Functional Ecology

Effects of elk and bison carcasses on soil microbial communities and ecosystem functions in Yellowstone, USA

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Author contribution

JKB conceived the study, JKB and ACR conducted the field work, BF the laboratory and bioinformatic analyses. ACR and AF analysed the data. ACR wrote the manuscript, AF, MS, BF, AWM and JKB contributed to the final manuscript.

Data availability

Raw sequences have been deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA550037. The rest of the data is available through the envidat.ch data portal of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL, https://doi.org/10.16904/envidat.152

Conflict of interest

The authors declare that they have no conflict of interest.

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Abstract

1. Carrion is long recognized as important to scavengers. How carrion may affect soil microbial biodiversity and ecosystem processes in natural systems is comparatively unknown, but important for the intersection of vertebrate food webs, belowground processes, and ecological heterogeneity.

2. We assessed *in situ* soil and plant responses to wolf-killed mammal carrion in Yellowstone National Park, USA.

3. Bison and elk carcasses increased soil respiration and vegetation nutrient concentration and altered bacterial and fungal communities on carcass compared to control plots. The "fingerprints" of soil microbial taxa associated with bison compared to elk carcasses differed considerably and taxa found depended upon abiotic gradients and soil properties.

4. We found evidence that soil microbial community changes associated with carcasses may not be as generalizable as previously thought, which is important for a mechanistic understanding of the links between carrion and soil heterogeneity and potentially for applications in forensic science.

5. This work demonstrates the importance of carrion studies in natural systems. Our findings show that carrion creates distinct ecological patterns that contribute to both above- and belowground biological heterogeneity, linking carrion distribution dynamics with soil microbial biodiversity and ecosystem functions.

Zusammenfassung

1. Dass Tierkadaver wichtig für Aasfresser sind, ist seit langem bekannt. Kaum bekannt ist jedoch, dass jeder Kadaver eine Stelle mit einmaligen ökologischen Eigenschaften darstellt, welcher die mikrobielle Biodiversität im Boden und Ökosystemprozesse beeinflusst und damit für grosse Heterogenität in natürlichen Ökosystemen sorgt.

2. Wir untersuchten im Yellowstone-Nationalpark, USA, in situ wie sich die Eigenschaften des Bodens und der Vegetation unter von Wölfen gerissenen Bison- und Wapiti-Kadavern wandeln.

3. Bison- und Wapiti-Kadaver erhöhten die Bodenatmung und die Nährstoffkonzentration der Vegetation und veränderten die Bakterien- und Pilzgemeinschaften im Boden im Vergleich zu den Kontrollflächen. Die mikrobiellen Lebensgemeinschaften im Boden unter Bison-Kadavern unterschieden sich jedoch auch erheblich von jenen unter Wapiti-Kadavern. Zusätzlich beeinflussten Bodeneigenschaften und die geografische Lage der Kadaver die Zusammensetzung der mikrobiellen Gemeinschaften.

4. Bisher wurde davon ausgegangen, dass Kadaver immer zu ähnlichen und voraussagbaren Veränderungen in den mikrobiellen Lebensgemeinschaften führen. Unsere Studie weist jedoch darauf hin, dass diese Veränderungen nicht so verallgemeinerbar sein dürften, wie bisher angenommen. Dies ist wichtig, um die Mechanismen zu verstehen, die zwischen Kadavern und der Bodenheterogenität wirken, auch im Hinblick auf mögliche Anwendungen in der forensischen Wissenschaft.
5. Diese Arbeit zeigt, welche Bedeutung Kadaver-Studien in natürlichen Ökosystemen haben. Unsere Ergebnisse zeigen, dass Kadaver unterschiedliche ökologische Muster erzeugen, die sowohl über- wie unterirdisch zu biologischer Heterogenität beitragen. Die grossräumige und langfristige Kadaver-Verteilung in natürlichen Ökosystemen dürfte deshalb mit der mikrobiellen Biodiversität und den Bodenfunktionen verknüpft sein.

Keywords: bacteria, biogeochemical cycling, carcass, carrion, decomposition, detritus, fungi, soil biodiversity

Introduction

Primary producers exploit solar energy and soil resources to produce tissues that feed the vast array of herbivores globally. By consuming this plant tissue, herbivores can alter plant species composition, plant physiological properties, and the amount of plant litter returned to the soil. These changes directly and indirectly affect the activity and abundance of soil organisms and therefore soil carbon (C) and nutrient cycling (Bakker, Ritchie, Olff, Milchunas, & Knops, 2006; Bardgett & Wardle, 2003; del-Val & Crawley, 2005; Forbes et al., 2019; Sitters & Olde Venterink, 2015; Wardle et al., 2004). Trampling and burrowing activities of herbivores and the deposition of dung and urine can also stimulate the activity of roots, microbes and/or soil arthropods and related soil processes (Barth, Liebi, Hendrickson, Sedivec, & Halvorson, 2014; Forbes et al., 2019; Risch et al., 2015; Schrama et al., 2013; Sitters et al., 2020; Sitters & Olde Venterink, 2015).

Herbivores, in turn, support a diversity of predators across multiple trophic levels (Loreau, 1995). In death, animal tissue usually is consumed, but also re-enters the ecosystem via decomposition, thereby dispersing and diffusing biologically limiting resources. Thus, dead animal matter (hereafter carrion; e.g., vertebrate carcasses) strongly affects and supports scavenger guilds, soils and plants, and increases the availability of biogeochemical hotspots (Barton et al., 2019; Barton, Cunningham, Lindenmayer, & Manning, 2013). While carrion effects are much more discrete compared to plant litter or frass, dung and urine of herbivores that returns to the soil system, their ecological consequences are analogous to a rhizosphere or drilosphere (Kuzyakov & Blagodatskaya, 2015; Pii et al., 2015); patches of soil are significantly affected by individual carcasses. Although a single carcass influences only a small proportion of terrestrial area, carrion input at the landscape scale creates a shifting mosaic of carcasses that can account for a biologically significant amount of heterotrophic activity and above-belowground interactions within an ecosystem (Barton et al., 2019; Carter, Yellowlees, & Tibbett, 2007).

Even mostly consumed carrion can result in strong ecological effects that can last multiple years, and shape biotic communities and ecosystem processes (Bump, Webster, et al., 2009; Bump, Peterson, & Vucetich, 2009; Macdonald et al., 2014). For example, several studies conducted in different ecosystems revealed that soil nutrient availabilities were elevated for multiple growing seasons at carrion locations (Barton et al., 2016; Bump, Webster, et al., 2009; Bump, Peterson, et al., 2009; Macdonald et al., 2014; Melis et al., 2007). This, in turn, can improve plant nutritional quality and affect plant community composition (Barton et al., 2016; Bump, Webster, et al., 2009; Bump, Peterson, et al., 2009),

creating “hotspots” of resources within the ecosystem. Carrion in general, and carrion amount in particular, was also shown to positively affect vertebrate and invertebrate scavenger diversity (Barton et al., 2013; Elbroch, O’Malley, Peziol, & Quigley, 2017; Nuria & Fortuna, 2007; Turner, Abernethy, Conner, Rhodes Jr., & Beasley, 2017).

