Reconstructing Paleoenvironmental Conditions of Northern Alabama Utilizing Guano Cores

by

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Abstract

The need to understand long-term precipitation patterns in the southeast United States are of great concern considering human population increase, climate change, agricultural production and environmental stressors. Current management strategies are typically based on records with short chronologies, spanning the past hundreds of years. One paleoclimate archive capable of tracking moisture regimes and other paleoenvironmental conditions over millennial timescales is guano deposits in cave systems. Guano deposits record moisture and precipitation and are commonly found in many caves systems throughout the southeastern United States. However, this potentially useful tool has received little attention. Here, I present a 9,000-year record of moisture and rainfall variability based upon stable isotopes systematics ($\delta^{15}N$, $\delta^{13}C$, δD) in a guano core collected from a cave system named Cave Springs, located near the city of Priceville, Alabama. Moisture variability was inferred from nitrogen and carbon stable isotope records. Deuterium was measured from bulk guano and used as a proxy of the evaporation to precipitation balance. Results show two periods of low moisture in the early (11,151 – 13,764 YBP) and late (0 – 4,177 YBP) Holocene separated by a period of high moisture during the middle (4,388 – 10,916 YBP) Holocene, which correspond to nearby pollen reconstructions. Furthermore, this study suggests that future research investigating guano deposits from caves can provide a reliable long-term record of local paleoclimate where other records such as lake sediments are not available.

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List of Abbreviations

AMS	Accelerator Mass Spectrometry
BACON	Bayesian Accumulation History
CAM	Crassulacean Acid Metabolism
C:N	Carbon:Nitrogen
C/N/S	Carbon/Nitrogen/Sulfur
D	Deuterium
D/H	Deuterium/Hydrogen
ENSO	El Nino Southern Oscillation
EPA	Environmental Protection Agency
H	Hydrogen
HCl	Hydrochloric Acid
HCO	Holocene Climatic Optimum
LIA	Little Ice Age
MWP	Medieval Warm Period
MYA	Million Years Ago

NOAA	National Oceanic Atmospheric Association
PCA	Principal Component Analysis
PVC	Polymerizing Vinyl Chloride
Spp	Species
TOC	Total Organic Carbon
TN	Total Nitrogen
V-SMOW	Vienna Standard Mean Ocean Water
YBP	Years Before Present

1. Introduction

1.1 Background Information

Long-term precipitation patterns in the southeastern United States are of great concern considering human population increase, climate change, agricultural production, and other environmental stressors. While forecasting models can be used to predict future precipitation changes, these models are frequently developed with historic data of <100 years (Fildes and Kourentzes 2011), creating a data gap for a longer environmental record. One strategy to extend historic climatological data is to utilize paleo-records of environmental change. However, most paleoenvironmental records are regional in scope, underscoring the need for additional records and extended timescales to be developed. An understanding of the drivers of long-term precipitation variability would enable predictions of the frequency of drought events in the future thus helping develop adaptation and mitigation policies, formulate municipal plans, and secure agricultural productivity. Multiple natural forces such as the Milankovitch cycles, solar forcings, and the El Niño Southern Oscillation (ENSO) can cause climate changes and impact precipitation. Anthropogenic effects, such as the emission of greenhouse gases, can also drive hydrological changes at the regional and global scales. Previous studies based on dendrochronology cores, lake sediment cores, pollen, and guano cores (e.g., Watts 1971, Mizutani et al. 1992, Waters et al. 2005, Therrell et al. 2006, Wurster et al. 2008, Wurster et al. 2010) have assessed paleoclimate reconstructions, but the southeastern United States has received less attention, especially regions inland from the coastal plain.

1.2 Statement of the Problem

Paleoclimate reconstructions in the southeastern United States have included a variety of measurements over a range of time scales. Modern records from this region, which come from environmental monitoring data, typically span the past ~50 years. Dendrochronology records are one reconstruction technique which provide high data, but for the southeastern United States, they only incorporate the last ~300 years (Stahle et al. 1998, Cleaveland et al. 2003, Therrell et al. 2006a, Therrell et al. 2006b). Both modern and dendrochronology records are precise and accurate due to high resolution over the temporal scales measured but have a limitation of not extending beyond the last few centuries. Pollen records obtained from lake sediments document local vegetation changes throughout the southeastern United States (Watts 1971, Watts 1975, Delcourt 1980, Grimm et al. 1993, Watts 2001, Mendieta et al. 2018) and identified species abundances and shifts of vegetation present over multiple time scales. These pollen records can be used in conjunction with other paleoenvironmental data such as stable isotopic changes. However, few other paleoclimate studies have been conducted for the southeastern United States outside of Florida. Lake sediments provide elemental, nutrient, and isotopic data over time but can be limited by lake location, temporal extent of the sediment core, and degradation of the paleoenvironmental proxies (Filley et al. 2001, Whitlock and Larson 2001, Waters et al. 2005, Waters et al. 2009). Stalagmites in cave systems provide paleoenvironmental data that can reconstruct precipitation throughout the Pleistocene, creating a large temporal scale. Unfortunately, there are few speleothem records for the southeastern United States (Medina-Elizalde et al. 2012, Medina-Elizalde et al. 2016, Dhungana and Aharon 2019). Therefore, a crucial knowledge gap exists to fill in climate information throughout the Holocene for the southeastern United States, especially for inland areas outside of the coastal plain. Few natural lakes exist in non-coastal areas making lake sediments and pollen records unattainable to study. However, the Appalachian mountain area is one of the most pervasive cave areas in the United States. Out of 45,000 caves that exist in the United States (Culver et al. 1999), over 3,000 are found in the Appalachian mountain region. Caves are also protected from surficial weathering processes and maintain constant environmental conditions, providing the ideal conditions for recording paleoenvironmental data (Cleary et al. 2016).

1.3 Bat Guano in Caves for Paleoclimate Studies

One paleoclimate archive capable of tracking moisture regimes, precipitation, and local vegetation over millennial timescales (Wurster et al. 2008, Wurster et al. 2010), but has received very little attention, is guano deposits in cave systems. According to the International Union for the Conservation of Nature (IUCN), guano is defined as the excrement produced by cave-dwelling bats (IUCN 2014). Guano deposits are common in many cave systems throughout the southeastern United States, where ~2,300 caves are located in Alabama alone (Culver et al. 2006). Most Alabama caves host migratory populations of *Myotis griesescens*, the endangered gray bat, which are distributed from its most southern point in Florida to the most northern point in Kentucky and Virginia of the Appalachian region (Decher and Choate 1995). Gray bats roost in southern states such as Florida where temperatures are warmer and migrate to caves in the northern Appalachian region where temperatures become colder in the winter (Decher and Choate 1995) to induce hibernation. Maximum migration distance has been measured to be 640 km (Decher and Choate 1995) which allows for a paleoclimate record representative of a regional scale. Due to migration patterns, gray bat annual cycles can be broken into distinct periods: spring migration, summer period, maternity period, fall migration, and hibernation (Decher and Choate 1995). Guano collection for paleoclimate studies s typically done during the maternity period, when female and male bats utilize separate caves. Bat guano deposits in cave systems have only recently been considered as a new paleoenvironmental data source that provides reliable and reproducible historic (Shahack-Gross et al. 2004, Wurster et al. 2010, Onac et al. 2014, Choa et al. 2016, Campbell et al. 2017).

Bat guano is a mixture of organic matter, feces, hair, pollen, and insect parts which can be analyzed for data such as stable isotopes, elements, and nutrients to provide unique paleoenvironmental data. In addition, the high organic matter content, lack of water, and constant bat presence allow for successful accelerator mass spectrometry (AMS) ^{14}C dating of guano cores. Paleoecological reconstructions from guano cores have been used in prior studies to infer alterations in climate (Wurster et al. 2007), bat ecology (Maher 2006), and other environmental parameters (Bird et al. 2007, Wurster et al. 2010) throughout the Holocene, but few standardized techniques have been established. Guano deposits are a new source of paleoenvironmental data, and this thesis further develops the application of guano core data to produce paleoclimate and paleoenvironmental records.

1.4 Guano Core Measurements

1.4.1 Pollen, Elements, Organic Matter

Pollen in bat guano can be a powerful tool in determining local vegetation. Gray bats consume pollen-laden insects and ingest pollen adhered to their fur during grooming (Maher 2006). The type(s) of pollen grains contained in the bat guano reflect the local vegetation and have direct implications of the environmental conditions at the time (Maher 2006). Minerals in cave sediments and bat guano can also be used to reconstruct the paleochemical conditions, thus providing insight into past environmental conditions (Shahack-Gross et al. 2004). Many minerals are found in bat guano such as complexes of phosphate, Aluminum (Al), Iron (Fe), Calcium (Ca), and Sulfur (S) among others (Shahack-Gross et al. 2004, Giurgiu and Tamas 2013).

1.4.2 Fractionation of Carbon Isotopes as a Proxy of Vegetation Changes

The relative abundance of C_3 and C_4 plants can be inferred from the carbon (C) stable isotope compositions of sampled bat guano. Multiple studies performed at many caves in the American Midwest have tracked vegetation over the course of the Holocene in this way (White 2007, Wurster et al. 2007, Batina and Reese 2010). However, only one paleoclimate reconstruction study using guano cores exists for the southeast United States (Campbell et al. 2017). Biologic processes such as photosynthesis prefer ^{12}C over the heavier isotopes of C (^{13}C, ^{14}C) because ^{12}C forms weaker chemical bonds that require less energy to break (O’Leary 1988). Values of $\delta^{13}C$ vary among primary producers with three different photosynthetic pathways: C_3, C_4, and Crassulacean acid metabolism (CAM). Each pathway produces a specific range of $\delta^{13}C$ ratios. C_3 plants fall between -32‰ and -20‰, C_4 plants fall between -17‰ and 9‰, and CAM plants fall between the two (Des Marais et al. 1980, Choa et al. 2016). The C_3 pathway is used by trees, bushes, and cold season grasses (Zhaoyan et al. 2003) and produces the lowest $\delta^{13}C$ values that are typical of more dry environments. The C_4 pathway is used by most tropical and warm season grasses (Zhaoyan et al. 2003) and produces higher $\delta^{13}C$ values. The isotopic values of plants will assimilate into chitinous insect tissue when they are consumed. Therefore, guano is assumed to reflect the $\delta^{13}C$ values of local vegetation as bats consume insects that pollinate and live within the terrestrial environment (Des Marais 1980, Onac et al. 2014, Wurster et al. 2007).

1.4.3 Fractionation of Hydrogen Isotope Ratios as a Proxy of Moisture

Atmospheric precipitation is the main source of hydrogen in the form of water for terrestrial plants. The hydrogen isotope composition of this water is determined by climate, altitude, and longitude and is reflected in the composition of leaf tissue (Hayes 2001, Chikaraishi et al. 2004, Gröcke et al. 2006,). Atmospheric circulation happens in response to solar forcing and the Earth's heat imbalance, which ultimately creates the seasonal changes experienced throughout the globe and is a balance between precipitation and evaporation. These processes cause differences in the hydrogen isotope composition of water throughout the globe due to fractionation (Gröcke et al. 2006). For example, when air masses cool, they lose moisture in the form of precipitation, which is D-enriched relative to H (Gröcke et al. 2006) therefore, precipitation from cooler temperatures and higher latitudes and altitudes will be deuterium depleted (Gröcke et al. 2006). Hydrogen from insect chitin is sourced solely their diet (Gröcke et al. 2006). Incorporating, the local D/H ratios in leaf water into their bodies (Gröcke et al 2006). About 77% of the hydrogen atoms in chitin are bonded strongly to the carbon atoms, which prevents later exchange with the atmosphere (Gröcke et al. 2006). This results in the organic hydrogen retention of D/H paleoclimate information over geologic time (Gröcke et al. 2006). Chitin has strong structural components with high preservation potential, providing long-term records of precipitation (Grocke et al. 2006, Wurster et al. 2008). During periods of flooding and increased precipitation, δD values will be lighter compared to periods of drought (Wurster et al. 2008). δD values will also be higher in warmer temperatures and lower relative humidity (Wurster et al. 2010). This allows the inference of moisture variation and rainfall for the southeastern United States.

1.4.4 Fractionation of Nitrogen Isotopes as a Proxy of Moisture

In addition to the carbon ad hydrogen systems, stable nitrogen also provide paleoenvironmental data when measured throughout cores of guano material (Cleary et al. 2016). Similar to carbon, specific dietary regimes produce distinct $\delta^{15}N$ values (Mizutani et al. 1992, Wurster et al. 2010, Royer et al. 2015) that arise from the nitrogen cycle via nitrogen mixing with soil, biomass, atmosphere, and consumers. Most studies of guano cores attribute δ^{15}N values of bat guano to diet, however, there is some evidence that δ^{15}N values provide a record of anthropogenic and climatic influence on the regional nitrogen pool such as changes in deforestation, fertilizer usage, and prescribed fires (Cleary et al. 2016). One potential drawback of using δ^{15}N data is that nitrogen isotopes can fractionate via volatilization (Mizutani et al. 1992), which will artificially produce elevated δ^{15}N values (Cleary et al. 2016). This volatilization occurs from microbial processes (denitrification, nitrification, etc.) and causes the bulk guano to be nitrogen deprived overall and ^{15}N enriched (Mizutani et al. 1992, Cleary et al. 2016). Another challenge is the complicated nature of the nitrogen cycle. One way to mitigate this issue is to analyze the C:N ratio, which can indicate whether the isotopic ratio has been altered (Cleary et al. 2016). The interpretation of δ^{15}N values varies among studies (Campbell et al. 2017); while some guano studies claim lower δ^{15}N values indicate dryer climates (Cleary et al. 2016), others suggest high δ^{15}N values are associated with more arid climates (Wurster et al. 2010, Royer et al. 2015).

1.5 Objectives

This thesis applies analysis of elemental compositions, organic matter, and stable isotope ratios to guano cores from the southern region of the Appalachian mountain range (Fig. 1). The three guano cores were collected from three different cave systems from northern Alabama and throughout Tennessee: Cave Springs Cave, Nunley Mountain Cave, and Cripps Mill Cave. Paleoclimate proxies were measured on all three guano cores in order to accomplish three objectives: 1) Apply paleoenvironmental measurements to each guano core to better understand diagenetic patterns and guano core variability for paleoclimate interpretations, 2) Reconstruct paleoclimate change throughout Holocene from the guano collected from Cave Springs Cave in Priceville, Alabama and, 3) Assess the Cave Springs record within the framework other paleoclimate reconstructions from the southeastern United States.

