Coyote (Canis latrans) mammalian prey diet shifts in response to seasonal vegetation change‡

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Drylands typically have strong seasonal variation in rainfall and primary productivity. This study examines the effects of seasonal change in grass-derived resource availability on the base of the food chain of a mammalian predator. Seasonal changes in live grass cover were measured in two vegetation types at the Sevilleta National Wildlife Refuge in central New Mexico, USA. Non-invasive genetic sampling of scat was used to identify individuals in the local coyote (Canis latrans) population. Stable carbon and nitrogen isotope analysis of hair removed from scats of 45 different coyotes was used to assess seasonal variation in the diet of mammalian coyote prey that came from C4 grasses. Live grass cover increased from the spring to the summer and fall; contribution of C4 grasses to the diet of mammalian coyote prey increased from the summer to the fall and was higher in grassland areas. There were significant differences in the seasonal patterns in the prey diet between grassland and shrubland areas.

Keywords: arid ecosystem; carbon-13; coyote prey; food chain; isotope ecology; microsatellite; nitrogen-15; seasonality

1. Introduction

Predators play an important role in many ecosystems and can produce top-down effects on the abundance, diversity, and growth of organisms in lower trophic levels [1–3]. They are also prone to bottom-up effects associated with changes in the abundance or productivity of primary producers and consumers [4–6]. Pulses in primary production and plant-derived food resources associated with rainfall in an arid environment lead to spikes in both primary and secondary consumer populations [5]. The populations of some predators, including coyotes and lynx (Lynx canadensis), have been shown to closely track the abundance of prey species, with peaks in prey population size leading to heightened predator abundance and population growth [4,7,8]. Overall, the decline of a top predator can have significant effects on a local community [1–3,9], and top predators are particularly sensitive to changes that occur at the base of the food chain. These factors make top predators ideal focal organisms for investigations of the bottom-up ecological effects of both long- and short-term vegetation changes on local faunal communities.

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Woody plant encroachment, a widespread process of land cover change, is occurring in grasslands and savannas in dryland areas globally. These areas include arid, semi-arid and dry-subhumid regions [10–14]. This vegetation change is characterized by the local proliferation of one or more native woody plant species in a grass-dominated region that results in a shift from grassland to shrubland or savanna to woodland over a period of 50–200 years [10,11,15,16]. This vegetation shift could very well have significant effects on the structure and composition of local food webs [17–19]. Dryland areas where woody plant encroachment occurs have low annual rates of precipitation: 100–1200 mm/yr [12]. In many dryland areas, droughts are common and rainfall events are concentrated during a relatively short winter or summer rainy season [12,20]. This seasonal climatic variation has bottom-up effects on the local biotic community as a result of the tight coupling between water availability and various ecological processes, including grass primary production [20–24]. Seasonal variation in both prey availability and consumer diet has been observed in dryland areas [24–27]. Of particular relevance is the observation of a sharp increase in net primary production of grasses and a corresponding increase in their use during the summer monsoon, or rainy season, by consumers in a woody plant-encroached area [23,24,28]. This highlights the impact that seasonal variation in primary productivity can have on the base of the food chain in dryland areas.

The coyote is a model species for a study on the impacts of seasonal vegetation change in a woody plant-encroached area on the base of a mammalian predator food chain. The coyote is a common generalist species [29] that can also be a top predator and play an important role in the dynamics of the local community [2]. Coyote diet varies geographically [30,31] and in response to both short- and long-term changes in food resource availability [4,25,32–34]. Of particular interest is the observation that coyote fruit consumption tracks changes in the availability of fruits from different woody plants both seasonally and over a period of nearly 20 years [25]. Additionally, potential prey species of the coyote (lizards and arthropods) have been found to shift their diet in response to an increase in grass primary production during the summer monsoon season [23,24]. These observations of variation in the diet of coyotes and their prey indicate that the base of the food chain of coyotes found in an arid woody plant-encroached area is likely to be affected both by the increase in woody plant-derived resources associated with woody plant encroachment and the spike in grass-derived resources that is driven by the seasonal variation in rainfall typical of arid environments.

Shifts in the base of the food chain in response to vegetation changes associated with seasonal variation in rainfall at a woody plant-encroached site can be analyzed using stable carbon isotope techniques. C₃ and C₄ plants have distinct carbon isotope signatures as a result of differences in their photosynthetic pathways [24,35–38]. At our field site, the encroaching woody plants use the C₃ photosynthetic pathway whereas the grasses are predominately C₄ plants [16,23]. Carbon isotope techniques have been used to determine whether various herbivores are browsers that eat C₃ plants or grazers that forage on C₄ grasses [39–41]. These techniques have also shown that the base of the food chain for both primary and secondary consumers in a woody plant-encroached area shifts from C₃ to C₄ plants during the summer monsoon season [16,24].

The primary objective of this study was to assess the impact that short-term vegetation change, driven by seasonal climatic variation in a woody plant-encroached area, has on the base of the food chain of a generalist predator, the coyote. The use of stable carbon and nitrogen isotopes enabled us to determine the extent to which coyote prey, particularly mammals, were feeding on C₃ and C₄ plants in different seasons. Our specific questions were: (1) Is there seasonal variation in the base of the coyote food chain in a dryland area impacted by woody plant encroachment? and (2) Does the nature of this variation differ between grassland and woody plant-encroached shrubland communities?

We expected that there would be a seasonal shift in the base of the food chain with the portion of the diet of mammals preyed on by coyotes that comes from C₄ grasses increasing from the
spring to the summer and fall in both grassland and shrubland areas in response to increased grass productivity during the summer rainy season. We also expected that the base of the coyote food chain would differ between grassland and shrubland vegetation types such that the contribution of C4 grasses to the diet of mammalian coyote prey would be lower for coyotes sampled in shrubland communities due to the lower grass cover in these areas.

