Identifying the diet of a declining prairie grouse using DNA metabarcoding

Authors: Sullins, Daniel S., Haukos, David A., Craine, Joseph M., Lautenbach, Joseph M., Robinson, Samantha G., et al.

Source: The Auk, 135(3) : 583-608

Published By: American Ornithological Society

URL: https://doi.org/10.1642/AUK-17-199.1

The Auk
Ornithological Advances
AmericanOrnithology.org

Volume 135, 2018, pp. 583–608
DOI: 10.1642/AUK-17-199.1

RESEARCH ARTICLE

Identifying the diet of a declining prairie grouse using DNA metabarcoding

Daniel S. Sullins,[1]* David A. Haukos,[2] Joseph M. Craine,[3] Joseph M. Lautenbach,[1a] Samantha G. Robinson,[1b] Jonathan D. Lautenbach,[1] John D. Kraft,[1] Reid T. Plumb,[1c] Jonathan H. Reitz,[4] Brett K. Sandercock,[5d] and Noah Fierer[6]

[1] Kansas Cooperative Fish and Wildlife Research Unit, Division of Biology, Kansas State University, Manhattan, Kansas, USA
[2] U.S. Geological Survey, Kansas Cooperative Fish and Wildlife Research Unit, Division of Biology, Kansas State University, Manhattan, Kansas, USA
[3] Jonah Ventures, LLC, Manhattan, Kansas, USA
[4] Colorado Parks and Wildlife Department, Lamar, Colorado, USA
[5] Division of Biology, Kansas State University, Manhattan, Kansas, USA
[6] Cooperative Institute for Research in Environmental Science, University of Colorado, Boulder, Colorado, USA
[a] Current address: Sault Ste. Marie Tribe of Chippewa Indians, Sault Ste. Marie, Michigan, USA
[b] Current address: Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, Virginia, USA
[c] Current address: California Department of Fish and Wildlife, Montague, California, USA
[d] Current address: Department of Terrestrial Ecology, Norwegian Institute for Nature Research, Trondheim, Norway
* Corresponding author: sullins@ksu.edu

Submitted October 10, 2017; Accepted February 24, 2018; Published May 16, 2018

ABSTRACT

Diets during critical brooding and winter periods likely influence the growth of Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) populations. During the brooding period, rapidly growing Lesser Prairie-Chicken chicks have high calorie demands and are restricted to foods within immediate surroundings. For adults and juveniles during cold winters, meeting thermoregulatory demands with available food items of limited nutrient content may be challenging. Our objective was to determine the primary animal and plant components of Lesser Prairie-Chicken diets among native prairie, cropland, and Conservation Reserve Program (CRP) fields in Kansas and Colorado, USA, during brooding and winter using a DNA metabarcoding approach. Lesser Prairie-Chicken fecal samples ($n = 314$) were collected during summer 2014 and winter 2014–2015, DNA was extracted, amplified, and sequenced. A region of the cytochrome oxidase I (COI) gene was sequenced to determine the arthropod component of the diet, and a portion of the *trn*L intron region was used to determine the plant component. Relying on fecal DNA to quantify dietary composition, as opposed to traditional visual identification of gut contents, revealed a greater proportion of soft-bodied arthropods than previously recorded. Among 80 fecal samples for which threshold arthropod DNA reads were obtained, 35% of the sequences were most likely from Lepidoptera, 26% from Orthoptera, 14% from Araneae, 13% from Hemiptera, and 12% from other orders. Plant sequences from 137 fecal samples were composed of species similar to *Ambrosia* (27%), followed by species similar to *Lactuca* or *Taraxacum* (10%), *Medicago* (6%), and *Triticum* (5%). Forbs were the predominant (>50% of reads) plant food consumed during both brood rearing and winter. The importance both of native forbs and of a broad array of arthropods that rely on forbs suggests that disturbance regimes that promote forbs may be crucial in providing food for Lesser Prairie-Chickens in the northern portion of their distribution.

Keywords: arthropods, diet, DNA metabarcoding, foraging, forbs, grasslands, grouse, invertebrates, Lesser Prairie-Chicken, *Tympanuchus pallidicinctus*

Identificación de la dieta de un urogallo de la pradera en disminución usando meta-códigos de barra de ADN

RESUMEN

La dieta durante los períodos críticos de incubación y de invierno probablemente influencian el crecimiento de las poblaciones de *Tympanuchus pallidicinctus*. Durante el período de incubación, los polluelos en rápido crecimiento de *T. pallidicinctus* tienen altas demandas de calorías y están restringidos a alimentos dentro del entorno inmediato. Para los adultos y los juveniles durante los inviernos fríos, alcanzar las demandas de termorregulación a partir de los ítems alimenticios con contenido limitado de nutrientes puede ser un desafío. Nuestro objetivo fue determinar los componentes principales de animales y plantas de la dieta de *T. pallidicinctus* en praderas nativas, cultivos y campos del Programa de Reservas de Conservación (PRC) en Kansas y Colorado, EEUU, durante la incubación y el invierno, usando un enfoque de meta-códigos de barra de ADN. Las muestras de heces de *T. pallidicinctus* (n = 314) fueron

colectadas durante el verano de 2014 y el invierno de 2014–2015 y el ADN fue extraído, amplificado y secuenciado. Una región del gen de citocromo oxidasa I (COI) fue secuenciada para determinar el contenido de artrópodos de la dieta y una porción de la región del intrón trnL fue usada para el componente de las plantas. El uso de AND de heces para cuantificar la composición de la dieta en contraposición con la identificación visual tradicional del contenido intestinal reveló una mayor proporción de artrópodos de cuerpo blando que lo registrado previamente. Entre 80 muestras de heces de las cuales se obtuvieron umbrales de lectura del ADN de artrópodos, 35% de las secuencias fueron probablemente de Lepidoptera, 26% de Orthoptera, 14% de Araneae y 13% de Hemiptera y 12% fueron de otros órdenes. Las secuencias de plantas a partir de 137 muestras de heces estuvieron comprendidas por especies similares a *Ambrosia* (27%) seguidas de especies similares a *Lactuca* o *Taraxacum* (10%), *Medicago* (6%) y *Triticum* (5%). Los forbes fueron la planta principal (>50% de las lecturas) consumida durante la crianza de la nidada y en el invierno. La importancia de los forbes nativos y de una amplia gama de artrópodos que dependen de los forbes sugieren que los regímenes de disturbio que promueven a los forbes pueden ser críticos para brindarle alimentos a *T. pallidicinctus* en la porción norte de su distribución.

Palabras clave: ADN, artrópodos, dieta, forbes, forrajeo, invertebrados, meta-códigos de barra, pastizales, *Tympanuchus pallidicinctus*, urogallo

INTRODUCTION

Knowledge of how starvation, predation, and thermoregulation interact to regulate Lesser Prairie-Chicken populations (*Tympanuchus pallidicinctus*) is limited, in part, by a lack of knowledge of diets during critical ecological periods (McNamara and Houston 1987, Newton 1998, Patten et al. 2005, Haukos and Zavaleta 2016). Lesser Prairie-Chicken populations have experienced long-term declines and continue to decline in areas that appear to provide good-quality habitat at broad scales (Garton et al. 2016, Rodgers 2016, Spencer et al. 2017). Minimizing the degradation of remaining available habitat will require a comprehensive understanding of Lesser Prairie-Chicken biology, including dietary needs. Lesser Prairie-Chicken diets have not been well described but appear to be variable throughout the year (Olawsky 1987, Haukos and Zavaleta 2016). Most diet information is based on information from individuals collected in autumn over a small part of the species' range (Crawford and Bolen 1976, Smith 1979, Riley et al. 1993, Haukos and Zavaleta 2016). However, availability of food resources during brood rearing and winter may be most limiting for galliforms (Sedinger 1997, Sandercock et al. 2008, Hagen et al. 2009). Rapidly growing Lesser Prairie-Chicken, and other grouse (Phasianidae), chicks have high calorie demands and are restricted to foods within their immediate surroundings (Bergerud and Gratson 1988, Lautenbach 2015). For adults and juveniles, meeting thermoregulatory demands with available food items of limited nutrient content may be challenging during cold winters (Moss 1983, Olawsky 1987, Sedinger 1997).

During the brooding period, adult Lesser Prairie-Chickens and chicks consume an array of invertebrate taxa and are thought to specialize on grasshoppers (Orthoptera; Jones 1964, Suminski 1977, Davis et al. 1980). Yet this conclusion is based on only a few studies that assessed diets from crop and fecal contents and from sampling available invertebrates at locations visited by Lesser Prairie-Chickens (Haukos and Zavaleta 2016). Sampled plant and arthropod abundance may not always be a good estimator of food availability, and diets cannot always be assumed on the basis of association (Jones 1964, Davis et al. 1980, Litvaitis 2000). At feeding sites, the size, mobility, and phenology of invertebrates should constrain which arthropods are considered available prey for Lesser Prairie-Chicken chicks. Variation in arthropod prey vulnerability and availability at feeding sites, even within species, must be considered to identify optimal diets; a lack of accounting for this association may lead to erroneous conclusions (Sih and Christensen 2001).

Although arthropods are important food sources for Lesser Prairie-Chickens during summer and fall, Lesser Prairie-Chickens typically rely on plant matter to fulfill energetic demands during winter and spring (Haukos and Zavaleta 2016). Several research efforts have assessed winter diets in sand shinnery oak (*Quercus havardii*) prairie, where Lesser Prairie-Chickens readily use oak catkins and acorns when available (Jones 1964, Suminski 1977, Pettit 1986, Riley et al. 1993). Outside of periods when acorns are produced, and outside of the sand shinnery oak prairie, winter foods are less known (Salter et al. 2005, McDonald et al. 2014). The reliance on persistent woody vegetation during the winter months is well documented for grouse species, and Lesser Prairie-Chickens can make use of woody vegetation other than sand shinnery oak (Schmidt 1936, Schwilling 1955, Bergerud and Gratson 1988). For example, budding willows (*Salix* spp.) and cottonwoods (*Populus deltoides*) can be used during winter, as can portions of sand sagebrush (*Artemisia filifolia*) and skunkbrush sumac (*Rhus aromatica*; Schwilling 1955, Jones 1963). However, consumption of budding woody vegetation may be minimal in prairie-chickens in comparison to other grouse (Schmidt 1936).

Compared to other grouse, prairie-chickens may specialize on forb seeds and waste grain during winter (Schmidt 1936). Waste grain (e.g., *Sorghum* spp., *Zea* spp.) can provide an energy-rich food source for adult upland gamebirds (Evans and Dietz 1974, Bogenschutz et al. 1995, Guthery 2000). Use of grain fields by Lesser Prairie-Chickens has been reported during fall through early spring (Jamison et al. 2002); however, occurrence of Lesser Prairie-Chickens in cultivated fields has not been correlated with the amount of waste grain or related to increased body condition, survival, or reproductive output (Salter et al. 2005, Haukos and Zavaleta 2016). In addition to corn and sorghum, alfalfa (*Medicago* spp.) may be an important food resource in early spring (Jamison 2000, Larsson et al. 2013). It has been suggested that Lesser Prairie-Chickens use alfalfa fields primarily for the moisture content, and provision of moisture may make alfalfa fields more attractive than wheat (*Triticum* spp.; Larsson et al. 2013). Additionally, alfalfa may be used by prairie-chickens because it is richer in protein than other herbaceous foods (Mowat et al. 1965). In portions of their range removed from cultivation, broom snakeweed (*Gutierrezia sarothrae*), annual buckwheat (*Eriogoum annum*), and Johnny-jump-up (*Viola* spp.) may be primary winter food sources for Lesser Prairie-Chickens (Jones 1963).

True impacts on demography and contributions of food sources in the diet are difficult to estimate using traditional methods based on crop contents or scat dissection. For example, analysis of crop contents usually requires the harvesting of individuals and thus precludes any estimated impact on survival. Such post mortem analyses are not practical for species of concern. Microhistological analyses of feces are another option that can provide inference, and are noninvasive, but may underestimate easily digestible items (Bartolome et al. 1995, Litvaitis 2000). Additionally, not all contents in the crop are ultimately digested. Some of the material stored in the crop can be regurgitated (Jordan 2005). Therefore, DNA metabarcoding of fecal samples might be the best option for linking avian diets to fitness because it can identify prey items for species of conservation concern when collection of individuals is not practical (Pompanon et al. 2012). Instead of collecting individual crop samples, a standardized DNA region, or barcode, is identified that varies among, but is neutral within, taxa of interest. The DNA barcode region is amplified from fecal samples and compared to sequences from a reference database; then the relative contribution of food items can be estimated, based on the frequency of sequences (Ratnasingham and Hebert 2007, Zeale et al. 2011, Craine et al. 2015). DNA metabarcoding can be a particularly useful method for identifying soft-bodied arthropod prey items, which can be detected only by expert examination of gut contents or by histology of fecal samples (Burger et al. 1999, Zeale et al. 2011, Trevelline et al. 2016).

To estimate the effects of food availability on Lesser Prairie-Chicken populations, a stronger foundational understanding of diets used during critical life stages is needed, particularly in the northern extent of the species' range, which supports approximately two-thirds of the extant population (Garton et al. 2016, McDonald et al. 2016). Therefore, we used DNA metabarcoding of Lesser Prairie-Chicken fecal samples to quantify arthropod and plant taxa consumed by Lesser Prairie-Chickens during the brooding period and winter. We further used vegetation and arthropod survey data collected among 4 study sites in Kansas and Colorado, USA, to verify results.

METHODS

Study Area

The study area encompassed the northern extent of the Lesser Prairie-Chicken's distribution in Kansas and Colorado and included 4 study sites spread among the Mixed-Grass Prairie (Red Hills, Clark), Short-Grass Prairie/CRP Mosaic (Northwest), and Sand Sagebrush Prairie (Colorado, Clark) ecoregions (McDonald et al. 2014; Figure 1). Although the Colorado study site occurred within the Sand Sagebrush Prairie ecoregion, this site was predominantly composed of Conservation Reserve Program grassland (CRP) and cropland on the border of Prowers and Baca counties. Dominant grasses, forbs, subshrubs, shrubs, mean annual precipitation, and soil texture varied among study sites (Appendix Table 5). For example, subshrubs (e.g., *Gutierrezia sarothrae* and *Amphiachyris dracunculoides*) were more abundant than forbs in northwest Kansas and more abundant than shrubs at the Red Hills study site (Appendix Table 5). Forbs were predominantly *Salsola tragus* and *Kochia scoparia*, which were 2 of the top 3 most abundant forbs at all sites, excluding the Red Hills.

Sample Collection

We collected fecal samples during the brooding period (May–September) and winter (November–March) from Lesser Prairie-Chickens captured at leks between early March and mid-May using walk-in funnel traps and drop nets (Haukos et al. 1990, Silvy et al. 1990). We sexed the birds on the basis of plumage coloration, length of pinnae, and tail pattern (Copelin 1963). We marked female Lesser Prairie-Chickens with either a 15 g VHF transmitter or a 22 g GPS satellite PTT transmitter. We obtained locations for each VHF-marked female 3–4 times wk^{-1}, whereas females marked with GPS PTT transmitters accrued 8–10 locations day^{-1}, contingent on available daily solar energy. GPS locations were recorded every 2 hr during the day, with a 6 hr gap between 2300 and 0500 hours.

