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Nitrogen and Carbon Isotope Study of Bat Guano Core from Eagle Creek Cave, Arizona, U.S.A.

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Nitrogen and carbon isotope ratios were studied in a stratified deposit of guano of Mexican Free-tailed bats in Eagle Creek Cave, Arizona, U.S.A. Little diagenetic change was observed over the 25-year time span of the guano deposit. High aridity and reduced circulation of air in the cave are hypothesized to have slowed the normally rapid decomposition of the excreta and the subsequent escape of resultant ammonia. The results suggest the high dependency of the speed of diagenetic change on specific physical and other conditions of the caves and indicate that great care need be exercised in the interpretation of the isotopic ecogeochemistry of old guano. Relative contribution of C_3 photosynthesis to the food chain leading to the bats was estimated to be more than C_4 photosynthesis.

1. Introduction

It is well known that in many caves containing large colonies of roosting bats, atmospheric ammonia concentrations can be very high. This is particularly well documented in caves occupied by Mexican Free-tailed bats (*Tadarida brasiliensis*) where population sizes can exceed 10 million bats and ammonia concentrations can reach 1,000 ppm.[1] These ammonia concentrations, coupled with relative humidities close to 100%, are sufficient to cause extreme respiratory distress in man and to bleach the hair pigments of the bats.[2]

A similar situation occurs at large seabird rookeries, where a distinct odor of ammonia may be present. For instance, Smith has documented ammonia production from a penguin rookery on subantarctic Marion Island (46°54′ S, 37°45′ E), Repubic of South Africa.[3] In a series of isotopic studies of seabird rookeries, Mizutani and his colleagues have found highly elevated $\delta^{15}N$ in rookery soils, and attributed the cause to a relatively high $\delta^{15}N$ in nitrogen entering the ecosystem from the food chain and to a large fractionation effect associated with ammonia volatilization.[4]~[6]

These authors have further proposed that elevated $\delta^{15}N$ values might provide an insight into past environments and food chains,[7,8] although suitable samples are not easily obtained. Recently, however, Mizutani *et al.* have successfully identified abandoned seabird colonies from the isotopic signature of the soils.[9] Bat guano deposits are promising ave-

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nues of investigation along this line, because the sheltered environments of caves could provide for the accumulation of deposits representing hundreds or even thousands of years of deposition.

DesMarais *et al.* have studied the carbon isotopic signature of individual hydrocarbons in modern guano of mostly *Tadarida brasiliensis* and *Myotis velifer* from a cave in the Carlsbad region, New Mexico, U.S.A., and shown that this approach can yield insights into the ecology of living bats.[10] However, before isotopic ratios in historic and fossil guanos can be interpreted, a thorough understanding of the diagenetic changes that follow deposition is necessary. This is particularly true with respect to nitrogen isotopes, because ammonia volatilization is accompanied by the large fractionation subject to several variables. Here we report a study on diagenetic change in stratified guano from Eagle Creek Cave, Greenlee County, Arizona, U.S.A.

2. Sampling Locations

Eagle Creek Cave is a maternity roost for several million Mexican Free-tailed bats. The bats are high, fast flying aerial insectivores whose principal prey items are Lepidoptera, Hymenoptera, Coleoptera, and Homoptera.[11] The cave is situated in a riparian canyon within the Sonoran desert ecosystem, receiving 320 mm of precipitation per year. However, the bats are long distance fliers and apparently forage over irrigated crop fields to the west. The principal crop in the fields is cotton, with a little alfalfa.

Bats occupy Eagle Creek Cave only during the spring and summer months, where they deposit approximately 100 m^3 of guano each year.[12] Early season guano accumulates as coarse pellets, but later season guano is extensively processed by coprophagous dermestid beetles (*Dermestes carnivorous*), resulting in recognizable annual double layers.[13] The cave was completely mined in 1954, but guano accumulation has been uninterrupted since that time. A coring technique originally developed by Altenbach and Petit,[13] and modified by McFarlane and Keeler[14] allowed for the removal of samples spanning 25 years of deposition.

