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METHODS AND ANALYSIS OF BAT GUANO CORES FROM CAVES FOR PALEOECOLOGY

Alexandra Tsalickis[1,C], Matthew N. Waters[1], and Joshua W. Campbell[2]

Abstract

Whereas bat guano is gaining viability in accurately reconstructing local paleoenvironmental and climatic conditions, overall reviews of methods for analyzing and collecting bat guano cores have received less attention. Guano cores have been collected from several locations (e.g., United States, Romania, Philippines, and southeast Asia), and the processing and collection methods are quite similar despite a lack of standardized techniques. Physical, chemical, and elemental analyses on guano samples have focused on the interpretation of precipitation changes over time, with additional applications from stable isotope analysis being used for other paleoenvironmental conditions. We obtained three bat guano cores from Alabama and Tennessee to evaluate the collecting and processing techniques of guano. Climatic temperature changes were not analyzed in this study. The purpose of this investigation was to summarize multiple techniques and approaches used to process and analyze bat guano cores with a focus on reconstructing paleoclimate in cave environments throughout the globe. From these three cores, we describe challenges and make recommendations for improving guano analysis.

INTRODUCTION

Traditional methods of assessing paleoclimate include analyzing samples from lacustrine sediments, ice, pollen, and even soils. However, these sample types are limited to specific geographic areas. For example, glacial lakes can provide Holocene climatic histories but are limited to upper latitudes or high altitudes. In areas such as the southeastern United States, glacial lakes do not exist. Ice cores can only be obtained from ice-covered regions such as polar areas and high-altitude glaciers and cannot provide local climate histories (Jouzel and Masson-Delmotte, 2010). Furthermore, lakes tend to occur clustered in specific regions, thus limiting extensive distribution. Similarly, caves are clustered in specific regions and provide variable conditions for habitation by bats and for the accumulation and preservation of guano deposits. Some species such as *Myotis grisescens* (gray bats), roost in the same caves each year depositing annual laminations of guano into mounds (Martin, 2007). Insectivorous bat guano has been useful in paleoecological studies by tracking moisture and precipitation through stable isotope analysis, elements, nutrients, and radiocarbon dates (Bird et al., 2007; Wurster et al., 2008, 2010; Onac et al., 2014; Forray et al., 2015; Choa et al., 2016; Cleary et al., 2016, 2018, 2019). Thus, bat guano can be key for reconstructing the paleoclimate in areas where traditional methods are not available.

Insectivorous bat guano is comprised of multiple constituents such as bat-associated materials (pollen, bat hair, skin cells, feces, and dander), as well as extraneous materials (guanivorous and fungivorous invertebrates that burrow through the guano, the invertebrates frass, fungal hyphae and spores, and microbes that grow in the guano) making analysis indirect and complicated. Bat hair accumulates in guano piles through grooming during roosting activity. Insectivorous gray bats produce chitin-rich guano, which decomposes over time causing older deposits to lack discernible chitin that is more prevalent in younger deposits. Ambient pollen grains stick to bat hair as they fly in search for insects to consume, which themselves carry pollen (Maher, 2006). While roosting on cave ceilings, pollen falls off bat hair and into guano piles. Bats also ingest pollen, which passes through their intestinal tract via grooming or consuming pollen-laden insects. Pollen, hair, and chitin can provide individual data when analyzed alone; bat hair provides phylogenetic insight; stable isotopes in chitin provide information about local vegetation and precipitation; and pollen provides information on local vegetation changes. Collectively, these tools create a robust paleoclimatic record.

Bat guano has been shown to track moisture regimes and changes in local vegetation over the duration of the Holocene (11,650 YBP) and even into the Pleistocene (2.6 Ma – 11,700 YBP) (Wurster et al., 2008, 2010; Campbell et al., 2017). Although bat guano has only recently been exploited as a paleoclimate tool, more studies are utilizing its potential in providing information about local paleoenvironmental conditions that are both reliable and reproducible (Shahack-Gross et al., 2004; Onac et al., 2014; Forray et al., 2015; Choa et al., 2016; Campbell et al., 2017, Gallant, et al., 2020). Bat guano found inside caves is protected from surficial weathering, providing a uniquely unaltered data source (Onac et al., 2014; Cleary et al., 2016). Bat guano can sometimes provide high-resolution age-depth models

[1]Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL
Current Affiliation: Clemson University, Department of Forestry and Environmental Conservation, 261 Lehotsky Hall Box 3403317, Clemson, SC
[1]Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL
[2] USDA-ARS, Northern Plains Agricultural Research Laboratory, Sidney, MT
[C]Corresponding author: atsalic@g.clemson.edu

with constant deposition rates as maternity colonies and/or hibernacula of bats roost in the same caves each year (Martin, 2007). To further guano research and expand on paleoclimatic records, we present the physical, chemical, and biological characteristics of three guano cores and potential methods to provide a reproducible procedure for processing guano. In the first portion of this paper, we will review methods and analyses performed in other bat guano studies (Table 1), and in the second portion we discuss methods and analyses used on our three bat guano coring experiments with recommendations for challenges confounding guano interpretation.

