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ABSTRACT Prairie deer mice are important predators in many agricultural systems, and through their diet they can help to regulate pest insect and weed populations. Our objective was to test whether fecal material is an effective means of detailing the foraging ecology of small mammals. We conducted three studies to evaluate the efficacy of this technique: 1) field-collected fecal material from unknown deer mice from late winter to early spring, 2) fecal material collected in an enclosure with mice fed a mix of C_3 and C_4 plant seeds, and 3) fecal material from tagged female mice in the field. We detected significant shifts in $\delta^{13}C$ in one study and $\delta^{15}N$ in another relative to spring thaw ($\delta^{13}C$: –13.34 vs. –10.72, $P = 0.01$, $\delta^{15}N$: 4.92 vs. 4.09, $P = 0.03$), a significant correlation between the relative amounts of two seed types and $\delta^{13}C$ (slope = 5.46, $SE = 1.82$, $P < 0.01$), and a significant decrease in $\delta^{15}N$ due to nursing (4.57 ± 0.19 vs 3.28 ± 0.47, $P = 0.02$). Use of this technique will help to clarify foraging of this economically important species in agroecosystems.

KEY WORDS agroecology, corn belt, ecosystem services, isotopic fractionation, *Peromyscus maniculatus bairdii*, prairie deer mouse, seed predation, stable isotopes

Fecal material is evidence of short-term diets because it contains several indicators of trophic ecology: DNA, food remains, and isotopes (Dickman and Huang 1988, Piggott and Taylor 2003, Salvarina et al. 2013) and has a fast turnover rate (gut passage time). Salvarina et al. (2013) determined that under controlled conditions, bat fecal isotopes are indicative of trophic shifts within a few hours, illustrating that fecal isotopes are representative of recent dietary behavior.

Ratios of stable heavy to light isotopes of nitrogen and carbon can be used to detect structure of food webs, diet shifts, and anthropogenic impacts (Dalerum and Angerbjorn 2005, Layman et al. 2007, Crawford 2008). Mammals tend to excrete depleted levels of heavy nitrogen isotopes (^{15}N) in urea relative to the proportion of such isotopes in their diet (Vanderklift and Ponsard 2003). Physiological fractionation of ^{15}N causes an enrichment (increase relative to food) of whole body and fecal heavy isotopes, resulting in an increase in $\delta^{15}N$ of approximately 3–4‰ (parts per thousand) compared to the diet, and thus an increase in each trophic level (Gaebler et al. 1966, DeNiro and Epstein 1981, Minagawa and Wada 1984, Owens 1987). Enrichment of $\delta^{15}N$ relative to diet can vary across taxa, but values are relatively similar for closely related organisms in similar environmental conditions (Vanderklift and Ponsard 2003, Crawford 2008).

One cause of variation in isotope fractionation (discrimination factor or diet-tissue isotopic difference, Δ) is digestion. For example, different digestive systems (e.g., hindgut versus foregut fermentation) result in different levels of heavy isotope enrichment (Sponheimer et al. 2003). In addition, food sources have variable decomposition in the digestive tract (e.g., soft-bodied versus hard-bodied invertebrates), which can affect isotope ratios, and thus field studies are necessary to determine if this source of variation overwhelms the dietary signal (Crawford 2008).

Unlike nitrogen isotopes, carbon isotopes ($^{12}C/^{13}C$) do not tend to fractionate between diet and many body tissues or fecal material (DeNiro and Epstein 1978). However, due to the kinetic isotope effects of different photosynthesis pathways, C_3 plants (e.g., soybean and velvetleaf) are relatively depleted in ^{13}C compared to C_4 plants (e.g., corn and foxtail), and the resultant lower value of ^{13}C in the consumer's tissues provides evidence of diet.

To test the efficacy of stable isotopes from field-collected fecal material, we evaluated whether stable isotopes in fecal material indicate the composition of a weed-seed diet, and whether field-collected samples are suggestive of known trophic relationships. Further, we evaluated whether the reproductive status of females alters isotope values. We used field collected fecal material to test for winter-spring diet shifts in $\delta^{15}N$ levels due to predicted shifts from plant to animal foods. We used the same fecal material to detect corn and soybean in the diet due to differences in $\delta^{13}C$ levels due to different metabolic pathways in C_4 and C_3 plants, respectively. In a separate study, we used fecal material from females of known reproductive status to detect if $\delta^{15}N$ values were changed due to milk production disproportionately removing ^{15}N from available nutrition. Moreover, we used mesocosm studies to test if quantified diet could be detected in fecal isotope values by recording proportions of a C_3 and C_4 plant consumed.

STUDY AREA

Our study was conducted in Ames, Iowa USA (Enclosures: 41º 59' N, –93º 40' W and surrounding fields) using

Peromyscus maniculatus bairdii (prairie deer mice) and seeds from local farms in Story County, Iowa. We studied mice that were located in corn or soybean fields managed as monocultures.