During the brooding season, we collected fecal samples from marked hens and chicks (separate vials for each)

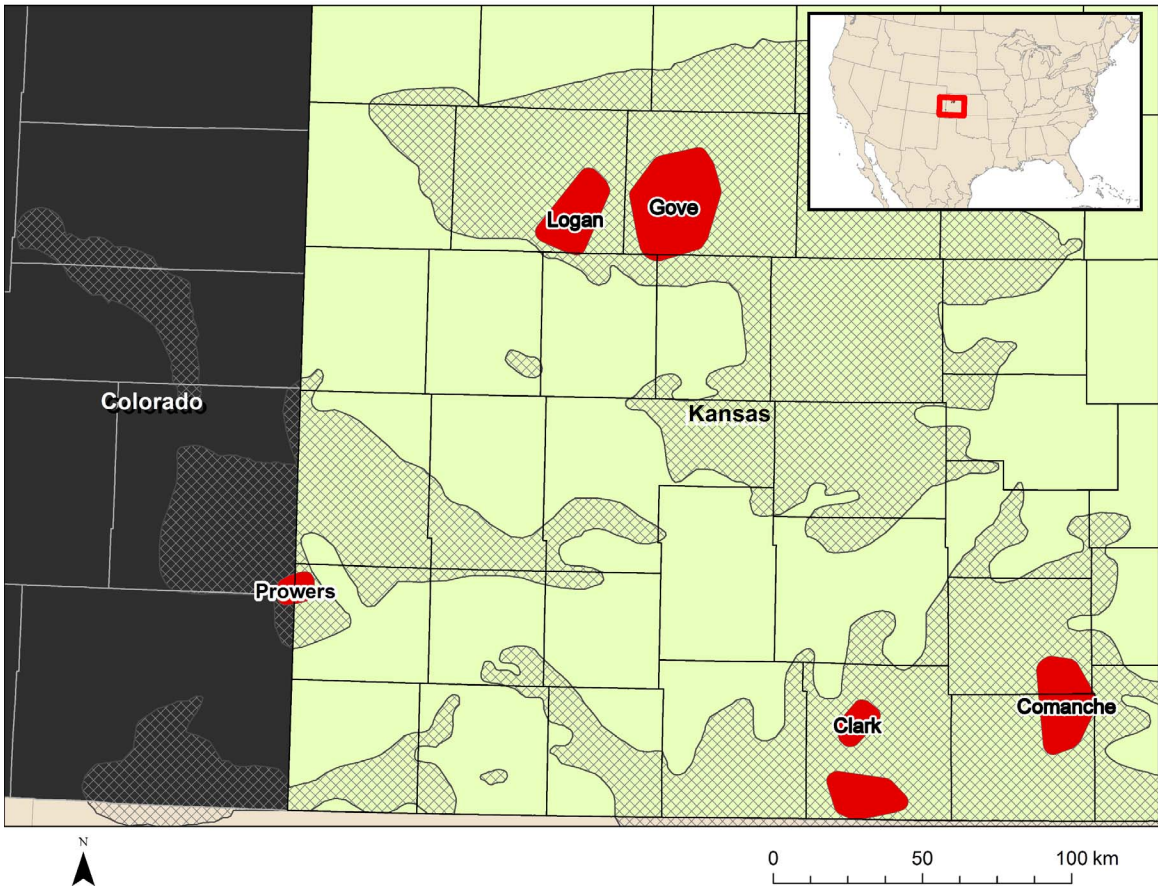


FIGURE 1. Extent of study area as determined by minimum convex polygons (shown in red) of VHF- and GPS-marked Lesser Prairie-Chickens in western Kansas and eastern Colorado, USA, 2014–2015. Study sites in Gove and Logan counties, Kansas, were combined for analyses and are referred to as "Northwest." The study site on the edge of Comanche and Kiowa counties, Kansas, is referred to as "Red Hills." The estimated current distribution of Lesser Prairie-Chickens is indicated by hatch marks (Hagen and Giesen 2005).

during brood capture and weekly flush counts occurring within 1 hr of sunrise (2–98 days old). We classified fecal samples as either chick or adult samples on the basis of their relative size differences. During winter and early spring (December–March), we collected fecal samples (≥1 pellet) at roost sites. Fresh fecal samples that were still moist and appeared to have been dropped the previous night were placed in 20 mL vials using small plastic sampling spoons to minimize DNA contamination. Vials labeled with the date, unique bird ID, and coordinates of the collection location were stored in a freezer at field sites and at Kansas State University before being shipped frozen overnight for laboratory analyses.

Sequencing

We extracted Genomic DNA from fecal samples using the PowerSoil-htp 96-well Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, California, USA). For arthropods, we amplified a fragment of the Folmer region of the cytochrome oxidase I (COI) gene using arthropod-specific primers (Bohmann et al. 2011, Zeale et al. 2011). To determine the contribution of plants to diets, a portion of the chloroplast *trn*L intron was PCR-amplified from each genomic DNA sample using the c and h *trn*L primers (Taberlet et al. 2007), but modified to include appropriate barcodes and adapter sequences for Illumina multiplexed sequencing. The barcodes used were 12 base pair (bp) error-correcting barcodes unique to each sample (Caporaso et al. 2012). Each 25 μL PCR reaction was mixed according to PCR Master Mix specifications (Promega, Madison, Wisconsin, USA), with 2 μL of genomic DNA template. For *trn*L, the thermocycling program used an initial step at 94°C for 1 min, a final extension at 72°C for 2 min, and the following steps cycled 36 times: 1 min at 94°C, 30 s at 55°C, and 30 s at 72°C. For COI, the thermocycling program used an initial step at 94°C for 5 min, a final extension at 72°C for 10 min, and the following steps cycled 45 times: 30 s at 94°C, 45 s at 45°C, and 45 s at 72°C. We cleaned amplicons from each sample and normalized them using SequalPrep Normalization Plates

(Life Technologies, Carlsbad, California) before pooling them for sequencing on a MiSeq (Illumina, San Diego, California) running the 2 × 150 bp chemistry.

Assignment of Reads to Arthropod Genera

For COI reads indicating arthropod taxa, we demultiplexed sequences using "prep_fastq_for_uparse.py" (Leff 2018). Read 2s were used for downstream analysis, due to higher-quality scores. Sequences were filtered and operational taxonomic unit (OTU) picking was performed using the UPARSE pipeline (USEARCH 7). Quality filtering included trimming sequences to the expected amplicon length (158 bp—only for 250 bp reads), filtering by quality score (maxee value of 1.5), removing sequences below the minimum expected amplicon length (90 bp), and removing singletons. We clustered sequences de novo at 99% similarity for OTU picking. We performed taxonomy assignment in QIIME, using the hierarchical naive Bayesian classifer RDP, retrained with a custom reference database curated from the Barcode of Life Database (version 3). Taxonomy was assigned at 99% similarity, with a 50% confidence threshold. We further filtered sequences to remove non-arthropod sequences by removing sequences that were not resolved to at least the family level. All samples with <10 COI reads were excluded from analysis for arthropods in diet.

We calculated the percentages of all sequences assigned to a given OTU for each sample. This is referred to as RRA (relative read abundance; Kartzinel et al. 2015). For COI, an average of 9.67% of all sequences were matched to genera in the order Diptera, almost exclusively during summer. Due to observations of contact between fecal material and dipterans, we assumed that dipteran DNA entered fecal material through secondary contact after defecation and before collection. Therefore, we excluded all dipteran reads from analyses. We limited assignment of OTU to genera present among all study sites as estimated from arthropod sweep-net survey (see details below).

Arthropod availability. We constrained assignments to taxa available for consumption in western Kansas and eastern Colorado. We used sweep-net surveys at brood locations from May to August in 2013 and 2014 to sample available arthropod prey. Sweep netting is an efficient method for sampling a wide array of invertebrate species (Yi et al. 2012). However, sweep netting can be biased toward capture of Araneae, Orthoptera, Lepidoptera, and Thysanoptera (Doxon et al. 2011, Spafford and Lortie 2013). Therefore, we didn't compare biomass estimates from sweep-net surveys directly to items detected in diet using a resource-selection type analysis. Instead, we restricted DNA metabarcoding assignments to taxa detected among all sites including genera within Orthoptera, families within Hemiptera, families detected within Coleoptera, families within Araneae, and all other taxa to the order resolution.

To perform sweep-net surveys, three 100-sweep surveys were conducted at sites where fecal samples were collected and at nearby paired random locations. Survey sweeps moved north to south, passing along 3 parallel transects 10 m apart, with the center transect passing directly through the bird location (Hagen et al. 2005). We compared cumulative biomass (g) of arthropod orders (broader taxonomic resolution) at study sites to help explain relative differences in diets among sites.

Spatial and temporal influence on the consumption of arthropods. After RRA was estimated for all arthropod (COI) reads indicative of potential foods available in the study area, we summed genus-specific RRA to estimate RRA at the order level. Using RRA, we documented the relative contribution of all orders to Lesser Prairie-Chicken diets during the brood-rearing period and winter, and then assessed orders as dependent variables in separate beta regression model sets.

We used a regression based on a parameterization of the beta distribution to examine differences in RRA for orders that were predominant in fecal samples. We evaluated the relationships of RRA values among independent variables including period (brooding period and winter), chick (yes or no) during the brood-rearing period, and study sites (Northwest, Red Hills, Clark, and Colorado; Ferrari and Cribari-Neto 2004). We developed box plots to depict the median, first, and third quartiles, and maximum and minimum values of RRA for the 4 predominantly consumed orders at each site. After screening for differences among period, site, and age class, we used a multimodel inference approach to examine how spatially and temporally related covariates influenced the composition of arthropods in the diet during the brood-rearing and winter periods, separately. We examined periods separately because of the differences in available foods based on phenology and because Lesser Prairie-Chickens use a greater abundance of arthropods in the brood-rearing period than in winter, regardless of the composition of arthropods consumed (Jones 1963).

Spatial covariates were based on the location of the fecal sample and included binary covariates (occurred in cover type = 1, otherwise = 0) for native grassland, CRP, and cropland. Also included in the model set was land cover type as a categorical covariate with multiple levels, including native grassland, CRP, and cropland as separate factors and a study-site model with multiple levels (Northwest, Red Hills, Clark, and Colorado). "Native grassland" refers to grasslands occurring on soil never previously tilled and that were typically maintained for cattle production (but note that all CRP grasslands assessed were planted with native grasses and forbs). Temporally related covariates included day since start of

period, chick age in days, and age class during the brood-rearing period (adult, juvenile). Day since start of period was set sequentially from 1, as the earliest date of bird use for a fecal sample collected, to the latest date of bird use for collected fecal samples in a period (brood rearing and winter). We conducted regression and performed multimodel inference using the packages "betareg" (Zeileis et al. 2016) and "AICmodavg" (Mazerolle 2016) in R (R Development Core Team 2016).

After fitting beta distribution regression models, we screened for period, age, and site effects based on informative beta coefficients. Beta coefficients were considered informative, or statistically meaningful, if not overlapping zero at the 85% confidence interval (CI; Arnold 2010). For multimodel inference, we ranked and selected the most parsimonious model based on Akaike's Information Criterion corrected for small sample sizes (AIC_c), for the 3 most abundant orders based on RRA. Models with $\Delta AIC_c \leq 2$ were considered equal in parsimony (Burnham and Anderson 2002, Arnold 2010).

Assignment of Reads to Plant Taxa and Functional Groups

Sequences were demultiplexed for *trn*L using a Python script (available from https://github.com/leffj/helper-code-for-uparse/blob/master/prep_fastq_for_uparse_paired.py). Paired end reads were then merged using "fastq_merge" pairs (Edgar 2010). We used "fastx_clipper" to trim primer and adaptor regions from both ends (https://github.com/agordon/fastx_toolkit) because merged reads often extended beyond the amplicon region of the sequencing construct. Sequences lacking a primer region on both ends of the merged reads were discarded. Sequences were quality trimmed to have a maximum expected number of errors per read of <0.1, and only sequences with >3 identical replicates were included in downstream analyses. BLASTN 2.2.30+ was run locally, with a representative sequence for each OTU as the query and the current National Center for Biotechnology Information (NCBI) nucleotide and taxonomy database as the reference. The tabular BLAST hit tables for each OTU representative were then parsed so that only hits with >97% query coverage and identity were kept, using the "usearch7" approach (Edgar 2013, Craine et al. 2015). The NCBI genus names associated with each hit were used to populate the OTU taxonomy assignment lists. All samples with <50 *trn*L reads were excluded from analyses of *trn*L RRA (Kartzinel et al. 2015). We estimated OTU-specific RRA and defined a representative genus for each OTU to describe composition in diet. We used the representative genera when summarizing OTU composition in diets. For example, OTUs were from species in genera similar to *Ambrosia*. We limited plant genera within OTU to those detected during extensive vegetation surveys among sites (Appendix A).

For *trn*L, an average of 4% of sequences was from *Pinus* (range: 0–51%). Because of the unlikelihood of *Pinus* biomass being consumed and the presence of *Pinus* DNA in the blanks, the one OTU that matched with *Pinus* species was removed from the dataset. For *trn*L, among the top 10 OTUs, OTU 23 did not match at 97% levels for coverage and identity for any species in the NCBI database. However, OTU 23 matched at 100% coverage and 95% identity with a *Chenopodium* species in the NCBI database and was considered a species similar to *Chenopodium* for the purposes of this study.

Functional group assignments. Because OTUs often encompassed multiple genera, we grouped RRA from different plant genera into functional groups including forbs, shrubs, subshrubs (mostly *Gutierrezia*), legumes, grasses, crops (not including alfalfa), and alfalfa. Placing genera into each functional group presented challenges because the OTUs frequently encompassed genera indicative of multiple groups (see below). However, linking plant foods consumed to specific functional groups was necessary to allow for comparisons among sites and to make direct connections to the utility of landscapes with an agricultural component. In some instances, OTUs that included genera related to both grass and crop as well as shrub and subshrub functional groups included repeat values and, therefore, added values could surpass 100%. For example, 17 of 33 OTUs that identified either grass or crop foods included both crop and native grass genera (e.g., *Triticum* and *Elymus*); 2 of 45 OTUs of genera including shrub, subshrub, and forb species included representatives of >1 functional group (e.g., *Artemisia* and *Ambrosia*); and 1 of 5 OTUs for genera of legumes included both cultivated and native species (e.g., *Medicago* and *Vicia*). To overcome functional-group overlap within OTUs, we constrained the use of crop and shrub foods to instances when each land cover type occurred within 48 hr home ranges; and we used the Bayesian approach, similar to regional assignments in Royle and Rubenstein (2004), to estimate RRA for each functional groups using identity values as a prior probability:

$$\mathrm{RRA}_{fg=k} = \left(\frac{I_{g=i}}{\sum_{\mathrm{OTU}=j}(I_g)} \times \mathrm{RRA}_{\mathrm{OTU}=j} \right)$$

We estimated an adjusted RRA for each functional group ($\mathrm{RRA}_{fg=k}$) by estimating the average identity value (I_g) among genera within an OTU and then dividing I_g by the sum of identity values for functional groups within each OTU. We then multiplied the quotient by the RRA estimated for each OTU ($\mathrm{RRA}_{\mathrm{OTU}=j}$). The adjusted RRA

accounts for the probability that each read is from a particular functional group based on the identity value. The identity value is a measure of the match between the OTU detected in the fecal sample and genus-specific reference sequence.

Plant availability. To limit plant forage possibilities to those available, and to minimize the overlap of certain OTUs encompassing multiple functional groups, we combined DNA metabarcoding inference with telemetry and extensive plant survey data. We limited native plant food availability to those genera detected during point-step transects among all study sites (Appendix A). At each study site, patches were delineated and digitized in ArcGIS 10.2 using aerial imagery from the Bing aerial basemap layer (product of ESRI, i-cubed, USDA FSA, USGS, AEX, GeoEye, Getmapping, Aerogrid, IGP) or the National Agriculture Imagery Program (NAIP) 2012 satellite imagery. Patches were identified as areas of homogeneous vegetation >2 ha in size, placed in categories (e.g., grassland, lowland, or CRP), and confirmed upon ground truthing. Within each patch, three 250 m point-step transects were conducted. Each point-step transect involved identifying the plant species for each pace (Evans and Love 1957). All delineated patches were surveyed during summer for each study site, and 20% of patches using a stratified random sample approach were surveyed during fall and winter.

To minimize overlap of certain OTUs that included multiple functional groups, we created home ranges encompassing the previous 48 hr period visited by each individual and identified the presence–absence of crop or shrub functional groups. We used minimum convex polygons for GPS-marked and buffered VHF-marked bird locations in ArcGIS 10.2 by maximum moved distance by GPS-marked birds during the 48 hr period. We used maximum distances to buffer sampled locations for VHF birds during each season. We excluded dispersing birds with straight-line movements >5 km from analyses. A 48 hr home range was used because it should encompass the spatiotemporal foraging extent incorporated into the fresh fecal sample. The 48 hr home interval encompassed a 9.9 hr fluid retention in Rock Ptarmigan (*Lagopus muta*), while providing enough locations to include foraging locations (Stevens and Hume 1998). We used occurrence of cultivated foods (row crops, alfalfa) and shrubs within an individual's home range to determine whether a bird had access to cultivated foods. We excluded cultivated crops as potential food items if there was no cropland in the 48 hr home range. After accounting for the availability of crop and shrub foods to each individual, we adjusted RRA to reflect availability by adding, or removing, functional-group possibilities. All home ranges included CRP or native grassland; therefore, forbs and grasses were included as possibilities for all individuals.

Spatial and temporal influence on the consumption of plants. After RRA was estimated for all plant functional groups (e.g., forbs, shrubs, subshrubs, legumes, grasses, and crops), we focused on univariate variation of specific functional groups among spatial and temporal independent covariates. Similar to methods described above, we used the package "betareg" in R to examine differences between periods (brooding period and winter) and among study sites (Northwest, Red Hills, Clark, and Colorado; Ferrari and Cribari-Neto 2004). Then we used a multimodel inference approach to test how differences in spatially and temporally related covariates influenced the composition of functional groups in the diet during the brood-rearing and winter periods separately.

We used the same spatially related covariates as we did for arthropods, including CRP, native grassland, crop, alfalfa, and land cover type. Temporally related covariates included day since start of period and the quadratic effect of day since start of period. We expected that the composition of functional plant groups may change later in the brood-rearing period and that plant composition of winter diets may change because only the most persistent shrub- and crop-based foods remain available during the coldest portions of winter. We followed the same multimodel inference protocol based on AIC_c and informative coefficients of beta regression models (85% CI) described above for arthropods (Burnham and Anderson 2002, Arnold 2010, Mazerolle 2016, Zeileis et al. 2016).

