RESEARCH ARTICLE

Reintroduced bison diet changes throughout the season in restored prairie

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Grazing as a management tool is often intended to alter plant community dynamics through preferential foraging. Bison diet in the western United States has been well studied, especially in short and mixed grass remnant prairies. However, there is little known about what bison consume in restored and tallgrass prairies. As bison reintroductions are used more commonly in eastern tallgrass prairies, it is important to understand their diet to predict future impacts on prairie plant communities. This study aims to understand bison diet across different seasons, and asks whether diet differs among male and female, and differently aged bison. We used stable isotope analysis to quantify $\delta^{13}C$ and $\delta^{15}N$ in plants and used a Bayesian isotope mixing model to estimate bison diet. We found bulls relied more heavily on C_4 plants and wetland plants than cows, which relied more heavily on forbs, but no differences in diet between ages. Our analysis shows that bison primarily grazed on C_4 grasses throughout the late spring and summer. However, bison foraged more on wetland species and forbs in the late summer and fall. This change in diet could have implications for wetland species and habitats, through dung inputs and trampling. The relatively high reliance on forbs for nearly one-third of bison diet could mean intended impacts of reintroduced bison such as increased plant diversity through preferential grazing on grasses could be dampened. Managers reintroducing bison to restored prairie ecosystems should ensure adequate wetland and forb species, in addition to a mix of grasses.

Key words: bison, diet, isotope, prairie, reintroduction, restoration

Implications for Practice

- Bison reintroductions are increasing in restored tallgrass prairies, which provide unique forage choices. Studying their diet in this new environment can help refine hypotheses about bison impacts on plant communities.
- Bison eat primarily C_4 grasses in late spring/summer but shift to wetland/other C_3 plants the rest of the year. Bison rely on nitrogen-fixing/other forbs for a substantial portion (32.5%) of their diet. Such patterns may weaken hypothesized increases in forb diversity through preferential C_4 grass grazing.
- Through grazing and trampling of wetland species, bison may create unintentional impacts on wetland ecosystems.
- Managers should consider bison stocking density by availability of specific plant communities such as wetlands. *Carex* spp. may be particularly important in all seasons besides summer.

Introduction

Grasslands are some of the most threatened ecosystems globally (Gibson 2009). Tallgrass prairie in the North American Midwest is especially threatened; 82–99% of it has been converted to agriculture over the past two centuries (Samson & Knopf 1994). Even what little prairie remains has been degraded due to invasive species, loss of native grazers, and altered burning patterns (Knapp et al. 1999; Fletcher & Koford 2003). To redress prairie loss, managers seed former agricultural lands and reinstate key ecosystem processes with prescribed fires and reintroduction of native grazers to create restored prairies (Knapp et al. 1999; Houdeshell et al. 2011).

Large grazers have important impacts on grassland ecosystems worldwide, and grazing is a common component in grassland restoration and management. In North American prairies, bison (*Bison bison*) are considered influential grazers. Although they were almost hunted to extinction in the late 1800s, populations are recovering in reserves and herds have recently been reintroduced to restored prairies for their ecosystem-engineering roles (Knapp et al. 1999). One main goal for reintroducing bison is to increase prairie plant diversity. Bison preferentially forage on Poales species (mainly grasses and sedges), which increases resource availability for otherwise less-competitive forb species and, in turn, increases plant diversity at a landscape scale (Knapp et al. 1999; Elson & Hartnett 2017). Bison dietary

preferences generally follow a seasonal trend from C_4 grasses in the summer and fall and then other C_3 Poales species until early summer (Chisholm et al. 1986; Steuter et al. 1995; Coppedge & Shaw 1998; Post et al. 2001). However, bison diet can vary across geographical region and forage availability (Chisholm et al. 1986; Larter & Gates 1991; Bergmann et al. 2015; Craine et al. 2015), and plasticity in bison diet makes forming predictions of how bison reintroduction will impact plant communities difficult.

The reintroduction of large grazers on restored prairies is an increasingly important and relatively new tool in grassland restoration. While some restoration sites use cattle to mimic historical grazing disturbances, grazer identity can drive plant responses (Tóth et al. 2018) such that cattle often differ from bison in their impacts on grasslands (Kohl et al. 2013; McMillan 2019). Thus, many managers are turning to bison to more accurately mimic historical prairie grazing regimes. One herd of bison was reintroduced to restored prairie at Neal Smith National Wildlife Refuge in Iowa, U.S.A., in 1996, and since 2014, two herds have been reintroduced to prairie restorations in Illinois, U.S.A. (Nachusa Grasslands and Kankakee Sands). Such reintroductions are likely to become more common as scales of restorations, and therefore potential bison reintroduction sites, increase. Until now, bison diet has never been studied in a restoration setting. Instead, research to date has focused on bison diet in grasslands that have never been plowed (hereafter referred to as remnants) and therefore yields insights that may not apply in restoration contexts. It is critical to understand bison diet in restoration contexts because bison grazing has the potential to shape the trajectory of plant community composition as restored prairies assemble. Restored prairies offer different forage options for reintroduced bison than the vegetation communities from which bison are sourced. Prairie restorations are often mosaics of prairie at different stages of succession, and are targeted for high plant diversity, especially driven by forbs (Rowe 2010). Bison herds are sourced from remnant prairies, which often have a higher proportion of grasses available for forage than typical prairie restorations do (e.g. Taft et al. 2006; Manning et al. 2017), so this new foraging landscape could yield bison diet that differs from diet in remnants.

Moreover, the extent to which bison diets shift throughout the seasons (Coppedge et al. 1998) could likewise impact restored plant communities in unexpected ways. It is well-documented that prairie restorations go through successional stages by shifts in dominance from annual weeds to native forbs and finally native C_4 grasses (Schramm 1990; Camill et al. 2004). Depending on forage preference, grazing in these different stages may result in different outcomes than in restored prairies that do not have bison grazing. For example, if bison are selecting N-fixing forb species within older restorations, this may reduce the number of forbs and counteract desired restoration goals (Craine et al. 2015). However, if bison are preferentially grazing C_4 grasses as forage in younger restored sites, this may help the forbs better compete against grasses and produce a more diverse restoration (Coppedge et al. 1998). Potential impacts to restorations become even more difficult to predict due to foraging and behavioral differences in spatial use based on bison age, sex, and

seasonal patterns (Post et al. 2001; Bergmann et al. 2015). Bulls consume higher proportions of C_4 grasses than cows and juveniles, which indicates bulls have a lower quality diet (Post et al. 2001). If females and juveniles are foraging on fewer C_4 grasses, they may be selecting areas with more C_3 species such as younger restorations, which could produce differential impacts on restoration outcomes. As bison reintroduction is increasingly used as a management tool in prairie restorations, it is critical to understand bison diet and its potential impact on plant communities.