METHODS

Fecal Material Collection and Processing

Mesocosm Study

In the first phase of the study, we evaluated the effectiveness of fecal isotopes in detecting the diet of *P. m. bairdii* when consuming naturally available foods. We placed Sherman live traps (7.62 × 8.89 × 22.86 cm) randomly in corn and soybean fields to collect *P. m. bairdii* during February and March 2010. We used approximately 20 traps to catch the four mice for the experiment. We were limited to four mice, because we only had enclosures for four mice at a time. Each of four mice were moved into a 3 m × 3 m enclosure. These enclosures consisted of a concrete floor, ~0.25 m deep of soil, 2 m high dark fiberglass and metal pipe walls with a 1 m high aluminum barrier around the inside perimeter to keep mice inside, and a metal roof. Within each room, there was a wooden nest box, a foraging tray, and water. The internal dimensions of the wooden nest boxes were 12 × 12 × 10 cm, had a removable lid, and a single opening with 2.5 cm corrugated plastic tubing to mimic a burrow tunnel. In each box, we added 2 cotton nestlets (PharmaServ, #NE3600) and provided the mouse with water and a supply of food that contained equal amounts of giant foxtail and velvetleaf seeds mixed in trays with sand. These trays were used as giving-up-density feeders (Brown 1988) with 1 liter of sand and ~42 g of seed total (twice as much as mice were known to use in three nights). Mice were allowed to forage in these trays for 1 night and 3 nights between fecal collection so that we could detect which time period was better reflected in the fecal isotopes. Due to the time of year and the ambient temperatures, mice had no access to animal food, and enclosures were inspected every three days for surface caches and new burrows. We collected all fecal samples after mice had been confined to enclosures for more than 3 days foraging on the same available seeds; all fecal samples were analyzed separately.

To evaluate the fractionation of diet to fecal isotopes and determine the consistency of results, we collected data on diet composition and sampled fecal material from individual mice. We weighed seeds before mixing them into the sand. After mice foraged for 1 or 3 nights, we collected seeds from the artificial burrows and sifted seeds from the sand with a 1mm sieve. From this combination of uneaten seeds and accounting for occasional caches in the enclosure, we could precisely evaluate the diet of mice. On the same day that we collected seeds, we collected fecal material from the foraging tray which was used for the isotope analysis (details below). When fecal material was collected on day 3, feces from all three days was present in the tray. All of the fecal material returned to the lab was either frozen and later heated or immediately heated to 190° C for 20 minutes to destroy pathogens. After heat treatment and drying, we used a seed separator to remove loose seed hulls. We processed samples with forceps to remove additional fecal material and other foreign objects prior to weighing seeds. For fecal analysis, an individual sample was comprised of a single fecal dropping from each mouse for a single date. The use of a single dropping allowed us to evaluate the usefulness of the smallest possible sample. Also, we analyzed the isotope composition of 8 foxtail and 8 velvetleaf seeds which were randomly selected from the source seed bags. This analysis gave us the benchmarks to assess the amount of fractionation in isotopes relative to their known diet.

Field Study

In the second phase of the study testing for seasonal diet changes, we evaluated whether fecal material that was present in seeds collected from GUD trays in a previous study would provide insight into trophic ecology of free-ranging mice. Therefore, we used fecal material from giving-up-density tray samples that were collected from cornfields in central Iowa. We conducted this previous foraging study in 2008, but separated the fecal material from the samples in 2011. The original study design was blocked, with 4 sample points at the corners of a 15 m square. At each corner were 2 buckets with 1 liter of sand and 3 g of foxtail or velvetleaf. We conducted the study from January to March 2008. There were 4 squares per field and 3 fields. Field A was a silage field (with corn cobs and stalks removed from the field in early fall) that had approximately annual additions of manure as a main source of nitrogen addition. Also, the field received synthetic fertilizers periodically. In fields B and C, synthetic fertilizers are the main source of nitrogen addition and fields were managed in a corn-soybean rotation. For fecal analysis, we collected a single fecal dropping from each seed sample in a field for a given date (i.e., total of 16 sample dates). We combined all fecal material from a field for a single date to detect a homogenized value for the diet being consumed in that field by multiple individuals which constituted a single sample.

Maternal Study

In the final phase of the study, we evaluated the diet and isotope fractionation of female mice. To evaluate the influence of reproductive status on isotopes, we collected fecal material directly from female mice in late winter to early spring 2012. This collection was facilitated by using artificial nest boxes that we placed in a harvested soybean field during fall 2011. This experimental setup was part of another project

to evaluate the effects of food addition on mouse reproduction and movement (Danielson et al., unpublished data). We arranged pairs of buried nest boxes along transects spaced 20 m apart and 4 burrow pairs (two burrow boxes with one side of each in contact with the other) per transect. We installed 5 parallel transects, for a total of 20 burrows. All transects were oriented in a north-south direction and were at least 40 m from the edge and 40 m apart As part of the study described above, we spread approximately 20 kg of corn around every other burrow pair in a 20 m radius in the late fall of 2011. During the sampling period in 2012, we visited nest boxes periodically and when we encountered a female, we recorded weight, nipple size, perforation, sperm plug, or pregnancy. Females were considered to be nursing if their nipples were large, or they were observed to be nursing young. For fecal analysis, we collected the first 2–3 fecal droppings to be excreted directly from each female mouse, and the single largest was analyzed. We collected corn and soybean samples from each transect during the sampling period, analyzed seeds for isotope composition, and compared values to fecal isotopes.

