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Publication Date

2017-06-01

DOI

10.1016/j.palaeo.2017.03.008

Peer reviewed



Pieces of the puzzle: Lack of significant C₄ in the late Miocene of southern California



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ARTICLE INFO

Article history:

Received 16 May 2016

Received in revised form 26 February 2017

Accepted 7 March 2017

Available online 9 March 2017

Keywords:

C₃/C₄ grasses

Mojave Desert

Carbon isotopes

Miocene

Herbivores

Dove Spring Formation

ABSTRACT

The stable carbon isotope ratios of tooth enamel from 89 herbivores from the Dove Spring Formation (DSF) of the El Paso Basin, western Mojave Desert, California, were analyzed to determine if C₄ plants may have been present in the late Miocene of southern California. Taxa analyzed include antilocaprids, castorids, camelids, equids, gomphotheriids, merycoidodontids, and rhinocerotids. The atmospheric CO₂-δ¹³C value has varied significantly in the geologic past, leading to changes in the δ¹³C values of plants and tooth enamel. The estimated δ¹³C value of atmospheric CO₂ in the middle to late Miocene is $-5.8 \pm 0.2\%$, making the pure C₃ diet cutoff value higher than the modern by ~2%. Given this, much of the evidence of an early spread of C₄, which inappropriately uses the modern C₃ diet cutoff value, may be a false signal from water-stressed C₃ plants and the amount of C₄ biomass in the geologic past may have been over-estimated. The enamel-δ¹³C values of all taxa sampled are $-10.1 \pm 1.1\%$ (n = 89). Accounting for the changes in the δ¹³C values of atmospheric CO₂, enamel-δ¹³C values from the DSF indicate that herbivores from that area had C₃ diets and lived in an environment dominated by C₃ plants. Serial enamel isotope data from the DSF revealed negligible intra-tooth variations in the δ¹³C values, confirming that these herbivores consumed C₃ plants year-round. If the C₄ photosynthetic pathway did spread to southern California prior to the rapid, global rise in C₄ in the late Miocene, the taxa sampled did not incorporate C₄ biomass into their diets. The results of the stable carbon isotope analysis, along with the evidence from the rock record and the life habits of the fossil taxa present, indicate that the El Paso Basin consisted of diverse habitats including C₃ grasslands, wooded C₃ grasslands, and riparian forests.

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1. Introduction

C₄ grasses do not exist on all continents, at all latitudes, or at all altitudes, but favor conditions in which they have the competitive advantage over other plant life. Studies of modern plant distribution (e.g. Teeri and Stowe, 1976; Mulroy and Rundel, 1977; Tieszen et al., 1979, 1997; Ehleringer et al., 1997; Edwards et al., 2010) show that C₄ plants have a clear advantage over C₃ plants in habitats with high solar radiation, high day-time growing season temperatures, limited water availability, warm season precipitation, and cool season dryness. Grasses in general are new to geologic history. The initial transition from woody C₃ plants to C₃ grasses likely took place because grasses tend to have a

higher tolerance to a wide range of soil factors and climatic extremes (Edwards et al., 2010). Influences such as herbivory and fires, which limit woody plant growth but sustain grasslands, likely played a role in this transition as well (Edwards et al., 2010).

During the Neogene there was a dramatic expansion of C₄ grasses (Cerling et al., 1997a, 1997b). The timing and mechanisms responsible for the expansion of C₄ grasses has been a topic of considerable debate over the last two decades. Most studies agree that approximately 6–8 Ma, in the late Miocene, there was a rapid, global increase in C₄ ecosystems (e.g. Cerling et al., 1997a, 1997b; Tipple and Pagani, 2007; Edwards et al., 2010). This marks the global expansion of C₄ grasslands after the initial spread of C₃ grasslands in the Oligocene and early Miocene (MacFadden and Cerling, 1996; Cerling et al., 1998; Ehleringer et al., 1997; Latorre et al., 1997; Edwards et al., 2010; Feranec and Pagnac, 2013). Various hypotheses have been proposed to explain this ecological change (e.g. Quade et al., 1989; Cerling et al., 1993, 1997a, 1997b; Wang et al., 1994; Huang et al., 2007; Tipple and Pagani, 2007). Early

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papers on this topic focus solely on the global event as causing the first appearance of a significant amount of C₄ grasses in localities such as Pakistan (Quade et al., 1989, 1992; Morgan et al., 1994; Cerling et al., 1997a, 1997b), East Africa (Morgan et al., 1994; Cerling et al., 1997a, 1997b), and the Americas (Cerling et al., 1993, 1997a, 1997b; MacFadden et al., 1996; Wang et al., 1994). This event can be correlated nearly worldwide (e.g. Cerling et al., 1997a, 1997b). Although abundant evidence indicates that there was a worldwide, rapid increase in C₄ ecosystems in the late Miocene, there have been several instances where the isotopic signature of C₄ plants has been found in paleosol carbonates and fossil teeth prior to the global C₄ expansion in the late Miocene (Morgan et al., 1994; Fox and Koch, 2003; Fox et al., 2012; Cotton and Sheldon, 2012; Chen et al., 2015; Feranec and Pagnac, 2013, 2017). This suggests an earlier regional spread of C₄ grasses, although the timing of such an event is not well constrained. These studies suggest that a significant amount of C₄ grasses existed in ecosystems of the middle Miocene in southern California (Feranec and Pagnac, 2013, 2017), as well as Pakistan and Kenya (Morgan et al., 1994), and in the early and middle Miocene in the Great Plains (Fox and Koch, 2003; Fox et al., 2012).

There was likely a dichotomy to the causes that led to the spread of C₄ grasslands in the Miocene, with some factors affecting the spread on a regional scale and others on a global scale. On a global scale, the two hypotheses mentioned most often in recent literature to explain this rise in C₄ biomass are: 1) the decrease in atmospheric CO₂ levels (Ehleringer et al., 1991; Quade et al., 1992; Cerling et al., 1993, 1997a, 1997b, 1998; Wang et al., 1994; MacFadden et al., 1996; MacFadden and Cerling, 1996; Edwards et al., 2010) and 2) global climatic changes leading to the increased low-altitude aridity, fire disturbance, and/or changing seasonality of precipitation regimes (Latorre et al., 1997; MacFadden et al., 1996; MacFadden, 1997; Pagani et al., 1999; Tiplle and Pagani, 2007). At low atmospheric CO₂ levels, C₄ plants are favored because of their ability to concentrate CO₂ in the bundle sheath cells while C₃ plants suffer from net photosynthetic loss due to photorespiration (Ehleringer, 2005; Tiplle and Pagani, 2007). Most proxy data do not seem to indicate a significant drop in pCO₂ in the middle or late Miocene, which raises speculation on the validity of this hypothesis for the cause of the spread of C₄ grasses (e.g., Pagani et al., 1999; Pearson and Palmer, 2000; Retallack, 2001; Royer et al., 2001; Tiplle and Pagani, 2007). Two recent studies (Kurschner et al., 2008; Tripati et al., 2009), however, show that atmospheric pCO₂ levels may have been close to the C₃–C₄ crossover threshold (~500–550 ppm) during the middle to late Miocene. The possibility of global climatic changes as a cause for the spread of C₄ grasslands is not well explored in the literature and would be difficult to prove through proxy records. Additionally, Cerling et al. (1997b) argued that high temperature alone would not be sufficient to cause the late Miocene global C₄ expansion because there have been other times in the geologic past with warm temperatures but no evidence of C₄ biomass.

