MULTI-SCALAR APPROACHES TO STABLE ISOTOPE ECOLOGY OF LATE QUATERNARY PROBOSCIDEANS IN THE MIDCONTINENT

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ABSTRACT

Stable isotopes of mammoths and mastodons have the potential to illuminate patterns in late Glacial landscapes and megafaunal population dynamics as these species approached extinction. The ecological factors at play in this extinction remain unresolved. Stable isotopes of bone collagen ($\delta^{13}C$, $\delta^{15}N$) and tooth enamel carbonate ($\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$) in late Glacial proboscideans from the Midwest, USA are leveraged to examine ecological and behavioral changes in these species during the last glaciation. Mammoth and mastodon $\delta^{13}C$ values suggest they are flexible mixed feeders with significant C3 contributions to their diets. Bone collagen $\delta^{15}N$ is also highly variable throughout this period. However some mastodons exhibit low $\delta^{15}N$ values during the B□lling-Aller□d. These low values could be explained by invoking changes in individual life histories (i.e., age at weaning), the expansion of available ecological niches, or both, metabolic compensation, or a mixture of these processes. Serial enamel samples show large differences between interglacial, mid-Wisconsin, and late Glacial mammoth oxygen isotope values. Finally, a new micro-milling approach to sampling mammoth tooth enamel is used to examine the life histories of two Missouri mammoths to provide nuanced, seasonally-resolved paleoecological information.

INTRODUCTION

The fate of mammoths and mastodons from the midcontinent of North America is key to understanding late Pleistocene extinctions. Whether extinctions are viewed as human-induced (Mosimann and Martin 1975; T. A. Surovell et al. 2016; T. Surovell, Waguespack, and Brantingham 2005; Alroy 2001; Fisher 2009), the result of late Glacial landscape changes (A. J. Stuart et al. 2004; Widga et al. 2017; Nogués-Bravo et al. 2008), a function of other ecological processes (Ripple and Van Valkenburgh 2010), or some combination, this region is unparalleled in its density of large, late Quaternary vertebrates and associated paleoecological localities. For these reasons, the midcontinent has proven instrumental in understanding ecological dynamics in proboscidean taxa leading up to extinction. In recent years, it has been recognized that multiple proboscidean taxa shared an ecological niche in late Glacial landscapes (Saunders et al. 2010) despite distinct extinction trajectories (Widga et al. 2017). Chronological studies indicate that proboscideans in this region experienced extinction *in situ*, rather than mobilizing to follow preferred niche space (Saunders et al. 2010). Despite this recent work leading to a more refined understanding of mammoths and mastodons in the region, many significant questions remain. Modern megafauna have a profound impact on local vegetation (Guldemond and Van Aarde 2008; Valeix et al. 2011), and it is unclear what effect late Glacial mammoths and mastodons would have had on canopy cover, nutrient cycling, and fruit dispersal in non-analogue vegetation communities. Furthermore, there are still issues of equifinality regarding the effect of human predators (Fisher 2009), or virtual absence of both human and non-human predators (Widga et al. 2017) in various scenarios of late Pleistocene ecosystem changes.

The high profile and acrimonious debate surrounding the cause of late Pleistocene megafaunal extinctions has spurred a number of productive regional studies to address the timing

and paleoecology of extinction in megafaunal taxa (Widga et al. 2017; Anthony John Stuart 2015; Anthony J. Stuart and Lister 2012; 2011; Pacher and Stuart 2009). The results of this research serve to constrain the number of possible extinction scenarios.

As in modern ecosystems, Proboscidea in late Pleistocene North America were long-lived taxa that likely had a profound impact on the landscape around them. Due to their size and energetic requirements, elephantoids are a disruptive ecological force, promoting open canopies in forests through tree destruction (Chafota and Owen-Smith 2009) and trampling vegetation (Plumptre 1994). Their dung is a key component of soil nutrient cycling (Owen-Smith 1992; Augustine, McNaughton, and Frank 2003), and was likely even more important in N limited tundra and boreal forest systems of temperate areas during the late Pleistocene. Even in death, it is probable that mammoths and mastodons wrought major changes on the systems within which they were interred (Keenan et al. 2018; Coe 1978). Precisely because proboscideans play such varied and important roles in the ecosystems they inhabit, they are good study taxa to better understand Pleistocene ecosystems. They are also central players in many extinction scenarios.

One of the major challenges to ecological questions is scale (Denny et al. 2004; Delcourt and Delcourt 1991; Davis and Pineda Munoz 2016). Processes that are acting at the level of an individual or a locality can vary significantly in space and time (Table 1; Figure 1). Paleoecological data collected from individual animals from local sites, is constrained by larger regional patterns that may or may not be apparent (e.g., taphonomic contexts, predator-prey dynamics). Paleoecological studies of vertebrate taxa often begin with the premise that the individual is an archive of environmental stimuli experienced in life. In long-lived taxa such as proboscideans, this window into past landscapes may span many decades. This longevity is both a benefit and a challenge to studies of proboscidean paleoecology.

Table 1. Scales of paleoecological analysis.

Scale	Method	Phenomena	Selected References
Small (Days)	Stomach contents, enamel micro-wear	Animal health, Landscape (local scale)	Green, DeSantis, and Smith 2017; Smith and DeSantis 2018; Rhodes et al. 1998; Teale and Miller 2012.
Meso (Weeks-Months)	Hair, Tusk/Tooth dentin, Tooth enamel	Seasonal diets, Reproductive events (e.g., musth, calving), Migration/Dispersal	Fox and Fisher 2001; Fisher 2018; Hoppe 2004; Hoppe and Koch 2007; Cerling et al. 2009; Metcalfe and Longstaffe 2012
Large (Years - Decades)	Bone collagen, tooth enamel, tooth mesowear	Climate/Vegetation trends, Large-scale land-use trends, Population-level trends in diet responses to landscape changes.	Iacumin et al. 2010; Szpak et al. 2010; Bocherens et al. 1996

Some approaches offer relatively high-resolution pictures of animal ecology at a scale that is of a short duration (days). Micro-wear analyses of dentin and enamel (Green, DeSantis, and Smith 2017; Smith and DeSantis 2018) are increasingly sophisticated, and have the potential to track short-term dietary trends. The remains of stomach contents also provide ecological information at this scale (Lepper et al. 1991; Teale and Miller 2012; Birks et al. 2019; van Geel et al. 2011; Fisher et al. 2012; Newsom and Mihlbachler 2006), which are essentially the 'last meal' representing a few hours of individual browsing. These techniques offer paleoecological insights that are minimally time-averaged, and at a time-scale that may be comparable to modern observations of animal behavior.

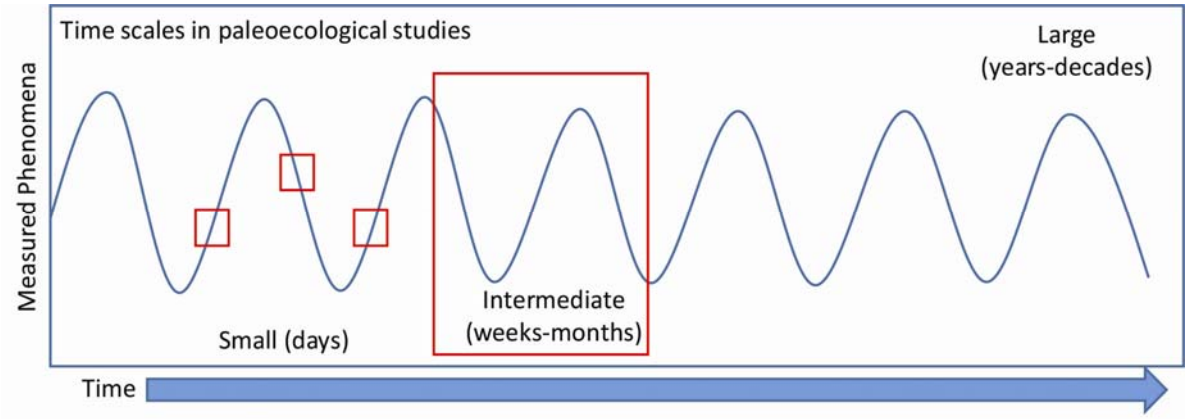

Figure 1. Scales of paleoecological analyses.