METHODS REVIEW

Field Collection Methods

Various extraction procedures have been utilized when coring bat guano. Wurster et al. (2010) used two 1 m length pieces of PVC (polyvinyl chloride) that had a diameter of 5 cm. The PVC pipes were driven into the guano pile and sealed causing the pipes to be removed with no excavation. It is ideal to avoid excavation to preserve the remaining guano pile and prevent any further disturbance to the natural cave setting. Similarly, Campbell et al. (2017) used aluminum tubes to core by manually pushing them into a guano pile. One drawback for these methods is compaction of the guano during collection. Campbell et al. (2017) noted compaction of the guano core by 59 % and Wurster et al. (2010) noted compaction of the guano core by 65 %. Compaction can cause the temporal resolution to alter and may negatively impact analysis when producing an age-depth model. One benefit of using PVC pipes or aluminum tubes to core guano is that it is a less expensive alternative to some other methods described further.

A standard Livingstone piston sampler has also been used in guano studies (Maher, 2006). To use a standard Livingstone piston sampler, the piston cable is anchored, and the corer is pushed into the guano mound. Maher (2006) wrapped the core barrel in plastic foil when it was removed to avoid handling adhering feces. Maher (2006) does note compaction (50 %) occurring with this type of corer. Additionally, a large coring system such as a piston sampler could be difficult to transport to the sample site in some cave systems.

A Russian peat corer is another method used to sample guano (Geantă et al., 2012; Forray et al., 2015; Cleary et al., 2016). Forray et al. (2015) notes their corers were one meter long and five cm in diameter. Studies that have used Russian peat corers for guano collection do not comment on any compaction that occurs. Therefore, to prevent compaction, a Russian peat corer could be ideal provided the system can be transported through the cave as mentioned above.

Sample Processing and Handling Methods

Different sampling preparations have been used in guano studies and all have been successful. Wurster et al. (2010) brought guano cores to the laboratory where they were later opened and sectioned by wetting the guano with deionized water to prevent mixing during the sampling process. Their guano cores were sectioned at four- and eight-mm intervals. Campbell et al. (2017) stored guano cores at -80°C until they were ready for sampling and sectioned their cores at 2 cm intervals. While some sampling methods occurred in laboratories (Mizutani et al., 1992; Maher 2006; Wurster et al., 2010; Campbell et al., 2017), other sampling methods occurred right after guano core extraction in the cave (Cleary et al., 2016) to reduce potential contamination of the core during transport. Forray et al. (2015) and Cleary et al. (2016) both sectioned guano cores at two cm intervals.

Table 1. Comparison of methods from existing literature.

Literature Explored	Core Collection	Isotopes Analyzed	Number of ^{14}C dates	Pollen Analyzed?	Oldest ^{14}C Date (YBP)
Cleary et al., 2019	Russian peat corer	$\delta^{13}C$, $\delta^{15}N$	12	Yes	810
Cleary et al., 2018	Russian peat corer	$\delta^{13}C$, $\delta^{15}N$	20	Yes	1,069
Campbell et al., 2017	Aluminum tubes	$\delta^{13}C$, $\delta^{15}N$	5	Yes	5,950
Royer et al., 2017	Excavation of cave floor	$\delta^{13}C$, $\delta^{15}N$	16	No	34,640
Cleary et al., 2016	Russian peat corer	$\delta^{15}N$	3	No	940
Forray et al., 2015	Russian peat corer	$\delta^{13}C$, $\delta^{15}N$	12	Yes	940
Royer et al., 2015	Plastic film placed below bat roosts	$\delta^{13}C$, $\delta^{15}N$	0	No	N/A
Onac et al., 2014	Excavated floor below bat roost	$\delta^{13}C$	3	No	670
Geantă et al., 2012	Russian peat corer	None	10	Yes	855
Wurster et al., 2010	PVC pipes	$\delta^{13}C$, $\delta^{15}N$, and δD	2	No	15,300
Bird et al., 2007	Excavated floor below bat roost	$\delta^{13}C$, $\delta^{15}N$	2	No	29,990
Wurster et al., 2007	Scooped surface of guano mound	$\delta^{13}C$, $\delta^{15}N$	0	No	N/A
Maher, 2006	Livingstone piston sampler	None	1	Yes	2,890

Prior to sample analysis methods, guano typically requires pre-treatments for various measurements. Most studies used dried guano samples and homogenized them in an agate mortar (Forray et al., 2015; Cleary et al., 2016; Campbell et al., 2017). Mizutani et al. (1992), used two different methods to dry guano: 1) samples were dried at 60 °C for 24 hours soon after collection; and 2) samples were dried after 17 days at ambient temperature by using a Labconco FDC-8 freeze dryer. Mizutani et al. (1992) noted neither drying method affected isotope values. Wurster et al. (2010) provides several different detailed extraction procedures used in their study to isolate organic matter components from bulk guano. Overall, the simplest way to prepare guano samples for analysis is to combine freeze drying with grinding in an agate mortar.