At the regional level, there are a variety of causes that have been suggested for the spread of C₄ grasses (Tiplle and Pagani, 2007; Edwards et al., 2010; Feranec and Pagnac, 2013). Tiplle and Pagani (2007) suggest that increased aridity, wind strength, and fire cycles may be the cause of the early spread of C₄ grasslands as evidenced by increased deposition of windblown dust and charcoal in ocean basins. The regional spread of C₄ grasses may have also been driven by changes in seasonality (Wang and Deng, 2005).

Isotope evidence from the middle Miocene Barstow Formation (southern California), which is near where the samples in this study were collected both geographically and temporally, reveals an apparent dietary intake of up to 18% C₄ plants by large herbivores in the Barstovian Land Mammal Age (middle Miocene, Feranec and Pagnac, 2013). Feranec and Pagnac (2013) called for the analysis of additional sites that pre-date the global rise in C₄, particularly those of Clarendonian Land Mammal Age (middle to late Miocene), suggesting that data from herbivore tooth enamel from such sites might support

the earlier regional spread of C₄ grasses. Knowing the abundance and geographic distribution of C₄ grasses prior to the late Miocene may help to discern the mechanism responsible for the global spread of C₄ ecosystems that took place between 6 and 8 million years ago (Feranec and Pagnac, 2013).

Here we examine the carbon isotope signatures of fossil tooth enamel of Clarendonian age herbivores of seven families—Antilocapridae, Camelidae, Castoridae, Equidae, Gomphotheriidae, Merycoidodontidae, and Rhinocerotidae—from the Dove Spring Formation of southern California to determine if they have an isotope signature indicative of the consumption of C₄ plants. The data are used to reconstruct the diets and environment of these herbivores and to determine if an early spread of the C₄ photosynthetic pathway took place in this region of southern California prior to the global C₄ expansion in the late Miocene (e.g. Cerling et al., 1997a, 1997b; Feranec and Pagnac, 2013). The results shed new light on the debate over the timing and extent of the C₄ expansion in the late Neogene.

2. Background

2.1. Carbon isotope fractionation in plants and herbivore tooth enamel

Approximately 85% of terrestrial plants utilize the C₃ photosynthetic pathway, including trees, most shrubs, and cool growing season grasses (MacFadden and Cerling, 1994; Cerling et al., 1997a, 1997b; MacFadden, 1997; Edwards et al., 2010). Warm growing season grasses, sedges, and some halophytic shrubs, consisting of around 10% of terrestrial plants, use the C₄ photosynthetic pathway (MacFadden and Cerling, 1994; Cerling et al., 1997a, 1997b; MacFadden, 1997; Edwards et al., 2010). Crassulacean acid metabolism (CAM) is the third photosynthetic pathway and is relatively uncommon. CAM plants, which are composed primarily of succulents and cacti in arid environments, make up only 5% of terrestrial plants (MacFadden and Cerling, 1994; Latorre et al., 1997; Fox and Fisher, 2004).

Due in part to varying biochemical reactions during photosynthesis and in part to environmental factors, the three photosynthetic pathways fractionate the stable carbon isotopes to different degrees (Ehleringer and Monson, 1993; Cerling et al., 1997a, 1997b; Fox and Koch, 2003; Fox and Fisher, 2004; Tiplle and Pagani, 2007). Modern C₃ plants have $\delta^{13}\text{C}$ values of -20 to -36% , averaging $-26.7 \pm 2.3\%$, while C₄ plants have $\delta^{13}\text{C}$ values between -9 to -17% , with an average of $-12.5 \pm 1.1\%$ (Cerling et al., 1997a, 1997b; Tiplle and Pagani, 2007; Kohn, 2010). The differences in $\delta^{13}\text{C}$ values for C₃ and C₄ plants can be explained by the affinity that the enzyme PEP-C, a key enzyme in C₄ photosynthesis, has for the heavier stable carbon isotope, ^{13}C (Tiplle and Pagani, 2007). Variation within the $\delta^{13}\text{C}$ values of C₃ plants is primarily a function of environmental factors (Tiplle and Pagani, 2007; Kohn, 2010). Water-stressed C₃ plants will tend to have higher (or less negative) $\delta^{13}\text{C}$ values, and thus, plants from open, savanna-like environments typically have higher $\delta^{13}\text{C}$ values relative to those from closed-canopy forest environments (Ehleringer and Monson, 1993; Kohn, 2010). In C₄ plants, the range of $\delta^{13}\text{C}$ values is primarily due to the different variations or enzymatic subtypes of the C₄ photosynthetic pathway (Tiplle and Pagani, 2007). CAM plants have $\delta^{13}\text{C}$ values that range between and slightly overlap that of C₃ and C₄ plants (Fox and Koch, 2003). CAM plants will not be considered further, however, because they are rarely found outside of deserts and are not considered to be a food source for mammalian herbivores (Fox and Koch, 2003; Fox and Fisher, 2004; Feranec and MacFadden, 2006).

When plants are consumed by herbivores, there is an enrichment in the heavy carbon isotope, ^{13}C , as the carbon fixed in photosynthesis is incorporated into the animal tissue due to the biochemical fractionation of carbon isotopes. For large herbivores, there is an enrichment factor ($\epsilon_{\text{enamel-diet}}$) of $+14.1\%$ for enamel relative to diet (Cerling and Harris, 1999; Tiplle and Pagani, 2007). The examination of tooth enamel is often the most reliable way to determine diet composition in animals

because enamel is not easily altered during diagenesis due to its high density and low porosity (Wang and Cerling, 1994; Tipple and Pagani, 2007). As a result of the enrichment factor, the carbon isotopic signature of tooth enamel that would represent a C₃ diet typically ranges from −8 to −16‰ and a C₄ diet from 0 to +4‰ for modern herbivores (Cerling et al., 1997a, 1997b). In most studies, modern or not, an enamel-δ¹³C value of −8‰ is used as the cut-off for a pure C₃ diet, with anything higher representing a measurable amount of C₄ plants being consumed (Cerling et al., 1993; Feranec and Pagnac, 2013). For large herbivores, this cut-off enamel-δ¹³C value corresponds to a diet-δ¹³C value of −22‰, reflecting a dietary intake of C₃ plants experiencing water stress (DeLucia and Schlesinger, 1991; Cerling et al., 1997a, 1997b; Ehleringer et al., 1998; Kohn, 2010; Wang et al., 2008; Zhang et al., 2012). A δ¹³C value of +2‰ is often used to represent the cut-off for a pure C₄ diet (Passey et al., 2002; Feranec and Pagnac, 2013).