Evaluation of Sampled Taxonomic Richness

To examine whether sample sizes were sufficient to detect all arthropod and plant foods used by Lesser Prairie-Chickens at each study site, we used species accumulation curves depicting the relationship between number of OTUs and number of fecal samples. Species accumulation curves were generated in the R package "vegan" with the "specaccum" function, and the "Lomolino" function was used to describe the curves (Oksanen et al. 2015). From the function, we estimated an asymptote and the number of OTUs achieving a midpoint of the asymptote. We also estimated extrapolated species richness using the function "poolaccum" within package "vegan" following Chao (1987).

RESULTS

We collected a total of 314 fecal samples from Lesser Prairie-Chickens during the brood-rearing period (n = 211) and winter (n = 103) of 2014–2015. The number of samples collected varied by site and season (Table 1). Among all sites and seasons, arthropod DNA were obtained from 96 of the 314 samples, and readable plant DNA was sequenced in 152 of the 314 samples. A total of 334 plant and arthropod OTUs (unique DNA groupings)

TABLE 1. Number of collected fecal samples and those with readable plant or animal DNA (in parentheses) at each study site in the northern portion of the Lesser Prairie-Chicken range in Kansas (KS) and Colorado, USA, during the brooding period and winter 2014–2015.

	Site	All seasons	Brood rearing	Winter
Animal DNA	Colorado	28 (13)	6 (3)	22 (10)
	Clark, KS	124 (29)	81 (17)	43(12)
	Northwest, KS	117 (27)	93 (25)	24 (2)
	Red Hills, KS	45 (11)	31 (5)	14 (6)
	Total	314 (80)	211 (50)	103 (30)
Plant DNA	Colorado	28 (28)	6 (6)	22 (22)
	Clark, KS	124 (51)	81 (9)	43 (42)
	Northwest, KS	117 (53)	93 (30)	24 (23)
	Red Hills, KS	45 (18)	31 (4)	14 (14)
	Total	314 (150)	211 (49)	103 (101)

were identified among all fecal samples. Among the 80 samples that produced ≥10 COI sequences, there was an average of 376 sequences per sample. An average of 4,591 sequences per sample were present among the 150 samples that produced ≥50 *trn*L sequences (plant DNA). During the brood-rearing period, 6% (4) of the 48 hr home ranges included CRP, 22% (15) included cropland, and 72% (48) included native grassland. Of the winter 48 hr home ranges, 15% (21) included CRP, 27% (38) included cropland, and 57% (79) included native grassland.

Arthropods

A total of 75 arthropod OTUs were identified in diets of Lesser Prairie-Chickens using COI analyses. Results from OTUs encompassed 4 classes: Insecta (63), Arachnida (9), Collembola (1), and Malacostraca (1). Among these 4 classes, 12 orders and 50 families were represented. Twenty-eight of the genera were Lepidoptera, 7 Araneae, and 6 Hemiptera (Appendix Table 6). On average, 35% of the RRA was from Lepidoptera, 26% from Orthoptera, 14% from Araneae, and 13% from Hemiptera (Appendix Figure 8 and Appendix Table 7).

Sweep-net transects indicated that arthropod communities varied among study sites. Orthoptera had the greatest percent biomass among taxa at each site (Clark = 90.2%, Red Hills = 71.5%, Northwest = 73.1%, and Colorado = 46.5%), followed by Lepidoptera, Phasmatodea, and Coleoptera (Appendix Figure 9). Lepidopterans comprised >4 times more of the arthropod community biomass in Northwest and Colorado sites than in the Red Hills site and 1.6 times more than in the Clark study site.

Beta regressions suggested no differences among Lepidoptera, Orthoptera, Hemiptera, and Araneae composition in diets between the brooding period and winter (winter $\beta = 0.054 \pm 0.303$, 0.269 ± 0.293, 0.210 ± 0.265, -0.265 ± 0.279, respectively; brooding period as reference intercept). However, average reads per sample were fewer in the winter than in the brooding period for all sites except Colorado (Appendix Table 7). Given our sample size, the power of detecting a difference at an 85% CI was 0.24, 0.43, 0.47, and 0.56, respectively. Chick and adult diets during the brood-rearing period did not differ in consumption of Lepidoptera, Orthoptera, Araneae, and Hemiptera (chick $\beta = 0.013 \pm 0.403$, 0.205 ± 0.386, 0.122 ± 0.388, -0.199 ± 0.370, respectively). Beta regressions also indicated no differential consumption of foods by age for Lepidoptera, Orthoptera, Araneae, and Hemiptera (age of chick days $\beta = -0.004 \pm 0.00779$, 0.00732 ± 0.00788, -0.000999 ± 0.007839, -0.00218 ± 0.00700, respectively). There was an indication of more complicated nonlinear trends in the consumption of Lepidoptera and Orthoptera with minimal use of Lepidoptera after 40 days of age and greater consumption of Orthoptera when chicks surpassed 40 days of age (Figure 2).

The lack of variation among periods and ages is further indicated by stronger model support for land cover (Native Prairie, CRP, cropland) and site-based covariates for Lepidoptera and Araneae, which suggest that variation in arthropod diet consumption is more influenced by landscape characteristics than by temporal factors (Table 2). For Orthoptera during brood rearing, the model including date as a covariate was ranked highest but was equally parsimonious ($\Delta AIC_c < 2$) with the native grassland, crop, and CRP models, and its beta coefficient overlapped zero at the 85% CI (Table 2). The combined effect of spatially related covariates in predicting the composition of each order during both brood rearing and winter carried an average model weight of 72% (Tables 2 and 3).

Spatial variation in dietary composition was indicated by RRA among sites (Figure 3). During the brood-rearing period, presence of native grassland had the greatest influence on arthropod diet composition among Lepidoptera, Orthoptera, and Araneae but carried, on average, 30% of model weight (Table 2), which suggests that several variables were likely influential. The contribution of Lepidoptera in diets during the brood-rearing period decreased in native grassland (native grassland $\beta = -0.657 \pm 0.405$; Table 2). Consumption of lepidopterans was 2.12× less in native grassland in comparison to cropland ($23.2 \pm 6.00\%$ vs. $49.2 \pm 11.8\%$; Figure 4). Similarly, the categorical native grassland covariate was the best predictor of the consumption of Araneae, based on AIC_c, and the beta coefficient did not overlap zero at the 85% CI (native grassland $\beta = 0.559 \pm 0.379$). Araneae contributed 65× more to diets in native grassland than in other cover types and was rarely consumed in cropland ($26.2 \pm 7.02\%$ vs. $0.04 \pm 0.004\%$; Figure 4). For Orthoptera, the model including native grassland as a covariate was not informative (native grassland $\beta = 0.154 \pm 0.361$). Despite not providing a statistically meaningful

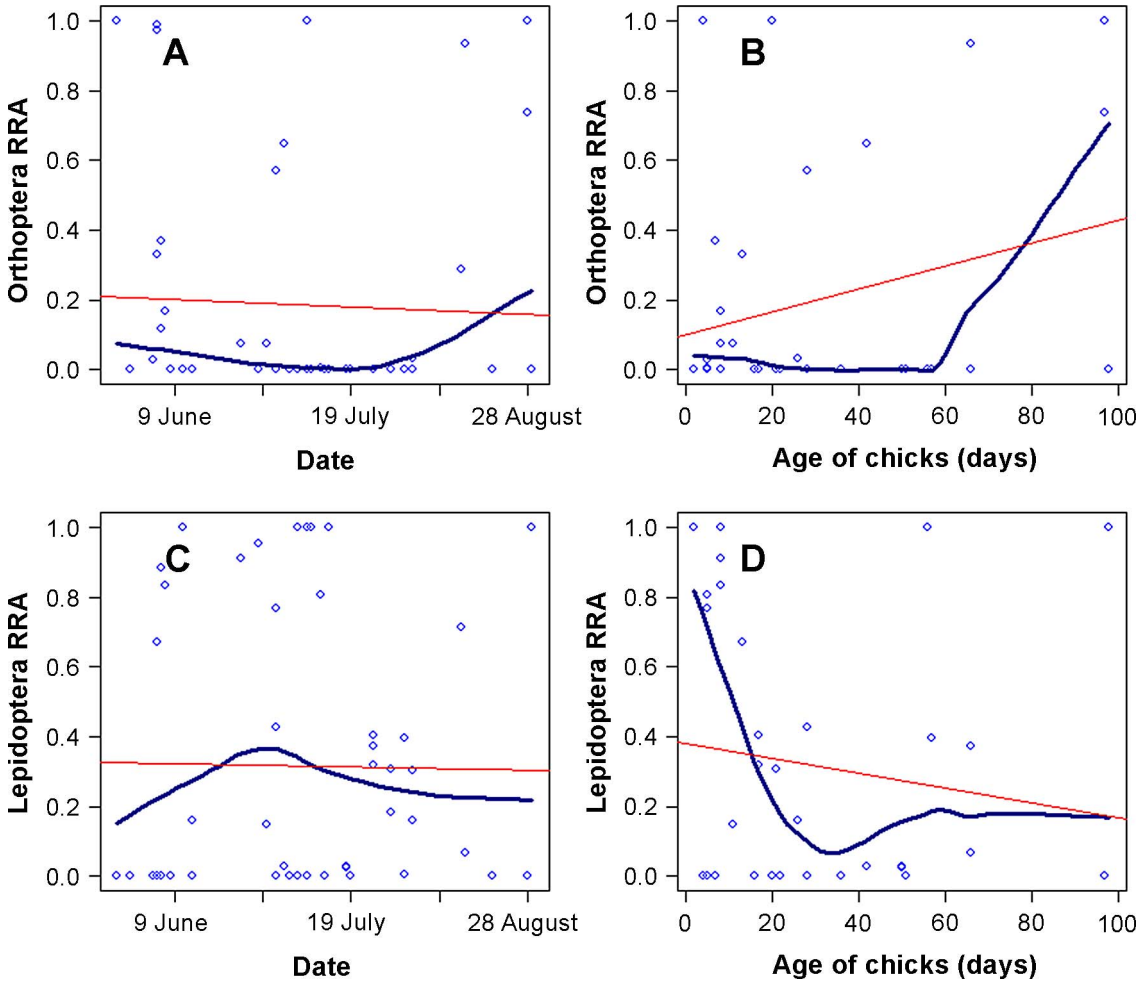


FIGURE 2. Scatter plots fitted with least squares (red) and locally weighted scatterplot smooth lines (blue) to depict patterns in the composition of Orthoptera (**A**, **B**) and Lepidoptera (**C**, **D**) in the diets of Lesser Prairie-Chicken chickens during the brood-rearing period of 2014 in Kansas and Colorado, USA. Days encompass May 27, 2014, to August 29, 2014; ages of chicks depicted range from 2 to 98 days.

difference, point estimates for Orthoptera RRA was 21.7 ± 6.50% in native grassland vs. 12.7 ± 6.71% in other cover types. Hemiptera contributed relatively equally to diets among Lesser Prairie-Chickens using CRP grassland, native grassland, and cropland (Figure 4).

In winter, Lepidoptera, Orthoptera, and Hymenoptera (most likely galls) contributed most to arthropod-based food for Lesser Prairie-Chickens (Appendix Figure 8 and Appendix Table 7). Of the top 4 orders contributing to winter diets, Orthoptera was the only order that changed (decreased) as the winter progressed, which was significant at the 85% CI (day since start of period $\beta = -0.035 \pm 0.0131$). Among sites, Clark birds had the greatest percentages of Orthoptera in their winter diet when compared to all other sites, and this was significant at the 85% CI (51.7 ± 12.6% in Clark vs. 18.3 ± 7.7% in Colorado vs. 0% in Red Hills and Northwest; Clark $\beta = 1.86 \pm 0.613$).

Plants

Metabarcoding of fecal samples indicated that Lesser Prairie-Chickens consumed foods encompassing 2 classes (Magnoliopsida and Liliopsida), 19 orders (predominantly Asterales, Poales, and Fabales), 30 families, and 90 genera. A total of 235 OTUs were found to represent ≥1% of the plant diet for a given bird at a given time. In contrast to the assignment of OTU to specific arthropod taxa, *trn*L OTUs were not genus specific and, on average, comprised 4.15 ± 4.79 genera, ranging from 1 to 28 potential genera that were present at all study sites combined. Of the 235 recorded OTUs, 70 represented ≥10% of the diet for ≥1 of the samples. The most abundant OTUs were from species in genera similar to *Ambrosia* (27% OTU-specific RRA of all reads), followed by species in genera similar to *Lactuca* or *Taraxacum* (10%), *Medicago* (6%), and *Triticum* (5%).

For the brood-rearing period, the 10 most abundant OTUs included species similar to *Ambrosia* (16.2%),

TABLE 2. Results of beta regression model for the consumption of Lepidoptera, Orthoptera, and Araneae by Lesser Prairie-Chickens in Kansas and Colorado, USA, during the brood-rearing period (June–September) of 2014. *K* is the number of parameters, AIC_c is Akaike's Information Criterion adjusted for small sample size, ΔAIC_c is the difference in AIC_c compared to the smallest value, and w_i is model weight. Models with beta coefficients not overlapping zero at the 85% confidence interval are in bold.

	Covariate [a]	*K*	AIC_c	ΔAIC_c	w_i
Lepidoptera	**Native grassland**	3	−66.03	0	0.38
	CRP	3	−64.98	1.05	0.22
	Crop	3	−63.68	2.35	0.12
	Land cover	4	−63.67	2.36	0.12
	Date	3	−63.21	2.82	0.09
	Site	5	−61.59	4.44	0.04
	Chick	3	−61.02	5.00	0.03
	Age	3	−37.34	28.68	0
Orthoptera	Date	3	−109.7	0.00	0.2
	Native grassland	3	−109.59	0.11	0.19
	Crop	3	−109.49	0.21	0.18
	CRP	3	−109.48	0.22	0.18
	Site	5	−108.62	1.08	0.12
	Chick	3	−107.42	2.28	0.06
	Land cover	4	−107.24	2.46	0.06
	Age	3	−65.88	43.82	0
Araneae	**Native grassland**	3	−133.12	0	0.34
	CRP	3	−132.42	0.7	0.24
	Date	3	−131.3	1.82	0.14
	Crop	3	−131.1	2.03	0.12
	Land cover	4	−130.76	2.36	0.1
	Chick	3	−129.09	4.03	0.04
	Site	5	−127.48	5.65	0.02
	Age	3	−76.71	56.41	0

[a] Covariates represent study site (site), day since start of period (date), adult or chick feces (chick), age in days of chick samples (age), and fecal sample located in cropland (crop), Conservation Reserve Program grassland (CRP), native working grassland, or each cover type (land cover).

TABLE 3. Beta regression model results for the consumption of Lepidoptera, Orthoptera, and Hymenoptera by Lesser Prairie-Chickens in Kansas and Colorado, USA, during winter 2014–2015. *K* is the number of parameters, AIC_c is Akaike's Information Criterion adjusted for small sample size, ΔAIC_c is the difference in AIC_c compared to the smallest value, and w_i is model weight. Models with beta coefficients not overlapping zero at the 85% confidence interval are in bold.

	Covariate [a]	*K*	AIC_c	ΔAIC_c	w_i
Lepidoptera	**Land cover**	3	−30.08	0	0.30
	Native grassland	3	−30.08	0	0.30
	CRP	3	−30.08	0	0.30
	Date	3	−27.66	2.42	0.09
	Site	5	−24.8	5.27	0.02
Orthoptera	**Date**	3	−41.49	0	0.86
	Site	5	−37.25	4.25	0.10
	Land cover	3	−32.75	8.74	0.01
	Native grassland	3	−32.75	8.74	0.01
	CRP	3	−32.75	8.74	0.01
Hymenoptera	Date	3	−62.4	0	0.24
	CRP	3	−62.4	0.01	0.24
	Land cover	3	−62.4	0.01	0.24
	Native grassland	3	−62.4	0.01	0.24
	Site	5	−57.91	4.49	0.03

[a] Covariates represent study site (site), day since start of period (date), and fecal sample located in Conservation Reserve Program grassland (CRP), native working grassland (native grassland), or each cover type (land cover).

Lactuca (8.5%), *Triticum* (5.5%), *Chenopodium* (4.3%), *Physalis* (3.9%), *Commelina* (3.1%), *Trifolium* (1.8%), and *Elymus* (1.4%). *Ambrosia* and *Triticum* were represented by 2 separate OTUs as part of the top 10 most abundant summer OTU foods. During winter, the 10 most abundant OTUs consumed included species similar to *Ambrosia* (21.0%), *Lactuca* (5.6%), *Medicago* (4.8%), *Triticum* (4.4%), *Bromus* (1.1%), *Oenothera* (0.9%), *Elymus* (0.7%), *Sorghum* (0.6%), and *Chenopodium* (0.6%). *Triticum* was represented by 2 separate OTUs as part of the top 10 most abundant winter OTUs.