2. Study area

The fieldwork for this study was performed at the Sevilleta National Wildlife Refuge (NWR) and Long Term Ecological Research site in central New Mexico (34.32°N, 106.81°W). The refuge encompasses 1000 km² [26], has roughly 320 km of road, and contains grassland, shrubland, and woodland vegetation types (Figure 1). The mean annual rainfall from 1988 to 2009 was 236 mm [42]. The summer monsoon typically extends from July to September [24]. In 2009, July, September, and October were the months with the highest amount of rainfall (Figure 2(a)).

Approximately 72% of the refuge is covered by either grassland or shrubland. Grassland is more prevalent in the northern half of the refuge and shrubland in the southern half. The eastern side of the refuge contains an active transition zone between grama (Bouteloua spp.) grassland and creosote (Larrea tridentata) shrubland. More specifically, creosote shrubs have been moving into grama grassland areas at the refuge over the past century [16]. These characteristics make the refuge an ideal setting for an assessment of the impact that seasonal climatic variation in a dryland area affected by woody plant encroachment has on the base of the coyote food chain.

Figure 1. Map of the Sevilleta NWR and Long Term Ecological Research study site in central New Mexico, USA. Black = shrubland, gray = grassland, white = other land cover types (modified from [56]). The path of the Rio Grande through the center of the refuge is indicated by a thick black line, while the boundary of the refuge is shown by a light gray line.
Figure 2. Seasonal differences in (a) rainfall and (b) live grass cover. See text for further details of statistical analyses described here (b). (a) Monthly rainfall (mm) recorded at one location in the northeastern part of the Sevilleta NWR in 2009 (based on [42]). Horizontal gray lines and labels indicate the months when vegetation and scat surveys were completed. For the summer season, all 2009 samples were collected in July but scats from 2008 were collected in both June and July. (b) Percent live grass cover is significantly different between vegetation types and increases between the spring and the summer and fall. Significant differences among seasons are denoted by letters such that average values for percent live grass cover are significantly different between seasons labeled with different letters (i.e. ‘a’ versus ‘b’). Thus, percent live grass cover is significantly different between the spring and the summer and the spring and the fall, but not between the summer and the fall. Error bars represent 95% confidence intervals.

3. Methods

3.1. Scat surveys

Carnivore scat samples were collected between June 2008 and November 2009. Three surveys of 20 road-based transects (10 in grassland, 10 in shrubland) were performed in the summer (June–July) of 2008. Six surveys of 22 transects (12 in grassland, 10 in shrubland) were conducted in 2009, two in each of three seasons: spring (April–May), summer (July), and fall (October–November; Figure 2(a)). Each transect was 1.6 km long and was separated from all other transects by at least 1.6 km. Transects were driven at slow speed (<16.1 km/h). Each carnivore scat that was encountered was sub-sampled for genetic analysis by placing roughly 0.4 mL of the fecal material from the outside of the sample in a 2 mL tube containing DETs (dimethyl sulfoxide (DMSO), Ethylenediaminetetraacetic acid (EDTA), Tris, salt) buffer [43]. A Global Positioning System (GPS) coordinate, acquired using a Garmin GPSmap 76S, was recorded before the remainder of the sample was collected for stable carbon and nitrogen isotope analysis.

3.2. Genetic analyses

All subsamples stored in DETs buffer were extracted using QIAamp DNA Stool Mini Kits (Qiagen Inc., Valencia, CA) and manufacture’s protocols. Extractions were performed in a laboratory space dedicated to low-quantity DNA samples that was separate from extraction of concentrated DNA and polymerase chain reaction (PCR) products, and a negative control was processed with each set of extracted samples. Primers that amplify a segment of the mitochondrial DNA (mtDNA) control region [44] were used to identify the species that had deposited each scat sample [45,46] and screen out any samples deposited by canids other than the coyote. The following PCR conditions, which are modified from [45], were used for these primers: (1) 95°C for 15 min, (2) 94°C for 30 s, (3) 44°C for 90 s, (4) 72°C for 1 min, (5) 60°C for 30 min. The second through fourth steps were performed 40 times. All samples from coyotes were run through a microsatellite analysis in order
to determine the minimum number of individual coyotes that had been sampled. In particular, coyote samples were screened using primers for eight canid-specific microsatellite loci (CXX173 and CXX250 [47]; CXX377 [48]; FH2001, CXX2010, FH2054, and FH2088 [49]; and CXX119) and per locus success rates were calculated based on the results. The following PCR conditions, which are modified from those in [46], were used for the microsatellite loci: (1) 95°C for 15 min, (2) 94°C for 30 s, (3) 63°C for 90 s, (4) 72°C for 1 min, (5) 94°C for 30 s, (6) 55°C for 90 s, (7) 72°C for 1 min, and (8) 60°C for 30 min. The second through fourth steps were performed 16 times, with the temperature in step three declining by 0.5°C in each cycle. Steps five through seven were performed 31 times. Samples for which five or more loci were successfully amplified were screened two to six more times with all eight primers, and negative controls were included to monitor for contamination. Replicate microsatellite PCR results were compared in order to obtain a consensus genotype for each sample. For a homozygous locus, one allele, and only that allele, had to be seen in all replicate PCRs (three or more) while, for a heterozygous locus, each allele had to be seen at least twice. All PCR products were separated via electrophoresis on an Applied Biosystems 3130xl capillary machine, and all microsatellite data were viewed using GeneMapper 3.7 [50].