Other approaches resolve time periods that are weeks to months in duration. Fisher's (Fisher 2018; 2009; Fisher and Fox 2006) work on incremental growth structures in proboscidean tusk and molar dentin reliably record weekly to monthly behaviors. The resolution of these methods may even include short-term, often periodic, life history events, such as reproductive competition (musth) and calving. Other researchers (Hoppe et al. 1999; Metcalfe and Longstaffe 2012; 2014; Pérez-Crespo et al. 2016) have explored incremental growth trends in proboscidean tooth enamel. Adult molars form over the course of 10-12 years with an enamel extension rate of ~1 cm/year in both modern elephants (Uno et al. 2013) and mammoths (Dirks, Bromage, and Agenbroad 2012; Metcalfe and Longstaffe 2012) providing the opportunity to understand meso-scale (potentially monthly) changes in diet and behavior.

Finally, some techniques measure animal diet and behavior over much longer scales (years-decades). In humans, bone collagen is replaced at a rate of 1.5-4% per year (Hedges et al. 2007). For equally long-lived proboscidean taxa, this means that stable isotope analyses of bone collagen is essentially sampling a moving average of ~20 years of animal growth. For younger age groups (<20 years), this average will be weighted towards time periods of accelerated maturation (adolescence), when collagen is replaced at a much greater rate (up to 15% in human adolescents) (Hedges et al. 2007). Tooth enamel can also be sampled at a resolution (i.e., "Bulk" enamel) that averages a year (or more) of growth (Baumann and Crowley 2015; Hoppe 2004), and it is likely that this is the approximate temporal scale that is controlling tooth mesowear (Fortelius and Solounias 2000).

Stable isotope studies are an important part of the paleoecological toolkit for understanding Quaternary proboscideans. Progressively larger, more complete datasets

characterize isotopic studies of Beringean mammoths, where stable carbon and oxygen isotopes in bone collagen and tooth enamel reliably track climate and landscape changes over the last glacial period (Iacumin et al. 2010; Bocherens et al. 1996; Szpak et al. 2010; Arppe et al. 2019), the place of mammoths in regional food webs (K. Fox-Dobbs et al. 2007; Kena Fox-Dobbs, Leonard, and Koch 2008), and characteristics of animal growth and maturation (Metcalfe and Longstaffe 2012; El Adli et al. 2017; Rountrey et al. 2012).

These studies have also been important to understanding both lineages of proboscideans in temperate North America. From the west coast (Coltrain et al. 2004; El Adli et al. 2015) to the southwestern (Metcalfe et al. 2011), and eastern US (Koch, Hoppe, and Webb 1998; Hoppe and Koch 2007), isotopic approaches have been very successful in understanding local to regional scale behavior in mammoths and mastodons. Although there have been efforts to understand mammoth and mastodon behaviors in the Midwest at relatively limited geographic scales (Baumann and Crowley 2015; Saunders et al. 2010), there is a need to systematically address long term isotopic trends throughout the region. In this paper, we approach this problem from a broad regional perspective, leveraging a new dataset of >100 ^{14}C dates with associated isotopic data on bone collagen. We also utilize an enamel dataset consisting of both serial bulk and micro-milled mammoth molar enamel samples (C, O, Sr isotope systems). Together, the results of these analyses offer a picture of mammoth and mastodon diets ($\delta^{13}C$, $\delta^{15}N$), late Quaternary paleoclimate ($\delta^{18}O$), and animal mobility ($^{87}Sr/^{86}Sr$) that is geographically comprehensive and spans the last 50,000 years.

MATERIALS AND METHODS

The proboscidean dataset utilized for this study (Figure 2; SM Table 1) was acquired with the goal of understanding mammoth and mastodon population dynamics in the late Glacial period, as these taxa approach extinction. Chronological and broad-scale paleoecological implications for this dataset have been explored in Widga et al. (2017) and more recently in Broughton and Weiztel (2018). However, in this paper we focus on the implications of these data for the stable isotope ecology of midwestern Proboscidea. We also discuss annual patterns in serially sampled mammoth teeth from glacial and interglacial settings. Finally, we sampled two mammoths from Jones Spring, Hickory Co., MO. One of these mammoths (305JS77) is from an interglacial-aged deposit. The other (64JS73) dates to the middle part of the last glaciation, ~50,000 ka.

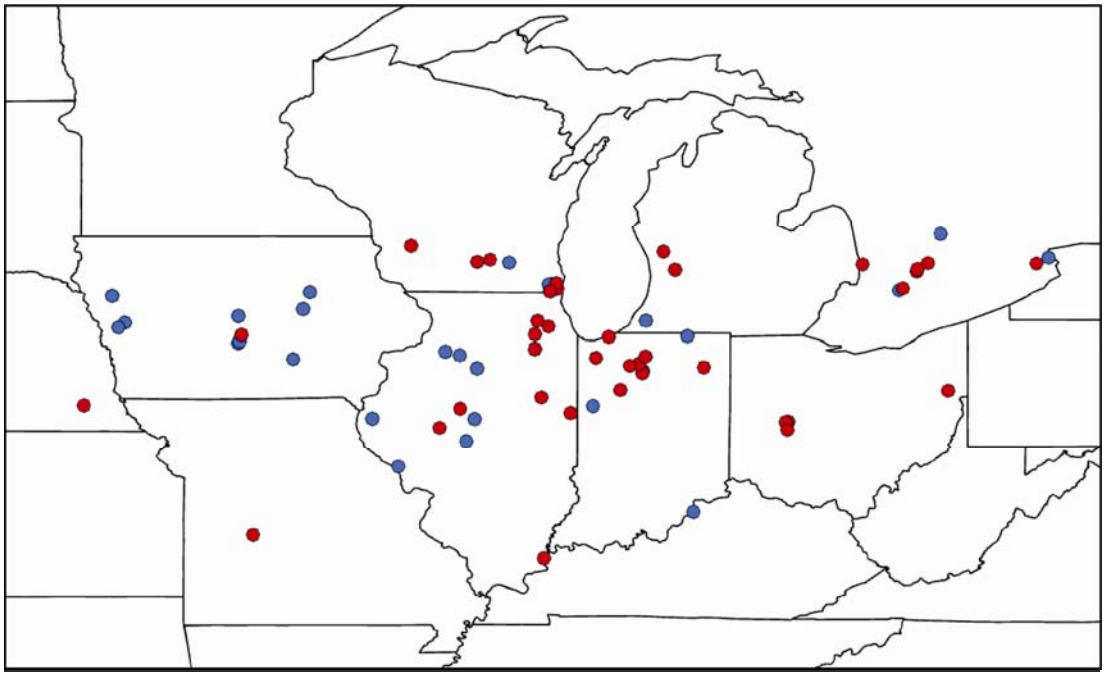

Figure 2. Map of dated midwestern mammoths and mastodons (from Widga et al., 2017). Mastodons=red; Mammoths=blue.

Mammoth and Mastodon bone collagen. Proboscidean samples were selected to widely sample midwestern Proboscidea, both stratigraphically and geographically (Widga et al. 2017). Due to extensive late Wisconsin glaciation in the region, this dataset is dominated by samples dating to the Last Glacial Maximum (LGM) or younger (<22 ka). Only 14 (15%) out of 93 localities predate the LGM.

