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A new stable isotope record of Neogene paleoenvironments and mammalian paleoecologies in the western Great Plains during the expansion of C$_4$ grasslands

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ABSTRACT

Stable isotopes in horse teeth from the North American Great Plains show that equids began to incorporate C$_4$ plants in their diets about 6.5 million years ago as C$_4$ grasslands expanded. However, the ecological response of many other ungulates to this expansion is poorly documented. We use stable carbon isotopes in mammalian tooth enamel to test whether other ungulates adapted by incorporating C$_4$ vegetation in their diets. The openness of habitats before the expansion of C$_4$ grasslands is uncertain, with plant phytolith studies suggesting a patchy environment with open-habitat grasses and forest, and pollen and macrofloral studies suggesting more forested habitats. To address this problem we use a model that predicts carbon isotope values for tooth enamel for a variety of environments, based on values in modern plants. Carbon and oxygen isotopes were analyzed in medium to large herbivores from three late Miocene and three Pliocene local faunas comprising a total of 13 and 7 genera, respectively, and 59 and 42 individuals. Results indicate that before the expansion of C$_4$ grasslands, taxa with high-crowned teeth were consuming predominantly C$_3$ vegetation. In contrast, by the late Pliocene most taxa studied were consuming a component of C$_4$ vegetation and only the peccary *Platygonus* sp. had a pure C$_3$ diet. C$_4$ consumption increased in the late Pliocene (~3.0 Ma) Big Springs local fauna probably in response to increased C$_4$ biomass. Most landscapes in the late Miocene of Nebraska were open, such as woodland-savanna or C$_3$ grassland, although low carbon values from the Pratt Slide local fauna suggest a denser, presumably forested area. This general pattern suggests an expansion of open-habitats no later than 12 Ma (early Clarendonian). Through the Miocene–Pliocene transition there was an overall shift to lower δ^{18}O enamel values, which parallels the long-term decrease in global mean annual temperature inferred from the marine record. Our results indicate that major changes in the diets of medium and large herbivores broadly corresponded with increased C$_4$ biomass and cooling climate from the latest Miocene to the late Pliocene.

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1. Introduction

Stable carbon isotope ratios from a variety of proxies, including soil carbonates, mammalian tooth enamel, and grass phytoliths document the global expansion of C$_4$ grasslands in the late Miocene and Pliocene (Passey et al., 2002; Fox and Koch, 2004; Edwards et al., 2010; McInerney et al., 2011; Strömberg, 2011; Strömberg and McInerney, 2011; Fox et al., 2012a). The timing of this expansion varied considerably among the continents. In the North American Great Plains, C$_4$ expansion began in the late Miocene at about 6.5 Ma and continued into the Pliocene (Cerling et al., 1993; Wang et al., 1994; MacFadden and Cerling, 1996; Cerling et al., 1997; Passey et al., 2002; Fox and Koch, 2004). Major faunal turnover occurred from the late Miocene to the

Pliocene in North America (Cerling et al., 1997; Janis et al., 1998, 2000, 2002, 2004) when a rich diversity of broad-leaf browsing species with low-crowned teeth transitioned to a lower diversity fauna with a greater percentage of higher-crowned grazers (Barry, 1995; Janis et al., 2002, 2004). The timing of changes in crown height do not, however, correspond well with the expansion of C$_4$ grasslands (e.g., Strömberg, 2006) and hypsodonty appears to be more of a function of habitat openness and aridity than of grass consumption (Mendoza and Palmqvist, 2008; Eronen et al., 2009; Strömberg, 2011). Mammalian species diversity in North America during the middle and late Miocene (Barstovian [16.0–12.5 Ma] and Clarendonian [12.5–9.0 Ma], North American land–mammal ages; Tedford et al., 2004) appears to have been relatively stable, followed by a progressive decrease in diversity with a loss of about two-thirds of the mammalian genera by the end of the Pliocene (Janis et al., 2000, 2002, 2004). Several mammalian groups that were previously well represented (e.g., oreodonts, chalicotheres, and rhinoceroses) disappeared from the Great Plains during this interval (Janis et al., 1998).

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Uncertainty exists about the types of biomes that were present in the Great Plains prior to the expansion of C_4 grasslands (Strömberg, 2011). Macrofloras and pollen records suggest that open, grass-dominated habitats did not spread until the middle to late Miocene (e.g., Thomasson, 1990; Bolick et al., 1995). Phytolith assemblages, however, suggest that open-habitat grasses expanded at the expense of trees and bamboos to form pooid-dominated savanna woodlands in the late Oligocene or early Miocene. Phytoliths suggest a mixture of grassy and wooded patches until the late Miocene when more uniformly open grasslands spread (Strömberg and McInerney, 2011). A trend of increasing hypsodonty in mammals began around 18 Ma but is not well synchronized with either the macrofloral–pollen or phytolith records, lagging the latter by some 4 myr (Strömberg, 2006).

Another factor complicating our understanding of the expansion of open grasslands is the stable carbon isotope record from soil carbonates that suggests the presence of 12–34% C_4 biomass (mean = 20%) through the early and middle Miocene, preceding the latest Miocene to Pleistocene expansion of C_4 grasslands (Fox and Koch, 2004; Fox et al., 2012a). However, Fox et al. (2012a) indicate that soil carbonate $\delta^{13}C$ values of Clarendonian age from Kansas do not necessarily require any C_4 biomass, nor do all of their Hemphillian samples. High individual $\delta^{13}C_E$ values from a horse tooth (Cormohipparion) and rhinoceros tooth (Teleoceras) from Ashfall Fossil Beds State Historic Park in Nebraska (Tipple and Pagani, 2007), which is early Clarendonian in age (~12 Ma), are the earliest unambiguous records of C_4 consumption by Great Plains mammals. However, because a large number of ungulates were sampled from the Ashfall locality (Clementz et al., 2008), as well as many horses from other Miocene localities (Passey et al., 2002), it appears that if 20% C_4 biomass were present in the late Miocene only a very small percentage of individuals were exploiting it. This is in spite of several mammal species possessing high-crowned teeth by the late Miocene, which should have enabled them to consume tough grasses.

The reconstruction of paleoenvironments and mammalian paleoecology in the Great Plains is critical for understanding how mammals adapted, or failed to adapt, to environmental change in the late Neogene. Cooling climate and expanding C_4 grasslands would have forced many mammalian groups to adapt to new ecological niches or perish. Previous studies found that horses adapted by incorporating C_4 vegetation in their diets (Wang et al., 1994; Passey et al., 2002), but it is not clear how other taxa responded to a changing ecosystem. With the expansion of C_4 grasslands and the opening of habitats, C_3-dominated environments may have become a minor component of the ecosystem. To test if other mammals adapted by incorporating C_4 vegetation in their diets we analyze the stable isotope composition of tooth enamel from a variety of medium- and large-bodied herbivores. Tooth enamel faithfully records the carbon isotope composition of an individual's diet with predictable C^{13}-enrichment (Cerling and Harris, 1999; Passey et al., 2005) and is used widely to recognize C_3, C_4, or mixed C_3/C_4 diets (e.g., DeNiro and Epstein, 1978; Wang et al., 1994; MacFadden and Cerling, 1996; Koch, 1998; Feranec and MacFadden, 2006). We also use stable isotopes to reconstruct late Miocene (late Clarendonian to middle Hemphillian, 9.5–7 Ma, Tedford et al., 2004) biomes to test whether the landscape contained patches of C_3 grassland and/or forest, as suggested by phytolith studies, or if forests were a more dominant component of the ecosystem before the expansion of C_4 grasses.

We address the following questions: (1) Was the late Miocene (Clarendonian and Hemphillian) landscape largely open with a major component of grasslands or were forests an important component? (2) Were late Miocene mammals consuming C_4 vegetation? The null hypothesis we are testing is that these mammals were consuming only C_3 vegetation. (3) How did late Miocene mammals partition habitats? (4) Did non-equid ungulates respond to the expansion of C_4 grasslands by adapting to a partial C_4 diet in a similar fashion to equids? (5) Is there a correspondence between faunal $\delta^{18}O_E$ values from the Great Plains

and the global temperature record inferred from benthic carbonates, as might be expected if cooling on the Great Plains mirrored cooling of the oceans?

2. Background

2.1. Geologic context

The geographic distribution of fossil localities and associated faunas used in this study and estimates of their geochronologic ages are shown in Fig. 1. Age estimates are based on the assignment of faunas to biochronologic subdivisions of the Clarendonian, Hemphillian, and Blancan (4.9–2.0 Ma, Bell et al., 2002) North American land–mammal ages by Repenning (1987) and Voorhies (1990). Land–mammal ages and their subdivisions are biochronologic units (Lindsay, 2003) usually based on the first appearance of a taxon or taxa (Wood et al., 1941; Woodburne, 1987, 2004). Boundaries are from Woodburne (2004) and Kelly and Secord (2009).

All teeth sampled in this study are from deposits of fluvial or fluvio-lacustrine origin. Although these deposits vary in their taphonomic histories, they appear to represent a limited range of fluvial floodplain settings in areas of low relief (Passey et al., 2002). Fossils were sampled from six localities: three from the Ogallala Group of late Miocene age and three from the Broadwater Formation of Pliocene age. The Ogallala Group contains the Pratt Slide and North Shore localities of late Clarendonian age, and the Cambridge locality of medial Hemphillian age (Voorhies, 1990; Passey et al., 2002; Tedford et al., 2004). The Broadwater Formation contains the Lisco locality of early Blancan age, the Broadwater locality of medial Blancan age, and the Big Springs locality of early late Blancan age (Repenning, 1987; Passey et al., 2002). Local faunas from these six sites bear the locality names.

