

ECOLOGICAL DIFFERENTIATION OF AMINO ACID STABLE ISOTOPE PROFILES REVEALS MICROBIAL CONTRIBUTIONS TO PROTEIN METABOLISM IN WILD SMALL MAMMAL COMMUNITIES

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Abstract

Amino acid stable isotope analysis offers an effective method to study ecological differentiation and metabolic differences within hosts in wildlife systems. Gut microbes can play a role in protein metabolism; however, there is limited information about their isotopic discrimination relative to mammals. The present study aimed to evaluate ecological differentiation in amino acid stable isotope profiles among wild small mammal groups and associated fecal microbial communities. A quantitative secondary-data design was applied to 70 observations containing ecological group information and amino acid carbon and nitrogen isotope variables. Non-detectable values were treated as missing data, isotope variables were converted into numeric format, and standardized values were used for multivariate analysis. Descriptive statistics, correlation analysis, principal component analysis, and analysis of variance were performed to assess isotopic structure and group-level differences. Stable isotope variables showed a clear multivariate structure. PC1 explained 41.999% of the total variance, while PC2 and PC3 explained 25.602% and 6.654%, respectively. Significant group differences were observed for PC1 ($F = 34.545, p < 0.001$) and PC3 ($F = 18.845, p < 0.001$), whereas PC2 was not significant. Carbon-associated amino acid markers, especially Leu13C, Glx13C, Ile13C, Val13C, and Pro13C, contributed most strongly to ecological separation. Amino acid isotope profiles distinguished mammalian groups from fecal microbial communities and highlighted the importance of carbon-linked metabolic pathways in ecological differentiation.

Keywords: stable isotope ecology, amino acid isotopes, small mammals, gut microbiota, principal component analysis

1. Introduction

Isotope ecology has grown significantly over the past decades as an essential tool for understanding biological interactions, trophic relationships, and nutrient cycling across ecosystems. Stable isotope methods make it possible to examine complicated ecological interactions and feeding relationships in wildlife communities where nutrient assimilation and metabolic interactions are usually very complex. The recent increased interest in isotope-based ecology is mainly driven by the ability of these techniques to combine ecological, biological, and geochemical data within a single system (Hobson, 2023). Stable isotope methods are currently used in various zoological studies concerning trophic ecology, physiology, migration, and ecosystem interactions. Furthermore, the interdisciplinary character of isotope applications contributes to broader ecological knowledge concerning both terrestrial and aquatic environments (Griffiths, 2020). Applications of isotopes in ecological studies have recently gained more prominence due to their importance in assessing biological adaptations to environmental change. Using stable isotopes enables researchers to identify ecological interactions caused by habitat modifications, species invasions, and new feeding relationships in complex ecological systems (McCue et al., 2020). In wildlife ecology, stable isotope techniques are used to differentiate wild animals from captive populations with distinct differences in their dietary composition and metabolism. Recent studies have revealed that isotopic profiling can reliably discriminate between wild and captive animal populations due to variations in dietary assimilation and metabolic processes (Brasileiro et al., 2023).

