Palaeogeography, Palaeoclimatology, Palaeoecology 266 (2008) 59–68

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo

Neogene forests from the Appalachians of Tennessee, USA: Geochemical evidence from fossil mammal teeth

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ARTICLE INFO

Article history:
Accepted 25 March 2008

Keywords:
Appalachians
Neogene
Paleoecology
Refugia
Stable isotopes
Rare earth elements

ABSTRACT

Neogene land-mammal localities are very rare in the northeastern U.S.; therefore, the late Miocene/early Pliocene Gray Fossil Site in eastern Tennessee can clarify paleoecological dynamics during a time of dramatic global change. In particular, the identification of ancient forests and past climate regimes will better our understanding of the environmental context of mammalian evolution during the late Cenozoic. Stable isotope analyses of bulk and serial samples of fossil tooth enamel from all ungulates present at the Gray site elucidate paleoecological reconstructions. The herbivorous megafauna include taxa of likely North American and Eurasian ancestry including: the tapir *Tapirus polkensis*, rhino *Teleoceras* cf. *T. hicksi*, camel cf. *Megatylopus* sp., peccary Tayassuidae, and proboscidean Gomphotheriidae. The tapir, rhino, camel, and peccary yield mean stable carbon isotope ($\delta^{13}C$) tooth enamel values of −13.0‰, −13.3‰, −13.8‰, and −13.1‰, respectively, suggesting forest-dwelling browsers. This range of $\delta^{13}C$ values indicates the presence of a C_3 dominated ancient local flora. Because $\delta^{13}C$ values decline with increasing canopy density, the ancient temperate forests from the Gray site were moderately dense. The lack of significant C_4 plant consumption (i.e., tooth enamel $\delta^{13}C$ values <-9‰) suggests the presence of forests large enough to independently support the continued browsing of sustainable populations of browsers from the Gray site. In contrast, bulk and serial $\delta^{13}C$ values ranging from −0.7‰ to 0.3‰ from a gomphothere tusk support a diet consisting of C_4 grasses, suggesting the presence of C_4 grasslands within the individuals home range. The rare earth element (REE) analyses of the gomphothere tusk and the teeth of *Tapirus* and *Teleoceras* indicates that these individuals shared similar depositional environments; thus, demonstrating the concurrent presence of C_3 forests and C_4 grasslands in the northeast. Stable carbon and oxygen serial sample variation of the tapir, rhino, peccary, and gomphothere is less than 1.5‰, suggesting minor differences in seasonal temperature and/or precipitation. These data support the possibility of a North American forest refugium in the southern Appalachians during a time typified by more open environments.

1. Introduction

Reconstructing the diet of ancient mammalian herbivores and their floral environment during the late Tertiary in eastern North America is necessary to understanding the context of mammalian evolution in this poorly understood region. Global climate change and C_3/C_4 transitions are interpreted to have taken place concurrently (Cerling et al., 1993; Wang et al., 1994; Cerling et al., 1997). While these transitions lead to dramatic increases in C_4 grasses in North America approximately 7 mya (Cerling et al., 1993; Wang et al., 1994; Cerling et al., 1997), it is unclear how eastern forests responded to such changes. It is possible that eastern North America sustained forest refugia, i.e., locations of relict populations of once widespread flora and fauna, during these transitions. The presence of a North American forest refugium has been proposed based on the Gray site's faunal and floral macrofossils including the abundance of forest-dwelling taxa (Wallace and Wang, 2004). Stable isotope sampling of the site's mammalian herbivores further clarifies our understanding of the paleoecology of this spatially and temporally rare site.

During the late Miocene to early Pliocene, herbivore diversity declined through a series of extinction events, with the once diverse short-crowned browsers experiencing proportionally greater declines than high-crowned (hypsodont), presumed grazing herbivores (Potts and Behrensmeyer, 1992; Janis et al., 2000, 2002, 2004). These declines are often attributed to the increase in C_4 grasslands resulting from increased aridity and/or reduced CO_2 levels globally (Potts and Behrensmeyer, 1992; Cerling et al., 1993; Wang et al., 1994; Cerling et al., 1997; Janis et al., 2000; Retallack, 2001; Janis et al., 2002, 2004; Stromberg, 2005). While it is clear that C_4 grasses increased in abundance throughout the late Miocene to early Pliocene (Potts and Behrensmeyer, 1992; Cerling et al., 1993; Wang et al., 1994; Cerling

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doi:10.1016/j.palaeo.2008.03.032

et al., 1997; Retallack, 2001), the driving mechanisms responsible for apparent global cooling and increased seasonality are still a matter of debate. Nevertheless, it is possible that some forested environments may have persisted as floral and faunal refugia within or in close proximity to C_4 grasslands during this time of transition. As morphologically inferred browsers appear to be more numerous at the Gray site, it is important to understand the true dietary feeding strategies of all ungulate taxa present. Reconstructing the diets of ungulates will also provide information on the associated flora and likely environments of the Gray Fossil Site and the broader ramifications for the ancient Appalachian forests.

The southern Appalachians have existed for the past ~250 million years, potentially providing a relatively stable environment for the resident flora and fauna (Graham, 1964, 1999). Dominated by tropical flora during the Cretaceous the vegetation present during the Paleocene to early Eocene suggests tropical rain forests and a megathermal (i.e. humid and warm with mean temperatures of ≥20 °C, *sensu* de Candolle, 1874) climate (based on mesophyllous, entire-margined leaves in the Lower Eocene Wilcox Formation, TN; Graham, 1964, 1999). Middle Eocene tropical dry forests subsequently transitioned to modern warm-temperate deciduous vegetation at lower elevations and montane coniferous forests at higher elevations, during the late Tertiary (Graham, 1964, 1999). These late Tertiary flora consisted of tropical vegetation (at the genus and family level) that were adapted to more temperate climates and interchanged with Asia and Europe (Graham, 1964). Additionally, molecular evidence of extant flora and fauna (e.g. eastern tiger salamander *Ambystoma tigrinum tigrinum*, red pine *Pinus resinosa*) suggests that southern Appalachian refugia maintained ancestral populations of taxa, requiring milder climates, during periods of glaciation (Crespi et al., 2003; Walter and Epperson, 2005). Thus, understanding the paleoecology of the southern Appalachians may help us to better understand present floral and faunal diversity.

The primary objective of our study is to reconstruct the ancient diets and paleoenvironments from the fauna of the Gray Fossil Site, from stable carbon and oxygen isotopes of ungulate tooth enamel. We also interpret the paleoclimatic records of serial samples from tooth and tusk enamel, determining seasonal variability. Additionally, we present rare earth element (REE) analyses of a subset of our ungulate taxa to determine the taphonomic context of the mammalian herbivores, determining if they represent a sympatric fauna (Trueman, 1999). The results of this study provide critical information to understanding the unique paleoecological dynamics occurring during the late Tertiary in the southern Appalachians.

