Past hydroclimate and vegetation variation in Romania inferred from isotopic geochemistry and pollen of cave bat guano

Daniel Martin Cleary
University of South Florida, dcleary1312@gmail.com

Follow this and additional works at: https://scholarcommons.usf.edu/etd

Part of the Climate Commons

Scholar Commons Citation

This Dissertation is brought to you for free and open access by the Graduate School at Scholar Commons. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Scholar Commons. For more information, please contact scholarcommons@usf.edu.
Past hydroclimate and vegetation variation in Romania inferred from isotopic geochemistry and pollen of cave bat guano.

by

Daniel Martin Cleary

A dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy with a concentration in Paleoclimatology
School of Geosciences
College of Arts and Sciences
University of South Florida

Major Professor: Bogdan P. Onac, Ph.D.
Jonathan G. Wynn, Ph.D.
Ioan Tanțău, Ph.D.
Phillip van Beynen, Ph.D.
H. Len Vacher, Ph.D.

Date of Approval:
May 28, 2019

Keywords: nitrogen and carbon stable isotopes, radiocarbon, guano, climate, Romania,

Copyright © 2019, Daniel Martin Cleary
DEDICATION

This dissertation is dedicated to my family that has supported and encouraged me throughout my graduate studies. I also dedicate this work to my uncle Tim Cleary who has always been there for me. Lastly to Bogdan P. Onac, whose guidance I will forever be thankful for.
ACKNOWLEDGMENTS

This research was supported through Romanian CNCS grant PN-II-ID-PCE 2011-3-0588 to Bogdan P. Onac. I thank Dr. Tudor Tâmaş and Alexandra Giurgiu who helped during coring and initial sampling activities associated with the Măgurici core. I acknowledge the administration of Porțile de Fier Natural Park, Mehedinţi Plateau Geopark, and the Romanian Speleological Heritage Commission for granting permission to recover guano profiles from Gura Ponicovei and Topolniţa caves, respectively. The assistance of Roxana Grindean with her comments and suggestions during the development of my pollen records is greatly appreciated. Special thanks to Dr. Ioan Coroiu for identifying the bats in both Gura Ponicovei and Topolniţa caves and Drs. Oana A. Dumitru and Ioan Povară for field assistance. I am also deeply grateful to Bogdan P. Onac and Jonathan G. Wynn for their thoughtful reviews that helped improve the Pollen, δ¹⁵N and δ¹³C guano-derived record of late Holocene vegetation and climate in the southern Carpathians, Romania manuscript.
# TABLE OF CONTENTS

LIST OF TABLES ............................................................................................................ iii

LIST OF FIGURES ......................................................................................................... iv

ABSTRACT ....................................................................................................................... v

INTRODUCTION ............................................................................................................... vii

CHAPTER 1: Evidence of long-term NAO influence on East-Central Europe winter precipitation from a guano-derived δ¹⁵N record ................................................................. 1

CHAPTER 2: A guano-derived δ¹³C and δ¹⁵N record of climate since the Medieval Warm Period in north-west Romania ......................................................................................... 2

CHAPTER 3: Pollen, δ¹⁵N and δ¹³C guano-derived record of late Holocene vegetation and climate in the southern Carpathians, Romania ........................................................................ 3

AUTHORS ....................................................................................................................... 3

ABSTRACT ....................................................................................................................... 3

INTRODUCTION ............................................................................................................... 4

STUDY AREA ................................................................................................................... 6

METHODS ....................................................................................................................... 7

Radiocarbon ................................................................................................................... 7

Isotopic analysis ............................................................................................................. 7

Pollen Preparation ....................................................................................................... 8

RESULTS .......................................................................................................................... 9

Radiocarbon ................................................................................................................... 9

Carbon and nitrogen isotopes: Gura Ponicovei ......................................................... 9

Carbon and nitrogen isotopes: Topolnița .................................................................. 10

Palynological and microcharcoal analysis Gura Ponicovei and Topolnița ............. 10

Elemental Data ............................................................................................................. 13

DISCUSSION ................................................................................................................... 13

Climatic information inferred from δ¹³C and δ¹⁵N .................................................... 14

δ¹⁵N winter precipitation reconstruction ..................................................................... 16

δ¹³C summer precipitation reconstruction .................................................................. 21

Vegetation dynamics and the link with climate conditions and human impact ....... 24

CONCLUSIONS ............................................................................................................. 28

ACKNOWLEDGEMENTS ............................................................................................... 29

LITERATURE CITED .................................................................................................... 29

FIGURES ......................................................................................................................... 42
TABLES .............................................................................................................................................48

APPENDIX A: Evidence of long-term NAO influence on East-Central Europe winter precipitation from a guano-derived $\delta^{15}$N record (Scientific Reports) ........................................................49

APPENDIX B: Copyright permission from Nature for use of this article in the dissertation ..................................................................................................................................................58

APPENDIX C: A guano-derived $\delta^{13}$C and $\delta^{15}$N record of climate since the Medieval Warm Period in north-west Romania (Journal of Quaternary Science) ................................................59

APPENDIX D: Copyright permission from John Wiley and Sons for use of this article in dissertation.............................................................................................................................................72
**LIST OF TABLES**

Table 1: Pollen, δ¹⁵N and δ¹³C guano-derived record of late Holocene vegetation and climate in the southern Carpathians, Romania.................................................................49
LIST OF FIGURES

Figure 1: Map of Romania with circles indicating locations referenced in text: Gura Ponicovei Cave (1; this study), Domogled National Park (2; Levanič et al., 2012), Topolnița Cave (3; this study), Drobeta-Turnu Severin (4; meteorological station), Zidită Cave (5; Forray et al., 2015), Măgurici Cave (6; Cleary et al., 2017, 2018), Tâul Muced Lake (7; Feurdean et al., 2015a), Bucharest (8) ..........................................................................................................................................................43

Figure 2: Age-depth models for Gura Ponicovei and Topolnița guano cores ........................................43

Figure 3: δ15N (red), δ13C (black), and Suess corrected δ13C (maroon) values of Gura Ponicovei guano; B) δ15N (blue), δ13C (black), and Suess corrected δ13C (purple) values of Topolnița guano ..................................................................................................................................................44

Figure 4: Pollen diagram from Gura Ponicovei Cave guano ..................................................................44

Figure 5: Pollen diagram from Topolnița Cave guano. .........................................................................45

Figure 6: A) %C (red) and %N (black) values from Gura Ponicovei core; B) %C (red) and %N (black) values from Topolnița core ..................................................................................................................................................45

Figure 7: A) Correlation analysis of δ15N and δ13C of Gura Ponicovei; B) Correlation analysis of Topolnița δ15N and Drobeta-Turnu Severin autumn/winter precipitation; C) Drobeta-Turnu Severin autumn/winter precipitation (black), Topolnița δ15N values (dotted blue) and with 3-year lag (solid blue) .........................................................46

Figure 8: A) Comparison of δ15N values from Gura Ponicovei guano, B) δ15N values from Măgurici guano, C) δ15N values from Topolnița guano; D) 3-year running mean of reconstructed winter precipitation in Europe, E) autumn/winter precipitation Cloșani Cave, F) NAO winter index. Vertical bars indicate similar dry/wet phases ...............................................................................................................................................47

Figure 9: A) δ13C values from Gura Ponicovei guano, B) δ13C from Topolnița guano, C) tree ring record of SPI from Domogled National Park D) Tree ring spring/summer precipitation from southern Czech Republic, E) 3-year running mean of European summer precipitation, and F) 3-year running mean of reconstructed European temperature anomaly. Vertical bars indicate similar dry/wet phases ...............................................................................................................................................48
ABSTRACT

While an abundance of paleo-records related to hydroclimate and vegetation exist in East-Central Europe, currently there is a scarcity of reconstructions that have the resolution to effectively capture the past 2000 years. A more complete understanding of this interval is important as it includes significant climatic events such as the Medieval Warm Period, Little Ice Age, and the post-industrial revolution human induced climate change. A solution to increasing our understanding of these events is the use of cave bat guano, a relatively underutilized source of climatic information.

Cave bat guano piles commonly have near annual deposition and in Europe can often be found to accumulate for over 1000 years. Additionally, as bats are primarily ingesting herbivorous insects, geochemical and palynological records sourced from guano can be linked to the local environment that lies within proximity to the cave. Variation of nitrogen and carbon stable isotopes can potentially occur in response to fluctuations in certain climatic parameters, such as temperature and water availability. These signals are then transferred from plant foliage to insect to bat and ultimately preserved in guano. Multiple factors also allow for the deposition of pollen grains in guano piles. Insectivorous bats can ingest pollen grains attached to insects, wind dispersed pollen can latch on to foraging bat’s fur later dislodging onto the guano pile, or cave ventilation. Therefore, through isotopic and palynological analysis of bat guano high-resolution late-Holocene records of climate and vegetation can be produced.

Guano cores were obtained from three caves along a north to southwest transect in Romania for analysis of carbon and nitrogen isotopes and pollen. In the Măgurici record
(northwestern Romania) a 2‰ increase in $\delta^{13}$C, 1.5‰ decrease in $\delta^{15}$N, and the presence of thermophilous species between AD 881 and 1240 indicate a warm and dry Medieval Warm Period occurred in the northwestern region of the country. This event is followed by the Little Ice Age (~AD 1240 – 1850), wherein a decrease from 13.9 to 8.5‰ in $\delta^{15}$N values and a 2.3‰ increase in $\delta^{13}$C values of Topolniţa guano (southern Romania) suggest a wetter summer and winter climate.

It was also demonstrated that the North Atlantic Oscillation (NOA) is the primary control of winter precipitation in at each study location. In AD 1970 a 4‰ decrease in $\delta^{15}$N values was found to correspond with a notable transition to a positive phase of the NOA (drier conditions). Additional positive phases of the NAO were found to be recorded in the Măgurici $\delta^{15}$N series between AD 1820 and AD 1855. In contrast, a 2‰ rise in $\delta^{15}$N values from AD 1940 until AD 1970 occurs contemporaneously with an increased frequency of negative NAO phases (wetter conditions).

Pollen extracted from aliquots of guano were investigated to reconstruct past environments within proximity of the caves. In doing so it was possible to discern whether fluctuations in primary forest taxa occurred in response to climatic and/or human impact. It was found that the development of patchy forests consisting of *Tilia*, *Quercus*, and *Carpinus betulus* following the Little Ice Age around Topolniţa and Gura Ponicovei caves is likely associated with the lower water availability at this time. Additionally, the significant contribution of ruderal pollen indicators since AD 1985 is suggesting an increase of agricultural practices around Topolniţa Cave.
INTRODUCTION

For this dissertation, three bat guano cores were recovered from caves in northwestern and southwestern Romania with the intention of reconstructing and better understanding the climatic and vegetal history since the Medieval Warm Period. Study sites were chosen in an effort to determine if there is a clear distinction between the western/northern of Romania and the southwest in terms of climatic influences. Fluctuations in temperature and precipitation in southwest Romania are often controlled by the Mediterranean thermal regime (mild winters), whereas the northern and western regions experience a more temperate-continental climate heavily impacted by the North Atlantic circulation systems.

One of the primary controls of winter precipitation in Europe is the North Atlantic Oscillation, a system for which very few paleo-records exist. **Paper 1** represents a nitrogen isotope analysis completed on a guano core obtained from Măgurici Cave located in northwestern Romania. In this study it is demonstrated that variation in $\delta^{15}$N values from guano can be related to the relative openness of the local nitrogen pool. It was found that the associated nitrogen cycle was primarily controlled by winter precipitation amount. This is explained by the impact of winter hydroclimate on leaching processes that control the amount of plant-available nitrogen produced via mineralization prior to the growing season. Positive phases of the North Atlantic Oscillation (drier conditions) corresponds with lower of $\delta^{15}$N values, whereas negative phases (wetter conditions) occur contemporaneously with increasing of $\delta^{15}$N. This is most recognizable during the 1970s (Fig. 1) where a shift from negative to positive phase occurred.
As winter precipitation in East-Central Europe is influenced by the North Atlantic Oscillation, past phases of this climatic system were then reconstructed since AD 1650 using the $\delta^{15}$N values of guano. These findings were important in further establishing the utility of $\delta^{15}$N values as a paleoclimatic proxy.

Building off the work presented in Paper 1, Paper 2 details the remainder of the 285 cm core from Măgurici Cave that was analyzed for $\delta^{13}$C, $\delta^{15}$N, and pollen. As guano deposition occurred from AD 881 until 1240 and from AD 1651 to 2013, interpretation of isotopic and palynological data were used to characterize the climate of the Medieval Warm Period and Little Ice Age (Fig. 2). This effort is important as the timing, duration, and amplitude of these events have been found to vary substantially across the European continent. Additionally, climatic and
environmental records of the Medieval Warm Period and Little Ice Age are particularly scarce in East-Central Europe, and especially within Romania.

Figure 2: The $\delta^{13}$C (blue) and $\delta^{15}$N (inverted axis; red) values from AD 881 to 2012 for Măgurici Cave guano. $\delta^{15}$N data are from Paper 1.

Unlike the $\delta^{15}$N-record of winter precipitation developed in Paper 1, this study showed that the variation in $\delta^{13}$C values are related to the effect of summer water availability on plant water use efficiency. This allowed for the production of a near annual reconstruction of summer hydroclimate. Key findings included the determination of a drier Medieval Warm period and progressively wetter conditions following the termination of the Little Ice Age.

Lastly, $\delta^{13}$C, $\delta^{15}$N, and pollen from two cores recovered from Topolniţa and Gura Ponicovei caves located in SW Romania were utilized in Paper 3 to interpret past climatic and environmental changes. With the location of the caves in the southwestern region of Romania, there was potential for a Mediterranean climate influence. Guano deposition began ~AD 1694 at Topolniţa and AD 1537 at Gura Ponicovei. Similar to Paper 2, $\delta^{13}$C values are interpreted to correspond with summer precipitation, whereas $\delta^{15}$N values are inferred, like in Paper 1, to record past phases of the North Atlantic Oscillation through its control on winter precipitation.

 ix
Differing from the previous two studies, this work placed more emphasis on the palynological records. The inclusion of pollen allowed to understand whether the forest dynamics were influenced by climate. Long term trends in the reconstructed seasonal hydroclimate were found to have some influence on variations in primary forest taxa identified in the pollen records. However, particularly with the Topolniţa record, human impact appears to had a significant effect on vegetation surrounding the cave.
CHAPTER 1: EVIDENCE OF LONG-TERM NAO INFLUENCE ON EAST-CENTRAL EUROPE WINTER PRECIPITATION FROM A GUANO-DERIVED $\delta^{15}$N RECORD

Note to Reader:

This chapter has been previously published: Cleary, D.M., Wynn, J.G., Ionita, M., Forray, F.L., Onac, B.P. - Evidence of long-term NAO influence on East-Central Europe winter precipitation from a guano-derived $\delta^{15}$N record. Scientific Reports, 7: 14095. See Appendix A for the PDF of the published document and Appendix B for permission from the publisher.
CHAPTER 2: A GUANO-DERIVED δ¹³C AND δ¹⁵N RECORD OF CLIMATE SINCE THE MEDIEVAL WARM PERIOD IN NORTH-WEST ROMANIA

Note to Reader:
CHAPTER 3: POLLEN, $\delta^{15}$N AND $\delta^{13}$C GUANO-DERIVED RECORD OF LATE HOLOCENE VEGETATION AND CLIMATE IN THE SOUTHERN CARPATHIANS, ROMANIA

Authors
Daniel M. Cleary, Angelica Feurdean, Ioan Tanțău, Ferenc L. Forray

Abstract
Two cores of bat guano were recovered from Topolnița and Gura Ponicovei Caves in the south-west region of Romania and analyzed for $\delta^{15}$N, $\delta^{13}$C and pollen. Deposition of guano began in AD 1537 at Gura Ponicovei and AD 1694 at Topolnița, allowing for the development and interpretation of climate and vegetation records since the Little Ice Age. $\delta^{15}$N values were found to record phases of the North Atlantic Oscillation through the effect of winter precipitation on the local nitrogen cycle. Alternatively, $\delta^{13}$C values are interpreted to correspond with variation in summer precipitation. Both records suggest progressively drier conditions during the termination of the LIA in the region at AD 1870. The transition to landscape openness, with patchy forests of *Tilia, Carpinus betulus,* and *Quercus* during this time could be related to the lower water availability at both sites. Wetter conditions inferred from a 3‰ increase in $\delta^{15}$N values between AD 1879 and AD 1970 occurred contemporaneously with a similar trend in European winter precipitation and increased frequency of positive NAO phases during this interval. The $\delta^{13}$C values between AD 1885 and AD 1968 suggest a trend towards a wetter summer climate as well.
Although there is at times evidence of forest dynamics being controlled by climate, vegetation appears to be more closely associated with human impact, particularly around Topolnița Cave. With contributions of ruderal pollen indicators from AD 1985 – present is likely related to agricultural practices that were absent around Gura Ponicovei.

