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Climate structures bison dietary quality and composition at the continental scale

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Abstract

Background: Nutrition is a key determinant of North American plains bison (*Bison bison*) weight gain and reproduction, yet little is known about what bison eat and the pattern of nutritional stress across their distribution.

Aims: In order to better understand patterns of bison nutrition across broad climatic gradients, bison fecal material was sampled from 50 sites across the United States in June and September of 2018.

Materials and Methods: For each sample, dietary composition and microbial composition were assessed with DNA metabarcoding, along with dietary quality via near-infrared spectroscopy and elemental composition.

Results: Herds in cool, wet sites typically had diets with high crude protein but not necessarily higher or lower digestible organic matter than in hot, dry sites. Independent of climate and season, on average, 57% and 48% of protein intake was derived from non-graminoids in June and September, respectively. Cool-season grass abundance in the diet was greater in cooler climates in June and September, while N_2-fixing plant abundance in the diet was greater in warmer climates in June. Among multivariate patterns, bison eating a high-quality diet had fecal material with higher concentrations of P, Mg, Mn, and Ca, and a higher relative abundances of cellulose-digesting *Ruminococcus*.

Discussion: By sampling across broad geographic gradients and across seasons, the drivers of different components of bison diet are becoming clear. Our results show that climate is integral to structuring bison diet quality, diet composition, elemental intake, and their gut microbiomes.

Conclusions: Although future work remains to better understand seasonal patterns and inter-annual variation in diet, North American plains bison should no longer be considered strict grazers. Also, given current geographic dietary patterns, bison in a warmer climate are likely to suffer increased nutritional stress unless actions are taken to increase protein availability.

KEYWORDS
bison, diet, fecal, herbivory, metabarcoding, near-infrared spectroscopy

1 | INTRODUCTION

The American bison (*Bison bison*) formerly roamed large expanses of the continental United States (US) with peak estimated numbers ranging from 30 to 60 million (Gates, Freese, Gogan, and Kotzman, 2010; Shaw, 1995). European westward expansion, drought conditions, hunting, and introduction of new diseases reduced bison numbers to less than 1,000 individuals by the late 1800s (Hill, 2014; Truett, 2003). The extinction of the American bison was prevented by early conservation efforts and private ownership of bison herds (Gates et al., 2010; Hornaday, 1889; Zontek, 2007). However, repercussions from the decimation of bison reverberated across the Great Plains. As a keystone species (Knapp et al., 1999), bison provided a food source for predators (Truett, Phillips, Kunkel, & Miller, 2001), maintained wallows that created ephemeral water pools necessary for reproduction of anurans (Gerlanc & Kaufman, 2003), and promoted prairie dogs and other wildlife to thrive (Fahnestock & Detling, 2002; Gersani & Sachs, 1992).

Reintroduction of bison on their native range has gradually occurred under private, public, and tribal hands. Over 350,000 individuals currently reside across North America (Aune, Jørgensen, & Gates, 2017). In order for bison to thrive, bison husbandry must be economically productive and sustainable. There are many factors that can influence productivity: genetics (Derr et al., 2012), disease (Gogan, Russell, Olexa, & Podruzny, 2013), parasite loads (Avramenko et al., 2018; Eljaki, Al Kappany, Grosz, Smart, & Hildreth, 2016), and nutrition (Craine, 2013). Of these, nutrition is one of the more important factors when assessing financial viability in livestock production (Hess et al., 2005). Forages with low crude protein (CP) or digestible organic matter (DOM) often have a slower passage rate that can lead to decreased intake which lowers weight gain and reproduction (Van Soest, 1994). The ability to extract energy from these low-quality forages has been linked to the rumen microbiome (Shabat et al., 2016). Specific microbial species thrive in the digestive tract and are influenced by diet and rumen digesta turnover time (Van Soest, 1994). Mineral nutrition is also important for animal performance (Van Soest, 1994). For example, deficiencies in selenium can cause white muscle disease, whereas toxicity can result in weakened hooves, hair, and acute myocardial necrosis (Davis et al., 2012; Hall & Kahn, 2010; Van Soest, 1994).

Although nutrition is known to be an important factor for bison performance, there is little understanding of how bison dietary quality and composition vary across broad geographic scales. Bison gain weight at a faster rate in cooler, wetter climates in the United States compared with warmer, drier climates (Craine, 2013), but there have been no comprehensive surveys of bison dietary composition, nutritional quality, or elemental intake that might underpin these patterns. In order to quantify bison diet across the United States, we organized synchronized collections of bison fecal material across the United States in June and September of 2018. Bison fecal material was analyzed for dietary composition, dietary quality, microbial assemblage composition, and elemental composition. We hypothesize that, similar to cattle (Craine, Elmore, Olson, & Tolleson, 2010), bison located in colder, wetter climates will have higher diet quality than their counterparts in drier, warmer climates.

2 | METHODS

2.1 | Sample collection

Known bison operations were contacted, and emails were sent to National Bison Association members to solicit participation. We created a website (www.whatbisoneat.com) that included a sign-up portal for participants to include metadata to ensure critical information was provided for each location. Each registered operation was sent a fecal collection kit, which included a sampling cup, instructions on sampling, a cooler, gloves, and an icepack.

Fresh bison fecal samples were taken from operations across the United States in June (June 18–22) and September (September 23–29) of 2018, with the majority of samples coming from the Great Plains (Figure 1). A total of 49 samples were collected from 43 different operations during the summer sampling, and 61 samples were collected from 47 individual operations during the fall sampling. A total of 40 operations participated in both sampling periods. Each sample consisted of ten fresh, individual fecal pats to avoid individual animal bias and represent variability within each herd.

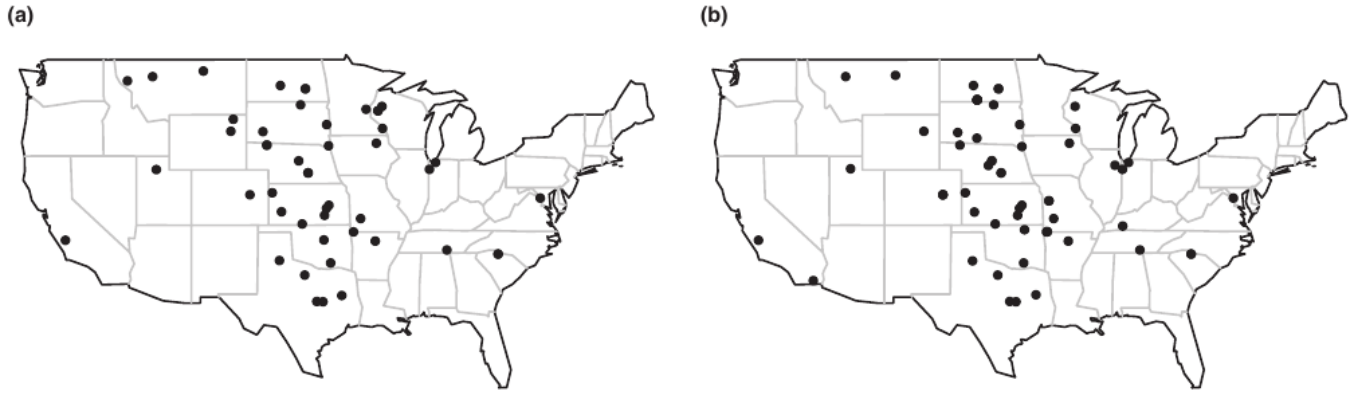

FIGURE 1 Location of operations participating in the Great American Bison Diet Survey during the summer sampling period in June (a), and the fall sampling period in September (b) for 2018

2.2 | Dietary quality

After collection, samples were frozen and sent to the Texas A&M Grazing Animal Nutrition Lab. The samples were subsequently dried at 60°C, ground in a Udy mill to pass a 1-mm screen, and analyzed using near-infrared reflectance spectroscopy (NIRS) to assess dietary quality parameters (crude protein [CP] and digestible organic matter [DOM]). NIRS is an accepted methodology used to obtain diet quality from free-ranging livestock and is a viable alternative to wet chemistry (Lyons & Stuth, 1992). Spectra (400–2,500 nm) are collected on a Foss® NIRS 6500 scanning monochromator with spinning cup attachment. Calibration curves were derived from NIRS spectra of cattle fecal material and directly measured forage quality (Showers, Tolleson, Stuth, Kroll, & Koerth, 2006) and applied to bison here as previously (Craine, Angerer, Elmore, & Fierer, 2016; Craine, Towne, Miller, & Fierer, 2015).