2.2. Carbon isotopes in mammals

Mammalian herbivore tooth enamel faithfully reflects the isotopic composition of ingested plants with predicable enrichment (DeNiro and Epstein, 1978; Vogel, 1978; Cerling and Harris, 1999; Passey et al., 2005). Plant carbon ingested by herbivores is incorporated into the mineralized tissues of the animal. The CO_3 component of tooth enamel therefore reflects the carbon isotope composition of the ingested plants. Both metabolism and biomineralization fractionate ingested carbon so that mammalian bioapatites are enriched in ^{13}C relative to the bulk diet (see Section 3.3).

Mammals feeding on C_3 vegetation can be readily distinguished from those consuming C_4 plants, or those that have mixed C_3/C_4 diets. Because C_4 plants have higher $\delta^{13}C$ values than C_3 plants (Vogel, 1978; O'Leary, 1988), with nearly non-overlapping ranges, pure C_3 and C_4 consumers will generally have non-overlapping ranges, while mammals with mixed C_3/C_4 diets will have intermediate $\delta^{13}C_E$ values (MacFadden and Cerling, 1996; Cerling et al., 1997, 1998; Koch, 1998; MacFadden et al., 1999a). Plants using the CAM photosynthetic pathway, characteristic of succulents, have values intermediate or partially overlapping with C_3 and C_4 vegetation (O'Leary, 1988; Ehleringer et al., 1991). However, CAM plants are not widespread (Ehleringer et al., 1991) and typically do not constitute a major part of ungulate diets.

The late Miocene faunas used in this study occurred before the expansion of C_4 grasslands and presumably lived in biomes dominated by C_3 vegetation. Isotopic variability among C_3 plants is caused by differences in light intensity, temperature, nutrient availability, and water stress (O'Leary et al., 1992; Koch, 1998). Generally, $\delta^{13}C$ values increase from denser and wetter forested parts of an ecosystem to more open or drier habitats (Ehleringer et al., 1986; Stewart et al., 1995; Heaton, 1999; Diefendorf et al., 2010; Kohn, 2010). These trends are reflected in the tooth enamel of herbivorous mammals with high $\delta^{13}C_E$ values representing more open, drier habitats and low values more closed,
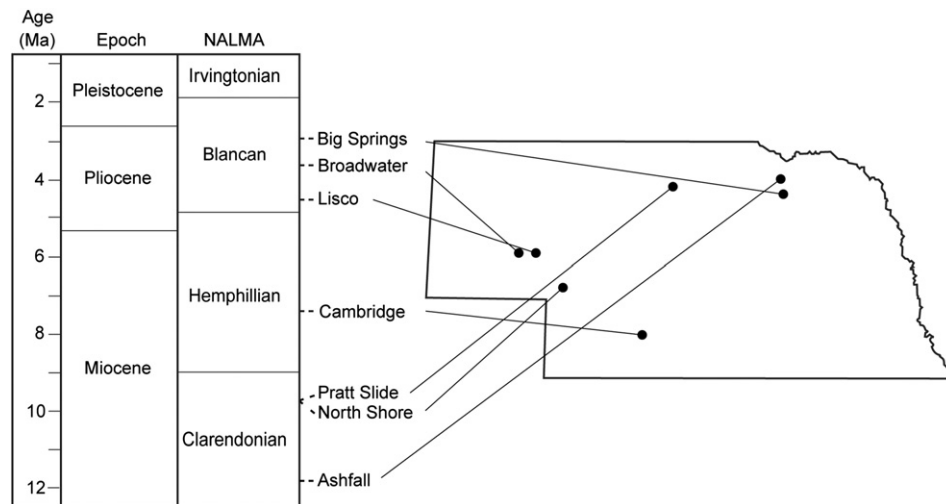
Fig. 1. Generalized diagram showing the geochronologic sequence of North American land-mammal ages and Nebraska fossil localities, and the geographic distribution of fossil localities sampled in this study.
Compiled from Repenning (1987), Voorhies (1990), Passey et al. (2002), and Kelly and Secord (2009).

wetter habitats (O'Leary et al., 1992; Koch, 1998; Cerling and Harris, 1999; Feranec, 2003; Feranec and MacFadden, 2006; Feranec, 2007; Nelson, 2007; Secord et al., 2008). Because $\delta^{13}C$ values in a C_3 plant species vary due to environmental conditions, and C_3 grasses growing in more closed areas are expected to have more negative values than those growing in more open areas, it is not possible to distinguish between individual ungulates consuming C_3 grasses and C_3 browse (broad-leaf dicots). However, we generally expect from studies of modern vegetation that the most open C_3 environments will be dominated by grasses and also have the highest $\delta^{13}C$ values. Therefore, mean $\delta^{13}C_E$ values for a taxon or fauna in the uppermost end of the range predicted for C_3 environments (see Section 3.3) likely reflect feeding in an environment with a grassland component.

2.3. Oxygen isotopes in mammalian tooth enamel

Oxygen isotopes in tooth enamel can be useful for understanding water usage and habitat preference among contemporaneous mammals (e.g., Kohn, 1996; Levin et al., 2006). The oxygen isotope composition of mammalian tooth enamel depends on the $\delta^{18}O$ value of ingested water, the fractionation of oxygen isotopes between body water and enamel, which is approximately constant in most mammals, and the physiology of the taxon (Land et al., 1980; Longinelli, 1984; Luz and Kolodny, 1985; Kohn, 1996; Kohn et al., 1996, 1998). The composition of ingested water, in turn, depends partly on whether that water is derived from drinking or from plant water. Water from both of these sources is ultimately derived from precipitation but leaf water tends to be ^{18}O-enriched due to evaporation, and enrichment increases with increasing aridity or solar radiation (Yakir et al., 1990; Yakir, 1992). Therefore, species with similar physiologies that derive a significant proportion of water from leaves should be ^{18}O-enriched compared to those that are obligate drinkers in the same local area (Balasse et al., 2003; Levin et al., 2006). Species that have physiological adaptations for retaining water in arid regions and/or get a large part of their water from plants are expected to have high $\delta^{18}O_E$ values relative to water-dependent species that rely heavily on drinking. In contrast, large semi-aquatic herbivores, such as hippopotami, are expected to have the lowest $\delta^{18}O_E$ values in a contemporaneous fauna (Clementz et al., 2008). Water-dependent herbivores that rely on drinking and consume vegetation in areas where water is readily available to plants should have low to intermediate values. Thus, relative mean $\delta^{18}O_E$ values within a fauna can be used to infer water dependence in a taxon (e.g., Levin et al., 2006).

In mixed C_3/C_4 biomes, another factor that could influence $\delta^{18}O_E$ values in herbivores is photosynthetic pathway. There is a tendency for the leaf water in C_4 grasses to be more ^{18}O-enriched than leaf water in C_3 grasses (Helliker and Ehleringer, 2002). Influences of C_3/C_4 differences appear to be greatest under low relative humidity growth conditions. While C_4 plants have higher $\delta^{18}O$ values than C_3 plants grown under the same conditions, the difference diminishes from an average ^{18}O enrichment of ~5‰ at low relative humidity to ~1‰ at high relative humidity (Helliker and Ehleringer, 2002). The influence on a herbivore's body water will be some fraction of these amounts depending on the portion of water an herbivore gets from food (e.g., Bryant and Froelich, 1995; Kohn, 1996).

Mammalian $\delta^{18}O_E$ values can also be useful for making paleoclimate interpretations, especially over geologically brief time intervals (e.g., Fricke et al., 1998; Secord et al., 2010, 2012). Mammals that get a large portion of their water from drinking should be best suited for tracking meteoric water values. Model results suggest that the portion of oxygen derived from water usually increases with body size and large mammals should best reflect surface water values (Longinelli, 1984; Bryant and Froelich, 1995). The composition of surface water is ultimately controlled by the composition of precipitation, although surface water reservoirs can vary substantially due to differing histories (e.g., Dutton et al., 2005).

The $\delta^{18}O$ values of precipitation are affected primarily by temperature at mid- to high-latitudes (Dansgaard, 1964; Rozanski et al., 1992; Gat, 1996). Warmer temperatures result in more positive $\delta^{18}O$ values in precipitation and cooler temperatures more negative ones due to Rayleigh fractionation and local atmospheric temperature at the time of precipitation (e.g., Sharp, 2007). Thus, warming and cooling trends in the geologic record are expected to positively covary with $\delta^{18}O$ values in precipitation, assuming no strong influences from other sources such as changes in vapor source through time or increased rainout from the uplift of mountains (e.g., Fricke and O'Neil, 1999; Koch et al., 2003; Kohn and Welker, 2005; Secord et al., 2010). In turn, large scale changes in the $\delta^{18}O$ value of meteoric water should be reflected in surface water consumed by mammals (Fricke and O'Neil, 1996; Iacumin and Longinelli, 2002; Balasse et al., 2003; Secord et al., 2010, 2012).

3. Methods

3.1. Sampling and pretreatment

Tooth enamel was bulk sampled from 101 specimens collected from six local faunas in Nebraska (Fig. 1). Areas of decalcified enamel were

avoided and only well preserved, hard enamel was sampled. Preference was given to sampling third molars and premolars (P_2–P_4) since these teeth are among the last ones to develop, mineralize, and erupt in mammals (Hillson, 2005), avoiding a potential weaning signal. 3–4 mg of enamel powder was drilled from each tooth along a non-occlusal surface parallel to the growth axis under a binocular microscope using a 1 mm diamond bit and a variable speed mounted dental drill.