**Introduction**

Recently, cave bat guano records have become more frequently utilized in reconstructing climate. Carbon and nitrogen isotopes records in guano have been served as a proxy for C$_3$ vs C$_4$ plant types (Mizutani et al., 1992a, b; Bird et al., 2007; Wurster et al., 2007; Choa et al., 2016), seasonal precipitation (Royer et al., 2015; Forray et al., 2015; Onac et al., 2015; Cleary et al., 2016, 2018), migration of the Inter-Tropical Convergence Zone (Royer et al., 2017), and phases of the North Atlantic Oscillation (Cleary et al., 2017). These records most commonly offer a view into the variability of climate over the past 2000 years. Bat guano is particularly useful when considering this interval of time as other resources of climate information (stalagmites, peat cores, etc.) lack the near annual resolution often expressed in large guano deposits. However, while guano-derived δ$^{15}$N and δ$^{13}$C provide an extensive amount of climatic information, pollen records (Carrión et al., 2006; Batina and Reese, 2011; Geantă et al., 2012; Forray et al., 2015) have the added benefit of describing the associated environment. Pollen enters guano piles in three possible ways; 1) through initial ingestion by bats and later defecation; 2) via deposition through cave air circulation if the cave passages permit (Leroy and Simms 2006); and 3) attached to the bat’s fur, from where grains can ultimately fall off onto the guano (Maher, 2006). Therefore, pollen grains preserved in guano deposits connect bats to the vegetation growing in the cave proximity (Navarro et al., 2001). Although these mechanisms may result in lower pollen concentrations in guano deposits by comparison to lake and peat
settings, guano offers the opportunity to record plant species that rely on insects more than wind for pollination. Particularly in Romania, palynological studies have demonstrated that bat guano can successfully provide insight into past environments (Geantă et al., 2012; Forray et al., 2015; Cleary et al., 2018). However, only a few studies overall have attempted to use both guano core pollen and $\delta^{13}C/\delta^{15}N$ records in guano to place the isotope derived climatic information within the context of the reconstructed vegetation composition (Forray et al., 2015; Cleary et al., 2018).

A vast majority of paleoclimate and paleoenvironmental records of Romania have been developed in the western Carpathian Mountains (Feurdean and Willis, 2008; Feurdean et al., 2009; Cleary et al., 2016), northern Carpathians (Popa and Kern 2009; Tanţău et al., 2011, 2014a; Feurdean et al., 2016; Cleary et al., 2018) and the eastern Carpathians (Tanţău et al., 2003, 2009, 2014b). A particularly important gap to fill in terms of paleoenvironmental records lies within the southwestern region of Romania. This area has a unique setting due to the potential influence of Mediterranean climate and the associated occurrence of many submediterranean plants (Cristea, 1993; Doniţă et al., 2005). The pollen records that do exist for this area of the country often come from high elevation peat and lakes that lack the resolution to properly consider the past 2000 years (Fărcaş et al., 1999; Rösch and Fischer, 2000; Magyari et al., 2018). Additionally, although climate records exist (Constantin et al., 2007; Drăguşin et al., 2014), very few provide consideration of climate variability in the region since the Medieval Warm Period (Onac et al., 2014; Warken et al., 2018).

In this study, two guano cores obtained from Gura Ponicovei and Topolniţa caves (Fig. 1) were analyzed for $\delta^{15}N$ and $\delta^{13}C$ and pollen with the aim to develop a paleoclimatic and paleoenvironmental reconstruction since the Medieval Warm Period. Through this approach we determine past vegetational dynamics and independently assess the role of climate on these
dynamics. Comparison with instrumental records nearby Gura Ponicovei and Topolnița caves are used to explore any existing relationship between the isotopic values and modern climate and therefore constrain the interpretation of paleoclimate signal extracted from the two cores.

**Study Area**

Gura Ponicovei Cave (Fig. 1.1) is located in the southwestern region of the Southern Carpathians, within the Poțile de Fier (Iron Gate) National Park. This 1,666 m long cave system (Goran, 1982) lies within the Danube Gorge just north of the Danube River at an elevation of 60 m above sea level (a.s.l.). Located within the upper part of the cave, approximately 267 m from the upper entrance is a small (55 cm) accumulation of guano that was deposited by bats on the floor of the Big Room.

An estimated 5 bat colonies of 1,000 individuals of *Rhinolophus euryale* are present within the Big Room. In addition to *Rh. euryale*, Murariu et al. (2004) described populations of *Miniopterus schreibersii*, *Myotis myotis*, *M. capaccinii* within the cave. All species are insectivorous with the exception of *M. capaccinii* that eats small fish (Levin et al., 2006) from the superficial layer of lakes. The foraging range of these bats is a function of the number of bats in the cave, however they can forage up to 32 km from the cave entrance (Egert-Berg et al., 2018). Gura Ponicovei is surrounded by dense forests containing *Carpinus betulus*, *C. orientalis*, *Tilia*, and *Quercus*. Present herbaceous taxa include Poaceae, Chenopodiaceae, Scrophulariaceae, *Plantago lanceolata*, *Filipendula*, and Asteroideae. Aquatic species, such as *Typha* are also common at lower elevations near the Danube.

Topolnița Cave (Fig. 1.3) is situated within the same southern section of the Carpathians, but on the Mehedinți Plateau. Relative to Gura Ponicovei, this cave system is positioned at a slightly higher elevation (~400 m a.s.l.), and has a total length of 22 km (Goran and Povară, 2019). The
guano heap is located in the Bat’s Gallery, approximately 100-150 m from the nearest entrance. The colony above the guano deposit is populated primarily by *Rh. euryale, Myotis daubentonii,* and *M. capaccinii,* all primarily insectivorous species (Coroiu, 2019). Due to the fact that both caves are located in the south-western region of the Southern Carpathians, the surroundings can potentially be influenced by Mediterranean climate. The seasonal climate includes temperatures averaging 20-22 °C during the hotter months and a mean of 1-3 °C during the winter (Murariu et al., 2004). Records from Târgu Jiu (203 m a.s.l.) and Drobeta-Turnu Severin (77 m a.s.l.) meteorological stations indicate that precipitation is around ~120-140 mm during the summer (Warken et al., 2018). In contrast, during the winter months this value is much lower (~ <40 mm).

**Methods**

*Radiocarbon*

An accelerator mass spectrometer at the Poznan Radiocarbon Laboratory (Poland) was used to measure the $^{14}$C age of bulk guano (Topolniţa 7 samples and Gura Ponicovei 5 samples). The calibrated years are expressed in years AD and the associated errors are 1 year for modern samples and 25-30 years for pre-modern samples.

*Isotopic analysis*

The guano deposits were sampled by a manually operated Russian corer (e.g. Forray et al., 2015). The cores were subsequently sampled at a 2-cm resolution for Gura Ponicovei and a 1-cm resolution for Topolniţa. The reason for a lower sampling resolution in Gura Ponicovei was the lack of visible changes in the sedimentology of the core. In contrast, the guano in the Topolniţa core was rather well laminated, thus sampled at higher resolution. Guano samples were first homogenized and treated with HCl to remove inorganic carbon (e.g. Onac et al., 2014).
Subsequently, 1–2 mg were weighed from each aliquot and then measured for $\delta^{13}$C, $\delta^{15}$N, %C and %N using a Costech ECS4010 Elemental Analyzer coupled with a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific) at the University of South Florida Stable Isotope Laboratory. A protein standard B2155 ($\delta^{13}$C: -27.09 ‰, $\delta^{15}$N: 5.94 ‰, %C: 46.5, %N: 13.3) and a glutamic acid (internal standard: $\delta^{13}$C: -16.49 ‰, $\delta^{15}$N: -6.26 ‰, %C: 41.37, %N: 9.54) were used during analysis. B2155 and IAEA-C7 were used to calibrate the $\delta^{13}$C value of the glutamic acid. Estimation of the precision of analysis ($\delta^{15}$N: 0.08 ‰; $\delta^{13}$C: 0.04 ‰) is based on replicate internal standards used during each analysis. Suess correction was applied for all $\delta^{13}$C data after AD 1850 (Forray et al., 2015) to correct carbon isotope “contamination” due to fossil fuel burning.

Pollen preparation

All 23 samples of the Gura Ponicovei core were analyzed for pollen at 2-cm intervals. Analysis of the Topolnița core was completed at 4-cm intervals between 0 and 56 cm and at 2-cm intervals between 56 and 94 cm, as the accumulation rate differs at the top and bottom of the core. Due to the samples being entirely organic material, a simplified procedure was used to isolate pollen grains. One cm$^3$ of each sample was first treated with HCl to remove carbonates and dissolve the Lycopodium, and then NaOH (10%) to remove humic acids and organic matter. Samples were sieved through a 250 µm mesh to remove coarse organic material, and preserved in glycerin. Pollen was counted under microscope (40 X) until a sum of ca. 200 grains was reached. The frequencies of pollen for each taxon were calculated as percentages of the total sum of arboreal pollen (AP) and non-arboreal pollen (NAP). The nomenclature for vascular plants follows Flora Europaea (Tutin et al., 1964–1980). Microscopic charcoal particles were counted
along pollen to reconstruct the fire history. The results are presented as a percentage pollen
diagram reconstructed in the Tilia software version 2.0.41 (Grimm, 1991).

**Results**

**Radiocarbon**

All radiocarbon ages used for radiocarbon dating are in stratigraphic order for both Topolnița and
Gura Ponicovei (Table 1) indicating no contamination of bulk guano samples.

Modern samples found to have high radiocarbon activity were calibrated using CaliBomb
(Reimer and Reimer, 2009) with calibration dataset IntCal13 (Reimer et al., 2013) and North
Hemisphere Zone 1 (NHZI) bomb curve extension (Hua, 2013). The remainder of the $^{14}$C ages
were calibrated with the Clam code using IntCal13 (Reimer et al., 2013). Ages were interpolated
between dated samples every 1 cm. The resulting age-depth models were produced using a linear
interpolation between all $^{14}$C ages using Clam 2.2 code (Blaauw, 2010) running in R software (R
Development Core Team, 2013).

The age depth model indicates that Gura Ponicovei guano sequence began to accumulate at AD
1537, whereas at Topolnița first deposition occurred beginning with AD 1694. At both sites the
guano accumulations were continuous up to present day with no hiatus.

**Carbon and nitrogen isotopes: Gura Ponicovei**

Low %N and %C prevented isotopic analysis of the lower 5 samples (46-55 cm; AD 1228-1490)
of the Gura Ponicovei core. Therefore, the $\delta^{13}$C and $\delta^{15}$N record begins at AD 1537. The $\delta^{13}$C
values remain fairly stable between AD 1850 and 1925, varying between -25.7 and -26.3‰ (Fig.
3A). In contrast, $\delta^{15}$N values express a decreasing trend beginning at AD 1537 (7.4‰) reaching a
low point at AD 1810 (6.5‰). Both $\delta^{13}$C and $\delta^{15}$N briefly increase at AD 1810 before a
substantial decrease beginning at AD 1989. A greater concentration of data occurs between AD
1989 and AD 1997 with large apparent variation within this 10-year interval. Starting at AD 1989 ($\delta^{15}$N: 6.9 ‰, $\delta^{13}$C: -26.3 ‰) both $\delta^{15}$N and $\delta^{13}$C time-series trend towards lower values ($\delta^{15}$N: 4.9 ‰, $\delta^{13}$C: -27.3 ‰).

**Carbon and nitrogen isotopes: Topolnița**

Between AD 1850 and 1786, $\delta^{13}$C values decrease by 2.8‰, a trend that terminates at the lowest value in the series (-28.2‰; Fig. 3B). The interval, AD 1786-1794 is characterized by a rapid 2.3‰ shift to higher values. Instead, $\delta^{15}$N values become progressively lower until AD 1876, decreasing from 13.9 to 8.5‰. At AD 1876 there is a transition towards higher values in both the carbon and nitrogen isotopic composition of guano (Fig. 3B). The $\delta^{15}$N values continue to increase for the remainder of the series reaching 15.5‰ at AD 2014. There is a brief departure from this trend at AD 2008 wherein $\delta^{15}$N decreases to 5.9‰. The $\delta^{13}$C of guano increases to -23.5‰ at AD 1985, after which decreases until AD 2008 by 1.4‰.

**Palynological and microcharcoal analysis Gura Ponicovei and Topolnița**

Pollen counts were too low (<150) in the lower 2 samples of the Gura Ponicovei record to be considered and are therefore removed from analysis. Based on cluster analysis of the pollen records, Local Pollen Assemblage Zones (LPAZ) for Gura Ponicovei (5) and Topolnița (5) were produced. Transitions between zones are indicative of significant changes in the pollen assemblages and therefore vegetation dynamics.

**Gura Ponicovei**

*AD 1395 – 1490 (LPAZ 1)*

The pollen record at the oldest interval of the Gura Ponicovei sequence indicates an environment characterized by open forest dominated by *Quercus*, *Tilia*, *Carpinus betulus*, and *Fagus sylvatica* (Fig. 4). Herbaceous species occurred in low percentages relative to arboreal pollen for most of
this interval. Notable constituents of the herbaceous assemblage include Poaceae, Geraniaceae, Verbascum, Caryophyllaceae, and Scrophulariaceae. Additionally, there were small frequencies of the aquatic species Potamogeton.

*AD 1490 – 1810 (LPAZ 2)*

This zone is characterized by a gradual decline in *Fagus sylvatica* to values below 10%.

*Quercus, Tilia, Carpinus betulus* continued to dominate forests in the area, however, *Corylus avellana* and *Fraxinus excelsior* also had a notable contribution. There was increased diversity and abundance of herbaceous taxa during this interval. Apiaceae, Chenopodiaceae, Poaceae, *Scrophulariaceae, Asteraceae* and *Artemisia* displayed the highest percentages.

*AD 1810 – AD 1990 (LPAZ 3)*

The pollen spectra of this zone demonstrated a sharp reduction in the tree pollen percentages. *Carpinus betulus* and *Tilia* remain the primary forest constituents while the percentages of *Quercus* and *Fagus sylvatica* declined. The abundance of herbaceous taxa increased markedly particularly for Chenopodiaceae, Scrophulariacea, *Verbascum, Artemisia*, Poaceae, and Asteraceae. *Typha* percentages increased in the late 1970’s.

*AD 1990 – 1996 (LPAZ 4)*

During this interval the frequency of arboreal pollen increased and consisted of *Carpinus betulus, C. orientalis, Quercus, Tilia, Salix, Fraxinus excelsior*, and *Fraxinus ornus*. Within herbaceous taxa, percentages of Poaceae pollen increased, contrasting a declined abundance of Scrophulariaceae and *Verbascum*. Significant expansion of Ranunculaceae at AD 1992 along with the presence of *Plantago lanceolata, Artemisia, Asteraceae, and Chenopodiaceae* could indicate increased land disturbance.

*AD 1996 – Present (LPAZ 5)*
Compared to the previous zone, there was a reduction of *Quercus, Corylus avellana,* and *Fraxinus excelsior,* and increased frequencies for *Tilia, Salix,* and *Ulmus.* The herbaceous assemblage was characterized by decreased herb diversity particularly taxa associated with land use, *Polygonaceae, Rubiaceae, Plantago lanceolata, Artemisia,* and *Asteroideae.*

**Topolnița**

*AD 1643 – AD 1790 (LPAZ 1)*

The beginning of the Topolnița sequence was characterized by forests composed of *Quercus, Fagus sylvatica, Betula, Tilia,* and with smaller occurrences of *Carpinus betulus* (Fig. 5). There was a high diversity and abundance of herbaceous taxa during this interval, primarily, *Poaceae, Asteraceae, Urticaceae, Plantago lanceolata, Artemisia, Cichorioideae, Centaureae,* and *Rubiaceae,* which suggests land disturbance and development of pastures/meadows in the area.