Sample Analysis Methods

Methods for Stable Isotopes

Isotopic analysis is important for determining changes in paleoenvironmental conditions. $\delta^{13}C$ and $\delta^{15}N$ are used to show changes in vegetation type through time while δD is used to show changes in hydrologic conditions over time. Some bat guano studies only performed isotopic analysis on $\delta^{13}C$, $\delta^{15}N$, or both (Mizutani et al., 1992; Forray et al., 2015; Royer et al., 2015; Cleary et al., 2016; Campbell et al., 2017; Royer et al., 2017; Cleary et al., 2018, 2019) while other studies performed isotopic analysis on all three isotopes $\delta^{13}C$, $\delta^{15}N$, and δD (Wurster et al., 2010). Some studies performed isotopic analyses at their own laboratory such as Wurster et al. (2010) using continuous-flow-isotope-ratio mass spectrometry (CF-IRMS) to determine $\delta^{13}C$ and $\delta^{15}N$. They used a ThermoFinnigan Flash 1112 Elemental Analyzer coupled by a ConFlo III to a ThermoFinnigan Delta XL Plus mass spectrometer. $\delta^{13}C$ and $\delta^{15}N$ values were determined for each section in the guano core. They also determined δD by using high-temperature flash pyrolysis CF-IRMS using a ThermoFinnigan High Temperature Conversion Elemental Analyzer (TC/EA) coupled through a ThemoFinnigan ConFlo III to a ThermoFinnigan Delta XL Pluss mass spectrometer. Other studies mailed subsampled sections of guano to other laboratories to be analyzed (Forray et al., 2015; Campbell et al., 2017).

Methods for ^{14}C

Obtaining ^{14}C values is necessary for determining the age of guano and creating an age-depth profile. ^{14}C values is something all guano studies have in common, however, what is not uniform is the quantity of radiocarbon dates collected. Wurster et al. (2010) collected only two ^{14}C dates: one at the surface of their guano core and the other at the bottom depth of 1.3 meters. Samples were sent to Beta Analytic (Miami, Florida). Campbell et al. (2017) used five samples from their core to establish a dating model with Beta Analytic performing the analysis. The five samples were chosen depending upon color changes or abrupt changes in guano texture. Forray et al. (2015) used twelve guano samples to obtain ^{14}C dates. While no set number is required, a greater number of samples analyzed provides a more robust dating model, but due to the cost of ^{14}C analysis, a minimum of three is recommended so that a trend line can be established showing age-depth relationships through time.

Methods for Elements/Nutrients

Elements and nutrients are not analyzed in every bat guano study. Those that do typically measure for C:N and N:H elemental ratios (Wurster et al., 2010) or TC (Total Carbon), TN (Total Nitrogen), and TP (Total Phosphorus) (Campbell et al., 2017). Campbell et al. (2017) measured bulk density by drying and weighing five mL aliquots of raw guano. They also measured loss on ignition by burning dried guano samples at 550 °C for three hours**.** Elemental analyses are typically measured on dried and homogenized guano.

Methods for Minerals

Few guano studies have thoroughly analyzed minerals in bat guano. Detailed methods for mineral analysis are explained in Giurgiu and Tămaş (2013). Giurgiu and Tămaş (2013) used X-ray diffraction, scanning electron microscopy, and electron dispersive spectroscopy to analyze mineral samples. Their analyses revealed the presence of multiple phosphate minerals: brushite, hydorxylapatite, leucophosphite, taranakite, variscite, gypsum, calcite, and illite-group minerals and quartz. Forray et al. (2015) analyzed minerals in their study by using a Bruker D8 Advance diffractometer with cobalt anode ($CoKa_1$ with ¼ 1.78897), Fe filter for the Kb line, and a one-dimensional LynxEye detector, using corundum (NIST SRM1976a) as the standard for instrument alignment.

Methods for Chitin Analysis

Chitinous insect fragments can be separated from guano to use for analysis and Wurster et al. (2010) provides a thorough description of their methods. They created solvent-extracted guano from bulk guano and neutralized the guano in H_2O. After neutralization, the guano was washed once in methanol and three times in chloroform/methanol with a 2:1 ratio. Finally, samples were lypholized. Wurster et al. (2010) used Fourier-Transform Infrared Spectroscopy to investigate chitin. Chitin can also be used to obtain stable isotope values, but no studies were found that performed such analysis.

Methods for Pollen

Pollen provides evidence for local vegetation types and can remain preserved for millions of years (Whitehead, 1973; Delcourt, 1980; Geantă et al., 2012). The pollen that accumulates in guano needs to be separated out from the other constituents through rinsing and repeated centrifugation (Rich, 1979). After pollen separation is complete, the pollen grains must be stained for slide preparation and identification under a microscope (Halbritter et al., 2018). Identifying pollen grains is a difficult task and should be accomplished by a trained individual. Pollen was not identified in guano cores collected for this project, but pollen analysis has been successfully applied to guano cores from the southeastern United States (Campbell et al. 2017). However, less is known concerning pollen delivery and the link between local vegetation and pollen occurrence is still needed.