In all studies, we returned samples to the lab where they were dried and sterilized in an oven at 190° C for 20 minutes. We homogenized the sample for subsampling using a mortar and pestle. We extracted a single subsample of ~0.5 mg from each pulverized sample and added this subsample to a tin cup. We stored subsamples in the tin capsule in a lab desiccator until isotope analysis.

Stable Isotope Analysis

To determine whether isotopes of diet and reproduction are detectable in field fecal isotopes, we processed subsamples via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer at Iowa State University (Department of Geological and Atmospheric Sciences). We used the international reference standards (Ammonium Sulfate [IAEA-N-1]; Ammonium Sulfate [IAEA-N-2]; Caffeine [IAEA-600], Sucrose [IAEA-CH-6; RM 8542], and Acetanilide [laboratory standard]) for isotope corrections. We used the same standards to assign the data to the appropriate isotope scale (12 total standards for the mesocosm study, 12 for the field study, and 23 for the maternal study).

Heavy isotopes are typically detected in low abundance (^{14}N, 99.64%; ^{15}N, 0.36%;Audi et al 2003), so their values are reported as parts per thousand (permil or ‰;Fry 2006). The typical equation used for reporting stable isotope values is:

$$\delta(\text{Heavy Isotope}) = ((R_{sample} - R_{standard})/ R_{standard}) * 1000$$

where R= $^{15}N/^{14}N$ and $^{13}C/^{12}C$ respectively, for N and C. Fractionation factor (Δ) is a measure of the difference between the stable isotope ratio of the diet of an animal and the ratio found in a particular tissue. Fractionation factor is calculated as $\Delta = R_{sample} - R_{diet}$. We calculated R_{diet} based on the R values of foxtail and velvetleaf for the time period a mouse was foraging (1 or 3 nights).

Statistical Methods

We conducted all analyses using JMP Pro 10.0.2 (SAS 2012). We used standard least squares analysis to evaluate the effects of time and multiple reproductive condition states on isotope composition. We analyzed the effect of sampling timeframes on fractionation of isotope ratios in two ways. We used one-way analyses of variance to evaluate samples collected before and after the spring thaw, as well as reproductive conditions (perforation, presence of sperm plug, nipple size, and pregnancy). We assessed assumptions of normality using Q-Q plots; our data showed no significant departures from normality. When repeated measures were analyzed, we assigned the identity of the mouse as a random variable to first remove variation due to identity.

RESULTS

Results from the analysis of standards resulted in a combined uncertainty (analytical uncertainty and average correction factor) for $\delta^{13}C$ is ± 0.21, 0.80, and 0.06‰ (VPDB) and $\delta^{15}N$ is ± 0.06, 0.20, and 0.17‰ (Air), respectively for the mesocosm, field, and maternal studies.

In the mesocosm study, we were able to assay 4 mice on known diets of velvetleaf and foxtail. In the field study, we analyzed 22 subsamples from 3 corn fields in late winter 2009. Finally, in late winter 2012 we used fecal material collected directly from female mice in soybean fields supplemented with corn, analyzing data from 33 mice over 4 weeks (2 before spring thaw and 2 after spring thaw).

Mesocosm Study

The entire range of isotope values for the $\delta^{15}N$ of fecal material was higher than the average values for foxtail and velvetleaf (4.63–6.94 for feces, 3.15 for foxtail, and 3.27 for velvetleaf, Table 1). Because the composition of diets was known in this phase of the study, we were able to determine that the average fractionation of $\delta^{15}N$ was enrichment by 1.91±0.13 ‰ (±SE) which was significantly greater than 0 (t = 13.22, df = 18, $P < 0.001$), but the fecal values of $\delta^{15}N$ were not significantly changed by seed diet composition ($P > 0.1$). The latter result was expected, as the $\delta^{15}N$ values of foxtail and velvetleaf were similar (Table 1). The range of $\delta^{13}C$ values overlapped with the average values of the weed seeds (Table 1). The average fractionation for $\delta^{13}C$ was –2.37±0.61, significantly less than 0 (t = 3.85, df = 16, P =0.001), and the ratio of foxtail:velvetleaf was a significant predictor of fecal $\delta^{13}C$ (slope = 5.46 ± 1.82, $P < 0.01$, Fig. 1). When we treated each subsample as independent, fractionation rates

Table 1. Isotopic values of food and fecal material. The reported values for each sample are the difference in relative abundance of the heavy stable isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) between the sample and an international standard. For known diets in the mesocosm study, fractionation between diet and fecal material is reported. Sample sizes are in parentheses after the sample type.

