\alpha = 0.05$.

Site	pH		Soil C mg kg^{-1}		Soil N mg kg^{-1}	
	Foraged	Unforaged	Foraged	Unforaged	Foraged	Unforaged
Gros Ventre	7.4	7.9	1.84	1.99	0.20	0.18
Lower Hoback	7.8	6.8	1.76	2.28	0.23	0.26
Miller Butte	6.8	6.7	3.89	3.79	0.44	0.44
Upper Hoback	7.7	7.9	1.52	1.27	0.22	0.17
Mean	7.4a	7.3a	2.25a	2.33b	0.27a	0.26a
SE	(0.23)	(0.33)	(0.55)	(0.53)	(0.06)	(0.06)

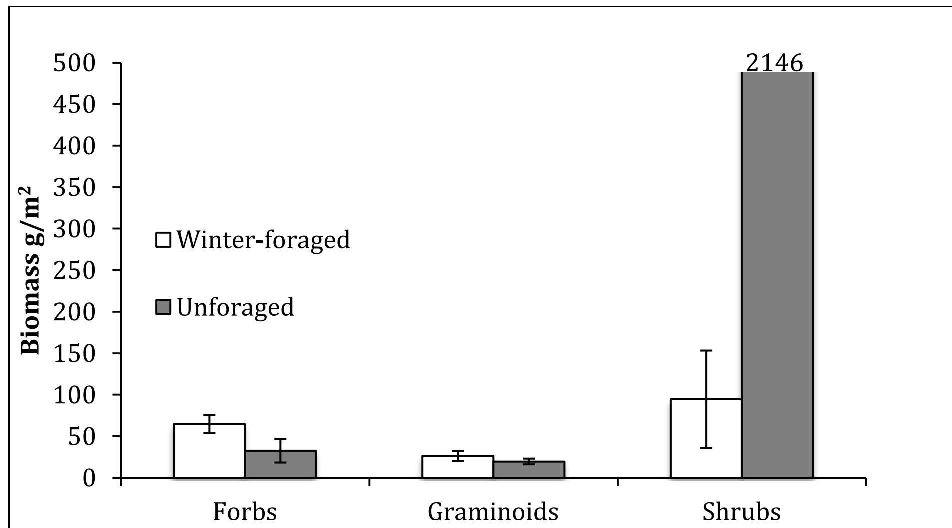


Fig. 1. Average biomass of shrubs, forbs, amd graminoids, in winter-foraged and unforaged treatment. Error bars indicate standard error. Forb and graminoid biomass were not significantly different between treatments (P = 0.12 and P = 0.51, respectively). Shrub biomass was marginally statistically different between treatments (P = 0.07) due to unusually low shrub biomass in the unforaged treatment at the Gros Ventre study site (316.7 g/m^2). When this site was removed from our analysis, shrub biomass became statistically significant (P = 0.026). We accepted statistical significance at $\alpha = 0.05$.