Pretreatment of samples followed that of Koch et al. (1997). Samples were treated with 2–3% reagent grade NaOCl for 24 h to remove organic matter, then rinsed five times with distilled water and dried overnight at 60 °C. Samples were reacted in 1 M buffered acetic acid for 24 h to remove nonstructural carbonates, and then rinsed and dried overnight. Our protocol differed from that of Koch et al. (1997) only in that samples were dried in a drying oven rather than being lyophilized.

3.2. Isotope analysis

Samples were reacted with phosphoric acid for 17 min at 77 ± 1 °C in a Finnigan MAT Kiel IV preparation device at the University of Michigan Stable Isotope Laboratory (UMSIL). Isotopic ratios of the resulting CO_2 were measured with a Finnigan MAT 253 triple collector isotope ratio mass spectrometer. Analytical precision at UMSIL is better than ± 0.1‰ (1 S.D.) for both $\delta^{18}O_E$ and $\delta^{13}C_E$, based on international standards for carbonate (NBS-18, NBS-19). Intra-lab enamel standards (LOX, from African elephant; MES, from fossil mammoth enamel from New Mexico) were used to monitor variance in enamel among batches. Mean values and variances were: $\delta^{18}O_E = 32.1 \pm 0.12$‰ and $\delta^{13}C_E = -5.7 \pm 0.04$‰ (1 S.D., n = 29) for LOX and $\delta^{18}O_E = 23.3 \pm 0.15$‰ and $\delta^{13}C_E = -9.7 \pm 0.03$‰ (1 S.D., n = 19) for MES showing that variance was higher in enamel than in the carbonate standards.

Isotopic results are expressed in standard δ-notation: X = $[(R_{sample}/R_{standard}) - 1] \times 1000$, where X is the $\delta^{13}C$ or $\delta^{18}O$ value, and R = $^{13}C/^{12}C$ or $^{18}O/^{16}O$, respectively. The $\delta^{13}C$ values are reported relative to the Vienna PeeDee Belemnite (VPDB) standard and $\delta^{18}O$ values are reported relative to the Vienna Standard Mean Ocean Water (VSMOW) standard.

3.3. Predicting carbon isotope values for late Neogene mammals

A model was developed following Secord et al. (2008) and Boardman and Secord (2013) to predict expected $\delta^{13}C$ values in herbivores for a variety of late Neogene biomes. We used data from extant plants as the basis for this model because the full range of $\delta^{13}C$ values from various biomes is not adequately documented in extant mammals. Values for modern vegetation were normalized to parameters for the late Neogene of Nebraska. Factors that were incorporated were: (1) The effects of latitude on $\delta^{13}C$ values in vegetation; (2) The effects of altitude on $\delta^{13}C$ values in vegetation; (3) Diet-enamel enrichment; and (4) Changes in the composition of atmospheric CO_2 between the late Neogene and present.

A mean increase of ~0.3‰/10° latitude in $\delta^{13}C$ values occurs in leaves receiving the same amount of light and water (Secord et al., 2008). To compensate, modern plant data were normalized to 42°N, the approximate latitude of Nebraska. A mean increase of 0.65‰/km altitude in $\delta^{13}C$ values occurs in leaves receiving the same amount of light and water (Secord et al., 2008). To compensate, modern plant data were normalized to 1.1 km, the approximate altitude of Nebraska. Adjusting for these factors allowed us to constrain predicted $\delta^{13}C$ values for various biomes including: closed forest, open canopy, mixed C_3/C_4, and C_4 grassland (Fig. 3).

The diet-enamel enrichment factor (ε* of Cerling and Harris, 1999) for ungulates is 14.1 ± 0.5‰ (1 S.D.) based on a large sample of African mammals while the absolute diet-enamel difference is 13.8 ± 0.6‰ (1 S.D.) (see Cerling and Harris, 1999 for discussion of enrichment factors versus absolute differences). Cerling and Harris found no significant difference between ruminant and non-ruminant ungulates. A later study (Passey et al., 2005) of cattle, pigs, rabbits, and voles found that the ruminant cattle had a significantly higher enrichment factor than the non-ruminants but because this study was based on a much smaller diversity of mammals and did not include horses, rhinos, or camels, as did the study by Cerling and Harris, the earlier study is more appropriate for use here. Notably, the mean enrichment factor for cattle and pigs (-14.0‰) is nearly identical to that of Cerling and Harris. We used estimated atmospheric $\delta^{13}C$ values reported by Tipple et al. (2010), based on benthic foraminifers, to approximate changes in $\delta^{13}C$ from the late Miocene to the Pliocene. Based on their estimates of atmospheric carbon, the Clarendonian to Blancan was ~0.5‰ more positive than during the pre-industrial Holocene. Considering the recent decrease of ~1.5‰ in $\delta^{13}C$ values caused by industrialization (Friedli et al., 1986), Clarendonian to Blancan atmospheric $\delta^{13}C$ was ~2.0% more positive than now. Error was propagated using standard error of the enrichment factor of Cerling and Harris (1999), $\delta^{13}C$ values from the modern vegetation biomes, and corrections for latitudinal differences. Model results indicate the following boundaries and ranges for mean $\delta^{13}C_E$ faunal values for Miocene and Pliocene localities: closed canopy forest (rainforest and monsoon forest): ≤ -12.1‰; open canopy biomes ("dry" and "wet" woodland-savanna, "dry" woody scrubland, "dry" forest, and "dry" C_3 grassland): > -12.1‰ to -7.7‰; mixed C_3/C_4 biomes: > -7.7‰ to 2.1‰; and pure C_4 grasslands: ≥ 2.1‰. Additionally, we calculate a boundary for closed canopy understory browsers based on Cerling et al. (2004) in which modern Ituri values are below -19%. With atmospheric and latitudinal corrections, $\delta^{13}C_E$ values for individuals in this study must be ≤ -15.7‰ to confidently indicate understory browsing in a densely canopied closed forest.

It is important to note that a component of C_4 vegetation could be included in the diets of mammals with values lower than -7.7‰, ranging down to about -10.0‰. However, since the null hypothesis we are testing is that the mammals we are studying *were consuming only C_3 vegetation*, to rigorously test this hypothesis a value on the upper end of the range for C_3 vegetation must be used for the boundary between C_3 and mixed C_3/C_4 diets. To calculate this boundary we used the mean $\delta^{13}C$ value of -24.6 ± 1.1‰ (based on 15 dicot species) reported by Passey et al. (2002) for water-stressed C_3 vegetation from Kenya sampled during a drought. With diet-enamel enrichment ($+14.1$‰) and atmospheric correction ($+2$‰) this yields an upper boundary of -7.7‰. The error reported by Passey et al. (2002) is presumably one standard deviation, although not explicitly stated, since one standard deviation was used for all plant values in the Cerling and Harris (1999) paper from which part of the drought data were drawn. Thus, mammals consuming water stressed C_3 vegetation could conceivably have values above -7.7‰ but because most herbivores consume a variety of plant species, values above -7.7‰ should be rare in herbivores feeding in a C_3 dominated environment. Thus, values that exceed -7.7‰ can be confidently attributed to a dietary component of C_4.

Our interpretations of C_4 consumption are also based on the observation that the amount of C_3 and C_4 vegetation consumed by grazers and mixed feeders is highly variable, and in environments where a considerable percentage of C_4 biomass is present some individuals will have $\delta^{13}C_E$ values that plot well into the mixed C_3/C_4 range while others may plot in the C_3 range (e.g., Passey et al., 2002, Pliocene faunas in this study).

4. Results

4.1. Results within faunas

The mean $\delta^{13}C_E$ and $\delta^{18}O_E$ values for individual taxa from North Shore, Pratt Slide, Cambridge, Lisco, Broadwater, and Big Springs are displayed in Fig. 3. Table 1 reports isotopic values and descriptive statistics. Because sample sizes are small for most taxa, we rely primarily on Tukey's post hoc test to determine significant differences among

Table 1
Descriptive statistics for $\delta^{13}C_E$ and $\delta^{18}O_E$ values from taxa examined in this study.