Compound-specific isotope analysis of amino acids has emerged as a powerful approach for understanding the nature of nutrient pathways and trophic interactions within biological systems. Unlike bulk isotope analyses, amino acid-specific methods allow the researcher to assess metabolic pathways and ecological interactions with greater accuracy. Amino acid-specific isotopic methods have proven successful in studying ecological interactions within complex food webs characterized by multiple trophic processes taking place simultaneously (Pollierer et al., 2019). Similarly, compound-specific isotopic analyses of amino acids have been used in marine studies to describe ecological differences among consumer groups inhabiting the same habitats but having unique niches (Larsen et al., 2020). Another area receiving considerable attention in modern ecology is related to the impact of gut microbial communities on the physiology of host organisms. These bacteria have a direct effect on the synthesis of amino acids, metabolism, and energy utilization in their hosts, which, in turn, impacts other ecological and physiological processes (Lindsay et al., 2020). Moreover, ecological interactions within the gut are influenced by trophic organization and nutrient pathways and shape microbial communities and their functions (Gralka et al., 2020). Ecological studies of microbial systems have indicated that gut microbial communities can produce evolutionary and ecological effects on their hosts through long-term metabolic interactions and adaptations (Moran et al., 2019). Recently, there has been increasing recognition of the role played by cross-feeding relationships in the functioning of gut microbial communities. Cross-feeding interactions play a significant role in shaping the metabolic pathways used by gut bacteria through the control of microbial coexistence, metabolic specialization, and resource allocation (Culp & Goodman, 2023). The results of such studies suggest the increasing relevance of considering microbial interactions in isotopic analysis of wildlife communities. Consequently, ecological differences among hosts could be represented by isotope variation in relation to microbial contributions to the metabolism of proteins. Multivariate methods have become more important in the interpretation of complex isotope datasets collected in the course of ecological studies. Usually, ecological systems involve many interacting biochemical variables that cannot be addressed in isolation. Principal component analysis and other multivariate methods are useful in detecting ecological gradients, clusters, and dominant variables in large isotope datasets. Research exploring ecological niche expansion and recovery processes in degraded ecosystems has shown that multivariate ecological variation can indicate important biological differences among populations and ecological niches (Jacobs et al., 2019). Even though stable isotopes became more prevalent in ecological studies over the past decade, certain limitations still exist in modern wildlife isotope analysis. Most existing studies concentrate only on analyzing trophic position and diets, but ignore microbial contributions to host-associated metabolism. Despite the demonstrated applicability of amino acid-based isotope methods in studying ecological interactions, the number of investigations comparing isotope distributions among mammalian ecological groups and microbes is limited. There is a predominance of controlled experimental or marine ecological studies, although ecological differences among wild small mammal populations have not yet been thoroughly explored. Finally, previous studies tend to consider each variable separately, even though multivariate approaches should be applied in such cases. Therefore, using the combination of principal component analysis, ecological groupings, and amino acid isotopes is still understudied in research on wildlife-associated microbial systems.

This study aims to examine ecological differences between wild small mammals and associated microbial communities in terms of the amino acid isotope profile. Furthermore, it aims to characterize the distribution patterns of carbon and nitrogen isotope variables, identify correlation structures between isotope markers, carry out ecological grouping using principal component analysis, establish the presence of isotopic differences between ecological groups, and find the isotope variables responsible for these differences.

2. Methodology

2.1 Research Design

The methodology used in this research to examine ecological variation in stable isotope composition was a secondary quantitative data approach. The examination centred on multivariate associations in the isotope composition of amino acids obtained from the wild community of small mammals and their respective microbes. The methodology utilized in analyzing ecological variations in group isotopic composition involved a cross-sectional approach.

2.2 Data Source

The dataset analyzed in the current study was sourced from the isotopic database released by Besser (2023). It comprised measurements of amino acids and isotopes of carbon and nitrogen that were found in relation to wild small mammals and their fecal microbiomes. Specifically, the original research focused on analyzing the role of gut microbes in protein metabolism in hosts by means of isotope measurements of amino acids among different ecological categories (Besser, 2023). The total number of measurements and variables in the dataset amounted to 70 and 36, respectively.

2.3 Data Preparation and Cleaning

Before any statistical calculations, the data set was first checked for missing values and nondetected values. The nondetected observations that appeared as text labels were changed to missing values to make them compatible with numerical computations. While all isotope variables were coded as numeric, categorical variables, such as ecological group and taxonomy, were left untouched for categorization purposes. Missing numeric values were imputed using mean replacement in multivariate calculations, including PCA computations.

2.4 Variables Included in the Study

The experimental analysis entailed the incorporation of amino acid-specific carbon and nitrogen isotope variables as the main variables used for measurement. Carbon isotope variables included Ala13C, Gly13C, Thr13C, Ser13C, Val13C, Leu13C, Ile13C, Pro13C, Asx13C, Glx13C, Phe13C, Tyr13C, and Lys13C. Nitrogen isotope variables included Ala15N, Gly15N, Thr15N, Ser15N, Val15N, Leu15N, Ile15N, Pro15N, Asx15N, Glx15N, Phe15N, Tyr15N, and Lys15N. Ecological grouping variables were additionally included to evaluate isotopic differentiation among Cricetidae, Heteromyidae, and fecal microbial communities.

2.5 Statistical Analysis

Descriptive statistical analyses were carried out to characterize the nature of distributions of isotope variables in terms of means, standard deviations, minimums, maximums, and missing data. Correlation analysis was then carried out to determine relationships between isotope variables as well as multivariate ecological patterns. Principal component analysis was employed to achieve a reduction of dimensions and determine clustering of isotopes between ecological classes. Isotope variables were standardized prior to conducting principal component analysis to eliminate any effect of scale on variables. The scores from principal component analysis were then utilized to represent differences between ecological classes. Analysis of variance was carried out on principal component scores to find out whether there were any statistically significant differences between isotope variables within ecological classes. Significance was determined at 95% level of confidence with a p-value less than 0.05.