3. Materials and Methods

3.1 Core sampling

Cores of stratified guano were recovered from Eagle Creek Cave on October 31, 1987, using 6 cm diameter plastic piping driven into the deposit and sealed in place with a plaster cap. A 1 cm strip was then cut from the wall of the core tube to expose the guano strata. Alternating light and dark strata were dated by reference to the uppermost pair (year: 1987A.D.).[14]

The dark, lower layer of each annual pair consists primarily of intact fecal pellets with undigested insect chitin. The light, upper layer of each pair contains a high proportion of bat hair, dermestid beeetle setae, and beetle frass. To avoid complications introduced by a further trophic level, analyses were concentrated on the dark, lower layers.

3.2 Guano preparations

3.2.1 HCl treatment

Bat guano often contains carbonates because of the capillary movement of water that leads to crust-like deposits of carbonate from limestone of the cave. Therefore, guano samples were decarbonated by the addition of an excess of organic-free 2 N HCl. The acidified slurry was then left overnight in a hood at am-

Table 1. Results from Different Drying Procedures

Year [A.D.]	Procedure	Kjeldahl nitrogen		Organic carbon	
		Content [mgN/(g dry sample)]	Isotope ratio [‰]	Content [mgC/(g dry sample)]	Isotope ratio [‰]
1982D	A	159	7.8	202	−24.6
	B	179	7.1	n.a.	n.a.
1978D	A	162	6.7	272	−23.5
	B	176	5.5	241	−23.0
	B	170	6.5	289	−23.6
1973D	A	162	7.3	245	−21.6
	B	166	6.7	237	−21.9
	B	168	6.9	321	−22.2
1973L	A	164	8.8	228	−20.4
	B	174	7.8	250	−21.1
1972D	A	178	8.4	222	−21.0
	B	182	8.1	n.a.	n.a.

Suffix attached to the year represents: D for dark layer and L for light layer. Letter A of procedure stands for the one at 60℃ for 24 hr soon after the collection, and letter B for vacuum-drying and direct Kjeldahl digestion as given in the text (3.2.2 Drying procedure of Materials and Methods). Letters n.a. signify "not analyzed."

bient temperature. After confirming that the pH of the top liquid of the slurry was less than 1, it was vacuum-dried and subjected to the analysis. No layer of the core samples showed a bubbling, indicating little presence of carbonates in the guano. In cases of some guanos reported separately,[15] however, a large number of bubbles evolved upon the addition of acid. In that event, the acid was slowly added until little bubbling was observed.

3.2.2 Drying procedure

Because of certain difficulties in transportation, samples had to remain at times as collected for a period, introducing a possibility of alterations of samples. The drying procedure to eliminate volatile components of guano to deter further alterations, however, would offer another opportunity to alter its nitrogen isotope ratio. Studying the extent of the artificial ^{15}N enrichment in soils of seabird rookery, Mizutani *et al.* found that the change caused by the drying is small.[7] The following examines the effect of drying on the isotope ratios for Eagle Creek Cave core samples.

To evaluate the effect of drying and long storage on the contents and their isotope ratios, some core samples were separated into two, and two different methods were examined: 1) To dry samples at 60℃ for 24 hr soon after the collection and to use the dried samples for both nitrogen and carbon isotope measurements; and 2) After 17 days at ambient temperature, to vacuum-dry a portion of samples by a Labconco FDC-8 freeze dryer for carbon isotope analysis after decarbonation and to directly put another portion without drying to Kjeldahl digestion for the purpose of nitrogen isotope measurement. In either case, dried guano was passed through a 2 mm stainless-steel sieve, homogenized to pass a 0.5 mm sieve, and subjected to the carbon isotope analysis.

Table 1 compares the results obtained

from the two procedures. In general, carbon content and $\delta^{13}C$ do not appear to be much different. A small decrease in nitrogen content and an increase in $\delta^{15}N$ seem to take place after drying at 60°C for 24 hr. The largest increase of 1.2‰ in $\delta^{15}N$ was obtained for the dark layer of 1978 core, while the smallest (0.2‰) was also from the same core. Probably, a variation of about 1‰ should be considered present not only for $\delta^{15}N$ but also for $\delta^{13}C$ in one layer of guano samples. Natural variation within a layer would be more than that caused by drying, though the $\delta^{15}N$ alterations during drying and/or prolonged storage may exist.

Unless otherwise noted, the method of 2) was employed in the following study, though the period of guano storage at ambient temperature was not necessarily 17 days but variable.