Local fauna	Genus	n	$\delta^{13}C$ (VPDB)					$\delta^{18}O$ (VSMOW)				
			Mean	Median	S.E.	S.D.	Range	Mean	Median	S.E.	S.D.	Range
			(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)	(‰)
NS	*Aphelops* sp.	3	−9.8	−9.4	0.6	1.0	−10.9 to −9.0	25.0	26.2	1.2	2.1	22.6 to 26.3
NS	*Cormohipparion occidentale*	5	−9.0	−9.2	0.2	0.4	−9.3 to −8.4	26.7	26.5	0.7	1.6	24.6 to 28.8
NS	*Megatylopus* cf. *M. primaevus*	3	−9.7	−9.7	0.4	0.6	−10.3 to −9.1	27.4	26.4	1.2	2.1	25.9 to 29.7
NS	*Serbelodon* cf. *S. barbourensis*	2	−10.5	−10.5	0.3	0.5	−10.8 to −10.2	23.8	23.8	0.7	1.0	23.1 to 24.5
NS	*Teleoceras major*	5	−9.1	−9.0	0.2	0.5	−9.6 to −8.4	25.5	26.0	0.4	0.8	24.4 to 26.3
PS	*Cormohipparion* sp.	2	−12.6	−12.6	0.4	0.5	−13.0 to −12.3	25.6	25.6	0.4	0.5	25.3 to 26.0
PS	*Cranioceras unicornis*	5	−12.4	−11.7	0.6	1.4	−14.5 to −11.4	27.1	27.2	0.3	0.7	26.3 to 27.8
PS	*Hipparion* sp.	3	−11.6	−11.3	0.5	0.8	−12.6 to −11.1	26.1	26.4	0.4	0.7	25.3 to 26.6
PS	*Procamelus grandis*	4	−11.2	−11.3	0.5	0.9	−12.2 to −10.2	27.5	27.1	0.4	0.8	27.0 to 28.6
PS	*Prosthennops* sp.	4	−11.5	−11.5	0.1	0.2	−11.7 to −11.3	22.4	22.4	0.2	0.3	22.0 to 22.7
PS	*Teleoceras major*	2	−11.3	−11.3	0.3	0.4	−11.6 to −11.1	24.9	25.0	1.5	2.1	23.5 to 26.4
C	*Alforjas* sp.	2	−10.1	−10.1	0.2	0.3	−10.3 to −9.9	28.3	28.3	0.6	0.9	27.7 to 29.0
C	*Aphelops mutilus*	2	−10.3	−10.3	0.2	0.3	−10.5 to −10.1	26.2	26.2	0.2	0.2	26.1 to 26.4
C	*Calippus* sp.	2	−9.6	−9.6	0.1	0.1	−9.7 to −9.6	29.0	29.0	1.0	1.4	28.1 to 30.0
C	*Hemiauchenia* cf. *H. vera*	3	−11.1	−11.2	0.4	0.7	−11.8 to −10.4	27.3	26.7	0.9	1.5	26.2 to 29.1
C	*Megatylopus* sp.	3	−10.2	−10.5	0.3	0.5	−10.5 to −9.6	28.1	27.7	0.4	0.6	27.6 to 28.8
C	*Neohipparion eurystyle*	5	−8.7	−8.8	0.2	0.5	−9.3 to −8.0	25.4	24.8	0.6	1.2	24.1 to 27.2
C	*Teleoceras fossiger*	4	−10.0	−10.2	0.5	1.0	−10.9 to −8.7	25.5	25.4	0.3	0.7	24.9 to 26.4
L	*Gigantocamelus spatulus*	5	−3.1	−2.9	0.5	1.1	−4.5 to −2.1	22.9	23.1	0.2	0.5	22.4 to 23.5
Bw	*Camelops* sp.	2	−7.5	−7.5	0.4	0.6	−8.0 to −7.1	20.8	20.8	0.9	1.3	19.9 to 21.7
Bw	*Equus simplicidens*	2	−5.1	−5.1	1.4	1.9	−6.5 to −3.8	19.9	19.9	2.2	3.1	17.7 to 22.1
Bw	*Hemiauchenia blancoensis*	6	−8.0	−8.4	1.1	2.7	−10.7 to −4.8	23.1	23.4	0.5	1.1	21.4 to 24.4
Bw	*Platygonus* sp.	4	−10.1	−9.9	0.4	0.8	−11.3 to −9.5	23.9	23.6	1.3	2.7	21.1 to 27.5
Bw	*Titanotylopus nebraskensis*	2	−7.9	−7.9	1.7	2.4	−9.6 to −6.2	21.2	21.2	0.7	1.0	20.5 to 22.0
BS	*Equus simplicidens*	6	−4.1	−3.6	0.9	2.1	−6.8 to −1.9	21.9	22.1	0.4	1.1	20.5 to 23.4
BS	*Gigantocamelus spatulus*	5	−4.7	−4.4	1.1	2.5	−8.8 to −2.5	22.9	23.4	0.9	2.0	19.5 to 24.4
BS	*Platygonus* sp.	3	−11.3	−11.7	0.4	0.6	−11.7 to −10.6	25.1	24.6	1.1	1.9	23.4 to 27.2
BS	*Stegomastodon* sp.	2	−1.7	−1.7	2.5	3.5	−4.2 to 0.8	22.4	22.4	2.8	3.9	19.6 to 25.2
BS	*Titanotylopus nebraskensis*	5	−3.8	−2.4	1.6	3.6	−9.0 to −0.1	24.7	26.0	1.3	2.9	19.8 to 27.1

NS = North Shore, PS = Pratt Slide, C = Cambridge, L = Lisco, Bw = Broadwater, BS = Big Springs.

taxa but also report values using Fisher's LSD test for comparison. There are no significant differences among taxa in the North Shore fauna (Table 2A). At Pratt Slide there are no significant differences in $\delta^{13}C_E$ values but *Prosthennops* is significantly lower in $\delta^{18}O_E$ values than all other taxa ($p < 0.001$ to 0.019, Tukey's test) and *Teleoceras* is significantly lower than *Procamelus* ($p = 0.025$, Tukey's test). In Cambridge, *Neohipparion eurystyle* has the highest mean $\delta^{13}C_E$ value (−8.7‰), which is significantly higher than in *Hemiauchenia* cf. *H. vera* ($p = 0.009$ Tukey's test). *Neohipparion eurystyle* also has the lowest mean $\delta^{18}O_E$ value (25.4‰), which is significantly lower than *Calippus* sp. ($p = 0.016$, Tukey's test), the other equid.

In the Pliocene, $\delta^{13}C_E$ values are generally much more variable than in the Miocene since most taxa are consuming an uneven mix of C_3 and C_4 vegetation (Fig. 3). *Gigantocamelus spatulus* was the only species sampled from Lisco and had a mean $\delta^{13}C_E$ value of −3.1‰ indicating a mixed C_3/C_4 diet (Fig. 3). However, both $\delta^{13}C_E$ and $\delta^{18}O_E$ values plot in a fairly narrow range relative to other taxa consuming C_4, suggesting a fairly specialized diet or possibly diagenetic compression of values. None of the Broadwater taxa were significantly different using Tukey's test. All individual $\delta^{13}C_E$ values for *Platygonus* sp. fall in the range expected for a C_3 feeder while values for the other taxa indicate C_4 consumption in at least some individuals (Fig. 3). All taxa from Big Springs except *Platygonus* sp. have mean $\delta^{13}C_E$ values that plot well into the range for mixed C_3/C_4 feeders. Nearly all individual values for mixed feeders also plot in the C_3/C_4 range, except for two that plot in the area of overlap between water-stressed C_3 and C_4. *Platygonus* sp. has significantly lower mean $\delta^{13}C_E$ values than all other taxa using Tukey's test ($p = 0.002$ to 0.032) and all individual values plot in the range expected for a non-water-stressed C_3 diet.

4.2. Whole fauna results

All individual carbon isotope values for the Miocene taxa fall within the range expected for a C_3 diet, although values in the upper end of the range would need to be from a water-stressed environment and could alternatively include a component of C_4 vegetation up to 15–20%. Nevertheless, no individual value exceeds the −7.7‰ threshold necessary to confidently indicate a component of dietary C_4 and refute our null hypothesis of no dietary C_4. With regard to the North Shore and Cambridge faunas, both have mean $\delta^{13}C_E$ values (−9.4‰ and −9.9‰, respectively) that plot in the range expected for open, dry C_3 environments such as grasslands or "dry" woodlands and are significantly higher than the modeled "wet" woodland-savanna biome (Fig. 4, Table 3). The mean Pratt Slide $\delta^{13}C_E$ value (−11.8‰) is significantly lower than the other faunal values ($p < 0.001$, Tukey's test), plots in the range expected for a wetter, more forested environment, and is significantly lower than values for the four modeled "dry" C_3 biomes (Fig. 4, Table 3). Mean $\delta^{13}C_E$ values for environments with a considerable portion of C_4 vegetation are more indicative of percent C_4 consumed than of a particular environment, although most values that plot well into the mixed range presumably represent an open biome with considerable grass. The Broadwater fauna has a mean $\delta^{13}C_E$ value of −8.1‰ and plots on the boundary between C_3 and mixed C_3/C_4 consumers. It is statistically indistinguishable from the "dry" scrubland, "dry" C_3 grassland, and "dry" forest, but is significantly different from the "wet" and "dry" woodland-savannas (Table 3). The Big Springs fauna has a mean $\delta^{13}C_E$ value of −5.0‰, plots in the region expected for mixed C_3/C_4 herbivores, and is significantly higher than mean $\delta^{13}C_E$ values for the modeled C_3 habitats (Table 3).

Of the Miocene local faunas, North Shore and Cambridge have the highest mean $\delta^{13}C_E$ values and are statistically indistinguishable ($p = 0.101$, T-test) while Pratt Slide has a significantly lower ($p < 0.001$, Tukey's test) mean $\delta^{13}C_E$ value. Of the Pliocene faunas, Big Springs has a significantly higher $\delta^{13}C_E$ value than Broadwater ($p = 0.005$, T-test). Mean $\delta^{18}O_E$ values for the Miocene faunas are statistically indistinguishable ($p = 0.196$, Kruskal–Wallis) as are mean values for the Pliocene faunas ($p = 0.224$, T-test). However, a large, significant

Table 2
Matrices of pairwise probabilities of mean differences in $\delta^{13}C_E$ (left) and $\delta^{18}O_E$ (right) values among genera. Values shown in bold indicate significance at $\alpha \leq 0.05$ using Fisher's LSD test. Pairs that are significant using Tukey's post hoc test ($\alpha \leq 0.05$) are indicated by an asterisk.