Herbivore tooth enamel represents a spatially averaged carbon isotope signal that is also affected to some degree by selective feeding (MacFadden et al., 1996). Studies tend to focus on only one or a few taxa, rather than a survey of all or most of the local fauna, which has the potential to bias paleoenvironmental reconstructions of C₃/C₄ biomass ratios (Tipple and Pagani, 2007). Uncertainties also exist in using enamel-δ¹³C values to reconstruct the proportion of C₃ and C₄ plants in the diets of herbivores depending on what end-member δ¹³C values are used for pure C₃ and C₄ diets. For example, an enamel-δ¹³C value of −8‰ could indicate either a C₃ diet consisting of water-stressed C₃ plants or consumption of a small amount of C₄ plants. However, the ambiguity in the interpretation of the carbon isotope data in terms of whether C₄ plants are a significant dietary component can be resolved by analyzing the isotopic compositions of serial enamel samples collected along the growth axes of individual teeth (Sharp and Cerling, 1998; Wang et al., 2008; Biasatti et al., 2010, 2012; Stacklyn et al., 2017). C₄ plants are primarily warm season grasses that can only grow in warm summer months in regions with sufficient summer precipitation (Ehleringer et al., 1991; Cerling et al., 1997a, 1997b). Thus, if C₄ plants are present in an environment, they are likely to appear as a seasonal signal in serially sampled tooth enamel (Sharp and Cerling, 1998; Wang et al., 2008; Biasatti et al., 2010). For herbivores consuming C₄ grasses, serial sample δ¹³C data are likely to show winters of C₃ consumption and summers of C₄ (or mixed C₃/C₄) consumption (Sharp and Cerling, 1998; Wang et al., 2008; Biasatti et al., 2010).

The carbon in plants and enamel is derived from the atmosphere, as is the ratio of stable carbon isotopes (Feranec and MacFadden, 2006). The δ¹³C value of atmospheric CO₂ has decreased from −6.5 to −8.0‰ since the beginning of the Industrial Revolution due to the burning of fossil fuels (Tipple et al., 2010). Proxy records show that the concentration and the δ¹³C values of atmospheric CO₂ have changed over time (e.g. Pagani et al., 1999; Tipple et al., 2010), which would have affected the δ¹³C values of plants (e.g. Feng and Epstein, 1995; Schubert and Jahren, 2012) and ultimately tooth enamel (e.g. Passey et al., 2002). δ¹³C data from benthic foraminifera suggest that Miocene atmospheric CO₂ δ¹³C values would have been even higher or more enriched in ¹³C (Passey et al., 2002; Tipple et al., 2010). Without accounting for the changes in the δ¹³C values of atmospheric CO₂ over geologic time, reconstruction of C₃/C₄ biomass from the δ¹³C values of enamel, and other means, could overestimate the amount of C₄ grasses in animals' diets and ecosystems.

2.2. Study area

The Dove Spring Formation (DSF, formerly the Ricardo Formation), is located in the El Paso Mountains of southern California (Fig. 1; Loomis and Burbank, 1988). The DSF is underlain by the dominantly volcanic middle Miocene Cudahy Camp Formation and overlain by Quaternary deposits representing bajadas and other unconsolidated sediment emanating from the Sierra Nevada mountains (Loomis and Burbank, 1988; Whistler et al., 2009). Whistler et al. (2009) broadly

separated the DSF into five lithofacies from which depositional environments are interpreted. The first is comprised of clay, silt, fresh-water limestone, and bedded cherts, which are interpreted as lacustrine deposits. The second lithofacies is the sandstone and conglomerate that make up the cliffs that give Red Rock Canyon its name and are interpreted to be fluvial channel deposits. The third lithofacies is primarily silt, thought to be floodplain and overbank deposits. The fourth is composed of alluvial fan deposits or alluvial fan conglomerates. The fifth lithofacies is made up of paleosols, caliche, and silcretes.

The DSF contains 18 volcanic air-fall vitric ashes that have been correlated throughout the formation and at least 11 have been regionally correlated and radiometrically dated (Fig. 2; Loomis and Burbank, 1988; Whistler et al., 2009). This allows the time of deposition of the DSF to be constrained to between 13.5 and 8.5 Ma, from Clarendonian to early Hemphillian, for members one through five and the lower third of member six (Whistler and Burbank, 1992; Whistler et al., 2009). Due to the poor exposure of member six, there are not as many reliable age dates associated with it (Loomis and Burbank, 1988). These ashes also serve as important chronostratigraphic event horizons used throughout the basin for correlation and outlining of fossil range zones (Whistler et al., 2009). These ashes were sourced from the southern Great Basin as well as the Yellowstone "Hot Spot" (Perkins et al., 1998; Perkins and Nash, 2002; Bonnicksen et al., 2008). The two basalt flow sequences, which lie within member four of the DSF, have a source in the Lava Mountains south of the Garlock fault in the Mojave block; the upper basalt sequence is the source of one of the most reliable radiometric age dates in the DSF (Whistler et al., 2009).

The DSF is known for having the most diverse vertebrate fossil assemblage of the southwestern United States, at least 88 genera in 37 families have been found and identified (Whistler et al., 2009, 2013), and the faunas within are largely of Clarendonian Land Mammal Age (Wood et al., 1941; Tedford et al., 1987, 2004). Fossils can be found throughout the formation but are most common in the silt deposits that characterize overbank and floodplain deposits and in the paleosols of member five (Whistler and Burbank, 1992; Whistler et al., 2009). Most fossils are found as a single element, whether it be bone or tooth, or a few associated pieces; partially articulated specimens are uncommon (Whistler et al., 2009). The Dove Spring Formation is also reported as the locality for the possible earliest C₄ macrofossil (Nambudiri et al., 1978; Tidwell and Nambudiri, 1989) as evidenced by Kranz anatomy and carbon isotopes in plant phytoliths.