2.3 | Climate data

Site location was used to obtain 30-year climate normal (1981–2010) from the Oregon State University PRISM Climate Group data explorer database (PRISM Climate Group). For sites measured in the summer, mean annual precipitation (MAP) averaged 752 mm and ranged from 311 to 1,566 mm. Mean annual temperature (MAT) averaged 10.9°C and ranged from 5.1°C to 19.3°C. For sites measured in the fall, MAP averaged 741 mm and ranged from 68 to 1,566 mm, while MAT averaged 11.2°C and ranged from 5.1°C to 22.9°C.

2.4 | Dietary composition

After dietary quality was assessed, dried samples were sent to the Jonah Ventures laboratory in Boulder, Colorado for DNA extraction. Diet composition was evaluated via DNA metabarcoding using the c-h primers of the trnL intron in plant chloroplast (Craine et al., 2015; Taberlet et al., 2007). Genomic DNA from samples was extracted using the MoBio PowerSoil-htp 96 well Isolation Kit (Cat#12955-4) according to the manufacturer's protocol. Genomic DNA was eluted into 100 µl and frozen at −20°C. Each 25 µl PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133) which included 0.4 µM of each primer and 1 µl of gDNA. Both forward and reverse primers also contained a 5′ adaptor sequence to allow for subsequent indexing and Illumina sequencing. DNA was PCR amplified using the following conditions: initial denaturation at 94°C for 3 min, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, and a final elongation at 72°C for 10 min. Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 min at 37°C following by inactivation at 95°C for 5 min and stored at −20°C. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction), and consisted of an initial denaturation of 95°C for 3 min followed by eight cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Five microlitre of indexing PCR product of each sample was visualized on a 2% agarose gel to ensure the success of the barcoding PCR. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies). Twenty-five microlitre of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5 µl of each normalized sample to the pool. Sequencing occurred on an Illumina MiSeq running the 2 × 150 bp chemistry with a v2 300-cycle kit.

Sequences were demultiplexed, and paired-end reads were merged, trimmed followed by a quality control step. Sequences were quality trimmed to have a maximum expected number of errors per read of less than 0.1, and only sequences with more than three identical replicates were included in downstream analyses. BLASTN 2.2.30+ was run locally, with a representative sequence for each OTU as the query and the current NCBI nt nucleotide and taxonomy database as the reference. Sequences were clustered into OTUs at the ≥97% sequence similarity level, and sequence abundance counts for each OTU were determined using the usearch7 approach (Edgar, 2013). The National Center for Biotechnology Information (NCBI) species names associated with each hit were used to populate the OTU taxonomy assignment lists.

Because of the strong relationship between chloroplast density and protein in leaves, results from this are considered to quantify the relative intake of protein from different species (Craine et al., 2015). DNA metabarcoding has been used on many different species to determine diet composition including large African herbivores (Kartzinel et al., 2015), horses (King & Schoenecker, 2019), and gray-sided voles (*Microtus oeconomus*) (Soininen et al., 2013).

The top 10 OTUs (operational taxonomic unit) for each sample were identified at the genus level using a taxonomic reference derived from publicly available sequences (Genbank, https://www.ncbi.nlm.nih.gov/genbank/), and plant tissue from voucher specimens sequenced by Jonah Ventures. Each OTU represents an individual plant species or group of plant species that have a minimum 97% base-pair matching to an individual gene sequence. Occasionally, more than one species or genus was matched to a specific OTU number. When this occurred, the USDA Plants Database (plants.usda.gov) was used to identify the genus of a representative species found in the study area. The top 100 OTUs across all sites were used for analyses. Each identified genus was also assigned to a functional group category: cool-season grass, warm-season grass, forbs, N_2-fixing plants, or woody species.

2.5 | Bacterial composition

In addition to sequencing with trnL primers, the 16S rRNA gene region was amplified with primers 515F–806R to target the V4 region of the 16S SSU rRNA and then sequenced to identify microbiome composition across participating herds (Bergmann, Craine,

Robeson, & Fierer, 2015; Liu, Lozupone, Hamady, Bushman, & Knight, 2007). Analyses were conducted at the Jonah Ventures laboratory, and the same procedures were used for 16S rRNA as trnL except with different primers. The top 50 OTUs overall were used for analyses. For each OTU, multiple species or genus with a 97% base-pair matching are listed as potential matches for the OTU of interest. Each gene sequence was input into the National Center for Biotechnology Information (NCBI) Nucleotide Blast sequencer using the 16S ribosomal RNA sequence database. The gene sequence was then assigned to a family or genus where possible.

2.6 | Elemental analysis

After DNA metabarcoding analysis, dried fecal samples were sent to Colorado State University's Soil, Water, and Plant Testing Lab for inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on a Perkin-Elmer Optima 7300 DV ICP after digestion with nitric acid. ICP-AES has proven to be a rapid and accurate method to assess elements from various substrates (Ioannidou, Zachariadis, Anthemidis, & Stratis, 2005; Nixon et al., 1986). Elements tested include the following: calcium, magnesium, sodium, potassium, zinc, iron, copper, manganese, and phosphorus. Selenium was also quantified using ICP—hydride generation (Layton-Matthews, Leybourne, Peter, & Scott, 2006).

2.7 | Statistical analyses

All statistical analyses and figures were conducted/produced in R Studio v. 3.5.2 (R Foundation for Statistical Computing, 2018). Mean values for temperature and precipitation were calculated for sites across both sampling periods and separately for the summer and fall sampling periods. Minimum and maximum CP, DOM, and DOM:CP were calculated across all sites, the summer sampling period, and the fall sampling period. For operations that participated in both the summer and fall sampling periods, a paired *t* test on dietary quality variables was conducted. The relationships between CP and DOM were assessed using a Type II regression model. A standard least squares model with effect leverage emphasis was used to assess CP and DOM with fixed effects of MAT and log-transformed MAP to understand the relationship between diet quality and climate.

Diet composition data were averaged across all herds to obtain the percentage of each functional group, with the exclusion of herds located in Alaska and California (n = 2 in summer sampling, n = 3 in fall sampling), which fell outside of the continental climate envelope we were primarily investigating. The relative abundance of different functional groups in the diet for summer and fall was calculated for the top 100 OTUs. A paired *t* test was conducted to assess differences in functional group consumption across herds participating in both the summer and fall sampling (n = 40).