How carrion affects soil microbial abundance and composition that is crucial for soil C and nutrient cycling and ecosystem functioning has, however, not been assessed in natural systems (Barton et al., 2013; Bump, Peterson, et al., 2009). Most of our knowledge stems from research on intact human or animal cadavers, mostly mice or domestic pigs, placed on the soil surface in controlled experiments (Finley, Pechal, Benbow, Robertson, & Javan, 2016; Keenan, Schaeffer, & DeBruyn, 2019; Keenan, Schaeffer, Jin, & DeBruyn, 2018; Metcalf et al., 2016; Singh et al., 2018). These experiments showed that soil bacterial community structures significantly changed and their functions decreased over time in soils below cadavers (Finley et al., 2016). However, in contrast to intact human or mammal cadavers left for complete decomposition, carcasses in natural systems are usually rapidly consumed and incorporated into higher tropic levels (Wilmers, Stahler, Crabtree, Smith, & Getz, 2003). Therefore, carrion in natural systems ostensibly contributes less direct resources to the soil system and could potentially have no or different impacts on soil biodiversity and ecosystem functioning compared to cadavers that experimentally decompose without realistic consumption (e.g., caged to exclude vertebrates).

Hence, assessments of *in situ* carrion effects in natural systems are required to understand how soil properties and ecosystem functioning might be altered via shifts in soil microbial (bacteria, fungi) biodiversity and community composition (see also Moleón & Sánchez-Zapata, 2015). Such assessments are key to develop a mechanistic understanding of carrion dynamics and to test whether or not carrion effects are generalizable. We therefore compared ecosystem functions (soil respiration, organic matter (OM) decomposition, vegetation nitrogen (N) concentration and C:N ratio), soil abiotic (soil C and N concentration, soil temperature, soil moisture), and soil biotic properties (bacterial and fungal abundance, richness, diversity, community structure) sampled on wolf-killed ungulate carcasses [bison (*Bison bison* Hamilton Smith), elk (*Cervus canadensis* Erxleben)] with proximate control sites in Yellowstone National Park (YNP), USA. When wolves kill an ungulate, all soft tissue (including hide) is consumed relatively quickly (days) and only bones, gut contents, hair, and teeth persist longer (months, years). The bodily fluids are, however, largely returned to the soil at the spot of carrion consumption.

We expected to find increased ecosystem functions and soil abiotic properties, i.e., increased decomposition and nutrient pulses at carcass sites (Bump, Webster, et al., 2009; Bump, Peterson, et al., 2009). In addition, we expected soil bacterial and fungal abundance to be higher, alpha-diversity to be lower, and bacterial and fungal community structures to be altered at carcass sites as only specialized organisms can process the carcass-derived OM (Finley et al., 2016). Finally, because bison exceed elk in body size and mass, we expected to detect differences in the microbial communities associated with the carcasses of the two species (Elbroch et al., 2017; Turner et al., 2017).

Methods

Study area and study sites

This study was conducted in YNP's Northern Range (NR), located in north-western Wyoming and south-western Montana, USA (~44.9163° N, 110.4169° W). The NR expands over ~1000 km^2 and features long cold winters and short dry summers. Grasslands and shrublands dominate the NR that is the home of large migratory herds of bison (winter counts 2017: ~3919 individuals; Geremia, Wallen, & White, 2017) and elk (~5349 individuals) as well as their main predators, approximately five packs of wolves with a total of 33 individuals (Smith et al., 2017). As part of a long-term research program within YNP, wolf predation has been studied since their reintroduction in 1995.

For our study, we received ground-truthed coordinates of bison and elk carcasses from winter 2016/17 (November 2016 through April 2017) from the YNP Wolf Project. Between June 20 and July 1, 2017, we visited 24 carcasses in total. At five sites, we could not sample as the carcasses were no longer found. In total we located remains (hairmats, rumen content, bones, teeth) of 19 adult male and female carcasses (7 bison, 12 elk; Supplementary Table 1). Live body weights of adult bison and elk are approximately 730 kg (male bison), 450 kg (female bison), 330 kg (male elk), and 235 kg (female elk, Meagher, 1973; Quimby & Johnson, 1951).

The kills and subsequent consumption happened between 34 and 173 days prior to our sampling (hereafter "days since kill", DSK), for which we accounted in our statistics. Note that wolves and other scavengers consumed the soft tissue of the carcasses quickly, hence, there is close to no soft tissue left for decomposition as compared to an intact body left on the soil surface. The 19 carcass sites covered the extent of YNP's NR, with both bison and elk carcasses showing similar distributions; elevation ranged from 1703 to 2884 m a.s.l. (Supplementary Fig 1 & Supplementary Table 1). The carcasses were all located in grassland

or sage-brush shrubland, with or without sparsely scattered trees, and both bison and elk carcasses showed the same distribution of DSK. At each study site, we selected a reference plot (hereafter "control") that was of comparable size, slope aspect and vegetation to the carcass location (hereafter "carcass"). The control was at least 10 m away (Danell, Berteaux, & Brathen, 2002; Melis et al., 2007) from the carcass itself to ensure the absence of potential direct and indirect carcass effects (paired design; (Bump, Webster, et al., 2009; Bump, Peterson, et al., 2009).

Ecosystem functions and soil properties

We randomly collected 50 g of mineral soil from three locations on both control and carcass plots to a depth of 5 cm with sterile techniques and gently mixed the material to obtain a composite sample. Half the soil sample was immediately bagged in plastic bags (whirl packs), stored in a cooler with ice packs (~5 ºC), sieved (2-mm) and frozen within 4-6 hours of collection to assess soil microbial communities. For this purpose, we extracted total genomic DNA from 0.5 g soil using the PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany). DNA concentrations were measured using PicoGreen (Molecular Probes, Eugene, OR, USA). PCR amplifications of partial bacterial small-subunit ribosomal RNA genes (region V3–V4 of 16S rRNA) and fungal ribosomal internal transcribed spacers (region ITS2) were performed as described previously (Frey et al., 2016). Each sample consisting of 40 ng DNA was amplified in triplicate and pooled before purification with Agencourt AMPure XP beads (Beckman Colter, Berea, CA, USA) and quantified with the Qubit 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicons were sent to the Genome Quebec Innovation Center (Montreal, Canada) for barcoding using the Fluidigm Access Array technology and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