		$\delta^{13}C$		$\delta^{15}N$	
Experiment	Sample	Average (±1SE)	Range	Average (±1SE)	Range
Mesocosm study: Wild mice in captivity	Velvetleaf (8)	–26.19 (0.56)	–30.80 to –11.50	3.27 (0.37)	–0.01 to 4.20
	Foxtail (8)	–12.52 (0.27)	–15.00 to –11.54	3.15 (0.18)	0.05 to 6.56
	Feces (20)	–14.04 (0.61)	–19.11 to –7.82	5.17 (0.15)	4.36 to 6.94
	Fractionation (17)	–2.37 (0.61)	–8.12 to 1.84	1.91 (0.13)	1.20 to 3.41
Field study: Wild mice in field	Feces (26)	–12.23 (0.54)	–16.63 to –7.99	6.68 (0.31)	3.99 to 8.80
Maternal study: Wild females sampled directly	Corn (6)	–11.51 (0.23)	–12.27 to –10.64	6.08 (1.61)	–0.67 to 11.42
	Soybean (6)	–27.35 (0.25)	–28.09 to –26.45	–0.28 (0.26)	–1.11 to 0.80
	Feces (49)	–23.03 (0.19)	–26.92 to –15.48	4.36 (0.19)	2.27 to 7.85

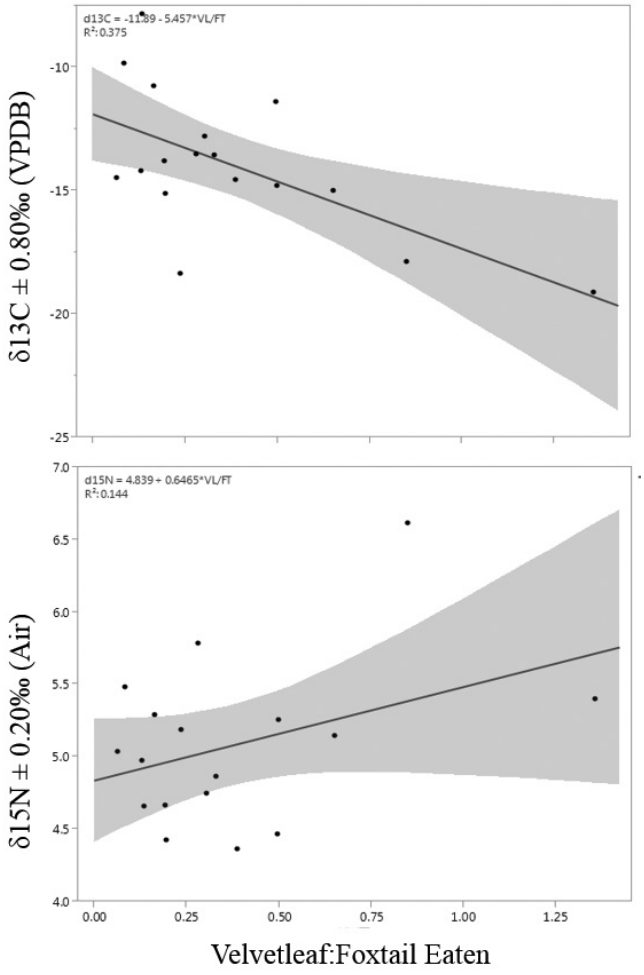

Figure 1. The effect of weed-seed diet composition of prairie deer mice on fecal isotope values. Graphs represent the least squares fit line of $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) as a result of diet composition (velvetleaf seed mass:foxtail seed mass). Shaded regions are the 95% confidence interval for each fitted line. Results are from 4 prairie deer mice captured in central Iowa, and kept on a controlled diet of foxtail and velvetleaf. Fecal material was collected repeatedly for these 4 mice, and the 17 data points represent repeated samples from these 4 individuals.

were significantly different between 1 night and 3 nights of foraging and fecal leavings ($\delta^{15}N = 1.68 \pm 0.11$ after 1 night and 2.24 ± 0.24 after 3 nights and $\delta^{13}C = -3.39 \pm 0.80$ after 1 night and -0.90 ± 0.65 after 3 nights: $F_{1,15} \geq 4.96$, $P \leq 0.04$). However, when we corrected for repeated measures within a single individual, the fractionation rates were similar ($F_{1,3} \geq 4.05$, $P \leq 0.09$).