3. Results

3.1 Descriptive Characteristics of Stable Isotope Variables

The set of stable isotopes had 70 records and comprised different ecological categories and isotope variables. There were significant variations for both the carbon and nitrogen isotope indicators. The dispersion of carbon isotope-related variables was greater compared to the dispersion of nitrogen isotope variables, meaning that there is significant heterogeneity in the isotopic compositions of biological samples. Some nitrogen variables had relatively high percentages of missing observations, especially in the amino acid-specific nitrogen isotope variables.

Table 1 presents the descriptive statistics of the stable isotope variables. In the carbon isotope variables, Leu13C had the minimum mean value (-26.964 ± 3.162), and the maximum mean value was for Thr13C (-9.033 ± 5.276). On the other hand, in the nitrogen isotope variables, Pro15N had the maximum mean value (13.825 ± 2.274) while Lys15N had the minimum mean value (4.904 ± 1.478).

Table 1. Descriptive statistics of stable isotope variables

Variable	Mean	Standard Deviation	Minimum	Maximum	Missing Values
Ala13C	-18.344	4.456	-26.3	-7.6	0
Gly13C	-13.833	5.586	-25.5	0.9	1
Thr13C	-9.033	5.276	-23.3	2.7	0
Ser13C	-9.771	4.084	-20.6	-0.9	1
Val13C	-23.004	4.216	-37.7	-12.4	0
Leu13C	-26.964	3.162	-34.1	-19.2	0
Ile13C	-19.949	4.076	-29.7	-11.4	0
Pro13C	-15.787	3.642	-28.3	-8.6	1
Asx13C	-16.643	5.095	-38.3	-3.4	0
Glx13C	-14.525	5.789	-32.1	-3.9	1
Phe13C	-22.054	2.991	-28.6	-15.0	0
Tyr13C	-24.133	3.526	-35.6	-18.6	1
Lys13C	-14.861	3.315	-24.5	-9.1	9

Ala15N	9.958	2.061	6.2	14.2	13
Gly15N	8.733	2.845	2.3	14.1	13
Thr15N	-7.691	5.134	-16.8	5.4	13
Ser15N	6.100	2.098	1.4	10.4	13
Val15N	10.707	1.674	6.5	13.8	13
Leu15N	9.196	1.615	5.2	12.3	13
Ile15N	9.751	2.160	6.1	14.4	13
Pro15N	13.825	2.274	8.1	18.3	13
Asx15N	11.988	1.641	7.2	14.8	13
Glx15N	11.789	1.689	8.4	15.7	13
Phe15N	9.712	1.328	6.2	12.6	13
Tyr15N	5.675	1.949	0.6	10.6	14
Lys15N	4.904	1.478	1.5	8.2	13

3.2 Distribution of Ecological Groups

The ecological makeup of the data set indicated an uneven sample representation among the different biological groups studied. The highest number of samples came from the heteromyids, followed by the cricetids and fecal microbe samples, which had relatively lower but equal numbers of samples. Even though the number of samples was unequal, there were isotopic values available in all ecological samples that could be analyzed using multivariate analysis.

Table 2 gives a breakdown of samples based on their ecological classification. The heteromyids consisted of 30 samples from three taxa, while the cricetids and fecal microbes had 20 samples each. The samples collected came from one site and from one year of collection.

Table 2. Sample distribution by ecological group

Ecological Group	Number of Samples	Number of Taxa	Number of Collection Locations	Number of Collection Years
Cricetidae	20	2	1	1
Heteromyidae	30	3	1	1
fecal microbes	20	4	1	1

3.3 Correlation Structure of Stable Isotope Variables

Significant correlation trends were noted between several isotope variables, especially carbon-based amino acid biomarkers. The majority of carbon isotope variables were positively correlated with each other, suggesting synchronous ecological and metabolic processes involved in the carbon acquisition process. Similarly, nitrogen isotope variables showed distinct positive clusters, but several isotope variables had inverse correlations.

As shown in Figure 1 below, the correlation plot for stable isotope variables is illustrated. Several carbon isotope variables like Leu13C, Glx13C, Val13C, and Ile13C show positive intercorrelation with each other. However, some of the carbon isotope variables, like Thr15N, have inverse correlation with multiple carbon isotope variables.