All samples were removed from dense bone, tooth or tusk dentin and submitted to the University of Arizona AMS laboratory. Collagen was extracted using standard acid-base-acid techniques (Brock et al. 2010), its quality evaluated visually, and through ancillary Carbon:Nitrogen (CN) analyses. Visually, well-preserved collagen had a white, fluffy appearance and C:N ratios within the range of modern bones (2.9-3.6) (Tuross, Fogel, and Hare 1988). Samples that had the potential to be terminal ages were subjected to additional analyses. The ABA-extracted gelatin was ultra-filtered (UF) through >30 kD syringe filters to isolate relatively undegraded protein chains (Higham, Jacobi, and Ramsey 2006). This fraction was also dated. All stable isotope samples were analyzed on a continuous-flow gas-ratio mass spectrometer coupled to an elemental analyzer at the University of Arizona. Standardization is based on acetanilide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}C$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}N$. Analytical precision (at 1σ) is better than ± 0.1‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. All $\delta^{13}C$ results are reported relative to Vienna Pee Dee Belemnite (VPDB) and all $\delta^{15}N$ results are reported relative to N-Air.

Serial and Micro-sampling of Mammoth tooth enamel. Mammoth enamel ridge-plates were sampled at two different scales. Serial sampling consisted of milling a series of 5-10 mg

samples of enamel powder with a handheld rotary tool equipped with a 2 mm diameter carbide bit along the axis of growth. Sample spacing was ~1 per centimeter of tooth growth. However, given the geometry of enamel maturation (Dirks, Bromage, and Agenbroad 2012), these samples at best, approximate an annual scale of dietary and water inputs.

Micro-sampling however, has the potential to illustrate sub-annual scale patterns in animal movement and behavior (Metcalfe and Longstaffe 2012). For this project, we built a custom micromill capable of *in situ*, micron-resolution, vertical sampling of a complete mammoth or mastodon molar. This micromill setup consisted of two Newmark linear stages coupled to a Newmark vertical stage to allow movement in 3-dimensions. These stages were controlled by a Newmark NSC-G 3-axis motion controller using GalilTools on a PC. A 4-cm diameter ball joint allowed levelling of a metal (Version 1) or acrylic (Version 2) plate for holding a specimen. The armature for V1 consisted of a 1971 Olympus Vanox microscope retrofitted with a stationary Proxxon 50/E rotary tool using a 0.5mm end mill. V2 has replaced this setup with a U-strut armature using a 3D printed drill mount to allow for greater vertical and horizontal movement to accommodate large, organically-shaped specimens. Specimens were stabilized on the mounting plate using heat-flexible thermoplastic cradle affixed to a metal plate with machine screws (V1). However, we later developed an acrylic mounting plate method where a mammoth tooth could be sufficiently stabilized using zip ties (V2). This micromill was developed to address the challenges of accurately micro-milling large specimens with minimal instrumentation costs. Complete plans for this micromill are available under an open hardware license at https://osf.io/8uhqd/?view_only=43b4242623a94a529e4c6ef2396345e9.

Micro-mill sampling resolution was 1 sample per millimeter along the growth axis of the tooth plate (Figure 3). Each sample was milled in 100µm-deep passes through the entire

thickness of the enamel. The lowest enamel sample in the series was used for isotopic analyses to minimize the effects of mineralization and diagenesis on the biological signal (Zazzo, Balasse, and Patterson 2006). Enamel powder was collected in de-ionized water to; 1) maximize sample recovery, and 2) lubricate the mill. These samples were too small for standard pretreatment of tooth enamel CO_3 (Koch, Tuross, and Fogel 1997). However, paired bulk enamel samples treated with 0.1 N acetic acid and 2.5% NaOCl show results that are the same as untreated bulk samples. Although this technique is both time- and labor- intensive, it is minimally invasive and is capable of sampling enamel growth structures at high resolution.

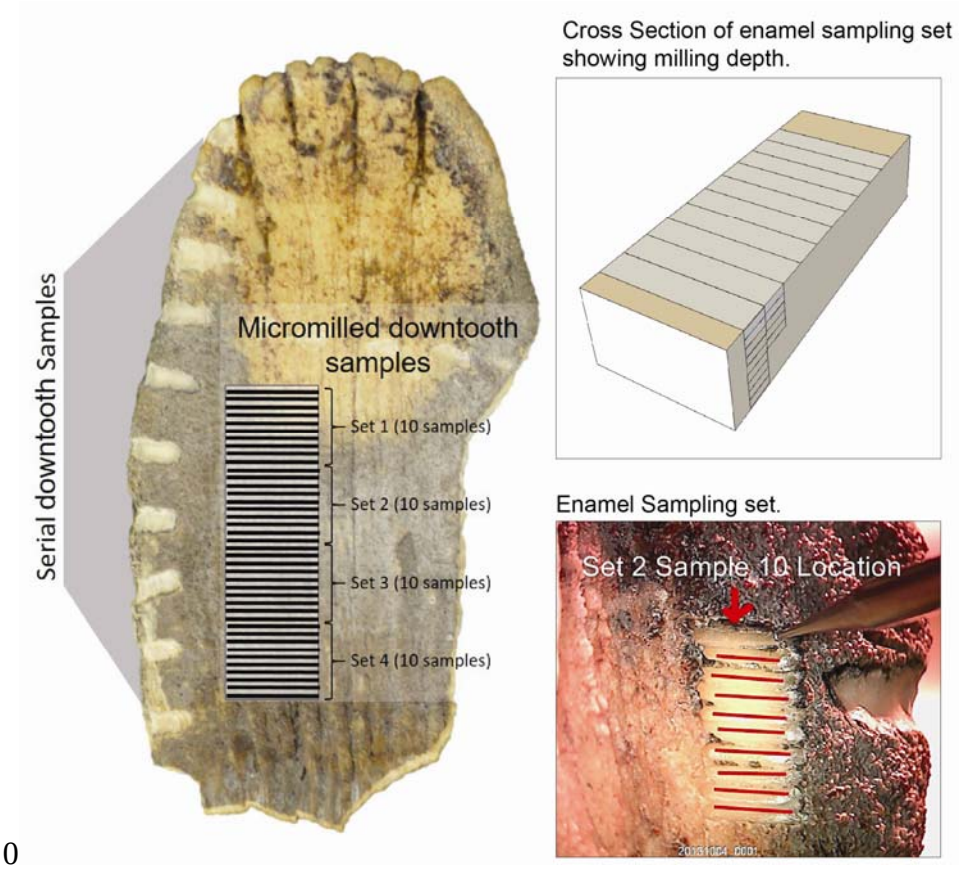

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Figure 3. Schematic illustration of serial and micro-sampling strategies.

All enamel powder samples were measured via a Finnigan Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler at the Iowa State University Stable Isotope lab (Department of Geological and Atmospheric Sciences. Reference standards (NBS-18, NBS-19) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Corrections were done using a regression method and isotope results are reported in per mil (‰). The long-term precision (at 1σ) of the mass spectrometer is ±0.09‰ for $\delta^{18}O$ and ±0.06‰ for $\delta^{13}C$, respectively, and precision is not compromised with small carbonate samples (~150 micrograms). Both $\delta^{13}C$ and $\delta^{18}O$ are reported VPDB.