*AD 1790 – AD 1957 (LPAZ 2)*

Herbaceous pollen types remained high relative to arboreal pollen throughout most of the 1800’s. A few instances of forest expansion notable for *Fagus sylvatica* and *Corylus avellana,* occurred at AD 1794, AD 1830, and AD 1922. A similar configuration of herbaceous taxa to Gura Ponicovei LPAZ 3 existed with higher percentages of *Asteraceae, Artemisia, Poaceae, Ranunculaceae,* and *Cannabis-type.* This and the smaller contributions of *Euphorbia,* *Polygonaceae, Rosaceae,* and *Fabaceae* suggest that plant diversity remained high around Topolnița Cave.

*AD 1957 – AD 1984 (LPAZ 3)*

This zone is marked by an expansion of *Fagus sylvatica* (30%) with additional increased percentages of *Salix* and *Corylus avellana.* The pollen percentages of *Carpinus betulus, Quercus,* and *Fraxinus excelsior* remained below 10%. There was a corresponding notable reduction in
herbaceous taxa in this zone, particularly for Poaceae and *Artemisia*, whilst others i.e.,
Brassicaceae, Chenopodiaceae, *Campanulaceae*, and *Verbascum* increased at this time.

**AD 1984 – AD 1991 (LPAZ 4)**

This zone is characterized by the decline of *Fagus sylvatica* at the expense of *Carpinus betulus*,
*Quercus*, *Tilia*, and *Betula*. Primary constituents of the herbaceous taxa are Poaceae,
Chenopodiaceae, Ranunculaceae, Scrophulariaceae, and *Verbascum*, however, the diversity of
herbaceous pollen is much lower than the previous zones.

**AD 1991 – present (LPAZ 5)**

The Topolnița region appears to be impacted by human activity since AD 1991 with increased
contributions from *Artemisia*, Urticaceae, Rosaceae, *Plantago lanceolata*, *Ambrosia*, and
*Cerealia*.

**Elemental Data**

The %C and %N values for the Gura Ponicovei core, notably mirror each other. Fresh surface
samples had values of 6.4 (%N) and 25.6 (%C) (Fig. 6A). Both values are lower than fresh
samples from other guano studies at Mâgurici Cave (%N: 11.3; %C: 36.4) and Zidită Cave (%N:
10.7; %C: 40.5 %), but are relatively stable around each respective surface value for the upper 13
cm of the core. Between 15 and 25 cm there is a distinct decreasing trend in both %N and %C.
At 25 cm %N reaches a minimum value of 3.4 %, whereas %C approaches 14.9%. After this
point both series appear to fluctuate around this point.

For the Topolniţa core, the lowest values of %C and %N are found in the upper 7 cm (%N: 6.7;
%C: 23.2; Fig. 6B). Both values for the remainder of the core remain relatively stable between 7
and 75 cm with average values of 10.65 % (%N) and 35.3 % (%C) over this interval.

**Discussion**
Climatic information inferred from $\delta^{13}C$ and $\delta^{15}N$

As all $\delta^{13}C$ values are between -23 and -28‰, variability within the time-series can be associated with processes that impact the fractionation associated with the C$_3$ pathway. When a climatic parameter is influencing the $\delta^{13}C$ values in C$_3$ plants, this signal can potentially be transferred from plant to insect to bat, and ultimately preserved in guano (Forray et al., 2015). While there is a potential ~1 ‰ enrichment that can occur between ingestion of plant and incorporation by the insect into chitin (Potapov et al., 2014), most mammals will have a whole body $\delta^{13}C$ value similar to that of their diets (DeNiro and Epstein, 1978). There is a small fractionation that occurs between transfer of bat whole-body and deposition of guano (DeNiro and Epstein, 1978), however the fractionation factor is relatively small with a constant offset to diet. Therefore, if demonstrated that a climate signal is recorded in the $\delta^{13}C$ values of Gura Ponicovei and Topolniţa bulk guano, a paleoclimate record based on the carbon isotopic composition can be produced.

A majority of $\delta^{13}C$ records have attributed carbon isotopic discrimination within the C$_3$ pathway to the variation within plant water-use efficiency (WUE) (Farquhar et al., 1982; Bodin et al., 2013). When there is a scarcity of water or aridity is high, vascular plants with the capability to reduce stomatal conductance are afforded a competitive advantage. Their ability to resist water loss results in less $^{13}C$-discrimination and $\delta^{13}C$-plant biomass more enriched in $^{13}C$ (higher $\delta^{13}C$ values). Alternatively, when water availability is not limiting, no competitive advantage occurs. The higher stomatal conductance and lower WUE under these conditions results in more $^{13}C$-discrimination and therein lower $\delta^{13}C$-plant biomass values.

However, $\delta^{13}C$ values are not a simple function of mean annual precipitation as factors such as annual water balance, evapotranspiration, seasonality, drainage conditions, etc. can also impact
WUE. In addition, carbon isotope discrimination within C₃ plants cannot always be associated with WUE (Tang et al., 2014). Variation within this photosynthetic pathway can also be attributed to changes in the concentration of atmospheric CO₂ (Silva et al., 2013) as well as light-use and canopy recycling of CO₂ (Lambers et al., 2008). Multiple environmental factors, such as temperature, salinity, and/or irradiance can also potentially influence plant-WUE irrespective of precipitation values (Farquhar et al., 1982). With respect to temperature, the effect of warm conditions can potentially outweigh the impact of rainfall received via its influence on evaporative demand during the growing season (Seibt et al., 2008). For example, Xu et al. (2015) found mean annual temperature to have such an influence on δ¹³C values in alpine areas. Additionally, Cleary et al. (2018) found evidence of local soil temperature having more impact on water availability than precipitation received locally between AD 1938 and AD 2012. Therefore, although water availability can often be directly linked with δ¹³C values, the potential multitude of additional environmental factors can create a less straightforward interpretation. Factors controlling the δ¹⁵N variability in guano are similarly complex. There are a multitude of nitrogen (N) transformations and transfers each with its own associated enrichment ranges that can occur within the soil reservoir (Högberg, 1997). Due to the fact that the significance of these processes cannot be determined based solely on the reservoir δ¹⁵N value, paleoclimate studies have largely avoided utilizing nitrogen isotopic records (Royer et al., 2015). However recent work has begun to circumvent these issues by interpreting δ¹⁵N values as an integrator of the N-cycle (Cleary et al., 2016, 2017; Wurster et al., 2017). The N pool mixing and any N gains/losses occurring within the soil reservoir can potentially contribute to the δ¹⁵N of the system (soil/biomass). This allows for the interpretation of the δ¹⁵N value of a system as it relates to the relative openness of the N-cycle (Robinson et al., 2001). A closed N-cycle occurs when N is a
limiting nutrient and therefore more tightly conserved, producing a decrease in $\delta^{15}$N values at each step of the N-cycle (soil, plant foliage, consumers) (Robinson et al., 2001; Szpak, 2014). Alternatively, when the N-cycle is more open due to abundant or sufficient N, there is an associated increase in $\delta^{15}$N values.

Past monitoring studies have found relationships between the N-cycle and mean annual precipitation (MAP: Austin and Vitousek, 1998; Handley et al., 1999; Amundson et al., 2003). Szpak (2014) also found the degree of anthropogenic influence to also have a potential control on relative openness. This can occur through the removal of N from the system (deforestation, burning) or the addition of synthetic fertilizer or manure. Paleo-studies have been able to use $\delta^{15}$N values to reconstruct winter precipitation via the effect of leaching during winter months (Cleary et al., 2016, 2017) and anthropogenic influence through palynological (Cleary et al., 2016) and historical records (Wurster et al., 2017).

The utility $\delta^{15}$N as a paleoclimate proxy is dependent upon evidence of a lack of denitrification and ammonia volatilization within the guano pile (McFarlane et al., 1995; Bird et al., 2007; Wurster et al., 2010). These microbial processes with high enrichment factors reduce the %N in the guano, ultimately altering the initial $\delta^{15}$N values. The %N values of the Topolnița core remain high throughout the entirety of the core, indicating $\delta^{15}$N values are likely to closely match the initial values upon deposition (Fig. 6B). While the %N decreases substantially towards the lower 20 cm of the Gura Ponicovei core (Fig. 6A), these values co-vary with %C. As the microbial processes responsible for N loss in a guano pile are irrespective of those that could remove carbon, we interpret this as evidence that the drop in %N is not due to loss of N following deposition. Therein both $\delta^{15}$N time series are appropriate for interpretation.

$\delta^{15}$N winter precipitation reconstruction
Statistical analysis of $\delta^{13}$C and $\delta^{15}$N values of Gura Ponicovei core indicates a correlation between the two isotopes ($R^2=0.65$; p-value <0.001; Fig. 7). This suggests a similar control of both $\delta^{13}$C and $\delta^{15}$N within the foraging range of the bats. Therefore, it is inferred that as water availability via plant WUE is responsible for the variation in $\delta^{13}$C values, water availability also controls the relative openness of the local N-cycle. This is consistent with results of the other Romanian guano studies in the northern and central parts of the country (Cleary et al., 2016, 2017) are hereafter interpreted as a proxy for precipitation. Due to the small sample number used for $\delta^{15}$N analysis from Gura Ponicovei between AD 1925 and present, a statistical analysis of nitrogen values and instrumental winter precipitation is not feasible. However, there is sufficient $\delta^{15}$N data from the Topolnița core. The best correlation ($R^2 = 0.45$; Fig. 7B) was found between $\delta^{15}$N values and winter/autumn precipitation. Notably, the application of a 3-year lag to the $\delta^{15}$N series (Fig. 7C) was necessary for this result. This lag is believed to be viable considering the errors associated with calibrated ages. In contrast with previous studies (Cleary et al., 2016, 2017), there is a lack of statistical correlation between the $\delta^{15}$N and $\delta^{13}$C values in the Topolnița core. However, this can be explained by a lack of contribution from summer precipitation (represented by $\delta^{13}$C values) to water availability during the winter months. Therefore, $\delta^{15}$N from both Gura Ponicovei and Topolnița cores are interpreted as a proxy for water availability during the autumn/winter seasons.

The Gura Ponicovei guano record was continuously accumulating through the Little Ice Age termination (LIA; ~AD 1250-1850). This climatic interval was characterized by cool and dry conditions in most parts of Romania (Popa and Kern, 2009; Feurdean et al., 2011 a, b; Haliuc et al., 2017), although milder conditions have been recognized in some areas (Forray et al., 2015). Interestingly, past work in southwestern Romania found the LIA to begin around AD 1230 based
on the deterioration of weather during the Medieval Warm Period/LIA transition (Onac et al., 2014). However, guano accumulation in Gura Ponicovei begins at AD 1228 (Fig 2A). The trend towards more negative $\delta^{13}C$ values and therefore dry climate conditions at Gura Ponicovei (Fig. 8) supports the determination of the LIA onset around AD 1230’s. This could also reflect a deterioration of climate during the termination of the Medieval Warm Period, and not necessarily a precise initiation of the LIA at AD 1230.

Warken et al. (2018) found the driest phase of the LIA in the nearby Cloșani Cave to be between AD 1675 and AD 1715 (Fig. 8E). At Gura Ponicovei and Topolnița, $\delta^{15}N$ values appear to indicate a trend towards drier winters beginning at AD 1500 and terminating at AD 1975. This milder gradual transition out of the LIA resembles that interpreted in the Apuseni Mountains (Forray et al., 2015). The wettest event recorded in Zidită Cave occurs near contemporaneously with a similar wet event found at Topolnița (Fig. 8). There are no such pulses towards wetter phases in the Gura Ponicovei record. This could be explained by the elevation differences of the caves. Topolnița Cave is positioned higher in the mountains and the surrounding environment is more subject to climate variation. Gura Ponicovei Cave positioned at low elevation is less likely to provide information on finer scale changes in climate, instead is archiving long term variation. This could also be simply attributed to insufficient data to capture short term variations in winter precipitation.

Past work suggests that negative and positive winter precipitation anomalies in Eastern Europe can often be attributed to East Atlantic/Western Russia system and the North Atlantic Oscillation (NAO; Bojariu and Paliu, 2001; Perșoiu et al., 2017). Indeed, the NAO is one of the primary influences on climate variability in the Northern Hemisphere (Hurrell et al., 2013). Positive phases of the NAO occur in response to a stronger Azores High and deeper Icelandic low (drier
winters in southern Europe). When NAO is in its negative phase, storm tracts migrate southwards which results in increased winter precipitation in the southern and eastern parts of Europe. Previous studies acknowledge that interferences with Mediterranean circulation can potentially affect the NAO signal in hydroclimate records (Constantin et al., 2007; Roberts et al., 2012). However, Warken et al. (2018) did find connectivity between North Atlantic forcing and autumn/winter precipitation in SW Romania on centennial to millennial timescales, but a weaker relationship at annual to decadal timescales. Higher resolution records of Topolniţa and Gura Ponicovei guano could potentially better capture the signal.

Most notably the drying trend indicated by a 3.2‰ decrease in the δ¹⁵N of Topolniţa guano (Fig. 8C; AD 1824 – AD 1841) occurs near contemporaneously with a progression towards more positive phases of instrumental record of the NAO (AD 1820 - AD 1855; Fig 8F). Additionally, there is a subsequent transition to a more open N-cycle (higher δ¹⁵N values) at AD 1879 that corresponds with a trend towards wetter winter conditions in Europe (AD 1882 - AD 1970; Fig. 8D). Interestingly the initiation of this event appears to precede an interval of more frequent negative phases of the NAO (AD 1923 - AD 1970). However, the Warken et al. (2018) appears to capture this shift towards increased winter precipitation more consistent with the instrumental record of the NAO (Warken et al., 2018; Fig 8E, F). The notable strong positive phase after the mid-1970’s is well recognized with decreasing winter precipitation in Romania (Tomozeiu et al., 2005; Cleary et al., 2017). Consistent with these findings, the 1.8 ‰ decrease in δ¹⁵N values (Topolniţa) beginning at AD 1973 also suggests a drier climate.

Unlike the previous interval, AD 1980 - AD 2014 is characterized by conflicting trends in terms of the associated precipitation. A higher frequency of negative phases of the NAO occurred during this interval, however δ¹⁵N values from Gura Ponicovei suggest a pronounced decrease in
winter precipitation (Fig. 8A). This is likely due to the fact that southern Romania is particularly vulnerable to drought relative to other areas of the country (Barbu and Popa, 2004). The most prolonged hydrological drought in this region was recorded between AD 1980 and 1995 (Stefan et al., 2004). In agreement, a closed N-cycle and prolonged dry phase is inferred by decreasing trend in $\delta^{15}$N values of Gura Ponicovei guano (Fig. 8A). The 1.7‰ decrease that occurs in the Gura Ponicovei record at AD 1991 overlaps with a similar decrease (3.1‰) inferred from Topolnița guano. Similar to the Măgurici record of the NAO (Cleary et al., 2017), $\delta^{15}$N values remain low at Gura Ponicovei after this event (Fig. 8A, B). This interval of ~AD 1970-present is characterized by more a pronounced positive phase of the NAO, agreeing with the sustained dry conditions indicated by the Gura Ponicovei and Măgurici records. Alternatively, increasing Topolnița $\delta^{15}$N values suggest progressively wetter conditions between AD 1991 and 2014 (Fig. 8C). This appears unrelated to the increased anthropogenic influence in proximity around Topolnița in the 1900’s (see below). Expansion of human indicator plants suggest deforestation and landscape openness that can result in a more closed N-cycle (lower $\delta^{15}$N values; Cleary et al., 2016; Wurster et al., 2017). An explanation could be associated with an influence of the East Atlantic/West Russia pattern of climate variability that trends towards more negative values (wetter conditions) between AD 1960 – present. The absence of a similar trend in the Gura Ponicovei record could be explained by the lack of data between AD 1960 and AD 2014 (Fig. 8A).