Field Study

The fecal material that we collected from the cornfield had $\delta^{13}C$ values that declined through time ($R^2 = 0.48$, $\beta = -0.09$, $SE = 0.02$; $t = 4.15$, $P < 0.001$; Fig. 2) and $\delta^{13}C$ values were significantly lower after spring thaw than before spring thaw (–14.19 [0.61] vs. –11.38 [0.59], $F_{1,24} = 7.28$, $P = 0.01$), indicating a shift toward C3-based foods. However, $\delta^{15}N$ values did not change through time ($R^2 = 0.01$ $\beta = -0.006$, $SE = 0.01$; $t = 0.38$, $P = 0.70$; Fig. 2) and values were similar after thaw to before (6.20 [0.66] vs. 6.86 [0.34], $F_{1,24} = 0.039$, $P = 0.85$), indicating a stable trophic level across this time period. The $\delta^{15}N$ values of the field A (primarily manure nitrogen fertilized), however, were significantly lower than the other two fields (primarily synthetic nitrogen fertilized) combined (4.87 (0.29) vs. 7.35 (0.28), $F_{1,24} = 25.47$, $P < 0.001$; Fig. 3), perhaps due to a different fertilizer regime. The ranges of $\delta^{13}C$ values were consistent across all three fields.

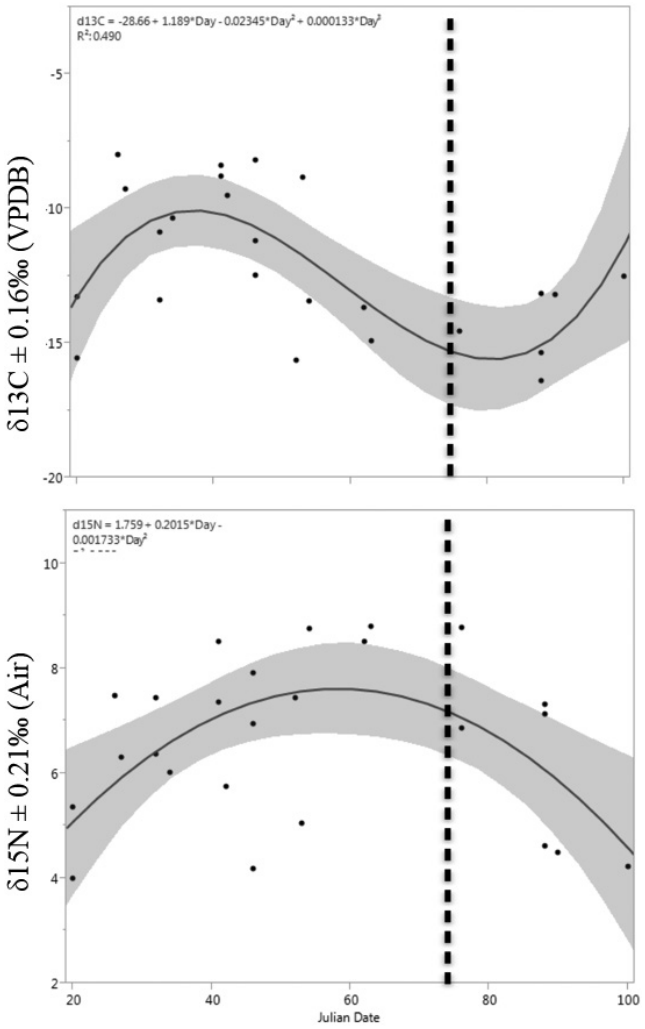

Figure 2. Isotope trophic patterns in prairie deer mouse diet through time. The isotope values are generated from fecal material collected between 1 January and 1 April 2009 ($n = 22$). All samples are from corn fields in central Iowa. The day of the year predicts $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values of feces. The fit line is the quadratic function of the effect of day on isotopic values, and the shaded region is the 95% confidence of the fit line. The $\delta^{13}C$ cubic line is significant for day, day^2, and day^3 ($P = 0.001$, 0.039, and 0.004), while the $\delta^{15}N$ quadratic line is significant for day^2 ($P = 0.004$), but not for day ($P = 0.24$). Lines shown had the lowest AIC_c values of up to third order polynomials. Data were generated from up to 120 buckets per sampling period (3 fields, 5 grids/field, 8 buckets/grid). Actual sample points varied, due to losses of buckets and lack of foraging in some grids. The material from a field was homogenized into a single sample from each sampling date. The fit line represents the fit across all three fields. The vertical line represents an approximation of spring thaw.