Quality control of bacterial and fungal reads was performed using a customized pipeline (Supplementary Table 2; Frey et al., 2016). Paired-ends reads were matched with USEARCH (Edgar & Flyvbjerg, 2015), substitution errors were corrected using Bayeshammer (Nikolenko, Korobeynikov, & Alekseyev, 2013) and PCR primers were trimmed (allowing for 1 mismatch, read length >300 bp for 16S and >200 bp for ITS primers) using Cutadapt (M. Martin, 2011). Sequences were dereplicated and singleton reads removed prior to clustering into operational taxonomic units (OTUs) at 97% identity using USEARCH (Edgar, 2013). The remaining centroid sequences were tested for the presence of

ribosomal signatures using Metaxa2 (Bengtsson-Palme et al., 2015) or ITSx (Bengtsson-Palme et al., 2013). Taxonomic assignments of the OTUs were obtained using Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) with a minimum bootstrap support of 60% implemented in mothur (Schloss et al., 2009) by querying the bacterial and fungal reads against the SILVA Release 128 (Quast et al., 2013) and UNITE 8.0 (Abarenkov et al., 2010) reference databases for 16S and ITS OTUs, respectively.

Abundances of the bacterial 16S rRNA gene and fungal ITS amplicon were determined by quantitative real-time PCR (qPCR) on an ABI7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) as described previously (Frossard et al., 2018). The same primers (without barcodes) and cycling conditions as for the sequencing approach were used for the 16S and ITS qPCR. Three standard curves per target region were obtained using tenfold serial dilutions of plasmids generated from cloned targets (Frey, Niklaus, Kremer, Lüscher, & Zimmermann, 2011). Data were converted to represent mean copy number of targets per gram of soil (dry weight).

The other half of the soil sample was bagged in paper, dried to constant weight at 60°C, passed through a 2 mm sieve and analyzed for total C and N concentration with a CE Instruments NC 2100 soil analyzer (CE Elantech Inc., Lakewood NJ, USA). We also collected 20 mature and undamaged leaves of the dominant grass species growing on control and carcass sites, but taxa were not recorded. The plant material was dried at 60°C, finely ground till homogenized and also analyzed to obtain total C and N concentrations. Soil temperature (10 cm depth) was measured with a waterproof digital thermometer (Barnstead International, Dubuque IA, USA) at three locations each at the control and carcass site. Soil moisture (0 – 10 cm depth) was measured with time domain reflectometry (Field-Scout TDR-100; Spectrum Technologies, Plainfield IL, USA) at five randomly chosen points on control and carcass sites. We measured soil respiration at five randomly chosen points at both control and carcass sites with a PP-Systems SRC-1 soil respiration chamber (closed circuit) attached to a PP-Systems EGM-4 infrared gas analyzer (PP-Systems, Amesbury, MA, USA). For each measurement the soil chamber (15 cm high; 10 cm diameter) was tightly placed on the soil surface, after clipping plants to avoid measuring plant respiration or photosynthesis. Measurements were conducted over 120 s.

In addition, we assessed the decomposition rates of standardized OM using the cotton strip assay (Latter & Howson, 1977; Latter & Walton, 1988). Cotton cloth tensile strength loss (CTSL) is a measure of decomposition, and an index to express the combined effect of soil microclimatic, physical, chemical and biological properties on decomposition while

accounting for OM quality (Latter & Walton, 1988; Risch, Jurgensen, & Frank, 2007; Withington & Sanford Jr., 2007). We placed five 20 cm wide x 13 cm long sheets of 100% unbleached cotton cloth (American Type SM 1/18'', Warp: 34/1, Weft: 20/1, Weave plain, 29.5 picks/cm warp, 22 picks/cm weft, 237 g/m^2; Daniel Jenny & Co., Switzerland;) at each carcass and control site vertically into the soil by making slits with a flat spade to a depth of 12 cm. We inserted each cloth with the spade, and then pushed the slit closed to assure tight contact with the soil. The cloths were retrieved after 18 to 27 days. After retrieval, the cloths were air-dried, remaining soil gently removed by hand, and 1.5 cm wide strips were cut at the 3.5-5.0 cm (top) and the 9-10.5 cm (bottom) soil depth. The strips were equilibrated at 50 % relative humidity and 20°C for 48 hours (climate chamber) prior to strength testing (Scanpro Awetron TH-1 tensile strength tester; AB Lorentzen and Wettre, Kista, Sweden). Cotton rotting rate (CRR) = $([CTS_{control} - CTS_{final}]/CTS_{final})^{1/3} * (365/t)$, where $CTS_{control}$ is the cotton tensile strength of a control cloth and CTS_{final} the cotton tensile strength of the incubated sample, t is the incubation period in days. Control cloths were inserted into the ground and immediately retrieved to account for tensile strength loss associated with cloth insertion. We averaged the CRR of top and bottom strips for further analyses as no difference was found between the two. All sampling and cloth insertion took place between June 20 and July 1, 2017, cloths were retrieved between July 17 and 20, 2017. Soil respiration, average CRR, vegetation N concentration and vegetation C:N ratio are defined as ecosystem functions, soil C and N concentration, soil temperature and moisture as soil abiotic properties, and bacterial and fungal richness (number of taxa), diversity (Shannon) and abundance as soil biotic properties.

Statistical analyses

Univariate analyses for ecosystem functions, soil biotic and abiotic properties

We tested whether individual ecosystem functions, soil biotic and abiotic properties differed between carcass and control ("Location"), bison and elk ("Species") and days since kill ("DSK"). For this purpose, we used linear mixed effect models (LMM, "*nlme*" package v 3.1 – 131.1 in R v 3.4.4; Pinheiro, Bates, DebRoy, & Sarkar, 2018; R Core Team, 2019) with Location, Species, Location x Species and DSK as fixed effects. Site was included as random effect to account for the paired design. We developed a separate model for all dependent variables. All but bacterial richness, fungal richness, fungal diversity and vegetation N concentration were natural-log transformed to meet model assumptions. For each LMM, we

calculated contrasts to assess the specific comparisons we were interested in with the "*lsmeans*" package v 2.27-62 (Lenth & Love, 2018): 1) carcass vs control, 2) carcass bison vs control bison, and 3) carcass elk vs control elk. We also tested whether we had differences between bison and elk carcasses or the sites where bison and elk were killed and included contrasts 4) carcass bison vs carcass elk and 5) control bison vs control elk.

We calculated the log response ratio (LRR = ln[carcass/control]) to obtain carcass effects for all variables for both species separately. LRR < 0 indicates higher value at control compared to carcass, LRR > 0 indicates higher values at carcass compared control. We used LRRs for visualization and to assess spatial patterns in carcass effects across YNP. For this purpose we calculated the Moran's I statistic for each ecosystem function, soil biotic and abiotic property based on a latitude-longitude matrix with the "*moran.test*" function in the "*spdep*" package version 1.1-3 (Bivand et al., 2019).