This study uses teeth of relatively large herbivores of only 7 of the 37 families present in the DSF: Antilocapridae, Castoridae, Camelidae, Equidae, Gomphotheriidae, Merycoidodontidae, and Rhinocerotidae. The fossil fauna present in the formation are primarily Clarendonian with some of the youngest being Hemphillian, younger than 9.0 Ma (Whistler et al., 2009). This study uses teeth found throughout the DSF. Only two samples were found stratigraphically high enough to be Hemphillian in age and 82 of the samples are Clarendonian age. Five samples have general stratigraphic localities which could denote either age. These fossil materials are an important archive of environmental changes in the southwestern United States. Reconstruction of the diets and habitats of fossil herbivores of the DSF fills in a gap in the global coverage of late Miocene C₄ expansion.

3. Methods

The carbon isotope ratios of 300 enamel samples from 89 fossilized herbivore teeth were analyzed. The fossilized teeth are primarily from the Clarendonian North American Land Mammal Age (12.5 to 9.0 Ma) in the middle and late Miocene of the Dove Spring Formation (Fig. 2; Whistler et al., 2009). The teeth come from the camelids (camels and relatives), antilocaprids (North American "antelopes"), gomphotheriids (distantly related to elephants), equids (horses), merycoidodontids (oreodonts), castorids (beavers), and rhinocerotids (rhinoceroses).

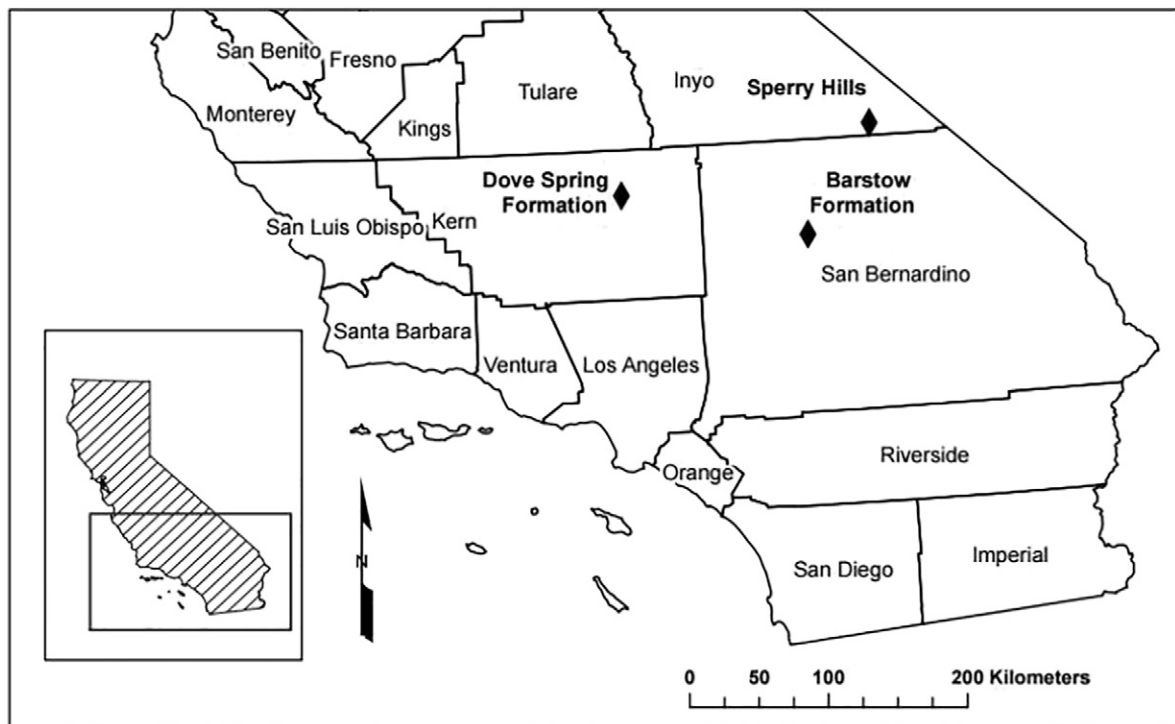


Fig. 1. Locality map showing the location of the Dove Spring Formation, the Barstow Formation, and the Sperry Hills in southern California.

Teeth were chosen in order to get permanent teeth (i.e. molars or premolars) from a wide variety of taxa. Bulk samples were taken by drilling along the growth axis of the tooth. Serial samples were taken by drilling in parallel bands perpendicular to the growth axis of the tooth. The enamel samples were then chemically treated using a procedure adapted from Wang and Deng (2005). Any pieces of enamel incorporated into the powder produced by drilling were ground to powder using a mortar and pestle. Approximately 2–3 mg of enamel powder was soaked in 5% sodium hypochlorite (NaOCl) overnight to remove organic contaminants then rinsed with distilled water. The last rinse was done with 1 M acetic acid (CH₃COOH), the samples were then drained and soaked in 1 M acetic acid overnight to remove non-structural carbonate. The samples were then rinsed with distilled water until a neutral pH was achieved. The treated enamel samples were reacted with 100% phosphoric acid (H₃PO₄) for 72 h at 25 °C to generate carbon dioxide. The carbon and oxygen isotopic ratios of the carbon dioxide were then measured using a Gas Bench II Autocarbonate device connected to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer at Florida State University. All results are reported in standard delta (δ) notation with units reported as parts per thousand or permil (‰) relative to the V-PDB (Vienna Pee Dee Belemnite) standard as:

$$\delta^{13}\text{C} = \left[\left(\frac{^{13}\text{C}/^{12}\text{C}}{\text{sample}} / \left(\frac{^{13}\text{C}/^{12}\text{C}}{\text{VPDB}} \right) - 1 \right) \times 1000 \right] \quad (1)$$

The analytical precision based on replicate analyses of NBS-19 and lab standards processed with each batch of samples is $\pm 0.1\%$ or better for $\delta^{13}\text{C}$.

For those teeth which were only sampled as serial samples, a proxy bulk sample- $\delta^{13}\text{C}$ value was calculated for the specimen by averaging isotopic ratios of all of the serial samples for the tooth. The mean, standard deviation, and range were calculated for each of the serially-sampled teeth and for the bulk samples of the seven families analyzed in this study. For each specimen, we estimated the average diet- $\delta^{13}\text{C}$ value for the herbivore using an enrichment factor ($\epsilon_{\text{enamel-diet}}$) of +14.1‰ given in Cerling and Harris (1999) for large herbivores. The calculated value represents the average $\delta^{13}\text{C}$ value of the plants each

herbivore consumed during the time that the hydroxyapatite in the tooth sampled was forming, not necessarily the $\delta^{13}\text{C}$ value of the environment.