All samples for which a consensus genotype was obtained at six or more loci were analyzed using Gimlet 1.3.3 [51] in order to determine the minimum number of individuals from which samples had been collected. The reliability of genotypes that were detected only one time in the dataset was tested using RELIOTYPE [52] and a reliability criterion of 95%. Per locus error rates, for both allelic dropout and false alleles, were determined using the first two successful microsatellite PCRs for samples that were included in the Gimlet analysis and were not excluded following analysis with RELIOTYPE. GENEPOP 4.0.10 [53,54] was used to determine if the genotypes were in Hardy–Weinberg (HW) equilibrium and if they met the assumption of linkage equilibrium (LE). Deviation from HW equilibrium or rejection of the null hypothesis of the LE test could indicate that there were errors in the consensus genotypes used to determine the minimum number of individual coyotes sampled [55].

3.3. Vegetation surveys and vegetation analysis

Data on percent live grass, forb, and woody plant cover were collected in 22 circular plots, one per scat transect. Each plot was 30 m in diameter and was located at a randomly selected site within 100 m of one of the road-based scat transects. Vegetation surveys were performed in each of the three seasons in 2009 in which scat surveys were conducted (spring, summer, and fall). For each survey, percent live cover data were collected in each circular plot along two orthogonal line intercept transects that were each 30 m long and were oriented north–south and east–west. Points at which live vegetation intersected transects were recorded to the nearest 0.1 m. Vegetation was considered to be alive if its leaves (woody plants, forbs, and grasses) or stems (grasses and forbs) were green. Percent live cover data were intended to represent average vegetation conditions in the three different seasons of interest. These data were not intended to provide information on conditions during the growth period of the hair samples removed from coyote scats (see later text). Small samples of the dominant woody plant species, one of the dominant grass genera, and a representative selection of the forb species found within each plot were collected in each season for carbon and nitrogen isotope analysis (see later text).

Scat samples were assigned to grassland or shrubland vegetation types at the field site using a Landsat Thematic Mapper (TM)-derived vegetation map [56] that was simplified from 13 to four vegetation types (grassland, shrubland, woodland, and other communities; Figure 1). This simplified vegetation map and tools in ArcGIS for Desktop 10.1 [57] were used to assess vegetation type within circular areas (5.6 km²; 1335 m radius) that were centered on scat sample collection
sites. The size of the circular areas was based on a radiotelemetry study that was performed in an arid environment in southern New Mexico comparable to that at the Sevilleta NWR. This study reported that, for territorial coyotes, the mean size of a core home range, or area of high use, is 5.6 km$^2$ [58]. Percent area covered by each vegetation type was calculated for each circular area, and thus for the land within a 1335 m circular radius of each scat sample. Each scat sample was assigned to the vegetation type (grassland versus shrubland) with the highest percent area within the circular area centered on the scat’s collection site. This approach entailed the following assumptions: (1) for a given scat, a coyote moved throughout the area surrounding the scat collection site while foraging and prior to depositing the scat; (2) the coyote’s movements were random with respect to the vegetation types within the area surrounding the scat sample, thus the coyote used the vegetation type that was more prevalent within this area more often; and (3) the area used while foraging was likely to be smaller than areas of high use identified using radiotelemetry data collected over the course of roughly two months in an arid environment in southern New Mexico similar to that at the study site [58]. The last assumption was tested by using circular areas of smaller (1.7 km$^2$) and larger (12.6 and 25.2 km$^2$) sizes and assessing the effect on the results obtained. The smaller area (1.7 km$^2$) is the mean size of a coyote home range as determined using GPS coordinates recorded for scat samples collected for this study, the noninvasive genetic sampling techniques described above, and a minimum convex polygon estimate of home range size [14]. The larger (12.6 and 25.2 km$^2$) areas are based on the radiotelemetry study from which the 5.6 km$^2$ value was drawn [58]. That study reported the mean size of a coyote’s total territorial home range as 12.6; 25.2 km$^2$ is notably larger than (i.e. twice the size of) this mean home range size for an arid environment.

3.4. Carbon and nitrogen isotope analyses

Prior to carbon and nitrogen isotope analysis, all samples of scat and vegetation were oven-dried for 24 h at 70°C and 60°C, respectively. Vegetation samples were ground with a Wiley Mill. For individuals sampled in more than one season in 2009, a small subsample of hair was removed from the first scat sample collected from each individual in the summer of 2008 and in each of the three seasons that surveys were performed in 2009. If the first scat sample did not contain hair and a second sample had been collected for that individual and season, then this second sample was searched for hair. Individuals that moved between scat transects that were in different vegetation types at any point during the study were excluded from this analysis. Each hair sample represented a random subsample of all mammalian prey present in a single scat and within a single coyote’s diet. Hair was selected as an appropriate tissue for assessing seasonal variation in the diet of mammalian coyote prey as it has carbon isotope incorporation rates that fall within the length of the seasons of interest. Incorporation rates for hair of small mammals, the most frequently consumed mammalian prey of coyotes at the study site [26], are shorter (e.g. 47.5 day half-life [59]) than the 90-day time periods that roughly characterize the seasons at the field site (March–May for spring, June–August for summer, September–November for fall). Individuals were chosen as the unit of sample in order to control for inter-individual differences in foraging patterns (e.g. [60]) and capture the variation in diet among coyotes sampled in a given vegetation type and season. Choosing a single sample for most individuals was done in order to lessen any bias in the results toward individuals that had been sampled many times (Figure 3(a) and 3(b)). Further, there is evidence that sampling an individual more than three times does not add greatly to the diet diversity observed at the population level, and that there is no significant intra-individual variation in diet composition [60]. However, to test the validity of this approach and ensure that it did not overlook any impact of intra-individual variation in diet, hair was removed from all samples collected within a single season from seven individuals sampled in different parts of the study
area. Each of the individuals was sampled along transects in only one vegetation type (grassland or shrubland). Exclusion of individuals that were sampled along transects in two vegetation types was done to minimize variation associated with potential differences in the composition of the small mammal community or diet of small mammals between vegetation types [61,62]. Further, this led to the exclusion of only seven individuals and 80 samples; the majority of individuals were sampled along transects in only one vegetation type and so this approach did not lead to a large reduction in sample size.