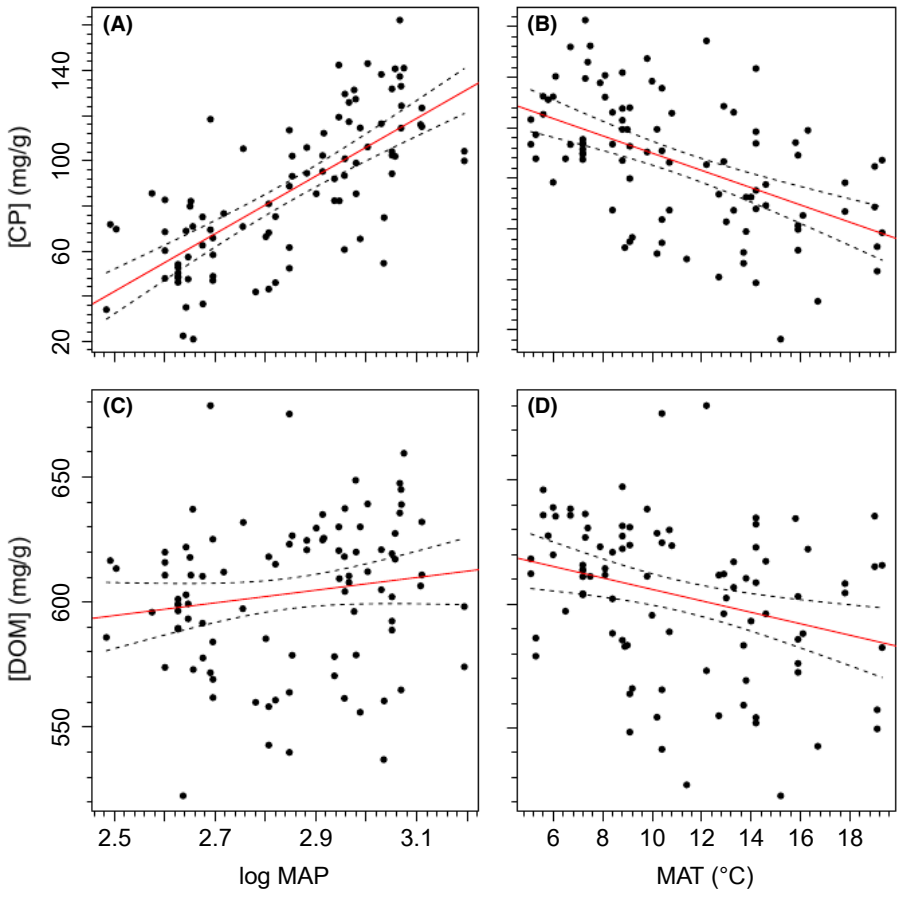

FIGURE 2 Leverage plot of dietary crude protein (mg/g) and DOM for bison sampled across different herds (n = 93 samples) in June and September of 2018 with log (MAP) and mean annual temperature (MAT, °C) as fixed effects

Elemental concentrations (Ca, Mg, Na, K, P, Fe, Mn, Cu, Zn, and Se) were calculated for sites across both sampling periods and separately for the summer and fall samplings. A paired *t* test of elemental concentrations was conducted for sites that participated in both sampling periods ($n = 40$). To assess relationships between elements, diet quality, and diet composition, a Type II regression model was created for each elemental variable between diet quality variables (CP and DOM) as well as between functional groups.

In order to understand the multivariate relationships among all the variables, a principal components analysis was conducted using MAP, MAT, CP, DOM, the top 50 OTUs from the 16S analysis of the microbiome grouped at the family or genus level, diet composition grouped at the functional group level, season (coded binary with summer = 0, fall = 1), and elemental data (Ca, Mg, Na, K, P, Fe, Mn, Cu, Zn, and Se). The first four PCA axes were rotated with varimax rotation.

For visualization purposes, we generated maps of modeled dietary quality based on relationships between forage quality (CP and DOM) and climate (MAP, MAT, and the interaction between the two) using coefficients derived from regression models. Maps were generated using R 3.5.2 and utilized the *raster* package for reclassifying and masking pixels. Forested areas were masked from the map based on data from the Commission for Environmental Cooperation, http://www.cec.org.

3 | RESULTS

3.1 | Diet quality and diet composition

Among the summer samples, dietary [CP] averaged 91.4 mg/g and ranged from 54.8 to 136.0 mg/g. Among fall samples, [CP] averaged 81.3 mg/g and ranged from 21.2 to 155.3 mg/g. Overall, bison consuming higher [CP] also had higher [DOM] levels ($r = .68$, $p < .001$). Overall, [CP] was highest in cool, wet climates (Figures 2 and 3; Tables 1 and 2). Based on the coefficients of the MAP and MAT × MAP interaction terms, the influence of MAP declined with increasing temperature until MAT of 19.7° (June) and 20.6° (September), beyond which increasing MAP decreased [CP] (Table 1). For both June and September, there was no significant influence of climate on [DOM] (Figures 2 and 3; Tables 1 and 2). Comparing dietary quality for the 40 sites that had been sampled in both periods, both CP (paired *t* test: 90 vs. 79 mg/g, $p = .02$) and DOM (614 vs. 587 mg/g, $p < .001$) were higher in summer than fall. The mean (±standard error) ratio of DOM:CP was 6.9 ± 0.22 during the summer and 8.8 ± 0.55 in the

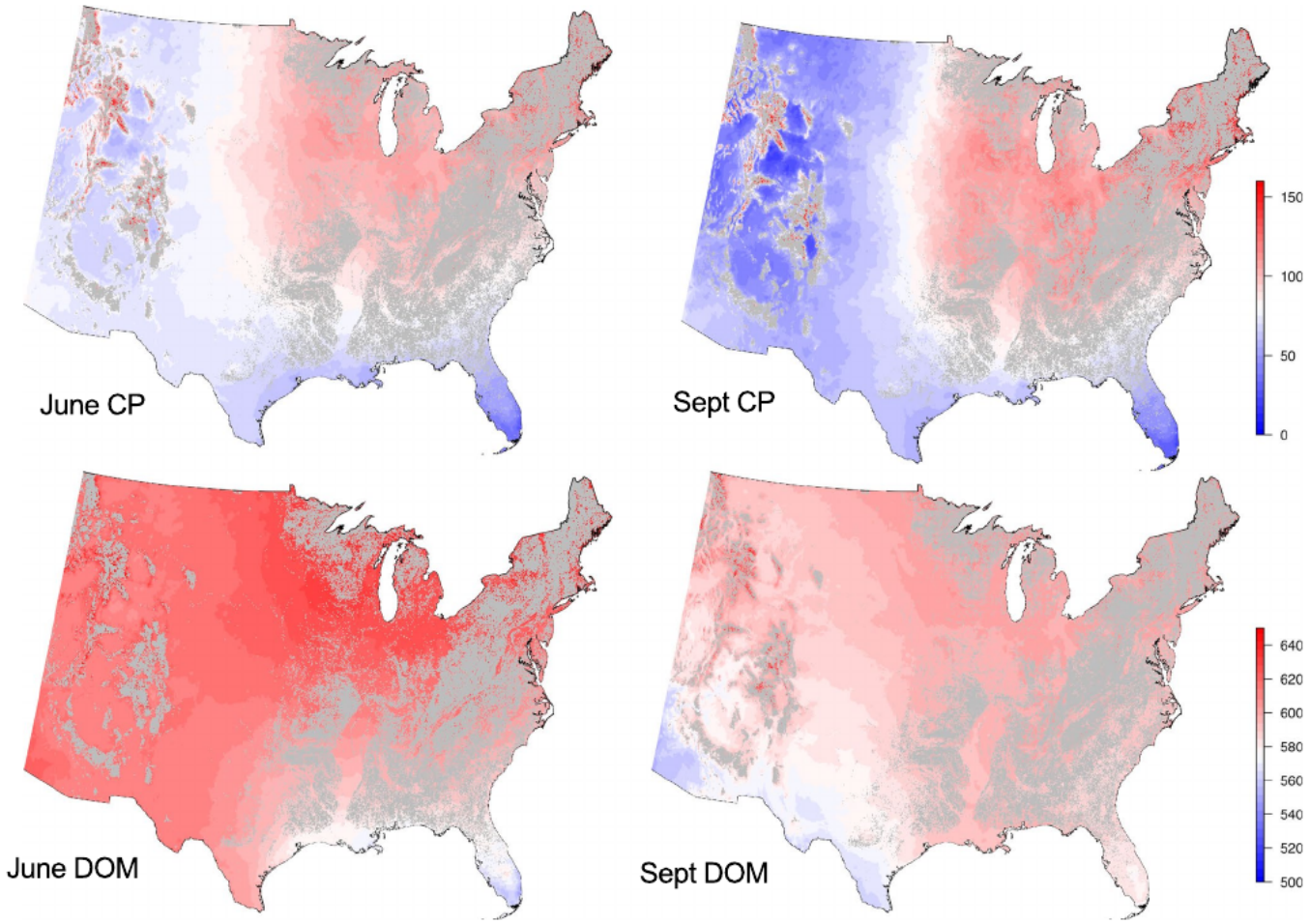

FIGURE 3 Map of modeled dietary quality in nonforested areas using regression relationships with climate (Tables 1 and 2). The projection of dietary quality was generated by using free software R (R Foundation for Statistical Computing, 2018)