$\delta^{13}C$ results are corrected -14.1‰ to approximate the $\delta^{13}C$ of dietary input ($\delta^{13}C_{diet}$) (Daniel Bryant and Froelich 1995), then converted to %C4 estimates with a dual endpoint mixing model using the distribution of $\delta^{13}C$ values in modern vascular plants to estimate end members (Post 2002; Lee-Thorp and Sponheimer 2003).

$$\%C4 = [\delta^{13}C\ diet-(-26.5)]/[-12.7-(-26.5)]*100$$

$\delta^{18}O$ results were converted from VPDB to SMOW using the equation

$$\delta^{18}O\ SMOW = (1.03086*\ \delta^{18}O\ VPDB)+30.86$$

The $\delta^{18}O$ of enamel phosphate ($\delta^{18}O_p$) for these samples was calculated from the $\delta^{18}O$ of enamel carbonate ($\delta^{18}O_c$) (Fox and Fisher 2001)

$$\delta^{18}O_p = (\ \delta^{18}O_c/1.106)-4.7288$$

Estimates of body water $\delta^{18}O$ ($\delta^{18}O_w$) were calculated following Dauxe et al. (2008)

$\delta^{18}O_w = (1.54* \delta^{18}O_p)-33.72$ (Daux et al. 2008)

The $^{87}Sr/^{86}Sr$ component of bioapatite reflects changes in the geochemical makeup of the surface an animal grazed across during tooth formation. In serial-scale analyses, enamel powder samples were split from the light isotope samples described above, and represent the same portions of mammoth teeth. Each ~5 mg sample of powder was leached in 500 μL 0.1 N acetic acid for four hours to remove diagenetic calcite and rinsed 3 times with deionized water (centrifuging between each rinse). In the micromilled series, small sample sizes prevented Sr analyses from being performed on the same samples as $\delta^{13}C$ and $\delta^{18}O$ analyses. Therefore, Sr from these growth series was analyzed opportunistically, or at the scale of 1 sample every 2mm.

All enamel samples were then dissolved in 7.5 N HNO_3 and the Sr eluted through ion-exchange columns filled with strontium-spec resin at the University of Kansas Isotope Geochemistry Laboratory. $^{87}Sr/^{86}Sr$ ratios were then measured on a Thermal Ionization Mass Spectrometer (TIMS), an automated VG Sector 54, 8-collector system with a 20-sample turret, in the University of Kansas Isotope Geochemistry laboratory. Isotope ratios were adjusted to correspond to a value of 0.71250 on NBS-987 for $^{87}Sr/^{86}Sr$. We also assumed a value of $^{86}Sr/^{88}Sr$ of 0.1194 to correct for fractionation.

The distribution of $^{87}Sr/^{86}Sr$ values in vegetation across the surface of the midcontinent is determined by the values of soil parent material. In a large part of this region, surface materials are composed of allochthonous Quaternary deposits such as loess, alluvium, and glacial debris. Therefore continent-scale Sr isoscape models derived from bedrock or water (Bataille and Bowen 2012) are not ideal for understanding first order variability in midwestern $^{87}Sr/^{86}Sr$. For these reasons, Widga et al. (2017) proposed a Sr isoscape for the Midwest based on surface

vegetation. At a regional scale, these trends in vegetation $^{87}Sr/^{86}Sr$ reflect the Quaternary history of the region, and are consistent with other, empirically derived, patterns in Sr isotope distribution from the area (Hedman et al. 2018; Slater, Hedman, and Emerson 2014). Sr isotope values in mammoth enamel are compared to a $^{87}Sr/^{86}Sr$ isoscape constructed from the combined datasets of Widga et al. (2017) and Hedman et al. (2009), and Hedman et al. (2018)(Figure 4).

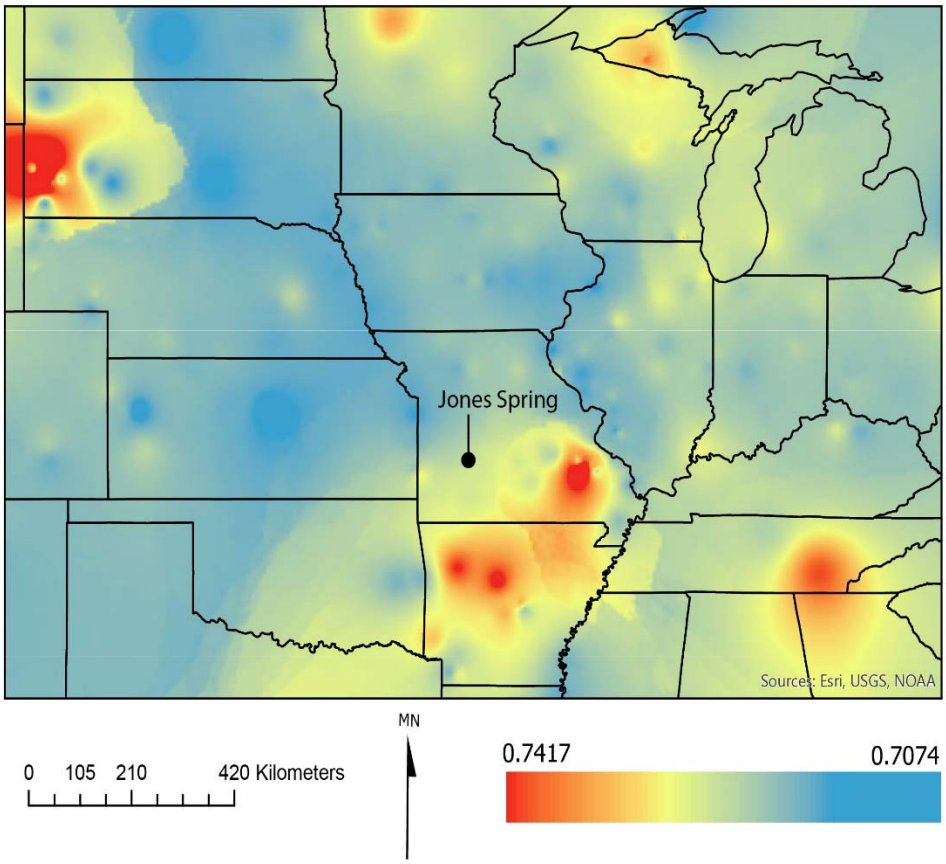

Figure 4. Midwestern Sr isoscape, data from Widga et al. (2017), Hedman et al. (2009, 2018).

RESULTS

When looking at large-scale changes in stable isotopes from mammoth and mastodon bone collagen, there is substantial inter-taxonomic overlap in the $\delta^{13}C_{coll}$ values throughout the last 30 ka (Figure 5, Table 2). Both taxa show average $\delta^{13}C_{coll}$ values around -26‰, consistent with a diet dominated by C3 trees, shrubs, and grasses. This is broadly consistent with variable, but shared diets during time periods when both taxa occupied the region.

However, there is a group of mastodons that show lower $\delta^{15}N$ values due to a significant, late expansion into a low $\delta^{15}N$ niche during the B□lling-Aller□d (B-A) when the average $\delta^{15}N$ values decrease to values <5‰. A similar shift is not evident in mammoths at this time.