2. Background

2.1. Stable isotope analysis: a theoretical foundation

Vertebrate fossil remains can clarify paleoecological hypotheses by allowing for an independent measure of habitat type, as inferred from stable isotope ratios. Stable carbon isotopes are incorporated into the lattice of enamel hydroxyapatite; therefore, retaining dietary isotopic signals that are reflective of plants consumed (DeNiro and Epstein, 1978; Krueger, 1991; Lee-Thorp and van der Merwe, 1991; Cerling et al., 1997; Cerling and Harris, 1999). Because ^{13}C /^{12}C ratios vary depending on a plant's photosynthetic pathway and do not decay with time (Ehleringer and Monson, 1993), the ratios of the past can be interpreted as similar to those of today (Cerling et al., 1997). Additionally, $\delta^{13}C$ signatures of C_3 and C_4 plants are incorporated into the tooth enamel hydroxyapatite of medium to large bodied herbivorous mammals with an enrichment factor of 14.1‰ (although non-ruminants may have an enrichment factor between 12 and 13‰; Cerling and Harris, 1999). However, due to modern atmospheric CO_2 enrichment, floral and faunal $\delta^{13}C$ values are an additional ~1.5‰ enriched today, as compared to the past (Cerling et al., 1997; Passey et al., 2002). Therefore, $\delta^{13}C$ values between −21‰ and −7‰ reflect a C_3 diet, whereas values between −2‰ and 4‰ indicate a C_4 diet (MacFadden et al., 1996; Cerling et al., 1997, 1999; Cerling and Harris, 1999; Cerling et al., 2004). Variation in $\delta^{13}C$ values within individual teeth can also indicate seasonal differences in diet, reflective of

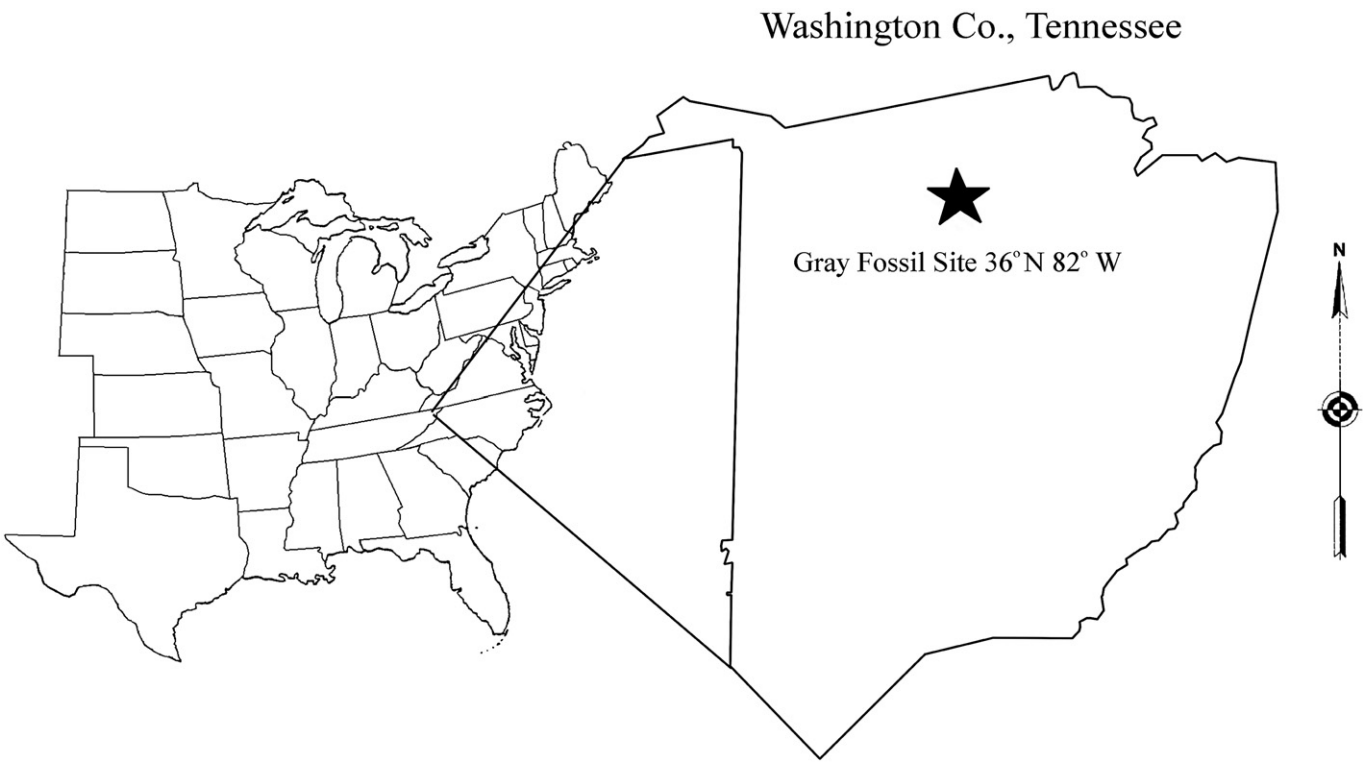

Fig. 1. Location of the Gray Fossil Site, Tennessee, USA.

seasonal changes in vegetation due to water stress (Roux et al., 2001; Ehleringer et al., 2002).

Stable carbon isotope data of extinct and extant taxa can also be used to reconstruct rainforest distributions due to greater ^{13}C discrimination occurring in dense closed canopies as compared to more open C_3 environments (van der Merwe and Medina, 1989; Cerling et al., 2004). Because $\delta^{13}C$ values increase with decreasing canopy density and/or increasing distance from dense forest edges (van der Merwe and Medina, 1989; Kapos et al., 1993; West et al., 2001), more negative $\delta^{13}C_{enamel}$ values of mammalian herbivores reflect the consumption of browse in forests with denser canopies (van der Merwe and Medina, 1989, 1991; Cerling et al., 2004). As temperate forest floral $\delta^{13}C$ values are typically more enriched in ^{13}C as compared to tropical forests (e.g. Cerling et al., 2004; Tu et al., 2004; BASIN Network, 2006), floral macrofossils and palynological evidence can further constrain interpretations of forest density. Therefore, $\delta^{13}C$ values of mammalian tooth enamel can indicate meaningful differences in habitat type within C_3 flora and therefore assist in determining relative canopy density.

Variation in stable oxygen isotopes of mammalian tooth enamel is a function of body water that reflects the response of meteoric water to changes in temperature and/or precipitation/humidity (Dansgaard, 1964; Bryant et al., 1994, 1996; Bocherens et al., 1996; Kohn, 1996; Sponheimer and Lee-Thorp, 1999; Higgins and MacFadden, 2004; MacFadden and Higgins, 2004; Hoppe, 2006). In terrestrial ecosystems, seasonal variation is recorded in tooth enamel with more positive $\delta^{18}O$ values indicating high summer temperatures as compared to more negative $\delta^{18}O$ values during cooler winters (Fricke and O'Neil, 1996; Feranec and MacFadden, 2000; MacFadden and Higgins, 2004).

Oxygen isotopes of mammalian tooth enamel can also vary between taxa occupying similar environments due to variations in the proportion of water ingested in the form of drinking water, as opposed to more evaporated plant water (Levin et al., 2006). By comparing the $\delta^{18}O$ values of evaporation sensitive taxa (i.e. $\delta^{18}O_{enamel}$ values increase with aridity) to evaporation insensitive taxa (i.e. $\delta^{18}O_{enamel}$ values track meteoric water) present at the same site, the $\delta^{18}O$ values of mammalian tooth enamel may be used as an index of terrestrial aridity (Levin et al., 2006). Because rhinos are evaporation insensitive (Levin et al., 2006), comparisons of the $\delta^{18}O$ values of rhinos to the likely evaporation sensitive camels (e.g. non-domesticated camelids typically acquire a large proportion of their water from plants), may elucidate relative aridity at the Gray site.