Past studies have analyzed bison dung for plant content (Putman 1984; Barker 1986), though this method can yield spurious results (Putman 1984; Barker 1986) and inaccurate diet proportions due to uneven digestion of plant species (Bartolome et al. 1995). Behavioral observations can be used for some herbivores in select ecosystems, but this is not possible in a tallgrass prairie with bison because the need to maintain a safe distance and the height and diversity of plants would together preclude accurate identification of diet items. Many studies have used microhistological analysis to quantify bison diet, which has been shown to overestimate the amount of Poales species in comparison to forbs (Vara & Holecheck 1980; Bartolome et al. 1995; Craine et al. 2015). Others have used only $\delta^{13}C$ to detect the shift between C_3 and C_4 plants in both modern and historical bison; however, this limits the ability to quantify forbs and different groups of Poales species (Steuter et al. 1995; Post et al. 2001; Feranec et al. 2009; Widga et al. 2010). To overcome such issues, some recent studies have used DNA sequencing to further investigate the role of forbs in bison diet (Bergmann et al. 2015; Craine et al. 2015; Leonard et al. 2017). DNA sequencing, unfortunately, can have the opposite result of microhistological analysis by overestimating the abundance of forbs due to the preferential degradation of grass DNA during digestion (Bergmann et al. 2015). Each of the aforementioned methods requires ongoing sample collection throughout the season to understand shifts in diet with time, as they only quantify diet in the small snapshot of time in which the samples were collected. Moreover, depending on the methods used, it can be difficult to ascribe single samples to known animals, making inferences about diet between sex and age more difficult. Alternatively, stable isotope analysis (SIA) of segmented hair collected just once during yearly health checks can be used to quantify seasonal trends in bison diet, as well as diet differences between sexes and ages. By incorporating isotopic signatures of potential forage species, isotope models can quantify the proportion of dietary sources in bison diet and allow better predictions of bison reintroduction impacts on plant communities. Stable isotope mixing models, using two biotracers ($\delta^{13}C$, $\delta^{15}N$), yield predictions of proportional dietary constituents if the diet groups are isotopically distinct and, when using segmented hair, can yield insights on seasonal shifts of bison diet.

In this study, we measured $\delta^{13}C$ and $\delta^{15}N$ in bison hair and in potential forage species to investigate seasonal changes in reintroduced bison diets in a mostly restored tallgrass prairie. Our goal was to determine if there are seasonal dietary trends or variation across both age and sex in a reintroduced herd of bison in restored prairie. We identified isotopically distinct plant groups of interest including C_4 grasses, C_3 grasses, and sedges collected from

upland prairies (hereafter upland C_3 Poales), forbs, N-fixing forbs, and wetland plants. We then used mixing models and bison tail hair isotopes to determine the proportion of bison diet constituents through time. We hypothesized that (1) younger bison and adult cows would eat plants with a higher nutritional content (lower C:N) than bulls, (2) seasonal shifts in diet would be consistent with changes in growing season forage availability, and (3) forbs would comprise a minimal amount of bison diet throughout the year (Coppedge et al. 1998; Post et al. 2001).

Methods

Study Site

We worked at Nachusa Grasslands (hereafter, Nachusa), an approximately 1,200 ha prairie remnant and restoration site in Franklin Grove, Illinois, owned by The Nature Conservancy (TNC). TNC began restoring former agricultural land at the site in 1987, and each year restores more of the landscape back from decades of rowcrop agriculture (corn and soybeans). Average annual temperature is around 8.5°C and average annual precipitation is roughly 950 mm. In 2014, TNC reintroduced bison to Nachusa and currently the herd totals approximately 125 individuals and has access to half the preserve (approximately 600 ha). The stocking rates in 2016–2017, the years considered in this study, were 0.30 and 0.34 animal units (AU) per acre, respectively. Stocking rate is calculated according to TNC's grazing plan (Considine et al. 2015) as:

$$(\Sigma \text{animal units}) \times \frac{6 \text{ months}}{\text{total acres}}$$

where animal units are as follows by life stage: calf = 0.5 AU; 1 year old = 0.8 AU; 2 years = 1 AU; cow >2 years = 1 AU; bull >2 years = 1.25 AU. The bison are free to roam the entire 600 ha and are not relegated to certain areas in the preserve. The accessible area is a mosaic of restored and remnant tallgrass prairie, wetland, and savanna. Remnant habitats of all three types experience brush thinning, regular prescribed fire, and invasive species control. Prairies were restored by planting native seed into agricultural fields. Where appropriate, subsurface drainage tiles were removed and landscapes sculpted to restore wetland habitat. More information on management and restoration methods at the site can be found in Kleiman (2016). Restorations make up approximately 76% of the bison unit area, or 460 ha, while remnant patches account for 24% of the area, or 148 ha. Plant composition not only differs across community types but also by years since restoration. Previous work has shown a decrease in forb abundance and diversity as the restorations age, which likely influences forage availability (Hansen & Gibson 2014; Barber et al. 2017; Klopf et al. 2017). The preserve is also managed with prescribed fire with more than half of the preserve area burned annually. The vast majority of sites average fire return intervals of every 1–3 years. Prescribed fire is applied in the fall and/or spring (nongrowing season) and varies by site and by year depending on TNC's land management goals for each site. Managers use relatively small (0.40–161 ha, median 38 ha)

burn units to contain fire to selected sites. Fire does not impact the stable isotopic signatures of plants at our study site (unpublished data), but bison are thought to prefer recently burned sites, a process known as pyric herbivory (Fuhlendorf et al. 2009), and resulting in a patch-burn grazing system.