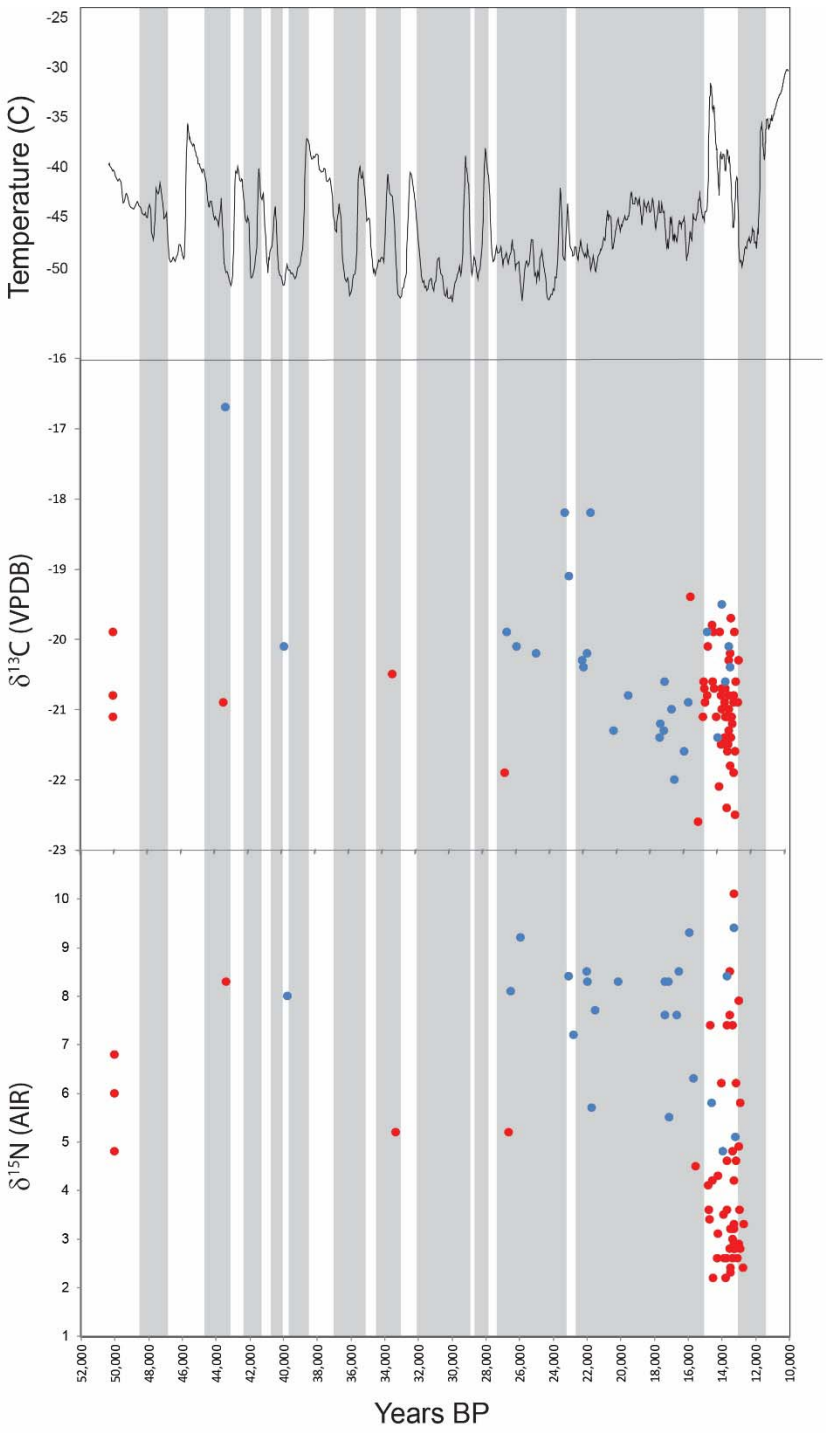

Figure 5. Changes in midwestern proboscidean collagen $\delta^{13}C$ and $\delta^{15}N$ through time. Mastodons=red, Mammoths=blue, Glacial stadials = grey bars. Temperature from GISP2 (Alley 2000).

Table 2. Mastodon and Mammoth collagen stable isotope values, by chronozone.

Taxon	Chronozone	N	$\bar{x}$ $\delta^{13}C$ (s.d)	$\bar{x}$ $\delta^{15}N$ (s.d)
Mammut	Aller□d	38	-21.1 (0.6)	4.2 (2)
	B□lling	6	-20.4 (0.5)	3.8 (1.5)
	Oldest Dryas	5	-20.5 (0.7)	4.6 (1.6)
	LGM II	0		
	LGM I	0		
	Pre-LGM	6	-20.9 (0.7)	6.1 (1.3)
	Average	57/55*	-21.0 (0.7)	4.4 (1.9)
Mammuthus	Aller□d	3	-20.6 (0.9)	6.1 (2.0)
	B□lling	1	-19.9	5.8
	Oldest Dryas	4	-21.4 (0.5)	7.9 (1.3)
	LGM II	4	-21.1 (0.4)	7.4 (1.3)
	LGM I	5	-20.1 (1.1)	7.6 (1.2)
	Pre-LGM	5	-19.6 (0.8)	8.2 (0.7)
	Average	25/21*	-20.4 (1.0)	7.5 (1.3)

*Available N for $\delta^{13}C$ is larger than for $\delta^{15}N$.

Stable isotope values of serially sampled mammoth molars are likely influenced by regional climate (Table 3; Figure 6). All serial series were between 9 and 16 cm in length and represent multiple years. Stable oxygen isotopes of three late Glacial mammoths (Principia College, Brookings, Schaeffer) overlap significantly, while mid-Wisconsin (234JS75) and interglacial (232JS77) mammoths from Jones Spring show elevated values indicative of warmer conditions. The amplitude of serial series from late Glacial mammoths (average amp=2.5) was not significantly different from that of the mid-Wisconsin individual from Jones Spring (amp=2.9). However, the interglacial animal shows a smaller amplitude (amp=1.1) over the course of tooth formation. Strontium isotope ratios of this animal indicate a home range in the southern Ozarks, throughout tooth formation, with a period of relatively elevated $^{87}Sr/^{86}Sr$ values also corresponding to low $\delta^{18}O$ values.

Table 3. Summary of micro-sampled tooth enamel series: $\delta^{13}C$, $\delta^{18}O$, and $^{87}Sr/^{86}Sr$.

ID	$\delta^{13}C$				$\delta^{18}O$				$^{87}Sr/^{86}Sr$			
Serial Analyses	X	Min	Max	Amp	X	Min	Max	Amp	X	Min	Max	Amp
Principia College (late Glacial)	-10.9	-11.3	-10.0	1.4	-7.9	-8.7	-6.7	2.0				
Brookings Mammoth (late Glacial)	-9.9	-10.5	-9.4	1.1	-9.2	-10.0	-7.8	2.2				
Schaefer Mammoth (late Glacial)	-9.0	-9.9	-7.0	3.0	-8.3	-9.9	-6.6	3.3				
Jones Sp. Mammoth, 243JS75 (mid-Wisconsin)	-1.7	-2.3	-0.2	2.1	-3.4	-4.5	-1.6	2.9				
Jones Sp. Mammoth, 232JS77 (interglacial)	-2.8	-3.8	-1.3	2.5	-0.5	-4.6	3.6	1.1	0.7162	0.7146	0.7177	-0.0031
Micro-Analyses												
305JS77 (interglacial)	-2.4	-3.5	-0.2	3.3	-3.9	-4.9	-2.9	2.0	0.7109	0.7106	0.7118	-0.0012
64JS73 (mid-Wisconsin)	-2.8	-4.3	-1.7	2.6	-4.9	-12.0	-0.4	11.7	0.7152	0.7140	0.7164	-0.0025

Two mammoth teeth from the Jones Spring locality in Hickory Co., MO were micro-sampled (Figure 7). These specimens are from beds representative of interglacial (MIS 5e) and mid-Wisconsin (MIS 4) deposits.