Functional groups. Home ranges (48 hr) averaged 45.06 ± 44.50 ha during the nonbreeding season and 11.17 ± 8.84 ha during brood rearing for GPS-marked birds. We then used the maximum-size home ranges of nondispersing GPS-marked individuals during each time period to estimate home ranges for VHF-marked Lesser Prairie-Chickens. Home ranges for VHF birds were derived from the higher-resolution GPS-marked bird data because GPS locations were obtained frequently enough to generate 48 hr home ranges. Maximum home range sizes during the nonbreeding and brooding periods were 191.52 ha and 32.83 ha, respectively, from which we derived 781 m and 323 m buffer distances around VHF fecal collection locations to account for all potentially used food sources.

In both the brood-rearing and winter periods, forbs were the predominant plant-based food source (winter 53.7 ± 3.7%, brooding 60.67 ± 5.5%; Appendix Figure 10). Differences in the overall use of functional groups among the winter and brood-rearing periods were minimal. However, subshrubs (e.g., *Gutierrezia* spp.) and grasses contributed 1.5 times (43.4 ± 3.7% vs. 29.8 ± 5.7%) more to Lesser Prairie-Chicken diets during winter than during brood rearing (winter $\beta = 0.564 \pm 0.220$, 0.287 ± 0.195). By contrast, there was no difference in the consumption of forbs, legumes, shrubs, and crops between periods (brooding $\beta = 0.198 \pm 0.230$, –0180 ± 0.209, 0.222 ± 0.175, –0.265 ± 0.185, respectively).

We assessed differences among all sites separately for each period. Within the brood-rearing period alone, foods in the forb, grass, and legume functional groups did not differ among sites. Shrub- and subshrub-based foods contributed more to diets during the brood-rearing period in the Red Hills and northwest Kansas compared to Clark

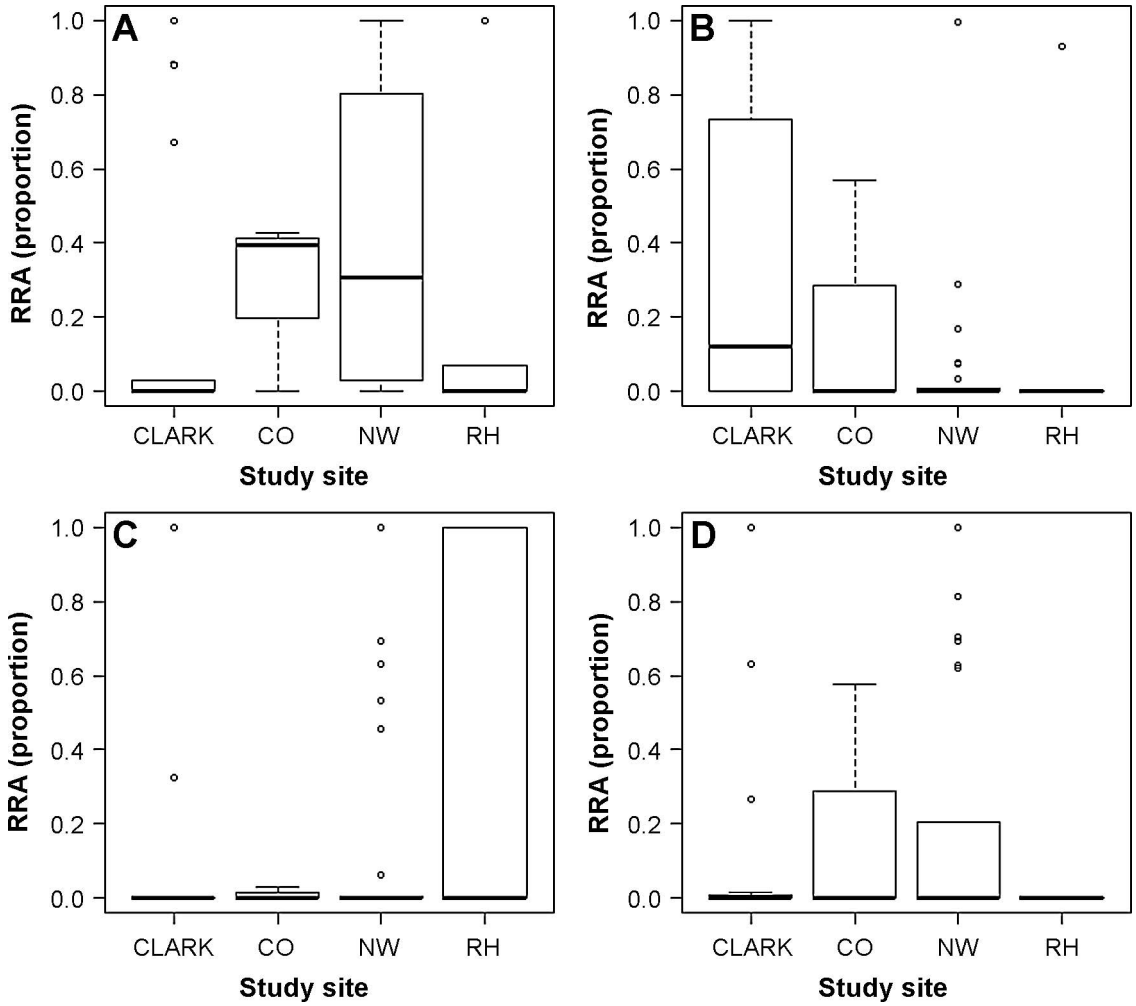


FIGURE 3. Relative readable abundance (RRA; proportion) of DNA within Lesser Prairie-Chicken fecal samples matching barcodes similar to arthropod orders (**A**) Lepidoptera, (**B**) Orthoptera, (**C**) Araneae, and (**D**) Hemiptera, grouped by study site. Fecal samples were pooled among study sites in Clark County, Kansas (Clark); Gove and Logan counties, Kansas (NW); Kiowa and Comanche counties, Kansas (RH); and Prowers and Baca counties, Colorado (CO), USA, and were collected during summer 2014 (hatch to 98 days old) from brooding females and chicks.

and Colorado (Red Hills $\beta = 1.82 \pm 0.782$, Northwest $\beta = 0.769 \pm 0.430$, Clark $\beta = 1.22 \pm 0.779$, Colorado $\beta = 0.836 \pm 0.444$). Crop-based foods provided a greater contribution to brood-rearing diets in Colorado compared to other sites ($\beta = 3.67 \pm 0.509$).

During winter, grass composition in diets varied among sites. More grasses were consumed during winter at the Northwest study than at the Clark study site (23.0 ± 2.6% vs. 11.0 ± 1.7%; $\beta = 0.855 \pm 0.289$; Figure 5). Shrub foods contributed more in winter at the Red Hills study site than at Clark ($\beta = 0.908 \pm 0.391$). Crop foods contributed more in winter to diets at the Northwest site than at Clark ($\beta = 0.443 \pm 0.288$). Last, subshrub foods contributed more in winter to diets at the Northwest and Red Hills study sites than at Clark ($\beta = 0.836 \pm 0.445$, 1.22 ± 0.779, respectively; Figure 5).

After screening for differences among periods and sites, we focused on winter diets, using a multimodel inference approach, because Lesser Prairie-Chickens predominantly consume plant material during winter (Jones 1963). Models including spatially related covariates carried, on average, 99% of model weight (AIC_c weight; Table 4). The top-ranking predictor for forb diet composition was occurrence in alfalfa and crop fields (Table 4). Forbs were consumed less in winter by Lesser Prairie-Chickens using alfalfa fields and crop fields in general ($\beta = -1.57 \pm 0.467$; identical beta values for alfalfa and crop). Forbs were more readily consumed in native grassland and CRP (Figure 6). The proportion of grass in diets was best predicted by site (Table 4; see differences above), with use of native grassland ranking second among models (native grassland $\beta = 0.386 \pm 0.238$). Birds using alfalfa and crop fields had

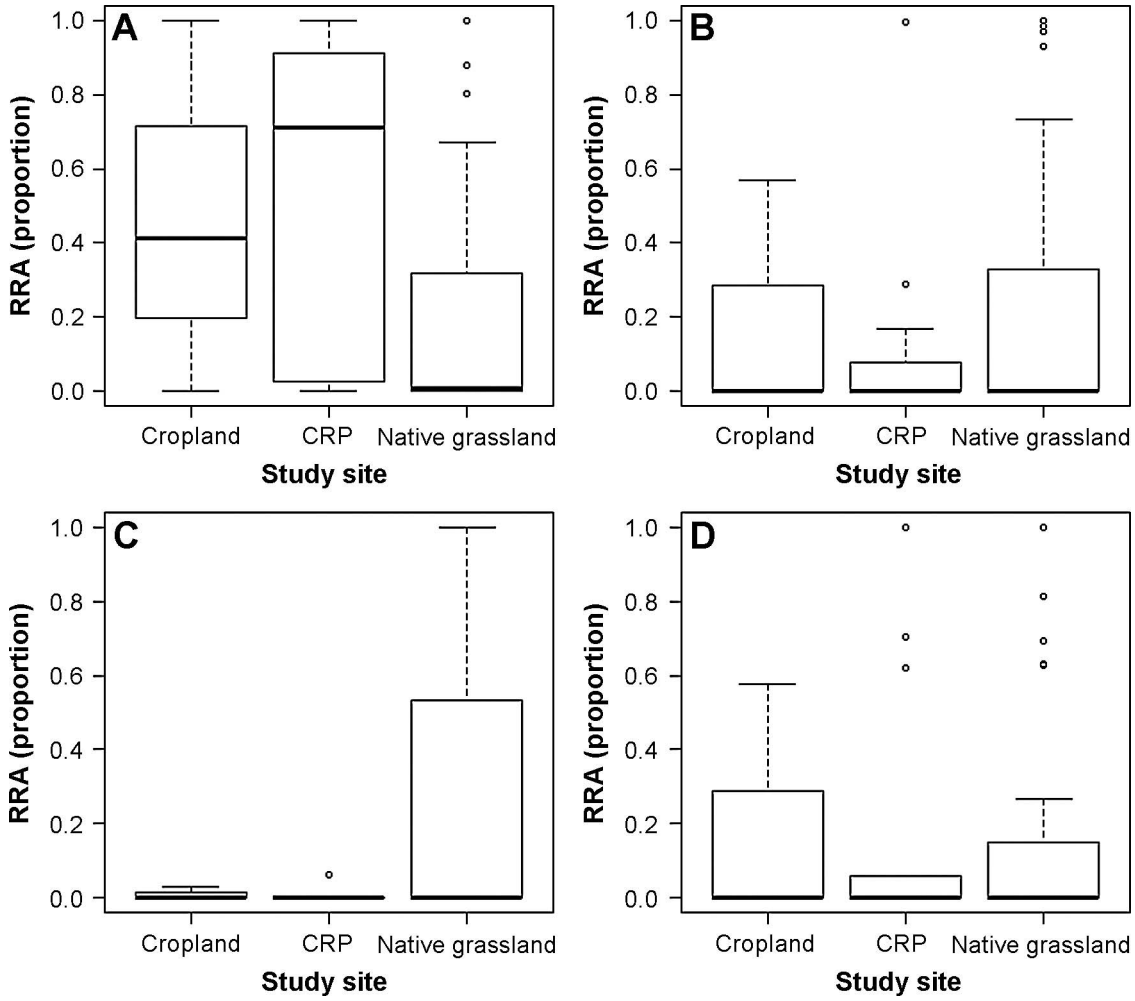


FIGURE 4. Relative readable abundance (RRA; proportion) of DNA within Lesser Prairie-Chicken fecal samples matching barcodes similar to arthropod orders (**A**) Lepidoptera, (**B**) Orthoptera, (**C**) Araneae, and (**D**) Hemiptera, grouped by land cover type where collected. Land cover types included cropland, Conservation Reserve Program grassland (CRP), and native working grassland (native grassland). Fecal samples were pooled among study sites in Clark County, Kansas; Gove and Logan counties, Kansas; Kiowa and Comanche counties, Kansas; and Prowers and Baca counties, Colorado, USA, and were collected during summer 2014 (hatch to 98 days old) from brooding females and chicks.

the greatest relative proportion of legumes in their diet ($\beta = 4.60 \pm 0.507$ for both alfalfa and crop). All fecal samples collected in cropland were collected in cultivated alfalfa, which confirms that birds can use alfalfa fields in winter as a food source. Shrubs contributed more to the diets of Lesser Prairie-Chickens using native grassland than to those in other cover types (native grassland $\beta = 1.55 \pm 0.254$; Table 4). The relative diet composition of subshrub appears to be most strongly influenced by use of crop fields, with consumption of subshrub lower in cropland ($\beta = -1.38 \pm 0.454$).

Evaluation of Sampled Taxonomic Richness

Among all sites, the arthropod species accumulation curve achieved an estimated asymptote at 156 OTUs, which suggests that we didn't sample all available forage; the midpoint for achieving an asymptote was estimated at 105 fecal samples (Figure 7). The extrapolated species richness at the OTU level (based on Chao 1987) was 101. The plant species accumulation curve achieved an estimated asymptote at 282 OTUs, which suggests that we sampled nearly all used plant forage at the OTU level. The midpoint for achieving the asymptote was estimated at 17 fecal samples (Figure 7). The extrapolated species richness at the OTU level (based on Chao 1987) was 262.

DISCUSSION

Using a combination of tools including DNA metabarcoding of fecal samples, telemetry data, and local plant and

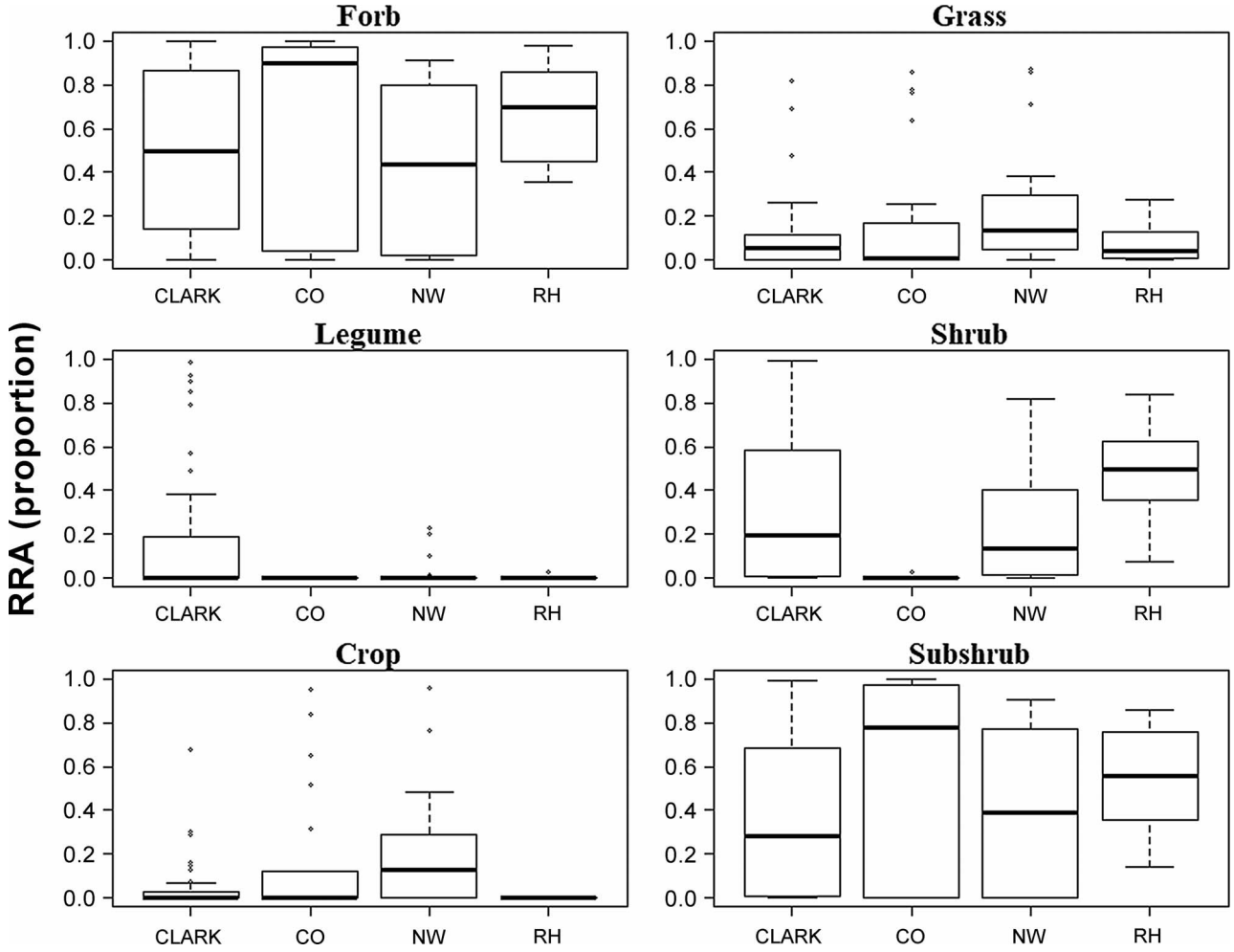


FIGURE 5. Adjusted relative readable abundance (RRA; proportion) of DNA within Lesser Prairie-Chicken fecal samples matching barcodes indicative of plant functional groups, including forbs, grasses, legumes, shrubs, crops, and subshrubs, grouped by study site. Fecal samples were collected from study sites in Clark County, Kansas (Clark); Gove and Logan counties, Kansas (NW); Kiowa and Comanche counties, Kansas (RH); and Prowers and Baca counties, Colorado (CO), USA, during winter 2014–2015 (November–March).

arthropod surveys, we identified foods consumed by Lesser Prairie-Chickens among 4 study sites. Lesser Prairie-Chickens that used native grassland maintained for cattle production consumed a greater diversity of arthropods and plant functional groups. In 48 hr home ranges that had a row-crop agriculture component, Lesser Prairie-Chickens largely used alfalfa when it was available during winter. Females and chicks, unexpectedly, preyed mostly on lepidopteran foods (likely larvae) during brood rearing. The use of shrub-based foods varied among sites but is likely not as important as in other regions (e.g., sand shinnery oak prairie) or in other grouse species (Schmidt 1936, Moss 1983, Olawsky 1987).