Plant Samples

To determine the isotopic signatures of bison diet constituents, a list of plant species was compiled to represent major plant groups or individual species that likely would be a part of bison diet (Table 1). Species were chosen either due to their abundance on the preserve, signs of grazing by bison, because they were common exotic species, or because literature reviews suggested preference for certain groups or species. The literature suggested C_3 Poales and C_4 grasses to be most likely primary components of diet (Larter & Gates 1991; Coppedge & Shaw 1998), but other evidence suggested the importance of nitrogen-fixing and other forbs and potentially shrubs (Painter & Ripple 2012; Craine et al. 2015). Therefore, we collected all possible grass and nitrogen-fixing species, the most common prairie sedges and rushes, and two shrubs that others have shown bison to browse (*Salix humix* and *Salix interior*). We also collected forbs upon which we saw direct evidence of bison grazing in upland prairie (*Eryngium yuccifolium* and *Tradescantia ohiensis*) and plants we saw in wetland communities that had direct evidence of grazing (*Carex vulpinoidea, Juncus effusus, Salix humilis, Scripus* spp., and *Typha angustifolia*).

We used plant surveys to determine the 10 most common forb species available as bison forage in addition to the species we collected based on observation and the literature. To determine the most abundant forbs within the preserve, we sampled plants across a chronosequence of 13 restorations and two remnant sites. Within each site, we randomly sampled 10 locations and recorded each species and the percent cover of those species within a 0.25 m² quadrat. Plants were identified and named according to Flora of the Chicago Region (Wilhelm & Rericha 2017). More details on plant surveys can be found in Barber et al. (2019).

After we selected which species to collect, we collected upland prairie plant tissue during August 2016 with supplemental sampling in October 2017 to sample wetland species. All species were collected within prairie or wetland restorations in areas accessible to bison. Three leaves of newest growth from three individuals within these areas were clipped and dried for at least 48 hours at 55°C. Following this, samples were ground into fine particles using a Wiley Mill in preparation for analysis. We also analyzed bison cubes, which are highly desirable bison treats fed to the bison by practitioners at the research site in low quantities the week prior to an annual health roundup and at the preserve's yearly public event in late September. Their isotope range fell within the upland C_3 Poales species in isotopic space.

Animal Samples

Bison tail hair was retrieved during the annual fall roundup on October 2, 2016, and October 18, 2017. Multiple hairs were pulled from the tail by land managers processing the animals and kept separate to distinguish individuals. Each individual

Table 1. Groupings for bison dietary items. All species included in the groups are listed along with the groups respective elemental and nutritional concentrations (±1 SE). High δ^{15}N forbs are forbs with δ^{15}N > 0, and low $\delta15$N forbs are forbs with δ^{15}N < 0. Nomenclature from Wilhelm and Rericha (2017). [e]Indicates exotic, nonnative species. [N-fix]Indicates species known to be nitrogen fixers.

Plant Group	Species	% N	% C	C:N
Upland C_3 Poales species	*Agrostis gigantea*[e], *Bromus inermus*[e], *Carex bicknellii*, *Dichanthelium oligosanthes* var. *scribnerianum*, *Dichanthelium villosissimum*, *Elymus canadensis*, *Juncus interior*, *Koeleria macrantha*, *Phalaris arundinacea*[e], *Phleum pratense*[e], *Poa pratensis*[e]	1.39 (± 0.24)	41.96 (± 0.77)	35.86 (± 3.23)
C_4 grasses	*Andropogon gerardii*, *Setaria viridis*[e], *Sorghastrum nutans*, *Sporobolus heterolepis*	1.75 (± 0.42)	40.52 (± 1.37)	25.54 (± 5.71)
High δ^{15}N forbs	*Astragalus canadensis*[N-fix], *Baptisia lactea*[N-fix], *Daucus carota*, *Euphorbia corollata*, *Penstemon digitalis*, *Salix interior*, *Solidago canadensis*, *Zizia aurea*	2.27 (± 0.39)	42.31 (± 1.27)	27.57 (± 5.31)
Low δ^{15}N forbs (mostly N-fixing forbs)	*Achillea millefolium*, *Anemone cylindrica*, *Baptisia leucophaea*[N-fix], *Chamaecrista fasciculata*[N-fix], *Dalea candida*[N-fix], *Dalea purpurea*[N-fix], *Desmodium illinoense*[N-fix], *Echinacea pallida*, *Eryngium yuccifolium*, *Lespedeza capitata*[N-fix], *Lotus corniculatus*[e, N-fix], *Medicago lupulina*[e, N-fix], *Melilotus alba*[e, N-fix], *Melilotus officinalis*[e, N-fix], *Ratibida pinnata*, *Symphyotrichum ericoides*, *Tradescantia ohiensis*, *Trifolium pratense*[e, N-fix], *Trifolium repens*[e, N-fix]	2.26 (± 0.32)	40.59 (± 1.02)	22.06 (± 4.26)
Wetland species	*Carex vulpinoidea*, *Juncus effusus*, *Salix humilis*, *Scripus* sp, *Typha angustifolia*	2.78 (± 0.34)	46.42 (± 1.11)	20.25 (± 4.64)

bison has a unique tag number and known age and sex. We retrieved hair from all processed individuals. Each strand was inspected using a Leica stereo zoom microscope to remove telogen phase hairs that would not have been in a period of growth during collection (Van Scott et al. 1957; Schwertl et al. 2003). To prevent the inclusion of bison milk in the potential sources, we excluded all individuals under the age of two because of known weaning patterns (Meagher 1986). Samples with less than 15 hairs were not included due to insufficient weights for sample processing. After all exclusions, 51 animals were processed (31 females and 20 males), but each individual had varying lengths of hair available for sampling each year (Table 2).