TABLE 1 Linear regression coefficients ± the standard error for response variables (diet quality, diet composition functional group, and microbiome) using climate variables (mean annual precipitation [MAP], mean annual temperature [MAT], and the interaction between MAP and MAT) as predictors for the June 2018 bison sampling period

	r^2	Intercept	MAP (100 mm^{-1})	MAT ($°C^{-1}$)	MAP × MAT (100 mm^{-1} $°C^{-1}$)
[CP] (mg/g)	.44***	30.01 ± 22.45	12.39 ± 3.16***	2.25 ± 2.07	−0.63 ± 0.26*
[DOM] (mg/g)	.13	592.65 ± 33.06***	6.57 ± 4.64	1.40 ± 3.05	−0.47 ± 0.38
Cool-season grass (%)	.31***	106.51 ± 30.37**	−5.20 ± 4.27	−7.82 ± 2.81**	0.55 ± 0.35
N_2 fixing (%)	.25**	−55.91 ± 36.99	8.53 ± 5.20	7.15 ± 3.42*	−0.60 ± 0.42
Warm-season grass (%)	.14	23.31 ± 17.55	−3.21 ± 2.47	−1.16 ± 1.62	0.28 ± 0.20
Forb (%)	.16*	27.52 ± 30.26	−1.79 ± 4.25	1.81 ± 2.80	−0.15 ± 0.35
Woody (%)	.06	−1.424 ± 13.806	1.667 ± 1.941	0.024 ± 1.275	−0.084 ± 0.158
[P] ppm	.23**	98 ± 1575	527 ± 221*	136 ± 146	−26 ± 18
[K] ppm	.18*	4,908 ± 2,280*	348 ± 321	−98 ± 211	−14 ± 26
[Mg] ppm	.20*	−223 ± 1,880	698 ± 264*	237 ± 174	−41 ± 22
[Mn] ppm	.26**	−60.4 ± 137.4	44.1 ± 19.3*	8.3 ± 12.7	−1.9 ± 1.6
[Z] ppm	.14	−48.75 ± 62.89	17.36 ± 8.84	5.00 ± 5.81	−0.96 ± 0.72
Acetitomaculum (%)	.04	0.40 ± 0.19*	−0.032 ± 0.027	−0.024 ± 0.018	0.0030 ± 0.0022
Olsenella (%)	.04	0.83 ± 0.36*	−0.058 ± 0.050	−0.044 ± 0.033	0.0050 ± 0.0041
Mogibacterium (%)	.08	0.99 ± 0.43*	−0.016 ± 0.061	−0.038 ± 0.040	0.0020 ± 0.0050
Christensenellaceae (%)	.03	0.32 ± 0.39	0.016 ± 0.055	0.0072 ± 0.036	−0.00037 ± 0.00449
Enterococcus (%)	.07	−0.15 ± 1.31	0.23 ± 0.18	0.096 ± 0.12	−0.019 ± 0.015
Methanobrevibacter (%)	.04	3.30 ± 3.12	0.19 ± 0.44	−0.064 ± 0.29	−0.0066 ± 0.0357
Ruminococcus (%)	.05	0.08 ± 0.24	0.03 ± 0.03	0.0065 ± 0.0218	−0.0020 ± 0.0027
Peptoclostridium (%)	.31***	−1.41 ± 0.45**	0.27 ± 0.06***	0.17 ± 0.04***	−0.022 ± 0.0051***
Paenibacillus (%)	.12	3.36 ± 1.57*	−0.45 ± 0.22*	−0.15 ± 0.15	0.029 ± 0.018
Thermobacillus (%)	.14	4.33 ± 2.47	−0.23 ± 0.35	−0.20 ± 0.23	0.012 ± 0.028
Ureibacillus (%)	.10	24.63 ± 15.61	0.11 ± 2.19	−0.83 ± 1.44	−0.0050 ± 0.1788
Herbinix (%)	.02	0.59 ± 1.28	0.035 ± 0.180	−0.015 ± 0.119	−0.0026 ± 0.0147
Lysinibacillus (%)	.25**	32.07 ± 20.13	−4.27 ± 2.83	−0.23 ± 1.86	0.30 ± 0.23
Solibacillus (%)	.05	−2.74 ± 4.71	0.74 ± 0.66	0.58 ± 0.44	−0.062 ± 0.054
Ruminococcaceae (%)	.07	2.90 ± 2.57	0.18 ± 0.36	−0.044 ± 0.245	−0.011 ± 0.029
Prevotellaceae (%)	.07	0.20 ± 0.42	0.053 ± 0.058	−0.0011 ± 0.0384	−0.0027 ± 0.0048
Rikenellaceae (%)	.09	2.49 ± 1.30	−0.095 ± 0.183	−0.11 ± 0.120	0.0067 ± 0.0149
Firmicutes (%)	.14	73.04 ± 13.80***	−0.024 ± 0.0194	0.076 ± 1.274	0.0013 ± 0.0016
Bacteroidetes (%)	.07	4.20 ± 2.49	−0.00081 ± 0.00351	−0.20 ± 0.23	0.00010 ± 0.00029
Euryarchaeota (%)	.04	3.31 ± 3.12	0.0020 ± 0.0044	−0.064 ± 0.288	−0.000066 ± 0.000357
Proteobacteria (%)	.08	−3.95 ± 5.22	0.010 ± 0.007	0.78 ± 0.48	−0.00102 ± 0.00060
Actinobacteria (%)	.04	0.84 ± 0.36*	−0.00058 ± 0.00050	−0.045 ± 0.033	0.000050 ± 0.000041

*$p < .05$.
**$p < .01$.
***$p < .001$.

fall across all bison herds sampled. During both seasons, 59 herds had a ratio above 7 which is considered the threshold for predominant protein limitation. None of the bison herds had a DOM:CP <4, which is considered the threshold for predominant energy limitation (Figure 4).

For dietary composition in summer, the most abundant species were included in OTUs that were represented by *Agrostis* (6.7%), *Medicago* (5.7%), *Aegilops* (4.5%), *Poa* (4.3%), and *Trifolium* (4.2%). On average, 32.7% of the summer protein intake was derived from N_2-fixing plants, 31.7% cool-season grasses, 19.9% forbs, 11.7% warm-season grasses, and 4% woody species. Among fall samples, the most abundant species were included in OTUs that had representative genera of *Agrostis* (6.3%), *Poa* (6.3%), *Medicago* (5.9%), *Ischaemum* (5.5%), and *Lotus* (3.5%). On average, 26.7% of the fall