Campbell et al. (2017) describes the typical routine of pollen analysis includes counting at least 300 identifiable grains and calculating percentages of individual taxa based on that sum. However, not all guano core samples will have abundant pollen, so a compromised version can be performed. To prepare guano samples for pollen analysis, Campbell et al. (2017) placed guano samples in 10 % KOH and immersed them in a boiling water bath for 10 minutes and then washed clean of water-soluble humic substances using distilled water and centrifugation. Once completed, residues were mixed with glycerine jelly and prepared on slides.

According to Maher (2006), guano can be processed as if it were lake sediment using standard palynology procedures without using the 10 % HCl and 10 % NaOH treatments because the guano is too acidic to contain much carbonate. This method has successfully been used in several guano studies (Geantă et al., 2012; Cleary et al., 2018, 2019). Maher (2006) recommends washing guano in warm water containing a trace amount of dish-washing detergent, followed by 5 to 10 minutes of acetolysis solution in a boiling water bath, staining, and mounting in glycerine.

Methods for Charcoal

Charcoal can be used to determine the presence of fire. We did not analyze microcharcoal in this experiment, however, it has been successfully analyzed in other bat guano studies (Forray et al., 2015). To analyze charcoal, add 5−10 mL of KOH to one gram of guano and then centrifuge for three minutes. Discard supernatant and dilute with H_2O_2 (4−6 %) and allow it to settle overnight. Mix and sieve through a 125µm sieve and rinse with deionized water. The sieve containing the guano is kept in a drying oven until all moisture is removed. Contents are emptied into a petri dish with graph paper underneath to visually inspect and count charcoal pieces under a binocular microscope. Charcoal accumulates in caves via water, wind, and bats (Forray et al., 2015). In congruence with a pollen record, charcoal can be used to assess fire histories, changes in vegetation structure, anthropogenic activities, and local climate (Forray et al., 2015). Additionally, Campbell et al. (2017) found evidence of Woodland Indian campfires within a guano core.

Data Analysis Methods

Age-Depth Model

A critical component to paleoclimate studies is to construct accurate age models from radioisotopic measurements. Radiocarbon dating provides an effective way to age a guano core since guano usually does not date back farther than 40,000 YBP. However, there are a few exceptions to this generalization: (1) ^{14}C dates cannot be obtained in brushite or mineral layers because dates will not be in a chronologic order and will show an inversion, and (2) as ^{14}C dates cannot date past 40,000 YBP, guano cores cannot be dated beyond this time limit, deeming ^{14}C ineffective.

Forray et al. (2015) used an age-depth model based on linear interpolation between all ^{14}C dates using the clam package in R software. Age-depth models can also be extended to the full length of the core, even if ^{14}C dates were not collected at the bottom of cores. Once ^{14}C dates are obtained, the Bayesian accumulation history (BACON) age-depth modelling package in R software can be used to project ages throughout guano cores with a 95 % confidence interval (Blaauw and Andrès, 2011). BACON works by dividing a core into multiple vertical sections and subsequently uses millions of Markov Chain Monte Carlo iterations to estimate the accumulation rate for each section in years/cm (Blaauw and Andrès, 2011).

CORING EXPERIMENT

To provide examples and compare the efficacy of a majority of the analyses mentioned above, three guano cores collected from the southeastern United States were analyzed to demonstrate the diversity of paleoenvironmental data in guano deposits. The three guano cores were collected from: (1) Cave Springs Cave in Priceville, Alabama, (2) Cripps Mill Cave in Cookeville, Tennessee, and (3) Nunley Mountain Cave in McMinnville, Tennessee.

Core Collection

Access for caves was granted by private landowners or filling out permits for access to protected caves on government property. The gray bat, *Myotis grisescens*, is the primary species in the three caves visited during this study in northern Alabama and Tennessee. Locations within each cave for guano coring were chosen based on availability and height of the guano pile. Ideal height for guano coring for this study was about two meters. Prior to coring, driving a

thin metal rod or wooden dowel was used to test depth of a guano pile, or ground penetrating radar, if available. Once chosen, one-meter sections of PVC pipes were hammered into the pile with a mallet. Diameter of the PVC pipes were about five cm to provide enough guano material for the analytical techniques applied to each sample. A minimum of five cm in diameter is our recommended size for core tubes. To decrease disturbance, guano core sections were dug out so as not to compromise lower guano core sections and to limit modern guano from falling into the core hole. In addition, hammering PVC pipe caused compaction. A Russian peat corer was not used in our studies, but it has proven to be successful in other studies (Geantă et al., 2012; Forray et al., 2015; Cleary et al., 2016). Cores must be kept upright after collection and frozen until ready to section, which can be challenging if cores are obtained long distances from cave entrances. Thus, extraneous material (e.g., newspaper, cotton, etc.) may be used to fill portions of the coring container that are unoccupied by guano to limit movement of the top sections of loose guano.