Multivariate analyses

Rare OTUs, defined as OTUs with a low abundance of reads, were retained in multivariate methods because they only marginally influence these analyses (Gobet, Quince, & Ramette, 2010). Bray–Curtis dissimilarity matrices were generated based on square-root-transformed matrices. We used Principal Coordinate Analyses (PCoA) to assess how soil bacterial and fungal communities differed between control and carcass of bison and elk ("*vegan*" package v 2.5-4, Oksanen et al., 2019). We then extracted PCoA axes scores 1 and 2 and used LMM ("*nlme*" package) with Location, Species, Location x Species and DSK as fixed effects. Site was, again, included as random effect. We again calculated the contrasts as described above using the "*lsmeans*" package. We also assessed how ecosystem functions, and soil abiotic and biotic properties were related to the soil bacteria and fungi community structure associated with bison and elk control and carcasses using the "*envfit*" function in the "*vegan*" package (Oksanen et al., 2019).

Indicator species analyses were performed using the *multipatt* function implemented in the "*indicspecies*" package version 1.7.6 with 100000 permutations (De Caceres & Jansen, 2016). This step allowed to identify OTUs that led to changes in multivariate patterns between control and carcass of both bison and elk separately (De Cáceres, Legendre, & Moretti, 2010). The *multipatt* function uses a point biserial correlation coefficient statistical test. Indicator OTUs were defined as bacterial and fungal OTUs with more than 50 sequences, i.e., removing rare taxa and taxa with low abundances containing little indicator information (Rime et al., 2015) and that were significantly correlated with Location ($p < 0.05$, correlation coefficient > 0.3). A heatmap of these OTUs were generated with the *vegan*

and *ggplot2* packages. The indicator analyses were performed in R version 3.3.3 (R Core Team, 2017).

Results

Our 19 sites differed considerably in soil abiotic properties (soil N concentration: 0.24 to 1.91%; soil C concentration: 2.85 to 29.31%; soil temperature: 8.7 to 20.6°C; soil moisture: 3.7 to 74.7% volumetric moisture content; Supplementary Table 1, Supplementary Fig 2&3). But there was no difference in ecosystem functions, and soil biotic and abiotic properties between the sites where elk and bison were found (Supplementary Table 3, contrasts for bison-elk, control).

Higher soil respiration and vegetation N concentration and lower vegetation C:N ratio characterized both bison and elk carcasses compared to the controls (Fig 1, Supplementary Table 3). In addition, bacterial diversity and richness and fungal richness were lower under elk carcasses compared to controls (Fig 1, Supplementary Table 3) and elk carcasses had a weak positive impact on soil moisture (Fig 1, Supplementary Table 3). The days since kill (DSK) did not affect our measures (Supplementary Table 4). Although all sites were relatively young (i.e., < 6 months since kill; Supplementary Table 1), only bones, gut contents, hair, and teeth remained.

We detected significant spatial gradients in the effects of bison carcasses on bacterial richness and elk carcasses on fungal diversity, fungal abundance richness and soil respiration across the Yellowstone landscape (Table 1). In addition, we found weak spatial patterns in the carcass effects for fungal richness, soil C and N concentrations under elk carcasses (Table 1).

A total of 1,019,422 bacterial and 1,402,378 fungal sequences were retrieved. After quality filtering we were able to distinguish 9048 bacterial (7431 bison, 7758 elk) and 5951 fungal OTUs (4126 bison, 4301 elk). The first two axes of our PCoAs explained 32.1% (bacteria) and 22.1% (fungi) of the total variability in soil community structure found on carcass and control sites associated with both bison and elk (Fig 2A, B). Bacterial and fungal communities were different between control and carcass soils for both bison and elk (Fig 2A, B, Supplementary Table 5), but did not differ between the control plots. Differences were not related to DSK (Supplementary Table 6). Additionally, soil bacterial and fungal communities differed between bison and elk carcasses (Fig 2 A, B, Supplementary Table 5).

Differences in bacterial communities detected along PCoA axis 1 were mainly related to differences in soil C and N concentration as well as soil moisture (Fig 2A, Table 2,

Supplementary Table 5) and seemed to be related to the differences in community composition found under bison compared to elk carcasses (blue versus orange arrows). In contrast, differences in soil biotic properties correlated strongly with bacterial community composition along PCoA axis 2: fungal abundances related positively with bacterial communities found under carcasses (pointing towards the arrow tips), while bacteria and fungi richness and diversity related positively with the bacterial communities of the control plots (pointing towards the arrow tails; Figure 2A, Table 2). Hence soil biotic properties seem to be more important for explaining differences between bacterial communities found under carcass and control, while soil biotic properties were more important in explaining differences in soil bacterial communities between bison and elk (Fig 2A, Supplementary Table 5). As observed for bacterial communities, soil biotic properties were more important for explaining the differences in fungal communities found between carcass and control soils (correlations with PCoA axis 1; Fig 2B, Table 2, Supplementary Table 5), while soil C and N concentration as well as soil moisture were related to differences in bison and elk (correlations with PCoA axis 2, Fig 2B, Table 2, Supplementary Table 5).

Indicator species analyses

Indicator OTUs that were associated with carcasses represented only a minority of the total bacterial or fungal OTUs. 40 bacterial indicator OTUs (0.54% of total bacterial OTUs; 2.47% of total bacterial sequences) were found at bison locations, whereas 74 (0.95% of total OTUs; 3.96% of total sequences) were found for elk (Fig 3). At both bison and elk carcass locations, indicator OTUs were mainly positively correlated with carcasses and were broadly distributed across taxonomic groups (Fig 3, Supplementary Table 7). Most indicator OTUs positively associated with bison or elk carcasses were found within the Proteobacteria, namely the orders Rhizobiales, Rhodospirillales, Burkholderiales and Caulobacterrales (Fig 3).

Large differences in the number of fungal indicator OTUs were observed between bison and elk carcass (Fig 4). Over 10 times more indicator OTUs were found at elk locations, reaching 53 indicator OTUs (1.23% of total fungal OTUs, 3.69% of total fungal sequences), whereas only 3 (0.07% of total OTUs, 0.24% of total sequences) were found at bison locations. Only one indicator OTU (OTU #162, Romboutsia sp., Firmicutes, Supp. Table 7) overlapped between elk and bison samples. In contrast to the bacterial indicator OTUs, the fungal indicators were mostly negatively correlated to carcasses for both bison and elk. At elk locations, fungal indicator OTUs were mainly scattered within the phyla

Ascomycota, with a majority of taxa from the Dothideomycetes, Leotiomycetes, Sordariomycetes and Eurotiomyceetes (Fig 4, Supplementary Table 8).