Hair strands were cleaned with ethanol. Each item (vegetation, 4 mg; hair, 1 mg) was weighed into 5×9 mm tin capsules. The entire hair shaft was used for hair samples removing a need for homogenization. The weighed samples were analyzed on an elemental analyzer (Costech 4010) coupled to an isotope ratio mass spectrometer (IRMS, DeltaPlux XP) at the Stable Isotope Laboratory at the University of New Hampshire (UNH). In general, four out of every 58 samples were replicated, and three standards and one known ‘unknown’ were run for every 10 samples. Twenty tins containing a second known material were mixed in with the first 277 samples analyzed. Using the data from the standards, the carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope values for the samples were corrected, when necessary, for both instrument drift and linearity (2010 conversation with and 2011 email from A. Ouimette, UNH; unreferenced). Absolute values of differences in carbon and nitrogen isotope values between replicate samples were calculated in order to evaluate consistency in the measurements as well as the extent to which samples were homogeneous and representative of the diet of a random subsample of the mammalian prey in a given coyote scat sample. Hair removed from a single coyote scat could potentially all come from a single small mammal. This is confirmed by the work of others at the study site; they rarely observed remains of more than one rodent in a single coyote scat [26].

### 3.5. Statistical analyses

Differences between isotope values of C$_3$ and C$_4$ plants were assessed using a Student’s $t$-test. IsotopeR [63] was used to estimate the proportional dietary contributions of C$_3$ and C$_4$ plants to the diets of mammalian coyote prey. The mean discrimination factors for hair ($\Delta^{13}$C = 2.9‰; $\Delta^{15}$N = 3.0‰) of rats (*Rattus norvegicus*; [64]) were added to the isotope values of C$_3$ and C$_4$ plants.
Variation associated with these discrimination factors ($\Delta^{13}$C: s.d. = 0.9‰; $\Delta^{15}$N: s.d. = 0.6‰ [64]) and the digestible concentrations ([C] and [N]) of plants were used to more accurately estimate dietary parameters [63,65,66]. The following statistical analyses were performed using SAS 9.1.3 [67] or SAS 9.3 [68], as was the Student’s $t$-test (PROC TTEST) described above. Vegetation survey data were used to assess differences in vegetation characteristics between grassland and shrubland vegetation types and among seasons. Percent live grass values were analyzed using a two-way Analysis of Variance (ANOVA) with season and vegetation type as independent variables. For each individual coyote, a single scat was randomly selected from all samples for which mean values for the contribution of C$_4$ grasses to the diet of mammalian coyote prey had been calculated using IsotopeR. This random selection was automated in R 3.0.1 [69] and repeated 100 times. This approach took advantage of data from individuals for which hair was removed from more than one sample while preventing the analysis from being biased towards these individuals (Figure 3(c)). In this way, all available isotope data were used rather than just considering the isotope value for one sample per individual and ignoring data obtained on additional scats from individuals sampled more than once. A one-way ANOVA (PROC GLM) was used to assess differences in the contribution of C$_4$ grasses to mammalian coyote prey diet among seasons for each of the 100 randomly selected sets of samples. A two-way ANOVA (PROC GLM) was used to assess difference in coyote prey diet between both vegetation types (grassland versus shrubland) and among seasons for each of these random sample subsets. Prey diet values for coyote scats collected in each season of interest (spring, summer, or fall), or associated with each of six possible season by vegetation type combinations, were averaged across individuals for each of the 100 random sample subsets. A one-way ANOVA was performed on the season averages, and a two-way ANOVA was performed on the averages for unique vegetation type and season combinations. A separate two-way ANOVA was performed for each size of circular area used to assign scats to a vegetation type. Means and 95% confidence intervals were calculated using the averages for each season and each combination of vegetation type and season.

4. Results

4.1. Scat surveys and genetic analyses

A total of 935 scat samples were sub-sampled for genetic analysis. The mtDNA species identification test indicated that just over two-thirds of these samples had been deposited by coyotes. Analysis in Gimlet 1.3.3 of the 520 scats for which consensus genotypes were obtained at six or more microsatellite loci indicated that a minimum of 81 individuals had been sampled. Per locus success rates for the microsatellite analysis ranged from 0.78 to 0.88. Per locus error rates ranged from 0.04 to 0.13 for allelic dropout and from 0.01 to 0.05 for false alleles. Results of the HW exact test and LE analysis indicated that, once related individuals were removed, one locus was out of HW equilibrium (FH2088, $P = 0.02$) and genotypes at one pair of loci (CXX250, CXX377, $P = 0.03$) were not independent of one another. If Bonferroni-corrected $P$-values are used (0.05/number of comparisons; based on [55]), then no loci were out of HW equilibrium and genotypes at all loci were independent of one another.