TABLE 2 Linear regression coefficients ± the standard error for response variables (diet quality, diet composition functional group, and microbiome) using climate variables (mean annual precipitation [MAP], mean annual temperature [MAT], and the interaction between MAP and MAT) as predictors for the September 2018 bison sampling period

	r^2	Intercept	MAP (100 mm^{-1})	MAT ($°C^{-1}$)	MAP × MAT (100 mm^{-1} $°C^{-1}$)
[CP] (mg/g)	.46***	−24.62 ± 23.39	18.95 ± 3.92 ***	4.23 ± 1.80*	−0.92 ± 0.27**
[DOM] (mg/g)	.07	594.93 ± 28.48***	2.60 ± 4.77	−1.82 ± 2.19	−0.021 ± 0.333
Cool-season grass (%)	.29***	57.61 ± 17.23**	−0.89 ± 2.86	−3.37 ± 1.32*	0.10 ± 0.20
N_2 fixing (%)	.05	21.94 ± 24.28	2.81 ± 4.03	−0.75 ± 1.86	−0.11 ± 0.28
Warm-season grass (%)	.19**	15.98 ± 22.47	−3.86 ± 3.73	1.21 ± 1.72	0.26 ± 0.26
Forb (%)	.08	−1.96 ± 15.72	1.96 ± 2.61	2.02 ± 1.21	−0.23 ± 0.18
Woody (%)	.04	−4.93 ± 8.42	0.67 ± 1.40	0.84 ± 0.646	−0.07 ± 0.10
[P] ppm	.38***	−1888 ± 1,109	788 ± 184***	233 ± 85**	−41 ± 13**
[K] ppm	.21**	−1967 ± 1,302	722 ± 216**	240 ± 100*	−43 ± 15**
[Mg] ppm	.41***	−523 ± 1,051	680 ± 175***	194 ± 81*	−32 ± 12*
[Mn] ppm	.38***	29.67 ± 67.35	19.81 ± 11.19	1.61 ± 5.16	−0.26 ± 0.78
[Z] ppm	.23**	−12.12 ± 21.35	7.85 ± 3.55*	1.43 ± 1.64	−0.31 ± 0.25
Acetitomaculum (%)	.28***	0.80 ± 0.18***	−0.068 ± 0.030*	−0.030 ± 0.014*	0.0032 ± 0.0021
Olsenella (%)	.14*	1.65 ± 0.50**	−0.11 ± 0.084	−0.081 ± 0.039*	0.0071 ± 0.0059
Mogibacterium (%)	.10	1.04 ± 0.55	0.037 ± 0.092	−0.052 ± 0.042	0.0011 ± 0.0064
Christensenellaceae (%)	.04	0.40 ± 0.70	0.11 ± 0.12	0.0070 ± 0.0540	−0.0046 ± 0.0082
Enterococcus (%)	.17	5.60 ± 1.51***	−0.48 ± 0.25	−0.28 ± 0.12*	0.029 ± 0.018
Methanobrevibacter (%)	.03	6.10 ± 2.55*	−0.24 ± 0.43	−0.24 ± 0.20	0.026 ± 0.030
Ruminococcus (%)	.07	−0.047 ± 0.180	0.056 ± 0.030	0.018 ± 0.014	−0.0039 ± 0.0021
Peptoclostridium (%)	.03	−0.20 ± 0.78	0.067 ± 0.130	0.067 ± 0.060	−0.0056 ± 0.0091
Paenibacillus (%)	.06	3.11 ± 2.60	−0.30 ± 0.44	0.0060 ± 0.1997	0.0055 ± 0.0305
Thermobacillus (%)	.02	−0.063 ± 1.033	0.0060 ± 0.1732	0.053 ± 0.079	−0.0027 ± 0.0121
Ureibacillus (%)	.08	20.69 ± 9.48*	−0.73 ± 1.59	−1.01 ± 0.73	0.055 ± 0.111
Herbinix (%)	.02	−0.041 ± 0.26	0.029 ± 0.044	0.0056 ± 0.0203	−0.0020 ± 0.0031
Lysinibacillus (%)	.02	22.86 ± 15.01	1.13 ± 2.51	0.22 ± 1.15	−0.033 ± 0.178
Solibacillus (%)	.10*	4.35 ± 3.33	−0.44 ± 0.56	0.088 ± 0.256	0.031 ± 0.039
Ruminococcaceae (%)	.11	−0.78 ± 1.45	0.38 ± 0.24	0.28 ± 0.11*	−0.032 ± 0.017
Prevotellaceae (%)	.14	0.0091 ± 0.1915	0.022 ± 0.032	0.035 ± 0.015*	−0.0028 ± 0.0022
Rikenellaceae (%)	.20	1.16 ± 0.52*	−0.076 ± 0.087	0.037 ± 0.040	−0.0011 ± 0.0061
Firmicutes (%)	.05	76.66 ± 9.51***	−0.0064 ± 0.0159	−0.88 ± 0.73	0.00061 ± 0.00111
Bacteroidetes (%)	.23**	0.70 ± 1.08425	0.00056 ± 0.0018	0.22 ± 0.08*	−0.00017 ± 0.00013
Euryarchaeota (%)	.04	6.10 ± 2.54710*	−0.0024 ± 0.00427	−0.24 ± 0.20	0.00026 ± 0.00030
Proteobacteria (%)	.13	−6.67 ± 3.95394	0.0140 ± 0.00662	0.72 ± 0.30	−0.00090 ± 0.00046
Actinobacteria (%)	.14*	1.65 ± 0.501800**	−0.0011 ± 0.000841	−0.08 ± 0.04*	0.000071 ± 0.000059

*$p < .05$.
**$p < .01$.
***$p < .001$.

bison diet was comprised of N_2-fixing plants, 24.6% cool-season grasses, 17.3% forbs, 27.6% warm-season grasses, and 3.6% woody species. Compared with the summer, a greater proportion of bison diet in the fall was from warm-season grasses, and less was from cool-season grasses and N_2-fixing plants. When comparing the 40 sites where diet was sampled in both periods, N_2-fixing plant abundance in the diet was higher in summer (33.8% vs. 18.7%, $p < .003$), while warm-season grass was lower (10.4% vs. 26.5%, $p < .001$; Table 3). There was no significant difference in the abundance of other functional groups between the two sampling periods ($p > .1$ for all other comparisons). In both June and September, cool-season grass abundance in the diet was greater in cooler climates, while N_2-fixing plant abundance was greater in warmer climates in June (Tables 1 and 2, Figure 5).

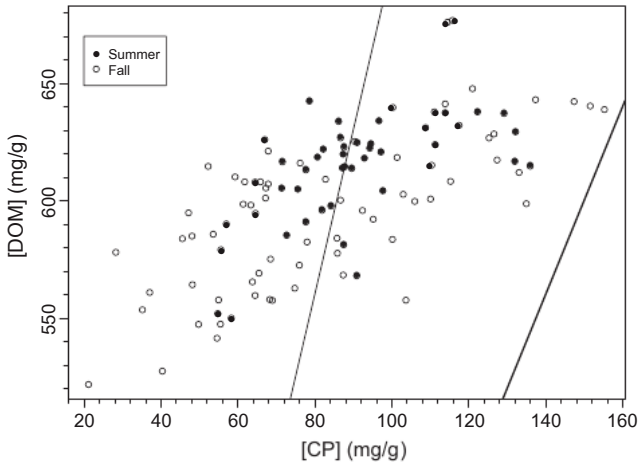

FIGURE 4 The relationship between digestible organic matter ([DOM]) and crude protein ([CP]) during the summer (closed symbols) and fall (open symbols) sampling periods for *Bison bison* in 2018. Thin line represents a ratio of DOM:CP of 7 and thick line a DOM:CP of 4. Samples to the left of the thin line are considered to indicate protein limitation, and samples to the right of the thick line would indicate energy limitation. Samples in between the two lines are considered to indicate diets that are generally balanced in protein and energy

3.2 | Microbial assemblage

Among the top 50 OTUs, Firmicutes comprised 87.3% of the microbial assemblage, Euryarchaeota 5.0%, Proteobacteria 4.4%, Bacteroidetes 2.7%, and Actinobacteria 0.6%. During the summer sampling period, Firmicutes comprised 87.9% of the microbial assemblage, Euryarchaeota 4.6%, Proteobacteria 4.0%, Bacteroidetes 3.0%, and Actinobacteria 0.5%. Fall microbial assemblage composition was similar to that of the summer. During the fall sampling period, Firmicutes comprised 86.8% of the microbial assemblage, Euryarchaeota 5.1%, Proteobacteria 4.8%, Bacteroidetes 2.6%, and Actinobacteria 0.7%. No significant differences in microbial components were found between seasons using a paired *t* test for sites participating in both sampling periods ($n = 40$; $p > .05$). Across all sites, the relative abundance of Firmicutes, Bacteroidetes, Euryarchaeota, and Proteobacteria was not associated with climate variables or season ($p > .05$ for MAP, MAT, and season). However, Actinobacteria was correlated with cooler sites and was more likely to be found in fall samples ($p < .05$ for MAT and season, $r^2 = .12$). However, when looking only at the fall samples across all sites, Bacteroidetes was positively correlated with MAT ($p < .05$, $r^2 = .23$), but was not correlated with other climate variables ($p > .05$ for MAP and MAP × MAT).