Discussion

Carcass effects on ecosystem functions, soil biotic and abiotic properties

Bison and elk carcasses increased soil respiration, vegetation nutrient concentrations, and altered soil microbial communities in YNP, USA. While other studies showed that carcasses are "hotspots" for specific plant and soil properties (Bump, Webster, et al., 2009; Bump, Peterson, et al., 2009), this study is, to our awareness, the first to extensively report how mammalian carcasses affect soil microbial communities in natural systems. We showed that elk, but not bison carcasses, negatively affected soil bacterial richness and diversity as well as fungal richness in YNP, which is partially in accordance to our hypothesis. This indicates, that elk carcasses may have added specific resources to the soil that favored some competitive bacteria at cost of a diverse community. Hence, carcasses inputs likely differs from sources, i.e., plant litter, dung, or urine, which are less discrete, but potentially lower in concentration.

Decreases in soil bacterial richness and diversity were also reported from soils sampled under intact human cadavers placed on top of the soil surface (Finley et al., 2016). Interestingly, buried human cadavers increased soil bacterial richness and diversity (Finley et al., 2016), which indicates that the soil microbial community response may depend on the degree of burial. If true, the behavioral ecology of consumption by top predators may influence soil microbial communities. For example, cougars (*Puma concolor*) and bears (*Ursus* sp.) bury or partially bury (i.e., short-term caching) their prey items after an initial feeding bout (Balme, Miller, Pitman, & Hunter, 2017). One explanation for this behavior is the *food-perishability hypothesis*, which postulates that short-term cachers store food to deter or delay food spoilage, i.e., to manipulate microbial growth (Bischoff-Mattson & Mattson, 2009). Given our results, a comparison of soil microbe response to cached versus un-cached carcasses is warranted.

As expected, our carcasses significantly affected ecosystem functions locally, but we also found a weak trend that elk carcasses affected soil C and N concentrations as well as soil respiration across YNP's NR. In addition, we found differences in our carcass effects across the landscape for bacterial richness (bison), fungal richness, diversity and abundance (elk). This is interesting because carrion decomposer communities among desert, subalpine forest and shortgrass grassland soils did not differ (Metcalf et al., 2016). This result, however, was

from boxed mouse carcasses placed on the soil surface using laboratory-controlled temperature and moisture conditions, and excluding all insects and other carrion consumers. Given these contrasting findings to our study, it may be premature to conclude or generalize that soil type does not affect soil microbial community and ecosystem response to carrion, especially given that so few soil types have been examined. Additional *in situ* research is needed to determine how microsite dynamics and soil properties shape the soil microbial decomposer response.

Differences in microbial communities depend on carcass identity

Carcasses from both bison and elk considerably affected the structure of both soil bacterial and fungal communities when compared to the control sites, and we also found different bacterial and fungal taxa to be associated with bison compared to elk carcasses. The differences in soil microbial communities between the carcasses of the two ungulate species could, as hypothesized, be related to differences in carcass body mass (Elbroch et al., 2017; Parmenter & MacMahon, 2009; Turner et al., 2017), or tissue content, for example, the amounts of polyunsaturated fatty acids (Meyer, Rowell, Streich, Stoffel, & Hofmann, 1998). Similarly, although both ungulate species are true ruminants, they have different dietary preferences; elk are typically classified as browsers and bison as grazer. As a consequence, the forage of elk likely contains more tannins compared to bison, thus, elk might harbor different microbiomes that affect soil responses differently than bison. We are not aware of any research that addressed the difference in soil microbial communities associated with different mammal carcasses. However, if mammals considerably differ in their microbial "fingerprint" during their decay, this might be of interest for forensic science.

Indicator species

The differences of bacterial and fungal communities found between carcasses and control soils could potentially be explained by the copiotroph–oligotroph trade-off, also known as r- and K-selection theory (Fierer, Bradford, & Jackson, 2007). This theory predicts that copiotrophic organisms thrive in soils with higher C mineralization rates, whereas oligotrophic groups dominate in soils of low C availability (Fierer et al., 2012, 2007; Fontaine, Mariotti, & Abbadie, 2003). In our study, OTUs of Proteobacteria for elk and Actinobacteria for bison tended to be more ubiquitous in the nutrient-rich soils under carcasses (positively correlated with carcasses). Members of both phyla are known to have copiotrophic lifestyles (Fierer et al., 2007). Also, almost all OTUs from Rhizobiales and Rhodospirillales were positively associated with carcass soils. These copiotrophic orders are able to fix N and therefore are fast growers and very strong competitors. However, also

several indicator OTUs belonging to Acidobacteria were positively associated with bison and elk carcasses, contrasting this theory. Generally, Acidobacteria are known to be ubiquitous, diverse, desiccation tolerant, and largely oligotrophic bacteria, abundant under low resource availability (Fierer et al., 2007; Kielak, Barreto, Kowalchuk, van Veen, & Kuramae, 2016). Yet, it is known that subgroups within a phylum show different responses than the one observed at the phylum level, potentially leading to misinterpretations. Indicator taxa in Proteobacteria for bison and Actinobacteria for elk did, indeed, showed a mixed response, suggesting that different subgroups might occupy different ecological niches (Hartmann et al., 2017; Navarrete et al., 2015).

Most of the fungal indicators showed a much weaker association to carcass than bacteria. Soil fungi have pivotal ecological roles as decomposers, pathogens, and mycorrhizal symbionts. Most of the fungal indicators that we identified (e.g., Dothideomycetes; Leotiomycetes) have a saprotrophic lifestyle, decomposing dead organic material (Goodwin, 2014; Zhang & Wang, 2015). However, the responses of these indicator taxa were mixed. Within one group some taxa were found to be associated with control soils and others with carcass soils suggesting a more ubiquitous distribution. Yet, it should also be noted that fungal indicators may lag in response to carrion introduction or decomposition (Bump, Peterson, et al., 2009).

Several of our indicator OTUs from Proteobacteria, including Phenylobacterium (Caulobacteraceae), Pesudolabrys and Variibacter (Xanthobacteraceae), were also found on human cadavers and are proposed as indicators in forensic science (Cobaugh, Schaeffer, & DeBruyn, 2015; Procopio et al., 2019). Similar, Firmicutes, indicators for both bison and elk carcasses, include many gut microbiome taxa found from human cadavers (Cobaugh et al., 2015; Finley et al., 2016; Metcalf et al., 2016). In contrast to bacteria, much less information is available concerning the association of fungal taxa with mammalian decomposition. Eurotiales (Ascomycota) fungi were identified as a strong driver of the microbial community associated with the decomposition of humans (Metcalf et al., 2016), however, in our study, they were mostly negatively associated with elk carcasses, contrasting previous findings.