4. Results

The mean bulk enamel- $\delta^{13}\text{C}$ value of all taxa sampled in this study is $-10.1 \pm 1.1\%$ ($n = 89$, 1 S.D.) with values ranging from -13.3 to -6.7% and a total range of 6.7‰ when serial samples have been averaged as a proxy for bulk samples (Table 1, see Supplementary Table 1 for complete results and sample details). Most of the fossil taxa sampled in this study are most reliably identified to the family level because many are only known from a few disarticulated fossil elements, generally teeth and jaw bones, which are not always sufficiently diagnostic to determine a genus and species. Of the seven families sampled, the gomphotheriids have the lowest (most negative) enamel- $\delta^{13}\text{C}$ values with a mean value of $-11.0 \pm 1.1\%$ ($n = 24$, 1 S.D.). The equids have a range of 4.5‰, which is the largest of any of the families sampled. The antilocaprids and equids have the highest enamel- $\delta^{13}\text{C}$ values with mean values of $-9.7 \pm 0.9\%$ ($n = 15$, 1 S.D.) and $-9.6 \pm 0.9\%$ ($n = 27$, 1 S.D.), respectively.

Comparing the sampled families, two-tailed *t*-tests show that the mean bulk enamel- $\delta^{13}\text{C}$ values of most families are not significantly different from one another at a 95% confidence level. A subset of the *t*-tests that were run is presented in Table 2, including the only tests that showed a significant difference. The mean of the enamel- $\delta^{13}\text{C}$ values of the gomphotheriids are significantly different from those of the antilocaprids ($t = 3.84$, $df = 37$, $p = 0.00047$), camelids ($t = 3.38$, $df = 37$, $p = 0.0017$), and equids ($t = 5.02$, $df = 49$, $p < 0.0001$). Possibly due to the fact that they had many fewer samples, the merycoidodontids were not found to be significantly different from any of the other families. The rhinocerotids and castorids were not included in the *t*-tests because there were very few samples from those families. Shapiro-Wilk normality tests were done on the five families included in the *t*-tests to determine the appropriateness of this statistical measure. The distribution of enamel- $\delta^{13}\text{C}$ values from each of the five

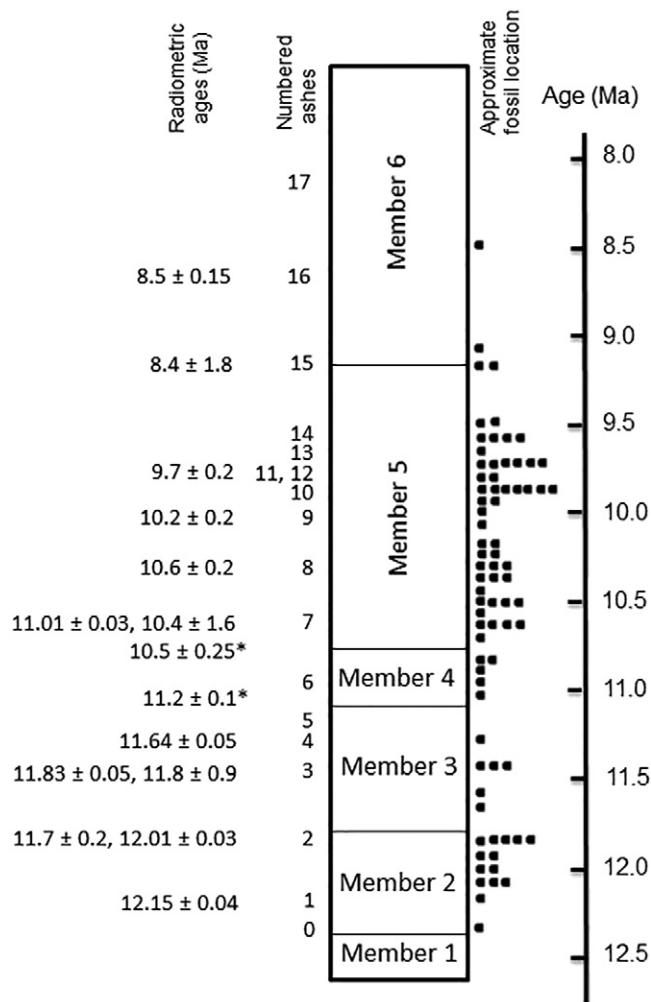


Fig. 2. Simplified stratigraphic column of the Dove Spring Formation denoting the positions of the 18 ash beds, numbered to the left of the column, correlated throughout the formation (see Loomis and Burbank, 1988 and Whistler et al., 2009 for detailed discussion of stratigraphy). Radiometric age dates (Whistler et al., 2009 and references therein) are to the left of the numbered ash beds, the * indicates those dates coming from the basalt flows that define the upper and lower boundaries of Member 4. Relative stratigraphic positions of the samples analyzed in this study are denoted under Approximate Fossil Location. Adapted from Whistler et al., 2009.

families were normal at 95% confidence interval. Results of these tests are detailed in Supplementary Table 2.

Using the enamel- $\delta^{13}\text{C}$ values and an enrichment factor (ϵ) of -14.1‰ given in Cerling and Harris (1999), we estimated the diet- $\delta^{13}\text{C}$ values for the DSF herbivores. The estimated diet- $\delta^{13}\text{C}$ values range from -27.3 to -20.7‰ , with a mean of $-24.1 \pm 1.0\text{‰}$ ($n = 89$, 1 S.D.) (Table 1).

Of the 89 teeth sampled in this study, 18 were serially sampled (Fig. 3). The serial samples come from six of the seven families sampled, including seven equid teeth, three each of camelid and gomphothere teeth, two each of antilocaprid and rhinoceros teeth, and one oreodont tooth. The serial samples allowed for the identification of seasonal variations in diet that may exist due to the varying growing seasons of C_3 and C_4 grasses (Sharp and Cerling, 1998; Wang et al., 2008; Biasatti et al., 2010). Seasonal signals in serially sampled oxygen isotope data, while not discussed here, served as proof that there has been little to no diagenetic alteration of these enamel samples.

There is no notable seasonal signal present in the carbon isotope data of the serial samples (Fig. 3). The mean intra-tooth variation of enamel- $\delta^{13}\text{C}$ values in the 18 teeth is $0.9 \pm 0.3\text{‰}$. Samples

LACM156567 and LACM55678, which were fossilized gomphotheriid and *Hipparion forcei* teeth, respectively, both have an intra-tooth variation of enamel- $\delta^{13}\text{C}$ values of 0.5‰ , which is the lowest of the 18 teeth. The highest intra-tooth enamel- $\delta^{13}\text{C}$ variation is 1.5‰ in LACM146573, an antilocaprid sample identified as *Paracosoryx furlongi*.