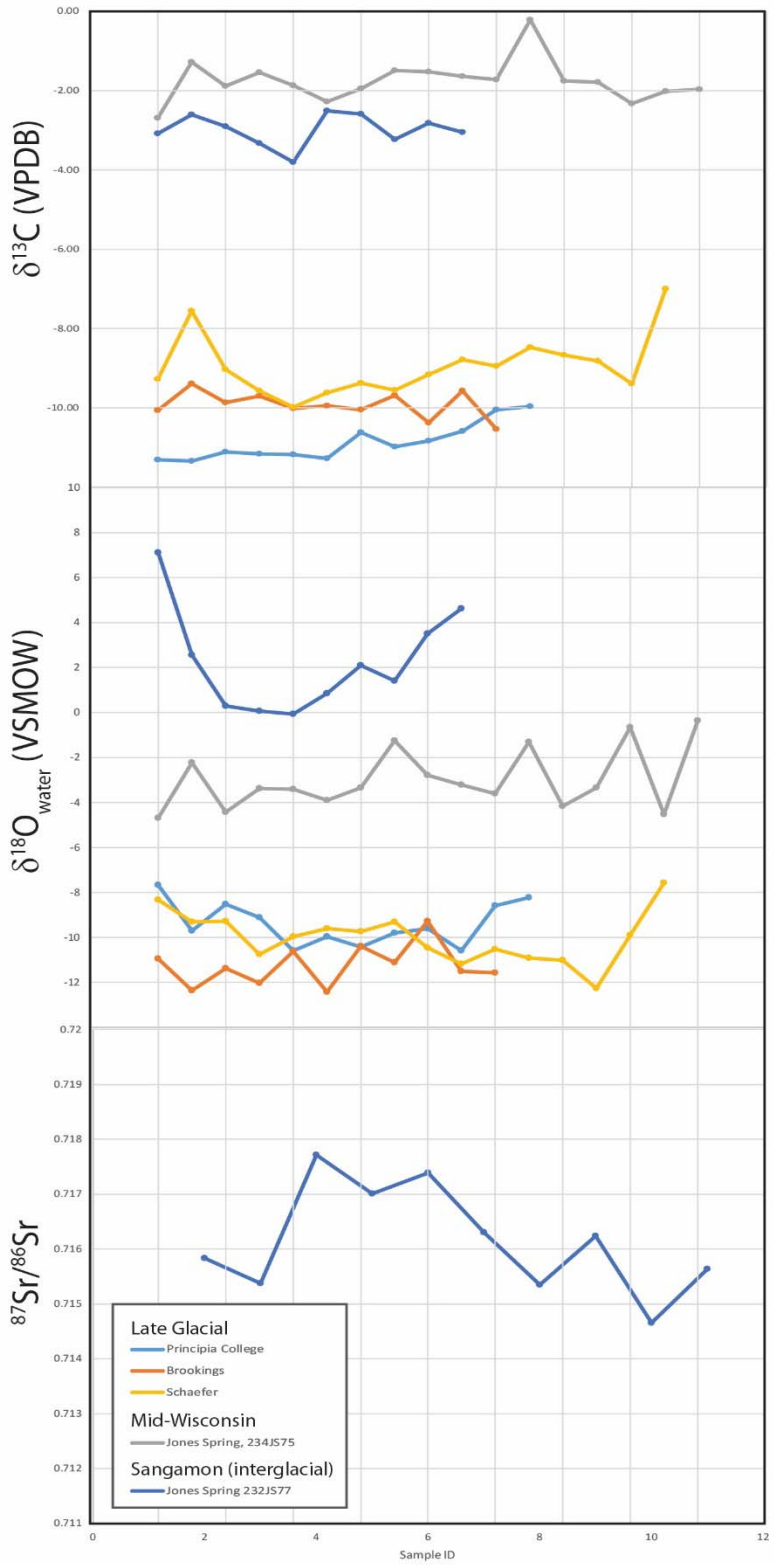

Figure 6. Time series, serial enamel $\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$.

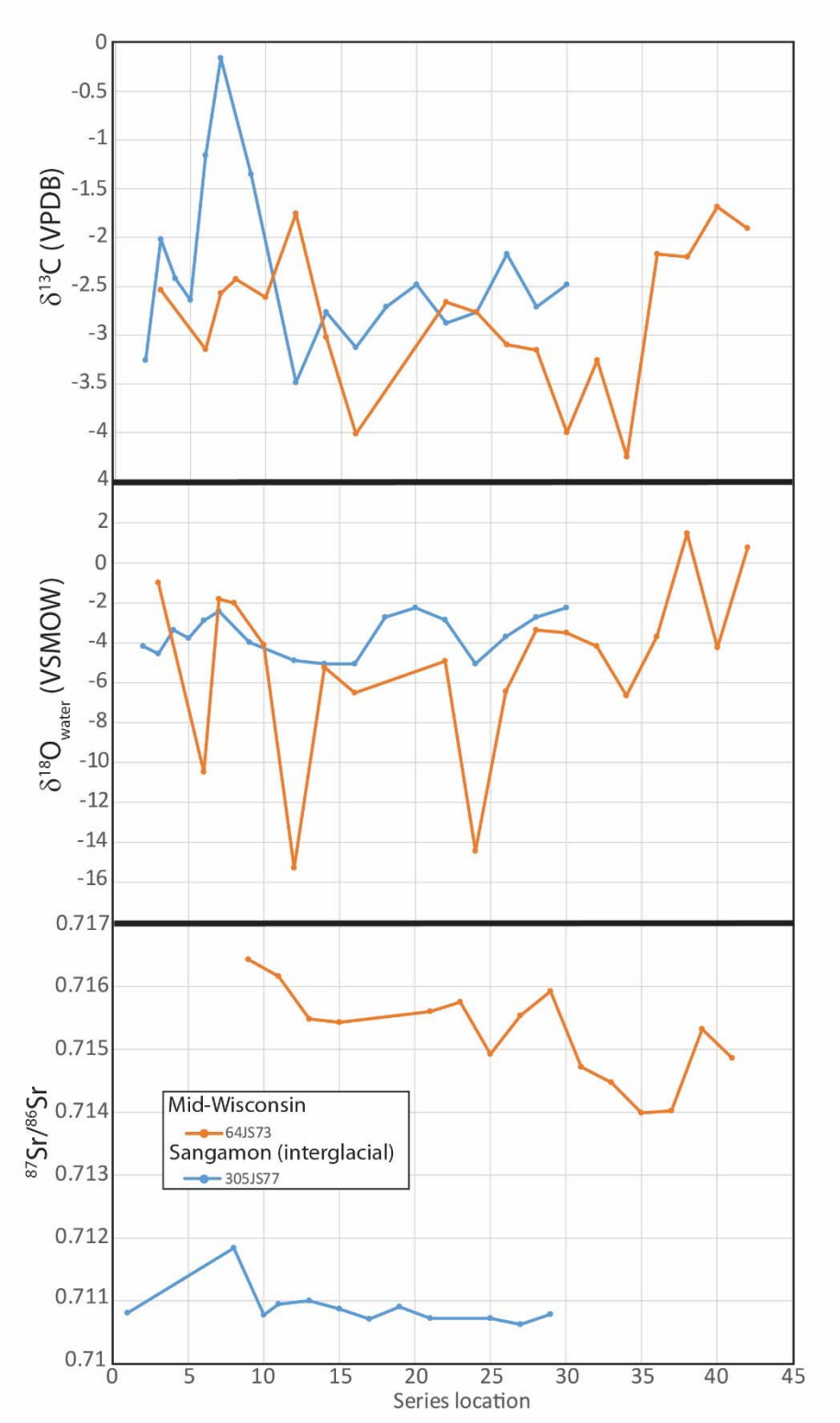

Figure 7. Time series, micro-sampled enamel $\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$.

The mid-Wisconsin molar (64JS73) shows regular negative excursions in the $\delta^{18}O$ values suggestive of seasonal temperature changes in ingested water. These excursions are relatively short-lived and extreme. Seasonal variation in enamel growth rate and maturation may account for the perceived short length of these periods. Maximum $\delta^{18}O$ values in both mammoths are similar however, the interglacial mammoth lacks these negative excursions and exhibits values that are more complacent through the length of the tooth.