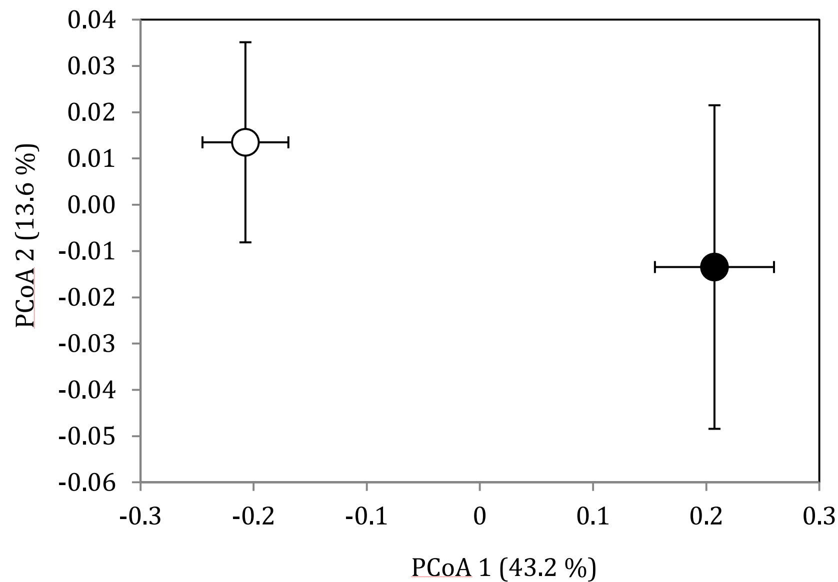
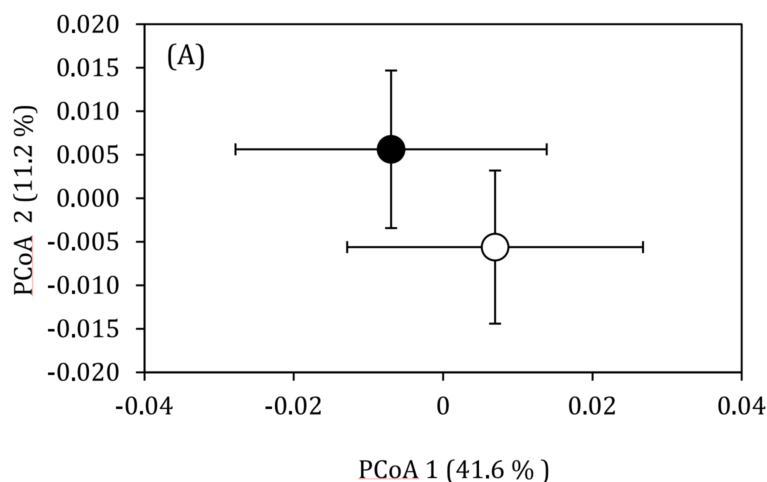


Fig. 2. Principal coordinate analysis visualizing shifts in plant community composition from winter foraging. Open symbol represents winter-foraged treatment and closed symbol designates unforaged treatment. Error bars indicate standard error. Percent variation accounted by each axis is enclosed by parentheses. PerMANOVA revealed plant composition between treatments was significantly different (P < 0.001). Shrub percent cover (r = 0.97; P = 0) was positively significantly correlated to PCoA axis 1. Mineral soil (r = -0.80; P < 0.0001), forbs (r = -0.50; P < 0.0001), and graminoids (r = -0.24; P < 0.05), were significantly and negatively correlated to PCoA axis 1. Graminoid (r = 0.70; P < 0.001), organic horizon (r = 0.56; P < 0.001) and forbs (r = 0.50; P < 0.001) were significantly and positively correlated with PCoA axis 2. Mineral soil (r = -0.55; P < 0.001) was negatively correlated with PCoA axis 2. Statistical significance was accepted at α = 0.05.