The hairs collected in 2016 were shorter than those collected in 2017, and yielded data for seven 1-cm increments between mid-April and mid-September, assuming a growth rate of approximately 1.9 cm/month (see below). Preliminary analysis of 2016 hair showed a clear trend between δ^{13}C and δ^{15}N from the first to last segment so three segments: 1 cm (approximately September), 4 cm (approximately July), and 7 cm (approximately May) were chosen for processing. Hairs collected in 2017 were longer, which allowed a longer window of potential seasonal shifts to be investigated. To expand seasonal inferences, we determined isotope values for the entire length of tail hair available, in 1-cm increments, for four individuals from which hair was collected in 2017 and 2016. We then visually inspected the results to find at which tail length the 2017 isotope values matched the 2016 values. We found the 24th centimeter of 2017 tail hair to overlap with the newest growth in the 2016 hairs (Figs. S1 & S2), indicating an approximate hair growth rate of 1.9 cm per month, given that 2016 and 2017 samples were collected 12.5 months apart. Visually inspecting trends in δ^{13}C and δ^{15} N for the four 2017 animals (Figs. S1 & S2), we

identified inflection points, or times where diet clearly shifted, and chose those time points as our foci for the remaining 47 animals for which we had tail hair. We ran the 1st, 5th, 12th, and 20th centimeters for all animals where the amount of hair available allowed from 2017, which according to our estimated growth rate are from approximately October 2017, July 2017, March 2017, and November 2016. Together with the 2016 tail hairs, that yielded data to examine seasonal shifts in diet from approximately May 2016 through October 2017 for 51 animals.

Once all samples were selected, we straightened the hairs with deionized water and aligned hairs for each individual from follicle to tip in tin (Sn) foil. We then folded the tin foil over the hairs and cut them into seven 10-mm segments. All samples were then rinsed in a 95% ethanol solution, dried for at least 48 hours at 55°C, and cut as small as possible into a homogeneous mixture (Kristensen et al. 2011; Burnik Šturm et al. 2017).

Stable Isotope Analysis

SIA is commonly used to reconstruct diets across taxa and biomes (Crawford et al. 2008; Boecklen et al. 2011; Layman et al. 2012). Mammals are the second most common taxon in SIA studies, with temperate grasslands being the third most commonly studied biome (Boecklen et al. 2011). Both δ^{13}C and δ^{15}N are the most commonly used stable isotope measures when reconstructing mammalian diets (Crawford et al. 2008). Carbon sources can be detected by using the δ^{13}C value due to a plant's photosynthetic pathway (DeNiro & Epstein 1978; O'Leary 1981; Cerling & Harris 1999; Inger & Bearhop 2008). During both C_3 and C_4 photosynthesis, CO_2 from the atmosphere is fixed, but different enzymes are used to catalyze the reaction. These enzymes discriminate ^{13}C differently and

Table 2. Sample sizes (numbers of individual bison) for the different time, sex, and age categories used to analyze hair samples. Months are approximate based on bison hair growth.

	May 2016	*July 2016*	*Sept. 2016*	*Nov. 2016*	*April 2017*	*August 2017*	*October 2017*
Sex							
Female	16	16	17	16	23	21	21
Male	12	12	12	11	17	15	15
Age							
2–3 year-olds	7	7	7	10	14	12	13
4–8 year-olds	9	9	10	8	12	10	12
>8 years	12	12	12	9	14	14	11

result in different $\delta^{13}C$ values (Marshall et al. 2007). The abundance of ^{15}N within individual plants and species can vary given a variety of factors such as nitrogen source, mycorrhizal associations, temperature, and precipitation (Adams & Grierson 2001; Evans 2001; Drucker et al. 2010). Such differences mean $\delta^{15}N$ can be used to further distinguish plant species or groups of plants in herbivore/grazer diet when each plant species or group of plants (e.g. C_4 vs. C_3) have unique isotopic signatures (Drucker et al. 2010; Kristensen et al. 2011).

Both plant and hair samples were weighed out to 5 mg and 1 mg, respectively, into tin capsules and processed in an isotope ratio mass spectrometer (DELTAplus Advantage Mass Spectrometer, Thermo Scientific, Wilmington, DE, U.S.A.) at the Stable Isotope Facility, Geology and Environmental Geosciences, Northern Illinois University. Samples were compared against NBS 22, USGS-25, IAEA-CH-6, IAEA-N1, and IAEA-N2 standards to calculate $\delta^{13}C$ and $\delta^{15}N$ values along with nitrogen and carbon concentrations as shown below (Michener & Lajtha 2007).

$$\delta(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

δ (‰) represents the isotopic value of C or N in parts per thousand. R is the ratio of heavy to light isotopes calculated from both the sample and the standard. Initial results for plants were plotted to determine the grouping of diet sources by both biological relevance and isotopic composition (Table 1). Accuracy of data was ensured by including duplicate samples and standards of known isotopic value.

Linear Models

We constructed linear mixed effects models using the package *lme4* (Bates et al. 2015) to test for differences in raw $\delta^{13}C$ and $\delta^{15}N$ isotope values by time, age category, sex, and the interactions between age category and time and between sex and time. To account for individuals being measured repeatedly over multiple samples, we included animal ID as a mixed effect in each of these models. We chose ages 2–3, 4–8, and >8 for age categories. At age three, females first become reproductively mature (Green & Rothstein 1991). Ages 4–8 represent the time males become reproductively mature and are most reproductively

active, and older animals are less reproductive (Maher & Byers 1987).

Mixing Models

The proportion of dietary sources in bison hair was investigated using Bayesian mixing models in the *MixSIAR* (Parnell et al. 2010, 2013) R package. MixSIAR allows users to customize models by selecting various input parameters (Stock & Semmens 2016). We built three models based on differences we found in linear models (Table S1): one that pooled all samples to estimate global bison diet, regardless of season; one that explored bison diet through time pooling all individuals; and one that explored bison diet through time separating males and females. We did not run models that separated age, because our linear models showed no differences. Each model quantified the proportion of each dietary group (Table 1) in bison diet and used time (as estimated from bison hair length segment) as a categorical variable. The seven segments of tail hair chosen from each individual were used to estimate isotopic values through time, which was imputed as a categorical variable. We treated time as a categorical variable because our data that explored the entire hair segments (Figs. S1 & S2) suggests nonlinear shifts in diet that continuous modeling would not accurately capture. Sample sizes for all models are listed in Table 2.

Trophic enrichment factors (TEFs) are used in isotope diet studies to account for enrichment in isotopic signature when one trophic level consumes another. TEFs from literature on domestic cattle were used because no information on bison TEFs currently exists. The TEFs for C and N were set at 2.7 ± 0.4 and 2.5 ± 1.7, respectively (Sponheimer et al. 2003). We chose domestic cattle because they were most similar to bison; similar reasoning has been used in muskox (Kristensen et al. 2011). Because our data suggested differences in elemental concentrations (i.e. %N, %C) between wetland and upland prairie plants, we used a concentration-dependent mixing model using the same approach as Parnell et al. (2010). To test for differences in elemental concentrations and to determine forage quality between plant groups, we constructed a linear model using %N, %C, and C:N as dependent variables and plant group as an independent variable, with each of the plant five plant

groups in Table 1 as levels. All results were reported using the predicted values from these models.