In summary, it appears that $\delta^{15}$N of guano from Gura Ponicovei and Topolnița track winter precipitation associated with the NAO through its influence on the local N-cycle. However, consistent with Warken et al. (2018), this study finds a non-persistent relationship between signals of the NAO in these records on annual timescales. Although 50 to 100-year trends agree
well, there is less frequent correspondence with 5-10 year variability. This is likely due to the smaller data size in comparison to the Măgurici record (Cleary et al., 2018). Therefore, the potential exists for a higher resolution record acquired from the region to provide a more complete record of NAO influence on winter precipitation in SW Romania.

$\delta^{13}C$ summer precipitation reconstruction

No statistically significant correlation was found between $\delta^{13}C$ values and instrumental records of temperature, soil temperature, and atmospheric CO$_2$ concentration between AD 1960 and 2014. However, Topolnița guano $\delta^{13}C$ values (Fig. 9B) were found to correspond well with the Levanić et al. (2012) summer standardized precipitation index (SPI) record (<50 km from study site; Fig. 9C). An apparent strong relationship exists between the two records, with lower $\delta^{13}C$ values occurring contemporaneously with drought conditions indicated by negative values of the SPI. Interestingly this is paradoxical to the generally accepted effect of water availability on plant-WUE and the associated carbon isotope fractionation. As mentioned above, typically higher $\delta^{13}C$ values of C$_3$ are associated with drought conditions and samples depleted in $^{13}C$ are interpreted to occur as a result of increased water availability (Lambers et al., 2008; Bodin et al., 2013). Although this type of relationship has been demonstrated to exist in peat mosses (Skrzypek et al., 2007), they function entirely different than vascular plants.

When considering temperature, the warmer European climate (Fig. 9F) between AD 1950 and 2014 as well as at AD 1782 does correspond with more negative values in $\delta^{13}C$. However, unique relative to other reconstructions of drought indices, an SPI record inherently is based solely on monthly precipitation data (McKee et al., 1993). There is no dissimilarity between the SPI and guano-$\delta^{13}C$ values during this period of similar trends with temperature. Therefore, while there is likely some influence of temperature on precipitation, the most consistent and
strong relationship exists between δ\textsuperscript{13}C values and the local SPI (Fig. 9E). This suggests that although carbon isotopic fractionation associated with WUE is counter to the previously established process, the local water balance is the source of variation in δ\textsuperscript{13}C values.

A primary control of precipitation on WUE rather than other environmental factors could be explained by the fact that the southern Romania is the most vulnerable to drought relative to other regions of the country (Levanič et al., 2012). Therefore, water availability would likely be the primary limiting ecological factor for plant growth in the region. Additional evidence to this point is provided by the good agreement between the summer SPI and δ\textsuperscript{13}C records of SW Romania and reconstructed summer precipitation in Europe (Pauling et al., 2006; Fig. 9E). Although we cannot offer an explanation as to why drought conditions would result in low WUE in plants, for the reasons listed, there is sufficient evidence of a precipitation control on the carbon isotopic composition of guano. This allows us to interpret the δ\textsuperscript{13}C values in terms of past water availability.

Although the Levanič et al. (2012) SPI record provides a detailed record of dry and wet years, guano δ\textsuperscript{13}C values can offer information regarding trends in precipitation. Gura Ponicovei δ\textsuperscript{13}C values indicate that climate in the region was becoming progressively wetter from AD 1537 until AD 1982 (Fig. 9A). However, an interval of decreased precipitation does occur between AD 1689 and AD 1781 in the Topolniţa record (Fig. 9B). This event appears to coincide with four extreme dry years (AD 1725, 1748, 1782, and 1784) in Domogled (Levanič et al., 2012; Fig. 9C). Lower water availability indicated by higher δ\textsuperscript{13}C values within this interval also occur contemporaneously with less precipitation in the Czech Republic (Fig. 9D) and overall European summer precipitation (Fig. 9E). Additionally, each record features a brief wet pulse that occurs within this interval at AD 1726. Spring precipitation in Europe was generally low throughout this
interval (Pauling et al., 2006) providing additional evidence that summer climate likely has more impact on plant water use efficiency around Topolnița cave. The SPI indicates that this area of SW Romania during the nineteenth century lacked extreme wet and dry years. Similarly, there is little variation in $\delta^{13}$C values of Topolnița guano between AD 1791 and AD 1878 (Fig. 9B). Summer precipitation in Europe during this interval also stabilized relative to prior and subsequent years with mean annual rainfall fluctuating between 150 and 232 mm (Fig. 9E). Additional evidence of this connection can be found in the gradual trend towards wetter conditions between AD 1795 and AD 1841. The higher summer water availability also occurred in the Metaliferi Mountains (Forray et al., 2015), on the Someș Plateau (Cleary et al., 2018), and in the Tâul Muced (Feurdean et al., 2015a).

As indicated by the precipitation data, the aforementioned interval of relative stability in terms of European summer water availability, is followed by 83 years of progressively wetter conditions data (AD 1885 – AD 1968). A corresponding increase in $\delta^{13}$C values over roughly the same interval (AD 1878 – AD 1978) suggests that summer precipitation also increased in SW Romania (Fig. 9B). A trend is not apparent in the SPI until a drought occurs in AD 1946, where after a higher frequency of extreme wet events occur (AD 1969, 1970, 1975, and 1982; Fig. 9C). This increase in wet events also corresponds with the highest precipitation received at both Topolnița and Gura Ponicovei caves (Fig. 9A, B). Interestingly, other European records of precipitation (Fig. 9F, G) indicate a drier climate over the same interval, in the Swiss Alps (van der Knaap et al., 2011), Northern Britain (Charman et al., 2006) and in the eastern Mediterranean (Touchan et al., 2005). However, the study by Briffa et al. (2009) of summer moisture availability found that dry and cool summer conditions were less widespread across Europe in the latter half of the nineteenth century. This is supported by geographically extensive European
records of precipitation (Pauling et al., 2006; Büntgen et al., 2011) and more localized records of Romania (Feurdean et al., 2015a) that indicate a wetter climate over the same interval. The initiation of this wet period appears to correspond with the termination of the LIA, a prolonged cold phase that affected many parts of Europe (Mann et al., 2009). The amplitude, duration, and climate associated with the LIA event (~AD 1240-1850) were variable at the regional level (Mann, 2002). At other sites in Romania the end of the LIA is usually recognized by a shift to drier conditions (Forray et al., 2015; Cleary et al., 2018). The contrasting results from Topolnița and Gura Ponicovei caves data could be attributed partly to the precipitation pattern altered by topography (Gura Ponicovei Cave less obstructed by mountains than Topolnița). The demise of the LIA in Romanian records occurred at varying times. Studies in the northwestern region (Cleary et al., 2018) and Metaliferi Mountains (Forray et al., 2015; Forray et al., 2019) found abrupt terminations at AD 1890 and AD 1870, respectively. Alternatively, other records in the Călimani Mountains (Popa and Kern, 2009) suggests the end of the LIA occurred earlier in the nineteenth century around AD 1840. In agreement with the former, a shift towards wetter conditions in this study, places the demise of the LIA at ~AD 1870 (Fig. 9B). This wet event is subsequently followed by a sharp change towards drier conditions. This shift appears to be inconsistent across Romania with wetter conditions existing in western (Forray et al., 2015) and northern (Popa and Kern, 2009) regions of the country. However, a decreased discharge of the Danube (Rîmbu et al., 2002) and droughts in AD 1987 and AD 2000 in Domogled (Levanič et al., 2012) provide evidence of a drier SW Romania during this period. This decreasing trend in δ13C values of Topolnița and Gura Ponicovei guano corresponds with a progressively drier and warmer climate in Europe (Pauling et al., 2006; Büntgen et al., 2011).

*Vegetation dynamics and the link with climate conditions and human impact*
AD 1395-1643: Open mixed deciduous forest

Our pollen record from Gura Ponicovei Cave near the Danube River, show abundant concurrence of thermophilous species such as *Quercus* and *Tilia* but also of *Fagus sylvatica*. As *Tilia* is an entomophilous species and bats are ingesting insects, insect pollinated species are better represented in guano-derived pollen records than in those from peat or lakes. The presence of *Fagus sylvatica*, a species that typically thrives in cool and moist summer and mild winters (Peterken and Mountford, 1996) suggests warm winters and moist summers between approximately AD 1395 and 1550 in the region. This agrees well with the stable wet winter and summer precipitation in Europe during this interval (Fig. 8D, 7E; Pauling et al., 2006).

AD 1643-1957: Increased landscape openness

Pollen records from both caves indicate a reduction in the forest cover beginning at AD 1643 around Topolniţa Cave and AD 1810 at Gura Ponicovei. In agreement with modern elevational distribution of the forest belts, the patchy forests were dominated by *Tilia* and *Carpinus betulus* admixed with *Corylus avellana* and *Quercus* at our low elevation cave (Gura Ponicovei) and by *Fagus sylvatica, Tilia, Carpinus betulus* at Topolniţa, our cave located closer to the *Fagus* forest belt (Fig. 1). Topolniţa Cave also features a stronger anthropogenic influence. The reduction in percentages of mesophilous species, and in particular of *Fagus sylvatica*, could be associated with a lower water availability, an interpretation also supported by the $\delta^{15}N$ and $\delta^{13}C$ values indicative of drier winter and summer seasons during the LIA (AD 1537 and AD 1781). As noted by Barbu and Popa (2004), southwestern Romania is particularly vulnerable to drought. Therefore, it appears that water availability rather than temperature is a primary control of vegetation dynamics around Topolniţa and Gura Ponicovei caves. Peak dry conditions in the region between AD 1775 and 1800 likely led to the near complete disappearance of *Fagus*
Fagus sylvatica from the Gura Ponicovei area, whilst more drought resistant species such as Tilia and Quercus were able to persist. The persistence of greater amount of Fagus sylvatica at Topolnița could indicate that the area was less susceptible to drought due to the higher elevation. On the other hand, the decreased tree cover and the parallel increase in anthropogenic pollen indicators expressed by our records partially overlaps with the pollen and map-based reconstruction of grasslands and agricultural land expansion in Transylvanian Depression between AD 1700 and 1850 (Feurdean et al., 2015b). This trend may suggest that tree cover decline may have also been the response to human impact not only to changing climate conditions.

AD 1957-1985: Open mixed deciduous foothill forest belt

Unique to the Topolnița record, a transition to a 28-year expansion of Fagus sylvatica dominated forests with contributions from Corylus avellana, Fraxinus excelsior and Alnus occurs contemporaneously with a reduction in the diversity and abundance of herbaceous taxa. Notably there is also an increased proportion of Typha, likely related to the construction of the Iron Gate I dam in AD 1971 (Orghidan and Negrea, 1979). The subsequent localized ~20 to 35m water level rise in the Danube river could have increased the potential of foraging bats to interact with Typha pollen through ingestion of aquatic insects. There is no sign of evident climatic change which can be traced by δ13C and δ15N values in guano samples.

However, the reduction in tree cover was very typical during the Socialist Period in Romania, when agricultural activities (pasture, croplands) intensified (Hartel et al., 2013; Feurdean et al. 2017). This reduction in herbaceous diversity could be attributed to the application of artificial fertilizers, which has been found to contribute to expanding grasses while repressing other herbs (Akeroyd and Page 2011; Wesche et al., 2012). While increasing agricultural practices had a significant impact on the relative proportions of tree versus herbaceous taxa, climate could
explain the proportion of primary forest constituents at this time. *Fagus sylvatica*, *Alnus*, and *Fraxinus excelsior* fair better with increased precipitation and soil moisture (Peterken and Mountford, 1996; Dobrowolska et al., 2011). Topolnița guano δ¹³C and δ¹⁵N values are indicative of progressively wetter climate from AD 1885 until AD 1968. In northern Romania, Popa and Kern (2009) found a brief cold spell in the 1910s, preceding the initiation of the warming trend at ~AD 1980.

**AD 1985-present: Forest expansion at Gura Ponicovei and human impact at Topolnița**

Forests around Gura Ponicovei consisting of *Carpinus betulus*, *C. orientalis*, *Quercus*, and *Tilia* are typical of modern vegetation for such a low elevation site (Abdi et al., 2009; Fărcaș et al., 2006). In the post Socialist period (AD 1989 to present) increased forest cover occurred in most of the Carpathian region as former croplands and pasturelands were abandoned (Griffiths et al., 2013). However, the pollen record from Gura Ponicovei suggests that agricultural practices were minimal in the region since the 1950’s at this location. Notably is the presence of *C. orientalis*, a species very indicative of a sub-Mediterranean environment and which thrive under warmer conditions. This type of forest assemblage with increased contribution of thermophilous and drought resistant tree species *e.g.* *Pinus*, *Salix*, and *Fraxinus ornus* is likely indicative of a warm/dry climate. Indeed, both δ¹³C and δ¹⁵N records feature a contemporaneous shift to drier winters and summers. Lower precipitation (Pauling et al., 2006) and higher temperatures (Büntgen et al., 2011) appear to have also impacted most of Europe at this time.

Contrarily to the Gura Ponicovei Cave, there is an overall reduction in arboreal taxa and increased contributions ruderal pollen indicators at Topolnița Cave (Fig. 5; 6). There does not appear to be substantial fluctuations in primary tree taxa (*Carpinus betulus*, *C. orientalis*, and *Quercus*) during this interval that could indicate a lack of deforestation activities. Forests
dominated by Quercus, Tilia, and Carpinus betulus are typical of lower elevation in Romania (> 300 m a.s.l.) and it appears that these thermophilous species replace the previously dominant Fagus sylvatica, in the pollen record. Human impact around the cave is likely attributed to agricultural practices that are not as prevalent around Gura Ponicovei. The microcharcoal spike around AD 1930 can be associated with controlled fire practices with the purpose of land cleaning (Whitlock and Larsen, 2001; Feurdean et al., 2013) that was also recognized by Forray et al. (2015) in the Mada region of the Apuseni Mountains.

Conclusions
The $\delta^{15}$N values of guano from Gura Ponicovei and Topolnița provide a record of winter precipitation in SW Romania. Variability in water availability seems to be associated with the NAO through its influence on the local N-cycle. It was also determined that $\delta^{13}$C values reflect the relationship between summer precipitation amount and plant WUE. These records indicate that drier winters and summers characterize the conditions of the LIA termination (AD 1870). Increased presence of thermophilous and drought resistant species in the vegetational assemblage agree with this climatic setting. Water availability appears to have increased in subsequent winter and summer seasons through the 1970’s. Expansion of Fagus sylvatica around Topolnița Cave can likely be attributed to these milder conditions. Gura Ponicovei and Topolnița $\delta^{15}$N and $\delta^{13}$C values indicate that droughts became more frequent in both seasons over the past 30-40 years. Both $\delta^{15}$N and $\delta^{13}$C reconstructions of precipitation compare more favorably with European records of precipitation than other Romanian data, indicating that climate within the country is more variable and well linked to a greater region than the country itself. Future work in SW Romania will be needed to better understand the climate disconnect between this region and more interior areas of the country.
Acknowledgments

The authors greatly appreciate the assistance of Roxana Grindean for her comments and suggestions during the development of these pollen records. We also thank Bogdan P. Onac and Jonathan G. Wynn for their insight and comments on this project and early drafts of this manuscript. Funding for this research was provided by Romanian CNCS grant PN-II-ID-PCE 2011-3-0588 to Bogdan P. Onac.

Literature Cited


palaeo-water table reconstructions from northern Britain. Quaternary Science Reviews. 25, 336-350.


Palaeohydrological changes during the mid and Late Holocene in the Carpathian area, 

Handley, L., Austin, A., Robinson, D., Scrimgeour, C., Raven, J., Heaton, T., Schmidt, S., 
Stewart, G., 1999. The $^{15}$N natural abundance ($\delta^{15}$N) of ecosystem samples reflects 

Hartel, T., Dorresteijn, I., Klein, C., Máthé, O., Moga, C.I., Öllerer, K., Roellig, M., von 

Phytologist. 137, 179-203.


Atlantic Oscillation: Climatic Significance and Environmental Impact-Geophysical 
Monograph. American Geophysical Union, 134, 1-35.


Leroy, S.A.G., Simms, M.J., 2006. Iron age to medieval entomogamous vegetation and 
Rhinolophus hipposideros roost in South-Eastern Wales (UK). Palaeogeography 
Palaeoclimatology Palaeoecology. 23, 4-18.