Conclusions

Soil microbial community changes were ungulate specific and varied across the YNP landscape and hence, may not be as generalizable as previously thought. Our findings should improve our understanding of carrion ecology (Barton et al., 2019) and its mechanistic impacts on soil microbial diversity, biogeochemical heterogeneity, conservation biology and potentially even forensic science. Although plant resource inputs (e.g., litter mass) outweigh

carrion inputs in most terrestrial systems by far, the life-limiting resources (e.g., N and micro-nutrients) from carrion are orders of magnitude higher (Barton et al., 2019; Carter et al., 2007). Hence, carrion has disproportional impacts relative to its input mass and drives soil microbial biodiversity and ecosystem functions.

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Figure captions

Fig 1. Differences in ecosystem functions, soil biotic and abiotic properties between control and carcass for bison and elk. ** $p < 0.01$, * $p < 0.05$. + $0.05 < p < 0.1$. Days since kill (DSK) had no effect on the difference between control and carcass. Error bars represent standard errors. Red = ecosystem functions, green = soil abiotic properties, blue = soil biotic properties. Values shown are log response ratios (LRR). For statistics see Supplementary Table 3 & 4.

Fig 2: Carcass-derived shifts in soil microbial communities and associated environmental properties. PCoA results for A) bacterial and B) fungal communities from control and carcass soils. The start of the colored arrows indicate the community composition in the control soils, the end of the arrows indicate the community composition at the carcass soils at each site. Orange indicates bison, blue elk. Days since kill (DSK) did not affect microbial community composition. Significantly correlating environmental drivers that explain differences in between community composition are displayed in the background. Length of grey lines indicates strength of correlations. Only environmental properties significantly correlated with community composition are shown. For statistics see Table 2, Supplement Table 5 & 6.

Fig 3: Bacterial indicator OTUs significantly correlated with carcass soils for both bison and elk. Heatmaps represent correlation coefficients to carcasses and OTU abundances (number of reads). External columns represent the phylum of the different taxa. p_ = phylum, c_ = class, o_ = order, g_ = genus. Number in [] represent OTU reference numbers as listed in Supplementary Table 7.

Fig 4: Fungal indicator OTUs significantly correlated with carcasses for both bison and elk. Heatmaps represent correlation coefficient to carcasses and OTU abundances (number of reads). External columns represent the phylum of the different taxa. p_ = phylum, c_ = class, o_ = order, g_ = genus. Number in [] represent OTU reference numbers as listed in Supplementary Table 8.