Results

Source Isotopic Signatures and Elemental Concentrations

Differences in $\delta^{13}C$ and $\delta^{15}N$ were used to group plant species into isotopically distinct dietary sources (Table 1; hereafter, plant groups). The range of $\delta^{13}C$ for bison diet items fell between $-32.51‰$ and $-11.32‰$ with a mean of $-26.69‰$ (Fig. 1). The main distinction between $\delta^{13}C$ was the difference in photosynthetic pathways with C_4 and C_3 plants having a mean of $-12.81‰$ and $-28.38‰$, respectively. C_4 species were therefore isotopically distinct and grouped together as a dietary source. C_3 plants could not be separated using only $\delta^{13}C$ because these values only differed from $-32.51‰$ to $-21.23‰$. To distinguish between C_3 plant groups, differences in $\delta^{15}N$ were used in conjunction with differences in $\delta^{13}C$. Forb species had very similar $\delta^{13}C$ values but varied in $\delta^{15}N$ from $-4.19‰$ to $3.30‰$. Forbs were assigned to two separate groups defined by whether they had high $\delta^{15}N$ ($\delta^{15}N > 0$) or low $\delta^{15}N$ ($\delta^{15}N < 0$) values. The majority of low $\delta^{15}N$ forbs ($n = 12$ of 19) were nitrogen-fixing forbs (hereafter called mostly nitrogen-fixing forbs; Table 1). All wetland species contained high values of $\delta^{15}N$ ranging from $4.37‰$ to $10.33‰$ and were clearly isotopically distinct from the other species. Finally, C_3 Poales species collected from upland prairies (hereafter, upland C_3 Poales) varied in both $\delta^{13}C$ and

$\delta^{15}N$ ranging from $-32.51‰$ to $-21.23‰$ and $-4.55‰$ to $1.70‰$, respectively. We tested several mixing models with different groupings of upland C_3 Poales species. However, the only model that converged ran with all upland C_3 Poales species grouped together so our final model grouped those species (Table 1).

Linear Models

We found no differences in isotopic composition of bison of different ages or interactions with time (Table 2). We did find $\delta^{15}N$ to vary through time and between bison sexes, which prompted us to run isotope mixing models that quantified diet shifts in bison through time and between males and females.

The plant groups differed in nutritional quality: elemental percentages of C ($F = 8.9744$, $df = 4$, 69; $p < 0.001$) and N ($F = 4.5861$, $df = 4$, 69; $p < 0.01$) and C:N ($F = 3.6086$, $df = 4$, 69; $p = <0.01$; Fig. 2) all varied between plant groups. Wetland species had 10% more C on average than the other groups, while the rest of the groups were similar to one another. Wetland species also had the highest N concentrations (2.78%) followed by high $\delta^{15}N$ forbs (2.27%), low $\delta^{15}N$ forbs (2.26%), C_4 grasses (1.75%), and finally upland C_3 Poales species (1.39%). Wetland species had the lowest C:N (20.25%), followed by the low $\delta^{15}N$ forbs (22.05%), high $\delta^{15}N$ forbs (27.57%), C_4 grasses (25.54%), and upland C_3 Poales species (35.86%). These results suggested that elemental concentrations vary with plant taxonomy, ecology, and physiology, and this
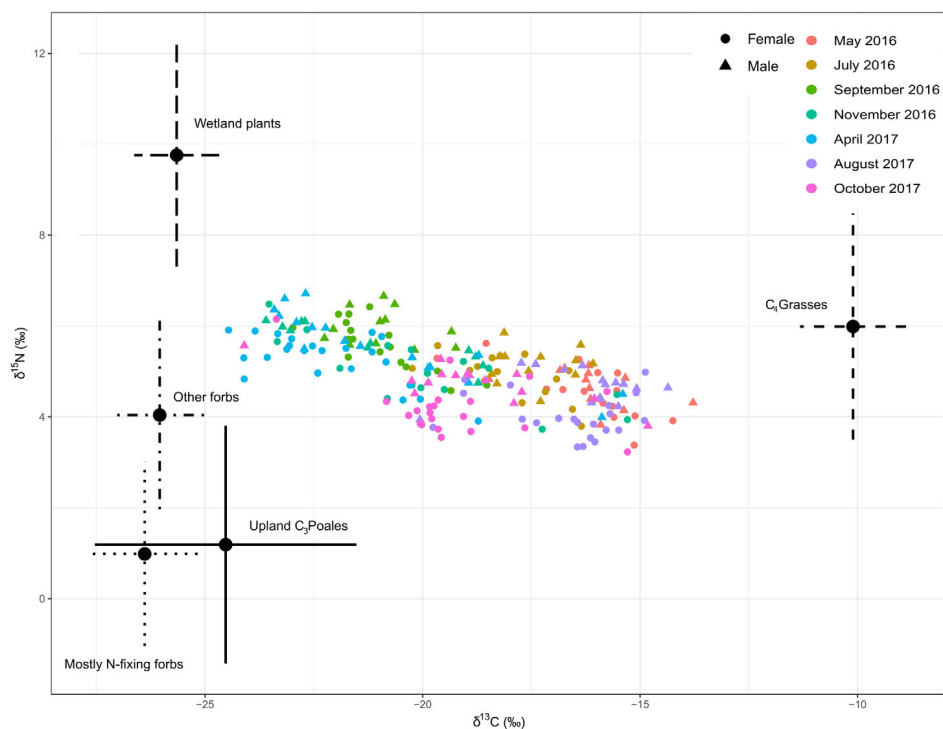