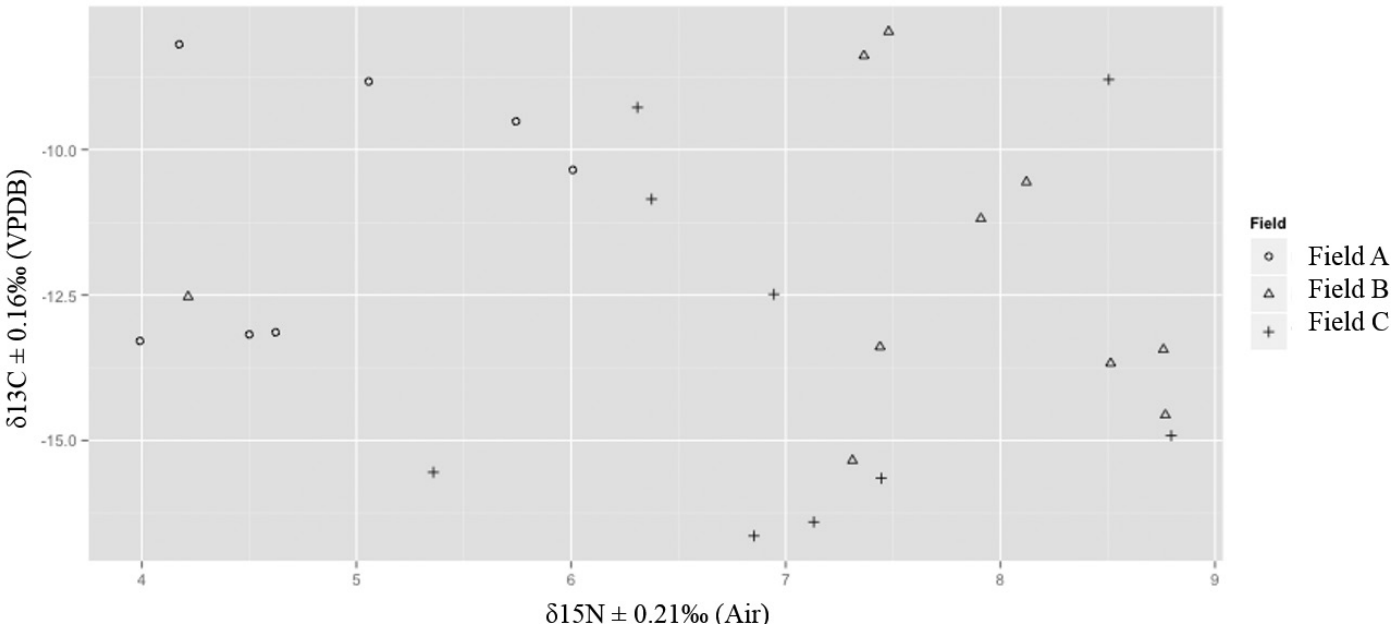

Figure 3. Trophic space of prairie deer mice in 3 corn fields in central Iowa. Values are generated from samples as described in Figure 2.

Maternal Study

The range of fecal isotope values for the maternal study indicated that individual females ranged from a complete reliance on soybean to a complete reliance on corn (Table 1, Fig. 4). $\delta^{15}N$ was significantly higher after thaw (4.92 [0.35] vs. 4.09 [0.21], $F_{1,47} = 4.72$, $P = 0.03$; Fig. 5). $\delta^{13}C$ was similar after thaw than before (–23.47 [0.60] vs. –22.82 [0.58], $F_{1,47} = 0.49$, $P = 0.49$). Additionally, mice with nipples rated as currently nursing had a significantly lower $\delta^{15}N$ value than those not nursing (3.28 [0.27] vs. 4.57 [0.20], $F_{1,41} = 6.17$, $P = 0.02$) while controlling for identity and time period (Fig. 6). When we corrected $\delta^{15}N$ values of nursing females by 1.29 (the average difference among nursing and non-nursing females), values after thaw were significantly higher, and to a greater degree than before the correction (5.50 [0.34] vs. 4.12 [0.33], $P = 0.008$). Conversely, perforation, sperm plug, and pregnancy were not significant predictors of isotope values (all $P > 0.05$).

DISCUSSION

We determined that fecal isotope values of *P. m. bairdii* reflect their diets when fed common weed seeds normally available in corn-soybean agriculture (Fig. 1). Our results builds on previous work that shows fecal material reflects diet in an array of animals in the wild (Sponheimer et al. 2003, Painter et al. 2009, Codron et al. 2011), as well as laboratory studies of small mammals (Hwang et al. 2007, Salvarina et al. 2013). Additionally, fecal isotope values of prairie deer mice matched predictions from these previous studies; especially that $\delta^{15}N$ was enriched by approximately 2‰ in fecal material relative to diet. In contrast, we found a small but significant fractionation of $\delta^{13}C$ that is not typically recorded in feces.

We noted ecologically relevant isotope values in free-ranging mice (Fig. 2). Across three fields, mice had biologically reasonable and consistent $\delta^{13}C$ and $\delta^{15}N$ values. As Ben-David (1997) illustrated, generalist predators switch dietary habits with the availability of food items, and this is detectable in isotope analyses. We determined that mice in unaltered, harvested cornfields relied on C_4-based foods (C_4 plants and herbivores of C_4 plants) throughout the study period, matching our expectations of their diets (Fig. 3). Also, we were able to detect unique nitrogen sources on fields [manure (low $\delta^{15}N$) vs. synthetic fertilizers (high $\delta^{15}N$); Fig. 3]. We determined an increase in consumption of primary consumer tissue near spring thaw, and a decrease thereafter (Fig. 2). Because spring thaw represents an opportunity for the emergence of insects and the opportunity to forage for them in the soil, the availability of animal protein should increase at this time. Other studies have shown that *P.m. bairdii* select insects when they are available, relative to the seeds that they subsist on through the winter (Whitaker 1966, Clark and Young 1986, Flick and Danielson, unpublished data).