A. North Shore $\delta^{13}C_E$, $\delta^{18}O_E$	1	2	3	4	5
1. *Aphelops*	1.000, 1.000				
2. *Cormohipparion*	0.088, 0.166	1.000, 1.000			
3. *Megatylopus*	0.849, 0.086	0.128, 0.555	1.000, 1.000		
4. *Serbelodon*	0.223, 0.424	**0.010**, **0.048**	0.170, **0.027**	1.000, 1.000	
5. *Teleoceras*	0.160, 0.685	0.691, 0.246	0.225, 0.121	**0.018**, 0.229	1.000, 1.000

B. Pratt Slide $\delta^{13}C_E$, $\delta^{18}O_E$	1	2	3	4	5	6
1. *Cormohipparion*	1.000, 1.000					
2. *Cranioceras*	0.808, 0.056	1.000, 1.000				
3. *Hipparion*	0.260, 0.513	0.257, 0.145	1.000, 1.000			
4. *Procamelus*	0.101, **0.022**	0.070, 0.485	0.564, 0.054	1.000, 1.000		
5. *Prosthennops*	0.186, **0.001***	0.159, **0.000***	0.863, **0.000***	0.661, **0.000***	1.000, 1.000	
6. *Teleoceras*	0.180, 0.456	0.173, **0.010**	0.716, 0.153	0.899, **0.004***	0.816, **0.003***	1.000, 1.000

C. Cambridge $\delta^{13}C_E$, $\delta^{18}O_E$	1	2	3	4	5	6	7
1. *Alforjas*	1.000, 1.000						
2. *Aphelops*	0.705, 0.066	1.000, 1.000					
3. *Calippus*	0.495, 0.527	0.296, **0.019**	1.000, 1.000				
4. *Hemiauchenia*	0.096, 0.307	0.195, 0.281	**0.023**, 0.098	1.000, 1.000			
5. *Megatylopus*	0.848, 0.780	0.824, 0.079	0.352, 0.337	0.098, 0.400	1.000, 1.000		
6. *Neohipparion*	**0.024**, 0.004	**0.010**, 0.332	0.111, **0.001***	**0.000***, **0.023**	0.007, 0.003	1.000, 1.000	
7. *Teleoceras*	0.869, 0.007	0.549, 0.423	0.532, **0.002***	0.036, **0.039**	0.678, **0.006**	**0.011**, 0.854	1.000, 1.000

D. Broadwater $\delta^{13}C_E$, $\delta^{18}O_E$	1	2	3	4	5
1. *Camelops*	1.000, 1.000				
2. *Equus*	0.281, 0.636	1.000, 1.000			
3. *Hemiauchenia*	0.788, 0.176	0.124, 0.066	1.000, 1.000		
4. *Platygonus*	0.186, 0.085	**0.020**, **0.032**	0.151, 0.494	1.000, 1.000	
5. *Titanotylopus*	0.863, 0.817	0.216, 0.485	0.954, 0.272	0.254, 0.134	1.000, 1.000

E. Big Springs $\delta^{13}C_E$, $\delta^{18}O_E$	1	2	3	4	5
1. *Equus*	1.000, 1.000				
2. *Gigantocamelus*	0.681, 0.474	1.000, 1.000			
3. *Platygonus*	**0.001***, 0.065	**0.004***, 0.209	1.000, 1.000		
4. *Stegomastodon*	0.293, 0.800	0.192, 0.784	**0.001***, 0.211	1.000, 1.000	
5. *Titanotylopus*	0.852, 0.058	0.569, 0.227	**0.001***, 0.829	0.370, 0.237	1.000, 1.000

(p < 0.001, *T*-test) decrease in mean $\delta^{18}O_E$ faunal values of 3.2‰ occurs from the late Miocene to the Pliocene (Table 4, Fig. 4).

5. Discussion

5.1. North Shore local fauna

All of the taxa in the North Shore local fauna have individual $\delta^{13}C_E$ values consistent with feeding in an open C_3 biome, such as "dry" woodland-savanna or C_3 grassland (Fig. 3). Both *Cormohipparion occidentale* and *Teleoceras major* have high mean $\delta^{13}C_E$ values (−9.0‰ and −9.1‰, respectively), supported by five samples each, that plot in the overlap zone for water-stressed C_3 and mixed C_3/C_4 vegetation. High hypsodonty indices (Table 5) for both of these species suggest that they were capable of grazing, and silicified grass remains (anthoecia) in the oral cavity of several articulated skeletons of *T. major* in the Ashfall local fauna strongly suggest that it consumed grass (Voorhies and Thomasson, 1979). Additionally, Tipple and

Pagani (2007) reported that both of these species contained individuals in the Ashfall local fauna with $\delta^{13}C_E$ values in the C_3/C_4 range. The high $\delta^{13}C_E$ values for these taxa from North Shore are consistent with feeding in open, water-stressed C_3 habitats or consuming a small amount of C_4 vegetation, although no individual has high enough values to confidently indicate C_4.

5.2. Pratt Slide local fauna

Pratt Slide appears to have been the most densely vegetated of the Miocene localities, based on its low mean $\delta^{13}C_E$ value plotting in the range expected for wet, open forest just above the closed forest boundary (Figs. 2 and 4). Notably, none of the individuals from Pratt Slide plot in the range expected for water-stressed C_3 vegetation, unlike the other Miocene local faunas. Three individuals of *Cormohipparion* sp. and *Cranioceras unicornis* have $\delta^{13}C_E$ values ranging from −13.0 to −14.5‰, suggesting feeding in a densely vegetated area. However, values are not low enough to confidently indicate the presence of a

Table 3
Pairwise probabilities of difference in the $\delta^{13}C$ values between the whole local faunal samples and possible vegetative biomes. Values in bold indicate significance at $\alpha \leq 0.05$ using a *T*-test.

Biomes	North Shore	Pratt Slide	Cambridge	Broadwater	Big Springs
"Wet" woodland-savanna, Texas	<**0.001**	0.354	<**0.001**	<**0.001**	<**0.001**
"Dry" woodland-savanna, South Africa	0.566	<**0.001**	0.196	<**0.001**	<**0.001**
"Dry" forest, Hawaii	0.372	<**0.001**	0.747	0.093	**0.002**
"Dry" C_3 grassland, Mongolia	0.293	**0.003**	0.865	0.096	**0.004**
"Dry" Woody scrubland, Utah	0.674	**0.001**	0.689	0.198	**0.011**

Table 4
Summary statistics for $\delta^{13}C_E$ and $\delta^{18}O_E$ values from the local faunas.

	$\delta^{13}C_E$ (VPDB)				
	North Shore	Pratt Slide	Cambridge	Broadwater	Big Springs
n	18.0	20.0	21.0	16.0	21.0
Median	−9.3	−11.6	−10.0	−8.8	−4.2
Mean	−9.4	−11.8	−9.9	−8.1	−5.0
Minimum	−10.9	−14.5	−11.8	−11.3	−11.7
Maximum	−8.4	−10.1	−8.0	−3.8	0.7
Range	2.5	4.4	3.8	7.5	12.4
S.D.	0.7	1.0	0.9	2.4	3.7
S.E.	0.2	0.2	0.2	0.6	0.8

	$\delta^{18}O_E$ (VSMOW)				
	North Shore	Pratt Slide	Cambridge	Broadwater	Big Springs
n	18.0	20.0	21.0	16.0	21.0
Median	26.0	26.4	26.4	22.1	23.4
Mean	25.9	25.7	26.8	22.4	23.3
Minimum	22.6	22.0	24.1	17.7	19.5
Maximum	29.7	28.6	30.0	27.5	27.2
Range	7.1	6.6	5.9	9.8	7.7
S.D.	1.8	2.0	1.6	2.2	2.4
S.E.	0.4	0.5	0.4	0.6	0.5

dense closed forest canopy, which would require individual values to be ≤− 15.7‰ (see the Methods section). The mean $\delta^{13}C_E$ value for *Cormohipparion* sp. from Pratt Slide (−12.6‰) is significantly lower (p < 0.001, T-test) than that of *C. occidentale* from North Shore (−9.0‰) with no overlap in individual values. This suggests that species within this genus were capable of exploiting a variety of habitats. The significantly low $\delta^{18}O_E$ value (22.4‰) in the peccary *Prosthennops* sp. at Pratt Slide is also notable and indicates that this species occupied a wetter habitat than the other taxa. This is in stark contrast to *Prosthennops* sp. from the late Miocene in California (Feranec and MacFadden, 2006) and the middle Miocene of Florida (Clementz et al., 2008) where its mean $\delta^{18}O_E$ values are not significantly different from other taxa. Our results imply that the Nebraskan species of *Prosthennops* sp. was living a different lifestyle than species in the other regions of North America.