Figure 1. Isotopic signatures for dietary groups (corrected for TEFs) and for bison consumers. Colored points represent sectioned hair clippings from each individual. Colors represent the estimated time of hair development.
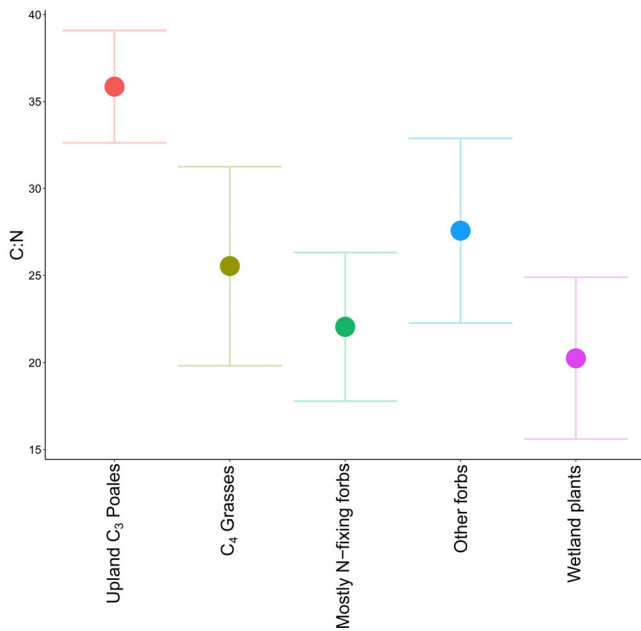
Figure 2. C:N values across plant groups. Upland C_3 Poales species have lower nutritional quality in relation to both wetland species and low $\delta^{15}N$ forbs. Circles represent mean values and error bars represent ± 1 SE.

influenced our decision to use a concentration-dependent mixing model.

Dietary Composition

Mixing models revealed that overall bison diet consisted primarily of C_4 grasses followed by high $\delta^{15}N$ forbs, mostly N-fixing forbs, upland C_3 Poales species, and wetland plants (means and SDs = 44.2% $\pm$ 0.089, 18.5% $\pm$ 0.12, 14.1% $\pm$ 0.088, 12.5% $\pm$ 0.89, and 10.8% $\pm$ 0.046, respectively). Dietary proportions changed through time (Fig. 3), and to a lesser extent, between sexes, with females eating slightly more N-fixing forbs than males and with males consuming higher proportions of C_4 grasses and wetland plants than females (Fig. 4). In the main growing season, bison diet closely mirrored the overall mean values. But moving toward fall, bison grazed on fewer C_4 grasses (mean proportions fell to 31%) and began relying more on upland C_3 Poales species, wetland plants, and high $\delta^{15}N$ forb species (mean proportions rose to 30, 22, and 11%, respectively by around November 2016).

Discussion

Large grazers are critical components of grassland ecosystems. The reintroduction of grazers, therefore, has important restoration implications. Bison reintroduction to tallgrass prairie restorations is becomingly increasingly common, but there is a dearth of information on bison diet in prairie restoration. Our findings show seasonal shifts in diet; some of these shifts matched our predictions based on plant growth patterns and forage availability and others were surprising. Bison strongly rely on C_4 grasses

during the late spring and into the summer, but they begin to shift to wetland and upland C_3 species and forbs in fall. These unexpected results have potential implications for management and the potential impacts of bison in restored prairies. Below we discuss these impacts and possible explanations for the patterns we document.

Because these results differ from previous studies, the intended management impacts from these bison may also differ. Instead of bison opening space for forb species by grazing primarily on grasses, they may be opening up space for C_3 Poales species by grazing on forbs in the fall. Through grazing heavily on wetland plants rather than on upland prairie plants, bison may create unintentional impacts on plant communities such as possible invasions and ecosystem impacts like soil erosion. For example, grazing by livestock in wetland areas has been shown to cause salination, soil erosion, and negative impacts on invertebrate communities in Patagonian wetlands (Epele & Miserendino 2015). Other research in central European habitats showed that wetlands were most sensitive to and negatively impacted by grazing by large-bodied cattle breeds (as opposed to smaller breeds), but grasslands were not degraded even by large breeds (Kovácsné Koncz et al. in press). Given that the size of bison is similar to those larger-bodied cattle breeds, this could indicate managers should expect wetland impacts from bison reintroduction. However, the grazing intensity of this study (0.61–0.68 AU/ha) is twice the intensity of grazing at our study's site (0.30–0.34 AU/ha). Nutrient inputs to wetlands via dung also are a possibility in herds that heavily graze in wetlands. Such impacts may be even further exacerbated in prairie restorations with a small total acreage of wetlands in comparison to the size of the herd. This indicates that managers interested in bison reintroduction may want to consider stocking density not only by total acreage of preserves, but also by the acreage of certain types of habitats including wetlands.

Beyond wetland impacts, bison reintroduction could contribute to invasive plant and/or weed establishment through trampling and wallowing behavior. Other studies have shown that even moderate grazing intensities, such as those seen in our study site, lead to a slight increase in weedy species (Deák et al. 2017) or that livestock and elk reintroduction can facilitate invasive plants (Hobbs 2001; Johnson & Cushman 2007), but still others find a neutral (Martin & Wilsey 2006) or positive effect of grazing on weed control (Milchunas et al. 1998; DiTomaso 2000). Given these mixed results, site-specific studies are important to ascertain whether bison reintroductions will impact weedy plant invasions. Wallowing behavior, in particular, could be a source for weed invasion (Collins & Uno 1983) because after wallows are abandoned, these bare ground patches may become invaded by weeds that require higher light and soil temperatures than they would otherwise have in dense tallgrass prairie vegetation.