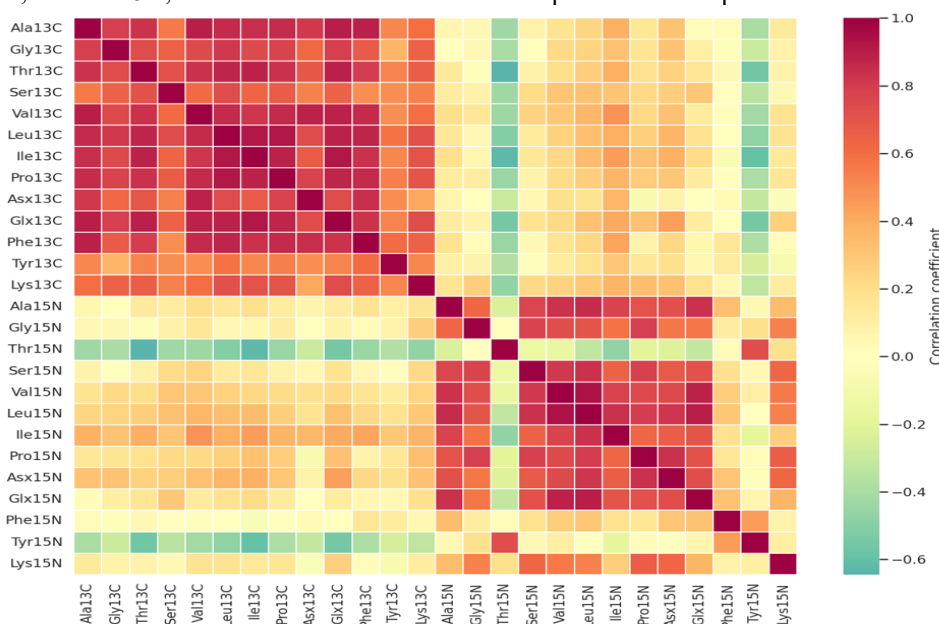


Figure 1. Correlation structure of stable isotope variables

3.4 Principal Component Analysis of Stable Isotope Profiles

PCA uncovered a significant multivariate structure in the isotope data set. While the first principal component accounted for the highest amount of variance, the other two principal components showed lower amounts of variance. All together, the first three principal components captured more than 74% of the variance, suggesting that the multivariate data can be compressed effectively while retaining ecological structure.

The ordination pattern generated based on PCA is presented in Figure 2. Clustering was observed between different ecological groups. Cricetidae individuals were largely located in areas with positive PC1 values. Fecal microbial communities were located in negative PC1 zones. Heteromyidae occupied intermediate positions with broader dispersion patterns.

