Comparison of small prairie mammals' dietary intake using carbon and nitrogen stable isotope data

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Abstract

Since small prairie mammals, such as voles and mice, strongly affect prairie plant communities, understanding their roles in restored (planted) and remnant prairies is of value. We used stable isotope ratios of carbon and nitrogen in fur samples of live trapped prairie mammals to explore their diets. Fur serves as a record of diet across months and provides a broad snapshot of the degree to which individuals are consuming C_3 and C_4 plants and the amount of animal matter in their diet. We used and compared two approaches, namely linear mixed random effects models and the integration of stratified random sampling and multilevel models, to increase the validity of our results. Together our analyses suggested significant differences in the isotope ratios of carbon for voles and mice and differences in nitrogen between individuals inhabiting restored as compared to remnant prairies.

Key Words: mixed random effects models, stratified random sampling, ecology, mammals

1. Introduction

Greater knowledge of the resource use of small prairie mammals is vital in understanding how they affect prairie plant communities and may also be important to their conservation (Howe et al. 2006). Rice County, Minnesota contains a wide range of remnant and restored prairies. Prairie remnants are fragments of presettlement prairie landscape that are often less disturbed and have maintained more of its original vegetation and soil. This contrasts with restored prairies, which are fragments of land that have been re-established and returned to prairie.

There are a wide range of plants found within prairie communities. Researches have shown that "each tribe has a specific diet, sometimes modulated by seasonality" (Calandra et al. 2015). High biomass, warm season grasses, such as Indiangrass (sorghastrum nutans), little bluestem (Schizachyrium scoparium), and big bluestem (Andropogon gerardi) comprise a large portion of the vole and mice diet. In addition, cool season, native plants and forbs, which are also main constituents in these mammals' diets. These two major classes of plants differ in their photosynthetic pathways and exhibit C_4 and C_3 photosynthesis, respectively (Figure 1). The higher price associated with C_3 plant seeds compared to C_4 plant seeds has resulted in restored prairies that contain larger degrees of C_4 plants, because they are more cost effective to plant. This results in possible differing prairie compositions between remnant and restored prairies.

Figure 1: Examples of C_3 (left) and C_4 (right) plants in prairies

A variety of small mammal species are found within the prairies of Rice County. Some of the most numerous include the harvest mouse (Reithrodontomys megalotis), meadow vole (Microtus pennsylvanicus), prairie vole (Microtus ochrogaster), and prairie deer mouse (Peromyscus maniculatus) (Figure 2). Two of these species, the prairie vole and harvest mouse, are of special concern (MN DNR) due to their declining numbers, which warrants careful monitoring of their status.

Figure 2: (left to right) Deer Mouse, Harvest Mouse, Prairie Vole, Meadow Vole

Due to mammals' small size and the difficulties associated with observing their eating behavior, researchers sometimes turn towards gut or feces content analyses to explore the diet of voles and mice. However, examining the fur may be a more advantageous method because it allows for a broad snapshot of the diet across several months. Dietary isotopic signatures are incorporated into the consumer's tissue and can be quantified using stable isotope mass spectrometry (Ben-David and Flaherty 2012). By analyzing the stable isotope ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in fur, we gain a record of the degree to which individuals have been consuming C_3 and C_4 plants, and the amount of animal matter in their diet. More specifically, high $\delta^{13}C$ reflect a greater reliance on C_4 plants compared to C_3 plants and higher $\delta^{15}N$ reflect a greater degree of carnivory. Need more information from the beginner's guide here.

In this work, we want to investigate if the species of the mammal or the types of prairies (remnant or restored) would result in a significantly different carbon or nitrogen level in mammals' diets with the consideration of other factors. We hypothesized that:

- Species in restored sites would have higher $\delta^{13}C$ values than mammals of the same species at remnant sites due to plant composition.
- Voles would have lower values of $\delta^{15}N$ in comparison to the omnivorous mice species due to their preference for grassy vegetation rather than

animal matter.

- Meadow voles and prairie voles would have a higher level of $\delta^{13}C$ (C_4 plants) in comparison to the harvest mice and prairie deer mice due to the structure of their crowned teeth and digestive systems.
- The two state registered species of special concern, prairie voles and harvest mice, would differ in diet from other more common mice and vole species.

In addition, we also assessed if different lab techniques (Paritte and Kelly 2009), specifically using acetone or chloroform as washing chemicals when preparing the fur samples, would give us different results. We expect no significant difference in carbon or nitrogen levels due to washing techniques.