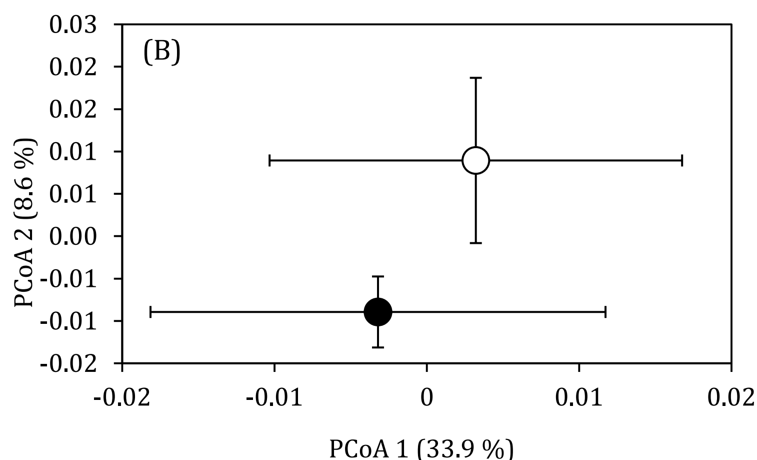


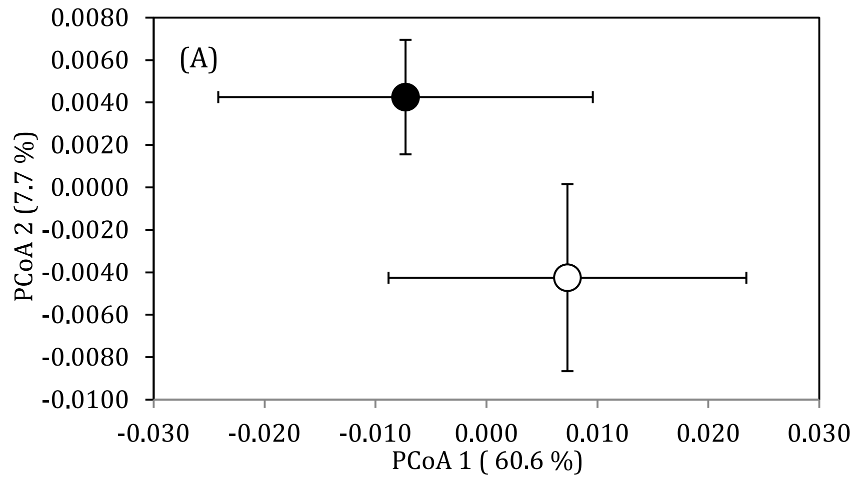
Fig. 3. Principal coordinate analysis visualizing shifts in A) bacterial and B) fungal taxonomic composition in response to winter foraging exclusion. Open symbols represent winter-foraged treatment and closed symbols represent unforaged treatment. Error bars indicate standard error. Percent variation accounted by each axis is enclosed in parentheses. Distance matrices were constructed using Bray-Curtis dissimilarity metric based on the presence/absence of all fungal and bacterial orders from functional assemblages. Pairwise comparison with PerMANOVA simulations found significant differences in treatment effects on bacterial taxonomic composition (P = 0.042), and marginally significant differences in fungal taxonomic composition (P = 0.075). We accepted statistical significance at α = 0.05.

Table 5. Bacterial orders significantly driving differences in taxonomic composition between treatments. Pearson's r correlation coefficient shows direction and degree of correlation of orders to PCoA axis 1 or 2.