3.3 | Elemental concentrations

The most abundant element in bison fecal material was Ca followed by Mg, Na, K, and P. Fecal [Ca] in the summer averaged 10,496 ppm (i.e., 1.05%) and ranged from 2,835 to 28,737 ppm. During the fall, [Ca] averaged 9,470 ppm and ranged from 4,112 to 22,637 ppm. Bison with higher [CP] also experienced higher fecal [Ca] ($r = .25$, $p < .05$). Higher [Ca] was associated with higher forb percentage ($r = .22$, $p < .05$) and higher woody percentage ($r = .25$, $p < .05$) in the diet, but was not associated with any other functional group or climate ($p > .05$). [Ca] did not differ across seasons ($p = .24$, paired *t* test). Magnesium concentrations were also associated with higher [CP] ($r = .68$, $p < .001$) and higher [DOM] ($r = .34$, $p < .001$) and averaged 4,003 ppm during the summer and 3,859 ppm during the fall. [Mg] was not different between seasons ($p = .61$), but increased with increasing precipitation ($p < .001$) (Tables 1 and 2).

Sodium levels averaged 477 ppm and varied from 81 to 1,267 ppm across locations in the summer. During the fall, Na averaged 47 ppm and ranged from 0.01 to 768 ppm. [Na] was not associated with [CP], but increased with increasing [DOM] ($r = .39$, $p < .001$). Diets with more cool-season grasses also had higher [Na] ($r = .28$, $p < .05$). Seasonal differences in Na levels were significant ($p < .0001$), but climate did not influence [Na] ($p > .05$ for MAP and MAT) (Tables 1 and 2).

Potassium levels averaged 5,184 ppm and varied from 1,473 to 10,143 ppm during the summer and averaged 2,319 during the fall with a range from 107 to 6,460 ppm. Significant differences occurred between seasons ($p < .0001$), and increased [K] was associated with increased [CP] ($r = .40$, $p < .0001$) and [DOM] ($r = .44$, $p < .0001$). Colder, wetter sites were correlated with higher [K] ($p < .05$ for both MAP and MAT) (Tables 1 and 2).

Phosphorus fecal concentrations varied from 676 to 6,469 ppm during the summer (averaging 3,250 ppm) and ranged from 1,345 to 7,677 ppm across sites in the fall (averaging 2,965 ppm), but were not different across seasons ($p = .61$). Increased fecal [P] was associated with both increased [CP] ($r = .75$, $p < .001$) and [DOM] ($r = .40$, $p < .001$). Increased MAP ($p < .001$) was associated with higher [P], but not MAT ($p = .33$).

Among the minor elements, manganese varied from 35 to 641 ppm during the summer and averaged 192 ppm. During the fall,

TABLE 3 Bison diet composition across the United States summarized by functional group (%) during the fall (September) and summer (June) sampling periods in 2018 for operations that participated in both sampling periods ($n = 40$)

Functional group	Summer (average %)	Fall (average %)	*p*-Value
Cool-season grasses	22.9	21.3	.56
N_2-fixing	33.8	18.7	<.003
Forbs	14.6	16.9	.57
Warm-season grasses	10.4	26.5	<.001
Woody	3.1	3.6	.75

FIGURE 5 Map of modeled dietary functional group composition in nonforested areas using regression relationships with climate (Tables 1 and 2). Included are cool-season grasses, warm-season grasses, forbs, and N_2-fixing plants. The projection of dietary quality was generated by using free software R (R Foundation for Statistical Computing, 2018)

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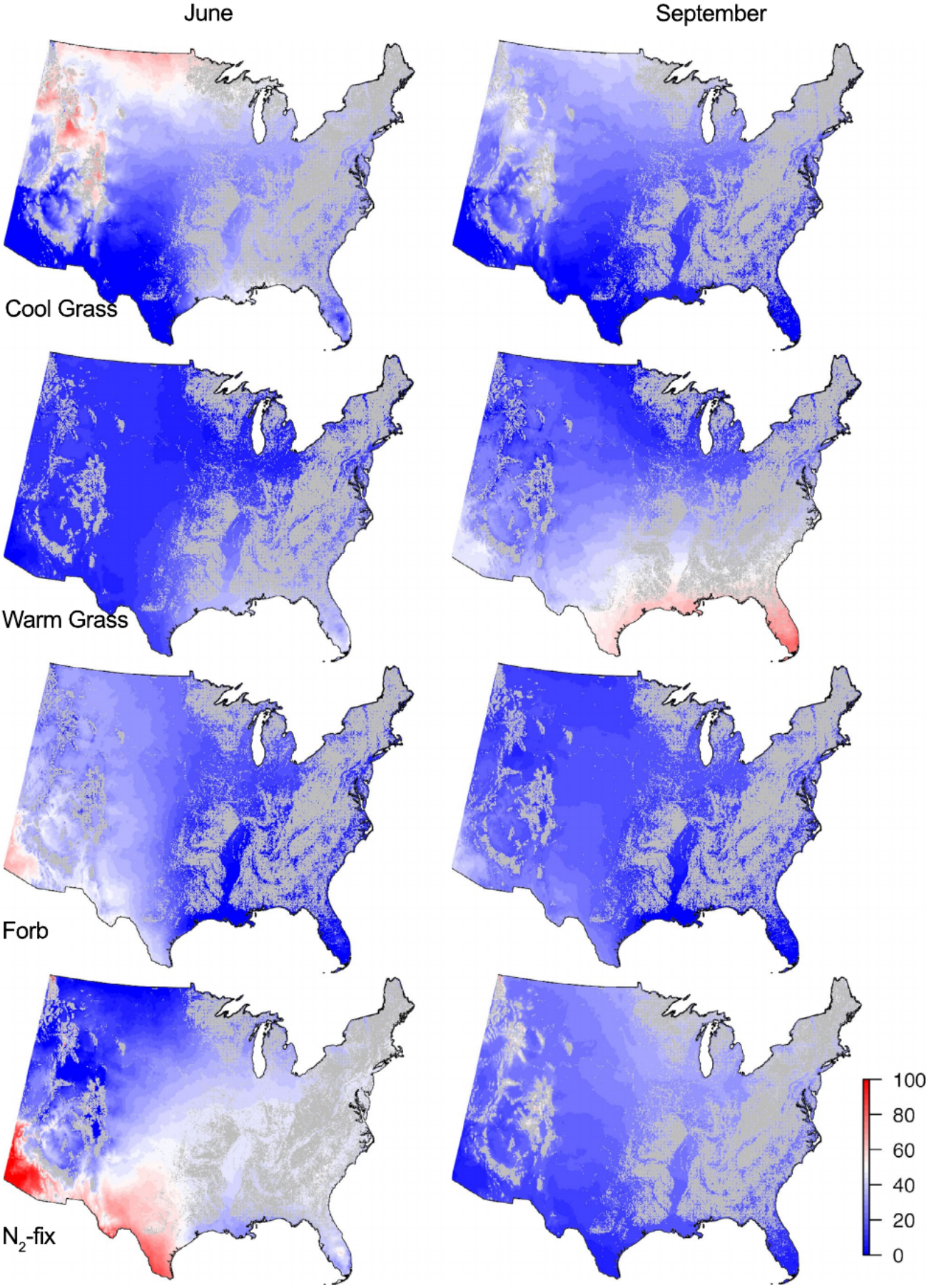

[Mn] averaged 173 ppm and ranged from 85 to 486 ppm. Sites with higher MAP ($p < .001$) and samples with higher [CP] had increased [Mn] ($r = .37$, $p < .001$).