Arthropods in Lesser Prairie-Chicken Diets

The greater consumption of Lepidoptera in this study than was found in past research is likely a product of both the limited detection of soft-bodied prey using traditional methods and inclusion of study sites that have a strong row-crop agriculture component. Lesser Prairie-Chickens are known to consume lepidopteran larvae, yet the results of previous research suggest minimal consumption of Lepidoptera in comparison to Orthoptera (Davis et al. 1980). The traditional use of fecal dissection may not be effective in detecting lepidopteran larvae (e.g., butterfly and moth caterpillars). No study using fecal dissection identified Lepidoptera as a prey item for Lesser Prairie-Chickens (Jones 1963, Doerr and Guthery 1983). Only studies that examined crop contents have reported consumption of lepidopteran larvae (Crawford and Bolen 1976, Suminski 1977, Smith 1979, Davis et al. 1980, Riley et al. 1993). However, not all studies examining crop contents have explicitly identified Lepidoptera as a food item, and foods from this order may be clumped as "other

TABLE 4. Akaike's Information Criterion adjusted for small sample size (AIC_c), difference in AIC_c compared to the smallest value (ΔAIC_c), and model weight (w_i) for beta regression models explaining winter plant diets of Lesser Prairie-Chickens in Kansas and Colorado, USA, 2013–2014. *K* is the number of parameters. Models with beta coefficients not overlapping zero at the 85% confidence interval are in bold.

	Covariate [a]	*K*	AIC_c	ΔAIC_c	w_i
Forb	**Alfalfa**	3	−139	0	0.42
	Crop	3	−139	0	0.42
	Land cover	4	−137	2.1	0.15
	Native grassland	3	−130	8.4	0.01
	CRP	3	−127	11.9	0
	Julian date	3	−127	11.9	0
	Site	5	−126	12.3	0
	Quad date	4	−125	13.2	0
Grass	**Site**	5	−398	0	0.73
	Native grassland	3	−393	4.4	0.08
	CRP	3	−393	4.5	0.08
	Land cover	4	−392	6.4	0.03
	Julian date	3	−391	6.5	0.03
	Alfalfa	3	−391	7	0.02
	Crop	3	−391	7	0.02
	Quad date	4	−390	7.8	0.01
Legume	**Alfalfa**	3	−249	0	0.42
	Crop	3	−249	0	0.42
	Land cover	4	−247	2.2	0.14
	Native grassland	3	−241	8	0.01
	Quad date	4	−241	8.3	0.01
	CRP	3	−239	9.8	0
	Julian date	3	−239	10.2	0
	Site not estimable [b]				
Shrub	**Native grassland**	3	−479	0	0.62
	Land cover	4	−478	1.5	0.3
	Site	5	−475	4.1	0.08
	Quad date	4	−461	18.4	0
	Date	3	−445	33.7	0
	Alfalfa	3	−443	36.2	0
	Crop	3	−443	36.2	0
	CRP not estimable				
Crop	Native grassland	3	−984	0	0.18
	Alfalfa	3	−984	0.08	0.17
	Crop	3	−984	0.08	0.17
	Site	5	−983	0.75	0.12
	CRP	3	−983	1.04	0.11
	Date	3	−983	1.35	0.09
	Land cover	4	−983	1.58	0.08
	Quad date	4	−982	1.99	0.07
Subshrub	**Alfalfa**	3	−249	0	0.42
	Crop	3	−249	0	0.42
	Land cover	4	−247	2.2	0.14
	Native grassland	3	−241	8	0.01
	Quad date	4	−241	8.3	0.01
	Site	5	−239	9.7	0
	CRP	3	−239	9.8	0
	Date	3	−239	10.2	0

[a] Covariates represent study site (site), day since start of period (date), and fecal sample located in cropland (crop), Conservation Reserve Program grassland (CRP), native working grassland (native grassland), alfalfa cropland (alfalfa), or each cover type (land cover).

[b] Some models were not estimable because they had too many zeros.

insects" (Olawsky 1987), making comparisons among other studies challenging. Overall, the soft-bodied nature of caterpillars likely makes them easier to digest and subsequently harder to detect using traditional dissection approaches (Trevelline et al. 2016). DNA metabarcoding may be the least biased tool for comparing dietary composition among soft- and hard-bodied prey.

In addition to palatability, use of lepidopteran larvae during the brood-rearing period may be related to the ease of capture by a small, 13–35 g chick. Lepidopteran larvae would be easy for Lesser Prairie-Chicken chicks to obtain when occurring within reach on the ground or in shorter vegetation. It is possible that soft-bodied larvae from other orders (e.g., Coleoptera) could also be consumed when available. Although we didn't expect a greater consumption of Lepidoptera than of Orthoptera by Lesser Prairie-Chicken chicks, we predicted that chicks would be restricted to smaller arthropod prey of limited mobility (following optimal diet theory; Suminski 1977, Sih and Christensen 2001). The use of lepidopteran larvae by Lesser Prairie-Chicken chicks supports this prediction. The potential dietary selection of lepidopterans further identifies the necessity of matching life histories among predator and prey. The life-history strategies of arthropod species may largely determine their importance as a prey item.

Although Lepidoptera were used as a food source among all land cover types and sites, specific lepidopteran genera were used in agricultural landscapes. Diets of Lesser Prairie-Chickens during the brooding period were largely supported by the genera *Euxoa* and *Dargida*. These 2 genera comprise several known agricultural pest species, including army cutworms (*Euxoa auxiliaris*). Dietary use of cutworms by Lesser Prairie-Chickens was also detected in fall by Crawford and Bolen (1976) in fragmented sand shinnery oak prairie. Consumption of agricultural pests provides evidence of one ecological service provided by Lesser Prairie-Chickens that could be used to gain conservation support in private working landscapes throughout their distribution (Wenny et al. 2011).

In contrast to the prevalent consumption of Lepidoptera in their northern range, the predominant use of orthopteran foods by Lesser Prairie-Chickens is well supported by other published research (Jones 1964, Suminski 1977, Davis et al. 1980, Doerr and Guthery 1983). The difference in predominant foods (Orthoptera vs. Lepidoptera) may be a result of spatial variation among study areas, in addition to potential biases in detecting soft-bodied prey using traditional methods. Even within the present study, we detected substantial variation in diets among study sites. The greater consumption of orthopterans at the Clark study site could be driven by the limited availability of lepidopterans and an increased abundance of grasshoppers in the genus *Melanoplus* at the Clark site (Appendix Figure

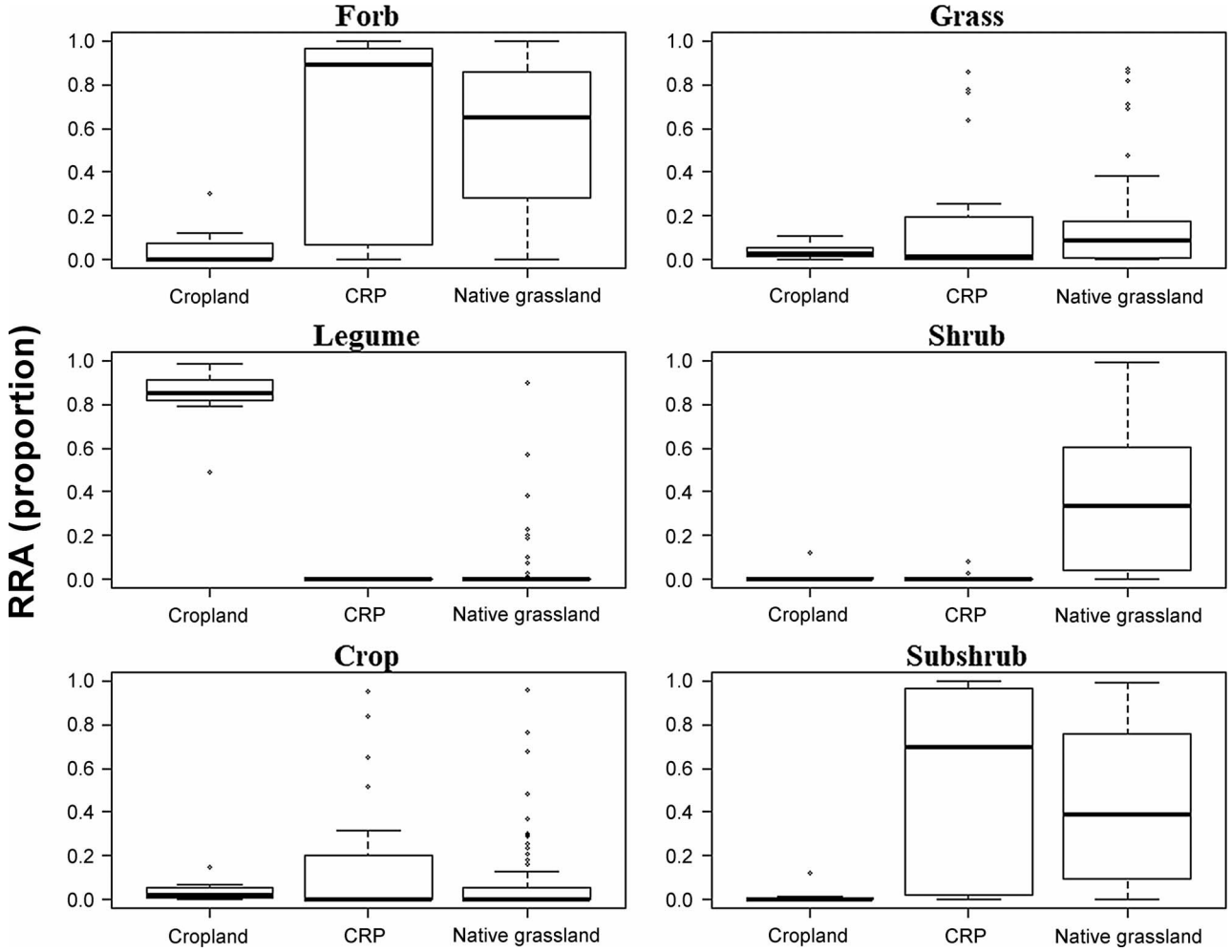


FIGURE 6. Adjusted relative readable abundance (RRA; proportion) of DNA within Lesser Prairie-Chicken fecal samples matching barcodes indicative of plant functional groups, including forbs, grasses, legumes, shrubs, crops, and subshrubs, grouped by land cover type. Land cover types included cropland, Conservation Reserve Program grassland (CRP), and native working grassland (native grassland). Fecal samples were pooled among study sites in Clark County, Kansas; Gove and Logan counties, Kansas; Kiowa and Comanche counties, Kansas; and Prowers and Baca counties, Colorado, USA, and were collected during winter 2014–2015 (November–March).

9; D. A. Haukos et al. personal observation). *Melanoplus* was the main genus of orthopterans used as a food across all sites. At the Clark study site, *Melanoplus sanguinipes* was substantially more abundant, and the roosting and morning basking of this species on bare ground may make it an easily obtainable prey item for Lesser Prairie-Chickens (Pfadt 1994, D. A. Haukos et al. personal observation).

The similar consumption of Orthoptera by Lesser Prairie-Chickens using grassland compared to cropland or CRP also doesn't provide any indication of difference in use of Lepidoptera vs. Orthoptera in grassland. Although Orthoptera composition was greatest in grassland, the RRA of Orthoptera was nearly identical to that of Lepidoptera in native grassland. Because RRA data are proportional among arthropod orders, an estimate close to 25% (split among 4 main foods) within one cover type would suggest that individuals using that cover type have more diverse diets. Although the split among the 4 orders was not perfectly uniform, Lesser Prairie-Chickens that used native grassland consumed a more diverse arthropod diet, which contrasts with our hypothesis that Lesser Prairie-Chickens would specialize on Orthopteran prey. Lesser Prairie-Chicken broods using native grassland may be opportunistic predators when diets are assessed during 0–90 days of age (Davis et al. 1980).

Despite the fact that brood diets appeared to be opportunistic when examining the brooding period as a whole, there was some indication of a nonlinear transition from Lepidoptera- to Orthoptera-dominated diets as

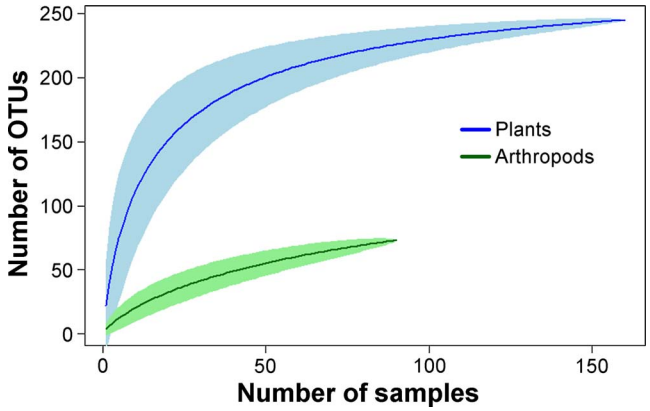


FIGURE 7. Species accumulation curves for plants and arthropods estimated using the R package "vegan" (Oksanen et al. 2015), depicting the relationship to number of operational taxonomic units (OTUs) detected in Lesser Prairie-Chicken fecal samples collected during brood rearing and winter, 2014–2015, in Kansas and Colorado, USA. Lomolino curves: plants 282.7/[1+17.1^log(2.3/*x*)]; arthropods: 156.0/[1+105.3^log (2.25/*x*)].

chicks surpassed ~40 days of age. We were particularly interested in diet during the first few weeks of a Lesser Prairie-Chicken's life. Knowledge of factors influencing survival during the first 21 days can be crucial for understanding what drives overall population growth rates (Hagen et al. 2009, McNew et al. 2012, Lautenbach 2015). The finite rate of population growth (λ) among prairie grouse and other galliformes has consistently been shown to be sensitive to variation in the 0- to 21-day-old survival bottleneck (*Tympanuchus* spp.; Wisdom and Mills 1997, Sandercock et al. 2008, Hagen et al. 2009, McNew et al. 2012, Taylor et al. 2012). Food availability may be particularly important for survival through this life stage, as indicated by strong variation in the mass of chicks and by observations of dead, undepredated chicks that may have died from starvation or thermal stress (Lautenbach 2015). Knowledge of the effects of food availability on chick survival is largely limited to inference from a closely related species within the subfamily Tetraoninane, the Greater Sage-Grouse (*Centrocercus urophasianus*). Sage-grouse chick survival can increase with the availability of Lepidoptera, slender phlox (*Phlox gracilis*), and total forb cover (Gregg and Crawford 2009). The influence of food availability on chick survival may contrast with the remainder of a grouse's life when there is strong support that predation poses the greater survival risk (Bergerud and Gratson 1988). However, if food availability drives passage through the most influential life stage and survival bottleneck, even if only lasting up to 21 days (the first 7 days may be most influential; Lautenbach 2015), the influence of food availability may be paramount and materialize in population level trajectories at much broader scales.

Comparative Nutrient Values of Lepidopterans and Orthopterans

Lepidopteran and orthopteran foods both provide greater concentrations of protein than any plant-based foods at the nutrient level (Lassiter and Edwards 1982, Savory 1989, Rumpold and Schlüter 2013). Protein in arthropod foods are also likely more digestible than that in plants (Stiven 1961, Savory 1989). On average, orthopterans can provide a food source that is 61% protein and 13% fat, whereas lepidopterans are 45% protein and 27% fat (Sugimura et al. 1984). Among protein estimates, there is interspecific variation and differences in digestibility. Furthermore, assimilation of protein from chitin-rich orthopterans and soft-bodied lepidopterans may be similar amid differences in nutrient composition (Sugimura et al. 1984). Mineral and amino acid composition provided by the 2 families appears to be similar, with variation among prey species (Rumpold and Schlütter 2013).