Levanič, T., Popa, I., Poljanšek, S., Nechita, C., 2012. A 323-year long reconstruction of drought 
for SW Romania based on black pin (Pinus nigra) tree-ring widths. International Journal 
of Biometeorology. 57, 703-714.


proxy, high-resolution record of peatland development and its drivers during the last millennium from the subalpine Swiss Alps. Quaternary Science Reviews. 30, 3467-3480.


Figures

Figure 1: Map of Romania with circles indicating locations referenced in text: Gura Ponicovei Cave (1; this study), Domogled National Park (2; Levanič et al., 2012), Topolniţa Cave (3; this study), Drobota-Turnu Severin (4; meteorological station), Zidită Cave (5; Forray et al., 2015), Măgurici Cave (6; Cleary et al., 2017, 2018), Tăul Muced Lake (7; Feurdean et al., 2015a), Bucharest (8).
Figure 2: Age-depth models for Gura Ponicovei and Topolnița guano cores.

Figure 3: A) δ¹⁵N (red), δ¹³C (black), and Suess corrected δ¹³C (maroon) values of Gura Ponicovei guano; B) δ¹⁵N (blue), δ¹³C (black), and Suess corrected δ¹³C (purple) values of Topolnița guano.
Figure 4: Pollen diagram from Gura Ponicovei Cave guano.

Figure 5: Pollen diagram from Topolniţa Cave guano.

Figure 6: A) %C (red) and %N (black) values from Gura Ponicovei core; B) %C (red) and %N (black) values from Topolniţa core.
Figure 7: A) Correlation analysis of $\delta^{15}N$ and $\delta^{13}C$ of Gura Ponicovei; B) Correlation analysis of Topolniţa $\delta^{15}N$ and Drobeta-Turnu Severin autumn/winter precipitation; C) Drobeta-Turnu Severin autumn/winter precipitation (black), Topolniţa $\delta^{15}N$ values (dotted blue) and with 3-year lag (solid blue).
Figure 8: A) Comparison of $\delta^{15}$N values from Gura Ponicovei guano, B) $\delta^{15}$N values from Măgurici guano, C) $\delta^{15}$N values from Topolnița guano; D) 3-year running mean of reconstructed winter precipitation in Europe, E) autumn/winter precipitation Cloșani Cave, F) NAO winter index. Vertical bars indicate similar dry/wet phases.
Figure 9: A) $\delta^{13}$C values from Gura Ponicovei guano, B) $\delta^{13}$C from Topolnița guano, C) tree ring record of SPI from Domogled National Park D) Tree ring spring/summer precipitation from southern Czech Republic, E) 3-year running mean of European summer precipitation, and F) 3-year running mean of reconstructed European temperature anomaly. Vertical bars indicate similar dry/wet phases.
### Tables

Table 1. Radiocarbon ages, calibrated years AD and the dates used in construction of the age depth models for Topolnița and Gura Ponicovei guano cores.

<table>
<thead>
<tr>
<th>Topolnița</th>
<th>Sample ID</th>
<th>$^{14}$C Activity (pMC)</th>
<th>yrs BP</th>
<th>Depth</th>
<th>Calibrated years (CE)</th>
<th>Age used (CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>-</td>
<td>0</td>
<td>2012-2015</td>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP14</td>
<td>104.88 ± 0.31</td>
<td>14</td>
<td>1980-1994</td>
<td>1987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP30</td>
<td>106.38 ± 0.36</td>
<td>30</td>
<td>1978-1992</td>
<td>1985</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP44</td>
<td>111.12 ± 0.36</td>
<td>44</td>
<td>1974-1988</td>
<td>1981</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP56</td>
<td>125.91 ± 0.36</td>
<td>56</td>
<td>1962-1976</td>
<td>1969</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP71</td>
<td>160 ± 30</td>
<td>71</td>
<td>1719-1788</td>
<td>1795</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP85</td>
<td>200 ± 30</td>
<td>85</td>
<td>1731-1809</td>
<td>1780</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP94</td>
<td>250 ± 30</td>
<td>94</td>
<td>1627-1690</td>
<td>1683</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gura Ponicovei</th>
<th>Sample ID</th>
<th>$^{14}$C Activity (pMC)</th>
<th>yrs BP</th>
<th>Depth</th>
<th>Calibrated years (CE)</th>
<th>Age used (CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>-</td>
<td>0</td>
<td>2012-2015</td>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI2</td>
<td>111.3 ± 0.33</td>
<td>2</td>
<td>1993-1999</td>
<td>1996</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI12</td>
<td>138.82 ± 0.37</td>
<td>12</td>
<td>1989-1995</td>
<td>1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI26</td>
<td>115.33 ± 0.35</td>
<td>26</td>
<td>1967-1990</td>
<td>1975</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI42</td>
<td>350 ± 30</td>
<td>42</td>
<td>1530-1608</td>
<td>1537</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI55</td>
<td>810 ± 30</td>
<td>55</td>
<td>1181-1269</td>
<td>1228</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX A: EVIDENCE OF LONG-TERM NAO INFLUENCE ON EAST-CENTRAL EUROPE WINTER PRECIPITATION FROM A GUANO-DERIVED $\delta^{15}$N RECORD

Note to Reader:

This chapter has been previously published: Cleary, D.M., Wynn, J.G., Ionita, M., Forray, F.L., Onac, B.P. Evidence of long-term NAO influence on East-Central Europe winter precipitation from a guano-derived $\delta^{15}$N record. Scientific Reports 7:14095. See Appendix A for the PDF of the published document and Appendix B to see permission from the publisher.
Evidence of long-term NAO influence on East-Central Europe winter precipitation from a guano-derived $\delta^{15}$N record

Daniel M. Cleary1, Jonathan G. Wynn1,6, Monica Ionita2,3, Ferenc L. Forray4 & Bogdan P. Onac5,4,5

Currently there is a scarcity of paleo-records related to the North Atlantic Oscillation (NAO), particularly in East-Central Europe (ECE). Here we report $\delta^{15}$N analysis of guano from a cave in NW Romania with the intent of reconstructing past variation in ECE hydroclimate and examine NAO impacts on winter precipitation. We argue that the $\delta^{15}$N values of guano indicate that the nitrogen cycle is hydrologically controlled and the $\delta^{15}$N values likely reflect winter precipitation related to nitrogen mineralization prior to the growing season. Drier conditions indicated by $\delta^{15}$N values at AD 1848–1852 and AD 1880–1930 correspond to the positive phase of the NAO. The increased frequency of negative phases of the NAO between AD 1940–1975 is contemporaneous with higher $\delta^{15}$N values (wetter conditions). A 4‰ decrease in $\delta^{15}$N values at the end of the 1970’s corresponds to a strong reduction in precipitation associated with a shift from negative to positive phase of the NAO. Using the relationship between NAO index and $\delta^{15}$N values in guano for the instrumental period, we reconstructed NAO-like phases back to AD 1650. Our results advocate that $\delta^{15}$N values of guano offer a proxy of the NAO conditions in the more distant past, helping assess its predictability.

Global atmospheric circulation has a number of preferred patterns of variability, all of which have expressions in surface climate. Regional climates may vary out of phase, due to the action of such teleconnections, which modulate the location and strength of the storm tracks and poleward fluxes of heat, moisture and momentum1–3. Over Europe, the strongest influence is given by the North Atlantic Oscillation (NAO)4–6. NAO has a substantial effect on the European climate by modulating the position of North Atlantic storm tracks, which in turn control short and long-term changes in precipitation and temperature. When NAO is in its positive phase (e.g., a stronger than normal Azores High and a deeper than normal Icelandic Low), winter storm tracks are deflected northward resulting in wet winters over the northern part of Europe (positive correlation between winter NAO index and winter precipitation) and dry winters over the southern and eastern part of Europe (negative correlation between winter NAO index and winter precipitation) (Fig. 1). During the negative phase of NAO, storm tracks are shifted southwards, thus bringing wet and mild winters over the southern and eastern parts of Europe and dry winters over the northern part of Europe.

At a regional scale, the Carpathian Mountains have a strong influence on NAO related precipitation and temperature anomalies in Romania7. Due to orographic effect of the Carpathians, the winter NAO related signal is stronger in the northwestern part of the country7 where Măgurici Cave (hereafter MC) is located (see Supplementary Fig. S1). While studies have demonstrated that the NAO is an important factor in winter climate variability in East-Central Europe (ECE)8–9, except a recent study reconstructing winter air temperature and...
whether the N-cycle is open or closed, a strong in

September-October-November (SON) NAO index that represents the strength of the system during the winter months. Thereafter, we use DJF meteorological records and a ECE reconstructed precipitation series from Pauling et al.,

that directly and indirectly relate to the NAO to further examine the impact of changes in vegetation and hydrology of ECE on the isotopic composition of guano.

Guano and Climate

Bat guano is primarily composed of loose organic material, such as insect chitin, with a geochemistry characterized by an abundance of transition metals. Although there are numerous organic compounds in guano, chitin is the most abundant in MC deposit. Therefore, measured δ15N values reflect chitin derived N, while the contribution of other N sources to bulk guano is likely insignificant. If bats are not changing their roost sites, guano can accumulate as thick deposits (3 m or more) over long time periods (centuries to thousands of years).

Depending on the morphology and hydrology of each cave system, flooding of underground passages may cause guano deposits to be interbedded with clay and silt layers. Such circumstances offer additional information with respect to local cave environmental changes while guano was being deposited.

Bat guano may provide an ideal record of past vegetation because the δ13C value of plants in the region is transferred from plant to insect to bat and ultimately recorded in guano. The δ13C values of foliage are distinct between the two main photosynthetic pathways (C3 and C4)

Additional variation in δ13C values occurs within C3 plants in response to the water use efficiency of photosynthesis. The preservation of these values within guano provides new and critical information on the changing vegetational assemblage through time. Although more complex, recent studies have demonstrated that δ15N values in bulk guano can be interpreted as an integrator of the nitrogen cycle (N-cycle). When nitrogen is a limiting nutrient, nitrogen is conserved and as a result less nitrogen is lost and δ15N values decrease (i.e., a relatively closed N-cycle). A relatively open cycle results in high δ15N values under the opposing conditions. When it can be demonstrated that a climatic influence controls whether the N-cycle is open or closed, δ15N values can also be used as a paleoclimatic proxy. Since the NAO has a strong influence on precipitation and temperature it is possible that the nitrogen isotopic composition of cave bat guano, which has already been shown to reflect changes in water availability could provide insight into the influence of the NAO beyond the historical record.

Results and Discussions

The nitrogen isotopic composition of bat guano may be affected by diagenesis after deposition via processes of ammonia volatilization or denitrification. The conditions under which alteration may have been significant are in part reflected in variation of %N along the profile (see Cleary et al.). The %N in the MC core (see Supplementary Dataset 1) shows little deviation from near surface values (~11.3%) to the lower most portion of the core (mean = 10.6%). Therefore, the increasing trend in δ15N values between AD 1650 and AD 1880 (see Supplementary Fig. S2) is likely unrelated to diagenesis and can be interpreted as primary variation, considered here as a proxy of the NAO variability. The subsequent interval of lower δ15N values (7.5 to 9.5‰; AD 1980 to 2012) represents a change in local N-cycle (see discussion below) as opposed to diagenetic processes.

It is difficult to elucidate past δ15N values at any stage of the food web (plant-insect-bat-guano), however it is possible to interpret δ15N as an integrator of the N-cycle. The isotopic fractionations, N pool mixing, and N gains/losses produce the δ15N value of the system (soil, biomass, consumers). Therefore this resulting value integrates these processes and can be used to interpret the state of the N-cycle. The fractionations occurring
during the metabolic processes within bats and insects as nitrogen is transferred from plant to guano remain fixed through time. Since these fractionations follow conservative pathways, variation in the nitrogen isotopic composition of guano can ultimately be related to changes that occur in the soil inorganic nitrogen reservoir from which plants access nitrate and ammonia. Such changes have been connected to the state of the N-cycle (open: more nitrogen loss and higher δ15N values; closed: less nitrogen loss and lower δ15N values) and attributed to hydrological influence on the state of the N-cycle.

Cleary et al. suggested variation in δ15N values of guano (ultimately related to those of foliage) are strongly correlated to instrumental record of winter precipitation. This may result from a lag between the preservation of δ15N values in foliage during the growth phase (spring–early summer), which reflect soil N conditions from months immediately prior to growth (late fall–winter). In temperate forests, the maximum plant-available N occurs just prior to the onset of the growing season due to limited plant uptake of N that has been produced largely by microbial mineralization in months prior. Although there may be significant wet deposition of N during the spring and summer months, this flux into terrestrial ecosystems is on average lower than that produced via mineralization. This pool of plant-available N is soluble; thus during the winter season it is very sensitive to leaching processes, which is in turn driven by winter climatic conditions such as snow melt and the type of winter precipitation (snow vs. rain) when temperatures that straddle the threshold of freezing. Since the state of the N-cycle is controlled by the amount of N in the system, we infer that any change in the N-cycle is in response to the impact of winter hydroclimate on leaching processes. During the subsequent growing season when plants begin to access the soil N-pool, the state of the N-cycle established by the amount of leaching in the winter is then recorded in the new foliage. Increased leaching would reflect a more open N-cycle and result in higher δ15N values at each level of the food chain and ultimately guano. Therefore, although bats forage in the summer months, nitrogen delivered to the soil via spring/summer precipitation will likely not influence the δ15N values of guano.

Cleary et al. compared δ15N values of bat guano to corresponding δ15N values and to meteorological data from a nearby weather station in the Mada region (Metaliferi Mountains, W. Romania), and interpreted that water availability is a primary control of δ15N values of guano. Given the well-documented relationship of δ13C values of leaf tissues to water-use efficiency the carbon isotopic composition of MC guano (~26.5 to ~21%) suggests variation within the C3 pathway that may ultimately be related to changes in water availability. Based on these observations, we test a hypothesis for a hydrological connection between δ15N and δ13C values of guano by examining the correlation between each proxy in the Măgurari Cave guano record. Excluding two outliers, the resulting statistical analysis indicates that the δ15N and δ13C values in the MC record between AD 1800 and 2012 show a negative correlation (p-value = 0.001; R² = 0.62; n = 105; see Supplementary Fig. 52a). This suggests some dependence of the N-cycle of the bats foraging area on water availability, as the latter can be interpreted as the primary control on δ15N and δ13C values of guano. Given the well-documented relationship of δ13C values in foliage during the growth phase (spring–early summer), which reflects hydrological conditions, it is likely that they are recording different seasonal influences on water availability. While δ15N values of foliage are related to water stress during photosynthesis (spring–summer), δ15N values of guano are related to water stress during leaching. Therefore, correlation between δ15N values and δ13C values is ultimately the result of the winter precipitation (control of N-cycle) contributing to the degree of water stress in the spring/summer (control of δ13C). Consequently, if there is an influence of the NAO on precipitation in ECE, the nitrogen isotopic composition of guano should retain this signal more accurately than δ13C values. In accordance, hereafter we focus our interpretations of δ15N values of guano on reconstructing DJF precipitation.

One of the most striking features of our δ15N reconstruction is the abrupt decrease (~4%) at the end of 1970’s, a reduced discharge of the Danube River, and decreasing winter precipitation over Romania. This shift at the end of 1970’s is clearly observed in the sea level pressure (SLP) and precipitation patterns (Fig. 2). The difference map in the SLP field between 1940–1970 and 1980–2010 is indicative of a period characterized by an increased frequency of positive NAO phases after the 1970s (Fig. 2b), which is associated with a strong reduction in winter precipitation over the southern part of Europe (Fig. 2c). The resulting precipitation anomaly that occurs between northern and southern Europe during the winter months is also recognized in instrumental records across these regions. This regional decrease in winter precipitation that occurs at the end of the 1970’s is demarcated by the most significant decrease in δ15N values of MC guano. Given the direct link between precipitation and the current phase of the NAO, we interpret the δ15N values to largely reflect a hydrologic component of the regional climate with a signal that can be related to the NAO.