5.8. Cambridge local fauna

In the Cambridge local fauna the equid *N. eurystyle* has the highest mean $\delta^{13}C_E$ value, significantly higher (p = 0.009 Tukey's test) than the camel *Hemiauchenia* cf. *H. vera*. Individual values of *N. eurystyle* plot in the range expected for water-stressed C_3 or mixed C_3/C_4 vegetation, but no values are high enough to confidently indicate C_4. The high $\delta^{13}C_E$ values in *N. eurystyle* suggest that it was living in an open environment and may have been a grazer, which is consistent with a previous interpretation based on hypsodonty and microwear in *N. eurystyle* from Florida (MacFadden et al., 1999b). Individual values of most other taxa plot in the lower end of the water-stress range or in the "normal" range for open habitats.
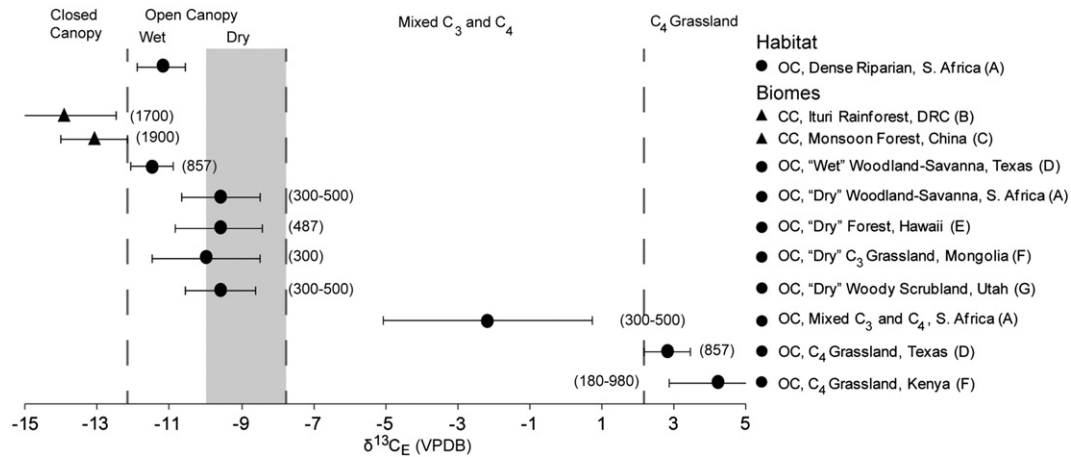
5.4. Lisco camel

Gigantocamelus spatulus is the only taxon sampled from the Lisco locality and plots in the region for a mixed C_3/C_4 diet (Fig. 3). The mean $\delta^{13}C_E$ value of −3.1‰ agrees with the interpretation by Voorhies and Corner (1986) that *G. spatulus* was a grazer but contrasts with the microwear study of Semprebon and Rivals (2010) that suggested it was a browser (Table 5). However, because of the narrow ranges in both $\delta^{13}C_E$ and $\delta^{18}O_E$ values and the unusual "rusty" color of teeth from Lisco, it is possible that these teeth have been diagenetically altered and data from Lisco may not be reliable.

## 5.5. Broadwater local fauna

Individual $\delta^{13}C_E$ values for taxa in the Broadwater fauna suggest habitats ranging from an open C_3 environment to mixed C_3/C_4 grasslands (Fig. 3). Individual $\delta^{13}C_E$ values for all taxa except *Platygonus* sp. fall in the range expected for mixed C_3/C_4 consumption, indicating a considerable component of C_4 grasses at this time. Both *Hemiauchenia blancoensis* and *Titanotylopus nebraskensis* contain individuals that plot in the C_3 and mixed C_3/C_4 ranges, suggesting either that these taxa consumed browse and graze, or were eating C_3 and C_4 grasses. Semprebon and Rivals (2010) reported that *T. nebraskensis* was a browser based on a low hypsodonty index of 2. Browsing is

Table 5
Previous dietary interpretations and hypsodonty indices for taxa used in this study.

Taxa sampled	Locality	Compared species from literature	Presumed diet	Method determined	HI (q)
Calippus sp.	C	*Calippus* spp. (a)	Grazer	Incisor arcade shape (a)	5
Cormohipparion sp.	PS	*Cormohipparion emsliei* (b),	Mixed feeder	Hypsodonty, microwear (b);	5
Cormohipparion occidentale	NS	*Cormohipparion* sp. (c)		stable isotopes (c)	
Equus simplicidens	BW; BS	*Equus* sp. (c), *Equus burchelli* (d)	Grazer	Stable isotopes (c); microwear (d)	5
Hipparion sp.	PS	*Hipparion* sp.	Mixed feeder	Microwear (e)	5
Neohipparion eurystyle	C	*Neohipparion eurystyle* (b)	Grazer	Hypsodonty & microwear (b)	5
Aphelops sp.	NS	*Aphelops* spp. (f)	Browser	Hypsodonty & stable isotopes (f)	4
Aphelops mutilus	C				
Teleoceras fossiger	C	*Teleoceras* spp. (f), *Teleoceras major* (g)	Mixed feeder/grazer	Hypsodonty, stable isotopes (f);	5
Teleoceras major	PS; NS			stomach contents (g)	
Serbelodon cf. *S. barbourensis*	NS	*Serbelodon* sp. (h)	Mixed feeder	Wear patterns on tusks (h)	1
Stegomastodon sp.	BS	*Stegomastodon* spp. (i)	Mixed feeder	Stable isotopes (i)	4
Alforjas sp.	C	*Alforjas* sp. (j)	Browser	Premaxillary shape (j)	3
Camelops sp.	BW	*Camelops* spp. (k)	Browser	Hypsodonty, microwear & mesowear (k)	4
Gigantocamelus spatulus	L; BS	*Gigantocamelus spatulus* (l, k)	Browser or grazer	Hypsodonty, microwear & mesowear (l, k)	4
Megatylopus cf. *M. primaevus*	NS	*Megatylopus* sp. (k)	Browser	Hypsodonty, microwear & mesowear (k)	3
Megatylopus sp.	C				
Hemiauchenia cf. *H. vera*	C	*Hemiauchenia* sp. (k), *H. vera* (m),	Mixed feeder/browser	Hypsodonty, microwear, mesowear (k);	3
Hemiauchenia blancoensis	BW	*H. blancoensis* (m)		stable isotopes (m)	
Procamelus grandis	PS	*Procamelus* sp. (k)	Browser	Hypsodonty, microwear & mesowear (k)	4
Titanotylopus nebraskensis	BW; BS	*Titanotylopus nebraskensis* (n)	Browser	Hypsodonty (n)	2
Cranioceras unicornis	PS	*Cranioceras* sp. (o)	Browser/mixed feeder	Microwear & mesowear (o)	1
Platygonus sp.	BW; BS	*Platygonus* sp. (c)	Mixed feeder	Hypsodonty & stable isotopes (c)	1
Prosthennops sp.	PS	*Prosthennops xiphodonticus* (p)	Browser	Hypsodonty & muzzle shape (p)	1

Abbreviations: C = Cambridge; PS = Pratt Slide; NS = North Shore; L = Lisco; BW = Broadwater; BS = Big Springs; HI = hypsodonty index. References: (a) Hulbert (1988); (b) MacFadden et al. (1999b); (c) MacFadden and Cerling, 1996; (d) MacFadden (2000); (e) Caprini (1998); (f) MacFadden (1998); (g) Voorhies and Thomasson (1979); (h) Lambert (1992); (i) Prado et al. (2005); (j) Dompierre and Churcher (1996); (k) Semprebon and Rivals (2010); (l) Voorhies and Corner (1986); (m) Feranec (2003); (n) Barbour and Schultz (1934); (o) Semprebon et al. (2004); (p) Mendoza (2007); (q) Jardine et al. (2012).