The Need for Ancillary Data

The potential benefits of using DNA metabarcoding to understand diets of wildlife species are numerous, but the current utility of the method hinges on ancillary data that can be used to constrain and evaluate the completeness of reference databases. We were unable to distinguish among certain plant foods that were from grass and crop functional groups using the primers we selected. The addition of 48 hr home range data allowed for greater inference on the use of cultivated foods. Additionally, reference DNA sequences for species that did not occur at any of the field sites sometimes matched sequences in fecal samples. To avoid inaccurate predictions, we constrained possible food sources to those detected during vegetation and arthropod surveys. The amplification of plant and arthropod DNA in only a proportion of the samples may be a problem unique to Lesser Prairie-Chickens and, potentially, other grouse species. For example, DNA was successfully amplified in all fecal samples from Louisiana Waterthrush (*Parkesia motacilla*), in 100% of bison (*Bison bison*) fecal samples, and in 74% of fecal samples from bats (Bohmann et al. 2011, Craine et al. 2015, Trevelline et al. 2016).

Plants

The predominant use of forbs as a food source during both brood-rearing and winter periods highlights the need to maintain disturbance regimes that support healthy forb populations (Hagen et al. 2004). Forbs provided a critical habitat component for Lesser Prairie-Chickens as food resources, even though they often comprised <10% of the available vegetation.

We detected greater RRA of forbs during brood rearing and winter, with specific forbs showing greater use during specific periods. During the brood-rearing period, forbs

consumed by Lesser Prairie-Chickens were largely from *Chenopodium*- and *Abutilon*-like species. *Chenopodium album* (lamb's quarters) was present at all field sites during summer. The leaves of *C. album* are known to be palatable and high in calcium, which may be particularly important for growing Lesser Prairie-Chicken chicks (Adedapo et al. 2011). The use of *Abutilon*-like species may indicate consumption of *Callirhoe involucrata* (purple poppy mallow) or *Sphaeralcea coccinea* (scarlet globemallow), both of which were present at all sites and actively growing during the brood-rearing period (D. A. Haukos et al. personal observation). Leaves of *S. coccinea* are high in vitamin A, calcium, and protein and can be selected as food by scaled quail (*Callipepla squamata*; Ault et al. 1983, Arthun et al. 1992). Although documentation of *C. involucrata* as food for grassland birds is limited, the plant has adequate phosphorus and crude protein content (*Odocoileus virginianus*; Everitt and Gonzalez 1981). It also functions as a known larval host and food source for several butterflies (Fernandez-Canero and Gonzalez-Redondo 2010, Scott 2014). Observations were made of several caterpillar larvae on the receptacles of *C. involucrate* flowers at the Clark study site during the brooding period (D. Sullins personal observation). The presence of *Abutilon*-like plants in Lesser Prairie-Chicken diets could be from either direct or indirect consumption mediated through lepidopteran herbivory. The presence of arthropod foods can be attained only by first providing necessary host plants.

Outside of the brooding period, plant matter becomes particularly important in Lesser Prairie-Chicken diets during winter and spring as available forage decreases, thermoregulatory needs are maximized, and stored energy becomes particularly important with approaching lekking and nesting seasons (Haukos and Zavaleta 2016). Winter diets of grouse are often limited to only a few items that can provide sustenance—typically high in fiber, low in nutrient content, and requiring longer digestive tracts to process (Moss 1983). In the present study, the greater consumption of forbs compared to all other functional groups suggests a reliance on noncultivated foods in the northern portion of the Lesser Prairie-Chicken range. Use of forbs by Lesser Prairie-Chickens contrasts with grouse of more ancestral Arctic and boreal origins that largely consume woody vegetation during winter (Schmidt 1936, Moss 1983, DeYoung and Williford 2016) but is consistent with comparatively greater predation of "weed seeds" by pinnated grouse (e.g., Greater Prairie-Chickens [*Tympanuchus cupido*]) in comparison to Sharp-tailed Grouse (*T. phasianellus*; Schmidt 1936). Forb DNA was nearly absent from fecal samples collected in cropland, which suggests that current use of herbicides may reduce the availability of forbs in cropland.

Although forbs were dominant plant foods used by Lesser Prairie-Chickens during brood rearing and in winter, the relative importance of crops, shrubs, legumes, and subshrubs as food sources increased from brood rearing to winter. The amount of grass consumed remained the same, in contrast to the results of Jones (1963), who documented a slight increase in grasses consumed during winter. The increased use of shrubs and subshrubs may be related to the persistence of shrub- and subshrub-based foods during winter. Broom snakeweed was present at all study sites. This subshrub maintains green basal leaves longer into the fall and winter compared to other plants in the region, thus providing a persistent source of leafy green vegetation (Ralphs and Wiedmeier 2004). Broom snakeweed is a known food for Lesser Prairie-Chickens and has protein and nutrient content similar to green grass, but numerous secondary metabolite compounds make broom snakeweed challenging to digest (Jones 1963, Davis et al. 1980, Ralphs and Wiedmeier 2004). Although subshrubs such as broom snakeweed may not be easy to digest, they may provide a food source, persistent throughout the winter, for which grouse have evolved advanced digestive systems to procure nutrients, as indicated by seasonal changes in gut morphology (Olawsky 1987, Sedinger 1997, Donaldson et al. 2006).

Shrub-based foods can be important for Lesser Prairie-Chickens (Jones 1964, Crawford and Bolen 1976, Suminski 1977, Olawsky 1987, Riley et al. 1993) and other grouse (Patterson 1952, Remington and Braun 1985). Most research indicating that shrubs are important for Lesser Prairie-Chickens has focused on the use of sand shinnery oak where available in Texas and New Mexico, USA (Suminski 1977, Olawsky 1987, Riley et al. 1993). Sand sagebrush, sumac (*Rhus* spp.), willow (*Salix* spp.), and cottonwood (*Populus* spp.) have also provided food for Lesser Prairie-Chickens (Schwilling 1955, Jones 1963, 1964). The increased use of shrub-based foods during winter corresponded with the increased consumption of sand sagebrush from December to February in northwest Oklahoma, USA (Jones 1963).

Outside of using persistent winter foods in the form of shrubs and subshrubs, cultivated crops can be used by Lesser Prairie-Chickens (Salter et al. 2005). Use of cultivated legumes during winter was largely restricted to the Clark study site, where the OTU containing alfalfa (*Medicago* spp., 100% identity and coverage) was consumed 1.95× more than the next leading OTU containing *Triticum*-like species. Cultivated alfalfa was available at the Clark study site and was consumed by Lesser Prairie-Chickens in distinct cropland areas. The use of alfalfa cropland at this site may explain differences in space use among regions (Robinson 2015).

Diversity and Food Stability

The greater diversity of forage in native working grassland may be key to food and nutrient stability in Lesser Prairie-Chickens. Lesser Prairie-Chickens occur in a region with the greatest variability of net primary productivity in the Great Plains (Sala et al 1998, Grisham et al. 2016). In such a variable environment, population viability may be more influenced by a stable presence of foods from year to year than by an abundance at any one time. Various arthropod and plant taxa can boom or bust in response to years of above-average precipitation or drought, and therefore food stability may be linked to a diversity of forage (Haglund 1980, Tilman and Downing 1994, Gutbrodt et al. 2011, Craine et al. 2013). Our results indicated that native working grassland provided forage for Lesser Prairie-Chickens, in addition to providing cover for reproduction and adult survival (Hagen et al. 2013). However, in some landscapes it is possible that the presence of small-scale row-crop agriculture adjacent to grassland could diversify food options (Rodgers 2016).

ACKNOWLEDGMENTS

We thank all the landowners who allowed access during the research project and all our technicians, including B. Anderson, S. Baker, S. Bard, G. Brinkman, K. Broadfoot, R. Cooper, J. Danner, J. Decker, E. Entsminger, R. Galvin, N. Gilbert, A. Godar, G. Gould, B. Hardy, S. Hoffman, D. Holt, B. Irle, T. Karish, A. Klais, H. Kruckman, K. Kuechle, S. Lane, E. Leipold, J. Letlebo, E. Mangelinckx, L. McCall, A. Nichter, K. Phillips, J. Proescholdt, J. Rabon, T. Reed, A. Rhodes, B. Ross, D. Spencer, A. Steed, A. Swicegood, P. Waldron, B. Walter, I. Waters, W. White, E. Wiens, J. Yantachka, and A. Zarazua for assisting with data collection. We greatly appreciate the logistic and technical support provided by J. Pitman, C. Hagen, J. Prendergast, K. Fricke, D. Tacha, D. Kraft, J. Ungerer, D. Krehbiel, A. Elliott, D. Dahlgren, G. Kramos, and A. Flanders. Thanks to J. Zavaleta for thought-provoking conversations related to Lesser Prairie-Chicken diets. We thank C. Conway for reviewing an earlier version of this publication.

Funding statement: Funding for the project was provided by Kansas Wildlife, Parks, and Tourism (Federal Assistance Grant KS W-73-R-3); USDA Farm Services CRP Monitoring, Assessment, and Evaluation (12-IA-MRE CRP TA#7, KSCFWRU RWO 62); and USDA Natural Resources Conservation Service, Lesser Prairie-Chicken Initiative.

Ethics statement: We complied with the *Guidelines to the Use of Wild Birds in Research* for all components of this research. We prepared protocols and obtained collection permits to capture and handle through the Kansas State University Institutional Animal Care and Use Committee protocol nos. 3241 and 3703; Kansas Department of Wildlife, Parks, and Tourism scientific collection permits SC-042-2013, SC-079-2014, and SC-001-2015; and Colorado Parks and Wildlife scientific collection license numbers 13TRb2053, 14TRb2053, and 15TRb2053.

Author contributions: D.S.S. and D.A.H. conceived the idea, design, and experiment. J.M.C., N.F., D.S.S., J.M.L., S.G.R., J.D.L., J.D.K., R.T.P., and J.H.R. performed the experiments (collected data and conducted the research). D.S.S., J.M.C., and N.F. developed or designed the methods. D.S.S. and J.M.C. analyzed the data. D.S.S., D.A.H., J.M.C., and B.K.S. wrote the paper.

LITERATURE CITED

Adedapo, A., F. Jimoh, and A. Afolayan (2011). Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album*. Acta Poloniae Pharmaceutica 68: 83–92.

Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's Information Criterion. Journal Wildlife Management 74:1175–1178.

Arthun, D., J. L. Holechek, J. D. Wallace, M. L. Galyean, M. Cardenas, and S. Rafique (1992). Forb and shrub influences on steer nitrogen retention. Journal of Range Management 45:133–136.

Ault, S. C., and F. A. Stormer (1983). Seasonal food selection by scaled quail in northwest Texas. Journal of Wildlife Management 47:222–228.

Bartolome, J., J. Franch, M. Gutman, and N. G. Seligman (1995). Technical note: Physical factors that influence fecal analysis estimates of herbivore diets. Journal of Range Management 48:267–270.

Bergerud, A. T., and M. W. Gratson (1988). Survival and breeding strategies of grouse. In Adaptive Strategies and Population Ecology of Northern Grouse, vol. 2: Theory and Synthesis (A. T. Bergerud and M. W. Gratson, Editors). University of Minnesota Press, Minneapolis, MN, USA. pp. 473–575.

Bogenschutz, T. R., D. E. Hubbard, and A. P. Leif (1995). Corn and sorghum as a winter food source for Ring-necked Pheasants. Journal of Wildlife Management 59:776–784.

Bohmann, K., A. Monadjem, C. Lehmkuhl Noer, M. Rasmussen, M. R. K. Zeale, E. Clare, G. Jones, E. Willerslev, and M. T. P. Gilbert (2011). Molecular diet analysis of two African Free-tailed Bats (Molossidae) using high throughput sequencing. PLOS One 6: e21441.

Burger, J. C., M. A. Patten, J. T. Rotenberry, R. A. Redak (1999). Foraging ecology of the California Gnatcatcher deduced from fecal samples. Oecologia 120:304–310.

Burnham, K. P., and D. R. Anderson (2002). Model Selection and Multimodal Inference: A Practical Information-Theoretic Approach, second edition. Springer, New York, NY, USA.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gromley, et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME Journal 6:1621–1624.

Chao, A. (1987). Estimating the population size for capture–recapture data with unequal catchability. Biometrics 43:783–791.

Copelin, F. F. (1963). Lesser Prairie Chicken in Oklahoma. Pittman-Robertson Project W-62-R. Oklahoma Wildlife Conservation Department, Oklahoma City, OK, USA.

Craine, J. M., T. W. Ocheltree, J. B. Nippert, E. G. Towne, A. M. Skibbe, S. W. Kembel, and J. E. Fargione (2013). Global diversity of drought tolerance and grassland climate-change resilience. Nature Climate Change 3:63–67.

Craine, J. M., E. G. Towne, M. Miller, and N. Fierer (2015). Climatic warming and the future of bison as grazers. Scientific Reports 5:16738.

Crawford, J. A., and E. G. Bolen (1976). Fall diet of Lesser Prairie Chickens in west Texas. The Condor 78:142–144.

Davis, C. A., T. Z. Riley, R. A. Smith, and M. J. Wisdom (1980). Spring–summer foods of Lesser Prairie Chickens in New Mexico. In Proceedings of Prairie Grouse Symposium (P. A. Vohs, Jr., and F. L. Knopf, Editors). Oklahoma State University Press, Stillwater, OK, USA. pp. 75–80.

DeYoung, R. W., and D. L. Williford (2016). Genetic variation and population structure in the prairie grouse: Implications for conservation of the Lesser Prairie-Chicken. Studies in Avian Biology 48:77–98.

Doerr, T. B., and F. S. Guthery (1983). Food selection by Lesser Prairie Chickens in northwest Texas. Southwestern Naturalist 28:381–383.

Donaldson, J. R., M. T. Stevens, H. R. Barnhill, and R. L. Lindroth (2006). Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). Journal of Chemical Ecology 32:1415–1429.

Doxon, E. D., C. A. Davis, and S. D. Fuhlendorf (2011). Comparison of two methods for sampling invertebrates: Vacuum and sweep-net sampling. Journal of Field Ornithology 82:60–67.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10:996–998.

Evans, K. E., and D. R. Dietz (1974). Nutritional energetics of Sharp-tailed Grouse during winter. Journal of Wildlife Management 38:622–629.

Evans, R. A., and R. M. Love (1957). The step-point method of sampling—a practical tool in range research. Journal of Range Management 10:289–297.

Everitt, J. H., and C. L. Gonzalez (1981). Seasonal nutrient content in food plants of white-tailed deer on the South Texas plains. Journal Range Management 34:506–510.

Fernandez-Canero, R., and P. Gonzalez-Redondo (2010). Green roofs as a habitat for birds: A review. Journal Animal and Veterinary Advances 9:2041–2052.

Ferrari, S. L., and F. Cribari-Neto (2004). Beta regression for modelling rates and proportions. Journal of Applied Statistics 31:799–815.

Garton, E. O., C. A. Hagen, G. M. Beauprez, S. C. Kyle, J. C. Pitman, D. D. Schoeling, and W. E. Van Pelt (2016). Population dynamics of Lesser Prairie-Chickens. Studies in Avian Biology 48:49–76.

Gregg, M. A., and J. A. Crawford (2009). Survival of Greater Sage-Grouse chicks and broods in the northern Great Basin. Journal of Wildlife Management 73:904–913.

Grisham, B. A., C. P. Griffin, and A. J. Godar (2016). Climate change. Studies in Avian Biology 48:221–242.

Gutbrodt, B., K. Mody, and S. Dorn (2011). Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120:1732–1740.

Guthery, F. S. (2000). On Bobwhites. Texas A&M University Press, College Station, USA.

Hagen, C. A., and K. M. Giesen (2005). Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*), version 2.0. In Birds of North Online (P. G. Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. http://bna.birds.cornell.edu/bna/species/364/articles/introduction

Hagen, C. A., B. A. Grisham, C. W. Boal, and D. A. Haukos (2013). A meta-analysis of Lesser Prairie-Chicken nesting and brood-rearing habitats: Implications for habitat management. Wildlife Society Bulletin 37:750–758.

Hagen, C. A., B. E. Jamison, K. M. Giesen, and T. Z. Riley (2004). Guidelines for managing Lesser Prairie-Chicken populations and their habitats. Wildlife Society Bulletin 32:69–82.

Hagen, C. A., G. C. Salter, J. C. Pitman, R. J. Robel, and R. D. Applegate (2005). Lesser Prairie-Chicken brood habitat in sand sagebrush: Invertebrate biomass and vegetation. Wildlife Society Bulletin 33:1080–1091.

Hagen, C. A., B. K. Sandercock, J. C. Pitman, R. J. Robel, and R. D. Applegate (2009). Spatial variation in Lesser Prairie-Chicken demography: A sensitivity analysis of population dynamics and management alternatives. Journal of Wildlife Management 73:1325–1332.

Haglund, B. M. (1980). Proline and valine—cues which stimulate grasshopper herbivory during drought stress? Nature 288: 697–698.

Haukos, D. A., and J. C. Zavaleta (2016). Habitat. Studies in Avian Biology 48:99–132.

Haukos, D. A., L. M. Smith, and G. S. Broda (1990). Spring trapping of Lesser Prairie-Chickens. Journal of Field Ornithology 61:20–25.

Jamison, B. E. (2000). Lesser Prairie-Chicken chick survival, adult survival, and habitat selection and movements of males in fragmented rangelands of southwestern Kansas. M.S. thesis, Kansas State University, Manhattan, KS, USA.