Figures and Tables

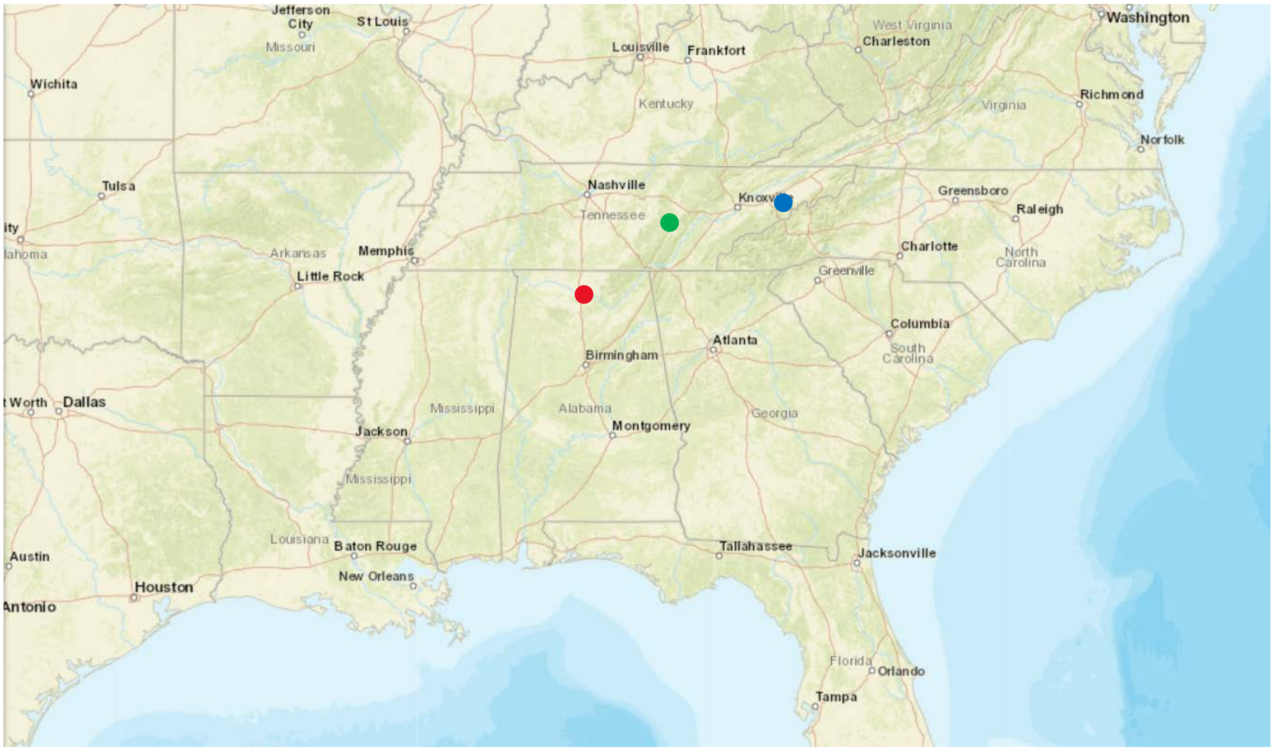

Figure 1. Locations of collected guano cores. Red) Cave Springs Cave, Priceville, AL; Blue) Cripps Mill Cave, Cookeville, TN; Green) Nunley Mountain Cave, McMinneville, TN

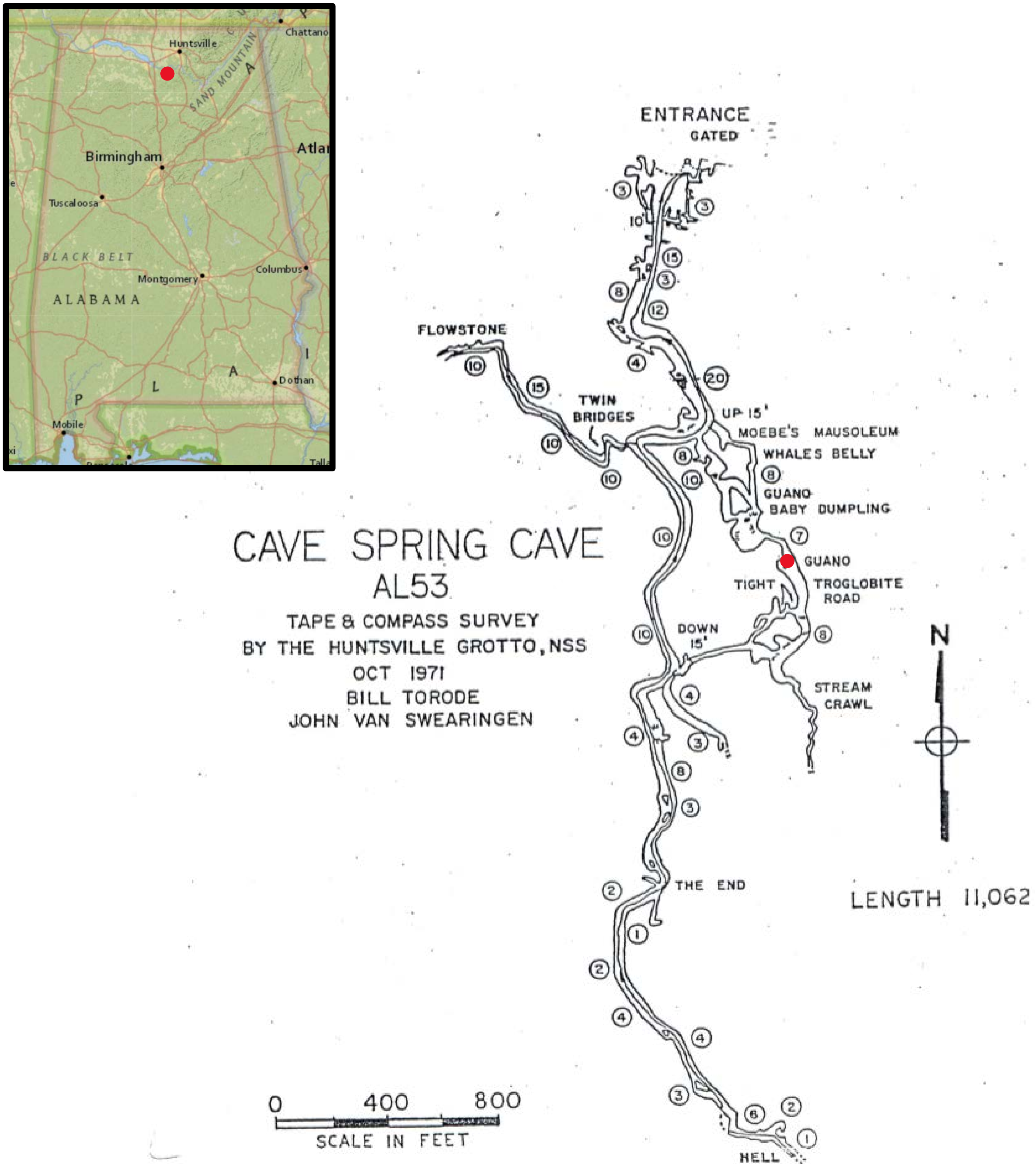

Figure 2. Map of Cave Springs Cave and its location in Priceville, Alabama, USA. 3,371 meters in total length. The guano pile that was cored is indicated with a red dot.

2. Paleoenvironmental Change throughout the Holocene in Northern Alabama, USA Inferred from Bat Guano

Abstract

The southeastern United States is experiencing a period of environmental change from population increases, climate change, and land use alterations creating the need to understand baseline conditions and environmental change prior to human impacts. While paleoenvironment data can be reconstructed from a variety of archives (e.g., lake sediments, tree rings, speleothems), some geographic areas lack such records of paleo-data. One scientific medium capable of tracking moisture regimes and other paleoenvironmental changes over millennial timescales, but has received little attention, is guano deposits in cave systems. Guano deposits reside in many cave environments in the southeastern United States and have been used as an archive of paleoclimate data including precipitation, vegetation, and other environmental change. Here, I present a 9,000-year record of moisture (wet/dry) and other periods of paleoenvironmental change based on stable isotopes (δ^{15}N, δ^{13}C, δD) in a guano core collected from Cave Springs Cave in Alabama, USA. Moisture was inferred from changes in deuterium while carbon and nitrogen stable isotopes were used as proxies of historic environmental changes on the landscape. Results showed that the early (11,151 – 13,764 YBP) and late (0 – 4,177 YBP) Holocene were drier than the middle (4,388 – 10,916 YBP) Holocene and the period of the Holocene climate optimum (5,000 – 9,000 YBP) (Mendieta et al. 2018, Watts, 1971). These changes strongly coincided with vegetation changes based on pollen records from nearby (~200 kilometers) lake records. Stable carbon and nitrogen are complex in interpretation thus limiting

inferences from stratigraphic change. This study suggests that future research investigating guano deposits from caves can provide a long-term record of local paleoclimate and paleoenvironmental change.

2.1 Introduction

Paleoclimate reconstructions using records from tree rings, pollen, and lake sediment cores have focused on the polar and tropical regions of the globe with fewer records from subtropical areas. The subtropical southeastern United States is home to environments typically useful for paleoreconstructions but most records contain short chronologies beginning in the mid Holocene or later (Larson and Schaetzl 2001). Furthermore, natural lakes in the southeastern United States are uncommon outside of the coastal plain compared to areas in temperate regions with histories of glaciers and ice sheets. The majority of paleoclimate data in the southeastern United States has resulted from dendrochronology records (Stahle et al. 1998, Stahle et al. 2019) which are limited to the most recent centuries (Therrell et al. 2006a). Current data from environmental monitoring (e.g., ambient temperatures, rainfall amount) and tree ring analyses provide accurate and high-resolution sources of data; however, they do not extend far enough into the past to provide paleoclimate reconstructions prior to human impacts (Stahle et al. 1998, Cleaveland et al. 2003, Therrell et al. 2006a).

One documented paleoclimate event that has been supported by pollen records from the southeastern United States is the Holocene Climatic Optimum (HCO;, Folland et al. 1990, Marcott et al 2013, Mendieta et al. 2018). This event was a warm period from 5000 – 9000 years before present YBP that coincided with a transition from *Quercus* (oak spp.) dominance to increased *Pinus* (pine spp.) population densities across the southeastern United States landscape as recorded in lake sediment cores (Watts 1971, Delcourt 1980, Grimm and Jacobson 1992, Grimm et al. 1993). Additional sediment studies have assessed pollen species and quantities to document environmental shifts in vegetation, moisture, and temperature changes over time in the southeast United States (Watts 1971, Watts 1975, Filley et al. 2001, Donar et al. 2009).

However, one lake sediment study in the southeastern United States shows no evidence of a *Quercus* to *Pinus* transition (Mendieta et al. 2018). The most likely explanation for this discrepancy is due to elevation changes in the landscape. Mendieta et al. (2018) utilized lake sediments to document the HCO collected from the highlands of Florida. The Florida highlands are ten meters higher in elevation than the lowlands where lake sediment cores were collected containing the HCO pollen change.

Past climate changes are typically reconstructed from core samples collected from lacustrine sediments, ice cores, speleothems, and soils (Grimm et al. 1993, Waters et al. 2009, Medina-Elizalde and Rohling 2012). One paleoclimate record that effectively tracks moisture regimes but has received less investigation is bat guano. Bat guano cores are collected from large, undisturbed deposits in cave environments and are a useful archive for paleoclimate reconstructions (Wurster et al. 2007, Onac et al. 2014, Campbell et al. 2017). Guano cores are collected from caves with constant bat presence and have been protected from surficial weathering processes (Onac et al. 2014, Cleary et al. 2016). In addition, many bat species are migratory and spend particular seasons in certain caves, making guano deposits a seasonally specific record (Campbell et al. 2017). Previous studies have shown that guano records past environmental conditions from large spatial distribution including tropical, semi-arid, and temperate locations (Wurster et al. 2017). Guano cores are readily dated using radiocarbon analysis and provide largely undisturbed depositional environments (Onac et al. 2013, Wurster et al. 2017). Primary analyses that have been applied to guano deposits include stable isotopes (Wurster et al. 2008, Wurster et al. 2010), organic matter (Shahack-Gross et al. 2004, Campbell et al. 2017), pollen (Campbell et al. 2017), elements such as magnesium, calcium, phosphorous, iron (Giurgiu and Tamas 2013), and nutrients (Hill et al. 1997, Giurgiu and Tamas 2013).

Bat guano research has been primarily conducted in the midwest United States (Wurster et al. 2008, Wurster et al. 2010) and in Europe (Giurgiu and Tamas 2013, Onac et al. 2014). Therefore, the southeastern United States is lacking in guano studies, despite having a large density of caves (over 3,000 in the Appalachian region) (Culver et al. 1999). Currently, only one guano core study has been published in this region (Campbell et al. 2017). Given the need for paleoreconstructions in the non-coastal southeastern United States, I analyzed $\delta^{13}C$, $\delta^{15}N$, and δD values, elements, and nutrients from a guano core collected from Cave Springs Cave in northern Alabama (Fig. 1) to provide one of the longest records of paleoclimate change for the southeastern United States. The guano core spanned the majority of the Holocene (11,650 YBP) and was collected from a maternity colony of gray bats (*Myotis grisescens*) that roost in this cave during summer months. From this data, I inferred changes of local vegetation from $\delta^{13}C$ and changes in precipitation and climate patterns from δD (Deuterium) via bulk guano and insect chitin over the majority of the Holocene. Three objectives were investigated: 1) to establish a dated record of isotopic chemistry and precipitation patterns from northern Alabama, 2) to compare the Cave Springs guano record to other paleoclimate records in the southeastern United States in order to examine their regional context, and; 3) to examine what causes the isotopic changes in $\delta^{13}C$, $\delta^{15}N$, and δD over time.

2.2 Methods

2.2.1 Study Site and Core Collection

Cave Springs Cave is located in northern Alabama near the city of Priceville (34.5251° N, 86.8947° W) and is part of the Wheeler National Wildlife Refuge. Based on available climate data from the Priceville meteorological station, the present-day climate of the area can be characterized as humid subtropical, with mean monthly precipitation ~ 107 millimeters for 2018 (NOAA 2018). Cave Springs Cave is 3,371 meters in total length (Fig. 1) and is well mapped. The guano pile was ~ 2 meters above the cave floor and was cored approximately 487 meters from the entrance. The core was retrieved in approximately two sections of polyvinyl chloride (PVC) pipe hammered into the ground with a mallet totaling around 87 cm. Core sections were returned to the lab and stored in a freezer until they were sectioned at 1 cm intervals.

2.2.2 Gravimetric Analysis, Nutrients, and Isotopes

Guano core sections were analyzed gravimetrically for bulk density and organic matter. For bulk density, 5 mL of raw guano was weighed, dried, and processed similarly to lake sediments (Brenner and Binford 1988, Campbell et al. 2017). Dried samples were burned at 550°C for three hours and organic matter was reported as percent loss on ignition. Bulk density is reported as g dry cm^{-3} wet.