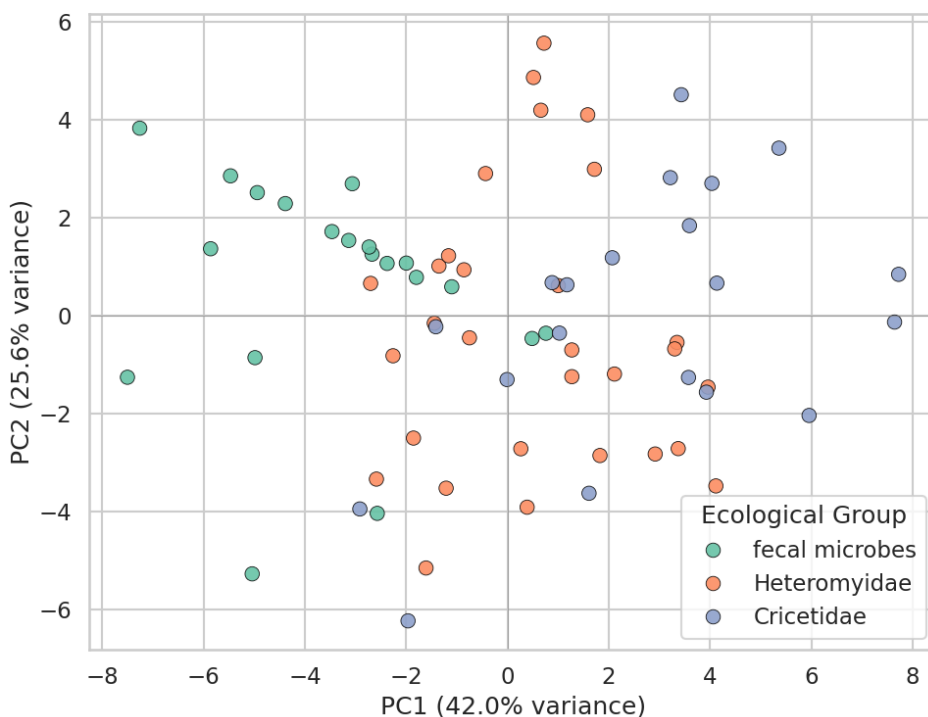


Figure 2. Principal component analysis of stable isotope profiles

3.5 Group Differences in Principal Component Scores

Differences were found among the ecological groups for the chosen principal components. Principal component one had pronounced differentiation between groups, while principal component two did not show any significant differences. Results from the analysis of variance are presented in Table 3 for the first three principal components. Principal component one displayed a highly significant difference between the groups ($F = 34.545, p < 0.001$). Likewise, principal component three displayed a significant difference between groups ($F = 18.845, p < 0.001$). However, no significant difference existed between the groups for principal component two ($p = 0.407$).

Table 3. ANOVA results for principal component scores

Principal Component	Explained Variance (%)	F Statistic	P Value
PC1	41.999	34.545	0.000000
PC2	25.602	0.912	0.406552
PC3	6.654	18.845	0.000000

3.6 Ecological Variation in Stable Isotope Profiles

Additional evidence for ecological segregation between different biological entities was provided using a visual representation of PC1 score values. The observed trend indicated distinct isotopic segregation of mammals and the fecal microbial communities.

The estimated differences between group PC1 scores are illustrated in Figure 3. While the PC1 values were positive in Cricetidae, those of fecal microbiota were clearly negative. Heteromyidae showed an intermediate position, indicating partially overlapping isotopic values with some ecological differences compared to other groups.

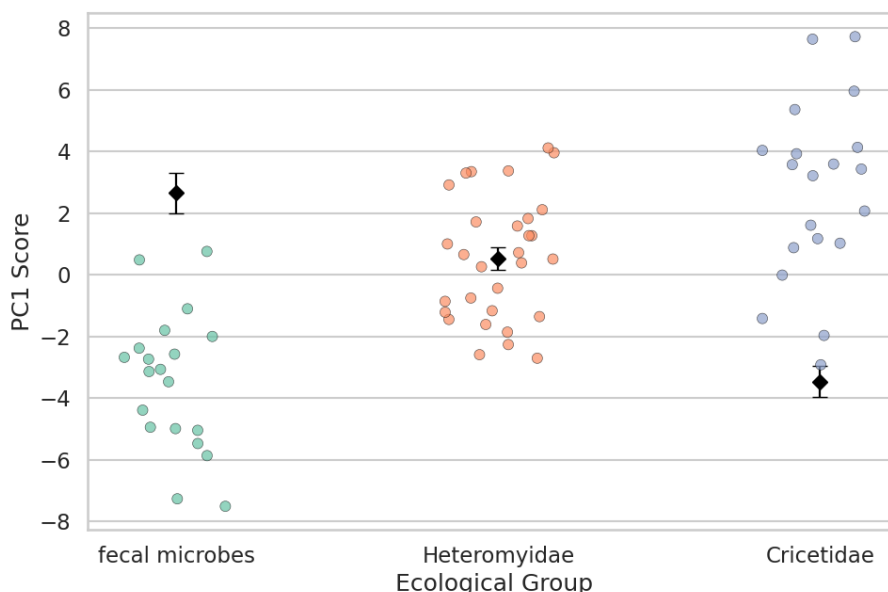


Figure 3. Estimated group differences in stable isotope profiles

3.7 Major Isotope Variables Contributing to Ecological Separation

From the loading pattern of the principal component analysis, it can be seen that the variables of carbon isotopes played an important role in ecological differentiation in the data set. A number of carbon markers from specific amino acids had higher loading values than those from nitrogen. The loading pattern of the most important isotope variables using the principal components analysis is shown in Figure 4. Leu13C, Glx13C, Ile13C, Val13C, and Pro13C were the most significant contributors to PC1. Moderate contributions were additionally observed for Ile15N and Leu15N, indicating partial involvement of nitrogen-associated ecological processes.