4.2. Vegetation surveys and stable carbon and nitrogen isotope analyses

The graph of monthly precipitation shows that rainfall was the highest in the fall season (Figure 2(a)). Results of the two-way ANOVA and Duncan’s test of percent live grass cover indicate that there are differences between vegetation types ($F_{1,58} = 4.59, P = 0.04$) and between the
spring and the summer and fall seasons \( (F_{2,58} = 9.73, P = 0.0002) \) but that there is no vegetation type by season interaction \( (F_{2,58} = 0.54, P = 0.58; \) Figure 2(b)). The \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) values for 18 samples (and one replicate) of dominant C4 grasses and 34 samples (and three replicates) from C3 plants were entered into IsotopeR to account for variation in the isotopic signatures of the dietary sources (Figure 4(a); Table S1). Grass samples represent species from six genera and have a mean (± 1 s.d.) \( \delta^{13}\text{C} \) signature of 14.8‰ ± 0.5 and \( \delta^{15}\text{N} \) signature of 1.7‰ ± 2.0. C3 plant samples include 14 samples of five dominant woody plant species, 16 samples of forbs, and four samples of two woody plant species which produce fruit or seeds found in coyote scat samples collected for this study and have a mean (±1 s.d.) \( \delta^{13}\text{C} \) signature of −25.5‰ ± 1.3 and \( \delta^{15}\text{N} \) signature of 1.7‰ ± 1.9. Isotopic signatures for C3 versus C4 plants were significantly different for \( \delta^{13}\text{C} \) (\( t_{51} = 43.89, P < 0.0001 \)) but not for \( \delta^{15}\text{N} \) (\( t_{54} = 0.09, P = 0.93 \)).

Carbon and nitrogen isotope values of hair taken from 143 scat samples obtained from 45 individuals (Figures 3(c) and 4(a)) were used to calculate values for the mean contribution of C4 grasses to the diet of mammalian coyote prey (Figure 4(b) and 4(c); Table S2). For the one-way ANOVAs run on 100 randomly selected sets of these 143 samples, there were significant differences \( (P < 0.05) \) among seasons 12% of the time (12 out of 100 trials; \( F \)-values ranged

![Figure 4](image-url)

Figure 4. (a) \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) values for all hair and vegetation samples used to assess the contribution of C4 grasses to the diet of coyote mammalian prey. Average values are shown for the hair (\( n = 8 \)) and vegetation (\( n = 4 \)) samples for which replicates were run. Vegetation data were corrected by adding the following discrimination factors to each value: 2.9‰ for \( \Delta^{13}\text{C} \) and 3.0‰ for \( \Delta^{15}\text{N} \). Mean contribution of C4 grasses to the diet of mammalian coyote prey for all samples in each (b) season and (c) season by vegetation type combination. Diamonds represent the per season (b) or per season by vegetation type combination (c) means of all mean contributions of C4 grasses to prey diet as determined for hair from 143 coyote scat samples using IsotopeR. Error bars indicate the full range of mean contribution values for each season (b) or season by vegetation type combination (c). Coyotes were assigned to vegetation types using 5.6 km² circular areas.
from 0.01 to 8.12; \( P \)-values ranged from 0.001 to 0.987). Results of two-way ANOVAs run on the 100 random sample subsets were relatively consistent regardless of the size of the circular areas used to assign the samples to vegetation types except there were more significant ANOVAs for the smallest circular area used (Table 1(a)). For the one-way ANOVAs of average values for each season within each random subsample, there was significant seasonal variation (\( F_{2,297} = 207.71, P < 0.0001 \)). The contribution of \( C_4 \) grasses to the diet of coyote prey declined from spring (mean \( ± \)95% confidence interval; 45.2% \( ± \)0.23) to summer (43.0% \( ± \)0.24) and increased from summer to fall (47.0% \( ± \)0.41). Results of the two-way ANOVAs of average mammalian prey diet values for each vegetation type and season combination for each of the 100 random subsamples indicate that, regardless of the size of the circular area used to assign scats to vegetation type, there were significant differences between vegetation types and among seasons, and there was a significant vegetation by season interaction as well (Table 1(b)). Specifically, prey diet from \( C_4 \) grasses was higher in grassland areas, increased from summer to fall (Table 1(b)), and

Table 1. Results of two-way ANOVAs performed on (a) data from 100 randomly selected subsamples of the data on the contribution of \( C_4 \) grasses to the diet of mammalian coyote prey and (b) on averages across individuals within unique vegetation type and season combinations for each of the 100 subsamples.

(a)

| Circular area | # of significant ANOVAs* | Source of variation | d.f. | \( F \)-value range | \( P \)-value range |
|---------------|--------------------------|---------------------|------|----------------------|---------------------|
| 1.7 km\(^2\)  | 22                       | Vegetation          | 1    | 0–9.45               | 0.004–0.978         |
| 1.7 km\(^2\)  | 5                        | Season              | 2    | 0.02–4.75            | 0.014–0.985         |
| 1.7 km\(^2\)  | 16(1)                    | Vegetation*Season   | 1 or 2 | 0–9.67               | 0.0004–0.996       |
| 1.7 km\(^2\)  | .                        | Error               | 39 or 40 |                      |                     |
| 5.6 km\(^2\)  | 8                        | Vegetation          | 1    | 0–5.93               | 0.02–0.99          |
| 5.6 km\(^2\)  | 3                        | Season              | 2    | 0.01–4.71            | 0.015–0.988        |
| 5.6 km\(^2\)  | 2                        | Vegetation*Season   | 1 or 2 | 0–3.86               | 0.03–0.994        |
| 5.6 km\(^2\)  | .                        | Error               | 39 or 40 |                      |                     |
| 12.6 and 25.2 km\(^2\) | 6                  | Vegetation          | 1    | 0–5.98               | 0.019–0.993       |
| 12.6 and 25.2 km\(^2\) | 4                  | Season              | 2    | 0.01–3.91            | 0.028–0.992       |
| 12.6 and 25.2 km\(^2\) | 2                  | Vegetation*Season   | 1 or 2 | 0.01–3.78            | 0.032–0.983       |
| 12.6 and 25.2 km\(^2\) | .                  | Error               | 39 or 40 |                      |                     |