Total phosphorus (P), sulfur (S), and other nutrients (Ca, Fe, Mg, K, Al, Na, S) were measured from dried sediments using an ARL 3560 ICP-AES following complete acid digestion using standard EPA methods (Waters et al. 2009). Total organic carbon (TOC) and total nitrogen TN were measured using a Costech Combustion Elemental Analyzer with an attached auto-sampler. Prior to analysis, samples were acidified for 12 hours in HCl vapors to remove inorganic carbon. $\delta^{13}C$ and $\delta^{15}N$ values were measured by an isotope ratio mass spectrometer coupled to a C/N/S (Carbon (C), Nitrogen (N), Sulfur (S)) analyzer at the University of California, Davis (Davis, California, USA). Carbon and nitrogen isotopic ratios were measured relative to an internal standard and calibrated to a reference standard of Pee Dee Belemnite and air, respectively. Isotopic ratios were calculated as standard delta notation and expressed as per mil (‰). $\delta\mathrm{mX}\ (‰) = \left(\frac{\frac{\mathrm{mX}}{\mathrm{LXsamp}}}{\frac{\mathrm{mX}}{\mathrm{LXstd}}} - 1\right) \times\ 1000$

For δD analysis, bulk dried guano was weighed out (~1 mg) and sent to the Cornell University Stable Isotope Laboratory (Ithaca, New York, USA). Deuterium isotopic measurements were calculated using an isotope ratio mass spectrometer (IRMS) relative to an internal standard and calibrated to the international reference standard of V-SMOW (Vienna Standard Mean Ocean Water). The standard is an average of different ocean water samples around the world. $\delta D\ (‰) = \left[\frac{\frac{\mathrm{D}}{\mathrm{Hsamp}}}{\frac{\mathrm{D}}{H_{VSMOW}}} - 1\right] \times\ 1000$

2.2.3 Radiocarbon Dating

Approximately 1 g of guano was sampled from 25, 36, and 51 cm depths in the core. All samples were dried for 24 hours in a dry oven and sent to the Center for Applied Isotope Studies, University of Georgia (Athens, Georgia, USA) for Accelerator Mass Spectrometry (AMS) ^{14}C analysis. AMS ^{14}C ages were calibrated using the CALIB ^{14}C calibration program (Stuiver et al. 1993) (Table 1). To calculate ages for depths where ^{14}C measurements were unavailable, the core top was marked as the date of collected and the ^{14}C dates were incorporated into the Bayesian Accumulation History (BACON) model package in R which uses Bayesian based modelling statistics (Blaauw and Andres 2011).

2.2.4 Statistical Analyses

Distinctions between early (11,151 – 13,764 YBP), middle (4,388 – 10,916 YBP), and late (0 – 4,177 YBP) Holocene were chosen utilizing the dating model and comparisons were calculated using the δD measurements from the guano core. Individual t-tests between early, middle, and late Holocene periods were not used in order to avoid inflating Type-1 error. There were not enough degrees of freedom between C, N, and D isotope ratios for a multivariate approach and data were not normally distributed, ruling out ANOVA or MANOVA as suitable tests. Instead, a Kruskal-Wallis non-parametric AOV was used for each isotope system using Statistix 9.0, Analytical Software (Tallahassee, FL). A Principal Component Analyses (PCA) was performed on C, N, and D isotope values as well as elemental concentrations (Fe, Al, K, Ca, P, Mg) in order to determine the ordination of isotopes and elements throughout each Holocene period. PCA was conducted using R statistical software.

2.3 Results

2.3.1 Core Description and Age

The Cave Springs guano core was 87 cm in length and did not show any visual signs of anthropogenic or biological disturbance. Based on microscopic analysis, the Cave Springs guano core consisted of chitinous insect pieces, bat hair, fecal material, and pollen. The bottom of the core was lighter in color and densely packed with clay-like consistency, while the top of the core had recent guano deposits and was dark brown in color with pellet-like consistency (Fig. 2). Other studies of guano also found lighter colorations at the bottom of core samples (Onac et al. 2015, Campbell et al. 2017). From 87 cm at the bottom of the core to 67 cm, guano was not observed, as this section of the core is inferred to be the mineral brushite, given the lack of organic matter and guano characteristics, high percentages of P and Ca, and the occurrence in other cave environments (Giurgiu and Tamas 2013, Onac et al. 2015, Stahle et al. 2019). Given the objectives of the study, the brushite part of the core (67-87 cm) is not included in the paleoreconstructions (Giurgiu and Tamas 2013, Stahle et al. 2019). From 66 cm to 42 cm, the guano showed light tan striations. Starting at 41 cm depth the guano consolidated and became lighter in color through 28 cm (Fig. 2). The top portion of the core (0-27 cm) was dark brown in color with pellet-like guano (Fig. 2).

The Cave Springs guano core provides a dated record of environmental change from present day to 9477±30 YBP at 51 cm based on calibrated AMS ^{14}C dates and a BACON model projected age of ~14,000 YBP at 87 cm (Fig. 3). The measured AMS C^{14} dates along with the top of the core were fitted with a best-fit line ($r^2=0.99$), a slope of $y=0.0054X + 0.3415$, and minimal error bars, suggesting constant deposition throughout the Holocene period.

2.3.2 Elements and Nutrients

Organic matter was measured as loss on ignition (LOI) and generally increased up the core (Fig. 4). Organic matter values are highest at 71% at the bottom of the core (66 cm) and lowest at 22% at the top of the core (20 cm). Bulk density decreased up the core from 66 cm to 22 cm due to decreasing age (Fig. 4). The total carbon (TC) increased up the core with minimal values of 1.15% at 66 cm and a peak value of 39.42% at 3 cm. Total nitrogen (TN) followed the same pattern of increasing upward with a peak value of 9.15% at 9 cm. Ca and P both rapidly increase at the bottom of the core reaching values of 90.53 mg/g and 54.24 mg/g, respectively. Ca is most concentrated at 37 cm as well (7,500 YBP) while P peaks at 57 cm (~12,000 YBP) (Fig. 5).

2.3.3 Stable Isotopes

From 67 cm to 53 cm, the δD values are consistently heavier (less negative) averaging at -79.97 ‰. From 52 cm to 20 cm, δD values are lighter (more negative) averaging around -116.31‰ (Fig. 6). From 20 cm to the top of the core at 1 cm, δD again are heavier with values average at -77.69 ‰. δD isotopes generally match the shifts of $\delta^{15}N$ isotopes at 53 cm (10,916

YBP) and 20 cm (3,712 YBP). The $\delta^{15}N$ values from the guano showed little stratigraphic change from 67 cm to 22 cm (Fig. 6). At 22 cm (4,598 YBP) $\delta^{15}N$ began to gradually decrease from 18‰ to 10‰ at 1 cm. The $\delta^{13}C$ values range from -26 to -29‰. Unlike $\delta^{15}N$, the $\delta^{13}C$ values do show stratigraphic change unlike the δD profile. From 67 cm to 28 cm, the $\delta^{13}C$ values only change by 1.95‰ ranging from -25.97‰ to -27.92‰. The $\delta^{13}C$ values rapidly increase to -26.86‰ at 28 cm. At 11 cm $\delta^{13}C$ decreases rapidly from -29.01‰ to -26.62‰ 8 cm.

The Kruskall-Wallis One-Way Nonparametric test determined there was significant difference between early, middle, and late Holocene time periods for δD ($p = 0.000$, $\chi^2 = 38.8193$) (Table 2). To determine which groups were significantly different from each other, I performed a Kruskall-Wallis All-Pairwise Comparisons Test for δD. Early and late Holocene were determined to be statistically similar ($\chi^2 = 48.8$ and $\chi^2 = 47.214$, respectively). Middle Holocene was significantly different ($\chi^2 = 18.5$) (Fig. 7). The PCA confirmed there were three distinct time periods (early, middle, and late Holocene) (Fig. 8).

2.4 Discussion

2.4.1 Dating Record

The Cave Springs guano core constitutes the oldest guano core record that has ever been collected and analyzed in the southeastern United States. The calibrated radiocarbon ages constrain multiple paleoclimatic events. The Medieval Warm Period (MWP) occurred during 800-1200 AD (Broecker 2001) and this would be confined to the upper portion of the core between 6-8 cm. The Little Ice Age (LIA) occurred 1400-1700 AD (Mann 2009) corresponding to the 4-5 cm section. Unlike other records, where paleo data can be extracted (lakes, speleothems, ice cores) guano cores contain few mechanisms for the disturbance of cave climate, bioturbation, or redox alterations. The cavern where the guano pile occurred did not show signs of historic flooding or water disturbance as noted in other areas of the cave. As a result, the bat colonies are assumed to have maintained a consistent migratory pattern and did not interact with the guano piles below the roosting areas.

2.4.2 Precipitation Changes in Inland Southeastern United States throughout the Holocene

The water from precipitation and consequently leaf tissue are known to reflect a distinct D/H value (Hayes 2001, Chikaraishi et al 2004, Gröcke et al. 2006) to provide a paleoclimatic moisture record (Wurster et al. 2008, Wurster et al. 2010). The δD values covaried with N isotope ratios, indicating the changes between values are likely attributable to moisture changes. Wurster et al. 2010 showed that changes in δD values in bat guano likely indicate a change in source or seasonality of precipitation or in plant assemblages which may have varying water-use preferences. Lighter δD values indicate more precipitation while heavier values indicate more aridity. The δD from Cave Springs recorded two dry periods throughout the Holocene that occurred during the early Holocene at 53-66 cm (11,000 – 14,000 YBP) and late Holocene at 0-20 cm (0-4000 YBP) based on the BACON dating model.

To better understand δD signals in the core, the data from Cave Springs were compared to D and oxygen (O) isotope ratios from rainfall collected between 2005-2015 in Tuscaloosa, Alabama (Joe Lambert unpublished data). D values from Tuscaloosa rainfall were substantially heavier (less negative) than Cave Springs guano (Fig.9). Hydrogen (H) isotopes can undergo fractionation through evaporation, condensation, and precipitation as well as through trophic interactions (Wurster 2008, Peters et al. 2012). According to Peters et al. (2012), evaporation favors light isotopes, the water in the bodies of animals tends to be deuterium enriched. This causes trophic enrichment of D as the H incorporated into animal tissues is from an enriched source. When considering this mechanism insectivorous bat guano, there is a three-tier trophic level system (plant tissue, insect chitin, and bat guano) allowing D to become enriched by the time it is deposited in bat guano. This may explain the increased D values from Cave Springs guano when compared to Tuscaloosa rainfall D (Fig. 9). D/H ratios have frequently been used as measures of paleoclimate (Birchall et al. 2005) despite a lack of understanding trophic discrimination of H isotopes between resources and consumer tissues (Peters et al. 2012). Many paleoclimate studies utilizing bat guano have also not incorporated D isotope fractionation through trophic levels into their interpretation (Wurster et al. 2008, Wurster et al. 2010). Wurster et al (2008) reported D values of -188‰ to -143‰ also showing fractionation processes in guano core samples. However, the relative shifts in D isotopes do appear to be related to overall precipitation patterns in the surrounding ecosystems of the cave as has been reported in other studies (Wurster et al. 2008, Wurster et al. 2010). Wurster et al (2010) and other studies have not applied effects of D isotopic fractionation in their guano cores, which is recommended for future guano analyses including deuterium.

H isotope ratios from Cave Springs guano were also compared to pollen data from the southeastern United States in various locations in northwest Georgia, Alabama, Florida, South Carolina, and North Carolina (Watts 1971, Watts 1975). Pollen records show evidence for a period of warming and increased aridity in the southeastern United States during 9,000 – 5,000 YBP referred to as the Hypsithermal event (Delcourt 1980, Delcourt and Delcourt 1980, Tanner et al. 2015, Driese et al. 2008) that coincided with the heavier values observed in the Cave Springs core and an inferred dry period. The shift from *Quercus* to *Pinus* has been frequently observed in previous cores collected from lake sediments across the Gulf coastal plain (Watts 1971, Watts 1975, Watts, 1970, Delcourt 1980, Delcourt and Delcourt 1980). The *Quercus* to *Pinus* shift is apparent in lake sediment cores from Bartow County, Georgia, USA when pine species began to dominate about 5000 YBP and replaced oak forests during the Holocene (Fig. 10). Priceville, Alabama is located approximately 241 kilometers away from Bartow County, Georgia and the *Quercus* to *Pinus* shift would also be expected to have occurred here. The most likely explanation for this phenomenon is a change in moisture or precipitation. *Pinus* are better adapted to wetter environments, allowing them to thrive for the last 4000 years since this is when an increase in moisture in the Cave Springs δD record is observed. *Quercus* dominated from 4000 YBP and throughout the middle Holocene due to a more arid climate (Delcourt 1980).

The δ^{15}N values from Cave Springs show a peak at ~5000 YBP which is congruent with the *Quercus* to *Pinus* shift documented during the Holocene Climatic Optimum (9000-5000 YBP) (Grimm et al. 1993) and the δD record from this study. The δ^{15}N values from Cave Springs dramatically increase at ~5000 YBP where the *Quercus* to *Pinus* shift also occurs indicating a dryer climate. The portion of the core from 0-4000 YBP indicate arid climates according to the δ^{15}N and δD values, possibly from anthropogenic effects. An alternative explanation would be that native Woodland Indians utilized this region of Alabama (Campbell et al. 2017). During this time, the Woodland Indians may have begun domesticating food plants which could have altered the local vegetation and influenced δ^{13}C values causing the sudden spike at 2000 YBP (Campbell et al. 2017). The lower portion of the Cave Springs core showed an additional wet period that coincided with *Pinus* increases in the bottom of the Bartow County lake cores around the same time period. This early Holocene wet period has received less attention due to the lack of records in the southeastern United States spanning this time, but isotopic profiles from Cave Springs suggest the climate was wetter during this time frame (Watts 1970).

Some sediment and pollen records show similar evidence for increased moisture in the southeastern United States during the same 4,000-8,000 YBP period, however, it is isolated to coastal areas (Gaiser et al. 2004, Goman and Leigh 2004, LaMoreaux et al. 2009). The Cave Springs guano core also shows evidence for a wet period during the Hypsithermal event. As northern Alabama is more inland, we can expect the climate pattern observed from the guano to match what was found from the pollen records taken in surrounding areas (i.e. Bartow County, GA, Watts 1970).