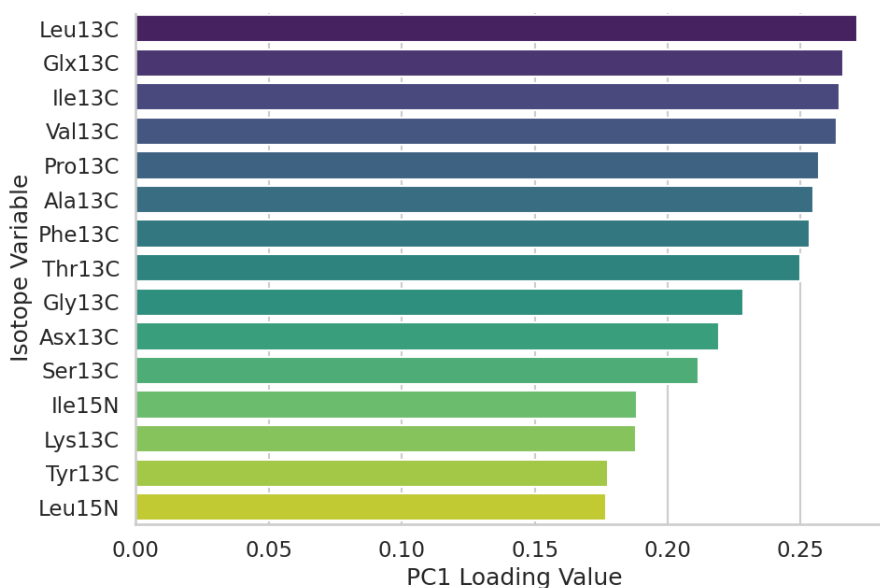


Figure 4. Major isotope variables contributing to PC1

4. Discussion

Significant ecological differentiation was demonstrated for stable isotopes of the examined groups in terms of their amino acids. The most pronounced difference was detected in respect of PC1, accounting for 41.999% of variance and being significant among the groups. This implies that the main isotopic gradient was non-randomly organized and followed the ecological pattern of biological samples. Cricetidae was characterized by high PC1 scores, while fecal samples of microbes had low PC1 scores, and Heteromyidae exhibited middle PC1 scores. PC1 could be interpreted as driven by carbon isotope variables such as Leu13C, Glx13C, Ile13C, Val13C, and Pro13C. As seen, carbon isotope markers seemed to play a more prominent role compared to nitrogen markers in the ecological differentiation of amino acids. This interpretation is supported by the correlation matrix, where many carbon isotope markers demonstrated a significant relationship among themselves. PC2 accounted for 25.602% of total variance but did not differ significantly among the groups, implying that it could represent intragroup variability. PC3 contributed even less but still differed significantly among the groups. Interpretation of these results is in line with the conclusions drawn above based on other analyses.

The separation of isotope profiles of host samples and fecal microbes can be viewed in light of evidence on the role of gut microbes in amino acid metabolism in mammals. Indeed, previous studies have shown that isotope markers and genetics could be used to trace amino acids produced by gut microbes within host bodies (Newsome et al., 2020). Such a connection between host amino acids and microbes has also been revealed in wild mammals, where differences in gut microbiota have been associated with specific physiology and biological stress responses (Stothart et al., 2019). The predominance of carbon markers in isotope profiles found in the current study agrees with previous research on the use of carbon isotope markers as a means of tracing metabolic state in animals. Stable isotopes in hair could be used to assess nutritional stress in wood bison (Funk et al., 2020). In addition, multi-isotope analysis could prove valuable in exploring the movement and biological activity of different animal species (Hobson & Kardynal, 2023).

The findings can be interpreted within the context of isotope use in ecological research involving wild animal populations. Long-term ecological research has noted that stable isotope analysis is valuable as long as it is combined with biodiversity data and thus enhances the interpretation of biological changes within animal populations (Turner et al., 2023). Stable isotope profiles in large animals can help determine seasonal changes in nutrition and environmental factors affecting them (Uno et al., 2020). The same holds true for research on trophic ecology since stable isotopes are capable of recording life history in various ecosystems. For example, a lifelong record of isotope profiles in narwhals' tusks demonstrates the animal's feeding habits and contamination levels (Dietz et al., 2021). Likewise, differences in isotope niches for seal- and fish-eating killer whales indicate that such data can be used to differentiate between ecologically distinct populations of animals (Jourdain et al., 2020).