As predicted, we found that bulls eat more C_4 grasses than cows, which favored higher-quality forage, especially in the fall. We also found our predicted shift in diet from late spring to early fall that coincides with the growing season and C_4 grass availability (Steuter et al. 1995). During the peak of the growing season, C_4 grasses are estimated to make up over half of the bison diet but
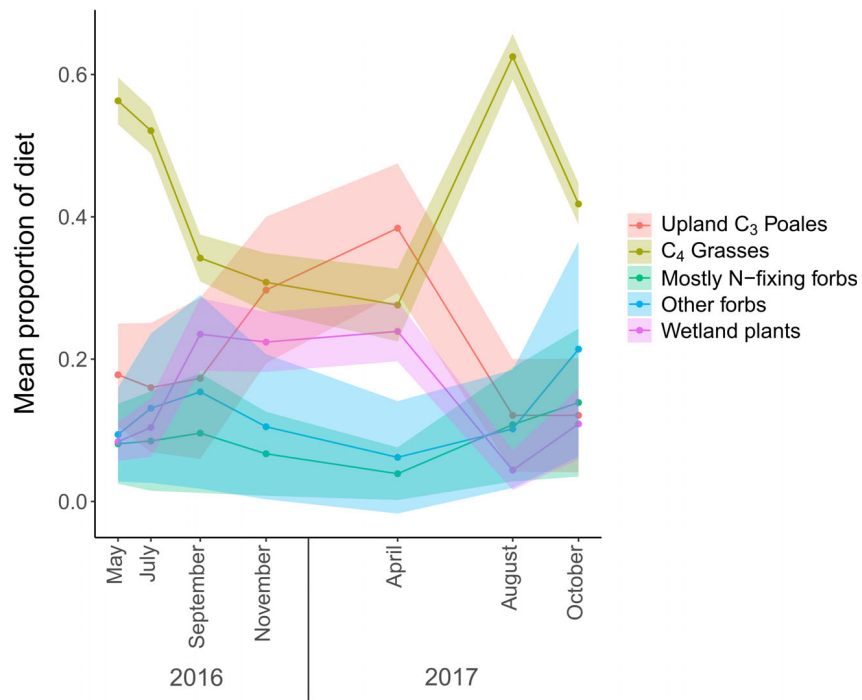
Figure 3. Seasonal trends of plant groups within bison diet. Solid lines represent median values of dietary proportions while the transparent ribbons represent ± 1 SD. C_4 grass consumption declined in late summer and was reduced during winter, when wetland species and upland C_3 Poales spp. increased in proportional consumption. Time points are May 2016 through October 2017; time points are approximate based on estimated growth rates of bison hair.

the proportion of C_4 grasses bison rely on for forage dips to a much lower proportion by early fall (around 30%). Bison shift to consuming largely upland C_3 Poales prairie species, wetland species, and a smaller percentage of nitrogen-fixing and other forb species. This is contrary to our predictions of forb species playing a minimal role in bison diet. Our results are in accordance with previous work that
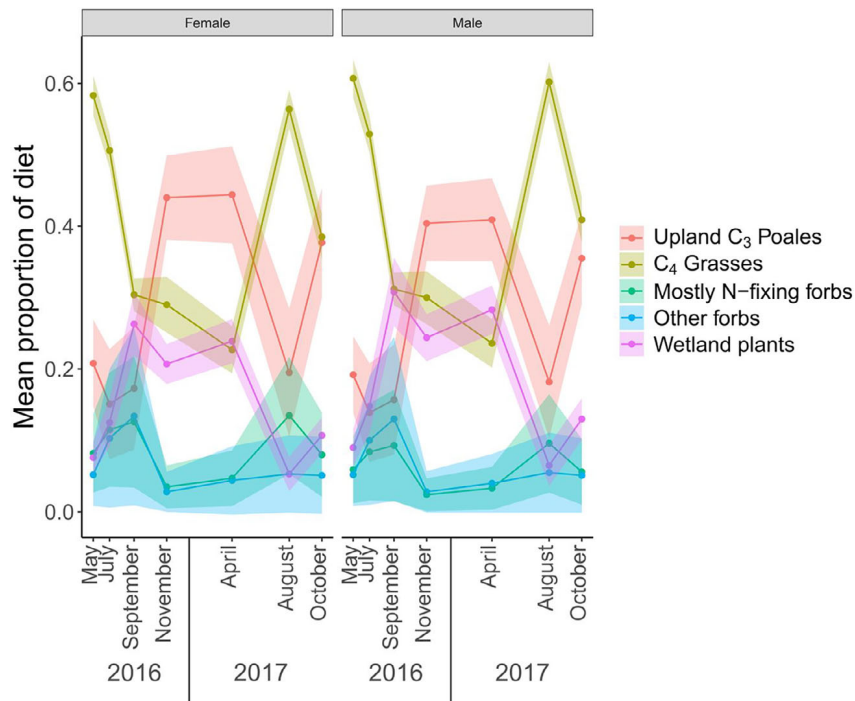

Figure 4. Seasonal trends of dietary groups within male and female bison. Solid lines represent median values of dietary proportions while the transparent ribbons represent ± 1 SD. Time points are May 2016 through October 2017; time points are approximate based on estimated growth rates of bison hair.

showed C_3 Poales species, specifically sedges, are a dominant dietary contributor from fall through spring (Steuter et al. 1995; Coppedge et al. 1998). The fact that upland C_3 Poales species and wetland species together make up well over half of bison diet in fall, winter, and spring suggests a strong reliance on C_3 species during this time. The wetland species group that makes up much of the diet in the early fall consists largely of C_3 Poales species. However, the increasing role of nitrogen-fixing and other forbs throughout the fall was unexpected. Bison reliance on these forbs may be explained by and aligned with our predictions of forage availability. As the C_4 grasses reach the senescent stage, wetland species and some forbs remain photosynthetically active. This may make them more palatable as grazers prefer to forage on new growth which tends to have a higher protein content (Bergmann et al. 2015). Contrary to our predictions, we did not see strong differences in diet between differently aged bison.

Bison were historically thought to be strict grass grazers (Peden et al. 1974; Coppedge et al. 1998), but our results and other recent studies (Craine et al. 2015; Leonard et al. 2017) suggest bison eat a wider range of diet items. Forbs have been shown to play a more important role in bison protein intake, especially those that are N-fixing (Craine et al. 2015), but the extent to which those findings translate to other bison herds remains unclear. At Nachusa, we show that together N-fixing and other forbs make up around 32% of bison diet throughout the season, with even greater reliance in the fall months. This could be driven by selective foraging of these higher-nitrogen plants, which could help animals build proteins and fat storage before winter. Bison from elsewhere have also shown plasticity in diet and potential selection of shrubs. For example, a herd of bison in Alaska were found to forage primarily on shrubs year-round (Waggoner & Hinkes 1986), while herds in Colorado short grass (Peden et al. 1974), wood bison in the Northwest Territories (Larter & Gates 1991), and Yellowstone bison (Painter & Ripple 2012) supplement their diet with shrub species when Poales species are less abundant. Nachusa's bison may consume shrubs, though they are scantly available and our methodology only included two potential shrubs—both willows (*Salix* spp.)—as diet items, which could not be separated from other isotopically similar forbs and wetland species.