2.4.3 Periods of Environmental Change in the Holocene

The stable carbon, nitrogen, and hydrogen isotopes from Cave Springs provide insight into the temperature and moisture levels throughout the Holocene. Carbon isotopes provide a proxy for vegetation changes between C_3, C_4, and CAM (Crassulacean Acid Metabolism) plants due to differences in photosynthetic pathways; with each pathway producing unique $\delta^{13}C$ values (Des Marais et al. 1980, Choa et al. 2016). C_3 plants range from -32‰ and -20‰, C_4 plants fall between -17‰ and 9‰, and CAM plants range between the two (Des Marais et al. 1980, Choa et al. 2016). Since vegetation changes are dependent on moisture and precipitation, $\delta^{13}C$ values are not a direct measure of moisture throughout history, however, $\delta^{15}N$ and δD have been shown in other guano cores to reflect changes in precipitation patterns (Wurster et al. 2010).

Multiple studies agree that higher $\delta^{13}C$ values indicate a drier climate while more negative $\delta^{13}C$ values indicate a wetter climate (Royer et al. 2015, Cleary et al. 2016). The $\delta^{13}C$ values throughout the Cave Springs core are highly negative (between -25‰ to -29‰) indicating drier conditions over the 9,000-year period. Our $\delta^{13}C$ values show a rapid change in vegetation between 1500 and 2000 YBP (Fig. 6). During drought or dry conditions, C_3 plants would become more numerous as their roots grow deep beneath the ground's surface to access water (Wurster et al. 2007), but C_4 plants are known to be more tropical thus supporting a more arid climate. As a result, further study is needed to distinctly apply carbon stable isotope analysis to guano core studies.

N isotopes have not been as commonly used in bat guano analyses as C and H isotopes, therefore, interpretation of the results varies between studies. Some studies claim N isotopes show no climatic parameters (Wurster et al. 2007) while others suggest $\delta^{15}N$ values record environmental changes (Mizutani et al. 1992). Studies also show disagreement about the interpretation of $\delta^{15}N$ values determining moisture (Campbell et al 2017). Wurster et al. 2010 claimed high $\delta^{15}N$ values are associated with more arid climates, whereas Cleary et al. (2016) claims the opposite, showing lower $\delta^{15}N$ values indicate dryer climates. The Cave Springs $\delta^{15}N$ profile demonstrates conflicting patterns with heavier values during the early Holocene dry period and lighter values during the late Holocene dry period. N isotopes can be impacted through post-depositional processes such as volatilization of N through denitrification and ammonification suggesting difficulty in $\delta^{15}N$ interpretation on longer timescales. Also, $\delta^{15}N$ values can be attributed to bat diet (Mizutani et al. 1992, Wurster et al. 2010, Royer et al. 2015). However, there is some evidence that climatic influences may affect the local N pool, allowing us to interpret the paleoenvironmental change of a region (Cleary et al. 2016).

Several possible explanations exist to explain the shifts in C and N isotope ratios. Wurster et al (2008), attributes fluctuations in their $\delta^{13}C$ record to be due to changes in atmospheric circulation and convective storms. The same may be true for the shifts in $\delta^{13}C$ and

δ^{15}N for Cave Springs. The drier periods in the early Holocene may have been due to glacial dynamics and release into the Gulf of Mexico (Grimm and Jacobson 1992, Grimm et al. 1993, Wurster et al. 2008).

2.5 Conclusions

The bat guano core from Cave Springs Cave in northern Alabama provided a well-dated record of paleoclimate change throughout the Holocene. Radiocarbon data reveals this core to be the oldest in the region, providing the longest guano record for the southeastern United States. The most reliable proxy from the Cave Springs core are hydrogen isotope ratios, the shifts in which correspond to dry periods during the early and late Holocene. In addition, the HCO determined to be a wetter period, with the deuterium record following closely to the nearby pollen record of Bartow County, Georgia. Techniques used for analyzing stable C and N isotopes in bat guano are still underdeveloped. However, growing research on the use of bat guano for paleoenvironmental reconstructions has proven guano to be a robust and complex resource for interpreting past local climates. In areas of the United States that contain caves with roosting bat colonies (e.g., southeastern United States), bat guano is an important and accurate proxy to use for paleoclimate reconstruction of the local area as well as the broader region.

Figures and Tables

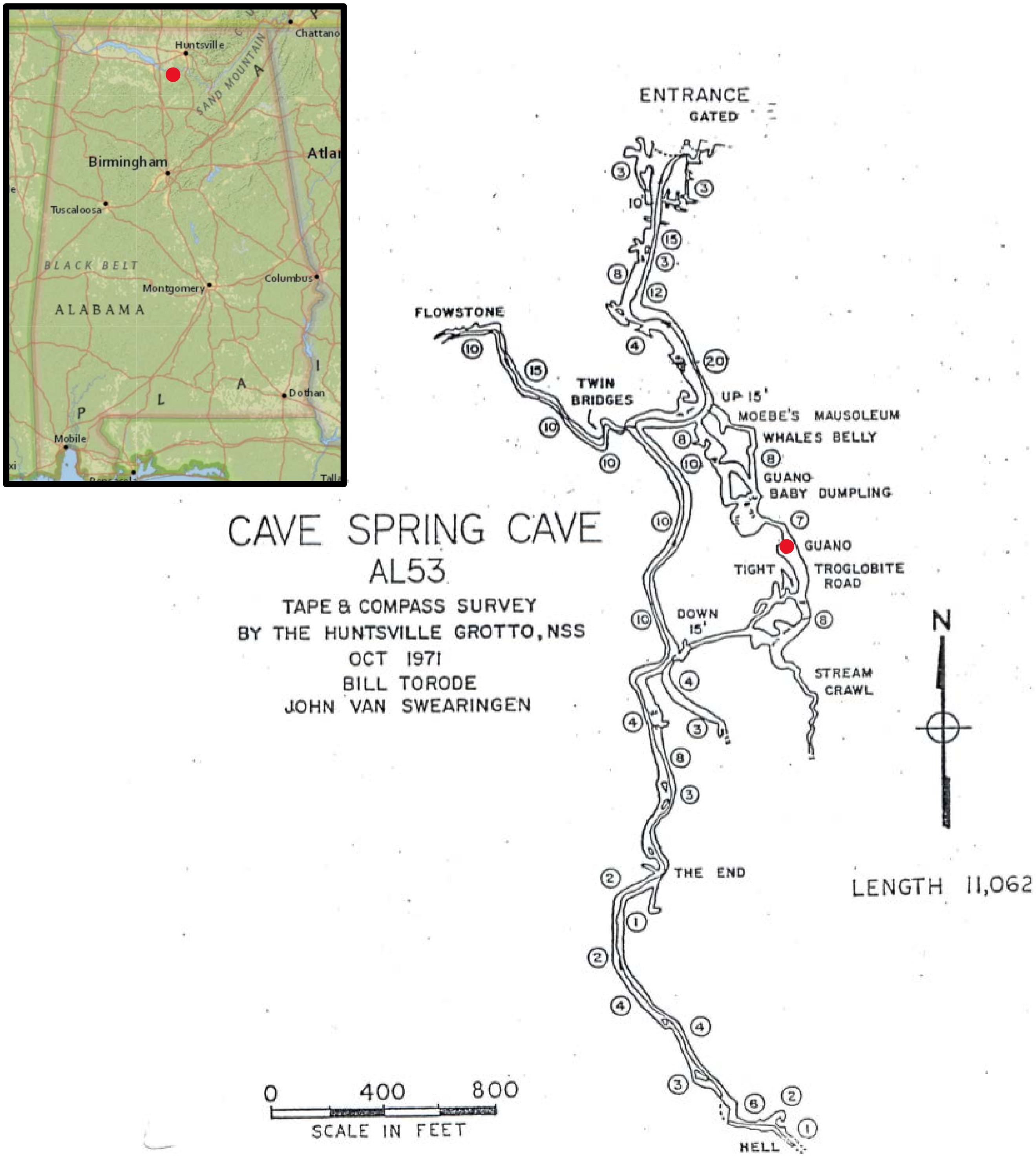

Figure 1. Map of Cave Springs Cave. 3,371 meters in total length. The guano pile that was cored is indicated with a red dot.

Figure 2. Cores retrieved from Cave Springs Cave. Cave Springs Cave is located in Priceville, Alabama. A) The top 0-32 cm the portion of the core is pellet-like and dark brown. B) 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.

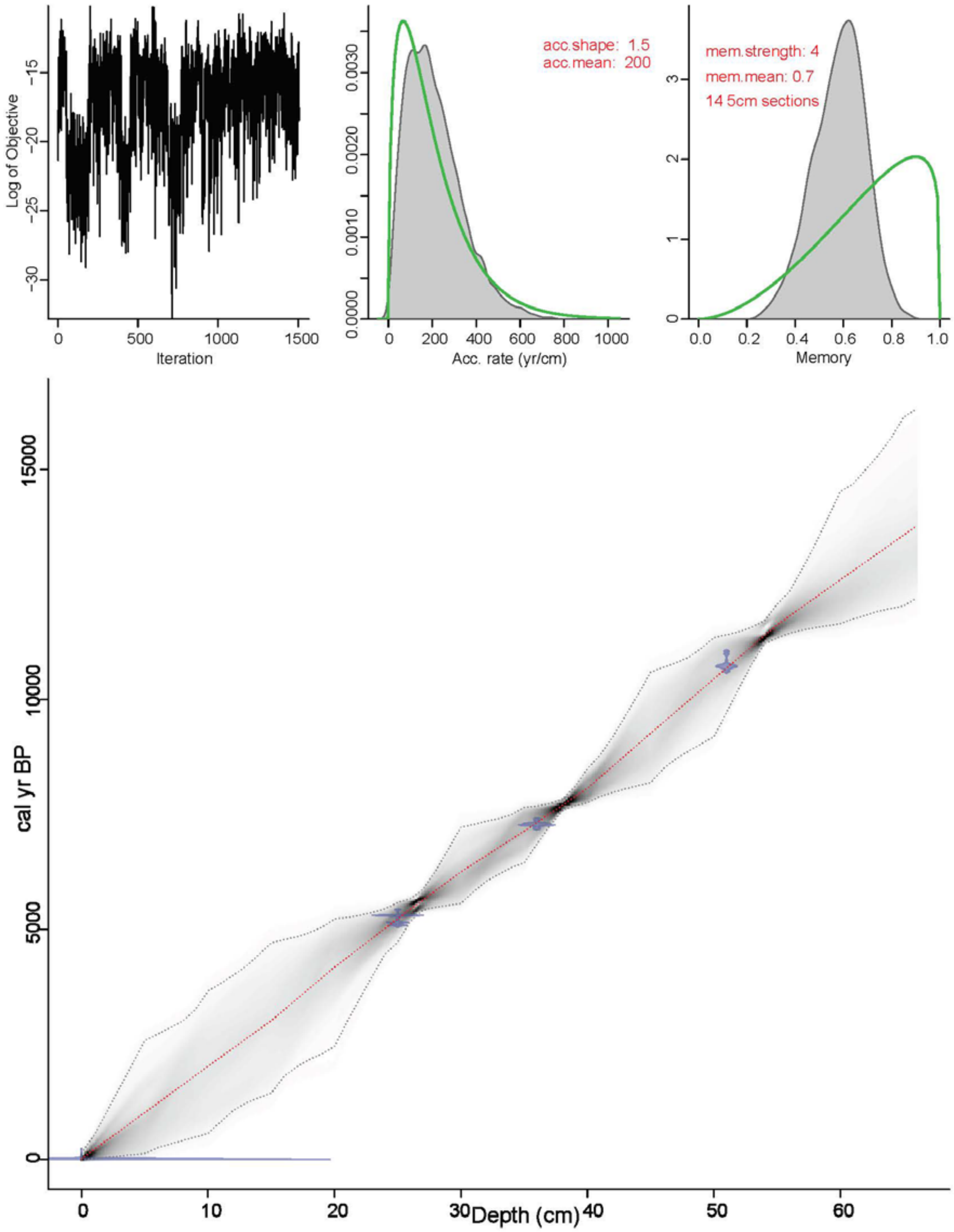

Figure 3. BACON age-depth model for Cave Springs Cave. Blue shaded regions indicate actual ^{14}C dates obtained. The gray area shows projected years to a 95% confidence interval. Red line indicates the correlation.

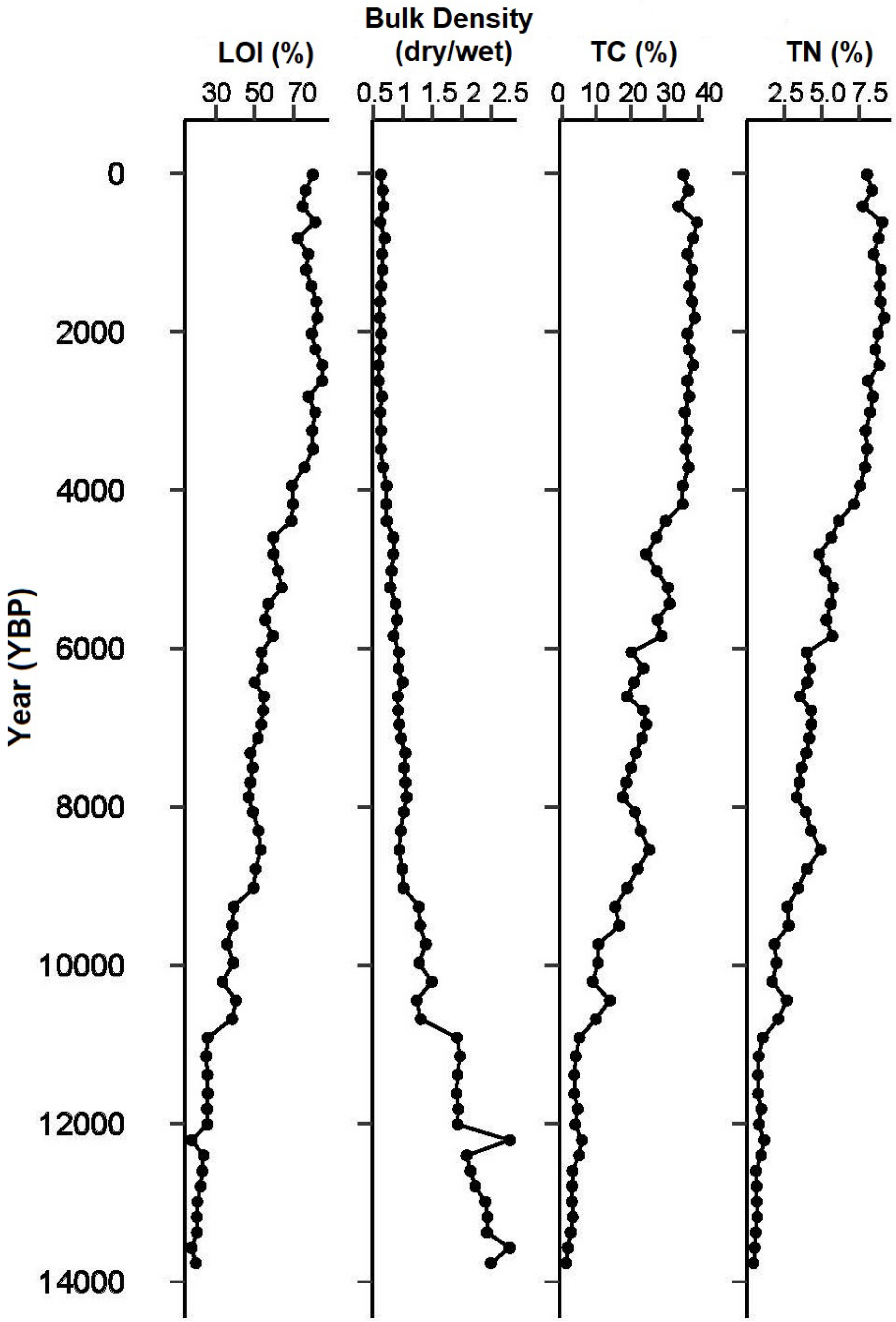

Figure 4. Organic matter content. Bulk density increases with depth. TC and TN decrease with depth. Loss on Ignition (LOI) decreases with depth.