Order	Axis correlation	*P*-value	Pearson's r
Actinomycetales	1(-)	<0.0001	-0.93
Pseudomonadales	1(-)	<0.0001	-0.93
Bacteroidales	1(-)	<0.0001	-0.93
Rhizobiales	1(-)	<0.0001	-0.92
Rhodobacterales	1(-)	<0.0001	-0.91
Xanthomonadales	1(-)	<0.0001	-0.89
Bacillales	1(-)	<0.0001	-0.88
Lactobacillales	1(-)	<0.0001	-0.87
Clostridiales	1(-)	<0.0001	-0.87
Burkholderiales	1(-)	<0.0001	-0.86
Enterobacteriales	1(-)	<0.0001	-0.85
Sphingomonadales	1(-)	<0.0001	-0.85
Chroococcales	1(-)	<0.0001	-0.84
Bdellovibrionales	1(-)	<0.0001	-0.84
Selenomonadales	1(-)	<0.0001	-0.83
Spirochaetales	1(-)	<0.0001	-0.83
Vibrionales	1(-)	<0.0001	-0.82
Deinococcales	1(-)	<0.0001	-0.80
Nitrospirales	1(-)	<0.0001	-0.79
Myxococcales	1(-)	<0.0001	-0.78
Erysipelotrichales	1(-)	<0.0001	-0.78
Bifidobacteriales	1(-)	<0.0001	-0.77
Fibrobacterales	1(-)	<0.0001	-0.77
Oscillatoriales	1(-)	<0.0001	-0.77
Cytophagales	1(-)	<0.0001	-0.77
Gloeobacterales	1(-)	<0.0001	-0.76
Caulobacterales	1(-)	<0.0001	-0.72
Desulfuromonadales	1(-)	<0.0001	-0.71
Flavobacteriales	1(-)	<0.0001	-0.69
Verrucomicrobiales	1(-)	<0.0001	-0.68
Thermotogales	1(-)	<0.0001	-0.65
Thermales	1(-)	<0.0001	-0.64
Sphingobacteriales	1(-)	<0.001	-0.60
Solibacterales	1(-)	<0.001	-0.60
Candidatus	1(-)	<0.001	-0.59
Caldilineales	1(-)	<0.001	-0.58
Nostocales	1(-)	<0.001	-0.57
Synergistales	1(-)	<0.001	-0.56
Rhodospirillales	1(-)	<0.001	-0.56
Chlorobiales	1(-)	<0.001	-0.55
Herpetosiphonales	1(-)	<0.001	-0.54
Methylococcales	1(-)	<0.001	-0.53
Neisseriales	1(-)	<0.01	-0.51
Phycisphaerales	1(-)	<0.01	-0.46
Oceanospirillales	1(-)	<0.01	-0.41
Thiotrichales	1(+)	<0.0001	0.70
Caldilineales	2(-)	<0.01	0.50
Chlamydiales	2(+)	<0.01	0.41
Ktedonobacterales	2(+)	<0.01	0.41
Rubrobacterales	2(+)	<0.01	0.43
Hydrogenophilales	2(+)	<0.01	0.51
Thermoanaerobacterales	2(+)	<0.001	0.52
Aeromonadales	2(+)	<0.001	0.58
Rhodocyclales	2(+)	<0.0001	0.64
Acidithiobacillales	2(+)	<0.0001	0.70

Table 6. Fungal orders significantly driving differences in taxonomic composition between treatments. Pearson's r correlation coefficient shows the direction and degree of correlation of bacterial orders to PCoA axis 1 or 2.

Order	Axis correlation	P-value	Pearson's r
Agaricales	1(-)	<0.0001	-0.90
Eurotiales	1(-)	<0.0001	-0.88
Sordariales	1(-)	<0.0001	-0.86
Onygenales	1(-)	<0.0001	-0.81
Glomerellales	1(-)	<0.0001	-0.81
Saccharomycetales	1(-)	<0.0001	-0.79
Hypocreales	1(-)	<0.0001	-0.76
Dothideales	1(-)	<0.0001	-0.74
Magnaporthales	1(-)	<0.0001	-0.74
Pleosporales	1(-)	<0.001	-0.68
Auriculariales	1(-)	<0.001	-0.67
Microascales	1(-)	<0.001	-0.66
Mucorales	1(-)	<0.01	-0.62
Helotiales	1(-)	<0.01	-0.54
Schizosaccharomycetales	2(+)	<0.05	0.45
Xylariales	2(+)	<0.01	0.57
Capnodiales	2(+)	<0.0001	0.77



