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Effect of diet, anthropogenic activity, and climate on $\delta^{15}N$ values of cave bat guano

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ABSTRACT

Few studies have attributed $\delta^{15}N$ values of guano to a factor other than diet. A $\delta^{15}N$ record obtained from a 1.5-m core of bat guano deposit from Ziditã Cave (western Romania) provides a record of anthropogenic and climatic influence on the regional nitrogen pool. Nitrogen content is nearly constant (%N > 9) for over 1 m of the core, indicating limited diagenesis. The $\delta^{15}N$ and $\delta^{13}C$ time series are compared and the $\delta^{15}N$ is also interpreted in light of previously published pollen assemblage from the same core. Using these comparisons the influence of anthropogenic activity and water availability is interpreted. Although some $\delta^{15}N$ variation can be attributed to major changes in anthropogenic activities, additional variation implies an alternative control. The correlation between $\delta^{15}N$ and $\delta^{13}C$ values is significant ($p < 0.01$), but not strong, suggesting that both variables are influenced by water availability, known to be a primary control of $\delta^{13}C$ values within C_3 ecosystems. Drier periods indicated by higher $\delta^{13}C$ values correspond with lower $\delta^{15}N$ values and vice-versa for wetter conditions. The instrumental climate record (precipitation amount) for the past 50 years nearby the cave supports this relationship. From 1000 to 1800 CE, the $\delta^{15}N$ values fluctuated between 11.5 and 14‰, then decreased in two abrupt steps, at 1800 and 1947 CE. The most significant decrease occurred after 1947 CE when values fell from 12.5 to below 7‰, suggesting the N-cycle transitioned towards a more conservative state. The correlations between $\delta^{15}N$ and $\delta^{13}C$, and the instrumental precipitation record, along with the apparent negligible effect of diet on long term $\delta^{15}N$ variation indicate that the $\delta^{15}N$ values of guano can be used as an integrator of the local N-cycle.

1. Introduction

Caves are frequently suitable sites for paleoclimate and paleoenvironmental studies because their deposits are protected from surficial weathering. A generally untapped archive is preserved in bat guano, which can be found in many caves and can be precisely dated using radiocarbon (McFarlane et al., 2002; Bird et al., 2007; Wurster et al., 2007; Onac et al., 2015; Royer et al., 2015). Guano deposits are primarily composed of unconsolidated organic material (insect remains), sometimes interbedded with clays (Mizutani et al., 1992a; Onac et al., 2014). When bioturbation and diagenesis are minimal, the original stratigraphy of the guano deposit may be preserved.

Paleoenvironmental reconstructions using guano have focused primarily on stable isotope records such as bulk $\delta^{13}C$ values, $\delta = [(R_{sample} - R_{standard}) / (R_{standard})] \times 1000$; $R = {}^{13}C/{}^{12}C$; Mizutani et al., 1992b; Wurster et al., 2007; Forray et al., 2015), pollen (Maher, 2006; Geantă et al., 2012), $\delta^{2}H$ in chitin (Wurster et al., 2010), as well as chemical composition (Bird et al., 2007; Onac et al., 2015; Wurster et al., 2015). In contrast, the nitrogen (N) isotopic composition of guano has received far less attention. Using the well-defined 3–4‰ increase in $\delta^{15}N$ values with each higher trophic position (e.g., DeNiro and Epstein, 1981; Peterson and Fry, 1987; Koch et al., 1994), guano N studies have often been limited to confirmation of the diet and trophic position of the bats (Mizutani et al., 1992a; Bird et al., 2007). However, Mizutani et al. (1992a) reported a latitudinal dependence of ^{15}N enrichment in guano at caves in Jamaica and the southwestern United States, reflecting trends in aridity or climate. Meanwhile, other organic materials used as paleoenvironmental proxies have shown that $\delta^{15}N$ values can provide additional resources to the more commonly used $\delta^{13}C$ and $\delta^{2}H$ values (Esmeijer-Liu et al., 2012; Szpak, 2014). In addition to records of long-term paleoenvironmental changes, $\delta^{15}N$ values in guano may also track the degree to which anthropogenic activities (deforestation, fertilizer usage, controlled fires, etc.) and climate may have impacted the local ecosystem.