The $\delta^{13}C$ series from both molars indicate these mammoths experienced a C4 diet throughout the year. However, deep dips in the $\delta^{18}O$ series of the mid-Wisconsin molar correspond to at least two temporary peaks in the $\delta^{13}C$ series.

The $^{87}Sr/^{86}Sr$ isotope series from both animals demonstrate an adherence to separate home ranges. The interglacial animal shows values similar to bedrock units outcropping locally in western Missouri. The mid-Wisconsin animal, however, had a home range across rock units with much higher $^{87}Sr/^{86}Sr$ values. The home ranges for these animals do not overlap, despite their recovery from different strata within the same locality.

DISCUSSION

Overall, mammoths and mastodons exhibit similar trends in isotopic ecology throughout the late Glacial. The two taxa have indistinguishable $\delta^{13}C_{coll}$ values, and there is no apparent chronological trend as these two species approach extinction in the Younger Dryas. Changes in moisture during the late glacial corresponded with relatively large changes in vegetation and the contribution of grasses to proboscidean diets (Voelker et al. 2015; Gonzales et al. 2009; Saunders et al. 2010). These changes may not be apparent in the proboscidean $\delta^{13}C_{coll}$ due to similar isotopic composition of C3 grasses and C3-dominated closed forests. Trends in mammoth

$\delta^{13}C_{coll}$ are similar across their range during the late Wisconsin. Siberian and European mammoths exhibit a similar range of $\delta^{13}C_{coll}$ values (Iacumin et al. 2010; Szpak et al. 2010; Arppe et al. 2019; Schwartz-Narbonne et al. 2019).

In both taxa, mean $\delta^{15}N_{coll}$ decreases slightly throughout the sequence, however, the minimum $\delta^{15}N$ values for B-A mastodons is significantly lower than either taxa that were sampled over the last 30 ka.

Stable nitrogen isotope values of these populations are generally similar to midwestern proboscideans that lived prior to the B-A. Although the anomalously low values of B-A mastodons are also shared with other mastodons in the Great Lakes region (Metcalfe, Longstaffe, and Hodgins 2013), they are significantly lower than Eurasian or Beringean mammoths (with some exceptions, Drucker et al. 2018).

The underlying ecological cause(s) of these low $\delta^{15}N_{coll}$ values is unclear. Flux in soil and plant N can be a function of plant-based N_2 fixation (Shearer and Kohl 1993), rooting depth (Schulze, Chapin, and Gebauer 1994), N loss related to climate factors (Austin and Vitousek 1998; Handley and Raven 1992), microbial activity, or mycorrhizal colonization (Michelsen et al. 1998; Hobbie, Macko, and Williams 2000). Furthermore, the role of large herbivore populations in N flux may be significant (Frank, Evans, and Tracy 2004). In N limited environments such as tundra, terrestrial plants may receive relatively more N from inorganic sources. Boreal forests like those of the late Glacial Midwest, however, exhibit relatively greater biological productivity, so plant shoots are more likely to take in volatized ammonia (low $\delta^{15}N$) from organic sources such as urea (Fujiyoshi et al. 2017).

At this time, it is difficult to distinguish which (if any) of these scenarios had an impact on late Quaternary $\delta^{15}N$ values of proboscideans. Although there is a wide range in $\delta^{15}N$ of

mammoths and mastodons throughout the late Glacial sequence, $\delta^{15}N$ values below 5‰ are only present in mastodons during the B-A in the lower Great Lakes (39-43 deg. latitude), throughout an area suggested to be vegetated by a disharmonius flora dominated by Black Ash and Spruce (Gonzales and Grimm 2009; Gill et al. 2009) (Figure 8). The low $\delta^{15}N$ values suggest that these mastodons occupied an undefined, local-scale, dietary niche that was not shared by contemporary mammoths, or by earlier mastodons. Isotopically light plant shoots can occur with increased utilization of volatized NH_4 from organic sources, including urea from herbivores. The B-A may have been a time of high mastodon populations in the Great Lakes region (Widga et al. 2017). Concentration of these populations around resources (e.g., water sources) may have had an impact on the $\delta^{15}N$ of browsed plants due to increased contributions of herbivore urea or were a function of an overall increase in biological productivity (Sah, Rita, and Ilvesniemi 2006).

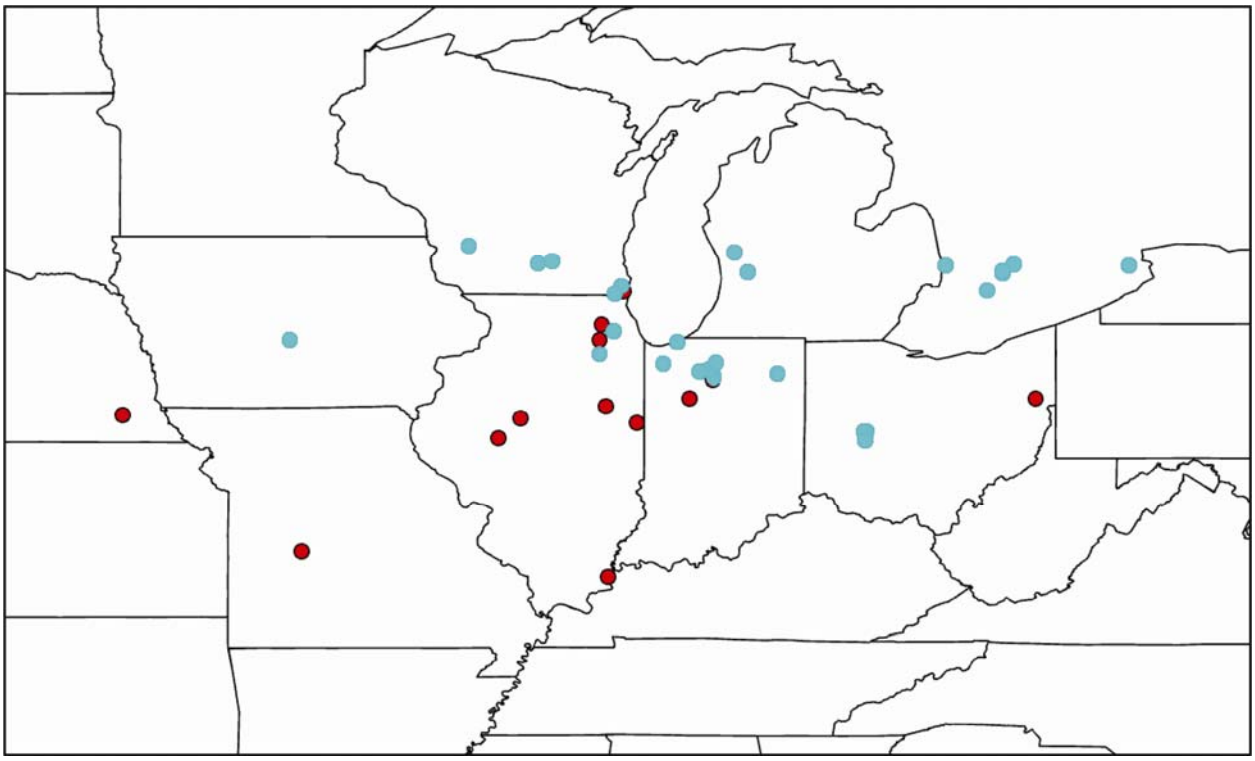

Figure 8. Mastodon distribution during the B-A. Blue circles indicate mastodon $\delta^{15}N < 5$ per mil. Dark red circles are mastodons with $\delta^{15}N > 5$ per mil.