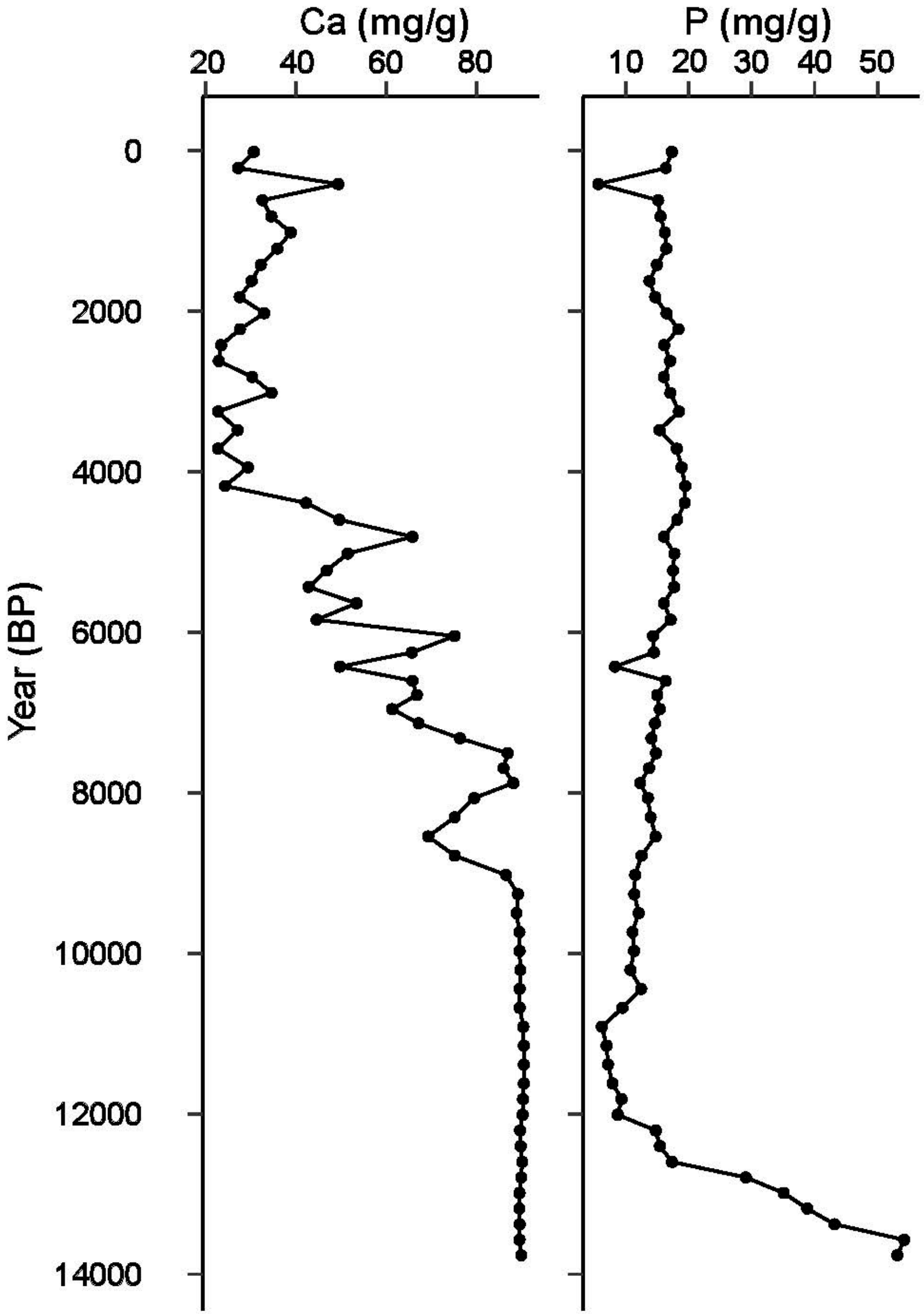

Figure 5. Calcium (Ca) and phosphorus (P) profiles. Both elements rapidly increase at the bottom of the core, but at different times. Calcium peaks at 9,024 YBP and phosphorus peaks at 12,794 YBP. Increases in both elements indicate brushite.

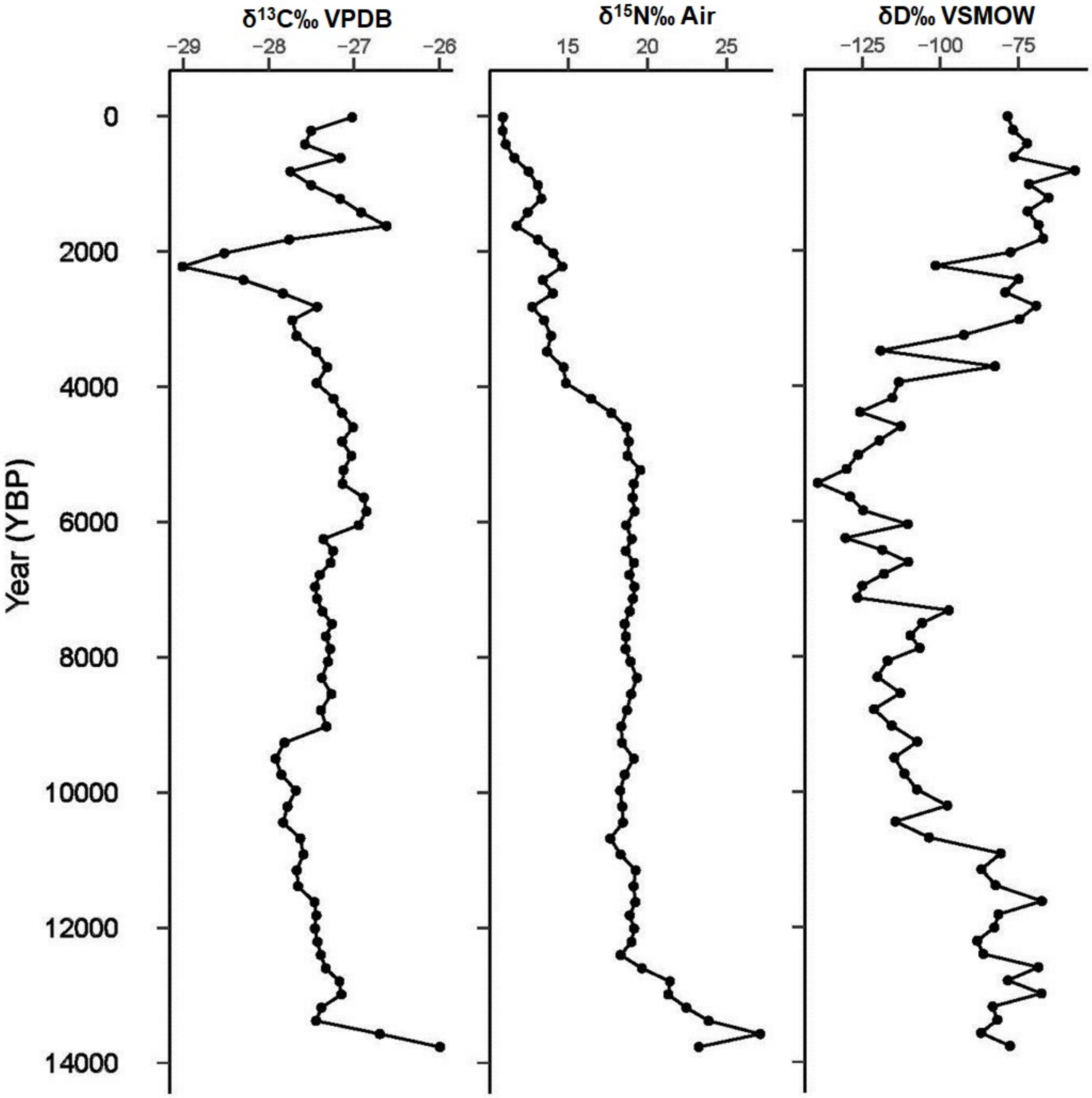

Figure 6. $\delta^{13}C$, $\delta^{15}N$, and δD values of Cave Springs Cave guano core. Nitrogen values show two major shifts at 4000 YBP and 13000 YBP, but relatively gradual. Carbon values show one peak at 2,222 YBP. Deuterium values show an early Holocene zone at 11,151 – 13,764 YBP, a middle Holocene zone at 4,388 – 10,916 YBP and a late Holocene zone at 0 – 4,177 YBP.

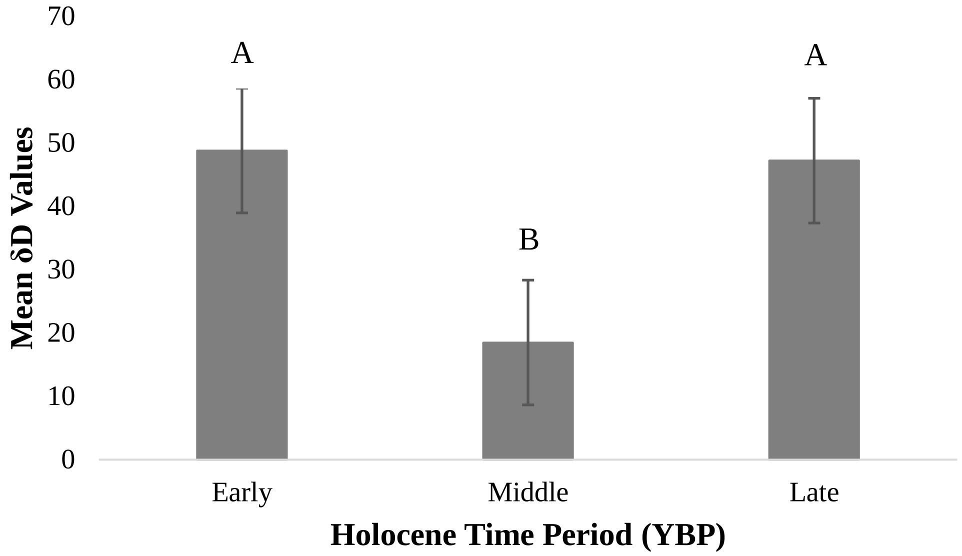

Figure 7. Mean δD values from Kruskall-Wallis One-Way Nonparametric AOV. Middle Holocene is significantly different from early and late Holocene ($\chi^2 = 18.5$). Columns with Holocene time period (early, middle, or late) with A and B as significantly different at $P \leq 0.05$.

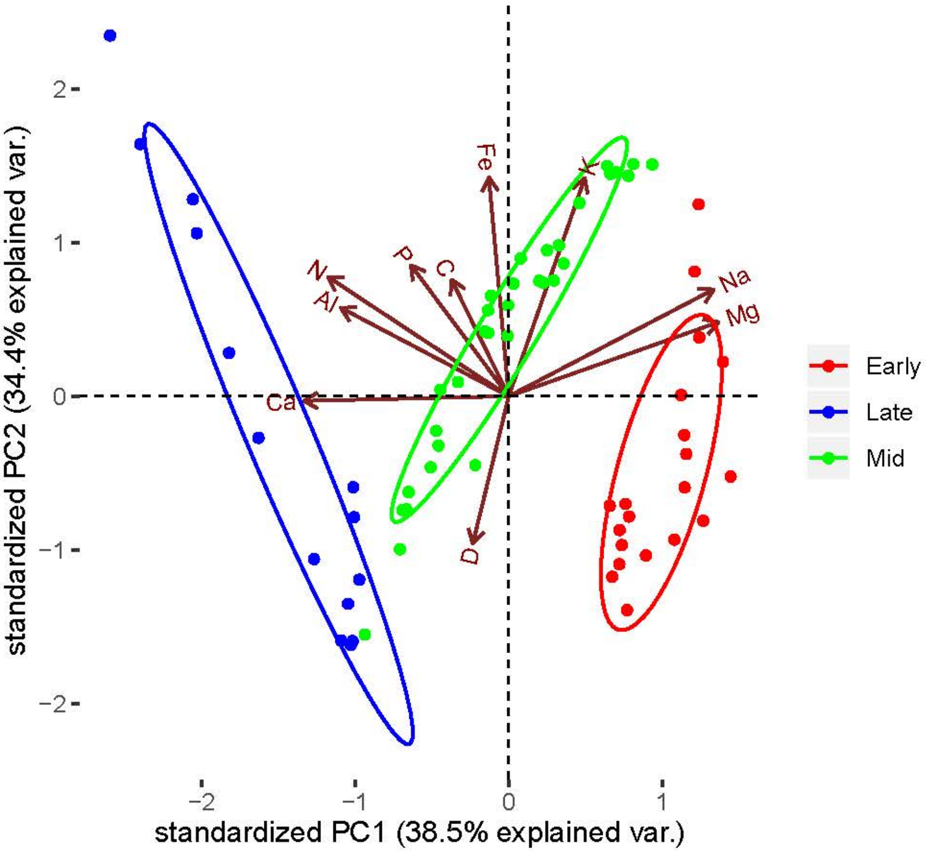

Figure 8. Principal Component Analysis of isotope systems ($\delta^{13}C$, $\delta^{15}N$, and δD) and elemental concentrations (Fe, Al, K, Ca, P, Mg) in the Cave Springs guano core. D represents δD, N represents $\delta^{15}N$, and C represents $\delta^{13}C$. Three distinct time periods are revealed (early, middle, and late Holocene).