Zinc averaged 52 ppm with a range from 9 to 341 ppm across sites during the summer sampling period and averaged 35 ppm with a range from 9 to 165 ppm in the fall with significant seasonal differences ($p < .01$). Increasing [Zn] was associated with increased [CP] ($r = .35$, $p < .001$), increased [DOM] ($r = .23$, $p < .05$), and higher MAP ($p < .001$).

Copper averaged 26 ppm and varied from 4 to 145 ppm across sites in June. In September, copper averaged 13 ppm and ranged from 3 to 69 ppm. Increasing [Cu] was associated with increasing [CP] ($r = .34$, $p < .001$) and [DOM] ($r = .23$, $p < .05$).

Selenium averaged 0.120 ppm and ranged from 0.001 to 1.469 ppm across all sites in June. In the fall, [Se] averaged 0.003 ppm and ranged from 0.001 to 0.010 ppm. [Se] increased with increasing [DOM] ($r = .24$, $p < .05$), but not [CP] ($p = .44$). [Se] and [Cu] were not associated with climate variables ($p > .05$). [Mg], [K], [P], [Mn], [Zn], [Cu], and [Se] were not associated with any specific functional groups in the diet ($p > .05$).

3.4 | Principal component analysis

In the principal component analysis that included climate, dietary functional group composition, sampling season, diet quality, elemental concentration, and microbial composition, the first 4 axes explained 43% of the variation in the included variables (Table 4). Axis 1 explained 12% of the variation in the dataset and was correlated with a high abundance of a variety of microbes with amylolytic, acetogenic, and methanogenic properties (*Olsenella*, *Mogibacterium*, *Acetitomaculum*, Christensenellaceae, *Enterococcus*, and *Methanobrevibacter*). Samples with the high *Olsenella*-suite of bacteria were more likely to collected in September and to have a higher proportion of cool-season grass species in the diet ($r^2 = .31$, $p < .001$).

Axis 2 also explained 12% of the variation in the dataset and separated samples with high dietary quality found in cooler, wetter locations from those lower dietary quality samples collected in warmer, drier locations. Samples associated with Axis 2 had higher [CP] and [DOM], as well as higher [P], [Mg], [Mn], [Zn], [Cu], and [Ca]. These high dietary quality samples were more likely to be found in cooler sites ($r^2 = .75$, $p < .001$). High dietary quality samples also had a greater proportion of *Ruminococcus* and *Peptoclostridium* bacteria and a lower proportion of *Paenibacillus* bacteria.

Axis 3 explained 10% of the variation in the dataset and separated samples based on a number of factors surrounding the proportion of cool-season grasses in bison diet. Samples that scored high on Axis 3 had a high proportion of cool-season grasses, were more likely to be collected during the June sampling period, and had higher [Na], [K], and higher [DOM]. Bison that ate more cool-season grasses also had higher abundances of *Thermobacillus*, *Ureibacillus*, and *Herbinix* coupled with lower abundances of *Lysinibacillus* and *Solibacillus*.

Axis 4 also explained 10% of the variation in the dataset and separated samples with a high abundance of cellulolytic bacteria (Ruminococcaceae, Prevotellaceae, Rikenellaceae, and *Bacteroides*) from those with low abundance of these taxa. Samples with high abundances of cellulolytic bacteria were more likely to have woody species in the diet ($r^2 = .25$, $p < .001$).

4 | DISCUSSION

By sampling across broad geographic gradients and across seasons, the drivers of different components of bison diet are becoming clear. For example, climate is integral to structuring bison diet quality, diet composition, elemental intake, and their gut microbiomes. In June, the diet of bison in a typical cool, wet site with MAT of 6°C and MAP of 800 mm would have a [CP] of 112 mg/g and a [DOM] of 631 mg/g. For these animals, 45% of the dietary composition would be C_3 grasses and 26% would consist of N_2 fixing species. Their fecals would average 4,829 ppm [Mg], 6,415 ppm [K], and 3,885 ppm [P]. In addition, 20.3% of the microbiome would consist of *Ureibacillus* and 11.0% *Lysinibacillus*. In contrast, bison in a typical hot, dry site (MAT = 19°C, MAP = 900 mm) in June would have [CP] that was 32% lower (76 mg/g) and [DOM] that was 5% lower (598 mg/g). Just 6% of the dietary protein would be derived from C_3 grasses, while 54% would be from N_2-fixing species. Their fecals would have 25% lower [Mg] (3,599 ppm), 42% lower [K] (3,718 ppm), and 23% lower [P] (2,999 ppm), and 9.0% of the microbiome would consist of *Ureibacillus* and 40.7% *Lysinibacillus*.

The climatic patterns of bison diet observed here reinforces similar findings in previous, more geographically restricted studies (Craine, Joern, Towne, & Hamilton, 2009; Craine et al., 2015; Craine, Towne, Tolleson, & Nippert, 2013). For example, comparing bison diet at two sites that differ in MAT by 6°C, bison in the cooler site also had higher dietary quality and depended more on graminoids and less on eudicots (Craine et al., 2015). As previously posited, the greater general reliance on N_2-fixing plant appears to compensate for generally lower dietary protein in other plants, but experimental manipulations would likely be necessary to support this assertion more definitively. These relationships between bison diet quality and climate appear to be general for ruminants in the Great Plains as since it also is seen in cattle across the United States (Craine et al., 2010). For example, for cattle, peak [CP] increased by 2.78 mg g^{-1} °C^{-1}, which was similar to 3.23 mg g^{-1} °C^{-1} for bison here. In addition, a different survey of cattle diet across the Great Plains also revealed greater reliance on grasses in high-latitude sites, with greater reliance on forbs in low-latitude sites (Craine et al., 2016). This study is the first to measure elemental concentrations in bison fecal material across climatic gradients, and future work will be necessary to further investigate and interpret elemental patterns. For example, bison in cool and wet sites had higher concentrations of Mg, K, and P in their fecal material than hot, dry sites, but no differences in the concentrations of other elements like Ca. Additional research is necessary to

TABLE 4 Principal components analysis with varimax rotation for four axes assessing differences in bison diet quality, composition (functional group level), fecal element concentrations, microbiome, and climate for June and September of 2018. The first four axes explained 43% of the cumulative variation in the dataset