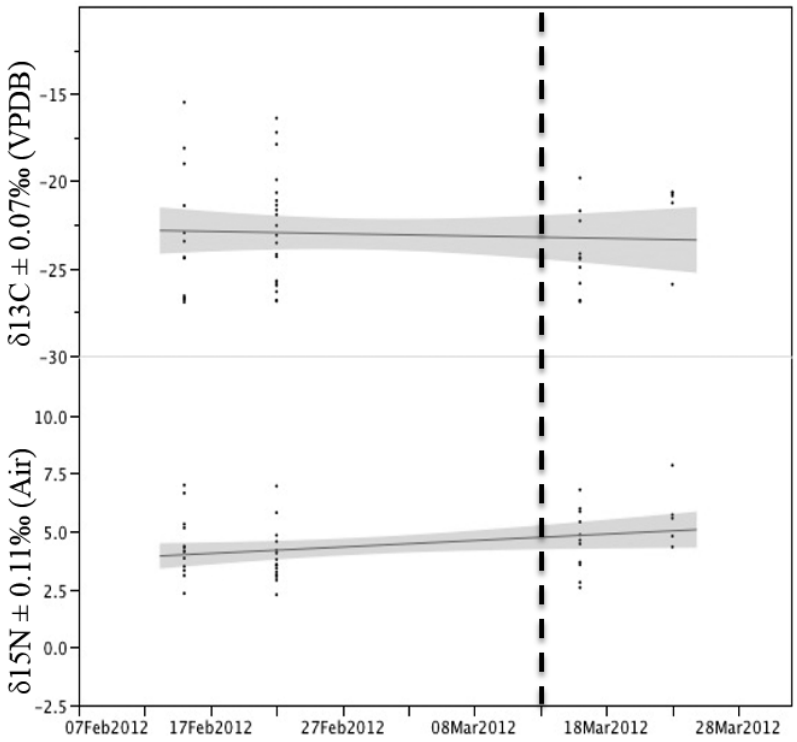

Figure 4. Stable isotopes for female prairie deer mice in central Iowa. The fit line is a first order approximation of the effect of time of year on isotope values, and the shaded area is a 95% confidence interval around that estimate. Fecal material was collected directly from 33 female mice in artificial burrows. Dashed line represents approximate spring thaw event.

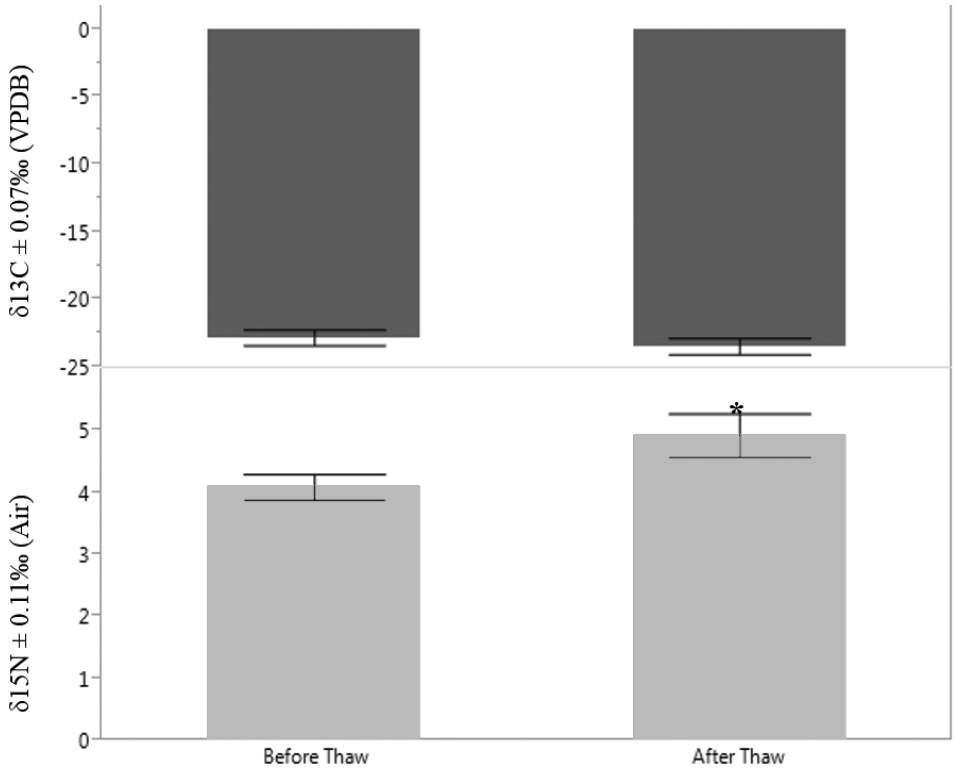

Figure 5. Comparison of stable isotopes before and after spring thaw. Values are from fecal material of 33 female prairie deer mice. Bars are means with error bars representing 1 standard error. An asterisk denotes a significantly higher value.