Due to the absence of δ15N values for certain years it is difficult to confidently utilize a statistical analysis to compare the record directly to the NAO index. However, there is a correlation (p-value = 0.002; R² = 0.43) between the first derivatives of time series of the NAO index and the δ15N values of MC guano (AD 1981–2012; interval of near annual ages of guano; see Supplementary Fig. 63). Additionally, since AD 1800, lower (higher) δ15N values, which are indicative of dryer (wetter) conditions, occur preponderantly during positive (negative) phases of the NAO (Fig. 3). The occurrence of more negative phases of the NAO (AD 1940–1975) corresponds with progressively wetter conditions expressed in the δ15N record. Likewise, trends towards drier conditions interpreted from δ15N values appear near contemporaneous with a higher frequency of NAO positive phases (AD 1848–1852; AD 1875–1930, and AD 1975–2012).

Our interpretation of δ15N values of MC guano as a NAO proxy (Fig. 3a) is supported by comparison to other precipitation proxies that are more directly influenced by the NAO. Lower δ15N values from MC correspond well with drier conditions indicated by a DIF reconstructed precipitation record for ECE (Fig. 3b), a long-term precipitation measurement in southern France (Marseille; Fig. 3c), and nearby Budapest and Baia
Mare meteorological stations\textsuperscript{45} (Fig. 3d,e). All three are located over regions strongly influenced by NAO (see the blue shaded band in Fig. 1a). Frequently there is contemporaneous overlap between a negative NAO index and wetter conditions indicated by precipitation records and the $\delta^{15}\text{N}$ values from MC guano (Fig. 3h). This agrees with an interpretation of a wetter NW Romania during the negative phase of the NAO. Thus, the state of the N-cycle that is preserved in $\delta^{15}\text{N}$ values of MC guano appears to be influenced by DJF precipitation, which in turn is modulated in ECE by the NAO.

A few of the general trends in hydroclimate that are expressed by the NAO index, meteorological records, and the nitrogen isotopic composition of MC guano can also be found in other Romanian and southern Europe paleo-records spanning the period AD 1850 to present. $\delta^{15}\text{N}$ values from the MC core trend towards wet conditions until AD 1800, fluctuates between 1800 and 1975, after which the climate abruptly became drier until present. Broadly, the $\delta^{15}\text{N}$ values from Ziditâa and Măgurici guano frequently show similar trends. The two records are well-correlated after AD 1900, when both feature a gradual trend towards drier conditions interrupted by a wetter interval between AD 1940 and 1975 (Fig. 3g,h). $\delta^{15}\text{N}$ values from Ziditâa, Măgurici, and $\delta^{13}\text{C}$ values in a *Sphagnum* core from the Tăul Muced\textsuperscript{46} (Rodnei Mountains, N. Romania; see Supplementary Fig. S1), all reflect a drying trend after –AD 1975 (Fig. 3f–h). However, the MC record appears to correspond more consistently with the NAO index, suggesting that the influence of the NAO is more prevalent in NW Romania than in other regions of the Carpathians.

Given that the signal of the NAO instrumental record is reflected in the $\delta^{15}\text{N}$ values since AD 1850, we extend our interpretation of this proxy to infer past phases of the NAO prior to the instrumental record. Indeed, using the ECE DJF precipitation reconstruction\textsuperscript{47} and measurements\textsuperscript{48} (Marseille) as additional evidence, $\delta^{15}\text{N}$ values suggest that the positive phase of the winter NAO dominated the circulation between AD 1650 and 1800. Over this 150-year interval, there were five prominent periods of at least 2 years each during which the negative phase was dominant (Fig. 3h). Our core reveals a major depositional hiatus, when guano accumulation ceased and a silty-clay layer was deposited between ~AD 1713 and 1715 (Fig. 3b). This period corroborates well with one of the highest reconstructed value of DJF precipitation across ECE\textsuperscript{12}, suggesting unusually wet winter seasons. Concurrent, historical hydroclimate records elsewhere in Europe\textsuperscript{46} document an increased in flooding frequency at this time. Interestingly, a testate amoeba record from Tăul Muced ombrotrophic bog located ~100 km NE of our cave documents a decline in water table depth over this period\textsuperscript{49}. Furthermore, the sharp decrease of the $\delta^{13}\text{C}$ values of *Sphagnum* at the same location (Fig. 3f) was interpreted to indicate prevalence of drier conditions\textsuperscript{44}. These site-specific contrasting precipitation patterns are not surprising since the winter NAO signal is stronger in the intra-Carpathian region\textsuperscript{9}.

Following the guano hiatus, the $\delta^{13}\text{N}$ time series suggests that beginning at ~AD 1720, a gradual transition to recurring positive phases of the NAO occurred, with the atmosphere remaining locked into this mode until
AD 1790. During this 70-year interval, significant negative phases of the pattern appeared only three times (AD 1760, 1775, and 1785), all coincident with wettest winters recorded at the Marseille weather station (Fig. 3c,h).

From AD 1800 to 1820, we identified an abrupt transition to more positive nitrogen isotopic values, which suggest increased regional moisture delivery mostly over the winter period. As inferred from our guano $\delta^{15}N$ values, this begins a period of ~20 years of mild and wet winters in the ECE relative to the earlier interval, which ends ~AD 1840 when precipitation started to decrease. The onset of this period of positive winter NAO phase is coeval with a marked phase of ice ablation in the St. Livre Cave (SW Switzerland), thought to represent almost three decades of warm and dry winters\(^{48}\). It also agrees well (but opposite sign) with a composite speleothem annual growth-rate record in NW Scotland, reflective of positive winter NAO states\(^{49}\). It is apparent from the discussion above that the changes in the phases of the NAO may partly explain the climate variability over the last part of the LIA.

**Conclusions**

A nitrogen isotopic proxy record of guano provides new information regarding the effect of DJF hydroclimate system on the N-cycle and the influence of the winter NAO on the ECE. The evidence of a NAO signal contained within the MC guano $\delta^{15}N$ series is the temporal strong correlation of the winter precipitation amount in the instrumental record. This study demonstrates that future guano research should consider not only precipitation, but also larger scale climatic systems when utilizing the nitrogen isotopic composition of guano. The use of nitrogen isotopic composition of bat guano is possible to add to, and improve the historical record of the NAO. As such, our results suggest that the $\delta^{15}N$ values of guano can be utilized to reconstruct past phases of the NAO beyond the instrumental record and demonstrates that the $\delta^{15}N$ values of guano can offer a proxy of the NAO in regions where instrumental or historical records are limited.

**Methods**

**Guano coring and sampling.** In October 2012, a Russian peat corer was used to extract a 287 cm core from a guano pile located in the Circular Room of MC (Fig. 1 in Johnston et al.\(^{50}\)). The coring site was adjacent (within 1 m) to the one investigated by Johnston et al.\(^{50}\). Both cores have similar stratigraphy, however, the lower 29 cm of the first one recovered only clay, whereas ours penetrated a 4-cm thick clay layer at 237 cm revealing an additional 46 cm of guano beneath it. Except for the clay layer, the entire core length was sampled for isotopic analyses and radiocarbon measurements.

---

**Figure 3.** Comparison of guano $\delta^{15}N$ with other hydroclimate proxy records between AD 1650 and 2012: (a) Winter (DJF) NAO index\(^{56}\); DJF precipitation data series from ECE\(^{12}\) (b), Marseille\(^{45}\) (c), Budapest\(^{45}\) (d), and Baia Mare\(^{45}\) (e); $\delta^{13}C$ of Sphagnum from Tăul Mucel\(^{46}\) (f); $\delta^{15}N$ values of guano from Zidit Cave\(^{26}\) (g), and Măgurici Cave (h; this study). The black smoothed lines in a–e represent the 3-year running mean.
Radiocarbon dating and age-depth models. Twenty aliquots of bulk bat guano from various depths of the Mālagućir core were submitted for radiocarbon dating by accelerator mass spectrometry (AMS) at the Poznan Radiocarbon Laboratory (Poland) and returned ages in stratigraphic order. Sample MG-15 was contaminated with young organic matter and thus discarded. Since guano is mainly composed of chitin (>95%), which makes it an excellent material for AMS age determinations, no sample preparation was needed.

The age-depth models (see Supplementary Fig. 54) are based on a linear interpolation between each 14C age in the upper 50 cm of the core, whereas for the rest of the sequence a type 2 smooth spline was applied. Both models were generated using Clam code16. The reasoning for employing a linear age-depth model for the upper 50 cm is because continuous observations since 1965 confirmed that the size of the bat colony has not changed, and therefore, it is expected that the guano accumulation remained constant. The default calibration curve utilized by Clam is the northern hemisphere terrestrial curve IntCal13.14C (cc = 1) from Reimer et al.17. The samples in the top 50 cm of the core are characterized by high radiocarbon activity (130.06 ± 1.4 and 132.46 ± 0.34 pMC) resulting in modern ages (1979–1980 and 1977–1978 cal. yrs). Guano began to accumulate in the Circular Room at ~ AD 881, shortly before the beginning of the Medieval Warm Period (MWP–AD 950–1300). One hiatus is inferred from the age depth model between AD 1237–1651, an interval that corresponds to the first half of the LIA. The raw 14C data are included in Supplementary Dataset 2, and the results of modeling in Supplementary Dataset 3).

Elemental and stable isotope analyses. Contiguous 1-cm bulk guano sub-samples were recovered for isotopic analyses along with a modern sample collected in 2012 to anchor the isotope chronology. Chitin is the dominant organic compound in MC guano, therefore, we considered compound specific extraction to be unnecessary. Due to the cave climate (see Supplementary Information) it is highly unlikely that any soluble guano-derived N-compound will survive and potentially impact the nitrogen isotopic composition.

All samples were prepared for δ15N and δ13C analysis following the procedures described in Forray et al.20 and Cleary et al.26. Out of these samples, 1–2 mg aliquots were weighed and placed in tin cups and then measured for δ15N, δ13C, δ15N, δ13C, and C/N. Analysis was performed using a Costech Elemental Analyser coupled to a Delta V Isotope Ratio Mass Spectrometer hosted in the Stable Isotope Laboratory (School of Geosciences, University of South Florida). A glutamic acid (internal standard), δ15N = −6.28‰, δ13C = −16.50‰, δ15N = 9.54‰, δ13C = 41.37‰) and a protein standard B2155 (δ15N = 5.94‰, δ13C = −26.98‰, δ15N = 13.32‰, δ13C = 46.5‰) were used during analysis. Certified reference materials, B2155 and IAEA-N1, were used to calibrate the δ15N value for the internal standard. B2155 and IAEA-G7 were used to calibrate the δ13C value of the glutamic acid. Estimation of the precision of analysis (δ15N = 0.08‰, δ13C = 0.04‰) was based on replicate internal standards during each run (Supplementary Dataset 1).

Statistical methods. Correlation analysis between δ15N values (mean = 10.4‰; std. dev. = 1.5) and δ13C values (mean = −24.5‰; std. dev. = 0.7) (n = 105) was completed using SPSS. This statistical test is appropriate as both data sets were extracted from the MC core with the same sampling and temporal resolution. Resulting p value and R value (p-value = <0.001; R = 0.62) from the 2-tailed test indicate statistical correlation. MATLAB was used to convert unevenly sampled data to evenly sampled δ15N values, which was then used to compute the first derivatives (1–year time steps). This step allowed for the examination of year-to-year changes in values. Correlation analysis (n = 26) using SPSS between the first derivatives of δ15N time series (mean = 8.4‰; std. dev. = 0.6) and DJF NAO index (mean = 0.5; std. dev. = 1.4) data sets was completed. This analysis was performed for years between 1981 and 2015 that included a respective δ15N value. The decision of testing the derivatives is appropriate due to the fact that the sensitivity of change being a more probable representation of influence of the NAO then raw values. The 2-tailed test resulted in a p-value = <0.002 and R2 = 0.43.

References
11. Peypouquet, J., Alados-Arboledas, L. & Remillard, J. Estimation of the precision of analysis (δ15N = 0.08‰, δ13C = 0.04‰) was based on replicate internal standards during each run (Supplementary Dataset 1).

55

Acknowledgements
We thank Dr. T. Tămaș (TT) and A. Giurgiu who helped during coring and initial sampling activities. We acknowledge the E-_OBS dataset from the EU-FP6 project ENSEMBLES (http://ensembles-eu.metoffice.com) and the data providers in the ECA&D project (http://www.ecad.eu). This research was funded by the Romanian CNCS grant PN-II-ID-PCE 2011-3-0588 to BPO.

Author Contributions
B.P.O. designed the project and B.P.O., T.T., and J.G.W. recovered the cave guano core. B.P.O., F.L.F., and T.T. sampled the core and D.M.C. ran the stable isotope analyses and constructed the age-depth model. M.I. analyzed present-day climate data and derived precipitation sources. D.M.C. analyzed the data and wrote the main manuscript text along with B.P.O. and J.G.W., and further contribution from M.I. and F.L.F.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-14488-5.

Competing Interests
The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017
APPENDIX B: COPYRIGHT PERMISSION FROM NATURE TO USE THE ARTICLE

EVIDENCE OF LONG-TERM NAO INFLUENCE ON EAST-CENTRAL EUROPE

WINTER PRECIPITATION FROM A GUANO-_DERIVED $\delta^{15}N$ RECORD IN

DISSERTATION

Rights and permissions

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.
APPENDIX C: A GUANO-DERIVED $\delta^{13}C$ AND $\delta^{15}N$ RECORD OF CLIMATE SINCE THE MEDIEVAL WARM PERIOD IN NORTH-WEST ROMANIA

Note to Reader:

This chapter has been previously published: Cleary, D.M., Onac, B.P., Tanțău, I., Forray, F.L., Wynn, J.G., Ionita, M., Tâmaș, T. A guano-derived $\delta^{13}C$ and $\delta^{15}N$ record of climate since the Medieval Warm Period in north-west Romania. *Journal of Quaternary Science* 33(6):677-688. See Appendix C for the PDF of the published document and Appendix D to see permission from the publisher.
A guano-derived δ^{13}C and δ^{15}N record of climate since the Medieval Warm Period in north-west Romania

DANIEL M. CLEARY,† BOGDAN P. ONAC,‡,§,‖,¶ IOAN TANȚĂU,∥,‡,§ FERENC L. FORRAY,∥ JONATHAN G. WYNN,¶ MONICA IONITA† and TUDOR TAMAȘ∥

†Kaust Research Group, School of Geosciences, University of South Florida, Tampa, FL, USA
‡Department of Geology, Babeș-Bolyai University, Cluj-Napoca, Romania
§Emil Racoviță Institute of Speleology, Cluj-Napoca, Romania
¶National Science Foundation, Alexandria, VA, USA
‖Paleoclimate Dynamics Group, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany

Received 14 February 2018; Accepted 25 April 2018

ABSTRACT: A 285-cm core of bat guano was recovered from Măgurari Cave in north-west Romania and analyzed for δ^{13}C, δ^{15}N and pollen. Guano deposition occurred from AD 881 until 1240 and from AD 1651 to 2013, allowing for the interpretation of summer variations in precipitation and temperature during the Medieval Warm Period (MWP) and the Little Ice Age (LIA). A 2% increase in δ^{13}C, 1.5% decrease in δ^{15}N, and the presence of Ulmus, Quercus and Carpinus betulus indicate a warm and dry MWP occurred in the region. The lack of deposition during the beginning of the LIA suggests a possible climate-induced change in prey availability resulting in bats vacating the cave. Variation of δ^{13}C values between −25 and −23% at AD 1650 (LIA) indicates similar drier conditions as at the end of MWP. However, a 2% decrease in δ^{13}C values that occurred between AD 1790 and 1900 suggests climate was trending towards wetter conditions at the end of the LIA. From AD 1938 to 2013, δ^{13}C values appear to be more influenced by temperature, indicating that this parameter had a more significant effect on carbon discrimination than water availability. Copyright © 2018 John Wiley & Sons, Ltd.

KEYWORDS: bats; paleoclimate; pollen; Romania; stable isotopes.

Introduction

The Medieval Warm Period (MWP) represents the most recent extended warm interval in the North Atlantic region that occurred before pre-industrial times (Trouet et al., 2009). In contrast, the Little Ice Age (LIA) was a lengthy period with lower temperatures that were experienced mainly in the Northern Hemisphere (Mann et al., 2009). However, the occurrence, amplitude and duration of these events have been found to vary distinctly over different regions. Additionally, although paleo-records of these recent major climatic events exist for Europe, they are scarce within Romania. Therefore, more information is needed to better characterize these climate intervals within this region in terms of precipitation, temperature and vegetation dynamics.