Core Processing

We stored our guano cores in a walk-in freezer to maintain their integrity and prevent any contamination. PVC pipes were cut open by using a circular saw and sliced down the pipe on opposing sides. Our cores were sectioned at one cm intervals. Based on our literature review, one cm is the smallest scale typically used in sectioning bat guano (Wurster et al., 2010; Geantă et al., 2012; Forray et al., 2015; Cleary et al., 2016; Campbell et al., 2017; Royer et al., 2017). If possible, a smaller scale can be used to create a higher resolution in the radiocarbon dating model and isotopic profile, but limitation of material could occur based on the number of analyses performed. Before sectioning the guano core, radiocarbon dates were chosen observationally in each of the guano cores. We picked samples at the top and bottom of each of the cores to retrieve the best age estimates. Our guano samples were analyzed by Beta Analytic Laboratories for radiocarbon dates.

Once sectioned, each centimeter was analyzed for organic matter as loss on ignition (LOI) when heated to 550 °C for four to five hours. Bulk density was calculated as (g dry cm^{-3} wet) using the proportion of dry/wet mass and organic/inorganic content like methods performed in limnology studies by Brenner et al. (1999) (Fig. 1). For chemical analyses one cm sections were freeze dried, ground with a mortar and pestle, and sent to Waters Agricultural Laboratories (Camilla, Ga.) for elemental and nutrient data using an ARL ICP instrument. Dried and ground guano samples were sent to UC Davis Stable Isotope Facility (Davis, CA) for $\delta^{13}C$ and $\delta^{15}N$ isotopes and to Cornell University Stable Isotope Laboratory (Ithaca, NY) for δD isotopes.

Sample Analysis

Methods for Insect Separation

To separate insect chitin, one gram of guano was put in a vial along with 10 mL of a 1 % solution of detergent to break apart the guano. The vial was then put on a vortex mixer for 10 seconds followed by a hot water bath for 10 minutes and sonication for 10 minutes. After this, the guano solution sat for 24 hours and the following day was sieved through a 120 µm screen with hot water followed by deionized water. After samples dried in an oven, they were brushed out of sieves and placed in petri dishes for separation using forceps under a dissecting microscope (Fig. 2). While this method could be used to reconstruct insect consumption through time by volume of identified chitin fragments recovered from samples and species (if able to identify from chitin pieces), it appears that application is limited to modern guano samples. Degradation of deeper insect samples showed a decomposition of insect parts where individual parts were not recognizable. Chitin fragments were not identified in this study but may be able to be identified to Order level in the top portion of cores where less degradation occurred. Not all types of

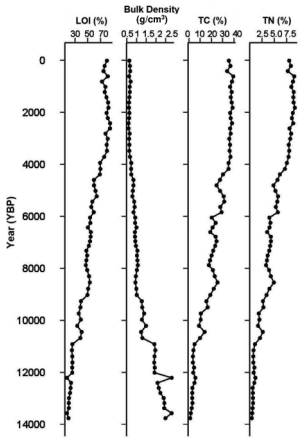

Figure 1. Organic matter content analysis in Cave Springs core. Bulk density increases with depth. Total carbon (TC), Total nitrogen (TN), and loss on Ignition (LOI) decrease with depth.

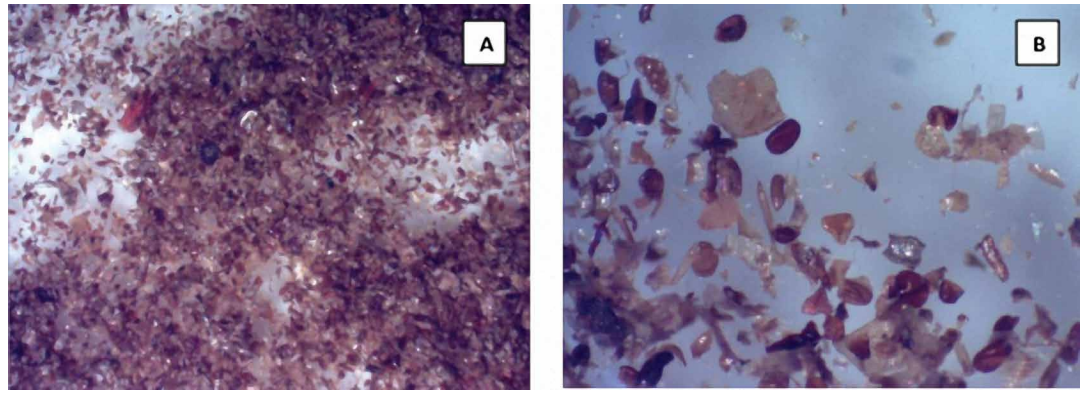

Figure 2. Insect chitin in bat guano. Pictures are taken under a dissecting microscope A) 20× magnification and B) 40× magnification.

insects were equally preserved and equally recognizable by isolated body parts, but undoubtedly, many soft-bodied insects are completely digested and will not leave any traces behind. This phenomenon may differ in caves in other regions that have a different climatic history or invertebrate fauna.

Climatic Temperature Analysis

While many studies have utilized bat guano as a proxy for moisture regimes (Wurster et al., 2007, 2010; Forray et al., 2015; Choa et al., 2016; Cleary et al., 2016; Campbell et al., 2017; Gallant et al., 2020), none have reconstructed paleotemperature conditions. It may be possible to use $\delta^{13}C$ in combination with a pollen record to determine the local vegetation type over time and what the temperature requirements would have been for those plants to thrive. In our studies, we have identified distinctions between C_3 and C_4 plant dominance throughout time by using $\delta^{13}C$ values. C_3 plants are between −32 ‰ and −20 ‰, C_4 plants are between −17 ‰ and 9 ‰, and CAM plants range between the two from −14 ‰ to −33 ‰ (Bender et al., 1973; Choa et al., 2016; Des Marais et al., 1980).