The variation in intra-tooth $\delta^{18}\text{O}$ values was larger compared to the intra-tooth $\delta^{13}\text{C}$ variability (Fig. 3). Serial sampling of an assortment of the teeth—particularly the equids, camelids, and rhinocerotids—show a strong seasonal signal in the oxygen isotope data, indicating that the enamel samples, and thus the carbon isotope data, have not been affected by diagenesis (Fig. 3).

5. Discussion

5.1. Diets and habitats of herbivores of the Dove Spring Formation

The presence of C_4 grasses in this ecosystem during the middle to late Miocene would lend support to previous work that found measurable amounts of C_4 in the diets of herbivore taxa of the nearby Barstow Formation (Feranec and Pagnac, 2013, 2017) which is several million years older than the DSF. Evidence of C_4 grasses would also offer further support for the early regional spread of C_4 plants prior to the late Miocene worldwide rapid increase in C_4 ecosystems. The absence of C_4 in the diets of the herbivore taxa analyzed in this study would imply that either the grasslands in this area in the middle to late Miocene were C_3 grasslands (Morgan et al., 1994; Cerling et al., 1997a, 1997b) or selective feeding by herbivores on C_3 plants has prevented a C_4 carbon isotopic signal from appearing in the tooth enamel (Feranec and Pagnac, 2013). Selective feeding as a cause of the absence of C_4 seems unlikely, however, given the diversity of taxa sampled.

The enamel- $\delta^{13}\text{C}$ values of all taxa sampled in this study range from -13.3 to -6.7‰ , with a mean of $-10.1 \pm 1.1\text{‰}$ ($n = 89$). If the modern cutoff enamel- $\delta^{13}\text{C}$ value of -8‰ for a pure C_3 diet was assumed to be applicable in the distant past as the end member for water-stressed C_3 plants (e.g. Feranec and Pagnac, 2013), these enamel- $\delta^{13}\text{C}$ values would indicate that most herbivores had C_3 diets and only three individuals possibly consumed a small, but measurable, amount of C_4 plants (Fig. 4). However, it has been well documented that the $\delta^{13}\text{C}$ value of atmospheric CO_2 , the carbon source for plants, has changed over time (e.g. Marino and McElroy, 1991; Passey et al., 2002; Tipple et al., 2010). These changes should have resulted in corresponding changes in the $\delta^{13}\text{C}$ values of plants and tooth enamel (Passey et al., 2002; Tipple et al., 2010). Proxy atmospheric CO_2 - $\delta^{13}\text{C}$ records suggest that the $\delta^{13}\text{C}$ values of atmospheric CO_2 during the time period of 8–13 Ma varied from -5.0‰ to -6.2‰ (Passey et al., 2002). After accounting for these changes in the carbon isotopic composition of atmospheric CO_2 , an end-member enamel- $\delta^{13}\text{C}$ value for water-stressed pure C_3 diets of $-5.8 \pm 0.2\text{‰}$ was calculated for the time period represented by the DSF (~ 8 – 12.5 Ma) using -8‰ as the cutoff $\delta^{13}\text{C}$ value for modern C_3 diets rather than -10.3‰ (Passey et al., 2002). The error of $\pm 0.2\text{‰}$ given for this value represents an error attributable to temporal variations of the estimated $\delta^{13}\text{C}$ value of atmospheric CO_2 during that time

Table 1

Summary statistics of the $\delta^{13}\text{C}$ data for the seven large herbivore families from the Dove Spring Formation, southern California.

Taxon	N	Mean $\delta^{13}\text{C}$ (‰)	S.D. (‰)	Range (‰)
Antilocapridae	15	-9.7	0.9	-7.4 to -10.7
Camelidae	15	-9.9	0.8	-8.6 to -11.1
Castoridae	1	-10.2	-	-
Equidae	27	-9.6	0.9	-6.7 to -11.2
Gomphotheriidae	24	-11	1	-9.1 to -13.3
Merycoidodontidae	5	-10.3	1.1	-8.9 to -11.6
Rhinocerotidae	2	-10.6	0.7	-10.1 to -11.0
Herbivore Overall	89	-10.1	1.1	-6.7 to -13.3
Estimated Diet		-24.2	1	-20.8 to -27.4

Table 2

Results of two-tailed *t*-test for unpaired data with equal variance for significant differences between mean isotopic values of different herbivore families from the Dove Spring Formation, southern California. *df*: degrees of freedom, *t*: *t*-value, *p*: *t* probability.

	Mean difference (‰)	<i>df</i>	<i>t</i>	<i>p</i>	Significant difference at 95%?
Antilocapridae- $\delta^{13}\text{C}$ vs. Merycoidodontidae- $\delta^{13}\text{C}$	0.6	18	1.18	0.25	No
Equidae- $\delta^{13}\text{C}$ vs. Antilocapridae- $\delta^{13}\text{C}$	-0.1	40	-0.26	0.79	No
Equidae- $\delta^{13}\text{C}$ vs. Camelidae- $\delta^{13}\text{C}$	0.3	40	1.12	0.26	No
Equidae- $\delta^{13}\text{C}$ vs. Gomphotheriidae- $\delta^{13}\text{C}$	1.4	49	5.02	<0.0001	Yes
Equidae- $\delta^{13}\text{C}$ vs. Merycoidodontidae- $\delta^{13}\text{C}$	0.7	30	1.53	0.14	No
Gomphotheriidae- $\delta^{13}\text{C}$ vs. Antilocapridae- $\delta^{13}\text{C}$	1.3	37	3.84	0.00047	Yes
Gomphotheriidae- $\delta^{13}\text{C}$ vs. Camelidae- $\delta^{13}\text{C}$	1	37	3.38	0.0017	Yes

period. Given the value of -5.8‰ as the cutoff enamel- $\delta^{13}\text{C}$ value for pure C_3 diets during this portion of the late Miocene, none of the herbivores sampled from the DSF consumed a measurable amount of C_4 plants (Fig. 4).