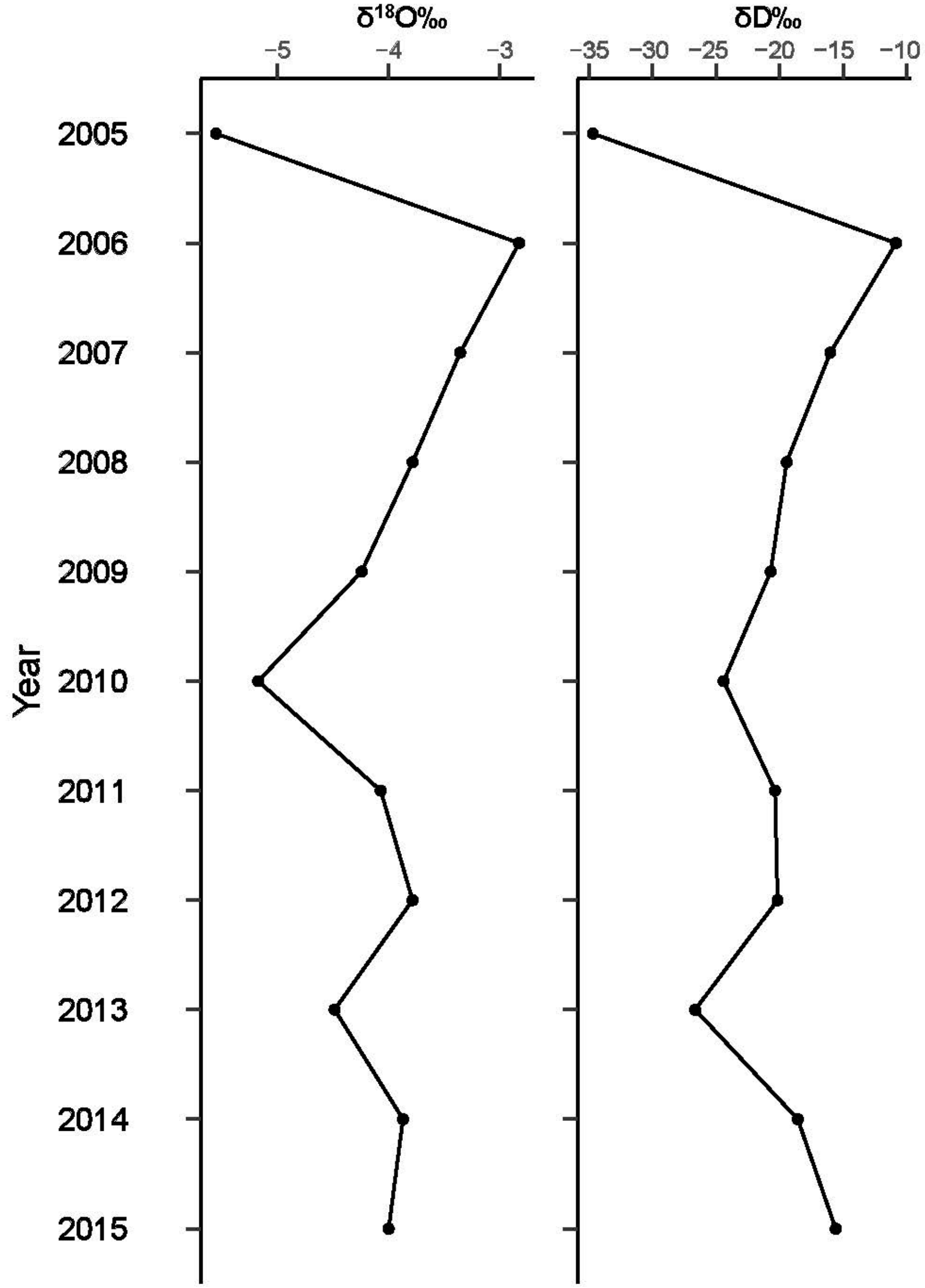

Figure 9. Stable isotopes from rainfall in Tuscaloosa, AL. Oxygen values measured by Joe Lambert, research scientist at the University of Alabama. Each point represents the average isotopic values in rainfall collected for that year.

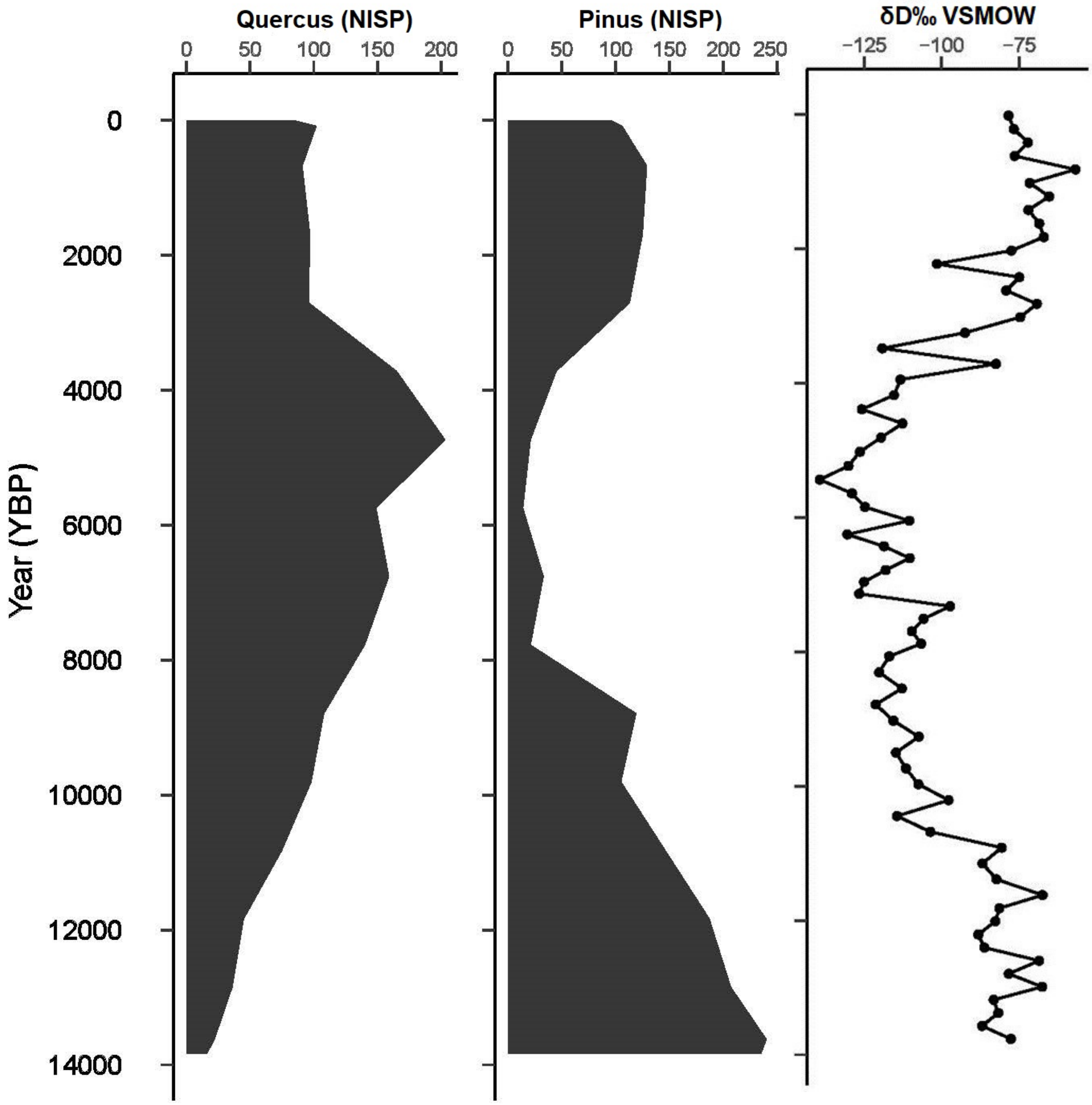

Figure 10. ***Quercus*** **and** ***Pinus*** **pollen record from small ponds in Bartow County, Georgia (Watts 1970) compared to the deuterium record from Cave Springs.** The units of measurement for Quercus and Pinus are number of individual specimens (NISP).

Depth (cm)	**δD**	**$\delta^{13}C$**	**$\delta^{15}N$**	**^{14}C Cal (YBP)**	**^{14}C Age (YBP)**	**UGAMS#**
25	-130.06	-27.12	19.55	4565.5	4070 ± 20	35051
36	-97.25	-27.37	18.89	6350	5560 ± 25	35052
51	-103.65	-27.63	17.66	9477	8190 ± 50	34438

Table 1. ^{14}C dates from Cave Springs cave in Priceville, Alabama and their respective isotope values. ^{14}C dates are in stratigraphic order.

Holocene time period	**Kruskall-Wallis X^2 value**	**Kruskall-Wallis All-Pairwise Comparisons test classification**	**Depths in the core**
Late	48.810	A	0-20 cm
Middle	18.500	B	21-52 cm
Early	47.214	A	53-66 cm

Table 2. Results from the Kruskall-Wallis One-Way Nonparametric AOV test and All-Pairwise Comparisons test for δD. Groups were separated by Holocene time period. Different letters are significantly different at $P \leq 0.05$.

3. Anatomy of a Guano Core to Improve Guano Applications in Paleoecology

Abstract

Although bat guano is gaining viability in accurately reconstructing local paleoenvironmental climates (Mizutani et al. 1992, Wurster et al. 2008, Wurster et al. 2010), an overall review of methods for analyzing and collecting bat guano cores has not been conducted. Despite the various locations of collected guano cores (i.e. western United States, Romania, Philippines, and southeast Asia), the processing and collection methods are quite similar. Physical, chemical, and elemental analyses are the three main tools used to interpret precipitation changes over time. Temperature changes may also be determined but were not analyzed in this review. The purpose of this review is to work toward community-accepted approaches to processing and analyzing bat guano cores in order to reconstruct paleoclimate in cave environments throughout the globe. From my own experiences, I describe challenges and make recommendations for improvement.

3.1 Introduction

Traditional methods of assessing paleoclimate include analyzing core samples from lacustrine sediments, ice, pollen, and even soils. However, these sample types are limited to specific geographic areas. For example, glacial lakes provide long climatic histories but, for example, glacial lakes in the tropics are limited to high altitude areas in mountainous regions. Ice cores can only be obtained from ice covered regions such as polar areas and high-altitude glaciers and cannot provide local climate histories. Furthermore, lakes tend to occur clustered in specific regions thus limiting extensive distribution. On the other hand, caves can be found globally and provide refuge to multiple bat species. Some species such as *Myotis grisescens* (Gray bats), roost in the same caves each year depositing annual laminations of guano into mounds (Martin 2007). Both insectivorous and frugivorous bat guano has been useful in paleoecological studies (Wurster et al. 2010). Thus, bat guano can be key for reconstructing paleoclimate in areas where traditional methods are not available.

Bat guano effectively tracks moisture regimes and changes in local vegetation over the duration of the Holocene (11,650 YBP) and even into the Pleistocene (2.6 million years ago (MYA) – 11,700 YBP) (Wurster et al. 2008, Wurster et al. 2010, Campbell et al. 2017). Although bat guano has only recently been exploited as a paleoclimate tool, more studies are recognizing its potential in providing information about local paleoenvironmental conditions that is 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). Bat guano is found inside caves that are protected from surficial weathering, providing a uniquely unaltered data source (Onac et al. 2014, Cleary et al. 2016). Bat guano also provides high-resolution age-depth models with constant deposition rates as maternity colonies of gray bats roost in the same caves each year (Martin 2007). To further guano research and expand on paleoclimatic records, I present the anatomy of three guano cores and methods to provide a reproducible procedure for processing guano. Here, I provide the anatomy of guano cores from insectivorous bats, review aspects of analysis, and make recommendations for improvement.

3.2 Constituents of Bat Guano

Bat guano is comprised of multiple constituents (bat hair, insect chitin, feces, and pollen) making analysis indirect and complicated. Bat hair accumulates in guano piles through grooming during roosting activity. The gray bats, *Myotis grisescens*, is the primary species in the three caves visited during this study in northern Alabama and Tennessee. Insectivorous gray bats produce chitin-rich guano, which decomposes over time causing older sections to lack discernible chitin while it is more prevalent in younger sections. Ambient pollen grains stick to bat's fur as they fly through the air in search for insects to consume, which themselves carry pollen (Maher 2006). While roosting on cave ceilings, pollen falls off their hair and into the guano pile. Pollen, hair, and chitin can provide individual data when analyzed alone; bat hair provides phylogenetic insight; the stable isotopes in the chitin provides information about local vegetation and precipitation; pollen provides information on local vegetation changes. Collectively, these tools create a robust paleoclimatic record.

3.3 Physical Characteristics and Elemental Composition of Guano Cores

Bat guano cores vary in their physical and elemental components. Three guano cores were collected for this study: 1) Cave Springs Cave in Priceville, Alabama, 2) Cripps Mill Cave in Cookeville, Tennessee, and 3) Nunley Mountain Cave in McMinneville, Tennessee. The Cave Springs Cave guano core contained many colored striations that varied from light tan to dark brown (Fig. 1). 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. 2). 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 striating up the core (Fig. 3). Cores commonly have a pellet-like consistency towards the top and become denser, becoming more clay-like with depth due to compaction. Bulk density increases with depth in the Cave Springs guano core while total carbon (TC) and total nitrogen (TN) decrease (Fig. 4). Even though Cave Springs and Cripps Mill cores were around the same length ~87 cm, they had dramatically different ages. Cave Springs guano was dated from radiocarbon to ~9000 YBP radiocarbon dating showed Cripps Mill guano to be modern—showing length of guano cores are no measure for age.

3.4 Brushite

In each guano core, lighter, tan-colored material was found at the bottom and was determined to be, a mineral found only in cave systems called brushite (see section 2.4; Giurgiu and Tamas 2013, Stahle et al. 2019). Calcium and phosphorus rapidly increase at the bottom of guano cores in congruence with the presence of brushite (Fig. 5). Brushite and other cave minerals contain high amounts of phosphorus and calcium and form after long periods of time (hundreds to thousands of years). Whether brushite is formed from a reaction with guano and cave minerals or formed from cave sediments is still up for debate. However, due to the lack of organic material in the brushite zones of the cores, these portions are not ideal for paleoenvironmental reconstructions.

Phosphate minerals are a common feature in caves containing bat guano accumulations (Giurgiu & Tamas 2013) and more than 100 have been identified in total (Onac 2012). The mechanisms responsible for phosphate formation in caves include digestion, dissolution, double replacement, and redox reactions (Onac and Forti, 2011b). Five phosphate minerals are considered common: hydroxylapatite, brushite, ardealite, taranakite, and variscite. Of these minerals, brushite, ($Ca(HPO_4)\bullet 2H_2O$), is one of the most important phosphate species occurring in the guano-bearing caves world-wide as it is most commonly found (Hill & Forti 1997).

Brushite is a cave mineral that was recently discovered; its first recorded appearance was in 1864 by Gideon E. Moore. In his communication, Moore (1865) only vaguely describes his physical observations of brushite. Since then, few studies have been published on the composition and formation of brushite, creating a need to understand its chemical composition and environmental conditions leading to its genesis (Dumitras et al. 2004).