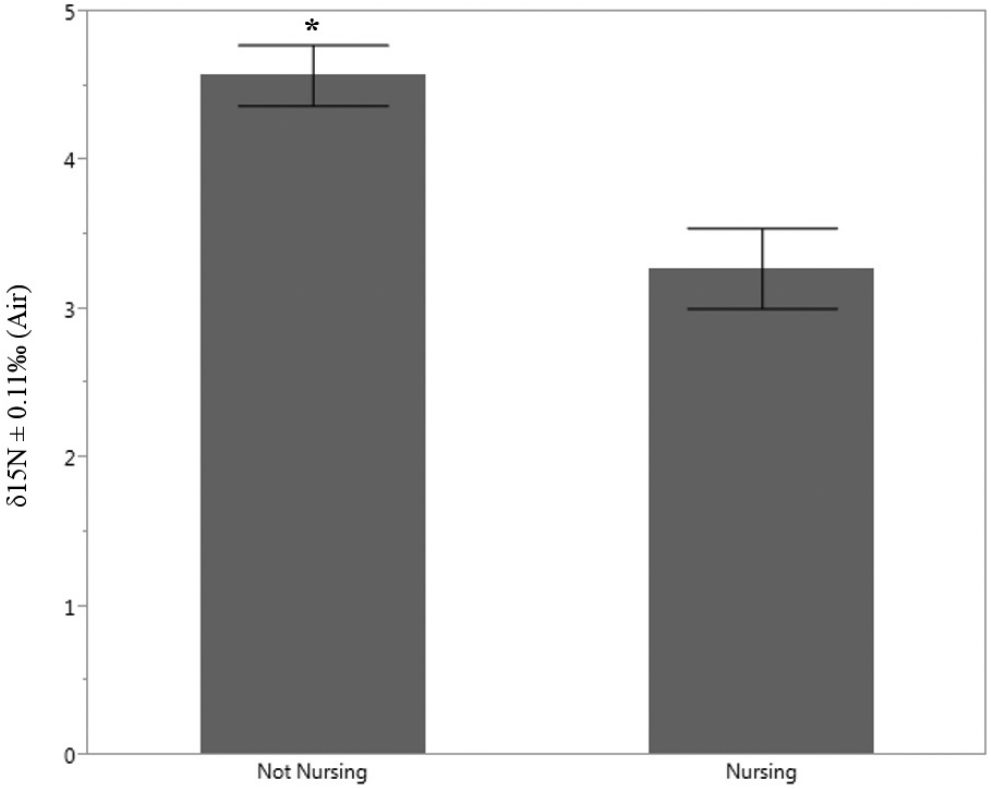

Figure 6. Comparison of $\delta^{15}N$ values based on nursing. Values are from fecal material of 33 female prairie deer mice. Bars are means with error bars representing 1 standard error. An asterisk denotes a significantly higher value.

We detected smaller shifts in diet during the maternal study (Table 1) than during the free-range study, though in the free-range study we were unable to track individuals. The small but significant enrichment in $\delta^{15}N$ over time and no change in $\delta^{13}C$ (Fig. 4) are best explained by the large amount of corn available for consumption (placed by us for another project). Even with the overwhelming amount of corn consumed, mice significantly increased their consumption of animal tissue following spring thaw (Fig. 5). In contrast, there was no evidence that mice in different reproductive states were consuming different diets despite the high energy requirements for lactating females and the strong correlation between litter size and energy consumption (Stebbins 1977). In addition, maturity and sexual activity were not associated with altered isotope values. However, our study found that successful reproduction and nursing of offspring did cause a decrease in $\delta^{15}N$ of fecal material (Fig. 6), similar to a study by Kurle (2002) that suggests that milk preferentially receives ^{15}N isotopes, reducing the values of $\delta^{15}N$ in other bodily tissues. Nevertheless, our results are in contrast to Ben-David et al. (2012), who determined that pregnancy and milk production did not produce any more fractionation of isotopes than those predicted by body mass change. A plausible explanation for this difference may be that Ben-David et al. (2012) were studying muscle and red blood cells. Rates of incorporation into different tissues, compared to the excretion of non-incorporated isotopes, could explain this difference (Rio et al. 2012), as could differential turnover times.

Despite strong effects of timing and nursing, we did not detect an interaction between the two effects. Nursing had a larger effect than time, but both were significant when included simultaneously in the model. Nevertheless, it is reasonable to think that in studies where nursing is not taken into consideration, significant depression of $\delta^{15}N$ values may occur, and subtle shifts in diet may go undetected. For instance, in our free-ranging mice, the lack of increase in $\delta^{15}N$ after spring thaw may result from a high percentage of nursing mice. If our lowest $\delta^{15}N$ values after spring thaw are from nursing females, then values would plateau rather than decline after spring thaw. In systems supplemented with food, as we see in agricultural fields, deer mice will reproduce more frequently and even during the winter (Taitt 1981). Further isotope research in this system should carefully account for this.

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