2.2. Rare earth element analysis: understanding taphonomic history

REE patterns of fossilized skeletal material within a deposit can be compared to determine whether a site has experienced significant mixing and/or reworking (Trueman, 1999). Because REEs are taken up in skeletal tissue in higher concentrations beginning shortly after death and continuing for approximately 10,000 to 30,000 yr during diagenetic recrystallization, REEs patterns of fossil enamel and dentin reflect the geochemistry of the local pore-water during that time (Henderson et al., 1983; Trueman, 1999; Patrick et al., 2001; MacFadden et al., 2007). As early recrystallization results in reduced porosity and flow, initial REE patterns are typically preserved throughout later digenesis (Trueman, 1999). Similar REE patterns indicate similarities in the geochemistry of the pore-water, and comparable depositional environments (Trueman 1999; Trueman et al., 2004). Therefore, it is possible to compare REE patterns of fossils from the Gray site to determine if they shared similar depositional environments or were reworked from a spatially and/or temporally distinct locality.

2.3. Gray Fossil Site, Tennessee, USA

The Gray Fossil Site, located in Washington, Co., Tennessee, USA (Fig. 1) is biostratigraphically dated between 4.5 and 7 Ma, based on the presence of the rhino *Teleoceras* and short faced bear *Plionarctos* (Wallace and Wang, 2004). The site is a sinkhole deposit consisting of finely laminated clays, silts, and fine sands with intermixed gravel lenses. The deposit resulted from a paleosinkhole lake that was approximately 2 ha in size and up to 39 m thick (Wallace and Wang, 2004; Shunk et al., 2006). The vertebrate taxa are of North American and, somewhat surprisingly, Eurasian ancestry (Table 1). The ungulate taxa include, the tapir *Tapirus polkensis*, rhino *Teleoceras* cf. *T. hicksi*, camel cf. *Megatylopus* sp., peccary Tayassuidae, and gomphotheriid proboscidean (Table 1). Additionally, the Gray site preserves a large population of the extinct tapirs (*T. polkensis*) represented by over 70 individuals, an order of magnitude larger than the total number of individuals of all other ungulate taxa. Given that fossil tapirs are robust indicators of ancient forests (DeSantis and MacFadden, 2007), the Gray site likely indicates the presence of forested environments.

Floral macrofossils and pollen likewise support a forested interpretation for the Gray site. Palynological evidence suggests a predominantly oak (*Quercus*) and hickory (*Carya*) forest, consisting of nearly 70% of the pollen (Wallace and Wang, 2004; Table 1). Along with pine (*Pinus*), representing 9% of the pollen analyzed, the remaining flora present occurs in even lower abundances (Wallace and Wang, 2004). Because grass pollen occurs in such low abundance (<2%), and anemophilous (wind dispersed) grass pollen is often over representative of a sites flora when present (Horrocks et al., 2000), it is unlikely that C_4 grasses made up a substantial portion of the vegetation present at the Gray site. Additionally, the $\delta^{13}C$ values of all bulk organic matter sampled from the site range in value from −28 to −24‰, representing only C_3 flora (Shunk et al., 2006).

3. Materials and methods

3.1. Morphological measurements

Selected dental measurements were taken to quantify relative crown height. Measurements of crown heights and widths of the

Table 1
Biota from the Gray Fossil Site, Tennessee. Compiled from Wallace and Wang (2004) and Schubert and Wallace (2006)

Fauna	Flora
Osteichthyes	Conifers
Amphibia	*Tsuga*
Anura	Anura
Plethodontidae	Deciduous
Ambystoma sp.	*Quercus*
Reptilia	*Carya*
Chrysemys sp.	*Ulmus*
Trachemys sp.	*Betula*
Terrapene sp.	*Fraxinus*
Chelydridae	*Celtis*
Alligator sp.	Shrubs
Viperidae	*Alnus*
Colubridae	*Salix*
Aves	Herbs
Passeriformes	*Ambrosia*-type
Mammalia	Cyperaceae
Soricidae	Gramineae
Talpidae	Umbelliferae
Lagomorpha	Caryophyllaceae
Rodentia	
Xenarthra	
Gomphotheriidae	
Tapirus polkensis	
Teleoceras cf. *T. hicksi*	
Tayassuidae	
cf. *Megatylopus* sp.	
Canidae	
Mustelidae	
cf. *Machairodus* sp.	
Plionarctos sp.	
Pristinailurus bristoli	
Arctomeles dimolodontus	

Table 2
Bulk carbon and oxygen isotopes of mammalian ungulate enamel, Gray Fossil Site

Taxon	Specimen no.*	Tooth position	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
Tapirus polkensis	291	LM2	−13.0	−3.7
T. polkensis	586	Partial RP4,M2,or M3	−13.9	−3.8
T. polkensis	587	Partial LP4,M2,or M3	−12.7	−3.5
T. polkensis	588	Rm3	−13.7	−4.2
T. polkensis	595	Lm3	−13.1	−2.8
T. polkensis	602	Lp4	−12.1	−4.5
T. polkensis	606	LM3	−13.4	−4.2
T. polkensis	607	RM3	−13.3	−4.1
T. polkensis	608	RM3	−11.2	−4.3
T. polkensis	623	RM3	−14.1	−4.6
T. polkensis	639	LM2	−12.7	−4.2
T. polkensis	652	RM2	−13.1	−4.5
T. polkensis	653	LM3	−14.0	−4.6
T. polkensis	661	LM2	−13.9	−2.3
T. polkensis	664	RM3	−11.5	−3.9
T. polkensis	666	LM3	−14.0	−5.2
T. polkensis	683	LM3	−13.7	−4.1
T. polkensis	3424	RM3	−12.9	−3.8
T. polkensis	3425	RM3	−13.4	−4.5
T. polkensis	3426	LM2	−13.7	−3.0
T. polkensis	3427	LM2	−13.6	−4.1
T. polkensis	2/20/04-027	Lm3	−10.9	−3.4
T. polkensis	2002-5-119	LM3	−12.4	−4.7
Teleoceras cf. *T. hicksi*	566	Rp3	−13.4	−3.9
Teleoceras cf. *T. hicksi*	609	Lm3	−13.2	−5.5
Teleoceras cf. *T. hicksi*	780	RM1	−13.0	−4.6
Teleoceras cf. *T. hicksi*	781	LM1	−13.6	−5.3
Tayassuidae	593	LM3	−14.0	−4.2
Tayassuidae	778	LM3	−12.4	−4.1
Tayassuidae	779	Rm3	−12.9	−4.9
cf. *Megatylopus* sp.	738	Deciduous premolar	−13.8	−1.7
Gomphotheriidae	305	Tusk	−0.3	−4.2

*All specimen numbers are East Tennessee Museum of Natural History catalogue numbers with the exception of two uncatalogued specimens (2002-5-119, 2/20/04-27).

lower third molars (m3) were taken from all specimens isotopically sampled with m3s present. A hypsodonty index (HI) was then calculated for all available m3s according to Janis (1988), by dividing the unworn crown height by the m3 width. Lower third molars with significant wear were excluded when determining mean HI values. These HI values were then used to determine if hypsodonty is predictive of diet, as inferred from stable carbon isotopes at the Gray Fossil Site.