One hypothesis of brushite formation speculates solutions percolating through guano deposits interact with the limestone bedrock and produce Ca-rich phosphates (brushite and hydroxylapatite) (Hill & Forti, 1997). Another hypothesis suggests brushite is formed by guano interacting with calcite at low pH (Frost and Palmer 2011).

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

3.5 Stable Isotopes Composition of Guano Cores

Stable isotopes 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). Studies are in disagreement whether $\delta^{15}N$ can provide accurate moisture data (Wurster et al. 2010, Cleary et al. 2016, Campbell et al. 2017). $\delta^{13}C$ and δD isotopes are more commonly used to determine paleoclimate. It is proposed that $\delta^{15}N$ accumulates from N gains/losses, N pool mixing, and isotope fractionations (Cleary et al. 2016) which ultimately allows the determination of moisture regimes through time. δD isotopes are a common, standard, and accurate way to determine moisture levels (Wurster et al. 2010). δD values of insect chitin reflect metabolic and drinking water sources and they are correlated with δD values in local precipitation (Grocke et al. 2006, Wurster et al. 2010).

Isotopes performed on bulk guano versus insect chitin yield varying results (Fig. 6). One drawback of performing analyses on insect chitin alone is the degradation of chitinous material. Distinguishable insect chitin pieces are 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 dated to be modern should be consistent in quantity.

3.6 Age-Depth Models

A critical component to paleoclimate studies is to making sure the sample dates are correct. 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. Once ^{14}C dates are obtained, the Bayesian accumulation history (BACON) age-depth modelling package in R software projects ages throughout the core with a 95% confidence interval (Blaauw and Andrès 2011). Linear correlation lines indicate more constant sedimentation rates (Fig. 7).

3.7 Methods of Analysis and Improvement

3.7.1 Obtaining the Core

Access for caves were granted by private landowners or filling out permits for access to protected caves on government property. Locations within each cave for guano coring were chosen based on availability and height of the guano pile. Ideal height for guano coring is ~ 2 meters. Once chosen, three-foot sections of PVC (polyvinyl chloride) pipes were hammered into the pile with a mallet. To decrease disturbance, guano core sections are 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, and I recommend using a Russian peat corer for better results. Other studies have used a Russian peat corer and have been successful (Forray et al. 2015, Cleary et al. 2016). After cores have been collected, they must be kept upright, and frozen until ready to section. Our cores were sectioned by one centimeter. If possible, a smaller scale can be used to create a higher resolution in the radiocarbon dating model. Once sectioned, each centimeter was analyzed for loss on ignition (LOI), organic matter content, and bulk density, using methods like those performed in limnology studies by Brenner et al (1999) (Fig. 4). After physical analysis is conducted, chemical analyses can be performed next. The one-centimeter sections are freeze dried, ground with a mortar and pestle, and sent off for elemental and nutrient data.

3.7.2 Insect Separation Procedure

Separating chitinous insect pieces from fresh guano provided different results than bulk guano alone (Fig. 6). To separate insects, ¼ tsp of guano was put in a vial along with 10 ml of a 1% solution of Citranox detergent. The vial was then put on a vortex mixer for 10 seconds followed by a hot water bath for ten 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 then deionized water. After samples dried in an oven, they were brushed out of sieves and placed in petri dishes for separation using forceps while under a dissecting microscope. While this method could be used to reconstruct insect consumption through time by volume and species (if able to identify from chitin pieces), it appears that application is limited to modern guano samples.

3.7.3 Pollen Analysis Difficulties

Pollen provides evidence for local vegetation types and contributes to paleoclimatic problems (Whitehead 1973, Davis 1976, Delcourt 1980). The pollen that accumulates in guano needs to be separated out from the other constituents in a tedious process. After pollen separation is complete, the pollen grains must be stained for identification under a microscope. Identifying pollen grains is a difficult task. Most methods involve using concentrated hydrofluoric acid (HF) (Lentfer and Boyd, 2000), causing more of a hazard and I did not have the identification skills or the knowledge to accomplish this. Pollen was not identified in guano cores collected for this project but could be a useful analysis for paleoclimatic reconstructions for future studies. However, less is known concerning pollen delivery and the link between local vegetation and pollen occurrence is still needed.

3.7.4 Charcoal Analysis

Charcoal is another possible avenue of study as bats collect charcoal on their fur while they fly in the air foraging for food. Once separated from guano, charcoal can be viewed under a dissection microscope and quantified to determine years of larger forest fires. The charcoal separation method is similar to insect separation. Guano will be rinsed, sieved, and dried as described above and then brushed into a petri dish. Once the guano sample is in the petri dish, grid lines are drawn on the cover and macrocharcoal pieces are counted and recorded for each grid block.

3.8 Conclusions

As more studies of bat guano are being explored, it will become more of a common practice to use guano in paleoclimate research. Guano studies have already been used across the globe in countries such as Romania (Onac et al. 2014), Guadeloupe (Royer et al. 2015), the Phillippines (O. Choa et al. 2016) and the western portion of the United States (Mizutani et al. 1992, Wurster et al. 2008, Wurster et al. 2010), however the majority of published studies have favored isotopic analysis over biological remains at this time.

Figures and Tables

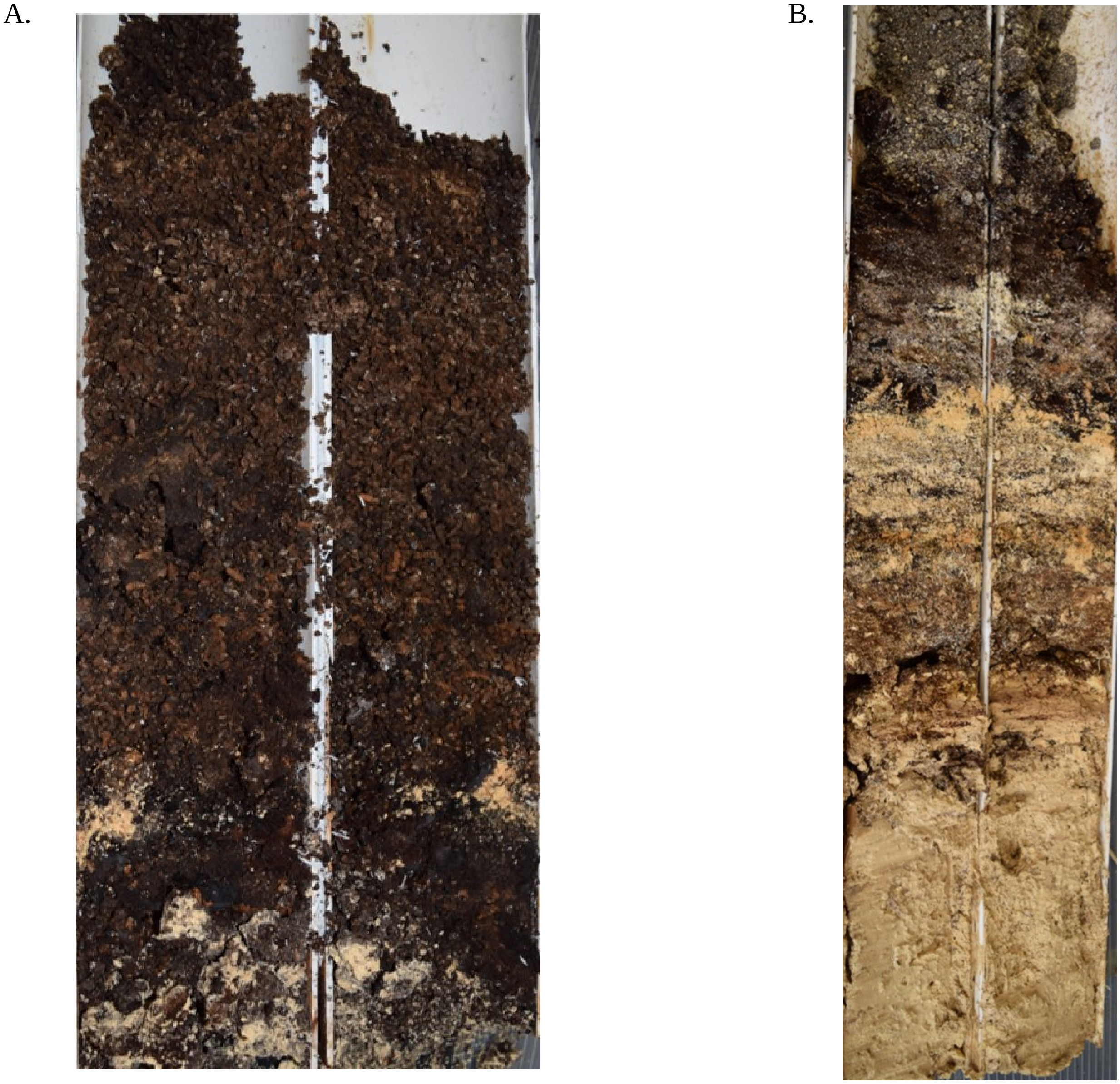

Figure 1. Cave Springs Cave guano core. Cave Springs Cave is located in Priceville, Alabama. A) The top 0-32 cm the portion of the core is pellet- like and dark brown. B) The bottom 33-87 cm portion of the core has many colored striations with layers of brushite intermixed with the guano. Guano core was 87 centimeters in total length.

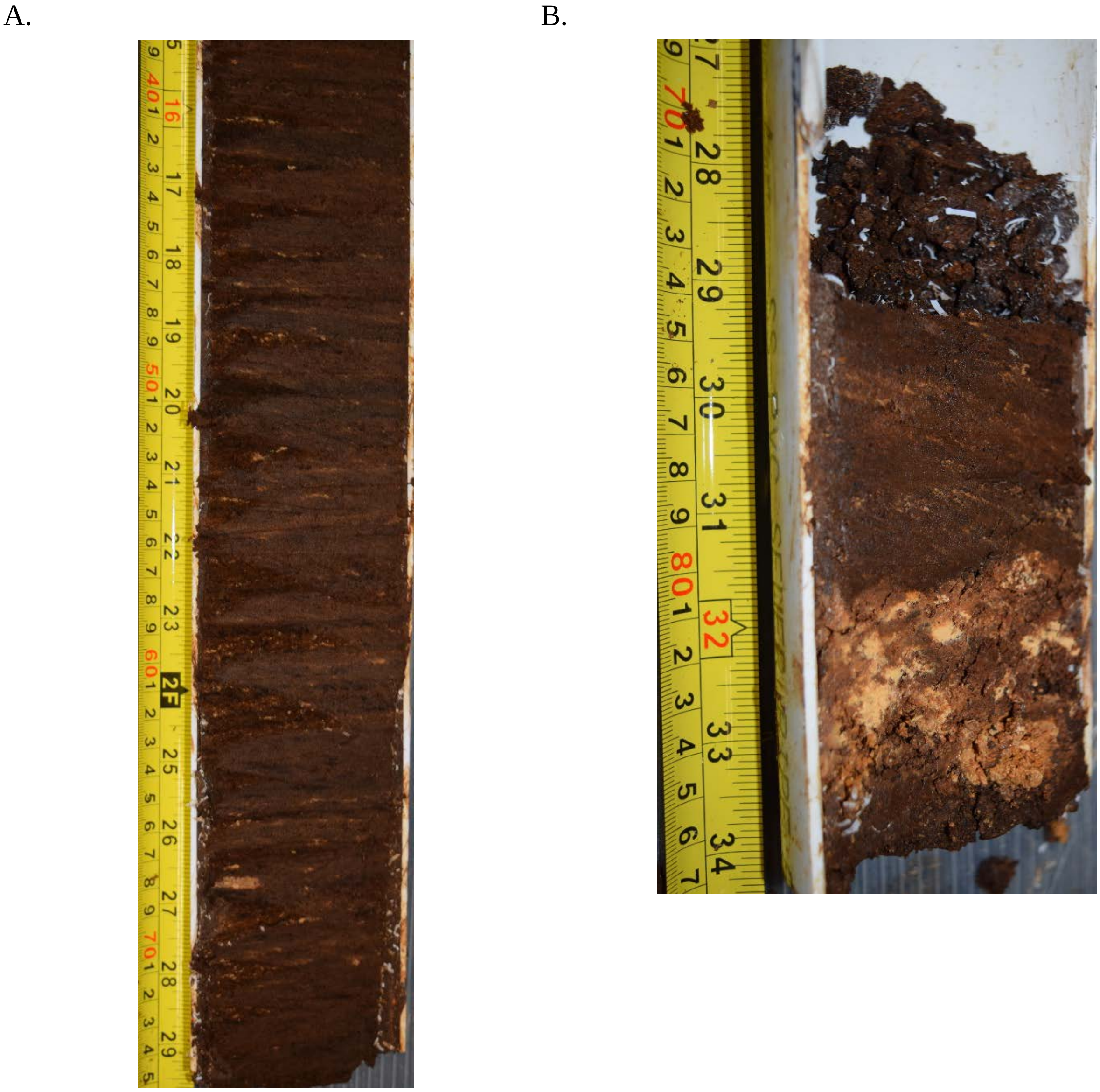

Figure 2. Cripps Mill guano core. Cripps Mill is located in Cookeville, Tennessee A) The top (0-86 cm) is consistent in texture and color. B) The bottom (81-87 cm) indicates brushite. Guano core was 87 centimeters in total length.

Figure 3. Nunley Mountain Cave guano core. Nunley Mountain Cave is located in McMinneville, Tennessee. Guano core was 71 centimeters in total length.