Jamison, B. E., R. J. Robel, J. S. Pontius, and R. D. Applegate (2002). Invertebrate biomass: Associations with Lesser Prairie-Chicken habitat use and sand sagebrush density in southwestern Kansas. Wildlife Society Bulletin 30:517–526.

Jones, R. E. (1963). Identification and analysis of Lesser and Greater prairie chicken habitat. Journal of Wildlife Management 27:757–778.

Jones, R. E. (1964). Habitat used by Lesser Prairie Chickens for feeding related to seasonal behavior of plants in Beaver County, Oklahoma. Southwestern Naturalist 9:111–117.

Jordan, M. J. R. (2005). Dietary analysis for mammals and birds: A review of field techniques and animal-management applications. International Zoo Yearbook 39:108–116.

Kartzinel, T. R., P. A. Chen, T. C. Coverdale, D. L. Erickson, W. J. Kress, M. L. Kuzmina, D. I. Rubenstein, W. Wang, and R. M. Pringle (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proceedings of the National Academy of Sciences USA 112:8019–8024.

Larsson, L. C., C. L. Pruett, D. H. Wolfe, and M. A. Patten (2013). Fine-scale selection of habitat by the Lesser Prairie-Chicken. The Southwestern Naturalist 58:135–149.

Lassiter, J. W., and H. M. Edwards, Jr. (1982). Animal Nutrition. Reston Publishing, Reston, VA, USA.

Lautenbach, J. (2015). Lesser Prairie-Chicken reproductive success, habitat selection, and response to trees. M.S. thesis, Kansas State University, Manhattan, KS, USA.

Leff, J. W. (2018). Helper-code-for-uparse. https://github.com/leffj/helper-code-for-uparse

Litvaitis, J. A. (2000). Investigating food habits of terrestrial vertebrates. In Research Techniques in Animal Ecology: Controversies and Consequences (L. Boitani and T. K. Fuller, Editors). Columbia University Press, New York, NY, USA. pp. 165–190.

Mazerolle, M. J. (2016). Package 'AICcmodavg', version 2.00. R Foundation for Statistical Computing, Vienna. http://cran.r-project.org

McDonald, L., G. Beauprez, G. Gardner, J. Griswold, C. Hagen, F. Hornsby, D. Klute, S. Kyle, J. Pitman, T. Rintz, D. Schoeling, and B. Van Pelt (2014). Range-wide population size of the Lesser Prairie-Chicken: 2012 and 2013. Wildlife Society Bulletin 38:536–546.

McDonald, L., K. Nasman, T. Rintz, F. Hornsby, and G. Gardner (2016). Range-wide population size of the Lesser Prairie-Chicken: 2012, 2013, 2014, 2015, and 2016. Technical report prepared for the Western Association of Fish and Wildlife Agencies. Western EcoSystems Technology, Laramie, WY, USA.

McNamara, J. M., and A. I. Houston (1987). Starvation and predation as factors limiting population size. Ecology 68: 1515–1519.

McNew, L. B., A. J. Gregory, S. M. Wisely, and B. K. Sandercock (2012). Demography of Greater Prairie-Chickens: Regional variation in vital rates, sensitivity values, and population dynamics. Journal of Wildlife Management 76:987–1000.

Moss, R. (1983). Gut size, woody weight, and digestion of winter foods by grouse and ptarmigan. The Condor 85:185–193.

Mowat, D. N., R. S. Fulkerson, W. E. Tossell, and J. E. Winch (1965). The in vitro digestibility and protein content of leaf and stem portions of forages. Canadian Journal of Plant Science 45: 321–331.

Newton, I. (1998). Population Limitation in Birds. Academic Press, San Diego, CA, USA.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner (2015). vegan: Community Ecology Package. R package version 2.4-2. http://CRAN.R-project.org/package=vegan

Olawsky, C. D. (1987). Effects of shinnery oak control with tebuthiuron on Lesser Prairie-Chicken populations. M.S. thesis, Texas Tech University, Lubbock, USA.

Patten, M. A., D. H. Wolfe, E. Shochat, and S. K. Sherrod (2005). Effects of microhabitat and microclimate selection on adult survivorship of the Lesser Prairie-Chicken. Journal of Wildlife Management 69:1270–1278.

Patterson, R. L. (1952). The Sage Grouse in Wyoming. Sage Books, Denver, CO, USA.

Pettit, R. D. (1986). Sand shinnery oak control and management. Management Note 8. Natural Resources Management, Texas Tech University, Lubbock, TX, USA.

Pfadt, R. E. (1994). Migratory Grasshopper: *Melanoplus sanguinipes* (Fabricius). Species Fact Sheet. Wyoming Agricultural Experiment Station Technical Bulletin 912.

Pompanon, F., B. E. Deagle, W. O. C. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet (2012). Who is eating what: Diet assessment using next generation sequencing. Molecular Ecology 21:1931–1950.

Ralphs, M. H., and R. D. Wiedmeier (2004). Conditioning cattle to graze Broom Snakeweed (*Gutierrezia sarothrae*). Journal of Animal Science 82:3100–3106.

Ratnasingham, S., and P. D. N. Hebert (2007). BOLD: The barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes 7:355–364.

R Development Core Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Remington, T. E., and C. E. Braun (1985). Sage Grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055–1061.

Riley, T. Z., C. A. Davis, and R. A Smith (1993). Autumn and winter foods of the Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) (Galliformes: Tetraonidae). Great Basin Naturalist 53: 186–189.

Robinson, S. (2015). Landscape ecology, survival, and space use of Lesser Prairie-Chickens. M.S. thesis, Kansas State University, Manhattan, KS, USA.

Rodgers, R. D. (2016). A history of Lesser Prairie-Chickens. Studies in Avian Biology 48:15–38.

Royle, J. A., and D. R. Rubenstein (2004). The role of species abundance in determining breeding origins of migratory birds with stable isotopes. Ecological Applications 14:1780–1788.

Rumpold, B. A., and O. K. Schlüter (2013). Nutritional composition and safety aspects of edible insects. Molecular Nutrition & Food Research 57:802–823.

Sala, O. E., W. J. Parton, L. A. Joyce, and W. K. Lauenroth (1988). Primary production of the central grassland region of the United States. Ecology 69:40–45.

Salter, G. C., R. J. Robel, and K. E. Kemp (2005). Lesser Prairie-Chicken use of harvested corn fields during fall and winter in southwestern Kansas. Prairie Naturalist 37:1–9.

Sandercock, B. K., W. E. Jensen, C. K. Williams, and R. D. Applegate (2008). Demographic sensitivity of population change in Northern Bobwhite. Journal of Wildlife Management 72:970–982.

Savory, C. J. (1989). The importance of invertebrate food to chicks of gallinaceous species. Proceedings of the Nutrition Society 48:113–133.

Schmidt, F. (1936). Winter food of the Sharp-tailed Grouse and pinnated grouse in Wisconsin. The Wilson Bulletin 48:186–203.

Schwilling, M. D. (1955). A study of the Lesser Prairie-Chicken in Kansas. Kansas Forestry, Fish and Game Commission, Pratt, KS, USA.

Scott, J. A. (2014). Lepidoptera of North America 13. Flower visitation by Colorado butterflies (40,615 records) with a review of the literature on pollination of Colorado plants and butterfly attraction (Lepidoptera: Hesperioidea and Papilionoidea). Contributions of the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, CO, USA.

Sedinger, J. S. (1997). Adaptation to and consequences of an herbivorous diet in grouse and waterfowl. The Condor 99: 314–326.

Sih, A., and B. Christensen (2001). Optimal diet theory: When does it work, and when and why does it fail? Animal Behaviour 61:379–390.

Silvy, N. J., M. E. Morrow, E. Shanley, and R. D. Slack (1990). An improved drop net for capturing wildlife. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 44:374–378.

Smith, R. A. (1979). Fall and winter habitat of Lesser Prairie Chickens in southeastern New Mexico. M.S. thesis, New Mexico State University, Las Cruces, NM, USA.

Spafford, R. D., and C. J. Lortie (2013). Sweeping beauty: Is grassland arthropod community composition effectively estimated by sweep netting? Ecology and Evolution 3: 3347–3358.

Spencer, D., D. A. Haukos, C. Hagen, M. Daniels, and D. Goodin (2017). Conservation Reserve Program mitigates grassland loss in the Lesser Prairie-Chicken range of Kansas. Global Ecology and Conservation 9:21–38.

Stevens, C. E., and I. D. Hume (1998). Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiological Reviews 78:393–427.

Stiven, A. E. (1961). Food energy available for and required by the Blue Grouse chick. Ecology 42:547–553.

Sugimura, K., E. Hori, Y. Kurihara, and S. Itoh (1984). Nutritional value of earthworms and grasshoppers as poultry feed. Japanese Poultry Science 21:1–7.

Suminski, H. R. (1977). Habitat evaluation for Lesser Prairie Chickens in eastern Chaves, New Mexico. M.S. thesis, New Mexico State University, Las Cruces, NM, USA.

Taberlet, P., E. Coissac, F. Pompanon, L. Gielly, C. Miquel, A. Valentini, T. Vermat, G. Corthier, C. Brochmann, and E. Willerslev (2007). Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35:e14.

Taylor, R. L., B. L. Walker, D. E. Naugle, and L. S. Mills (2012). Managing multiple vital rates to maximize Greater Sage-Grouse population growth. Journal of Wildlife Management 76:336–347.

Tilman, D., and J. A. Downing (1994). Biodiversity and stability in grasslands. Nature 367:363–365.

Trevelline, B. K., S. C. Latta, L. C. Marshall, T. Nuttle, and B. A. Porter (2016). Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (*Parkesia motacilla*). The Auk: Ornithological Advances 133: 415–428.

Wenny, D. G., T. L. DeVault, M. D. Johnson, D. Kelly, C. H. Sekercioglu, D. F. Tomback, and C. J. Whelan (2011). The need to quantify ecosystem services provided by birds. The Auk 128:1–14.

Wisdom, M. J., and L. S. Mills (1997). Sensitivity analysis to guide population recovery: Prairie-chickens as an example. Journal of Wildlife Management 61:302–312.

Yi, Z., F. Jinchao, X. Dayuan, S. Weiguo, and J. C. Axmacher (2012). A comparison of terrestrial arthropod sampling methods. Journal of Resources and Ecology 3:174–182.

Zeale, M. R. K., R. K. Butlin, G. L. A. Barker, D. C. Lees, and G. Jones (2011). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. Molecular Ecology Resources 11:236–244.

Zeileis, A., F. Cribari-Neto, B. Gruen, and I. Kosmidis (2016). Package "betareg". https://cran.r-project.org/web/packages/betareg/betareg.pdf

APPENDIX A

Plant Genera Detected ($n = 257$) during Vegetation Surveys at Study Sites in Western Kansas and Eastern Colorado, USA, 2013–2016

Acer	*Asclepias*	*Catalpa*	*Croptilon*
Achillea	*Aster*	*Celtis*	*Croton*
Achnatherum	*Asteraceae*	*Cenchrus*	*Cryptantha*
Aegilops	*Astragalus*	*Cephalanthus*	*Cucurbita*
Agrostis	*Atriplex*	*Ceris*	*Cuscuta*
Allium	*Baccharis*	*Chaeropyllum*	*Cynodon*
Amaranthus	*Baptisia*	*Chaetopappa*	*Cyperaceae*
Ambrosia	*Bassia*	*Chamaecrista*	*Cyperus*
Amorpha	*Boltonia*	*Chamaesaracha*	*Dalea*
Amphiachyris	*Bothriochloa*	*Chamaesyce*	*Delphinium*
Andropogon	*Bouteloua*	*Chenopodium*	*Descurainia*
Androsace	*Brickellia*	*Chloris*	*Desmanthus*
Anemone	*Bromus*	*Cirsium*	*Dianthus*
Antennaria	*Buchloe*	*Cleome*	*Dichanthelium*
Aphanostephus	*Calamovilfa*	*Comandra*	*Digitaria*
Apocynum	*Callirhoe*	*Commelina*	*Distichlis*
Argemone	*Calylophus*	*Convulvulus*	*Draba*
Aristida	*Cannabis*	*Conyza*	*Echinacea*
Artemisia	*Carduus*	*Coreopsis*	*Echinochloa*
Aruncus	*Carex*	*Cornus*	*Elaeagnus*
Asclepia	*Castilleja*	*Corydalis*	*Eleocharis*

Elymus
Engelmannia
Equisetum
Eragrostis
Ericameria
Erigeron
Eriochloa
Eriogonum
Erioneuron
Escobaria
Eupatorium
Euphorbia
Euphorbiaceae
Evolvulus
Fabaceae
Ferocactus
Froelichia
Gaillardia
Galium
Geum
Glandularia
Gleditisia
Glycyrrhiza
Gomphrena
Grindelia
Gutierrezia
Haplopappus
Helianthus
Hesperostipa
Heterotheca
Hibiscus
Hoffmannseggia
Hordeum
Hybanthus
Hydrocotyle
Hymenopappus
Hypericum
Indigofera
Ipomoea
Ipomopsis
Iva
Juglans
Juncus
Juniperus
Krameria
Lactuca
Lepidium
Lespedeza
Liatris
Linum
Lithospermum
Lotus
Lygodesmia
Machaeranthera
Maclura
Marsilea
Medicago
Melampodium
Melilotus
Menispermum
Mentzelia
Microseris
Mimosa
Minuartia
Mirabilis
Monarda
Muhlenbergia
Nama
Nothoscordum
Nuttallanthus
Oenother
Oenothera
Opuntia
Oxalis
Oxytropis
Packera
Panicum
Paronychia
Parthenocissus
Pascopyron
Paspalum
Pediomelum
Penstemon
Phemeranthus
Phyla
Physalis
Physaria
Phytolacca
Plantago
Poa
Poaceae
Polanisia
Polygala
Polygonaceae
Polygonum
Polytaenia
Pomaria
Populus
Portulaca
Proboscidea
Prunus
Psilostrophe
Psoralidium
Pyrrhopappus
Pyrus
Quincula
Ranunculs
Ranunculus
Ratibida
Rayjacksonia
Rhus
Ribes
Robinia
Rudbeckia
Rumex
Salix
Salsola
Salvia
Sambucus
Sanguisorba
Sapindus
Schedonnardus
Schedonorus
Schizachyrium
Schoenoplectus
Scirpus
Securigera
Senecio
Setaria
Silphium
Sisymbrium
Sisyrinchium
Smilax
Solanum
Solidago
Sophora
Sorghastrum
Sorghum
Spartina
Sphaeralcea
Sporobolus
Stellaria
Stenaria
Stenosiphon
Stillingia
Streptanthus
Symphyotrichum
Tamarix
Taraxacum
Tephrosia
Tetraneuris
Thelesperma
Townsendia
Toxicodendron
Tradescantia
Tragia
Tragopogon
Tribulus
Tridens
Trifolium
Triodanis
Tripsacum
Triticum
Typha
Ulmus
Urtica
Verbascum
Verbena
Vernonia
Vicia
Viola
Vitus
Vulpia
Yucca
Zea

APPENDIX TABLE 5. Proportional abundance of the most common grass, forb, subshrub, and shrub species estimated from point-step transects by study site for the northern portion of the Lesser Prairie-Chicken range in Kansas and Colorado, USA, 2014–2015. Mean annual precipitation (PRISM climate group averaged from a PRISM 800 m resolution raster file) and dominant soil textures (Soil Survey Staff 2016) are also included.