(b)

| Circular area | Source of variation | d.f. | \( F \)-value | \( P \)-value | Difference in prey diet |
|---------------|---------------------|------|---------------|---------------|-------------------------|
| 1.7 km\(^2\)  | Vegetation          | 1    | 256.42        | <0.0001       | 3                       |
| 1.7 km\(^2\)  | Season              | 2    | 116.68        | <0.0001       | 4                       |
| 1.7 km\(^2\)  | Vegetation*Season   | 2    | 212.52        | <0.0001       | 8                       |
| 1.7 km\(^2\)  | .                   | .    | 593           |               |                         |
| 5.6 km\(^2\)  | Vegetation          | 1    | 147.23        | <0.0001       | 3                       |
| 5.6 km\(^2\)  | Season              | 2    | 32.64         | <0.0001       | 3                       |
| 5.6 km\(^2\)  | Vegetation*Season   | 2    | 72.28         | <0.0001       | 7                       |
| 5.6 km\(^2\)  | .                   | .    | 583           |               |                         |
| 12.6 and 25.2 km\(^2\) | Vegetation                         | 1    | 118.49        | <0.0001       | 3                       |
| 12.6 and 25.2 km\(^2\) | Season                           | 2    | 14.43         | <0.0001       | 2                       |
| 12.6 and 25.2 km\(^2\) | Vegetation*Season                     | 2    | 77.97         | <0.0001       | 6                       |
| 12.6 and 25.2 km\(^2\) | .                                                 | .    | 578           |               |                         |

Note: In (a), ANOVAs with a \( P \)-value of less than 0.05 were considered to be significant and one ANOVA for the smallest circular area had a \( P \)-value less than the Bonferroni-corrected value of 0.0005. In (a) and (b), identical results were obtained for the two largest circles (12.6 and 25.2 km\(^2\)) and d.f. = degrees of freedom. In (b), difference in prey diet was calculated by subtracting average values for contribution of \( C_4 \) grasses to mammalian prey diet for coyote scat samples assigned to grassland versus shrubland vegetation types (vegetation; grassland minus shrubland); samples collected in fall versus summer (season; fall minus summer); samples collected in fall and assigned to a grassland area versus in spring and assigned to a shrubland area (vegetation*season; fall grassland minus spring shrubland). Thus, for the analysis done using 5.6 km\(^2\) circles, the consumption of \( C_4 \) grasses by mammalian coyote prey is 3% higher, on average, in the grassland than the shrubland and in the fall than the summer. Similarly, \( C_4 \) grass consumption is 7% higher in the fall in grassland areas than it is in the spring in shrubland areas.

*Count of ANOVAs with a \( P \)-value <0.0005 shown in ()’s if count >0.
Figure 5. The graph provides information on intra-individual variation in values for the mean contribution of C4 grasses to the diet of coyote prey. Prey diet values were determined using carbon and nitrogen isotope analysis of hair \((n = 38)\) samples removed from coyote scat samples collected during spring 2009. Each individual coyote \((n = 7)\) deposited three to eight samples along transects located in either grassland (individuals 1, 14, and 16; indicated by a*) or shrubland (individuals 15, 30, 38, and 55) areas. Diamonds represent per individual means of mean C4 contributions to prey diet as determined using IsotopeR. Error bars represent 95% confidence intervals.

went down in the summer in grassland areas (mean ± 95% confidence interval; scats assigned to vegetation type based on 5.6 km² areas but similar results were obtained when vegetation type was assigned using circular areas of different sizes; spring = 45.8% ± 0.26; summer = 42.9% ± 0.23; fall = 49.0% ± 0.43) but up in the summer in shrubland areas (spring = 42.3% ± 0.48; summer = 43.8% ± 0.88; fall = 42.5% ± 1.01). Regardless of the circular area used in vegetation type assignment, mean percent coyote prey diet from C4 grasses was 42% or higher in both vegetation types and all three seasons, and ranged from 43 to 51% across seasons in the grassland areas and from 42 to 44% in shrubland areas. On average, grasses represented 77–90% of total live cover, including grasses, woody plants, and forbs, in grassland areas and 51–73% of total live cover in shrubland areas. There was very little difference between \(\delta^{13}C\) and \(\delta^{15}N\) values of replicate samples of hair pieces taken from a single scat sample (mean ± 1 s.d.; -0.4‰ ± 0.3 for \(\delta^{13}C\) and 0.1‰ ± 0.1 for \(\delta^{15}N\)). Variation in the diet of mammalian prey consumed by a single coyote in a single season was fairly high (Figure 5), as was variation in diets of prey based on all scat samples (Figure 3(c)) from all coyotes sampled in each season (Figure 4(b)) and each season by vegetation type combination (Figure 4(c)).