3.3 Determination of Kjeldahl nitrogen content and organic carbon content

Organic nitrogen was converted to ammonia by Kjeldahl digestion.[5] The ammonia thus produced was steam-distilled and collected as ammonium sulfate. The nitrogen content was determined by using its aliquot, with the remainder used for nitrogen isotopic measurement. The phenol-hypochlorite method[16] was used for the determination. Some additional details of the measurement are elsewhere.[7]

A sample was combusted to form carbon dioxide as described elsewhere.[17] The amount of gas thus generated was measured manometrically. Organic carbon content was calculated from its volume, and the gas was later used for carbon isotopic measurement.

3.4 Isotope measurement

The ammonium sulfate solution was converted *in vacuo* to N_2 gas by a modification of Rittenberg.[18] The gas thus produced was purified by either passing it through or circulating it for 30 min in a CuO furnace with Pt wire heated at 700°C and a Cu furnace heated at 400°C. The nitrogen gas thus purified was introduced for ratiometry to either a Hitachi RMU-6R mass spectrometer with dual inlet and double collector systems or a Finnigan MAT-251 mass spectrometer with dual inlet and triple collector systems. Additional details are elsewhere.[7]

In accordance with convention, the nitrogen isotope ratio was expressed in ‰ deviation from atmospheric nitrogen as defined by the following equation:

$$\delta^{15}N(‰) = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{air}}{(^{15}N/^{14}N)_{air}} \times 1{,}000$$

Working standards were two ammonium sulfate solutions with $\delta^{15}N$ of −3.4‰ and 1.3‰. Standard deviation of the nitrogen isotope measurements was 0.2‰.

To determine the carbon isotope abundance, the gaseous carbon dioxide was introduced to the mass spectrometers. The data for carbon were expessed in the same manner as for nitrogen, and reported as ‰ deviation from the PDB carbonate standard, which is a Cretaceous belemnite (*Belemnitella americana*) from the Peedee Formation of South Carolina, U.S.A. The carbon isotope data from the Hitachi RMU-6R were corrected for ^{17}O. Results from the triple-collecting Finnigan MAT-251 were compared with those from the Hitachi RMU-6R to modify the correction equation of Craig.[19]

Working standards of carbon were calibrated against U.S. National Bureau of

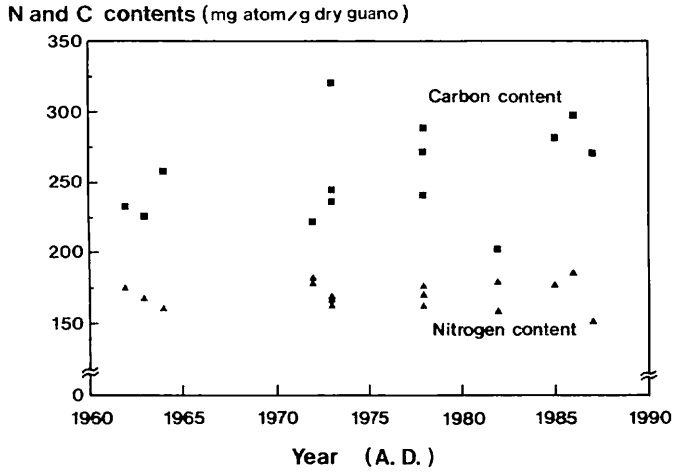

Fig. 1. Nitrogen and carbon contents of the dark layers and the year of deposition. Each datapoint represents the analysis of a single layer. Symbols used are: ▲, nitrogen content; and ■, carbon content. The following data were obtained from samples dried at 60°C for 24 hr: nitrogen and carbon data for the years 1962~1964; one set of nitrogen and carbon data for years 1973 and 1978 (*cf.* Table 1); and carbon data for years 1972 and 1982.

Standards isotope reference material No. 20 and No. 21. The $\delta^{13}C$ values for the two working standards were −19.4‰ and −12.0‰. Standard deviation of the carbon isotope measurements was less than 0.1‰.