The significant effect of amino acid carbon markers can also be interpreted on the basis of studies in compound-specific isotope analysis. The use of ^{13}C values in glycolytic amino acids helps estimate carbohydrate consumption by carnivorous fish (Wang et al., 2019). While the biological object may differ, the logic remains the same: amino acid isotope markers can be used to assess metabolic substrate consumption and the corresponding ecological patterns. Emerging techniques of compound-specific isotope analysis, such as hydrogen isotope analysis of fatty acids, prove that there is a great potential in using biochemical isotope markers to study the movement and trophic ecology (Pilecky et al., 2021). Finally, gut microbial ecology gives grounds to discuss the ecological implications of amino acid profiles in different groups of mammals. Small mammals have been shown to demonstrate a link between gut microbiota and their thermoregulation (Zhang & Wang, 2022). Furthermore, natural microbial communities of small mammals can vary depending on the host's evolutionary history and physiology (Brown et al., 2023).

The findings demonstrate the potential of multivariate isotope analysis in amino acids as a way to differentiate ecological patterns in small mammalian animals. The PC1 gradient shows the strength of the approach in tracing biological variability that cannot be captured by individual isotope values. The importance of carbon isotope markers in the PC1 gradient suggests that carbon-linked amino acids can serve as valuable biochemical markers. The ecological difference between fecal microbes and the host implies the importance of microbe-induced metabolism in the host's physiology. As a result, fecal microbial samples do not merely provide background noise but rather important information on the isotope-based metabolic pattern in the hosts. This conclusion is important for zoological, nutritional ecology, and other ecological studies that explore animal-microbe interactions in the wild.

Several limitations need to be taken into account. First, the sample size is rather modest, and all samples have been collected during a single season at a single location. Second, some nitrogen isotope variables contain missing values, necessitating the imputation procedure. Future research should include larger sample sizes, multiple habitats, seasonal sampling, and integration with microbiome sequencing to clarify how microbial composition directly relates to isotope-based metabolic differentiation.

5. Conclusion

Ecological differentiation was apparent in amino acid stable isotopes between the different small mammal taxa and their respective microbial community in the feces. Differentiation between clusters was more evident on PC1, where the Cricetidae, Heteromyidae, and the fecal microbial samples exhibited different isotopic positions. It is interesting to note that the main carbon-based amino acids, including Leu ^{13}C , Glx ^{13}C , Ile ^{13}C , Val ^{13}C , and Pro ^{13}C , were the main discriminators between groups, thus indicating that carbon metabolisms are essential for group classification. In summary, this study indicates that the application of compound-specific isotope analysis is useful in discerning the metabolic and ecological structures in wildlife studies. Also, the differentiation of the microbial community in the feces is indicative of the importance of microbial community activity in protein metabolism of hosts. Overall, the analysis demonstrates that multivariate isotope approaches can provide meaningful insight into animal-microbe ecological relationships and may support future work in zoological ecology, nutritional ecology, and wildlife metabolic research.

References

1. Besser, A. (2023). Supporting isotopic data for: Amino acid isotope analysis reveals variation in gut microbial contribution to host protein metabolism in a wild small mammal community [Data set]. Zenodo. <https://doi.org/10.5061/dryad.tdz08kq49>
2. Brasileiro, L., Mayrink, R. R., Pereira, A. C., Costa, F. J. V., & Nardoto, G. B. (2023). Differentiating wild from captive animals: an isotopic approach. *PeerJ*, *11*, e16460.