5. Discussion

Our first objective was to determine whether there was seasonal variation in the base of the coyote food chain at the study site. Our expectation that there would be a shift in the base of the food chain from C3 plants to C4 grasses from spring to summer and fall in both grassland and shrubland areas was partially met as there was significant seasonal variation in average percent diet of mammals consumed by coyotes from C4 grasses. The observed increase in consumption of C4 grasses (increase of 4% on average; 43% versus 47%) occurred between summer and fall, which was later than expected. This increase is slightly smaller than an increase in consumption of C4 grasses (increase of 12–17%) by different small mammal species between dry and wet
periods in eastern Africa [70] and by lizards at the study site between spring (May) and the end of summer/early fall (September; [24]). Seasonal variation in percent live grass cover followed an expected trend in both vegetation types, with percent cover values increasing significantly between the spring and the summer and remaining high in the fall (Figure 2(b)). Additionally, grass accounted for a fairly consistent percentage of the diet of mammalian coyote prey (43–47% on average) in all seasons. Percent diet of mammalian coyote prey from C4 grass in grassland areas (43–51% on average) was low relative to the percentage of total live cover accounted for by grasses (77–90%). These results indicate that seasonal changes in vegetation characteristics lead to significant changes in the base of the coyote food chain, specifically the diet of the mammals preyed on by the coyotes. Grass is a consistent food resource for mammals preyed on by coyotes regardless of vegetation type and season, though its use is not as high as its availability in the grassland areas. However, it is important to note that live grass cover may have been slightly lower during the time period for which the analyzed hair samples recorded the diet of mammalian coyote prey. Specifically, the hair recorded information on prey diet for a time period that preceded the dates of the vegetation surveys, and therefore during which cumulative annual rainfall was lower (Figure 2(a) and 2(b)). These results do not mirror the definitive increase in C4-derived food resource use from spring to summer documented by Warne et al. [24] for both primary (arthropods) and secondary (lizards) consumers at the Sevilleta NWR; rather they document a delayed increase from spring to fall. This delay matches the rainfall patterns in 2009 (Figure 2(a)), as well as the slower isotope incorporation rates of the tissue considered in this study (hair; e.g. 47.5 day half-life [59]) versus Warne et al. [24] (blood plasma; e.g. 25–44 day retention time [71]), and thus the longer time required for the isotopic composition of the tissue to change in response to an increase in grass availability. Conversely, this slower incorporation rate of hair may mean that prey tissues removed from scats collected in early summer reflect prey diet in late spring, and those from early fall provide information on prey diet in late summer. Given this observation, our results may be more similar to those of Warne et al. [24] than originally thought. Warne et al. [24] also observed that, in a year of high C4 plant production, the percentage of C4-derived carbon in the blood of secondary consumers (40%) was low relative to C4 plant availability (87% of total annual net primary production). This could be explained in part by differences in nutritional quality between C3 and C4 plants; other researchers have indicated that C3 plants tend to be a higher quality food resource than C4 plants [72–74]. In particular, C3 plants tend to have higher protein, nonstructural carbohydrate, and water content while having lower carbon to nitrogen ratios, less fiber, and being overall less tough than C4 plants [73,74]. Thus, mammals preyed on by coyotes may select for C3 over C4 plants.

Our second objective was to determine whether seasonal variation in the diet of mammalian coyote prey differed between vegetation types at the study site. On average, and regardless of the size of circular areas used in assigning scats to vegetation type, there was a significant decline of roughly 3% in the percentage of C4 grasses in the diet of mammalian coyote prey between grassland and woody plant-encroached shrubland areas (Table 1(b)). This decline is small in comparison to differences between small mammals that eat primarily C4 grasses (grass specialists; >75% C4 diet) or a mixture of C3 and C4 plants (grass-browse generalists; <40–55% C4 grasses), but large compared to an observation of no significant difference in the diet of individual species between a savanna and bush habitat in eastern Africa [70]. Interestingly, C4 grasses still accounted for a fairly high percentage (42–44% on average) of the diet of coyote prey in shrubland areas, though the percentages are still somewhat low when compared to the percentage of total live cover accounted for by grasses in shrubland areas (51–73%). This indicates that mammals preyed on by coyotes still depend on grass as a food resource following the encroachment of C3 woody plants into native grassland areas. The coyote prey may actually be using grass in proportion to its availability in shrubland areas given that grass cover may have been lower during the time period over which prey diet was recorded in the analyzed hair samples.
The significant vegetation by season interaction for all sizes of circular areas used in vegetation type assignment (Table 1(b)) was unexpected; the use of C4 grasses by mammalian coyote prey declined slightly from spring to summer (3% on average) in grassland areas and from summer to fall (2% on average) in shrubland areas. The decline from spring to summer in grassland areas, and indeed for seasonal results regardless of vegetation type (2%; 45% versus 43%), might be due in part to an increase in the availability of seeds or fruits from C3 plants. For example, juniper (Juniperus monosperma) fruit is available starting in the summer [75], though juniper berries were observed in coyote scats collected at the field site more often in the fall than the summer of 2009. Further, other researchers found more fruits in coyote scats in fall than any other season [26]. However, coyote consumption of juniper berries and resources from other C3 plants may lag behind that of their mammalian prey. Small mammals, including both rodents and lagomorphs, occur frequently in the diet of coyotes at the Sevilleta NWR [26]. The rodent species that was most abundant, on average, from 1989 to 2009 (spring) and 1994–2009 (fall) in grassland areas at the Sevilleta NWR (silky pocket mouse; Perognathus flavus; [61]) consumes a high percentage of forb seeds and some shrub seeds, but no grass seeds, in the summer months in the short-grass steppe found in the northeastern part of the refuge [62]. This dietary preference for C3 forbs of the rodent that is typically most abundant in grassland areas may also help to account for the decline in C4 grasses in the diet of coyote prey in the summer months, though it is important to note that there is evidence that coyotes at the Sevilleta NWR do not forage opportunistically (i.e. in proportion to prey availability [26]). The decline in the use of C4 grasses in the fall in shrubland areas may be explained in part by the spike in forb (i.e. C3 plant) production during this season (Figure 6) and the aforementioned availability of fruits and seeds from C3 plants. This may also account for the surprising observation of a slight decline in the use of C4 grasses by coyote prey from the spring to the fall (2%) in shrubland areas when scats were assigned to vegetation type using the two largest circular areas.