RESULTS AND DISCUSSION

Physical Characteristics of Guano Cores

Bat guano cores vary in their physical and elemental components. The Cave Springs Cave guano core contained many colored striations that varied from tan to dark brown (Fig. 3). The Cripps Mill guano core from Tennessee showed no striations in color and was consistently dark brown even though it was equal in length to Cave Springs Cave (87 cm) (Fig. 4). The Nunley Mountain guano core had many color striations as well. The bottom of the core was gold in color while becoming darker shades of brown towards the top (Fig. 5). Variation in colors is caused by differences in mineral composition. Cores commonly have a pellet-like consistency towards the top and become denser and more clay-like with depth due to compaction. Bulk density increased with depth in the Cave Springs guano core while total carbon (TC) and total nitrogen (TN) decreased (Fig. 1). Even though Cave Springs and Cripps Mill cores were around the same length ~87 cm, they had dramatically different ages (Table 2). Compaction of guano also occurs over time resulting in the bottom portion to be more densely compacted than the top of a guano pile. Cave Springs guano was dated from radiocarbon to ~9000 YBP whereas radiocarbon dating showed Cripps Mill guano to be modern—showing length of guano cores alone do not provide an accurate measure of age. Guano from Nunley Mountain Cave was dated from radiocarbon to ~36,000 YBP (Fig. 5).

Elemental Composition Analysis

In each guano core, tan-colored material with a clay-like consistency was found at the bottom and was determined to be a mineral called brushite ($Ca(HPO_4)\cdot 2H_2O$) (Giurgiu and Tamas, 2013; Stahle et al., 2019). It is thought that brushite forms from reaction with guano calcite and clay at low pH values (Anthony et al. 2000). Calcium and phosphorus rapidly increase at a

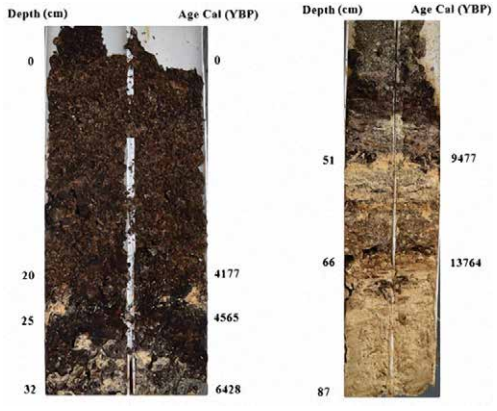

Figure 3. Cave Springs Cave guano core. Cave Springs Cave is located in Priceville, Alabama. Left: The top 0−32 cm the portion of the core is pellet-like and dark brown. Right: The bottom 33−87 cm portion of the core has many colored striations with layers of brushite intermixed with the guano. Guano core was 87 centimeters in total length.

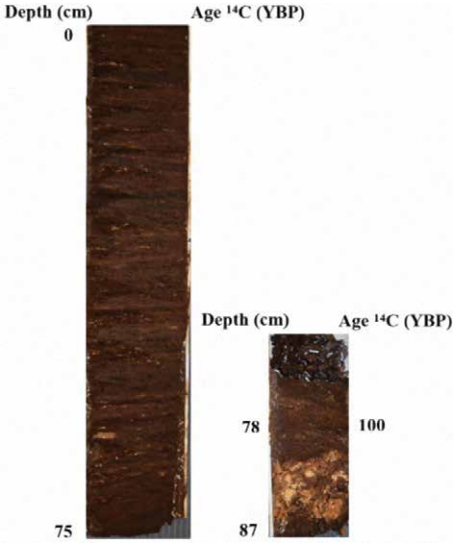

Figure 4. Cripps Mill guano core. Cripps Mill is located in Cookeville, Tennessee. Left: The top (0−86 cm) is consistent in texture and color. Right: The bottom (81−87 cm) indicates brushite. Guano core was 87 centimeters in total length.

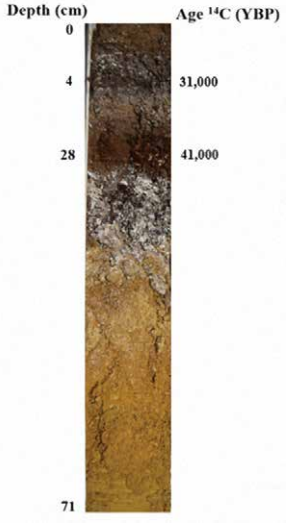

Figure 5. Nunley Mountain Cave guano core. Nunley Mountain Cave is located in McMinnville, Tennessee. The top (0−28 cm) is a dark brown color with some white deposits in between. The middle of the core (30−36 cm) contains white deposits while the bottom of the core (37−71 cm) is cave sediment. Guano core was 71 centimeters in total length.