	Colorado		Kansas					
	Prowers		Clark		Northwest		Red Hills	
Mean annual precipitation (cm)	43.4		58.6		49.4		69.2	
Dominant soil textures	Loam		Loamy fine sands, fine sandy loams, fine sands		Silt loams		Sandy loam, clay loam, and clay	
Grasses	*Bouteloua curtipendula*	0.453	*Sporobolus airoides*	0.037	*Bouteloua curtipendula*	0.266	*Schizachyrim scoparium*	0.064
	Bouteloua gracilis	0.063	*Sporobolus cryptandrus*	0.037	*Bouteloua gracilis*	0.163	*Bouteloua curtipendula*	0.046
	Schizacyrim scoparium	0.057	*Bouteloua gracilis*	0.032	*Pascopyrum smithii*	0.099	*Bouteloua gracilis*	0.026
Forbs	*Kochia scoparia*	0.110	*Ambrosia psilostachya*	0.037	*Ambrosia psilostachya*	0.027	*Artemisia ludiviciana*	0.053
	Salsola tragus	0.090	*Salsola tragus*	0.033	*Salsola tragus*	0.019	*Ambrosia psilostachya*	0.037
	Convolvulus arvensis	0.028	*Kochia scoparia*	0.014	*Kochia scoparia*	0.013	*Pediomelum spp.*	0.006
Subshrubs	*Gutierrezia sarothrae*	0.005	*Amphiachyris dracunculoides*	0.002	*Gutierrezia sarothrae*	0.033	*Amphiachyris dracunculoides*	0.011
			Gutierrezia sarothrae	0.002			*Gutierrezia sarothrae*	0.003
Shrubs	*Yucca glauca*	0.011	*Artemisia filifolia*	0.011	*Artemisia filifolia*	0.013	*Artemisia filifolia*	0.004
	Artemisia filifolia	0.001	*Prunus angustifolia*	0.002	*Yucca glauca*	0.004	*Prunus angustifolia*	0.002
	Ericameria spp.	0.001	*Rhus aromatia*	0.001			*Rhus glabra*	0.001

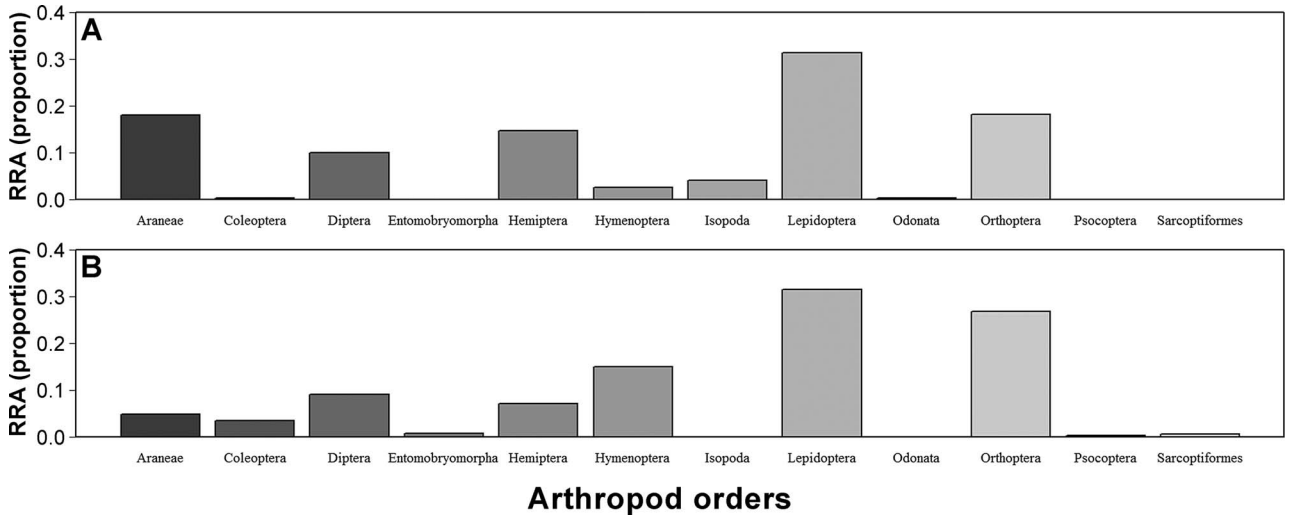
APPENDIX TABLE 6. Families and genera of arthropods detected using DNA barcoding in fecal samples of Lesser Prairie-Chickens during brood rearing and winter at 4 study sites in Kansas and Colorado, USA, 2014–2015.

Northwest		Clark		Red Hills		Colorado	
(n = 27 fecals, 29,073 reads)		(n = 29 fecals, 8,064 reads)		(n = 14 fecals, 5,810 reads)		(n = 13 fecals, 833 reads)	
Family	Genus	Family	Genus	Family	Genus	Family	Genus
Acrididae	***Melanoplus***	**Acrididae**	***Melanoplus***	**Acrididae**	***Melanoplus***	**Acrididae**	***Melanoplus***
Noctuidae	***Dargida***	**Noctuidae**	***Dargida***	**Noctuidae**	***Dargida***	**Noctuidae**	***Dargida***
Pentatomidae	***Thyanta***	**Pentatomidae**	***Thyanta***	**Pentatomidae**	***Thyanta***	**Pentatomid**	***Thyanta***
Pieridae	***Pieris***	**Pieridae**	***Pieris***	**Pieridae**	***Pieris***	**Pieridae**	***Pieris***
Araneidae	*Argiope*	Acrididae	*Arphia*	Agaonidae	*Valisia*	Braconidae	*Cotesia*
Braconidae	*Cotesia*	Aphididae	*Aphis*	Araneidae	*Argiope*	Crambidae	*Loxostege*
Braconidae	*Microplitis*	Caeciliusidae	*Valenzuela*	Cynipidae	*Andricus*	Cynipidae	*Andricus*
Caeciliusidae	*Valenzuela*	Cicadidae	*Tibicen*	Noctuidae	*Halysidota*	Dermestidae	*Anthrenus*
Carabidae	*Cyclotrachel*	Coreidae	*Leptogloss*	Philodromid	*Ponometia*	Erebidae	*Halysidota*
Chrysomelid	*Leptinotarsa*	Cynipidae	*Andricus*		*Philodrom*	Erebidae	*Spilosoma*
Coccinellidae	*Harmonia*	Delphacidae	*Muirodelpha*			Gryllidae	*Allonemob*
Crambidae	*Loxostege*	Diplopoda	*Brachyiulus*			Gryllidae	*Gryllus*
Culicidae	*Psorophora*	Entomobryid	*Entomobrya*			Miridae	*Lygus*
Dermestidae	*Anthrenus*	Gryllidae	*Allonemobius*			Noctuidae	*Agrotis*
Erebidae	*Caenurgina*	Gryllidae	*Gryllus*			Noctuidae	*Athetis*
Erebidae	*Pyrrharctia*	Muscidae	*Musca*			Noctuidae	*Dargida*
Geometridae	*Narraga*	Noctuidae	*Athetis*			Noctuidae	*Spodoptera*
Gryllidae	*Gryllus*	Noctuidae	*Euxoa*			Proctophyll	*Monojoube*
Libellulidae	*Sympetrum*	Noctuidae	*Noctua*			Salticidae	*Phidippus*
Miridae	*Lygus*	Noctuidae	*Sunira*			Sphingidae	*Hyles*
Noctuidae	*Chrysodeixis*	Notodontidae	*Dunama*			Tineidae	*Tinea*
Noctuidae	*Helicoverpa*	Philosciidae	*Burmoniscus*				
Noctuidae	*Leucania*	Ptinidae	*Stegobium*				
Noctuidae	*Ponometia*	Salticidae	*Phidippus*				
Noctuidae	*Psectrotarsia*	Tenthredinidae	*Dolerus*				
Noctuidae	*Spodoptera*	Tetragnathidae	*Leucauge*				
Notodontidae	*Dunama*	Theridiidae	*Latrodectus*				
Nymphalidae	*Chlosyne*	Theridiidae	*Parasteatoda*				
Proctophyll	*Monojouber*	Thomisidae	*Xysticus*				
Pterophoridae	*Emmelina*	Tineidae	*Tinea*				
Ptinidae	*Stegobium*						
Pyralidae	*Phycitodes*						
Salticidae	*Phidippus*						
Sphingidae	*Hyles*						
Sphingidae	*Manduca*						
Theridiidae	*Latrodectus*						
Tineidae	*Tinea*						

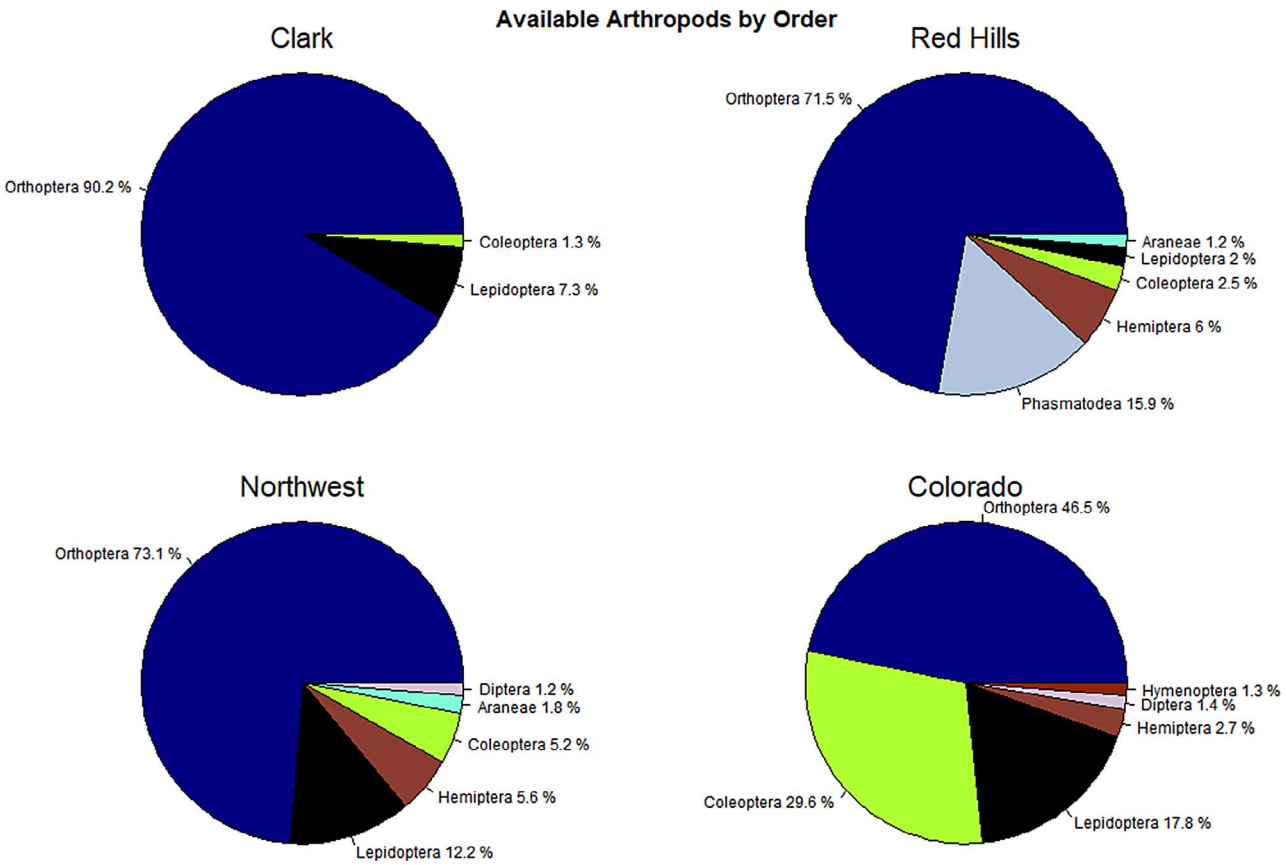
Notes: All fly-related taxa (Diptera) were removed because they likely reflect post-defecation contamination. Taxa in bold are those common among all study sites.

APPENDIX TABLE 7. Relative read abundance (sample size, mean, and SD) of arthropod orders in the diets of Lesser Prairie-Chicken chicks and adults during the brooding period, and of adults during winter, from 4 study sites in Kansas and Colorado, USA, 2014–2015. Only one brood sample had readable DNA from Colorado.

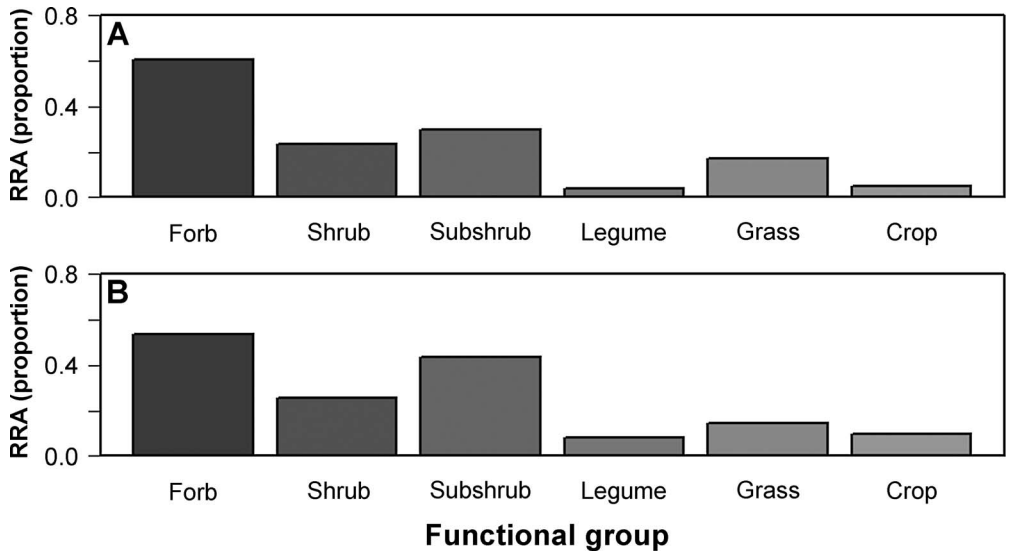
	Northwest			Red Hills			Clark			Colorado		
Order	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Brood rearing		28,879 reads			1,722 reads			4,283 reads			178 reads	
Araneae	25	0.135	0.283	5	0.400	0.548	17	0.196	0.392	3	0.009	0.003
Coleoptera	25	0.007	0.017	5	0.000	0.000	17	0.000	0.000	3	0.000	0.000
Diptera	25	0.151	0.327	5	0.200	0.447	17	0.002	0.007	3	0.000	0.000
Entomobryomorpha	25	0.000	0.000	5	0.000	0.000	17	0.000	0.000	3	0.000	0.000
Hemiptera	25	0.207	0.320	5	0.000	0.000	17	0.113	0.280	3	0.193	0.070
Hymenoptera	25	0.010	0.037	5	0.000	0.000	17	0.000	0.000	3	0.333	0.149
Isopoda	25	0.000	0.000	5	0.000	0.000	17	0.116	0.326	3	0.000	0.000
Lepidoptera	25	0.416	0.385	5	0.214	0.441	17	0.217	0.393	3	0.274	0.035
Odonata	25	0.008	0.038	5	0.000	0.000	17	0.000	0.000	3	0.000	0.000
Orthoptera	25	0.066	0.205	5	0.187	0.417	17	0.364	0.425	3	0.190	0.085
Psocoptera	25	0.000	0.001	5	0.000	0.000	17	0.000	0.000	3	0.000	0.000
Sarcoptiformes	25	0.001	0.003	5	0.000	0.000	17	0.000	0.000	3	0.000	0.000
Winter		194 reads			410 reads			1,527 reads			655 reads	
Araneae	2	0.000	0.000	6	0.167	0.408	12	0.025	0.069	10	0.020	0.054
Coleoptera	2	0.375	0.530	6	0.000	0.000	12	0.023	0.057	10	0.002	0.007
Diptera	2	0.500	0.707	6	0.000	0.000	12	0.046	0.113	10	0.120	0.313
Entomobryomorpha	2	0.000	0.000	6	0.000	0.000	12	0.021	0.073	10	0.000	0.000
Hemiptera	2	0.000	0.000	6	0.167	0.408	12	0.046	0.105	10	0.058	0.183
Hymenoptera	2	0.000	0.000	6	0.333	0.516	12	0.112	0.287	10	0.114	0.314
Isopoda	2	0.000	0.000	6	0.000	0.000	12	0.000	0.000	10	0.000	0.000
Lepidoptera	2	0.125	0.177	6	0.333	0.516	12	0.188	0.305	10	0.495	0.383
Odonata	2	0.000	0.000	6	0.000	0.000	12	0.000	0.000	10	0.000	0.000
Orthoptera	2	0.000	0.000	6	0.000	0.000	12	0.518	0.438	10	0.184	0.244
Psocoptera	2	0.000	0.000	6	0.000	0.000	12	0.010	0.035	10	0.000	0.000
Sarcoptiformes	2	0.000	0.000	6	0.000	0.000	12	0.011	0.026	10	0.007	0.022



APPENDIX FIGURE 8. Arthropod orders detected, using DNA metabarcoding, in Lesser Prairie-Chicken fecal samples collected (**A**) from brooding females and chicks during summer 2014 (hatch to 98 days old; $n = 50$ samples; $n = 35{,}062$ sequences) and (**B**) from adults during winter 2014–2015 (November–March; $n = 30$ samples; $n = 2{,}786$ sequences) in Kansas and Colorado, USA. Fecal samples were pooled among study sites in Clark County, Kansas; Gove and Logan counties, Kansas; Kiowa and Comanche counties, Kansas; and Prowers and Baca counties, Colorado.



APPENDIX FIGURE 9. Composition of arthropod orders available to Lesser Prairie-Chicken chicks in Clark County, Kansas (Clark); Gove and Logan counties, Kansas (Northwest); Kiowa and Comanche counties, Kansas (Red Hills); and Prowers and Baca counties, Colorado (Colorado), USA, during the summers of 2013 and 2014. The composition of orders was estimated using sweep-net surveys at each study site and is based on the biomass of each arthropod order.



APPENDIX FIGURE 10. Adjusted relative readable abundance (RRA; proportion) of DNA within Lesser Prairie-Chicken fecal samples matching barcodes indicative of plant functional groups, including forbs, grasses, legumes, and crops. Fecal samples were collected (**A**) from brooding females and chicks during summer 2014 (hatch to 98 days old; $n = 49$ samples; $n = 223{,}660$ sequences) and (**B**) from adults during winter 2014–2015 (November–March; $n = 101$ samples; $n = 516{,}960$ sequences) in Kansas and Colorado, USA.