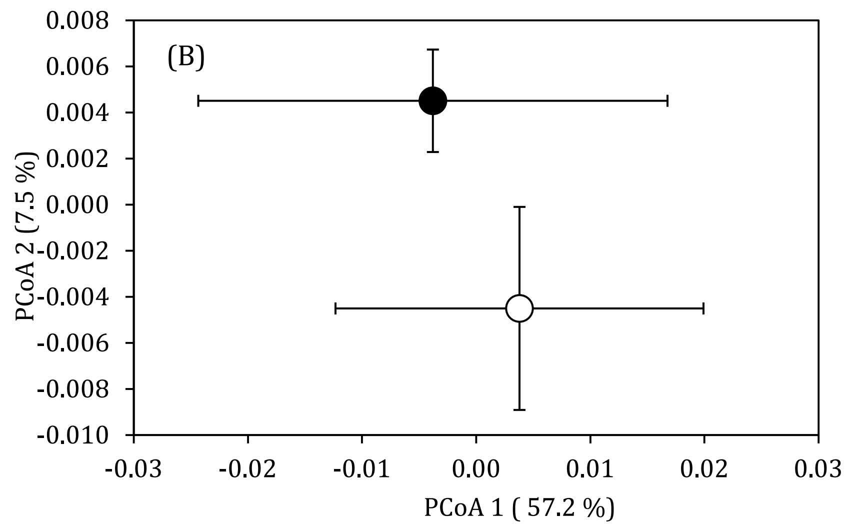


Fig. 4. Principal coordinate analysis visualizing shifts in A) bacterial and B) fungal functional composition in response to winter foraging exclusion. Open symbols indicate winter-foraged treatment and closed symbols indicate unforaged treatment. Error bars represent standard error. Percent variation accounted by each axis is enclosed in parentheses. Distance matrices were calculated using Bray-Curtis dissimilarity metric based on the normalized signal intensity of all relative gene variants responsible for leaf litter decay. PerMANOVA found significant differences in treatment effects for bacterial functional genes (P = 0.015) but not for fungal functional genes (P = 0.153). We accepted statistical significance at α = 0.05.

Table 7. Bacterial gene variants responsible for functional dissimilarity between treatments. Pearson's r correlation coefficient shows the degree and direction of correlation of a gene variant to PCoA axis 1 or 2.

Enzymes	Gene family	PCoA axis	P-value	Pearson's r
alpha-amylase	starch	1(-)	<0.001	-0.97
cellobiase	cellulose	1(-)	<0.001	-0.96
alpha –L arabinofur-anosidase	hemicellulose	1(-)	<0.001	-0.95
xylose isomerase	hemicellulose	1(-)	<0.001	-0.93
D-alanine—(R) lactate ligase	vanillin/lignin	1(-)	<0.001	-0.93
phenol oxidase	lignin	1(-)	<0.001	-0.92
pullulanase	starch	1(-)	<0.001	-0.92
endoglucanase	cellulose	1(-)	<0.001	-0.91
isoamylase	starch	1(-)	<0.001	-0.89
cutinase	cutin	1(-)	<0.001	-0.85
glucoamylase	starch	1(-)	<0.001	-0.80
neopullulanase	starch	1(-)	0.022	-0.46
exoglucanase	cellulose	2(-)	0.004	-0.56
glyoxal oxidase	lignin	2(-)	0.005	-0.55

Table 8. Fungal gene variants responsible for functional dissimilarity between treatments. Pearson's r correlation coefficient shows the degree and direction of correlation of a gene variant to PCoA axis 1 or 2.

Enzymes	Gene family	PCoA axis	P-value	Pearson's r
phenol oxidase	lignin	1(-)	<0.0001	-0.94
xylanase	hemicellulose	1(-)	<0.0001	-0.93
exoglucanase	cellulose	1(-)	<0.0001	-0.92
alpha –L-arabinofuranosidase	hemicellulose	1(-)	<0.0001	-0.90
endoglucanase	cellulose	1(-)	<0.0001	-0.90
glucoamylase	starch	1(-)	<0.001	-0.89
glyoxal oxidase	lignin	1(-)	<0.0001	-0.89
cellobiase	cellulose	1(-)	<0.0001	-0.88
alpha-amylase	starch	1(-)	<0.001	-0.87
acetylxylan esterase	cellulose	1(-)	<0.0001	-0.87
mannanase	hemicellulose	1(-)	<0.001	-0.71
ligninase	lignin	1(-)	<0.0001	-0.69
cutinase	cutin	1(-)	<0.001	-0.63
vanillin dehydrogenase	vanillin/lignin	1(-)	<0.01	-0.59

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