Figures

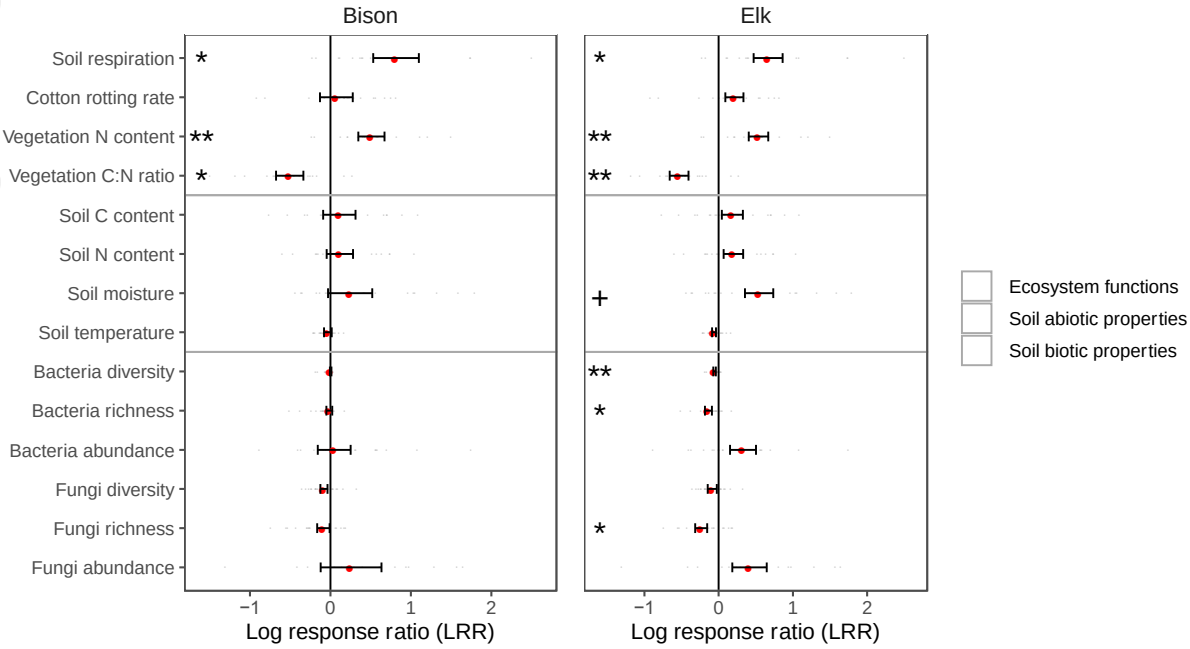

Figure 1

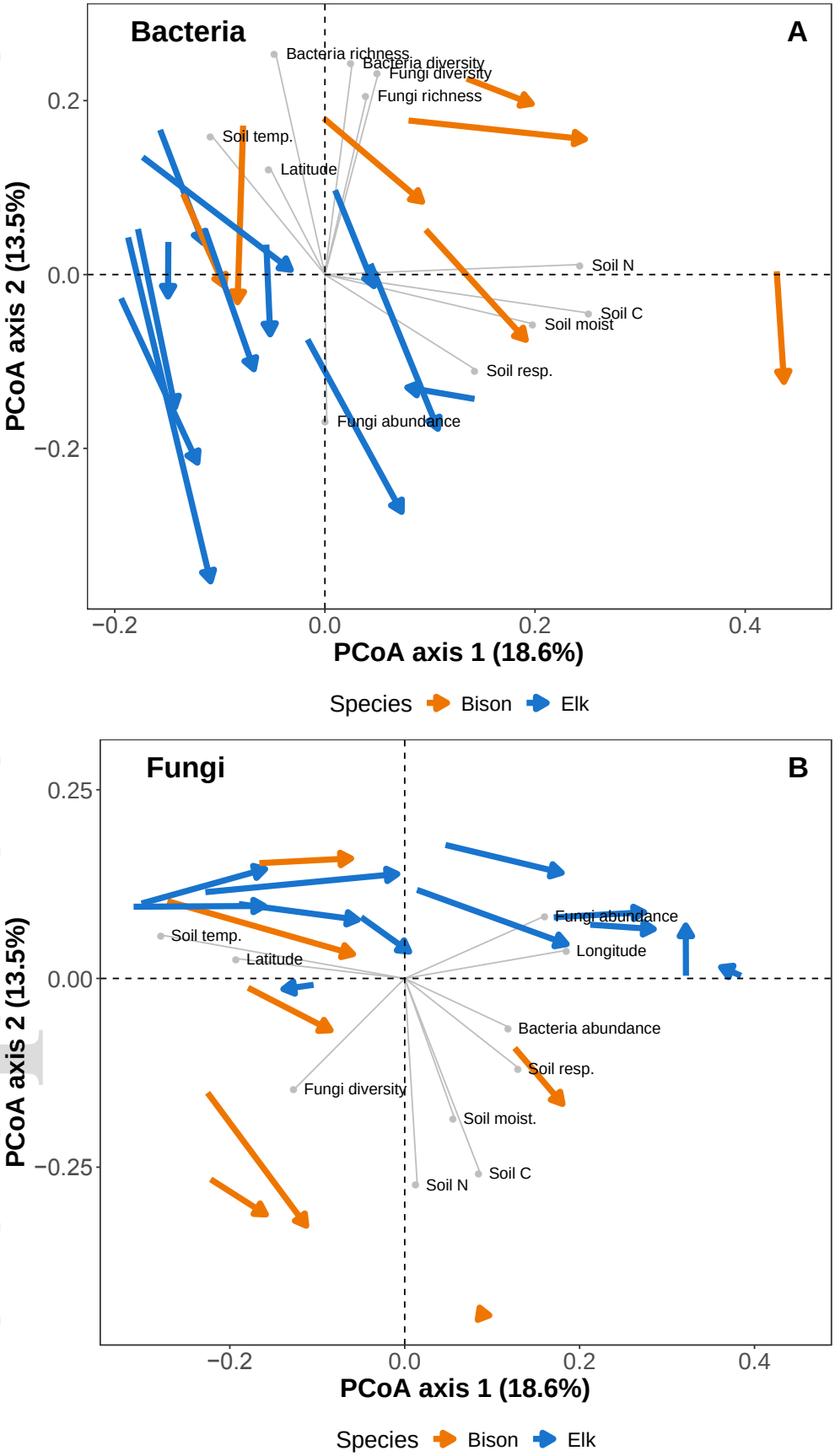

Figure 2

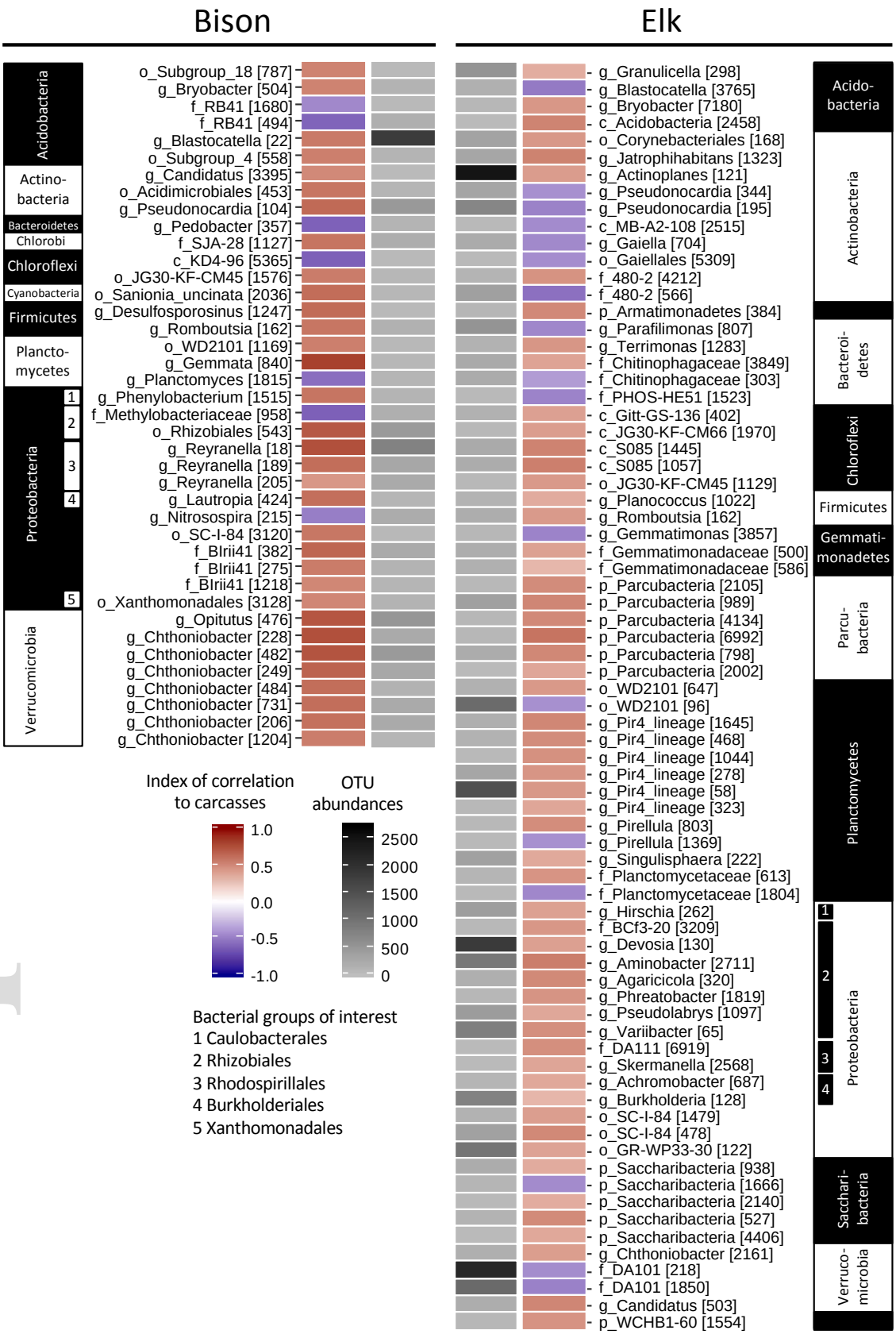

Figure 3

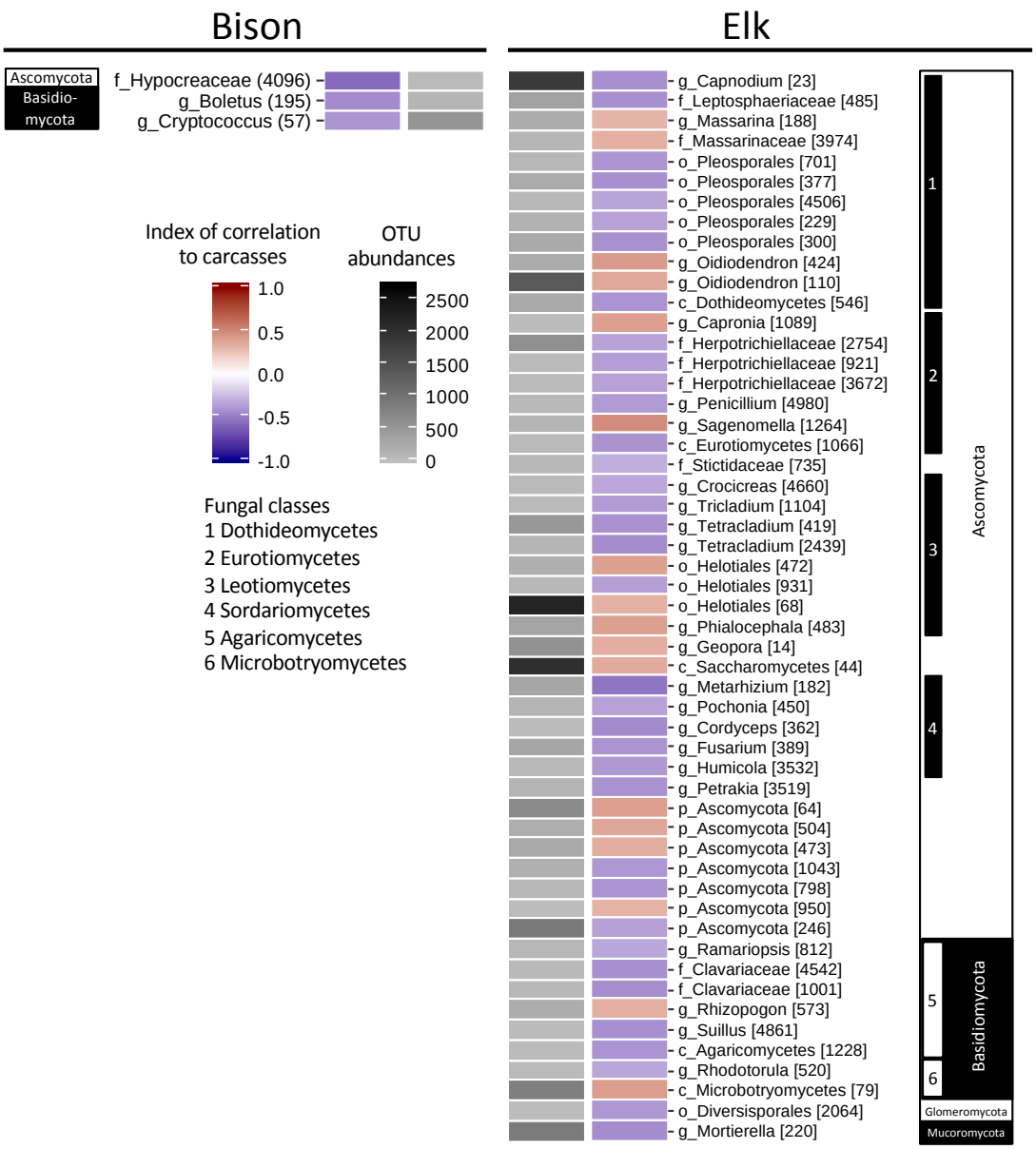

Figure 4

Tables

Table 1: Spatial autocorrelation (Moran's I) of carcass effects (log response ratios, LRR) across the YNP landscape for each ecosystem function, soil abiotic and biotic properties for bison and elk separately (z-scores, p-values). Red = ecosystem functions, green = soil abiotic properties, blue = soil biotic properties. richness = number of OTUs found, diversity = Shannon diversity index. Bold = significant landscape effects $p < 0.05$, italic = significant landscape effects $0.1 > p > 0.05$. CRR = cotton rotting rate

	Bison		**Elk**	
	z-score	**p-value**	**z-score**	**p-value**
Soil respiration	-0.012	0.51	**1.607**	**0.05**
CRR	0.243	0.40	0.086	0.47
Veg N	-0.346	0.64	-1.306	0.90
Veg C:N	-0.405	0.66	-1.230	0.89
Soil C	0.057	0.487	*1.311*	*0.10*
Soil N	0.160	0.41	*1.301*	*0.10*
Soil moisture	-1.036	0.85	-0.155	0.56
Soil temperature	-0.294	0.62	1.061	0.14
Bacteria richness	1.874	**0.03**	-0.220	0.59
Bacteria diversity	-0.075	0.53	0.066	0.47
Bacteria abundance	-0.073	0.53	-0.010	0.50
Fungi richness	-0.799	0.79	*1.260*	*0.10*
Fungi diversity	-0.441	0.67	**2.543**	**0.01**
Fungi abundance	0.701	0.24	**2.397**	**0.01**