The use of $\delta^{15}N$ in guano has eluded paleoenvironmental science for mainly two reasons: 1) the potential of digenetic alteration of $\delta^{15}N$ signal in guano and 2) the complicated nature of the N cycle (Bird et al., 2007; Wurster et al., 2007). The first condition relates to microbial

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processes (i.e. denitrification, nitrification, etc.), which result in the remaining bulk guano becoming deprived of N, while ^{15}N-enriched (Robinson, 2001). Therefore, as shown by Bird et al. (2007) and Wurster et al. (2007), the carbon (%C), nitrogen (%N) contents, and C:N ratio can be indicative to whether the isotopic composition of guano has been changed following its deposition. Given that the degree of decomposition typically increases with depth and age (Tiunov, 2007), the most recent guano typically presents the greatest potential for interpreting past changes of environment and climate.

The N cycle (soil, biomass, atmosphere, consumers) is complicated due to the potential mixing of different N-pools and various N transformations with wide ranges of enrichment factors (Högberg, 1997; Robinson, 2001). These processes may mask the δ^{15}N composition of the primary signal, such as the source from which plants access N, making traditional isotopic tracer studies problematic. However, a new paradigm is emerging in ecological applications, which makes use of fundamental rules governing N isotopic fractionation in complex systems, whereby δ^{15}N values of soil, plants, and consumers within the N-cycle can be utilized as an integrator, rather than a simple tracer of the N-cycle (Robinson, 2001). The δ^{15}N value of a system is the result of any N gains/losses, N pool mixing, and isotope fractionations and therefore this value integrates these processes (Robinson, 2001). This approach allows for the development of paleoenvironmental records within the context of the state of the N-cycle. For example, recent work has begun to elucidate some of the local to regional scale climatic controls on N cycling and δ^{15}N values of soil and foliage (Austin and Vitousek, 1998; Aranibar et al., 2003; Swap et al., 2004). In addition, δ^{15}N studies can be routinely used to address the roles of anthropogenic activities, such as deforestation, fertilizer usage, and controlled fires in the global N-cycle (Kendall et al., 2007). Other applications include the ability to interpret the "openness" of an ecosystem's N-cycle, which is reflected in the δ^{15}N of soil and plants (Robinson, 2001). From this approach one can interpret temporally and spatially patterns of more closed vs. more open N-cycling, which varies at several scales with mean annual precipitation (MAP; Austin and Vitousek, 1998; Handley et al., 1999; Amundson et al., 2003) and the degree of anthropogenic influence (Szpak, 2014).

The fractionation occurring during metabolic processes within bats and insects follow conservative pathways and remain fixed. Therefore, in the absence of post-depositional diagenesis, bulk guano δ^{15}N values can be interpreted within the constraints of terrestrial ecological processes affecting δ^{15}N of the source area. When instrumental records are not available to compare with the δ^{15}N values in older guano deposits, other proxies (e.g., pollen or δ^{13}C) can be utilized.

While there is presently a resurgence of studies using δ^{15}N values from a variety of sources and to understand climatic and environmental processes, the δ^{15}N signature of guano has not yet been considered in any European caves. Here we report stable isotope and elemental analysis of a 900-year old guano core from Zidită Cave (Romania) with the aim to examine primary controls of δ^{15}N values of bat guano. The guano-derived δ^{15}N record is compared to previously published pollen and δ^{13}C data from the same core to conclude whether δ^{15}N data reflect the state of the local N-cycle (both from a climatic and anthropogenic perspective) and/or changes in trophic level.