3.2. Stable isotope analysis

A total of 32 bulk and 58 serial enamel samples from 32 individuals were analyzed from the Gray Fossil Site in eastern Tennessee (Table 2, Appendix A). Samples were primarily collected from late erupting teeth (e.g. third molars or fourth premolars) in order to avoid sampling teeth that mineralized while nursing or weaning. Bulk samples were collected from one area perpendicular to the growth axis of the tooth. Serial samples were acquired by sampling the teeth with parallel grooves at intervals of 2–3 mm perpendicular to the growth axis of the tooth. When sampling the gomphothere tusk, a total of 14 samples, each 1.5 mm wide in the growth direction and 15 mm across the enamel band, were taken every 5 mm in order to acquire data representative of at least 1 yr of growth (Fox and Fisher, 2004). Between 2 and 10 mg of tooth enamel sample was collected using a low speed FOREDOM™ drill and carbide dental burrs. Samples were chemically pretreated prior to isotopic analysis with H_2O_2 to remove organics and weak acetic acid (0.1 N, CH_3CO_2H) to remove secondary carbonates (Koch et al., 1997). Approximately 1 mg of treated sample was then analyzed using a VG Prism mass spectrometer in the Department of Geological Sciences at the University of Florida.

Stable isotope data are reported in conventional delta (δ) notation for carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$), where $\delta^{13}C$ (parts per mil, ‰)= $((R_{sample}/R_{standard})-1)*1000$, and $R=^{13}C/^{12}C$; and, $\delta^{18}O$ (parts per mil, ‰)=$((R_{sample}/R_{standard})-1)*1000$, and $R=^{18}O/^{16}O$. Analyzed samples were calibrated to NBS-19 and then to V-PDB (PeeDee Belemnite) following the Vienna (V−) convention (Coplen, 1994).

3.3. Rare earth element analysis

Enamel and dentin from the gomphothere tusk, 1 rhino tooth, and 3 tapir teeth (each from different individuals), were sampled for REE analysis. Approximately 5–10 mg of sample was removed using a FOREDOM™ drill and carbide dental burrs. These samples were cleaned in Savillex™ vials with 1 ml of 3 M HNO_3, dissolved and heated overnight on a hot plate. After samples were dried until all liquid was evaporated, the samples were weighed and dissolved in 2 ml of 5% HNO_3 and left overnight on a hotplate. Approximately 3 ml of 5% HNO_3 was added to each sample and the sample weights were calculated in order to achieve a dilution factor of 2000. All samples were then run on a Thermo Finnigan ELEMENT2 inductively Coupled Plasma Mass Spectrometer in the University of Florida Department of Geological Sciences for bulk REE concentrations. Internal standards and bone ash (NVS SRM 1400) were run with the samples, enabling corrections to be made due to instrument drift. All REE concentrations were normalized to PAAS (Post-Archean Australian Shale; McLennan, 1989). The REEs analyzed, range from La (Z=57) to Lu (Z=71). We excluded europium (Eu) from the analysis *post hoc*, due to anomalous Eu enrichment and depletion spikes found in the Gray specimens. These anomalies are likely due to Eu partitioning under closed conditions and irrelevant for our comparisons (Trueman et al., 2004). These methods follow Trueman et al. (2004) and MacFadden et al. (2007).

4. Paleoecology of the Gray Fossil Site: isotopic results and interpretations

4.1. Bulk carbon isotope analysis

The bulk carbon isotope analyses of *Tapirus polkensis* indicates a diet consisting entirely of C_3 plants ranging in $\delta^{13}C$ values from −14.1 to −10.9‰ with a mean of −13.0‰ (1σ=0.9‰; Fig. 2, Tables 2 and 3). The bulk $\delta^{13}C$ values of *Teleoceras* sp., a morphologically presumed grazer (MacFadden, 1998), range from −13.6 to −13.0‰ with a mean of −13.3‰ (1σ=0.3‰; Fig. 2, Tables 2 and 3). Peccary (Tayassuidae) bulk $\delta^{13}C$ values range from −14.0 to −12.4‰, with a mean of −13.1‰ (1σ=0.8‰; Fig. 2, Tables 2 and 3). These taxa are therefore interpreted to be obligate browsers, consuming only C_3 vegetation. While only one tooth was available for isotopic analysis of the camel, cf. *Megatylopus* sp., the $\delta^{13}C$ value of −13.8‰ is consistent with a predominantly C_3 diet (Fig. 2, Tables 2 and 3). The carbon isotopic niches of *Tapirus*,

Table 3
Stable carbon and oxygen values for the taxa of the Gray Fossil Site, Tennessee

Taxon	Common name	N[a]	$\delta^{13}C$ (‰)			$\delta^{18}O$ (‰)		
			Mean	SD	Range	Mean	SD	Range
Tapirus polkensis	Tapir	23	−13.0	0.9	−14.1 to −10.9	−4.0	0.7	−5.2 to −2.3
Teleoceras cf. *T. hicksi*	Rhino	4	−13.3	0.3	−13.6 to −13.0	−4.8	0.7	−5.5 to −3.9
Tayassuidae	Peccary	3	−13.1	0.8	−14.0 to −12.4	−4.4	0.4	−4.9 to −4.1
cf. *Megatylopus* sp.	Camel	1	−13.8	–	–	−1.7	–	–
Gomphotheriidae	Gomphothere	1[b]	−0.3	–	–	−4.2	–	–

[a] N=the number of different individuals sampled, the descriptive statistics do not include more than one tooth from an individual (no teeth were included that could have been missing teeth from an included individual).
[b] Gomphothere tusk enamel was only available for isotopic analysis.

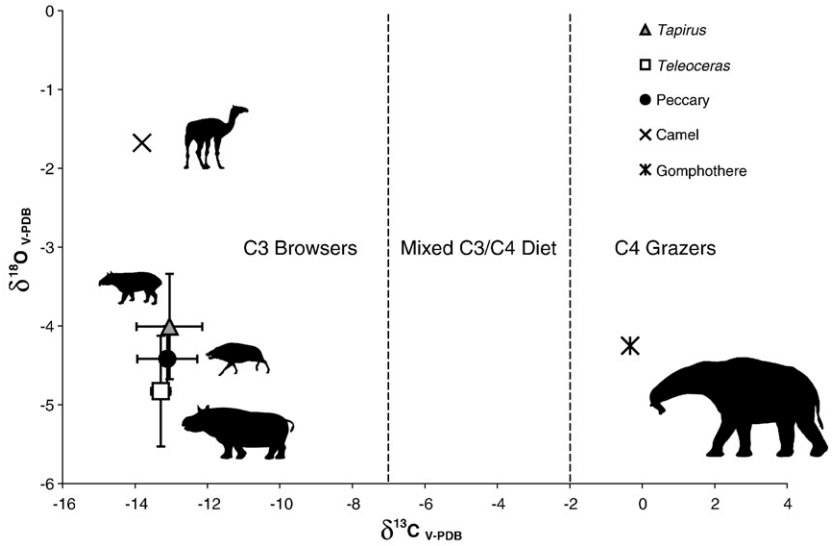

Fig. 2. Stable carbon and oxygen isotope values from the ungulate taxa at the Gray Fossil Site. Symbols represent the mean value, while the error bars correspond to 1 standard deviation.