Table 2. Comparison of ^{14}C dates and core lengths for each collected guano core. Basal ^{14}C dates listed are uncalibrated.

Cave and Location	Basal Age ^{14}C date (YBP)	Length of Core (cm)
Cave Springs Priceville, AL	8,440 ± 30	87
Nunley Mountain Cave McMinneville, TN	36,430 ± 130	71
Cripps Mill Cave Cookeville, TN	100 ± 20	87

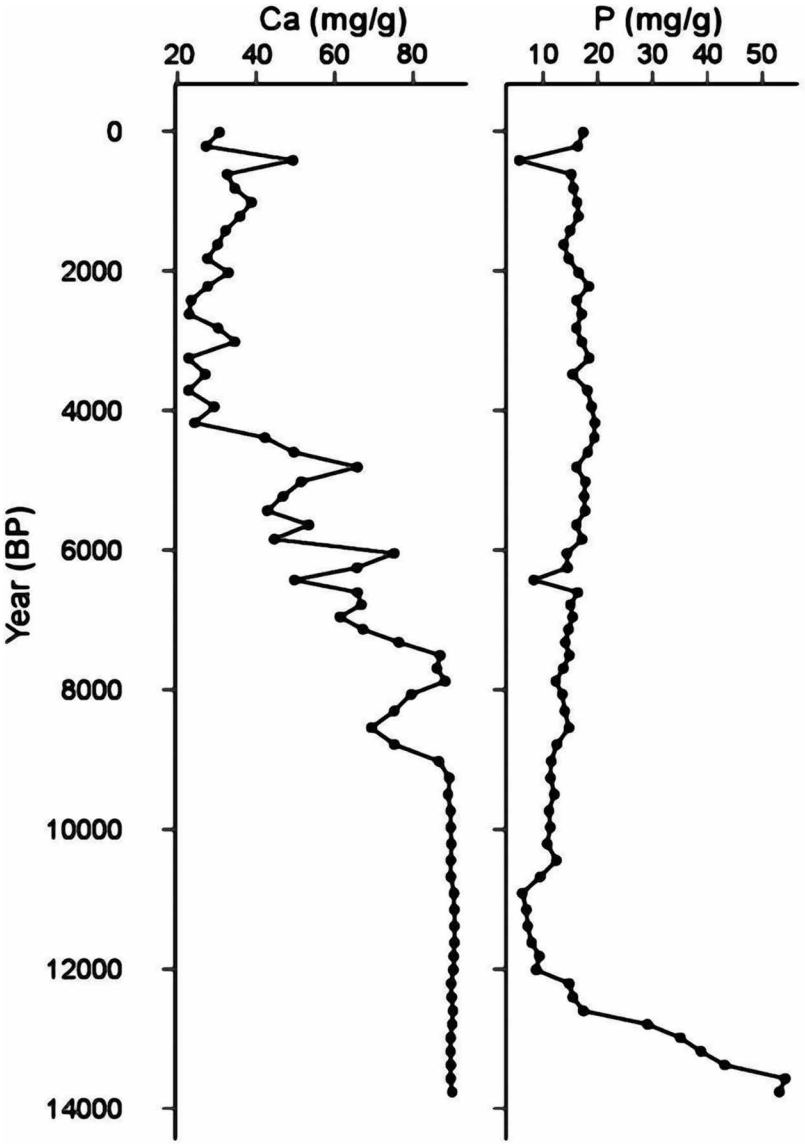

Figure 6. Elemental concentrations measured throughout Cave Springs guano core. Both elements rapidly increase at the bottom of the core, but at different times. Calcium peaks at 9,024 YBP and phosphorus peaks at 12,794 YBP. Increases in both elements indicate brushite.

the bottom of guano cores in congruence with the presence of brushite (Fig. 6). Brushite and other cave minerals contain high amounts of phosphorus and calcium and form after long periods of time (hundreds to thousands of years). However, due to the lack of organic material in the brushite zones of the cores, these portions are not ideal for paleoenvironmental reconstructions.

From our collected cores, brushite was found at the bottom of those extending throughout the Holocene, which indicates it is commonly occurring. Even though brushite has been found in older cores collected from this project, those samples do not produce accurate radiocarbon dates and resulted in inversions of the dating model. As a result, brushite cannot establish chronology in guano cores and was not included in any geochemical analyses.

Stable Isotopes Analysis

Stable isotopes (e.g., $\delta^{13}C$, $\delta^{15}N$, and δD) do not decay as radio isotopes do, allowing them to be quantified throughout a core despite their age. In addition, the stable isotope composition (i.e., isotopic ratio) of a material is the result of differences in the behavior of individual isotopes in response to natural processes. $\delta^{13}C$ isotopes provide information on the local vegetation type of a landscape due to the specific photosynthetic pathways that plants use (Des Marais et al., 1980). With $\delta^{13}C$ isotopes it can be determined whether C_3, C_4, or CAM plants dominated a region at a specific time, and this can give insight into the moisture content as C_3, C_4, and CAM plants each require unique water levels to survive (Cleary et al., 2016). Cleary et al. (2016) proposed that $\delta^{15}N$ accumulates from N gains/losses, N pool mixing, and isotope fractionations which may vary depending on local environment and climatic regimes. The interpretation of $\delta^{15}N$ isotopes from bat guano has changed over time. Recent contributions in the literature of $\delta^{15}N$ isotopes have built upon previous studies resulting in the now accepted interpretation from Cleary et al. (2016) suggesting low $\delta^{15}N$ values indicate periods of dry or arid climate. δD isotopes from bat guano are a common, standard, and accurate way to determine moisture levels of local environments (Wurster et al., 2010). δD values of insect chitin reflects metabolic and drinking water sources and they are correlated with δD values in local precipitation (Gröcke et al., 2006; Wurster et al., 2010).