2. Materials and methods

2.1. Study site

Zidită Cave (hereafter ZC) is located on the left bank of the Geoagiu River in the Metaliferi Mountains (western Carpathians; Fig. 1A) and its guano mound lies within the Bat Room (Fig. 1B). The present-day climate around the cave is temperate continental, with a mean annual temperature and precipitation of ~10 °C and 600 mm, respectively (data since 1961). At present, the vegetation in the ZC area consists of forests, meadows, orchards, and agricultural land. Along the Geoagiu River, the main species of trees are *Alnus glutinosa* and *Salix fragilis*. The primary trees and shrub taxa in forested locations are *Quercus pubescens*, *Q. frainetto*, *Q. robur*, *Fagus sylvatica*, *Tilia tomentosa*, *T. cordata*, *Acer campestre*, *A. pseudoplatanus*, *Crataegus monogyna*, and *Corylus avellana* (Forray et al., 2015). Species of *Viburnum* and *Rhamnus* are also present. Shrubs and grassland cover the hills of whose southern portion has been affected by deforestation (since late 1970s). Agriculture is also prominent at lower elevations where crops, hay fields, and pastures cover sizable areas. Herbaceous taxa at these locations are *Apiaceae*, *Caryophyllaceae*, *Chenopodiaceae*, *Fabaceae*, *Scrofulariaceae*, *Poaceae*, *Plantaginaceae*, and *Urticaceae*, while the main tree taxa are *Alnus glutinosa* and *Salix fragilis* (Pop and Hodişan, 1957).

Although a record from 1952 indicates the presence of a *Miniopterus schreibersii* maternity colony (Forray et al., 2015), systematic observations conducted between 1960 and 2014 showed the dominant species of bat in ZC was *Rhinolophus euryale* (Mediterranean Horseshoe Bat). This species feeds during the spring and summer eating mostly Lepidoptera (moths), such as *Colotois pennaria* as well as Coleoptera (beetles), Clucidae (mosquitoes), and in smaller amounts Diptera (flies), at distances of 5 to 10 km from the roosting location (Goiti et al., 2006; Miková et al., 2013). However, in March 2015 a maternity colony formed by *Myotis myotis* was the overwhelmingly dominant species inhabiting the cave, with only a few *Rh. euryale* individuals and a single *M. schreibersii* observed (I. Coroiu pers. comm.). Some *Myotis* species are known to eat larger prey such as fish (Aizpurua et al., 2013) but, similarly to *Rh. euryale*, has a primarily insect based diet (Arlettaz, 1996). These insects of the bat's diet feed on the surrounding vegetation during their larvae stage, which typically spans up to 90 days.

2.2. Sample collection and methodology

A 1.5 m core was recovered in 2012 from a guano pile in the Bat Room (Fig. 1B) using a Russian peat corer (Forray et al., 2015). Guano sampling was conducted within the cave to reduce the potential of contamination during transport. Sample aliquots were taken at 2 cm resolution and stored at 4 °C until analyzed. Upon starting the procedure, samples of guano were first homogenized in an agate mortar that was cleaned between samples with H_2O_2 to remove organics. The samples were then placed in a drying oven (at 40 °C) overnight. After drying, each sample was rehomogenized and then stored in glass vials to avoid contamination. Aliquots of 1–2 mg were weighted and placed in tin cups and then measured for δ^{15}N, δ^{13}C, %C, and %N, using a Costech Elemental Analyzer coupled to a Delta V Isotope Ratio Mass Spectrometer hosted in the Stable Isotope Laboratory (University of South Florida). A protein standard B2155 (δ^{15}N: 5.94; δ^{13}C: −26.98‰; %C: 46.5, %N: 13.3) and a glutamic acid (internal standard; δ^{15}N: −6.28‰; δ^{13}C: −16.5‰; %C: 41.37, %N: 9.54) were used during analysis. The δ^{15}N value for the internal standard was calibrated using the certified reference materials, B2155 and IAEA-N1. B2155 and IAEA-C7 were used to calibrate the δ^{13}C value for glutamic acid. Precision of analysis (δ^{15}N: 0.08‰; δ^{13}C: 0.04‰) was estimated based on replicate internal standards during each run.

The δ^{13}C values on this core are already published in Forray et al. (2015), however, while measuring δ^{15}N, δ^{13}C values were obtained simultaneously. Although the differences between the two-δ^{13}C data sets are insignificant (±0.02‰), we preferred to use our results since they were obtained from coeval but different aliquots.

Rh. euryale and *M. myotis* fresh guano samples were recovered within the Bat Room near the core site in May 2013 and June 2015, respectively. Vegetation samples were collected in June 2015 from the area surrounding the cave where bats are expected to feed. *Timarcha*