3. Brown, B. R., Goheen, J. R., Newsome, S. D., Pringle, R. M., Palmer, T. M., Khasoha, L. M., & Kartzinel, T. R. (2023). Host phylogeny and functional traits differentiate gut microbiomes in a diverse natural community of small mammals. *Molecular Ecology*, 32(9), 2320-2334.
4. Culp, E. J., & Goodman, A. L. (2023). Cross-feeding in the gut microbiome: ecology and mechanisms. *Cell host & microbe*, 31(4), 485-499.
5. Dietz, R., Desforages, J. P., Rig  t, F. F., Aubail, A., Garde, E., Ambus, P., ... & Sonne, C. (2021). Analysis of narwhal tusks reveals lifelong feeding ecology and mercury exposure. *Current Biology*, 31(9), 2012-2019.
6. Funck, J., Kellam, C., Seaton, C. T., & Wooller, M. J. (2020). Stable isotopic signatures in modern wood bison (*Bison bison athabascae*) hairs as telltale biomarkers of nutritional stress. *Canadian Journal of Zoology*, 98(8), 505-514.
7. Gralka, M., Szabo, R., Stocker, R., & Cordero, O. X. (2020). Trophic interactions and the drivers of microbial community assembly. *Current Biology*, 30(19), R1176-R1188.
8. Griffiths, H. (Ed.). (2020). *Stable isotopes: the integration of biological, ecological and geochemical processes*. Garland Science.
9. Hobson, K. A. (2023). Stable isotopes and a changing world. *Oecologia*, 203(3), 233-250.
10. Hobson, K. A., & Kardynal, K. J. (2023). Multi-isotope (δ 2H, δ 13C, δ 15N) feather profiles and morphometrics inform patterns of migratory connectivity in three species of North American swallows. *Movement Ecology*, 11(1), 48.
11. Jacobs, A., Carruthers, M., Eckmann, R., Yohannes, E., Adams, C. E., Behrmann-Godel, J., & Elmer, K. R. (2019). Rapid niche expansion by selection on functional genomic variation after ecosystem recovery. *Nature Ecology & Evolution*, 3(1), 77-86.
12. Jourdain, E., Andvik, C., Karoliussen, R., Ruus, A., Vongraven, D., & Borg  , K. (2020). Isotopic niche differs between seal and fish-eating killer whales (*Orcinus orca*) in northern Norway. *Ecology and Evolution*, 10(9), 4115-4127.
13. Larsen, T., Hansen, T., & Dierking, J. (2020). Characterizing niche differentiation among marine consumers with amino acid δ 13C fingerprinting. *Ecology and Evolution*, 10(14), 7768-7782.
14. Lindsay, E. C., Metcalfe, N. B., & Llewellyn, M. S. (2020). The potential role of the gut microbiota in shaping host energetics and metabolic rate. *Journal of Animal Ecology*, 89(11), 2415-2426.
15. McCue, M. D., Javal, M., Clusella-Trullas, S., Le Roux, J. J., Jackson, M. C., Ellis, A. G., ... & Terblanche, J. S. (2020). Using stable isotope analysis to answer fundamental questions in invasion ecology: Progress and prospects. *Methods in Ecology and Evolution*, 11(2), 196-214.
16. Moran, N. A., Ochman, H., & Hammer, T. J. (2019). Evolutionary and ecological consequences of gut microbial communities. *Annual review of ecology, evolution, and systematics*, 50(1), 451-475.
17. Newsome, S. D., Feeser, K. L., Bradley, C. J., Wolf, C., Takacs-Vesbach, C., & Fogel, M. L. (2020). Isotopic and genetic methods reveal the role of the gut microbiome in mammalian host essential amino acid metabolism. *Proceedings of the Royal Society B: Biological Sciences*, 287(1922).
18. Pilecky, M., Winter, K., Wassenaar, L. I., & Kainz, M. J. (2021). Compound-specific stable hydrogen isotope (δ 2H) analyses of fatty acids: a new method and perspectives for trophic and movement ecology. *Rapid Communications in Mass Spectrometry*, 35(16), e9135.
19. Pollierer, M. M., Larsen, T., Potapov, A., Br  ckner, A., Heethoff, M., Dyckmans, J., & Scheu, S. (2019). Compound-specific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. *Ecological Monographs*, 89(4), e01384.
20. Stothart, M. R., Palme, R., & Newman, A. E. (2019). It's what's on the inside that counts: stress physiology and the bacterial microbiome of a wild urban mammal. *Proceedings of the Royal Society B: Biological Sciences*, 286(1913).
21. Turner, T. F., Bart Jr, H. L., McCormick, F., Besser, A. C., Bowes, R. E., Capps, K. A., ... & Welicky, R. L. (2023). Long-term ecological research in freshwaters enabled by regional biodiversity collections, stable isotope analysis, and environmental informatics. *BioScience*, 73(7), 479-493.
22. Uno, K. T., Fisher, D. C., Schuster, G., Wittemyer, G., Douglas-Hamilton, I., Omondi, P., ... & Cerling, T. E. (2020). High-resolution stable isotope profiles of modern elephant (*Loxodonta africana*) tusk dentin and tail hair from Kenya: Implications for identifying seasonal variability in climate, ecology, and diet in ancient proboscideans. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 559, 109962.
23. Wang, Y. V., Wan, A. H., Krogdahl,   ., Johnson, M., & Larsen, T. (2019). 13C values of glycolytic amino acids as indicators of carbohydrate utilization in carnivorous fish. *PeerJ*, 7, e7701.
24. Zhang, X. Y., & Wang, D. H. (2022). Gut microbial community and host thermoregulation in small mammals. *Frontiers in Physiology*, 13, 888324.