In order to test the consistency and validity of our results, we used multiple tests including crossed random effects models, stratified random sampling to approach our research questions.

2. Methods

2.1 Data Collection and Laboratory Methods

Small mammals were live trapped during the summer of 2010, 2012, 2015, and 2017 at three remnant prairies and three reconstructed prairies planted within or near Rice County, Minnesota. Reconstructed prairies were planted starting in 1993 and, combined, are over 150 acres in area.

Fur was clipped dorsally from adults, soaked in de-ionized water for two hours, rinsed with acetone or chloroform:methanol (2:1), and dried at room temperature. Samples were run through a Costech Elemental Analyzer coupled to a Delta V Advantage isotope ratio mass spectrometer at St. Olaf College.

2.2 Data Management and Cleaning

2.2.1 Original Variables

Explanatory variables we did not manipulate include: year of data collection (Year, categorical), month the sample was run (Month, categorical), location of the prairie that the sample came from (Location, categorical), remnant prairie or restored prairie (Remnant/Restored, categorical), date the sample was run (Date, categorical), the specific line of traps that the fur sample came from (Flag, categorical), washing technique of acetone or formaldehyde (Washteq, categorical), tray in which the fur sample was run (Tray, categorical), sex of animal (Sex, categorical), species of animal (Species, categorical), and year the sample was run (Test Year, categorical). Response variables we did not manipulate include: carbon isotope readings from fur samples (Carbon, numerical) and nitrogen isotope readings from fur samples (Nitrogen, numerical).

2.2.2 Revision of Existing variables, Duplicate

The existing categorical variable, "duplicate" needed revision. "Duplicate" represented whether the collected fur sample was run through the spectrometer multiple times. The data was collected in all years except 2017. For our research we wanted to add an indicator for replicated data that were outside of normal variability. The

categorical variable was encoded as follows: 0 for no duplicate, 1 for one duplicate, 2 for two duplicates, etc. There is no preference in this assignment process. In the case of a run outside of the expected variation, per the notes by the primary researchers, the duplicate is assigned the last duplicate number multiplied by -1. For example, if a single fur sample has four duplicates, the one run outside of normal variability, will be assigned the number -4. This facilitates removal of errant data before analysis and we can also account for experimental duplication.

2.2.3 Creation of New Variables

Fur ID

To see how many unique fur samples we had in our dataset, we created a new variable called "FurID". This assigns an ID number for each fur sample and is repeated if there are multiple duplicates of that sample. We used variables Species, Sex, Date, Location, and Flag to decide if the samples were from the same mammal. For each year of data, we add a 100 in front of the FurID so we can also have an idea of how many individuals are collected each year.

Species Group

To compare Mice and Voles, the new variable "SpecGroup" was created. All mice (prairie deer mouse (n=132), harvest mouse(n= 70)) are put into the category "mouse" and all voles (prairie vole(n= 96), meadow vole(n= 100)) are put into the category "voles".

2.3 Statistical Models

2.3.1 Approach 1: mixed random effects models

In this approach, we kept all valid runs from all years. Our dataset has 16 different trays for 398 observations. There are 251 independent full samples without duplicates. We used FurID (indicating unique mammals) and Tray (indicating which tray the sample was ran) as our random effects. FurID can account for the correlations of samples that are taken from the same mammal, while Tray is a proxy of the measurement of all experimental errors including the moisture level of the test day, the scientists who were operating the mass spectrometer, the performance of the mass spectrometer, etc. We found that samples who were run in the recent years (representated by a bigger tray number) tend to have a higher nitrogen and carbon value even after adjusting for species (Figure 3).

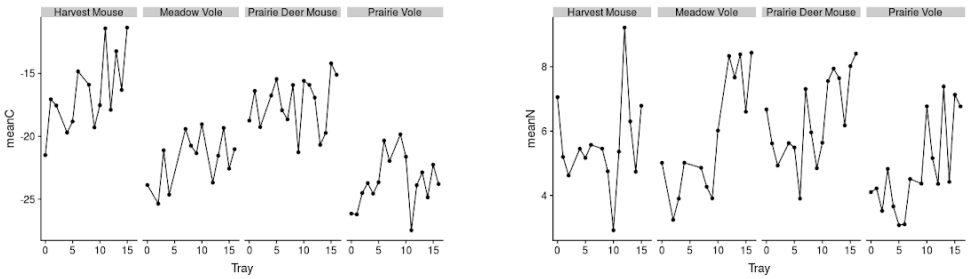

Figure 3: Mean carbon and nitrogen values by tray and species

However, since a few furs were run multiple times in different years thus different trays, FurID and trays are not nested within each other (Figure 4), so a three-level

multilevel model dosen't fit our data structure. Therefore, we decided to use a mixed effect model for our analyses with FurID and Tray as the random effect variables but on the same level.