4. Results and Discussion

4.1 Dark and light layers

The presence of the alternating dark and light layers which facilitated the dating of the Eagle Creek guano also permitted an examination of the isotopic effects of beetle coprophagy. Table 1 provides relevant data for the year 1973. In addition, the light layer from 1978 was also studied and yielded a carbon content of 283 mgC/(g dry guano) with a $\delta^{13}C$ of −22.7‰ and a nitrogen content of 160 mg N/(g dry guano) with a $\delta^{15}N$ of 4.9‰. In both years, there appear to be no consistent differences in the nitrogen isotopic signatures between the dark and light layers. In case of carbon, isotope signatures seem a little higher for light layers. At Eagle Creek Cave, dermestid beetle coprophagy may be accompanied by only a little isotopic fractionation of nitrogen and carbon.

4.2 Early diagenetic change in $\delta^{15}N$ and $\delta^{13}C$

Mizutani[4] and Ishizuka *et al.*[20] reported a rapid decomposition of avian guano in seabird rookeries. This was accompanied by a highly elevated value of $\delta^{15}N$ in the rookery soil.[7] More recently, Mizutani *et al.* reported that the extent of the enrichment is related to the latitude of rookery locations.[21] The return rate of the elevated $\delta^{15}N$ of soil to native value was shown in subantarctic Campbell Island (52°33′ S, 169°09′ E), New Zealand, to be quick in the first 10 years and to slow down in later years.[9] It is, therefore, of interest to search for early diagenetic changes in bat guano deposits, and how it compares with avian guano.

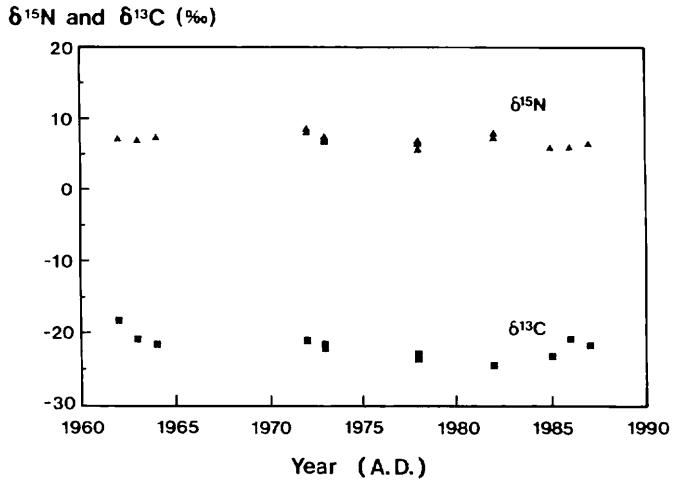

Fig. 2. Nitrogen and carbon isotope ratios for the dark layers and the year of deposition. Each datapoint stands for the analysis of a single layer. Symbols used are: ▲, $\delta^{15}N$; and ■, $\delta^{13}C$. The following data were obtained from samples dried at 60℃ for 24 hr: nitrogen and carbon data for the years 1962~1964; one set of nitrogen and carbon data for years 1973 and 1978 (*cf.* Table 1); and carbon data for years 1972 and 1982.

Figure 1 summarizes results of nitrogen and carbon contents from the dark layers of the Eagle Creek guano cores. Figure 2 shows those of isotopic ratios. Although there is considerable annual variation, there is no apparent pattern of long-term change during the 25-year record. This is in marked contrast to the situation in the seabird rookeries.

4.3 Aridity and ^{15}N enrichment

The high ammonia content[1] in the atmospheres of humid, large colony bat caves evidences rapid decomposition of organic nitrogen. Conditions in major roosts in the southwestern USA and in the tropics may necessitate respiratory equipment for human investigators.[22] However, at Eagle Creek Cave, 6 weeks after the departure of the bats, no ammonia odor was detectable. Eagle Creek guano analyzed in this study was found to contain 4.0±0.6% water (19 dark layer samples from 1971 to 1987), a condition that might be described as mummified.

In contrast, more than half the weight of seabird rookery soils was commonly water, even when collected after several days of dry weather.[23] Our study on fresh bat guanos from other regions showed that most of them contain *ca.* 40% water, the highest containing 75% water of its wet weight: guano of another insectivorous bat in Wondrous Cave, Jamaica.[15]

The Eagle Creek guano shows no evidence of decomposition to the eye or to the scanning electron microscope during its 25-year history in the cave.[24] We hypothesize that the rate of bat guano decomposition is controlled by moisture availability, and is virtually arrested under the extremely dry conditions prevalent in Eagle Creek Cave.