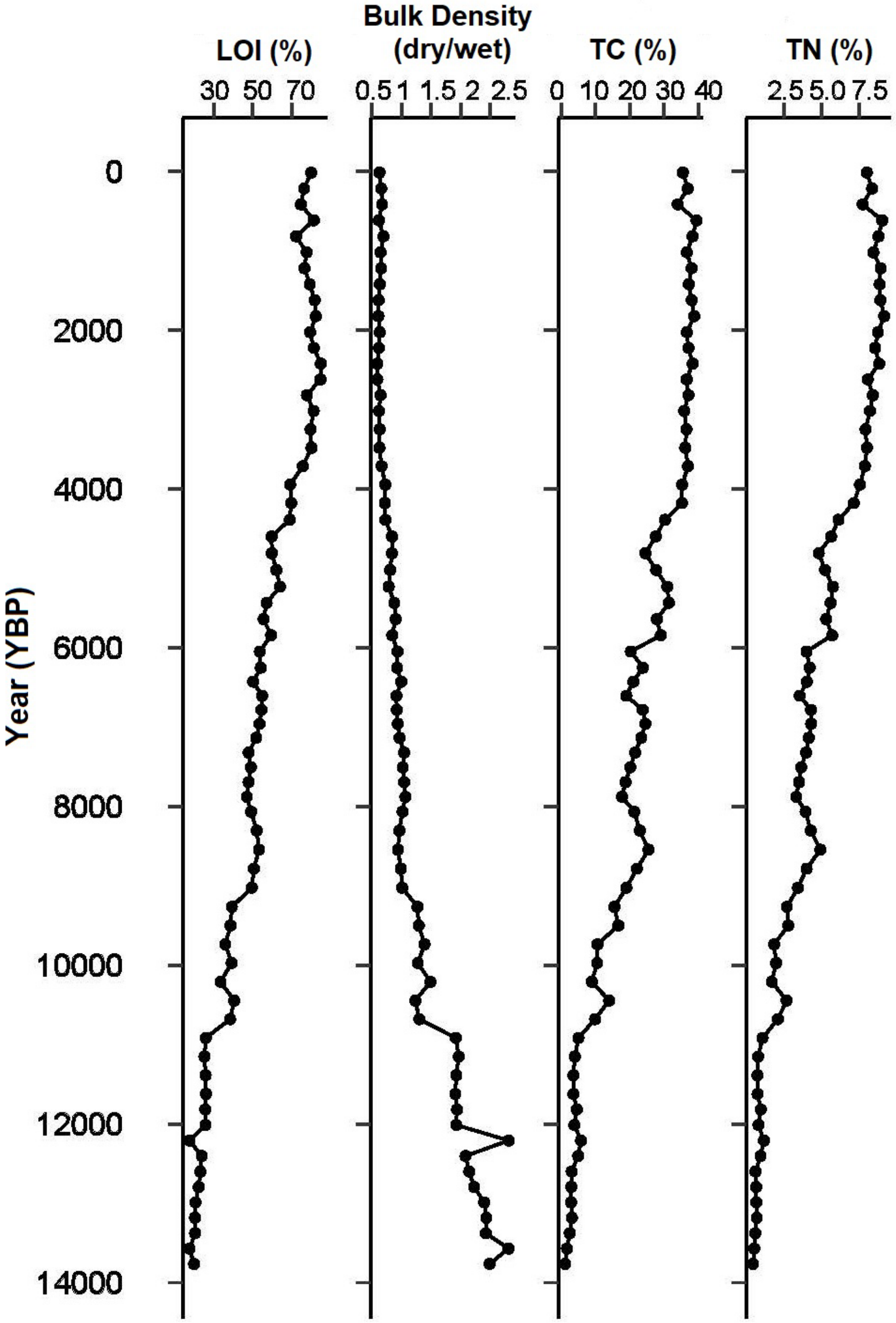

Figure 4. Organic matter content analysis in Cave Springs core. Bulk density increases with depth. TC and TN decrease with depth. Loss on Ignition (LOI) decreases with depth.

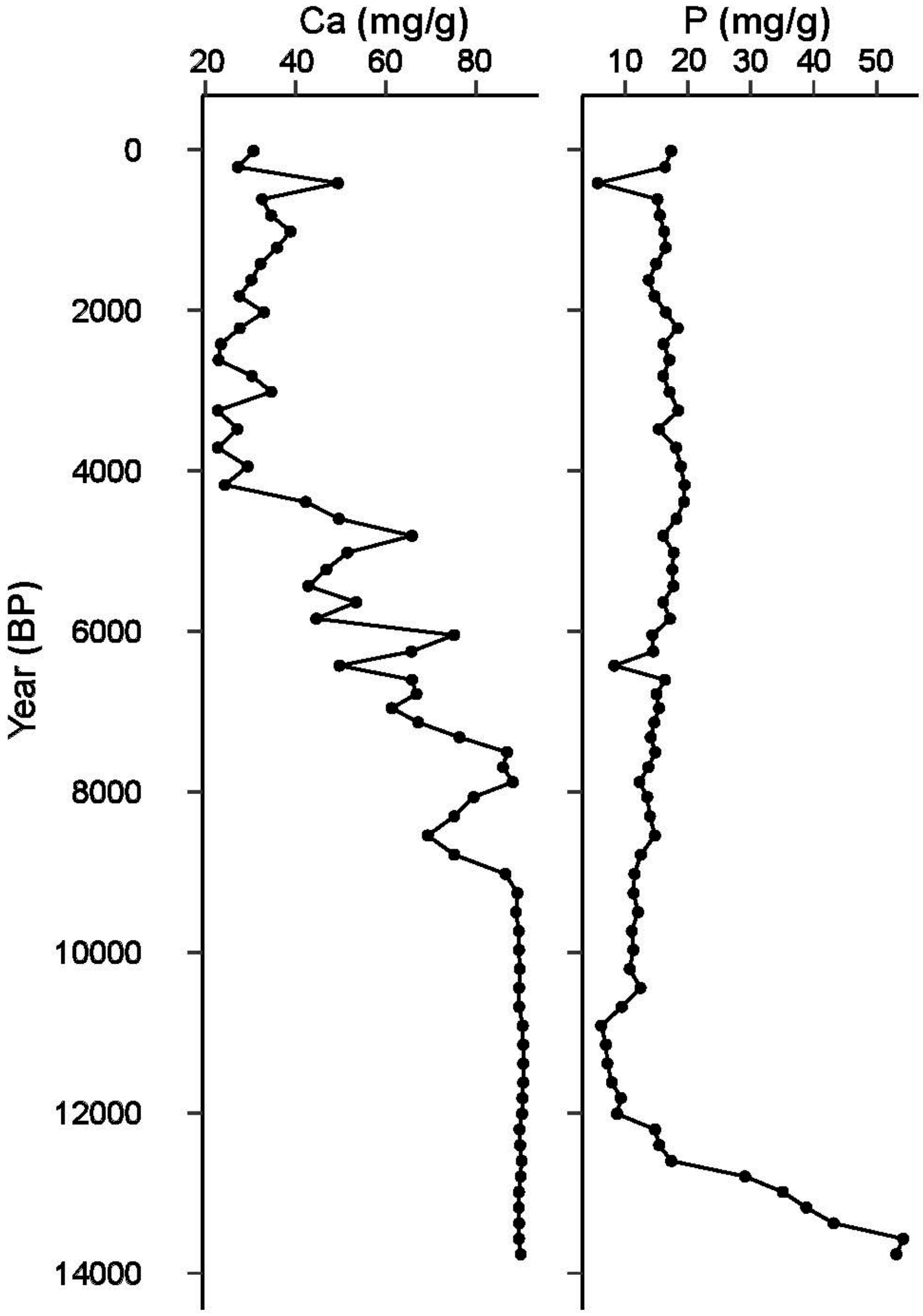

Figure 5. Elemental concentrations measured throughout Cave Springs guano core. Both elements rapidly increase at the bottom of the core, but at different times. Calcium peaks at 9,024 YBP and phosphorus peaks at 12,794 YBP. Increases in both elements indicate brushite.

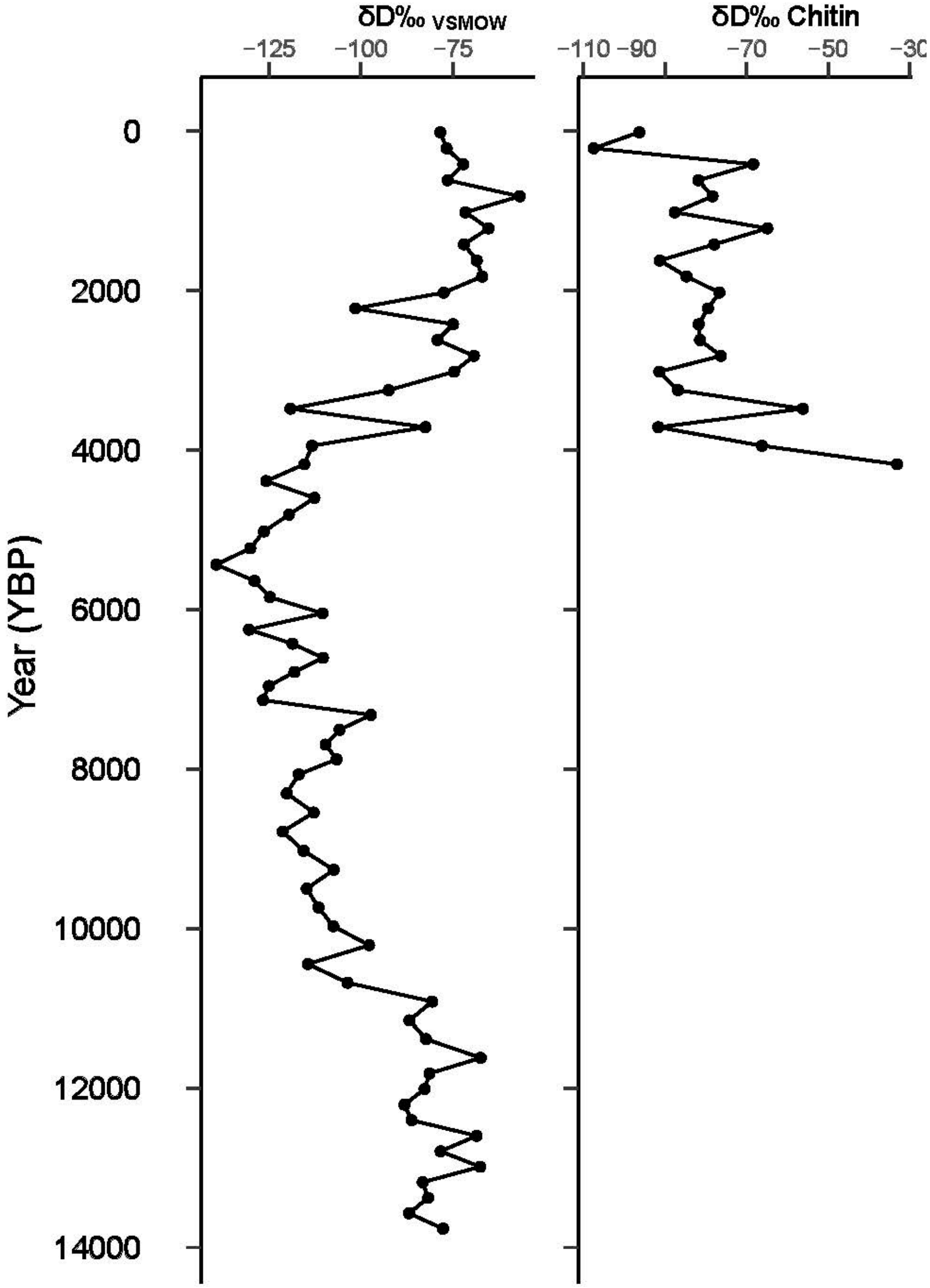

Figure 6. δD values of bulk guano (left) and insect chitin (right) from Cave Springs core. Fewer values were obtained from chitin due to degradation.

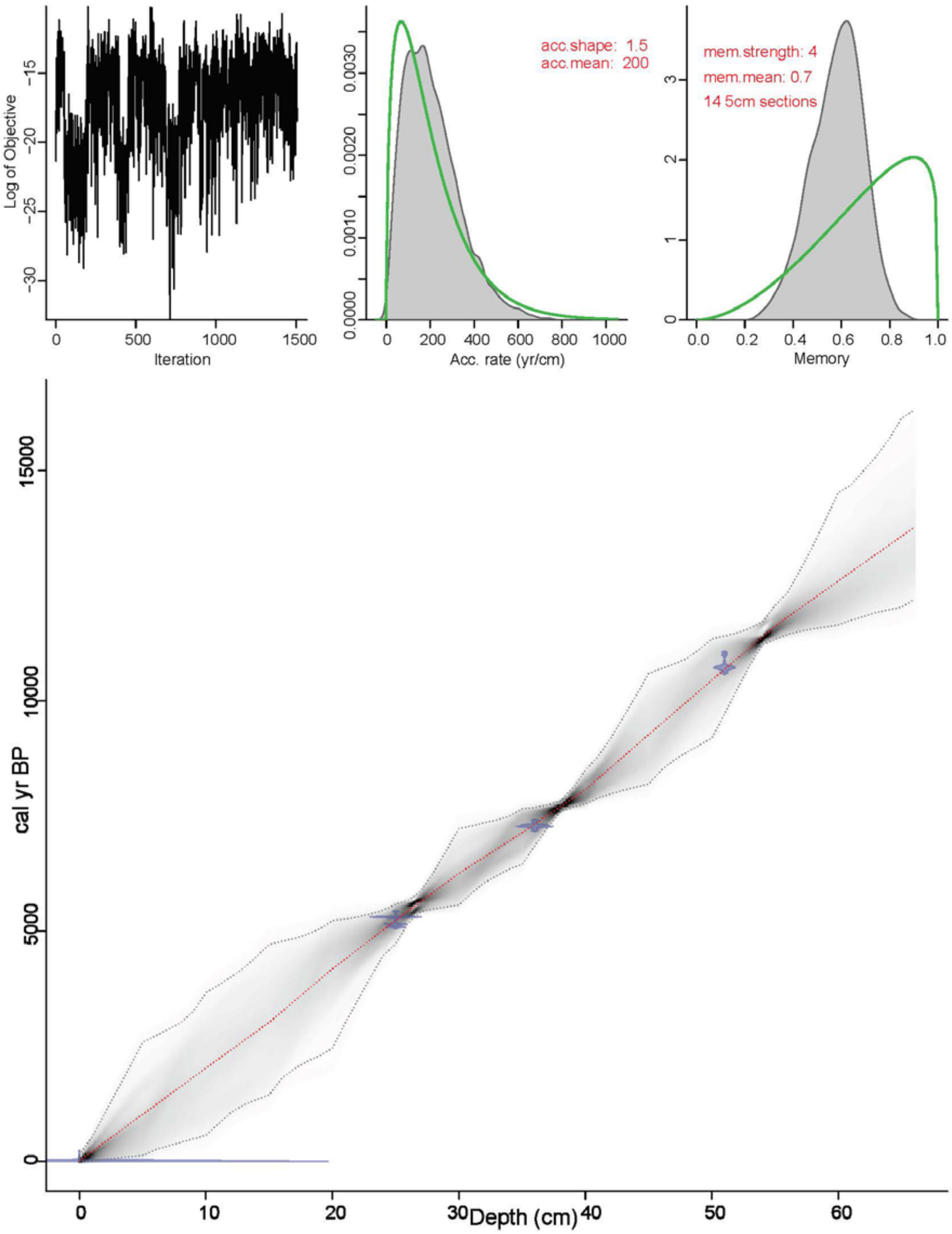

Figure 7. BACON age-depth model for Cave Springs. Blue shaded regions indicate actual ^{14}C dates obtained. The gray area shows projected years to a 95% confidence interval. Red line indicates the correlation.

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

Table 1. Comparison of ^{14}C dates and core lengths for each guano core collected.

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