FurID <int>	Tray <dbl>	Test.year <chr>
179	12	2016 Spring
179	4	2015 Summer
201	12	2016 Spring
201	7	2015 Fall
206	7	2015 Fall
206	12	2016 Spring
216	12	2016 Spring
216	6	2015 Fall
227	6	2015 Fall
227	12	2016 Spring
265	2	2015 Summer
265	12	2016 Spring
282	1	2015 Summer
282	11	2016 Spring

Figure 4: Non-nested structure: as we can see, samples with with the same fur ID can be run in multiple trays which makes FurID and Tray not nested

The type of prairies (Remnant or Restored), the species of mammals, the interaction term between them and the gender of the mammal are our primary explanatory variables of interest and, thus, are the fixed effect variables in the mixed effect model. Nitrogen and carbon isotope values are our two response variables.

2.3.2 Approach 2: stratified random sampling

In this approach, we used stratified random sampling to randomly select one of the duplicates within each FurID (mammal). Then we used similar multilevel models but with only Tray on the random level. We repeated the process 1000 times and compared the results with approach 1.

2.3.3 Lab techniques

In year 2017, biologists tried to use two different washing techniques, namely acetone or chloroform:methanol (2:1), when preparing a few fur samples from remnant prairies. Therefore, we filtered out the fur samples with different washing techniques to investigate if they have significant different effects on the nitrogen or carbon isotope levels of species. There are 40 dependent observations from 14 distinct fur samples. Since all samples are from remnant prairie, we only include the effect of different species on the isotope levels and washing techniques as fixed effects. FurID and Tray are still used as the random effect variables but on the same level for our analysis.

3. Results

3.1 Approach 1: mixed random effects models

3.1.1 Final model:

$$\textbf{Nitrogen} = \alpha_0 + \alpha_1 * \text{Remnant}_i + \alpha_2 * \text{Species}_i + \alpha_3 * \text{Remnant}_i * \text{Species}_i + u_i + v_k + \epsilon_1$$

$$\textbf{Carbon} = \beta_0 + \beta_1 * \text{Remnant}_i + \beta_2 * \text{Species}_i + w_i + \tau_k + \epsilon_2$$

i indicates the FurID level. For each individual mammal, we gave it a FurID. Many duplicate samples were collected from a single mammal, so we created this variable to represent the mammal from which the fur was taken.

k indicates the tray level. Samples with the same tray number mean they were ran at the same time. There are 16 trays in our dataset. Tray is basically a measurement of experimental errors including the moisture level of the time when the fur samples were run, the researcher who ran the samples, errors in all sample handling procedures, the status of the mass spectrometer, etc. However, we don't have any variable (measurement) on this level.

j indicates the sample level. Again, samples can be taken from the same mammal. We don't have any variables on this level.

We did not find gender to be a significant factor in our models for nitrogen nor carbon, and the same species doesn't have significantly different carbon level between remnant and restored prairies.

3.1.2 Final Model Output:

For stable isotope ratios of nitrogen, prairie voles were significantly different from the harvest mice (t=3.835) and prairie deer mice (t=6.273). The effect of the type of prairie on $\delta^{15}N$ for prairie voles was different from that for the harvest mice (t=-2.751). For prairie voles, $\delta^{15}N$ is significantly higher for those in restored prairies than in remnant prairies (t=3.538).