Table 2: Relationship (correlations) between environmental variables and soil bacterial and fungal community composition based on the "envfit" function in the "vegan" package. Bold indicates significant correlations ($p < 0.05$), italic values indicate variables tending to be correlated (p-value between 0.05 and 0.1). Significant correlations are shown as light grey lines in Fig 2. Red = ecosystem functions, green = soil abiotic properties, blue = soil biotic properties, white = landscape gradients and days since kill (DSK). richness = number of OTUs, diversity = Shannon diversity index, CRR = cotton rotting rate, DSK = days since kill

	Bacteria		**Fungi**	
	r2	**p**	**r2**	**p**
Soil respiration	**0.295**	**0.003**	**0.283**	**0.003**
CRR	0.114	0.13	0.014	0.77
Veg N	*0.139*	*0.09*	0.075	0.27
Veg C:N	0.074	0.27	0.060	0.34
Soil C	**0.591**	**0.001**	**0.664**	**0.001**
Soil N	**0.538**	**0.001**	**0.670**	**0.001**
Soil moisture	**0.386**	**0.002**	**0.337**	**0.002**
Soil temperature	**0.332**	**0.001**	**0.720**	**0.001**
Bacteria richness	**0.601**	**0.001**	0.098	0.16
Bacteria diversity	**0.543**	**0.001**	0.117	0.10
Bacteria abundance	***0.141***	***0.09***	**0.167**	**0.039**
Fungi richness	**0.397**	**0.001**	***0.148***	***0.06***
Fungi diversity	**0.520**	**0.001**	**0.334**	**0.001**
Fungi abundance	**0.254**	**0.013**	**0.298**	**0.003**
Longitude	*0.151*	*0.06*	**0.325**	**0.002**
Latitude	**0.159**	**0.05**	**0.335**	**0.002**
DSK	0.089	0.20	0.098	0.162