	Axis 1	Axis 2	Axis 3	Axis 4
MAP	−0.17	0.62	−0.32	−0.13
MAT	−0.32	0.27	−0.53	−0.04
Season (binary)	0.34	−0.22	−0.69	−0.12
[CP]	0.15	0.83	0.15	−0.04
[DOM]	0.13	0.43	0.52	0.05
Cool-season grass	0.36	−0.06	0.59	0.00
Warm-season grass	−0.26	−0.03	−0.45	0.12
Forb	−0.01	−0.08	0.14	0.01
N_2-fixing	−0.21	0.07	−0.06	−0.29
Woody	0.12	0.20	−0.03	0.49
[Ca]	−0.10	0.49	0.02	0.06
[Mg]	0.15	0.81	0.04	−0.11
[Na]	−0.24	0.18	0.64	0.23
[K]	−0.31	0.40	0.57	0.23
[P]	−0.03	0.82	0.04	−0.04
[Mn]	−0.15	0.62	−0.09	−0.08
[Cu]	−0.19	0.51	0.28	−0.04
[Zn]	−0.23	0.61	0.12	0.03
Acetitomaculum	0.65	−0.22	0.01	−0.02
Acinetobacter	−0.01	0.21	−0.05	−0.23
Bacillus	−0.31	0.15	0.07	−0.32
Bacteroides	−0.14	−0.08	−0.11	0.83
Brevibacillus	−0.45	−0.18	0.00	−0.38
Christensenellaceae	0.62	0.15	−0.31	0.08
Clostridium	−0.33	0.14	−0.03	0.10
Comamonas	−0.16	0.05	−0.3	0.06
Enterococcus	0.61	−0.19	0.10	−0.07
Escherichia	−0.10	0.01	−0.10	−0.02
Herbinix	−0.15	0.02	0.58	−0.12
Kurthia	0.38	−0.26	0.13	−0.14
Lysinibacillus	−0.56	−0.22	−0.45	−0.42
Methanobrevibacter	0.61	0.17	0.00	0.18
Mogibacterium	0.76	0.26	0.02	−0.09
Olsenella	0.80	−0.07	−0.03	−0.01
Paenibacillus	−0.28	−0.43	−0.10	−0.23
Peptoclostridium	0.20	0.34	−0.15	0.11
Prevotellaceae	−0.22	−0.04	−0.08	0.89
Rikenellaceae	−0.18	−0.26	0.17	0.85
Romboutsia	0.20	0.27	−0.09	0.07
Ruminococcaceae	−0.03	0.08	0.04	0.91
Ruminococcus	0.50	0.42	0.12	−0.01
Solibacillus	−0.37	−0.14	−0.42	−0.10
Streptococcus	0.11	0.20	0.05	0.29
Thermobacillus	−0.15	−0.08	0.61	−0.07
Ureibacillus	0.51	0.19	0.59	0.13
Variance explained	5.477	5.40	4.34	4.30
Percent explained	12	12	10	10
Cumulative percent	12	24	34	43

understand whether the differences in elemental concentrations along climate gradients reflect differences in elemental availability in plants to bison or differences in elemental supplementation through provision of supplementary mineral salts.

Beyond climatic differences, seasonal differences in diet were also apparent in this study. In general, fall diets were lower in [CP] and [DOM] than in the summer and had greater abundance of warm-season grasses and lower abundance of cool-season grasses. In the fall, both [K] and [Na] were lower and bison gut microbiomes had higher abundances of *Lysinibacillus* and *Solibacillus*, and lower abundances of *Thermobacillus*, *Ureibacillus*, and *Herbinix*. Typically, both bison and cattle dietary quality decrease as the growing season progresses and plants begin to mature or senesce (Craine et al., 2016, 2015; Tieszen, Stretch, & Kooi, 1998). The mechanism responsible for seasonal differences in [K] and [Na] across seasons could be correlated with supplementation strategies or plant elemental concentrations, but more research is necessary to make definitive conclusions.

Independent of climates and season, sampling the diets of bison broadly rejects the generalization that bison are strict grazers (Coppedge & Shaw, 1998; Knapp et al., 1999; Peden, Van Dyne, Rice, & Hansen, 1974; Reynolds, Hansen, & Peden, 1978; Steuter & Hidinger, 1999; Van Vuren & Deitz, 1993). On average, about half of bison protein comes from grasses with herbaceous forbs, N_2-fixing species, and woody species comprising the majority of protein intake. At some sites, very little protein came from graminoids. For example, at one site in Indiana, over 60% of the protein intake came from woody species in the *Salix* genus alone, while at one site in Montana, 88% of the protein intake in June came from forbs. The greater reliance on nongrasses in this study compared with previous studies can be attributed in part to the DNA metabarcoding approach which sequences chloroplast DNA that represents protein intake as opposed to biomass intake. Yet, the technique is not biased by greater degradation of cell wall components of high-quality plants as with microhistology (Bartolome, Franch, Gutman, & Seligman, 1995; Craine et al., 2015; King & Schoenecker, 2019). Thus, previous estimates of bison dietary components with microhistological analysis underestimated amounts of forbs and woody species actually consumed. Because most ruminants are limited by protein rather than energy, specifically when consuming low-quality forages, assessing protein composition can be helpful in detecting plants that play an important role in overall dietary quality.

The main microbial populations found in the rumen of bison were in the phylum Firmicutes. This phylum comprised 87% of the sequences found on average across all the sites and seasons in our study after relativizing to the top 50 OTUs, which is higher than >53% of Firmicutes sequences previously found in bison gut microbiome (Bergmann et al., 2015). Actinobacteria were more likely to be found in the fall at cooler sites, and Bacteroidetes were more likely to be found in warmer sites, but this may be due to the additional sites sampled in the fall rather than an actual correlation to season since differences in Actinobacteria or Bacteroidetes across sites sampled during both the summer and fall sampling periods were not significant.

The abundance of a variety of microbes (e.g. amylolytic, acetogenic, and methanogenic) and the higher proportion of cool-season grasses in the fall diet indicate that cool-season grasses likely contain more nonstructural carbohydrates compared with other functional groups during this time period and could be linked to a secondary regrowth in cool-season grasses (Reuter, 2000; Zhao, MacKown, Starks, & Kindiger, 2008). Samples collected from cooler sites also had higher diet quality, were correlated with higher levels of critical macro- and micro-elements, and had a higher abundance of cellulolytic and acetogenic microbes (Henderson et al., 2015; Yutin & Galperin, 2013). These samples also had a higher percentage of cool-season grasses. Given the regional locations of these samples, they are likely derived from areas with a northern mixed-grass prairie ecosystem. Grasses in this region have also been found to have [Ca], [Mg], and [Mn] that exceed minimum requirements for beef cattle during the summer and fall seasons (Munshower & Neuman, 1978). In areas with lower precipitation and lower temperatures, we found a higher abundance of *Thermobacillus*, a hemicellulolytic microbe (Rakotoarivonina, Hermant, Monthe, & Rémond, 2012). This may indicate that forages in the June diet at these locations have a higher content of hemicellulose. As expected, when bison consume more woody species, they are also more likely to have an abundance of cellulolytic microbes in the rumen (Girija, Deepa, Xavier, Antony, & Shidhi, 2013; Henderson et al., 2015).

As a result of this work, we now have the beginnings of baselines for diet quality and composition for US bison herds. Dietary quality is expected to be lowest in warm, dry climates with a greater percentage of eudicots than in cool, wet climates. Yet, the high dietary quality of bison in cool, wet climates where bison gain weight at the fastest rate also sets a baseline for potential nutrition. Whereas historical bison could migrate to take advantage of geographic gradients in forage quality, bison with diets that fall below this potential baseline can be improved through promotion of high-quality plant species, fertilization, or supplementation with protein. Diet quality still needs to be linked mechanistically to bison weight gain to determine whether variation in bison weight gain and/or reproduction are directly being caused by nutrition or other factors. With climate change, forage quality is expected to decline (Augustine et al., 2018; Craine et al., 2009). This research is further evidence that warming will increase nutritional stress in bison unless actions are taken to directly or indirectly increase protein availability.

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CONFLICT OF INTEREST

JC is the co-owner of Jonah Ventures, LLC, which is a commercial enterprise that sequences DNA for clients. All other authors declare no competing interests.

AUTHOR CONTRIBUTIONS

JC and TJ: wrote the main manuscript text and analyzed the data. JC: prepared figures. All authors contributed to the writing of the text and have reviewed the manuscript in its entirety.

DATA AVAILABILITY STATEMENT

All data and analysis code are available on Dryad at [https://doi.org/10.5061/dryad.jwstqjq4v].

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