The carbon level of prairie voles was significantly different from that of meadow voles (t=2.40), harvest mice (t=10.38) and prairie deer mice (t=10.22). The type of prairie was not a statistically significant predictor for carbon isotope level.(Table 1)

	Nitrogen		**Carbon**	
	coef	**T-stats**	**coef**	**T-stats**
Intercept	4.182	12.133*	-23.996	-40.680*
Remnant/Restored				
Restored (Ref)				
Remnant	1.634	3.538*	0.427	0.610
Species				
Prairie Vole (Ref)				
Meadow Vole	-0.349	-0.861	1.890	2.400
Harvest Mouse	1.453	3.835*	7.577	10.380*
Prairie Deer Mouse	2.047	6.273*	6.627	10.220
Interaction Terms				
Remnant/Restored × Prairie Vole (Ref)				
Remnant/Restored × Harvest Mouse	-2.035	-2.751*	-	-
Remnant/Restored × Prairie Deer Mouse	-0.482	-0.681	-	-

Table 1: Multilevel models results for nitrogen and carbon

3.2 Approach 2: Stratified random sampling

As we can see, the results are consistent among 1000 repeated trials (Table 2). They are also consistent with the crossed random effect models. Harvest mouse and prairie deer mouse are significantly different from prairie vole in all 1000 trials which is consistent with t-values greater than 2 in our mixed random effect model. The t-statistics for meadow vole when looking at carbon isotope level is 2.400, which is borderline significant in our mixed random effect model. Among simulated trials, the min of t-statistics of meadow vole for carbon is 1.55 while the max is 2.78. We need more data to test if there is a difference between two vole species in their carbon isotope levels.

Variables	**Nitrogen**		**Carbon**	
	Min	**Max**	**Min**	**Max**
Remnant/Restored				
Restored (Ref)				
Remnant	2.87*	3.86*	-0.34	1.06
Species				
Prairie Vole (Ref)				
Meadow Vole	-1.49	-0.60	1.55	2.78*
Harvest Mouse	3.33*	3.90*	9.21*	10.19*
Prairie Deer Mouse	5.24*	5.96*	8.26*	9.73*
Interaction Terms				
Remnant/Restored × Prairie Vole (Ref)				
Remnant/Restored × Harvest Mouse	-2.97*	-2.08*	-	-
Remnant/Restored × Prairie Deer Mouse	-1.48	0.21	-	-

Table 2: The minimum and maximum t-statistics for a given variable among 1000 trials. Note: we did not include simulated t-statistics for intercept since they are not our main interest.

3.3 Lab techniques

We did not find the use of different washing solutes to be a significant factor in nitrogen nor carbon isotope levels.

4. Discussion

In this study, we investigated factors that affect the diet of small prairie mammals by examining carbon and nitrogen isotope levels in their fur samples. We used two approaches to test statistical significance, namely mixed random effects models and stratified random sampling. The consistency in our findings in using these two approaches ultimately strengthens the validity of our results.

Beginning with carbon isotopes, we found that both prairie deer mice and harvest mice included more C_4 plants in their diet as indicated by the higher $\delta^{13}C$ in both, which was in opposition to our hypothesis. These findings led us to believe that since these mice do not have specialized teeth and specialized digestive systems to consume the fibrous, silica rich leaf material, they may instead be consuming the

seeds of C_4 plants rather than consuming C_3 plants as we had originally hypothesized. Looking at nitrogen isotopes, we found higher $\delta^{15}N$ in mice compared to voles may indicates the inclusion of more insects and other invertebrates in their diet. This similarity in $\delta^{15}N$ between the prairie vole and meadow vole indicates they occupy a similar dietary niche and may compete with one another when found in the same location. This may explain their population declines relative to the other mice and voles we studied. Additionally, there is a higher $\delta^{15}N$ content in restored prairies, which may be a result of historical use of fertilizers on fields that were once used for agriculture, but there is no found differences in $\delta^{13}C$ between remnant and restored prairies.

Limitations in our research include that we did not catch meadow vole in remnant sites, which may bias our use of an interaction term in the model. Our sample size is small (N=398) which may influence the accuracy of our results. Further research can involve data sets with larger sample sizes across larger time frames. Other methods can additionally be used to adjust for the batch effect caused by the use of trays and to account for use of other laboratory methods recorded in the handling of samples.

This study reveals that stable isotope data analysis can be useful in the study of small mammal populations. While obtaining of stable isotopes requires substantial field work and technological resources, its implementation within the biological community could provide useful information about population diets. This method additionally may result in highly variable results, but in using our techniques we have shown that certain forms of variation and grouping can be properly accounted for. Through the creation of these models, we are confident that the differences we observed between mice and voles as well as species level differences reflect the actual differences found in the fur samples as opposed to being procedural artifacts. We hope that this analysis of stable isotope data informs future research on the topic of prairie communities as it could be potentially useful in informing restoration and conservation efforts.

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