Teleoceras, the peccary and the camel appear to overlap and are not statistically different from each other (ANOVA, $p=0.81$; Kruskal–Wallis, $p=0.79$; Fig. 2), although it is difficult to comment on the degree of niche overlap due to the limited number of *Teleoceras*, peccary, and camel teeth sampled. The bulk $\delta^{13}C$ value of the tusk from the gomphotheriid proboscidean is −0.3‰ (Fig. 2, Tables 2 and 3), significantly different from all other taxa sampled under parametric analyses of ANOVA ($p<0.0001$) and all subsequent Fisher LSD multiple comparisons ($p<0.05$). However, non-parametric Kruskal–Wallis analysis yielded insignificant differences ($p=0.44$), likely because the gomphothere tusk represents only one sample. This inconsistent $\delta^{13}C$ value prompted a comparison of enamel and dentin REEs from the proboscidean with those of the tapirs and rhinos. This analysis, discussed later, enables us to determine if the tusk was subjected to a similar depositional environment as the more abundant forest-dwelling taxa (i.e. *Tapirus* and *Teleoceras*).

As prior pollen analyses have identified the Gray Fossil Site to indicate a predominantly oak–hickory deciduous forest, we can constrain our interpretation of canopy density. Average $\delta^{13}C$ values of modern temperate deciduous flora of approximately −26‰ and −30‰ are consistent with an open forest canopy and dense forest canopy, respectively (Garten and Taylor, 1992; Tu et al., 2004; BASIN Network, 2006). Accounting for both dietary (14.1‰ enrichment between large bodied ungulates and their diet; Cerling and Harris, 1999) and atmospheric enrichment (1.5‰; Cerling et al., 1997; Passey et al., 2002), all taxa with the exception of the proboscidean have mean $\delta^{13}C$ values consistent with a moderately dense, temperate forest ($\delta^{13}C \sim -13‰$). The bulk $\delta^{13}C$ values from the tapirs, rhinos, peccaries, and the camel do not support the presence of C_4 grasses at the Gray site. These data agree with the palynological evidence that documents low grass abundance (Wallace and Wang, 2004), of which the Gramineae pollen that is present may be from C_3 grasses. However, the bulk $\delta^{13}C$ value from the gomphothere indicates the presence of C_4 grasses at a distance within this species migration/home range and in large enough abundance to support a population of this herbivore with a pure C_4 diet, assuming that our gomphothere sample is representative of a population. Given that roughly contemporaneous sites below 37° latitude are thought to have undergone transitions to C_4 grasslands during the late Miocene/early Pliocene (Cerling et al., 1993; Wang et al., 1994; Cerling et al., 1997), the data presented here suggest the presence of a forested environment at the Gray Fossil Site that may have served as a refugium to taxa requiring forested habitats among C_4 grasslands.

The relationship between relative tooth crown height (hypsodonty index values) and $\delta^{13}C$ values are explored here. The average HI values of *Tapirus*, *Teleoceras*, and peccaries are 0.7, 1.3, and 0.5, respectively. Because the sample sizes of rhinos and peccaries are small, these HI values should be viewed as preliminary. These HI values are not predictive of $\delta^{13}C$ values. Instead, the most hypsodont taxon *Teleoceras* has some of the most negative $\delta^{13}C$ values. While high-crowned teeth are no longer synonymous with the grazing of C_4 grasses (MacFadden et al., 1999; Feranec, 2003, 2004; MacFadden, 2005), the Gray fauna likewise provides additional evidence that hypsodont teeth do not indicate C_4 grazing. Additionally, the browsing of C_3 vegetation by the high-crowned *Teleoceras*, a morphologically presumed grazer and isotopically classified mixed feeder/C_4 grazer in Florida during the early Miocene (MacFadden, 1998), demonstrates further support of the absence of significant C_4 flora at the Gray site.

4.2. Bulk oxygen isotope analysis

Bulk $\delta^{18}O$ values of *Tapirus polkensis* range from −5.2 to −2.3‰ with a mean of −4.0‰ (1σ =0.7‰; Fig. 2, Tables 2 and 3), not significantly different from *Teleoceras,* peccaries, and the gomphothere (ANOVA, $p=0.15$; Kruskal–Wallis, $p=0.21$). The bulk $\delta^{18}O$ values of *Teleoceras* sp. range from −5.5 to −3.9‰ with a mean of −4.8‰ ($1\sigma=0.7‰$), this is the most negative mean $\delta^{18}O$ value of all ungulates sampled (Fig. 2, Tables 2 and 3). The bulk $\delta^{18}O$ values of the peccaries range from −4.9 to −4.1‰ with a mean of −4.4‰ ($1\sigma=0.4‰$; Fig. 2, Tables 2 and 3). Additionally, the bulk $\delta^{18}O$ value of the gomphothere tusk falls within the range of the tapirs, rhinos, and peccaries at −4.2‰. The $\delta^{18}O$ of −1.7‰ for the camel (cf. *Megatylopus* sp.) is the most enriched in ^{18}O (Fig. 2, Tables 2 and 3), significantly different from all other taxa sampled under parametric analyses of ANOVA ($p<0.01$) and LSD multiple comparisons ($p\leq0.01$). No significant differences are observed when using the non-parametric Kruskal–Wallis analysis ($p=0.13$); however, this is likely a sample size issue because only one camel tooth is included in the analysis. The higher $\delta^{18}O$ value from the camel, a probable evaporation sensitive taxon, may be compared to evaporation insensitive rhinos to quantify relative aridity (Levin et al., 2006). *Teleoceras* has the most depleted bulk $\delta^{18}O$ value of −5.5‰ and the camel has the most enriched $\delta^{18}O$ value of −1.7‰; therefore, the total bulk $\delta^{18}O$ range of all taxa is 3.8‰. Comparing the difference between the evaporation insensitive rhino and likely evaporation sensitive camel may further allow for estimates