Life Histories and Landscape change in Jones Spring Mammoths

Fine-scale treatments of mammoth life histories are possible through analyses of incremental growth structures, such as molar enamel or tusk dentin. In the case of the two mammoths from Jones Spring, MO, isotopic trends in each animal illustrates different life histories. The interglacial mammoth occupied the western Ozarks as indicated by $^{87}Sr/^{86}Sr$ values deposited throughout the development of the sampled portion of the tooth. It occupied a landscape where C4 vegetation was common, with mild winter temperatures. The mid-Wisconsin mammoth from Jones Spring occupied the central core of the Ozark uplift indicated by $^{87}Sr/^{86}Sr$ values deposited during the formation of the sampled tooth. However, between the cessation of enamel formation and death, this animal moved ~250 km to the western Ozarks where it was preserved in the Jones Spring deposits. The oxygen isotope series of this animal suggests greater seasonality during the mid-Wisconsin when compared to the interglacial mammoth, with deep negative excursions during the cold season. It also had a diet composed primarily of C4 vegetation.

These results suggest a similar scale to landscape use strategies employed by late Quaternary mammoths in the Midwest as those suggested by Hoppe (Hoppe et al. 1999; Hoppe 2004) for mammoths in the Great Plains and Florida. Further, although $\delta^{13}C_{coll}$ values in mammoths clearly suggest a niche that, at times, included C3-dominated diets in a region that would be dominated by forest, not grasslands, both Jones Spring mammoths were mixed feeders with C4 grasses making up a significant part of their diet.

Testing scenarios of late Pleistocene Proboscidean population dynamics

Can this stable isotope dataset illuminate trends in late Proboscidean population dynamics during the late Glacial period in the Midwest? It is possible that this shift represents the colonization of a novel, $\delta^{15}N$ niche that also coincides with the climate and vegetational shift to the B-A. However, it is unclear what this niche might be. Drucker et al. (2018) noted anomalously low $\delta^{15}N$ values in LGM mammoths from Mezyhrich in central Europe, and attributed this pattern to a large, but unspecific, shift in dietary niche. Counter-intuitively, boreal forests typically have increased amounts of N fixing microbes relative to tundra environments, which would increase $\delta^{15}N$ in forage overall.

Some studies have suggested that the growth rate of an individual is inversely correlated with $\delta^{15}N$ (Warinner and Tuross 2010). This would be consistent with some scenarios of late Glacial mastodon population dynamics. Fisher (Fisher 2018; 2009) suggests that predator pressure from paleoindian groups who were megafaunal specialists would have caused mastodons to mature at a younger age. A decrease in the age at weaning would mean a shorter period of nursing-related, elevated dietary $\delta^{15}N$ in young mastodons. If this were the case, we would expect an overall decrease in $\delta^{15}N$ of bone collagen in animals in their first and second decade of life. In our dataset, there is no significant change in maximum or mean $\delta^{15}N$ in mastodon bone collagen, despite a subset of mastodons who have lower $\delta^{15}N$ values. If predator pressure is contributing to faster maturation and shortening the time of nursing, then it is only occurring in some areas. However, even if this were the case in these areas, it is still uncertain what ecological processes might drive an increase in growth rate. Depending on local forage conditions experienced by an animal, an increase in growth rate may be caused by increased predator pressure (Fisher 2009) or a decrease in population density (Wolverton et al. 2009).

It is possible that low $\delta^{15}N$ values in late mastodons may represent a systematic change in predator avoidance strategies among some mastodon populations (Fiedel et al. 2019). Mastodons may have been attracted to low $\delta^{15}N$ areas as a response to new predators on the landscape (i.e., humans). However, taphonomic modification of late Glacial proboscidean materials by humans or other predators is extremely rare in this dataset (Widga et al. 2017), so we see this scenario as an unlikely driver of late Glacial mastodon landscape use.

If proboscidean populations are dense on the landscape (Widga et al. 2017) there may be rapid, significant, and long-term related changes to N cycle. An influx of N via urea as would be expected in high-density areas, would cause isotopically heavy plant roots and isotopically light shoots. Under intense grazing pressure, changes to N contributions change quickly from inorganic soil mineral N reservoirs to N from urea (McNaughton, Ruess, and Seagle 1988; Knapp et al. 1999). Individuals with lower $\delta^{15}N$ values during the Aller□d lived in areas with high populations, which may have contributed to isotopically light forage (due to ammonia volitization, Knapp et al. 1999)).

CONCLUSIONS

Stable isotopes of proboscidean tissues in the midwestern US illustrate the variability inherent in modern paleoecological analyses. Traditional approaches to understanding animal diets and landscape use through morphology and a reliance on modern analogues is inadequate for understanding paleoecological changes within megafaunal populations, or during times of rapid ecological change such as characterizes the late Quaternary.

Stable isotopes from bone collagen of midwestern proboscideans suggest consistently C3-dominated diets over the last 50 ka. During the LGM, mammoth diets may have included C3 grasses, but the prevalence of C3 flora during the post-LGM period is likely due to a landscape shift to more forest (Gonzales and Grimm 2009; Saunders et al. 2010) with grasses making up very little of flora in the southern Great Lakes.

Despite the strong C3-signal to mammoth and mastodon diets during this period, the range of dietary flexibility and the degree of overlap between these two taxa is striking. The isotopically defined dietary niche of mammoths and mastodons shows extensive overlap and is consistent with overlapping ecological niches in these megafaunal taxa as they approach extinction. This is also supported by assemblages where both taxa co-occur (Saunders et al. 2010; Widga et al. 2017).

Stable isotopes of mammoths and mastodons have the potential to illuminate patterns in late Glacial landscapes and megafaunal population dynamics as these species approach extinction. Ecological factors at play in this extinction remain unresolved. Stable isotope of bone collagen ($\delta^{13}C$, $\delta^{15}N$) and tooth enamel carbonate ($\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$) in late Glacial proboscideans from the Midwest are leveraged to examine ecological and behavioral changes in these species during the last glaciation. Mammoth and mastodon $\delta^{13}C$ suggest they are flexible mixed feeders with significant C3 contributions to their diets. Bone collagen $\delta^{15}N$ is also highly variable throughout this period. However some mastodons exhibit low $\delta^{15}N$ values during the B-A. These low values could be explained by invoking changes in individual life histories (i.e., age at weaning), expansion of available ecological niches, in situ change in an extant niche, or a mix of these scenarios. Serial enamel samples show large inter-annual differences between interglacial, mid-Wisconsin, and late Glacial mammoth oxygen isotope values. Finally, a new

micro-milling approach to sampling mammoth tooth enamel is used to examine the life histories of two Missouri mammoths in great detail.

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Figures

Figure 1. Scales of paleoecological analyses.

Figure 2. Map of dated midwestern mammoths and mastodons (from Widga et al., 2017).

Figure 3. Schematic illustration of serial and micro-sampling strategies.

Figure 4. Midwestern Sr isoscape, data from Widga et al. (2017), Hedman et al. (2009, 2018).

Figure 5. Changes in midwestern proboscidean collagen $\delta^{13}C$ and $\delta^{15}N$ through time.

Figure 6. Time series, serial enamel $\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$.

Figure 7. Time series, micro-sampled enamel $\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$.

Figure 8. Mastodon distribution during the B-A. Blue circles indicate mastodon $\delta^{15}N < 5$ per mil. Dark red circles are mastodons with $\delta^{15}N > 5$ per mil.

Tables

Table 1. Scales of Paleoecological Analyses.

Table 2. Mammoth and mastodon collagen stable isotope values, by chronzone.

Table 3. Summary of serial and micro-sampled tooth enamel series: $\delta^{13}C$, $\delta^{18}O$, $^{87}Sr/^{86}Sr$.

Supplemental information

SM Supplemental Material. Word File

SM Table 1. Mammoth and Mastodon 14C dates, $\delta^{13}C$, $\delta^{15}N$ data.

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