Fig. 2. Predictive model for δ¹³C values of herbivore enamel in various biomes and habitats of the late Miocene and Pliocene. Predictions are based on δ¹³C values from modern floras normalized to 42°N latitude in North America and adjusted for diet-enamel enrichment and change in atmospheric δ¹³C values (see text). Mean annual precipitation values (mm/yr) for modern biomes are given in parentheses by error bars. Error bars show 95% confidence of mean (±1.96 S.E.). CC = closed canopy (black triangles), OC = open canopy (black circles), DRC = Democratic Republic of the Congo.
Data sources: A, Codron et al. (2005); B, Cerling et al. (2004); C, Ehleringer et al. (1987); D, Jessup et al. (2003); E, Sandquist and Cordell (2007); F, Cerling and Harris (1999); G, Williams and Ehleringer (1996).
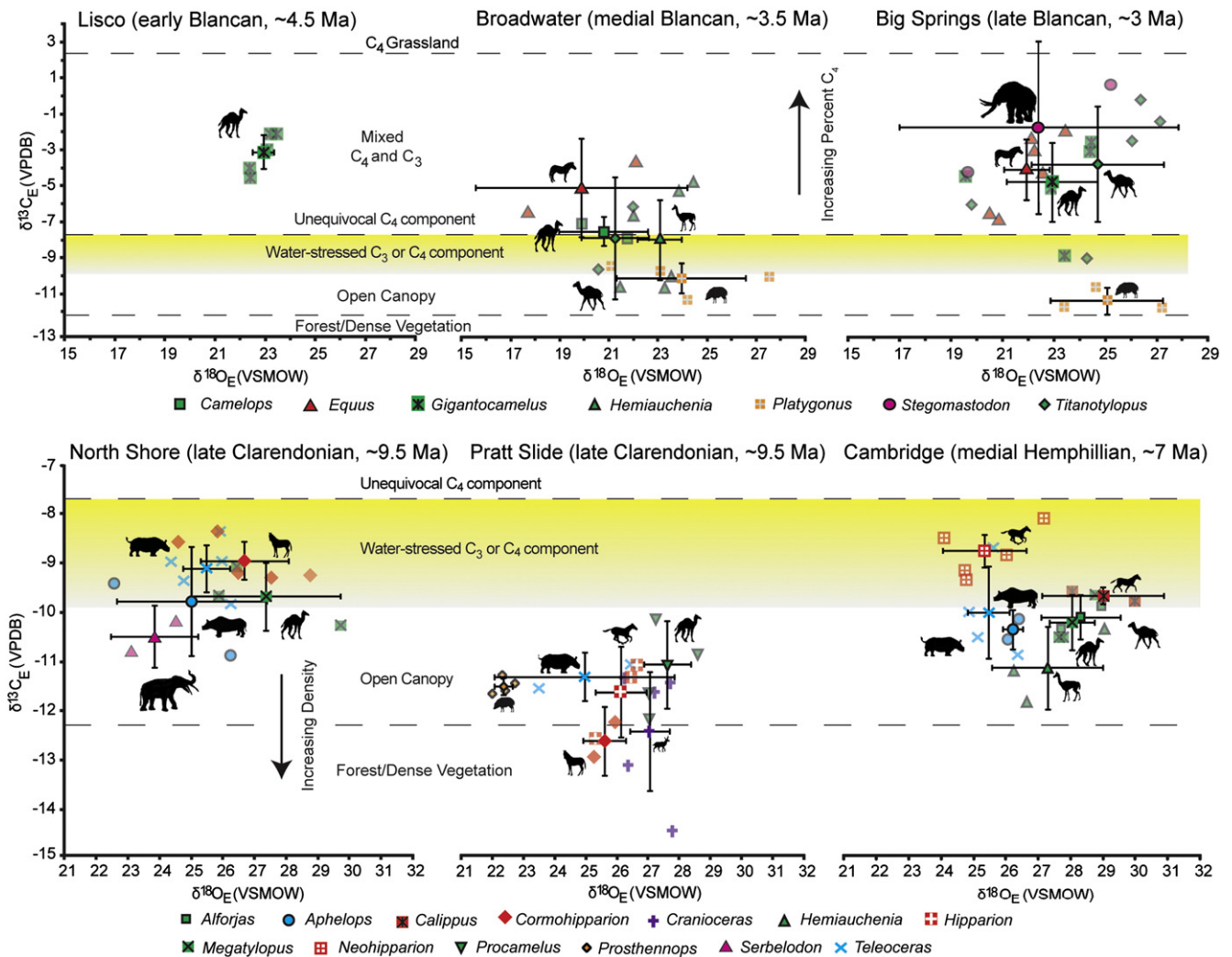
Fig. 3. Mean and individual δ¹³C$_E$ and δ¹⁸O$_E$ values for taxa in local faunas and boundaries between vegetation types. Means are designated by error bars representing 95% confidence of mean.

possible for one of the individuals we sampled (−9.6‰) but the higher value (−6.2‰) in the other one suggests some C_4 grazing. Only two teeth of *Equus simplicidens* were sampled, but both have $\delta^{13}C_E$ values (−3.8‰, −6.5‰) in the range expected for a mixed C_3/C_4 diet (Fig. 3). This is consistent with a previous work suggesting that it was a grazer (Table 5), as are most modern species of *Equus* (e.g., Janis, 1990).

Individual $\delta^{13}C_E$ values for the peccary *Platygonus* sp. in the Broadwater (and Big Springs) local fauna all plot in the range expected for C_3 consumption, although three are close to the lower end of the range for overlapping water-stressed C_3 and C_3/C_4. Nevertheless, no value is high enough to conclusively indicate anything but a C_3 diet. This contrasts with work on *Platygonus* sp. from Florida that showed that some individuals consumed a portion of C_4 or CAM vegetation (MacFadden and Cerling, 1996). The Florida and Nebraska samples could belong to different species or a single generalist species consuming different diets in different areas, possibly including CAM plants in Florida (see also *Platygonus* sp. at Big Springs).

5.6. Big Springs local fauna

All of the taxa from Big Springs, except *Platygonus* sp., yielded individual $\delta^{13}C_E$ values indicating that they consumed considerable portions of C_4 vegetation (Fig. 3) and were grazers or mixed feeders. Only two individuals among the mixed C_3/C_4 taxa plot in the C_3 range, one of *T. nebraskensis* and one of *G. spatulus*. The latter values both fall in the overlap range for water-stressed C_3 and mixed C_3/C_4, and thus even these individuals may have consumed some C_4 vegetation. Voorhies and Corner (1986) suggested that *G. spatulus* was a grazer based on hypsodonty, consistent with our results. However, our finding that *T. nebraskensis* was a grazer or mixed feeder is counter to previous hypsodonty work that suggested it was a browser (Barbour and Schultz, 1934).

Platygonus sp. has a significantly lower mean $\delta^{13}C_E$ value (−11.3‰) at Big Springs than all other taxa (Table 2E). Like the Broadwater local fauna, it plots in the range expected for C_3 feeders but unlike that fauna individual values are all below the range for water-stressed C_3 feeders (Fig. 3). The apparent prevalence of C_4 grass at Big Springs and lack of a C_4 signal in *Platygonus* sp. suggest that it may have been primarily a browser in Nebraska, and that the higher $\delta^{13}C_E$ value (−4.6‰) in *Platygonus* sp. from Florida (MacFadden and Cerling, 1996) was the result of consuming CAM vegetation that was not present in Nebraska.

A significant increase (p = 0.005, *T*-test) in mean faunal $\delta^{13}C_E$ values of 3.1‰ occurs between the Broadwater and Big Springs faunas, indicating greater C_4 consumption. This could potentially be tied to warmer conditions that favored C_4 vegetation. Studies have suggested a correlation between warmer temperatures and increased C_4 biomass during the Quaternary (DeSantis et al., 2009; Kohn and McKay, 2012). Warmer conditions for the late Blancan are generally in agreement with reconstructions of CO_2 concentrations for the Pliocene, which suggest that most of the Pliocene was warmer than today, with the warmest temperatures occurring from ~3.2 to 3.0 Ma, followed by rapid cooling after 3.0 Ma (Seki et al., 2010). The Big Springs local fauna was assigned to the early late Blancan by Passey et al. (2002), which corresponds to Blancan IV (~3.0 to 2.6 Ma) of Bell et al. (2004). If this correlation is correct, the Big Springs local fauna may correspond to the early part of the late Pliocene warm interval just before the onset of cooling. Better geochronologic controls are needed, however, to confidently tie the fauna to this interval.

5.7. Late Miocene and Pliocene biomes of Nebraska

Carbon isotope values from the late Miocene local faunas (North Shore, Pratt Slide, Cambridge) show that all individuals plot in the range expected for C_3 consumers or in the overlap range

between water-stressed C_3 vegetation and mixed C_3/C_4 vegetation. However, no individual value is high enough to confidently indicate a dietary component of C_4 vegetation (Fig. 3). The North Shore and Cambridge local faunas have high mean $\delta^{13}C_E$ values (−9.4‰ and −9.9‰, respectively) consistent with open biomes and are statistically indistinguishable from the "dry" woodland-savanna, "dry" forest, "dry" C_3 grassland, and "dry" woody scrubland (Table 3). This contrasts with Pratt Slide, which yielded a significantly lower $\delta^{13}C_E$ value (−11.8‰) and is statistically indistinguishable from the "wet" woodland-savanna (Table 3). Several individual values plot in the range expected for at least partially forested habitats in northern Nebraska in the late Clarendonian (Fig. 3). However, a markedly higher mean $\delta^{13}C_E$ value (~−8.9‰) from the Ashfall local fauna from the same latitude in northern Nebraska (Figs. 1 and 4), calculated from data in Clementz et al. (2008; Table 1, middle Miocene 11.8 Ma), suggests an open landscape in the early Clarendonian. These differences could indicate either a patchy forest in northern Nebraska during the Clarendonian, or be explained by a change from open to forested conditions from the early to late Clarendonian. However, a change from open to closed biomes across Nebraska would be difficult to explain without a change in climate, but a significant change in climate has not been recognized during this time interval. Thus, our results suggest that the lower $\delta^{13}C_E$ values at Pratt Slide resulted from local conditions. Our findings suggest that some forested areas were present in Nebraska in the late Miocene but that other areas were open. Overall, environments in late Miocene of Nebraska appear to have been open with some localized areas of denser, more forested habitats. This is most consistent with the phytolith record that suggests patches of grass and forest (Strömberg, 2004, 2006, 2011), although it is not clear if the geographic scales of "patchiness" suggested by the phytolith and isotope records are equivalent. The more forested conditions suggested by pollen and macrofloras (e.g., Thomasson, 1990; Bolick et al., 1995) may be the result of the preferential preservation of tree fossils in poorly drained areas.