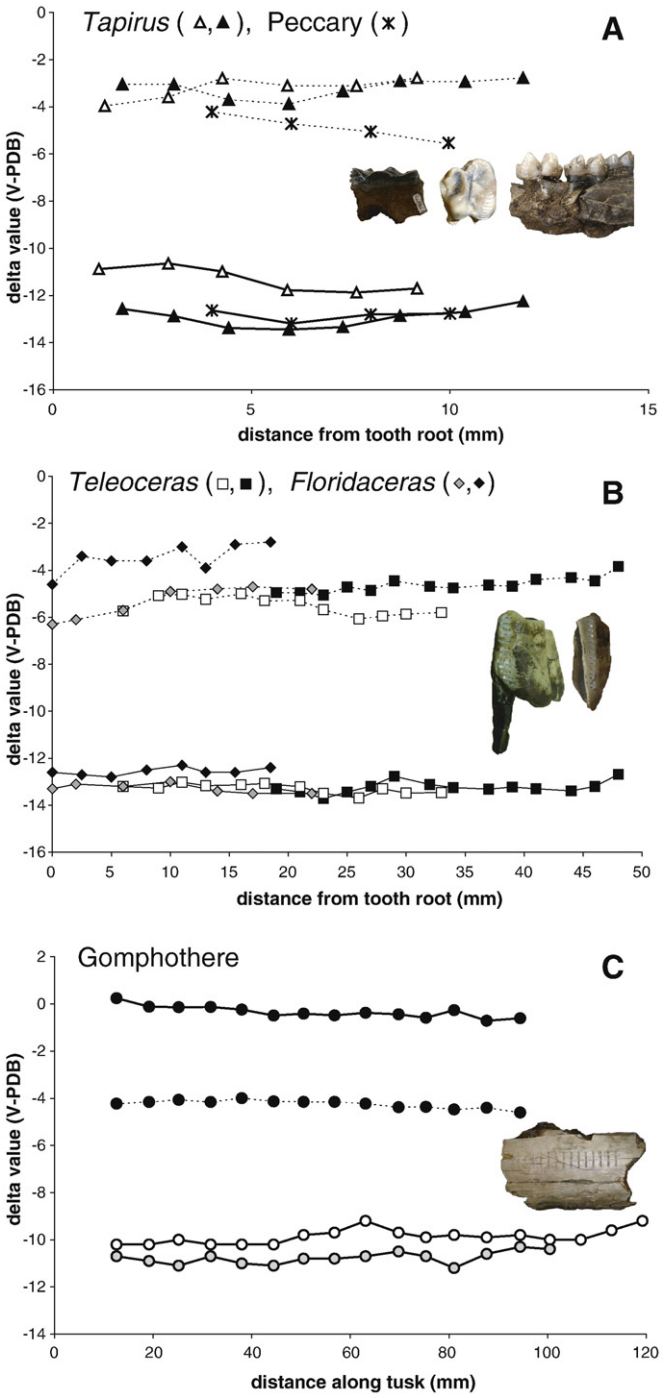

Fig. 3. Serial samples of the ungulate taxa from the Gray Fossil Site, including: *Tapirus* (△,▲), peccary (*), *Teleoceras* (■,□), and the gomphothere (●). *Floridaceras* (◇,◆) from the 15 Ma Gaillard Cut Local Fauna in Panama (MacFadden and Higgins, 2004) and late Miocene Gomphotheres (○,●) from the Port of Entry Pit in Oklahoma, USA (Fox and Fisher, 2001), are included for comparisons. Dashed lines represent oxygen values and solid lines indicate carbon values, while like colors correspond to the same individual per taxon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of relative aridity (Levin et al., 2006); however, additional samples of both the evaporation insensitive and sensitive taxa are first needed.

4.3. Seasonal reconstructions: serial sample analysis

The serial samples of *Teleoceras, Tapirus* and peccary teeth can reveal seasonal differences in monthly temperatures and/or precipitation. *Teleoceras* serial samples (N=12, 14) from two high-crowned teeth yield total $\delta^{13}C$ ranges of 0.6‰ (−13.7 to −13.1‰) and 1.0‰ (−13.7 to −12.7‰), with $\delta^{18}O$ ranges of 1.1‰ (−6.1 to −5.0‰) and 1.3‰ (−5.1 to −3.8‰; Fig. 3B, Table 4, Appendix A). Serial samples from two tapir individuals (N=8, 6), yield total $\delta^{13}C$ ranges of 1.1‰ (−12.3 to −13.4‰) and 1.3‰ (−10.6 to −11.9‰), with $\delta^{18}O$ ranges of 1.1‰ (−3.9 to −2.8‰) and 0.8‰ (−3.6 to −2.8‰; Fig. 3A, Table 4, Appendix A). One peccary tooth (N=4) was sampled to determine if it demonstrates the same pattern of little $\delta^{13}C$ and $\delta^{18}O$ variation. The range of $\delta^{13}C$ and $\delta^{18}O$ variation was 0.6‰ (−12.6 to −13.2‰) and 1.4‰ (−5.6 to −4.2‰), respectively (Fig. 3A, Table 4, Appendix A). All serial samples of *Teleoceras*, *Tapirus*, and the peccary demonstrate less than 1.5‰ variation in both carbon and oxygen isotopes, indicating the lack of significant seasonal variation.

Serial samples of the gomphothere tusk (N=14) were taken at intervals representing over 1 yr of growth (as per Fox and Fisher, 2004), varying ≤1‰ in both $\delta^{13}C$ and $\delta^{18}O$ values. The gomphothere $\delta^{13}C$ values ranged from −0.7 to 0.3‰ (Fig. 3C, Table 4, Appendix A), indicating the consumption of C_4 grass throughout the course of a year with the absence of seasonal variations in diet. The lack of significant oxygen variation (approximately 0.6‰, −4.6 to −4.0‰) likewise confirms the lack of significant seasonal changes in meteoric water due to temperature and/or precipitation. The variation of tusk $\delta^{13}C$ seen here is similar to late Miocene gomphotheres with equable serial sample records of approximately 1‰; however, those gomphotheres were C_3 browsers and/or mixed feeders (Fox and Fisher, 2001, 2004; Fig. 3C). The absence of considerable $\delta^{13}C$ and $\delta^{18}O$ variation is consistent with the isotopic patters found in the tooth enamel of the ungulates sampled. However, the lack of seasonal variation may be an artifact of the gomphothere's behavior, if it actively migrated to areas were C_4 grasses were abundant (i.e. migrating south during the winter to consume C_4 grasses under similar temperature/precipitation conditions as summer grazed C_4 grasses of northern latitudes). Additionally, we cannot infer that the gomphothere only consumed C_4 vegetation during its life including while at or near the Gray Fossil Site. Instead, we can only state that the gomphothere sampled consumed a diet indicative of a pure C_4 diet for at least 1 yr of its life.

The lack of significant variation in both the carbon and oxygen isotopes from all taxa sampled, suggests minor differences in monthly temperatures and/or precipitation during the Neogene in eastern Tennessee. The Gray fauna experienced a more equable climate than today (Climate Zone, 2006; U.S. Department of Commerce and NOAA, 2006; Waterisotopes.org, 2006; Fig. 4). Even though $\delta^{18}O$ variation is damped in mammalian tooth enamel due to the buffering of water sources and/or time averaging (Passey and Cerling, 2002), the *Teleoceras, Tapirus,* and peccary $\delta^{18}O$ ranges of variation are more similar to fossil taxa from the aseasonal Gaillard Cut Local Fauna assemblage in Panama (MacFadden and Higgins, 2004; Fig. 3A and B), than to taxa from highly variable climates. The serial samples of the rhinos *Floridaceras whitei* from the 15-million-year-old site in Panama have $\delta^{13}C$ variation of 0.5‰ (MacFadden and Higgins, 2004), similar to 0.7‰ and