Coyotes appear to have broad dietary niches in terms of their consumption of mammalian prey that eat C4 grasses versus C3 plants (Figure 4(b) and 4(c) and Figure 5). This is true for individual coyotes within a season (Figure 5) and across individuals within a season or within a particular vegetation type and season (Figure 4(b) and 4(c)). Regardless of whether they are consuming a greater diversity of prey species that are specialists, or are consuming prey that are generalists; this breadth both within and across individuals within a single season could obscure seasonal variation, and inter-vegetation type differences, in grass availability and its use by primary consumers.

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Figure 6. Variation in percent live forb cover among seasons was significant ($F_{2,58} = 8.11, P < 0.001$; two-way ANOVA performed on arcsine-transformed percentages; significant differences between seasons in live forb cover denoted by letters ‘a’ and ‘b’). Specifically, forb cover was significantly different between the spring and the fall and the summer and the fall, regardless of vegetation type. Further, forb cover was the highest in the fall season in both grassland and shrubland areas. Error bars represent 95% confidence intervals.
These fairly wide niches may help account for the relatively small magnitude of the increase in consumption of C₄ grasses from the summer to the fall (4% increase on average ignoring vegetation type; 43% versus 47%) and decrease in consumption of grasses from grassland to shrubland areas (3% decrease on average; Table 1(b)). There is some evidence that coyotes at the Sevilleta NWR frequently consume lagomorphs [26]. Lagomorphs at the refuge include desert cottontails (Sylvilagus auduboni) [26], which can inhabit both grassland and shrubland areas and consume both C₃ and C₄ plants [76], thus making them habitat and dietary generalists. This may contribute to the fairly wide dietary niches observed for the coyotes.

Only one aspect of coyote prey ecology was evaluated in this study, specifically that provided by the use of stable carbon and nitrogen isotopes. Our analyses allowed for an assessment of the diet of coyote mammalian prey, and thus the base of the coyote food chain, through a differentiation between C₄ grasses and C₃ plants, including woody plants and forbs. However, this approach did not account for other aspects of coyote prey ecology that may have been affected by seasonal variation in the availability of grass-derived resources. In particular, it did not account for seasonal shifts in prey availability or in the consumption of different prey species by the coyote. Surveys conducted in spring and fall of 2008 and 2009 in grassland and shrubland areas at the Sevilleta NWR indicate that there are inter-vegetation type differences, and sometimes inter-seasonal differences, in the relative abundances of species in the rodent community [61] and thus in a key component of the potential mammalian prey base of the coyote. Merriam’s kangaroo rat (Dipodomys merriami) was the most abundant species in both years and seasons in the shrubland vegetation type. The most abundant species in the grassland vegetation type were the silky pocket mouse (fall 2008; spring and fall 2009) and Ord’s kangaroo rat (Dipodomys ordii; spring 2008; [61]). The diets of these three species can vary among vegetation types and seasons [62]. The inter-vegetation type differences in the small mammal community observed at the Sevilleta NWR are supported by other studies; for example, Schroder and Rosenzweig [77] found that the Ord’s kangaroo rat selects for grass-dominated communities, while the Merriam’s kangaroo rat tends to use areas dominated by creosote shrubs. Furthermore, information on the body condition and fitness of both primary and secondary consumers with plants of different photosynthetic pathways and functional types at the base of their food chain (C₃ versus C₄) would help to clarify the impact that both seasonal and longer-term shifts in the availability of C₃ versus C₄ plants has on local mammalian populations. Several studies have assessed the use of C₃- versus C₄-derived food resources by primary and secondary consumers (e.g.[24,39,41,78–80]) and considered the nutritional quality of the diets of herbivores that eat C₃ versus C₄ plants [40]. In contrast and to our knowledge, very little is known regarding the effect that differences in the quality of these two food resources can have on the growth and reproductive rates of mammalian species (but see [81] for information on insects).

6. Conclusions

Overall, seasonal changes in vegetation characteristics, specifically percent live grass cover, do appear to have bottom-up effects on coyote prey at the Sevilleta NWR, and there is a decline in grass resource use from grassland to shrubland sites. The magnitude of seasonal variation and inter-vegetation type differences in the base of the coyote food chain may have been constrained by the broad ecological niche of the coyote and one of their most frequent prey items; even within a season, there was broad variation in the diet of mammalian prey of just seven of the 45 coyotes for which prey diet was evaluated. Mammalian coyote prey species utilize grass-derived food resources in both grassland and shrubland areas; the use is lower than availability in both vegetation types, though it may be roughly proportional to availability in the shrubland. Thus,
C₃ plants, which include both forbs and woody plants, may be an important food resource for mammalian coyote prey in this arid environment. This is likely the result of these plants being of higher nutritional quality for coyote prey than C₄ grasses. There are also inter-vegetation type differences in seasonal patterns in the use of C₄ grasses; consumption of C₄ grasses declines slightly in the summer in grassland areas and in the fall in shrubland areas. Given that rodents are a frequent component of coyote diet at the study site, the decline in grassland areas may be partly due to the consumption of seeds from C₃ shrubs and forbs, but not from grasses, in the summer by the rodent species that is typically most abundant in grassland areas at the Sevilleta NWR. The decline in C₄ grass consumption by mammalian coyote prey in shrubland areas may be the result of a spike in the production of C₃ forbs in the fall. More information is needed regarding the effects of different food resources (C₃ versus C₄) on the fitness and ecology of primary and secondary consumers.

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Supplemental data

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