Fox and Koch (2004) suggested the presence of 12–34% C_4 biomass (mean = 20%) through the early and middle Miocene preceding major C_4 expansion, based on carbon isotopes in soil carbonates from Nebraska and other states. If this much C_4 biomass was present in the late Miocene there is no clear indication that any of the high-crowned herbivores (Table 1, *Calippus* sp., *Cormohipparion* spp., *Hipparion* sp., *N. eurystyle*, *Aphelops* spp., *Teleoceras* spp.) were exploiting it to any meaningful extent. This is consistent with previous results from Miocene horses (Passey et al., 2002) and with samples from the Ashfall fauna in which only two of twenty-two high-crowned individuals yielded $\delta^{13}C_E$ values that could be confidently attributed to a component of C_4 vegetation (Tipple and Pagani, 2007; see Clementz et al., 2008, for summary data). Although some studies have used data from
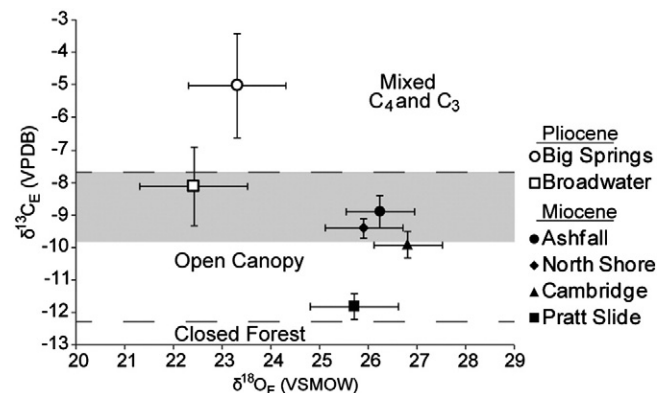

Fig. 4. Mean $\delta^{13}C_E$ and $\delta^{18}O_E$ values for local faunas with inferred biome boundaries. Error bars show 95% confidence of mean (± 1.96 S.E.). Ashfall data from Clementz et al. (2008; Table 1, 11.8 Ma).

Passey et al. (2002) to suggest that mammals were consuming up to 20% C_4 biomass in the middle and late Miocene (e.g., Edwards et al., 2010; Strömberg, 2011), $\delta^{13}C$ values in this range could also result from feeding in water-stressed C_3 habitats, as noted by these authors. Moreover, grazers in biomes with well documented components of C_4 vegetation show a high degree of variability in the percent of C_4 consumed among individuals. This is not the pattern seen in the late Miocene of Nebraska. If an average of 20% C_4 were being consumed by grazers, several individuals in a large sampling should have been consuming much higher percentages, yielding unambiguous C_4 values, while some would be consuming only C_3. Thus, if high-crowned taxa (Table 5) in the late Miocene were grazers or mixed C_3/C_4 feeders, which seems highly likely, they appear to have been largely avoiding C_4 vegetation, a conclusion reached by Fraser and Theodor (2013). Other possibilities are that the amount of C_4 vegetation was closer to the lower range (12%) reported by Fox and Koch (2004) or atmospheric $\delta^{13}C$ values were higher in the Miocene than estimated in their model, causing an over estimation of C_4 biomass.

By the middle Pliocene, $\delta^{13}C_E$ values in the Broadwater fauna indicate that horses and camels were including a component of C_4 vegetation in their diets (Table 1, Fig. 3, Appendix). The percentage of consumed C_4 increased further in the late Pliocene Big Springs fauna where *Stegomastodon* sp. was consuming the greatest percentage (Fig. 3). Mean $\delta^{13}C_E$ values are significantly correlated with hypsodonty index for the Blancan taxa (r = 0.698, p = 0.016, least squares linear regression) indicating a positive relationship between amounts of C_4 vegetation consumed and tooth crown height, as might be expected.

5.8. Climate change from the late Miocene to the Pliocene

Late Neogene faunal turnover in North America (Janis et al, 1998, 2000, 2002, 2004) was presumably linked to ecosystem change and the expansion of grasslands as global climate cooled from the middle Miocene through the Pliocene, as indicated by oxygen isotopes in benthic foraminifera (e.g., Zachos et al., 2001, 2008). However, although the marine record provides estimates of climate change, these estimates may not accurately reflect changes taking place in continental interiors (e.g., IPCC, 2007). Thus, climate proxies such as $\delta^{18}O$ values in mammals are of particular interest.

Marked negative shifts of −5.0 and −3.8‰ in $\delta^{18}O_E$ values occur in both horses and camels, respectively, from the late Miocene faunas to the Pliocene faunas (Fig. 4). The smaller decrease in the camels could be explained by a shift to more arid conditions if these camels were less water-dependent than horses. We note that in all cases but one (*Calippus* sp., Fig. 3), camels have higher mean $\delta^{18}O_E$ values than horses, which is consistent with lower water dependence in camels (e.g., Levin et al., 2006). However, although this pattern is generally consistent, caution is warranted since variability in $\delta^{18}O_E$ values is high and intra-faunal differences between horses and camels are not significant using Tukey's post hoc test (Table 2). Passey et al. (2002) also found a large negative shift in Nebraskan horses from the late Miocene to the Pliocene. They suggested that this shift was due to climate change and possibly taxon specific changes in water usage among horses. We concur that a component of this decrease could be due to changes in water usage among the horses but our new data show that a large decrease also occurs in camels, suggesting that most of the decrease was the result of a shift to more negative $\delta^{18}O$ values in Pliocene surface water. A decrease in the $\delta^{18}O$ values of soil carbonate nodules was also reported through this temporal interval in the western Great Plains, implying a decrease in surface water values (Fox and Koch, 2004; Fox et al., 2012b).

The dramatic drop in $\delta^{18}O_E$ values implies a major shift to cooler climate in the continental interior of North America during the Pliocene. Quantification of this shift is complicated by several variables, but we note that the expected direction for two of these variables would result

in a dampening of the measured change in $\delta^{18}O_E$ values, not amplification. A shift to a drier climate in the Pliocene, as has been inferred from the spread of grasslands (Axelrod, 1985), and the addition of C_4 grasses that have elevated $\delta^{18}O$ values relative to C_3 plants under dry conditions (Helliker and Ehleringer, 2002), should result in an increase in $\delta^{18}O_E$ values in Pliocene herbivores. Additionally, the oceans and atmosphere would have become more ^{18}O-enriched as lighter ^{16}O was preferentially incorporated in glacial ice (Lear et al., 2000; Fox et al., 2012b). This should result in higher $\delta^{18}O$ values in precipitation. These factors would have a dampening effect on the magnitude of change. Another factor to consider is a possible change to a more ^{18}O-depleted vapor source for Nebraskan precipitation in the Pliocene, such as a change in water vapor source from the Gulf of Mexico to the Pacific Ocean, which could conceivably be driven by cooling climate. This scenario is difficult to test but a regional analysis of mammalian $\delta^{18}O_E$ values from discrete time intervals could be employed. Nevertheless, the large decrease in $\delta^{18}O_E$ values reported here is consistent with a large decrease in temperature in the interior of North America from the late Miocene to the middle Pliocene as suggested by Passey et al. (2002), Fox and Fisher (2004), and Fox and Koch (2004).

6. Conclusions

The late Miocene faunas in this study largely or exclusively exploited C_3 vegetation and lived primarily in dry, open habitats. The presence of more densely vegetated, partially forested habitats is suggested, however, by low mean and individual $\delta^{13}C_E$ values in the Pratt Slide local fauna. With regard to our first question (Question 1, see the Introduction section), our overall results suggest predominantly open biomes in the late Miocene, consisting of grasslands or woodland-savannas, with a small component of more heavily forested areas. With regard to Question 2, no individual $\delta^{13}C_E$ value for the late Miocene ungulates we sampled is high enough to confidently indicate consumption of C_4 vegetation. This contrasts with an estimated 12–34% C_4 biomass (mean = 20%; Fox and Koch, 2004) in the late Miocene and suggests that either C_4 abundance was near or below the lower end of the reported range or that taxa in the late Miocene were largely avoiding C_4 vegetation. However, because $\delta^{13}C_E$ values indicating a component of C_4 vegetation (−7‰ to −4‰) were reported in individuals of *Cormohipparion* sp. and *T. major* from the Ashfall local fauna (Tipple and Pagani, 2007), and because we sampled these taxa in our Miocene faunas, the former hypothesis appears to be more likely.

With regard to Question 3, habitat partitioning within the late Miocene faunas is difficult to distinguish because mean $\delta^{13}C_E$ and $\delta^{18}O_E$ differences are fairly small, as are sample sizes for most taxa. Using Tukey's post hoc test there is only one significant difference among taxon pairs using mean $\delta^{13}C_E$ values and eight differences using mean $\delta^{18}O_E$ values (Table 2). While significant differences were not present in the North Shore fauna, *Prosthennops* sp. in the Pratt Slide fauna had significantly lower $\delta^{18}O_E$ values than all other taxa. This implies that it got most of its water from drinking and probably lived in a wetter habitat than the other species. In the Cambridge fauna, only *N. eurystyle* and *Hemiauchenia* cf. *H. vera* had significantly different $\delta^{13}C_E$ values. The higher values in *N. eurystyle* suggest that it was living in a more open habitat.

With regard to Question 4, species within several mammalian clades independently adapted to mixed C_3/C_4 feeding in response to the spread of C_4 grasslands in the Pliocene. These include several camelids, an equid, and a gomphothere. Although a component of C_4 vegetation was consumed by most of the taxa sampled, C_3 vegetation remained an important part of all diets and was abundant enough in the Blancan to support *Platygonus* sp., which fed predominantly or exclusively on C_3 plants. An increase in C_4 consumption occurs from the mid Blancan Broadwater local fauna to the late Blancan Big Springs local fauna. This increase parallels an increase in soil carbonate $\delta^{13}C$ values

(Fox and Koch, 2004), suggesting greater expansion of C_4 biomass in the late Pliocene.

With regard to Question 5, there is a marked shift to lower mean $\delta^{18}O_E$ values from the late Miocene to the Pliocene in both horses and camels. This decrease in $\delta^{18}O_E$ values suggests major cooling in the continental interior of North America in the Pliocene, which parallels global cooling in the oceans at this time. Our results indicate major changes in both climate and vegetation from the late Miocene to the late Pliocene in the Great Plains of North America.

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