Table 4
Serial carbon and oxygen isotope variation, per individual

Taxon	ETMNH no.	Tooth	N	$\delta^{13}C$ (‰)			$\delta^{18}O$ (‰)		
				Min.	Max.	Range	Min.	Max.	Range
Teleoceras cf. *T. hicksi*	609	Lm3	12	−13.7	−13.0	0.7	−6.1	−5.0	1.1
Teleoceras cf. *T. hicksi*	781	LM1	14	−13.7	−12.7	1.0	−5.1	−3.8	1.2
Tapirus polkensis	595	Lm3	6	−11.9	−10.6	1.2	−4.0	−2.8	1.2
Tapirus polkensis	3424	RM3	8	−13.4	−12.3	1.2	−3.9	−2.8	1.1
Tayassuidae	779	Rm3	4	−13.2	−12.6	0.6	−5.6	−4.2	1.4
Gomphotheriidae	305	tusk	14	−0.7	0.2	1.0	−4.6	−4.0	0.6

1.0‰ of *Teleoceras* from the Gray site (Fig. 3B, Table 4). However, the $\delta^{18}O$ variation of 1.6‰ and 1.8‰ for *Floridaceras* from the Gaillard Cut L.F. (MacFadden and Higgins, 2004) appears greater than the range of 1.1‰ and 1.2‰ in *Teleoceras* (Fig. 3B, Table 4). Additionally, annual variation in carbon and oxygen isotope values can yield differences as great as 4‰ in tooth enamel of taxa that are present in seasonally variable climates (as seen in bison, horses, and mammoths in Feranec and MacFadden, 2000); therefore, variation of <1.5‰ supports a relatively aseasonal climate (with regard to precipitation and/or temperature). Despite the presence of a deciduous temperate flora that are typically present in highly seasonal environments, the limited range of variation between serial samples likely represents a warmer and less seasonally variable climate than currently present in modern eastern North American temperate forests. Therefore, the floral environment of the Gray site may resemble more equable broadleaf forests than those present in the Appalachians today.

4.4. Evidence of a forest refugium

REE analysis of the gomphothere tusk, and *Tapirus* and *Teleoceras* teeth allow for a comparison of patterns of REEs obtained post-mortem. Normalized REE patterns of enamel and dentin from the gomphothere tusk are nearly identical to, and closely parallel, those of sampled enamel and dentin from *Tapirus* and *Teleoceras*, despite differences in concentrations of REEs (Fig. 5). Because similar REE patterns indicate comparable depositional environments (Trueman, 1999), it is likely that the tusk was deposited at the Gray site at a similar time to the rhinoceros and tapirs sampled for REEs, as opposed to being reworked. Therefore, the gomphothere's REE patterns indicate that it died at or in close proximity to the Gray Fossil Site.

The bulk and serial $\delta^{13}C$ values of the gomphothere tusk provide conclusive evidence that C_4 grasses were at least present within a distance no larger than the migration/home range of the individual sampled. While we are unable to determine the home range of gomphotheres or if they migrated, using modern proboscideans as analogs migration/home ranges may have been as large as 7000 km^2 (Stuwe et al., 1998). Therefore, C_4 grasses may have been present adjacent to the Gray site or at the fringes of the gomphothere's home range, potentially hundreds of kilometers away. As our gomphothere did consume a primarily C_4 diet over the course of at least 1 yr, it is likely that C_4 grasses occurred in abundances large enough to support both itself and a population of gomphotheres with pure C_4 diets, if our gomphothere's diet is representative of a population. While the gomphothere sampled has anomalous $\delta^{13}C$ values in relation to its browsing adapted dentition, gomphotheres have been described as isotopically inferred mixed C_3/C_4 feeders and C_4 grazers, in addition to C_3 browsers (MacFadden and Shockey, 1997; Cerling et al., 1999; Sánchez et al., 2003, 2004; Todd, 2006). Because the bulk $\delta^{13}C$ values from the tapirs, rhinos, peccaries, and the camel do not support the presence of C_4 grasses at the Gray site and palynological evidence indicates the low abundance of grass pollen (Wallace and Wang, 2004), it is improbable that C_4 grasses were present at significant levels at the site. Instead, the Gray Fossil Site likely represents a forested environment that may have served as a refuge to taxa in the southern Appalachians requiring C_3 browse, present concurrently with C_4 grasslands. The presence of forest-dwelling taxa such as the tapirs and red panda (*Pristinailurus bristoli*) at the Gray site demonstrates additional support that this forested environment may have provided unique habitat to taxa that were more widespread previously.

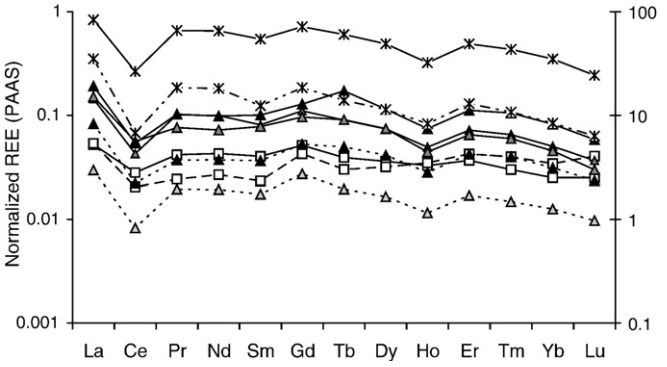

Fig. 5. Normalized $REE_{(PAAS)}$ concentrations of the (asterisk) gomphothere tusk, (triangles) *Tapirus*, and (square) *Teleoceras* teeth. Dentine and enamel REE concentrations are noted with dotted lines and solid black lines, respectively. Because gomphothere tusk enamel and dentin have higher absolute concentrations of REEs, they are plotted on the secondary *y*-axis (right).

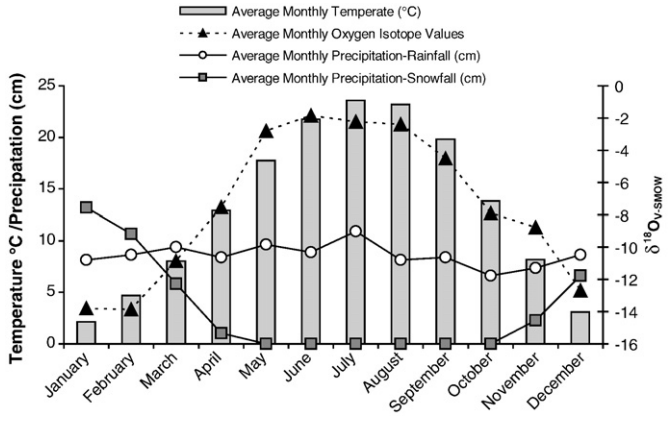

Fig. 4. Average monthly temperate (°C), monthly oxygen isotope values, and monthly precipitation (rainfall, snowfall) in Johnson City/Bristol Tri-City Area, Tennessee. Oxygen isotope data is from Waterisotopes.org, temperature data is a 10-yr mean taken from station 401094/13877 and provided by the U.S. Department of Commerce and NOAA, http://www.ncdc.noaa.gov/oa/climate/climatedata.html. Remaining precipitation data (rainfall, snowfall) is from Climate Zone www.climate-zone.com.