The greater reliance on forbs and N-fixing forbs than anticipated could be due to the higher prevalence of such plants at this restoration site compared to what previously studied herds had available for forage. Nachusa is known for its high-diversity prairie restorations compared to the prairie remnants that are well studied with respect to bison diet, with Nachusa's diversity being driven primarily by forb species. For example, average Shannon diversity of five plantings at Nachusa Grasslands is 2.5 (Taft et al. 2006), whereas the average Shannon diversity of remnant grasslands at Konza Prairie, Kansas is around 1.1 (Manning et al. 2017). Thus, it could be that bison prefer forbs but they aren't available in high enough quantities to make up a larger component of their diet at other locations. If this explanation holds, it could be important for managers to select high-diversity prairies and restorations for future bison reintroductions. Alternatively, bison may prefer to eat primarily Poales species, but Nachusa has such a high proportion of forbs that

bison inadvertently consume forbs as they are grazing on preferred grass species. Forbs constitute a high proportion of bison diet, even in spring after much of the site has been burned. Spring green-up makes for easy selection of diet items because the preserve has mostly bare ground with new shoots of growth, which can easily be selected by bison. We thus view this explanation as less likely than bison potentially selecting forbs for their diet. Indeed, we have seen evidence of bison grazing forbs in recently burned sites in the spring, in sites where Poales species were plentiful. Future studies comparing diet to available forage could help determine the extent to which diet selection plays a role in the results of our study.

Bison at Nachusa, which have access to mostly restored prairie, show similarities and differences with other tallgrass prairie herds on remnants. Bison on tallgrass prairie remnants in Oklahoma rely much less on forbs (<2% of diet; Coppedge et al. 1998) than Nachusa's bison do, but both show a peak of reliance on tall grasses in summer, with a higher reliance on C_3 Poales species in fall through spring. Bison on tallgrass prairie remnants in Kansas (Konza prairie) show a considerably higher reliance on C_4 grasses in winter and on N-fixing shrubs (*Ceanothus americanus*) in spring and fall (Craine et al. 2015) than our results suggest that Nachusa's bison do. Though our methods do not allow us to identify exact species in the diet, the evidence presented here, when combined with other studies, suggests that like other tallgrass prairie herds (Coppedge et al. 1998; Craine et al. 2015) Nachusa's bison are likely to rely strongly on *Carex* spp. throughout the winter.

The nutritional quality of plants could play an important role in forage selection by bison. Craine et al. (2015) found that bison protein intake largely came from N-fixing forbs in the spring and fall. Our results suggest that something similar may be happening with Nachusa's bison herd. The same dietary contributors that remain green longer also have similar nutritional content (C:N) to C_4 grasses. Therefore, bison could preferentially supplement their diets with equally nutritious forage, such as wetland species and high $\delta^{15}N$ forbs, as C_4 grasses senesce in the fall. Bison target wetland species and forbs later in the season, in higher amounts than they eat upland C_3 Poales species. Cows conceive during the rut in July or August, and have around a 10-month gestation period, so are pregnant through the winter (Meagher 1986). By reducing their reliance on more fibrous and less nutritious upland C_3 Poales species in the fall, bison maintain a diet of equivalent nutrition in a time where building mass for winter months and maintaining the health of pregnant females is imperative. Our evidence for cows eating more nitrogen-fixing forbs and less C_4 grasses than bulls provides further evidence that females specifically may seek out forage with higher nitrogen in their early stages of pregnancy. Further studies are needed to tease apart the mechanisms behind the selection of these plant groups and whether bison are using the more nutritional species preferentially to prepare for winter and fetal development, opportunistically as their preferred forage dies off, or if they congregate and forage near water resources during drier portions of the year.

We used a unique approach to quantify bison diet; our approach has promise to be used in future conservation and restoration

contexts. Isotope analysis overcomes some of the drawbacks of over- or underrepresentation of certain plant types in microhistological, DNA, and dung plant content analyses (Vara & Holecheck 1980; Putman 1984; Barker 1986, 19; Bartolome et al. 1995; Steuter et al. 1995; Post et al. 2001; Feranec et al. 2009; Widga et al. 2010; Bergmann et al. 2015; Craine et al. 2015; Leonard et al. 2017). Moreover, isotope analysis allows a look at diet over a longer temporal scale than other methods, which are limited to assessing diet of each single sample on the day it was collected. Through the collection of a few hairs in a single day, we were able to quantify diet changes of individual bison of known age and sex throughout 1–2 years—making this method logistically simpler and less time-intensive than other methods. However, isotope analysis is not a panacea; it lacks the ability to identify specific species in diets, and can only parse out isotopically distinct groups. Future studies regarding seasonal changes in bison diet in restored systems should focus on a more complete picture and employ a variety of methodologies. In this study, the potential range of the diet proportions within each plant group is quite large. Although the general trends we have observed are valid, the true dietary proportions of these groups are not as certain. Data from another method of diet sampling, such as microhistological or DNA analysis, could be incorporated as a priori proportional data within mixing models to produce more precise results. Pairing diet analyses with field observations of grazing behavior and spatial use would further strengthen inferences made. The assumption that bison and domestic cattle also have the same TEFs may introduce error into isotope models (Wolf et al. 2009). However, enrichment of isotopes into hair has been shown to be less variable than other tissues (Kristensen et al. 2011). Lab analysis of bison-specific TEFs should be pursued in future studies to produce more accurate results.

Our work provides new evidence that bison may be more reliant on forbs than previously thought, which aligns more closely with the recent studies emerging from herds in the short-grass prairies of the North American west (Bergmann et al. 2015; Craine et al. 2015). Our study found that bison mostly graze on C_4 grasses during the late spring but then transition to rely more heavily on a mix of wetland species and forbs in the fall. This shows that bison are not strictly reliant on Poales species and can broaden the scope of their diet either opportunistically or preferentially to maintain their nutrient intake. The information here is critical for managers that are considering bison reintroduction into prairie restorations and underscores additional considerations such as wetland plant and forb availability. Research on other herds reintroduced to restored prairie will help determine if these results are generalizable across a wider array of reintroduction sites.

Acknowledgments

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Linear mixed effects model output for both δ^{15}N and δ^{13}C.
Figure S1. δ^{15}N values of four bison consumers as measured from 2016 (left) and 2017 (right).
Figure S2. δ^{13}C values of four bison consumers as measured from 2016 (left) and 2017 (right).