For these guano core experiments, we only analyzed δD isotopes. Isotopes were compared with a paired t-test between bulk guano and insect chitin alone from Cave Springs Cave for the first 20 cm of the core to determine if insect chitin provides the same or distinct δD values compared to bulk guano (Fig. 7). No difference between the bulk guano

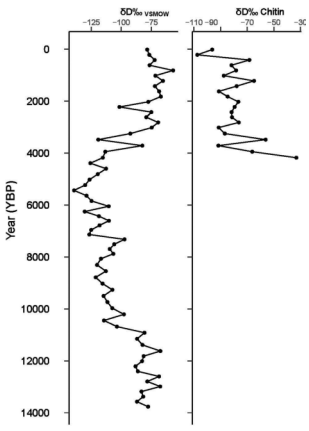

Figure 7. δD values of bulk guano (left) and insect chitin (right) from Cave Springs core. YBP measurements are calibrated years. Fewer values were obtained from older chitin due to degradation.

and insect chitin was detected (t = −0.34, df = 20, p = 0.74) despite some deviating values (e.g., −119.15δD ‰ bulk guano and −56.19δD ‰ insect chitin at 3,480 YBP). Deviating values may indicate hydrogen isotope fractionation which has not been analyzed in other studies reconstructing paleoclimate from guano cores (Bird et al., 2007; Wurster et al., 2008, 2010; Onac et al., 2014; Forray et al., 2015; Choa et al., 2016; Cleary et al., 2016). We suggest less negative δD indicates wet hydroclimatic periods while more negative δD values indicates dry hydroclimatic periods, thus, isotopes are coeval with precipitation over time. Results from the paired t-test are also unique to the limited number of samples (n = 20) and isotopes from Cave Springs Cave and may vary when analyzed from guano cores collected from other locations. One drawback of performing analyses on insect chitin alone is the degradation of chitinous material. Distinguishable insect chitin pieces were not found throughout the entirety of the core due to degradation making analysis only possible for the top to middle of the core, depending on the age. Older guano cores will have less chitin and possibly none at the bottom of the cores while cores that are modern should be consistent in quantity. Therefore, analyzing isotopic values of bulk guano may be the most efficient and productive method.

Age-Depth Models

Each of the three collected cores had unique dating structures. We sampled five radiocarbon dates from Cave Springs Cave and the oldest calibrated age at 67 cm was 12,380 YBP (Fig. 3). The BACON model was applied to this core and had linear correlation lines that indicate sedimentation rates were constant over time. The Cripps Mill Core was sampled at the bottom of the core (78 cm) for a radiocarbon date and revealed a modern age throughout the core (Fig. 4). Nunley Mountain Cave had the oldest radiocarbon date of 36,000 YBP (Fig. 5). However, Nunley Mountain Cave experienced a hiatus in bat presence as there were no modern or recent ages towards the top of the core when calibrated using the BACON model. Hiatuses can occur when bats undergo migratory changes or population changes.

Currently, it is not possible to date modern guano deposits since limnological dating methods such as 210 Pb and 137 Cs are not effective when used independently (McFarlane and Lundberg 2021). However, ^{137}Cs could be a possible method to date recent guano deposits, although it has not been tested.

CONCLUSIONS AND FUTURE IMPROVEMENTS

From three guano cores collected in various locations of Alabama and Tennessee, we analyzed physical, chemical, and elemental components. All three cores showed varying results and ages were drastically different despite having similar lengths. The goal of this study was to provide results for multiple guano cores and review methods and findings from other studies to allow advancement for studying bat guano.

With the potential to determine paleoclimatic and paleoenvironmental conditions, bat guano will gain notice as an accurate and viable proxy. As more studies of bat guano are being implemented around the globe, the interpretations of stable isotope ratios will become a more widely accepted method of paleoclimatic change throughout history, and a comprehensive procedural analysis may be created. Attention still needs to be focused on pollen pathways and potential δD isotope fractionations in bat guano. Currently, isotopic analysis has been favored over biological remains. In the future, biological aspects of guano may also be studied to determine paleoclimatic conditions. Guano studies have already been used across the globe in countries such as Romania (Onac et al., 2014), Guadeloupe (Royer et al., 2015), the Philippines (Choa et al., 2016) and the United States (Mizutani et al., 1992; Wurster et al., 2010; Campbell et al., 2017) and would be a useful tool for filling data gaps where paleoclimatic records currently do not exist.

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