Very low moisture content would also prevent the elevation of $\delta^{15}N$ even if decomposition continued to produce ammonia, since the ammonia would be tightly absorbed into the deposit and not vola-

tilized. Adequate moisture is known to be a factor in the volatilization of ammonia from topsoil in semiarid environments, and a rapid, surface drying reduces these losses.[25] Furthermore, the circulation of air may be another factor in determining the extent of the fractionation.[21] As there is a cave whose physical features are such that rapid ammonia volatilization and restricted air flow coexist,[1] the relative contribution of these variables to ^{15}N enrichment could be quantified.

4.4 Contribution of C_3 and C_4 fixations to bat food chain

There are two basic pathways of photosynthesis, C_3 fixation and C_4 fixation, which can be distinguished by $\delta^{13}C$ values for fixed carbon. Plants that engage in C_3 fixation are called C_3 plants and those engaged in C_4 photosynthesis are C_4 plants. CAM plants, another group of terrestrial plants, primarily incorporate CO_2 at night into C_4 acids,[26] though many may combine nocturnal C_4 fixation and mid-day C_3 fixation.

Studying the carbon isotope biogeochemistry of bats in the Carlsbad regions, DesMarais *et al.* reported $-26.0\pm2.2‰$ for 27 C_3 plants and $-13.1\pm1.1‰$ for 31 C_4 and CAM plants.[10] The present results give an average $\delta^{13}C$ of $-21.7\pm1.7‰$ for bulk guano. This value should reflect relative contributions of these primary producers to food chain leading to the bats.

The immediate surrounding environment of Eagle Creek Cave is arid Sonoran desert. The C_4 and CAM plants are more suited to sunny, semiarid environments than C_3 plants and often predominant in such environments.[27] However, the bats apparently forage over irrigated crop fields to the west, where C_3 plants are grown (principally cotton, with a little alfalfa). Using DesMarais *et al.*'s results as endpoint values for the two types of photosynthesis, the relative contributions of the primary producers can be estimated. This is of interest because many agricultural plants employ C_3 photosynthetic pathways and the significance of bats as predators of agricultural pests may be estimated.[10]

To evaluate the relative contribution, two steps have to be considered: a) from plants to insects and b) from insects to bat guano. Mizutani and Wada[8] found a statistically insignificant difference in the $\delta^{13}C$ values between the average diet and fresh guano of the Black-tailed Gulls (*Larus crassirostris*). However, in case of food chain effect between plants and insects, little data are available.

In particular, bats do not always consume whole insect body, and there exist differences in the isotope ratios among different tissues of various animals.[28]~[31] Though little study on the difference among insect tissues had been reported, wing and body of the White-lined Sphinx Moth (*Celerio lineata*) showed a $\delta^{13}C$ difference of more than 2‰,[15] and DeNiro and Epstein reported a large difference between a whole body of insects and their chitin,[32, 33] a predominant macromolecule in certain tissues.

Though the present state of knowledge comes with a large uncertainty, DeNiro and Epstein in the same report[33] states that the isotopic composition of the whole body of an animal is on average enriched in $\delta^{13}C$ by about 1‰ relative to the diet. It would give a rough estimate of the relative contributions. As the estimated, average $\delta^{13}C$ for diet of insects is $-22.7‰$, it can be inferred that the bats at Eagle Creek Cave on average consume more

insects that live on C_3 plants than those foraging over C_4 and CAM plants.

5. Conclusions

Statified deposit of bat guano in Eagle Creek Cave, Arizona, U.S.A., showed little diagenetic change in both contents and isotope ratios of nitrogen and carbon during recent 25 years. High aridity would have slowed the normally rapid decomposition of the excreta. The reduced circulation of air in the cave might also have contributed to the observation. As atmospheric ammonia concentrations are often high in other major occupied bat caves, the diagenetic fate of guano deposits may depend critically on local physical and other conditions. Careful precaution would be needed to interpret, in particular, the nitrogen isotope ratio of old guano.

Relative contribution of C_4 and CAM plants to Eagle Creek bat food chain was roughly estimated to be one third of C_3 plants. For more detailed analysis, the isotopic behavior along food chain should be better known; in particular, selection of insects and their tissues by bats, and the food chain effects between plants and insect tissues.

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