Even disregarding that the cutoff enamel- $\delta^{13}\text{C}$ value for a water stressed C_3 diet in the late Miocene was likely closer to -5.8‰ rather than -8‰ , only 3 out of 300 enamel samples analyzed had an

enamel- $\delta^{13}\text{C}$ value higher than -8‰ . These samples included LACM146616 (-7.8‰), the serial sample closest to the root of the tooth, and LACM146623 (-7.4‰), both from antilocaprid specimen identified as *Paracosoryx furlongi*, and LACM126135 (-6.7‰) from an equid specimen identified as *Megahippus* sp. It is highly unlikely that these three samples represent consumption of C_4 grasses. All three of these samples were from Member 2 of the DSF (Fig. 2). *Paracosoryx*

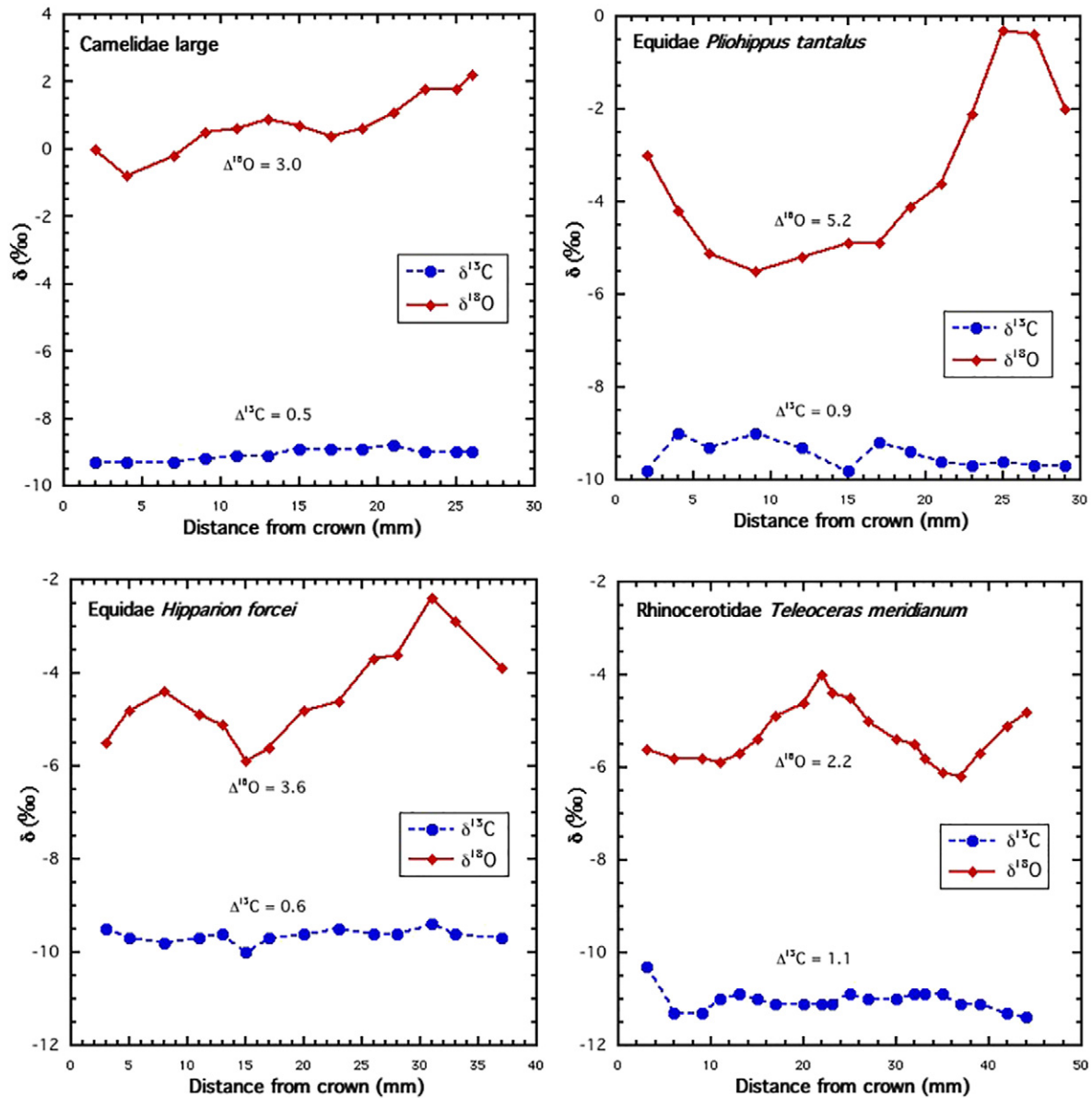


Fig. 3. Intra-tooth carbon and oxygen isotopic variations in four of the serially sampled teeth from the Dove Spring Formation, southern California. Lack of seasonal signal in the $\delta^{13}\text{C}$ data gives further evidence that no C_4 grasses were being consumed in this environment in the middle to late Miocene. The pronounced seasonal signal in the $\delta^{18}\text{O}$ data make it unlikely that the enamel samples have been affected by diagenesis.

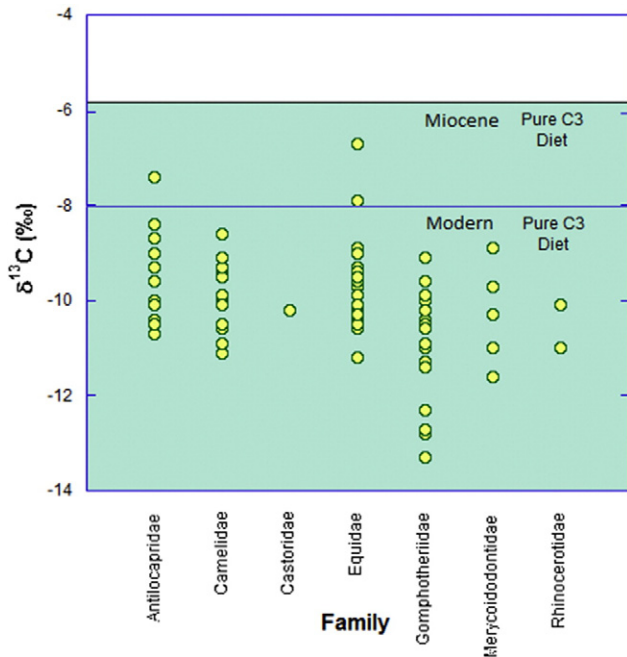


Fig. 4. $\delta^{13}\text{C}$ values of tooth enamel from the seven herbivore families examined in this study. Each circle represents a bulk sample or an averaged serial sample value with 89 total. The shaded area below -5.8% represents all carbon isotopic values that correspond to a middle to late Miocene pure C_3 diet, the additional line at -8% represents the modern cutoff value for pure C_3 diets (see text for explanation).

furlongi had hypsodont, or high-crowned, teeth and therefore would have been a grazer adapted to eating grasses, but that does not preclude from grazing on C_3 grasses. *Megahippus* sp., however, had brachydont, or low-crowned, teeth that would have been adapted to browsing and would have been worn down significantly by the consumption of grasses. As a likely browser, *Megahippus* sp. having the highest enamel- $\delta^{13}\text{C}$ value is anomalous, this sample was a bulk sample from a complete tooth; perhaps this individual survived on a diet of relatively water-stressed C_3 plants when this particular tooth was formed (during a drought year).