Cave bat guano has the potential to be a very useful resource for paleoclimate and paleoecological reconstructions. Studies using δ^{13}C values have provided information related to the shift between C_{3} and C_{4} plants as well as variation in plant water-use efficiency (Des Marais et al., 1980; Mizutani et al., 1992a,b; Bird et al., 2007; Wurster et al., 2007; Forray et al., 2015; Royer et al., 2017). Guano-derived δ^{15}N values have also been used to reconstruct regional winter precipitation (Cleary et al., 2017) and to assess the anthropogenic and climatic influence on a regional nitrogen pool (Cleary et al., 2016). In addition, pollen preserved in guano has been shown to be a reliable resource for reconstruction of vegetation dynamics (Carrion et al., 2006; Geantă et al., 2012). This type of proxy record is ideal for the study of the MWP and LIA in Romania as deposits have frequently been found to be as old as AD 800 (Geantă et al., 2012; Onac et al., 2014) with guano accumulation often occurring through parts of the LIA (Forray et al., 2015).

The connection between vegetation and guano is such that the carbon and nitrogen isotopic signature is transferred from plant foliage, to insects, to bats, and ultimately preserved in guano. Pollen can reach guano in one of three ways: (i) by pollen injection and subsequently defecation by bats, (ii) by air circulation/wind transport, and (iii) trapped on bat skin/hair (Carrion et al., 2006).

In locations where it can be shown that C_{3} and CAM plants are absent, and thus most of the vegetation follows the C_{3} photosynthetic pathway (δ^{13}C values typically between −24 and −32‰) plant water-use efficiency (WUE) may largely be responsible for variation in δ^{13}C variation of plant biomass. Plant WUE describes the ratio of net photosynthesis to transpiration (Farquhar et al., 1980; Nobel, 1980). Plants have some capacity to reduce stomatal conductance and/or increase photosynthetic capacity, which allows for some optimization of conditions resulting in higher WUE in conditions of low water availability and/or high CO₂ concentrations (Manzoni et al., 2011). In more arid conditions, these plants can minimize water loss (Farquhar et al., 1982) and as a result discriminate less against the heavier δ^{13}C and produce less negative δ^{13}C values in foliage. Less water-stressed conditions create the opposite effect, wherein little competitive advantage is afforded to plants with high WUE, leading to higher carbon isotopic discrimination (lower δ^{13}C values). This variation in plant foliage δ^{13}C values may be transferred to bat guano and can therefore be used to reconstruct the hydroclimate within the foraging range of the bat colony (Forray et al., 2015).

Although δ^{13}C values of guano have been used more frequently in paleoclimate studies, recent work has demonstrated that δ^{15}N values can provide information related to changes in precipitation (Cleary et al., 2016, 2017) and vegetation assemblage (Wurster et al., 2017). Because...
nitrogen pathways are more complex than those of carbon, nitrogen isotopic composition can alternatively be used as an integrator of the local nitrogen cycle (N-cycle) rather than as a simple tracer (Robinson, 2001). In this context, $^{15}$N values in guano can be associated with shifts between an open and closed N-cycle. In environmental conditions under which a multi-proxy approach suggests that climatic or environmental factors control the amount of nitrogen in the system, $^{15}$N values of guano can be used as a paleoclimate proxy (Cleary et al., 2016).

In this study, a 285-cm-long core was extracted from a guano heap in Măgurici Cave (north-west Romania; Fig. 1) with the aim to use carbon and nitrogen stable isotope values ($^{13}$C and $^{15}$N) along with the pollen record to interpret changes in the vegetation assemblage and the temperature and hydroclimatic regime of the MWP and LIA. Records of temperature and precipitation from the instrumental period of climate history are used to confirm or reject how these factors influence $^{13}$C and $^{15}$N values and if they have local or regional significance.

**Background**

**Study area**

Măgurici Cave is located in north-west Romania (Fig. 1) and consists of 641 m of galleries with a total vertical range of 30 m (Borda et al., 2004; Onac and Tămaș, 2018). Speleogenesis proceeded in the fossiliferous limestones (Coozla Formation) of Eocene–Oligocene age that outcrop on the Parcăreț-Boi Mare Karst Plateau in the north-east part of the Someșu Plateau (Onac and Todoran, 1987; Bucur et al., 1989; Prică, 2001). The climate of the region can be described as temperate-continental with mean annual temperatures of 7.4 °C in the higher altitudes (>300 m a.s.l.) and 8.5 °C at lower elevations. Annual average precipitation is between 700 and 900 mm in the region (Sandu et al., 2008). The current climatic regime is ideal for the main taxa present in the forests within proximity of the cave. Primary constituents include Quercus petraea, Fagus sylvatica, Carpinus betulus, Populus and Salix (Geantă et al., 2012). Agriculture is more prevalent at lower elevations with prominent crops, fields and pastures. The most common herbaceous pollen families encountered in the area are Chenopodiaceae, Plantaginaceae, Caryophyllaceae, Poaceae, Urticaceae and Apiaceae (Răștiu, 1966; Drăgulescu and Macalik, 1999).

The 285-cm guano accumulation is located in the Circular Room (See fig. 1 in Johnston et al., 2010) where a large nursing bat colony is currently present. The underground climate conditions in this sector of the cave are characterized by a mean annual temperature of 11.8 °C and relative humidity ranging between 85.6 and 98.5% throughout the year (Borda and Racoviță, 2000–2001). The stable climatic conditions documented in the Circular Room are due to the unique cave morphology, wherein a mud bank at the

![Figure 1. Map of Romania with circles indicating locations referred in the text: Tăuľ Muced (1; Feurdean et al., 2011a,b), Măgurici Cave (2; Calimani Mountains (3; Popa and Kern, 2009), Cluj Napoca (4; Lake Ighiel (5; Halluc et al., 2017), Zălabi Cave (6; Forray et al., 2015), and Gaura cu Mască Cave (7; Onac et al., 2014). The locations of Czech Republic (8; Brázda et al., 2002) and Budapest (9, Kiss et al., 2011) studies, and position of Romania (red outline) within Europe are indicated on the inset.](image-url)
entrance of the gallery constricts the passage way and limits airflow into the deeper parts of the cave. This could explain the continued presence of the bat colony within the Circular Room between April and September as the existing climatic regime is ideal for bats. Common species present in Măgurița Cave are Myotis mystis, Miniopterus schreibersii and Myotis blythii (Borda et al., 2004).

Core description

Guano cored for this work was completed within 50 cm of the core used by Johnston et al. (2010) and Geantă et al. (2012). The 271-cm core from the aforementioned studies has a largely similar lithostatigraphy to the guano analyzed here, which can be described as loose disaggregated insect remains for the upper 236 cm of the core. Visually this younger material appears as dark brown guano. In the previous work, coring was completed until a silty clay sequence was reached (Johnston et al., 2010). However, in this study a silty clay layer at 236–241 cm was cored through revealing an additional 45 cm of guano. Below this detrital layer, guano is more compact and drier than in the upper section of the core.

Methods

Radiocarbon

An accelerator mass spectrometer at the Poznan Radiocarbon Laboratory (Poland) was used to measure the radiocarbon age of 20 samples of bulk guano. The ages obtained are in stratigraphic order, although sample MG-15 was contaminated with younger organic material and thus omitted from the constructed age-depth model (Cleary et al., 2017). Due to associated errors with the radiocarbon dating (±1 year for modern samples and 2.5–30 years for pre-modern samples), the expressed years are not exact dates but rather approximations.

Isotopic and elemental analysis

After coring, sampling was completed at 1-cm resolution. Sample preparation followed the method described in detail by Forray et al. (2015). Guano samples of 1–2 mg were weighed from each aliquot and then measured for δ13C, δ15N, %C and %N using a Costech ECS4010 Elemental Analyzer coupled with a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fischer Scientific) at the University of South Florida Stable Isotope Laboratory. A protein standard B2155 (δ13C: −26.98%, δ15N: 5.94%, %C: 46.5, %N: 13.3) and a glutamic acid (internal standard: δ13C: −16.5%, δ15N: −6.20%, %C: 41.37, %N: 9.54) were used during analysis. B2155 and IAEA-C7 were used to calibrate the δ13C value of the glutamic acid. Estimation of the precision of analysis (δ13N: 0.08%; δ15C: 0.04%) is based on replicate internal standards during each analysis.

Pollen preparation

Palynological work was previously completed on a different Măgurița guano core (see Geantă et al., 2012), but did not capture the lowermost part of the deposit. Samples for pollen analysis were taken at 1-cm intervals from this section located between 250 and 285 cm. Due to the samples being entirely organic material, a simplified procedure was used to isolate pollen grains. One cm³ of each sample was treated with NaOH (30%) solution, centrifuged and washed repeatedly with distilled water. This in effect removed humic acids and organic matter, excluding pollen and spores. Samples were then staged on glass thin slides and pollen was counted until a sum of ca. 300–400 pollen grains was reached. The frequencies of pollen for each taxon were calculated as percentages of the total sum of arboreal pollen (AP) and non-arboreal pollen (NAP). The nomenclature for vascular plants follows Flora Europaea (Tutin et al., 1964–1980). Microscopic charcoal particles were also counted to reconstruct the fire history. The results are presented as a percentage pollen diagram reconstructed in the Tilia software version 2.0.41 (Grimm, 1991).

Results

%N and %C content of guano

There is a ~8% decrease in %C between 0 and 96 cm depth (36.4–28.1%), after which values stabilize around 34% (average %C: 31.5; Fig. 2). In contrast, excluding minor fluctuations, %N appears to be stable throughout the sequence (average %N: 10.6). There are two notable minima in %N that occur at 259 (8.5%) and 280 cm (8.1%), suggesting a possible loss of nitrogen at these depths. These minima are mirrored in the %C plot by values of 24.3 and 21.0%, respectively.

δ13C and δ15N of guano

The entire δ13C time series features values that fall between −26 and −21% (Fig. 3), suggesting that plants with a C3 photosynthetic pathway have been dominant in the vegetation assemblage since AD 881. During the MWP the δ13C values are the lowest for the entire core (~26%), before trending towards less negative values at AD 1076. This course continues until the beginning of the hiatus at AD 1240 where δ13C reaches −24.3% (Fig. 3). There are a few slight differences between the two time series as δ15N values first increase by 1% between AD 881 and 933, whereas δ13C values lack variation until AD 970. After this interval values decrease and plateau at ~12.4% (AD 962–1052) before briefly trending towards more positive δ15N values at AD 1076.

Following the lengthy hiatus that starts just after the beginning of the LIA, deposition resumed at AD 1651. The δ13C values following this interval are relatively stable until the end of the LIA (δ13C: −24.9 to −22.9%; mean: −23.9%). However, two notable variations occur during this apparent stability. The first is the short increase of 1.5% in δ13C values between AD 1651 and 1687 that is followed by a rapid approach to more negative values at AD 1696 (~24.6%). The second trend visible in this interval is the gradual increase in δ13C values from this minimum to more positive values at AD 1785 (δ13C = −23.1%). This is followed by another peak at AD 1821 (δ13C = −23.3%) before a ~80-year decrease in δ13C values to 24.5% punctuates the end of the LIA (AD 1850). Next, there is a rapid increase in δ13C values until AD 1938 where the most positive value is achieved (δ13C = −21.4%). After this maximum peak, two sudden drops mark the return to lower δ13C values, which remain below ~25% until AD 2012. The δ15N values display a gradual increasing trend from AD 1651 until 1850. After this interval, there is a prolonged stability before a drastic decrease in δ15N values at AD 1960 (Cleary et al., 2017).

Pollen and microcharcoal

Upon completion of palynological analysis, two local pollen assemblage zones (LPAZs) were defined in the lower 35 cm of guano (Fig. 4). The first occurred during the MWP...
(AD 899–1168; LPAZ 1), whereas the second encompasses a short period (AD 1651–1700; LPAZ 2) within the LIA. Analysis of the two LPAZs suggests that hiatus (AD 1240–1650) corresponds to significant changes in the pollen record, and thus probably also in the vegetation cover. The primary forest constituents in LPAZ 1 were *Betula*, *Alnus*, *Ulmus*, *Quercus*, *Carpinus betulus* and *Fagus sylvatica* with the prominent herbaceous families being *Apiaceae*, *Rubiaceae*, *Ranunculaceae* and *Scrophulariaceae*. In LPAZ 2 there is a decrease in most of the aforementioned forest taxa with the exclusion of *Fagus sylvatica*, which appears to expand greatly. There is also a significant increase in *Poaceae* during this interval. Other herbaceous plants appear to continue at the approximate same percentages across the LPAZs, although *Asteraceae* and *Plantago lanceolata* become more prevalent.
Figure 4. Pollen diagram for the lower 35 cm of the guano core.
Although there was an expansion of *Fagus sylvatica*, the proportion of tree pollen decreases across LPAZ 2 while herbaceous plants increase. The entire period is characterized by low microcharcoal percentages, except for the level from 258 cm (AD 1675) to 260 cm (AD 1670).

**Discussion**

**Suitability of guano for $\delta^{15}N$ analysis**

The $\delta^{15}N$ values of bat guano can be altered after deposition through denitrification, which preferentially removes...
$^{15}$N, resulting in a $^{15}$N-enriched residual guano. Due to the range of the enrichment factor associated with this process (28–33‰; Robinson, 2001) and the inability to determine the quantity of N that was denitrified, it is not possible to account for the amount of decomposition that may have occurred. Other enriching processes such as ammonia- volatilization have been shown to affect only urea and not the nitrogen isotopic composition of bulk guano (McFarlane et al., 1995). The degree to which denitrification may have altered $^{15}$N values can be screened by observing the variation in $^{15}$N with depth. Typically, denitrification increases with age, resulting in a trend towards lower $^{15}$N values with depth (Bird et al., 2007; Cleary et al., 2016). If $^{15}$N shows little variation and values are near the value for modern guano accumulation, we may consider the associated guano as acceptable for use in interpretation of $^{15}$N values as a proxy for paleoclimate, anthropogenic influence and trophic position (Cleary et al., 2016).

The $^{15}$N in the MC core shows little deviation from near surface values between 0 and 240 cm depth (Fig. 2). Beyond 240 cm depth, there are intervals of %N values that remain high, but there are a few instances of anomalously low %N. The associated $^{15}$N values for the guano are two low %N values between 250 and 285 cm (Fig. 2) are considered here but their interpretation should be treated with caution.

In contrast, the %C values of guano display moderate variation throughout the core. However, there are no major diagenetic processes that result in fractionation of carbon isotopes in guano, with only a 0.8‰ enrichment between diet and animal feces (Den Marais et al., 1980). Even when %C is low, the $^{13}$C values of the corresponding guano are interpreted to reflect the original value upon deposition (Forray et al., 2015). Therefore, because no shifts between C3 and C4 plants were expected in the region, the $^{13}$C values from the entire series are appropriate for interpretation of past water-use-efficiency.

**Pre-and Medieval Warm Period**

There is a contrast in trends between the carbon and nitrogen isotopic values from AD 881 to 940, where $^{13}$C values are relatively stable and $^{15}$N values become progressively more positive. This is probably related to the fact that the first five values of $^{15}$N from the base of the core show low %N (7.1–9.9‰; Fig. 2) that could indicate a possible alteration of the nitrogen isotopic composition. This alteration can occur in relatively stable and high, but there are a few instances of anomalously low %N.

 따른. The increasing $^{13}$C values and decreasing $^{15}$N isotopic records since AD 1075 suggest that the trend in climate was towards drier conditions (Fig. 5A,B). The same event is visible in the lower $^{13}$C values from the Gaura cu Muscă guano record (Fig. 5C; Onac et al., 2015), deeper water table in Taul Muced (Fig. 5D; Feurdean et al., 2015), and lower Ti count at Lake Ighiel (Fig. 5E; Haliuc et al., 2017). However, the initiation of the drying trend appears to begin only around AD 1110 at Gaura cu Muscă and Taul Muced (Fig. 5C). The onset of this drying event appears to have occurred later at more southern and eastern locations in Romania. These results are consistent with a drier Europe during the MWP, with precipitation values fluctuating approximately ±80 mm around 220 mm between AD 1050 until AD 1250 (Fig. 5C; Büntgen et al., 2011). With mean annual rainfall at 100–250 mm, this can be classified as a semi-arid environment (Dean, 2004). There are also a few wet events during this semi-arid period that occur contemporaneously around AD 1100, AD 1168 and AD 1142 in the Calimani, Gaura cu Muscă and European precipitation records (Fig. 5C,F,G) that are not visible in the Măgurić $^{13}$C record (Fig. 5A). The absence of these events in this record could be explained by the lower resolution in ages of the Măgurić core. It is also possible that less rain and higher summer temperatures occur in the Măgurić Cave region in contrast to the Zidată Cave, due to its lower elevation and landscape openness.