5. Discussion and concluding remarks

The mammalian herbivores from the Gray Fossil Site provide evidence of moderately dense ancient forests, flanked by distal C_4 grasslands. Both bulk and serial carbon isotopes indicate that all ungulate taxa sampled, with the exception of the gomphothere, consumed a diet of C_3 vegetation. Because all of the $\delta^{13}C$ values of *Tapirus*, *Teleoceras*, the peccaries, and the camel are less than −10‰, there is no isotopic evidence that suggests the consumption of C_4 grasses by these taxa. Therefore, the Gray site represents a forested environment large enough to support sustainable populations of its browsers (tapir *Tapirus polkensis*, rhino *Teleoceras* cf. *T. hicksi*, camel cf. *Megatylopus* sp., peccary Tayassuidae). The majority (63%) of specimens sampled have depleted $\delta^{13}C$ values of ≤−13‰, further suggesting that this dominant oak–hickory forest had a relatively dense canopy for a temperate forest.

The presence of organisms that currently live in humid mesothermal (i.e. moderate moisture and heat with mean temperature between 15 and 20 °C, *sensu* Wolfe, 1975 modified from de Candolle, 1874) and/or megathermal areas such as tapirs and alligators provides evidence for a warmer and more equable climate than today. Serial samples of tooth and tusk enamel document $\delta^{13}C$ and $\delta^{18}O$ variation of <1.5‰, demonstrating negligible seasonal changes in temperature and/or precipitation. Assuming that the climate was warmer (as inferred from the taxa present) and that the relatively constant annual precipitation patterns seen today (Sankovski and

Appendix A

Carbon and oxygen isotope values for serial samples of ungulate enamel, Gray Fossil Site, Tennessee

Sample	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
Teleoceras cf. *T. hicksi*	ETMNH 781	LM1
LGD-ETR1-S1	−13.3	−4.9
LGD-ETR1-S2	−13.4	−5.0
LGD-ETR1-S3	−13.7	−5.1
LGD-ETR1-S4	−13.4	−4.7
LGD-ETR1-S5	−13.2	−4.9
LGD-ETR1-S6	−12.8	−4.5
LGD-ETR1-S7	−13.1	−4.7
LGD-ETR1-S8	−13.2	−4.8
LGD-ETR1-S9	−13.3	−4.6
LGD-ETR1-S10	−13.2	−4.7
LGD-ETR1-S11	−13.3	−4.4
LGD-ETR1-S12	−13.4	−4.3
LGD-ETR1-S13	−13.2	−4.5
LGD-ETR1-S14	−12.7	−3.8
Teleoceras cf. *T. hicksi*	ETMNH 609	Lm3
LGD-ETR2-S1	−13.2	−5.7
LGD-ETR2-S2	−13.3	−5.1
LGD-ETR2-S3	−13.0	−5.0
LGD-ETR2-S4	−13.2	−5.2
LGD-ETR2-S5	−13.1	−5.0
LGD-ETR2-S6	−13.1	−5.3
LGD-ETR2-S7	−13.2	−5.3
LGD-ETR2-S8	−13.5	−5.7
LGD-ETR2-S9	−13.7	−6.1
LGD-ETR2-S10	−13.3	−5.9
LGD-ETR2-S11	−13.5	−5.9
LGD-ETR2-S12	−13.5	−5.8
Tapirus polkensis	ETMNH 3424	Lm3
LGD-ETT5-S1	−10.9	−4.0
LGD-ETT5-S2	−10.6	−3.6
LGD-ETT5-S3	−11.0	−2.8
LGD-ETT5-S4	−11.8	−3.1
LGD-ETT5-S5	−11.9	−3.1
LGD-ETT5-S6	−11.7	−2.8
Tapirus polkensis	ETMNH 608	RM3
LGD-ETT15-S1	−12.6	−3.0
LGD-ETT15-S2	−12.9	−3.0
LGD-ETT15-S3	−13.4	−3.7
LGD-ETT15-S4	−13.4	−3.9
LGD-ETT15-S5	−13.3	−3.3
LGD-ETT15-S6	−12.9	−2.9
LGD-ETT15-S7	−12.7	−2.9
LGD-ETT15-S8	−12.3	−2.8
Tayassuidae	ETMNH 779	Rm3
LGD-ETP1-S1	−12.6	−4.2
LGD-ETP1-S2	−13.2	−4.7
LGD-ETP1-S3	−12.8	−5.0
LGD-ETP1-S4	−12.8	−5.6
Gomphotheriidae	ETMNH 305	tusk
LGD-ETG1-S1	0.2	−4.2
LGD-ETG1-S2	−0.1	−4.2
LGD-ETG1-S3	−0.1	−4.1
LGD-ETG1-S4	−0.1	−4.2
LGD-ETG1-S5	−0.2	−4.0
LGD-ETG1-S6	−0.5	−4.1
LGD-ETG1-S7	−0.4	−4.2
LGD-ETG1-S8	−0.5	−4.1
LGD-ETG1-S9	−0.4	−4.2
LGD-ETG1-S10	−0.4	−4.4
LGD-ETG1-S11	−0.6	−4.4
LGD-ETG1-S12	−0.3	−4.5
LGD-ETG1-S13	−0.7	−4.4
LGD-ETG1-S14	−0.6	−4.6

The taxa, specimen IDs, and tooth positions are noted.

Pridnia, 1995; Climate Zone, 2006; U.S. Department of Commerce and NOAA, 2006; Fig. 4) occurred in the past, we would expect to see minor differences in $\delta^{18}O$ values. Additionally, relatively constant precipitation and warmer mean annual temperatures could explain why C_3 and C_4 floras do not experience seasonal water stress (as inferred from the lack of seasonally enriched $\delta^{13}C$ values), due to greater evaporation during periods of increased temperature and/or aridity. Currently, the southern Appalachians are relatively warm and humid at low elevations, while precipitation shows little seasonality. Therefore, the Gray site may have served as a refugium to taxa requiring C_3 vegetation and more equable/warmer environments than may have been available at other geographical localities during the Miocene/Pliocene C_4 transition.

Due to limited numbers of *Teleoceras*, peccary, and camel specimens sampled, it is too early to speculate on the true degree of niche overlap present at this site. Continued excavation of the Gray Fossil Site will provide larger samples from which future analyses of isotopic niche overlap can be clarified. Additionally, microwear analysis of the ungulates sampled isotopically will provide further resolution to whether C_3 consumers were feeding on C_3 grasses and/or C_3 browse. The continued sampling of the vertebrate fauna present at the Gray site will further clarify ecological niches and seasonal variation. In particular, additional gomphothere samples will shed light on population level dietary variation including estimates of percent C_4 grass consumption. These mammalian herbivores from the Neogene of eastern North America provide unique opportunities to understand paleoecological phenomenon occurring during a period of dramatic global change.

Acknowledgements

We thank B. Schubert, R. Hulbert, B. MacFadden, J. Curtis, G. Kamenov, and J. Labs-Hochstein for their contributions. Additionally, we would like to thank R. Feranec, P. Higgins, and an anonymous reviewer for helping to improve this manuscript. Financial support was provided by the Tennessee Department of Transportation, East Tennessee State University, the Don Sundquist Center for Excellence in Paleontology, Florida Museum of Natural History, University of Florida's Department of Zoology, the National Science Foundation Southeast Alliance for Graduate Education and the Professoriate, a Miss Lucy Dickinson Scholarship in Vertebrate Paleontology, a Britt Paleobiology Award, and a Geological Society of America Research Award. Karen Carr provided the images included in the second figure. This is the University of Florida Contribution to Paleobiology 596.

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