The presence of Ulmus, Quercus and Carpinus betulus is consistent with this interpretation as they are reliant on warm and dry conditions (Abdi et al., 2009; Feurdean et al., 2010a,b). Although Fagus sylvatica and Alnus, by contrast, prefer wetter conditions (Peterken and Mountford, 1996) the aforementioned species are typically confined to lower elevations and are low pollen producers. Therefore, their relatively higher percentages at this mid-elevation site suggests that there were probably larger forests of these taxa at low elevations due to the warmer and drier overall climatic regime.

Both this guano pollen record (Fig. 4) and that of Geanăță et al. (2012) indicate that F. sylvatica forests began to expand ~AD 1100. This is a highly competitive species that thrives under wetter conditions and cooler summers (Kutzbach and Webb, 1993). However, the gradual increase in $^{13}$C values (decrease in $^{15}$N) suggests drier conditions before the hiatus. It is likely that the region was progressing towards less water availability, although the cooler conditions led to a decrease in the warm reliant taxa (Ulmus, Quercus and Carpinus betulus). The dominance of F. sylvatica indicates that precipitation was still at an amount that allowed for the expansion of this species, although the greater impact on this change in vegetational assemblage is probably due to the shift to cooler conditions.

**Little Ice Age**

Based on the gap between the interpolated radiocarbon ages at 268 and 269 cm in our age model (Cleary et al., 2017), a depositional hiatus is interpreted to occur between AD 1240 and 1651. The absence of guano could be the result of (i) a flash flood within Măgurić Cave preventing access to the Circular Room or (ii) a change in climatic conditions just before the onset of the LIA (AD 1250) wherein the bats left the cave. Climate would seem to be a more viable explanation as there is no evidence of a flood (associated silt or clay deposits). Additionally, isotopic (Fig. 5) and palynological results (Fig. 4) also suggest that the climate was transitioning towards drier and cooler conditions at AD 1075 and this trend continued until AD 1240. Therefore, the cause of this hiatus (AD 1240–1651) may more likely be associated with this change. The gap in this record covers more than half of the LIA, representing a lengthy period with no bats roosting in the Circular Room (Johnston et al., 2010). The fact that the hiatus occurs contemporaneously with the presumed timing of the LIA in Europe provides further evidence of the climate event in north-western Romania (Johnston et al., 2010; Geanăță et al., 2012). The absence of the bats may be related to the cooler conditions associated with the early parts of the LIA (Geanăță et al., 2012), which would have reduced the availability of prey for the bats (Dietz et al., 2006). With less food present, the bats would probably have vacated Măgurić Cave for a region that experienced milder climatic conditions over this period. Based on the age of the guano, bats probably returned to the cave when conditions became warmer and wetter around AD 1651 (Fig. 3). Although we cannot infer the climate of the LIA due to the absence of guano for this interval, the $^{13}$C values just before the
Depositional hiatus suggest a trend towards drier conditions. The interpreted climate from the Măgurici guano record at AD 1651 adds to previous work which found alpine areas of Romania to be cooler during the LIA (Popa and Kern, 2009; Feurdean et al., 2011a, b). Cool and dry conditions may have been less hospitable to bats in this region of Romania in comparison with the Mada region (Fig. 1; locality 6) where there was a gradual transition to the LIA (possibly due to

Figure 6. Comparison of $\delta^{13}$C values from Măgurici guano (A), Ti influx into Lake Ighiel (B; Haliuc et al., 2017), $\delta^{13}$C of Zdîta guano (C; Forray et al., 2015), depth to water table at Tâu Muced (D; Feurdean et al., 2015), precipitation values from southern Czech Republic (E; Brázdi et al., 2002), 10-year running mean of reconstructed precipitation totals in Europe (F; Büntgen et al., 2011), temperature anomaly for Calimani Mountains (G; Popa and Kern, 2009), and the temperature record in Budapest, Hungary (H; Kiss et al., 2011) from AD 1600 to AD 2012. Brown bar at AD 1712–1715 represents the occurrence of the silty clay layer.
milder conditions) without the cessation of guano deposition (Forray et al., 2015). A possible reason for these contrasting conditions between the Măguri and Mada regions could be due to the northern areas being on the rain shadow side of the Carpathians.

Following AD 1651, forests were still dominated by *Fagus sylvatica* in addition to a larger *Picea* component (Fig. 4). This suggests that this vegetational assemblage probably persisted through the LIA and continued to AD 1700. The δ13C values indicate that the hydroclimate was relatively stable between AD 1651 and 1900. However, a brief drying trend did occur from AD 1651 to 1687 that was followed by a rapid shift to wetter conditions at AD 1696. Following this interval and until AD 1785, the δ13C values progressively became less negative, suggesting that climate was drier for nearly 100 years. This interpretation is consistent with the findings in the Rodna Mountains (1360 m a.s.l.) where the water table in a swampy lake was much lower during this time (Feurdean et al., 2015; Diaconu et al., 2017). Popa and Kern (2009) also found this interval to be drier in the Câlimani Mountains (~1820 m a.s.l.), opposed to other areas of Europe that featured cooler and wetter summers (Bradley and Jones, 1993).

Notably, the silty-clay layer interbedded within the guano pile (AD 1712 and 1715) occurs within this period of relatively stable climate. Its occurrence probably suggests the presence of a lake originating from increased precipitation that temporarily submerged the lower 45 cm of the guano pile within the Circular Room. Previous studies have also linked the occurrence of detrital layers to wetter periods during which cave passages could have been partly or totally inundated (Forbes and Bestland, 2006; Onac et al., 2014). During the interval when the silty-clay accumulated in Măguri Cave, the weather appears to have deteriorated throughout many parts of Europe causing severe flooding events (Marusek, 2011). The suggested increased precipitation for the interval of AD 1712-1715 is contemporaneous with the wettest period in the southern Czech Republic (Brázdil et al., 2002; Fig. 6E). This interval also coincides with one of the highest reconstructed precipitation values (Büntgen et al., 2011; Fig. 6F). The event would probably have resulted in the ceasing of guano deposition due to the development of a temporary lake within the chamber. The reason the lower 45 cm of guano was not washed away probably relates to the absence of last moving/turbulent water during this event. Therefore, the lower part of the sequence was preserved when guano deposition resumed. This was confirmed by the fact that radiocarbon ages in this section of guano were found to be in stratigraphic order, lacking any depositional hiatus.

The δ13C values indicate that the climate towards the end of the LIA (AD 1785–1850) in north-west Romania was gradually becoming wetter. There is a notable wet period that of the LIA (AD 1785–1850) in north-west Romania was greatly reduced, with one of the higher reconstructed precipitation values. The δ13C values suggest wetter conditions during this event. Therefore, the lower part of the sequence was preserved when guano deposition resumed. This was confirmed by the fact that radiocarbon ages in this section of guano were found to be in stratigraphic order, lacking any depositional hiatus.

The δ13C values over the following ~80 years (AD 1823–1892) indicate that there was a sustained trend towards wetter climatic conditions. This agrees with other Romanian studies that found wetter conditions in the Apuseni Mountains (Forray et al., 2015; Cleary et al., 2016), Tâul Mare-Răbdău peat bog (Cristea et al., 2014), and Tâul Muced (Feurdean et al., 2015). Temperature records from the Câlimani Mountains and Hungary indicate climate was progressing towards warmer conditions (Popa and Kern, 2009; Kiss et al., 2011). Increased precipitation in Romania is also consistent with the relatively higher precipitation throughout Europe, which transitioned towards a wetter climate as well (Fig. 6F; Büntgen et al., 2011).

There is debate over the precise initiation and demise of the LIA in Europe, with many studies concluding differing intervals of time (Matthews and Briffa, 2005). With respect to Romania, the LIA appears to have covered AD 1370 until ~AD 1840 in the Câlimani Mountains (Popa and Kern, 2009). In the Apuseni Mountains (western Romania), Forray et al. (2015) found this event to begin at around AD 1200 and persist until an abrupt termination between AD 1870 and 1900. Previous studies on Măguri and Gaura cu Muncă guano (Geantă et al., 2012; Onac et al., 2015) determined the onset of the LIA at AD 1240 and 1285, respectively, with conclusion at ~AD 1810 in the first site. However, it is difficult to interpret the climatic conditions through the pollen record at this time due to periods of substantial deforestation. A better indicator of the LIA termination in the region is the δ13C record. Here we interpret the demise of the LIA to have occurred at AD 1892 where a clear transition to drier conditions occurred. The drier conditions are consistent with the expansion of *Quercus* and decrease of *Fagus sylvatica* found by Geantă et al. (2012).

### 1935 to Present

Following the cessation of the LIA, δ13C values indicate that climate shifted rapidly towards drier conditions until AD 1938. The extremely low values between AD 1935 and 1939 suggest this was one of the driest periods experienced at the site. Severe droughts are recorded in other regions of Eastern Europe (Majer et al., 2000; Bündgen et al., 2011; Ionita et al., 2015) and agree with the drier conditions suggested by the δ13C record of Forray et al. (2015) in the south-eastern Apuseni Mountains (Fig. 1; locality 6). Additionally, this event follows a large increase in charcoal accumulation in the Rodna Mountains at AD 1920 and higher values overall between AD 1900 and 1950 (Feurdean et al., 2015). The increased burning suggests warmer and drier conditions during this interval, consistent with the δ13C values of this study and historical records (Sandu et al., 2008).

However, in disagreement with other climate reconstructions for Romania are the progressively wetter conditions that occurred since AD 1938 inferred from the Măguri δ13C record (Fig. 6A). The δ15N record from this core also suggests a drier winter climate during this interval (Cleary et al., 2017). While the δ13C values suggest wetter conditions since AD 1938, this is highly unlikely due to the aforementioned reduced precipitation across other European regions. This discrepancy can be explained by a shift in the control of plant WUE within the region. Although the δ13C values differ from both local (Fig. 7) and European (Fig. 6F,E) precipitation records, there are similarities between the Măguri record and the warming trends of European temperature reconstructions (Fig. 6D,G) and local soil temperature (Fig. 7). Increasing temperatures can influence evaporative demand, therein controlling water available to plants during the growing season (Selb et al., 2008). If temperatures reach a sufficient magnitude this climatic parameter could become a more
important factor influencing plant WUE than the amount of water received during the growing season. Although infrequently considered, there is evidence of mean annual temperature having a significant impact on carbon isotope discrimination in alpine areas (Xu et al., 2015). The atmospheric and soil temperatures in the region reaching maximum values of ~19 and 22°C could represent this magnitude (Fig. 7). Because temperature is one of the most critical factors that controls plant growth (Xu et al., 2015), and the primary forest constituents have been trees that prefer cool and wet conditions (F. sylvatica; Geantă et al., 2012) it is plausible that a warming climate could become the most influential on plant WUE. The soil temperature, at Baia Mare meteorological station, shows a positive and significant trend (99% significance level) over the last ~40 years (Fig. 7), while the total precipitation amount shows no trend. Therefore, we interpret the $\delta^{13}C$ record to indicate a warming trend beginning at AD 1938 until AD 2012. During this interval the climate in the region during the growing season became progressively warmer, consistent with other Romanian and European observations and reconstructions (Ionita et al., 2016). Within the area around Zidită Cave, plant WUE continued to be controlled by water availability (Forray et al., 2015), which could be due to its position at a higher altitude (410 m a.s.l.) within the Apuseni Mountains where annual temperatures are lower. Alternatively, Măgurici Cave lies at a lower elevation onto the Someș Plateau, where temperatures are comparatively higher during the growing season. The inferred warming trend after AD 1938 at Măgurici is consistent with the temperature increase at Călimani Mountains (Fig. 6G) and Budapest (Fig. 6H). Thus, temperature must be considered as a possible influence of plant WUE in future guano-derived $\delta^{13}C$ studies, particularly with respect to samples deposited after the industrial revolution.

Conclusions
These results indicate that the MWP and LIA had a substantial impact on north-west Romanian climate. The $\delta^{13}C$ record from Măgurici guano suggests the MWP to have occurred from AD 881 until AD 1240, consistent with other studies in the region (Feurdean et al., 2015; Onac et al., 2015). The inferred drier conditions during this interval agree with other records in Romania and Europe. While the timing of the LIA in Romania appears to vary with location within the Carpathian Mountains, the presence of the hiatus within our record suggests an initiation at AD 1250. From AD 1600 until the conclusion of this climatic event, $\delta^{13}C$ values suggest progressively wetter conditions. The abrupt shift towards drier conditions at AD 1893 is interpreted here to signify the termination of the LIA in the region. From AD 1938 to 2012, the $\delta^{13}C$ values suggest that temperature had a greater effect on plant WUE than precipitation. The addition of these significant findings to other guano studies indicates that the proxy will be valuable in future studies attempting to decipher past climate and environments.

Acknowledgements. We thank A. Giurgiu who helped during coring and initial sampling activities. This research was supported through Romanian CNCS grant PN-II-ID-PCE 2011-3-0588 to B.P.O.

Abbreviations. AP, arboreal pollen; CAM, crassulacean acid metabolism; LIA, Little Ice Age; LPAZ, local pollen assemblage zone; MWP, Medieval Warm Period; NAP, non-arboreal pollen; WUE, water-use-efficiency.

References


Onac BP, Hutchinson SW, Geanta A et al. 2015. A 2500-yr Late Holocene multi-proxy record of vegetation and hydrologic changes


APPENDIX D: COPYRIGHT PERMISSION FROM JOHN WILEY AND SONS TO USE THE ARTICLE A GUANO-DERIVED $\delta^{13}C$ AND $\delta^{15}N$ RECORD OF CLIMATE SINCE THE MEDIEVAL WARM PERIOD IN NORTH-WEST ROMANIA IN DISSERTATION
This Agreement between Daniel Cleary ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number 4536081235531
License date Feb 25, 2019
Licensed Content Publisher John Wiley and Sons
Licensed Content Publication Journal of Quaternary Science
Licensed Content Title A guano-derived δ13C and δ15N record of climate since the Medieval Warm Period in north-west Romania
Licensed Content Author Daniel M. Cleary, Bogdan P. Onac, Ioan Tanțău, et al
Licensed Content Date Jul 26, 2018
Licensed Content Volume 33
Licensed Content Issue 6
Licensed Content Pages 12
Type of use Dissertation/Thesis
Requestor type Author of this Wiley article
Format Print and electronic
Portion Full article
Will you be translating? No
Title of your thesis / dissertation Past hydroclimate and vegetation variation in Romania inferred from isotopic geochemistry and pollen of cave bat guano
Expected completion date May 2019
Expected size (number of pages) 85
Requestor Location Daniel Cleary
407 Monterey BLVD NE
SAINT PETERSBURG, FL 33704
United States
Attn: Daniel Cleary
Publisher Tax ID EU826007151
Total 0.00 USD

TERMS AND CONDITIONS
This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work.
(collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at http://myaccount.copyright.com).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.

- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, and any CONTENT (PDF or image file) purchased as part of your order, is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.

- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner. For STM Signatory Publishers clearing permission under the terms of the STM Permissions Guidelines only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts, You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding
("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto.

- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.
This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.

Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.

These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.

WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

**WILEY OPEN ACCESS TERMS AND CONDITIONS**

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

**The Creative Commons Attribution License**

The [Creative Commons Attribution License (CC-BY)](https://creativecommons.org/licenses/by/) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

**Creative Commons Attribution Non-Commercial License**

The [Creative Commons Attribution Non-Commercial (CC-BY-NC) License](https://creativecommons.org/licenses/by-nc/) permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)
Creative Commons Attribution-Non-Commercial-NoDerivs License
The Creative Commons Attribution Non-Commercial-NoDerivs License (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations
Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee. Further details can be found on Wiley Online Library http://olabout.wiley.com/WileyCDA/Section/id-410895.html

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.