The antilocaprids are represented in the DSF by the genera *Cosoryx* and *Paracosoryx* as well as two other genera that were not identified in the specimen sampled for this study (Whistler et al., 2009). Their hypsodont teeth combined with the stable carbon isotope values ($-9.7 \pm 0.9\%$, $n = 15$) from samples in this family imply that these grazers were feeding on C_3 grasses. Camelids are represented by as many as four genera—*Hemiauchenia* sp., *Procamelus* sp., *Megatypolus* sp., and *Aepycamelus* sp.—in the Dove Spring Formation; the former two are thought to have been grazers, the latter two browsers (Whistler et al., 2009). The camelids are not able to be sufficiently identified by the isolated teeth and jaws that are common to the DSF and many of the specimens from this family that were sampled in this study were identified as ‘small,’ ‘medium,’ or ‘large,’ which may or may not be diagnostic of different genera. The enamel- $\delta^{13}\text{C}$ values do not lend any diagnostic assistance in assigning the specimen sampled to different groups. Enamel- $\delta^{13}\text{C}$ values for all of the samples were clustered around the mean at $-9.9 \pm 0.8\%$ ($n = 15$). With the mix of life habits present in this family, this would imply that the grazing genera, *Hemiauchenia* and *Procamelus*, were feeding on C_3 grasses. The castorids are only represented by one individual in this study, and the enamel- $\delta^{13}\text{C}$ value of that specimen is -10.2% . This individual, an ancestor to the modern beaver, had hypsodont teeth (Sues, 2000) but is thought to have been a browser (Rybczynski, 2008).

The equids exemplified by six genera in the DSF, some of which have been identified to a species level. Taxa sampled in this study include *Megahippus* sp., *Cormohippus* sp., *Pliohippus tantalus*, and *Hipparion*

forcei, as well as some specimens that are only identified as equids. All of these genera, except *Megahippus*, are hypsodont (Sues, 2000) and assumed to be primarily grazers. The enamel- $\delta^{13}\text{C}$ values of the equid specimens are $-9.6 \pm 0.9\%$ ($n = 27$) and are clustered tightly around the mean with the exception of three outliers at -6.7% (*Megahippus* sp.), -7.9% (unidentified equid), and -11.2% (*Hipparion forcei*). All of the enamel- $\delta^{13}\text{C}$ values are within the range for pure C_3 diets, suggesting that the equids in this area most likely grazed on C_3 grasses. The unidentified equid with the enamel- $\delta^{13}\text{C}$ value of -7.9% was not discussed previously as being higher than -8% because the difference between the values is within the analytical uncertainty ($\pm 0.1\%$) for carbon isotope analysis and was not considered to be conclusive evidence of the inclusion of C_4 plants in the diet of this individual.

The gomphotheriids are represented by two genera in the DSF, *Gomphotherium* and *Amebelodon* (Whistler et al., 2009). The enamel- $\delta^{13}\text{C}$ values of the gomphotheriids are $-11.0 \pm 1.0\%$ ($n = 24$). This family has one of the largest ranges of carbon isotope values (4.3%) of the seven families sampled, second only to the equids (Table 1). The enamel- $\delta^{13}\text{C}$ values range from -9.0 to -13.3% . An enamel- $\delta^{13}\text{C}$ value of -13% in the Miocene is equivalent to a modern value of approximately -15% (given the difference in the carbon isotope composition of atmospheric CO_2 between the late Miocene and the present), which is typical of herbivores feeding on plants grown in the understory of a forest. The plotted data appear as though they could represent two groups, one centered around -10% and another centered around -13% (Fig. 4). This seems to be misleading, however, as members of both genera are present in the former and both *Gomphotherium* and unidentified gomphotheres are present in the latter. Both genera are thought to have been browsers or mixed feeders, but individuals with the low enamel- $\delta^{13}\text{C}$ values may have preferred the more forested habitats while the others the more open habitats.

The merycoidodontids are represented by one genus, *Merychyus*. The enamel- $\delta^{13}\text{C}$ values of the merycoidodontids are $-10.3 \pm 1.1\%$ ($n = 5$) and are all within the range for a pure C_3 diet. Microwear studies of other Miocene oreodont teeth suggest that this genus was likely one of browsers and mixed feeders (Mihlbachler and Solounias, 2006). The rhinocerotids are represented by two genera in the DSF, *Aphelops* and *Teleoceras*. Only one individual from each genus was sampled with enamel- $\delta^{13}\text{C}$ values of -10.1% and -11.0% , respectively, both values representing pure C_3 diets. *Aphelops* was a relatively long-limbed, brachydont rhino comparable to the modern day browsing black rhino (MacFadden, 1998). *Teleoceras* was a hypsodont, short-limbed rhino originally thought to be an amphibious grazer due to its hippo-like morphology (MacFadden, 1998). Based on enamel- $\delta^{18}\text{O}$ data from both *Aphelops* and *Teleoceras*, MacFadden (1998) suggested that neither was primarily aquatic, i.e., *Teleoceras* was most likely a mixed feeder or a C_3 grazer in this environment.

Further proof of a lack of C_4 plants in this ecosystem comes from the serial enamel- $\delta^{13}\text{C}$ data from individual teeth (Fig. 3) showing little or no intra-tooth carbon isotopic variations. In environments where C_4 biomass is being consumed, a seasonal change in diet would be expected because C_4 grasses are warm growing season grasses. This should result in a noticeable seasonal signal in the serially sampled enamel of grazers as they alternate between feeding on C_3 plants in cooler seasons and C_4 plants in warmer seasons (Sharp and Cerling, 1998; Wang et al., 2008; Biasatti et al., 2010). The lack of significant variability in the stable carbon isotope data for the serial samples, particularly in the known grazers such as the equids and antilocaprids, lends further support for the inference from the bulk enamel- $\delta^{13}\text{C}$ data that C_4 grasses were not present in this environment—or at the very least, C_4 grasses were not being consumed. The small amount of intra-tooth variability present in serial samples is more likely due to changes in water-stress and/or differences in $\delta^{13}\text{C}$ values of different C_3 plants being consumed, rather than consumption of C_4 vegetation.

The reconstructed diet- $\delta^{13}\text{C}$ values for the DSF herbivores are $24.1 \pm 1.0\%$, varying from -27.3 to -20.7% . After accounting for

changes in the $\delta^{13}\text{C}$ values of atmospheric CO_2 (Tippie et al., 2010), these values are equivalent to modern diet- $\delta^{13}\text{C}$ values of -29 to -23‰ (averaging $-26 \pm 1\text{‰}$) and well within the $\delta^{13}\text{C}$ range of modern C_3 plants (Kohn, 2010). Aridity is a commonly cited cause in the literature for the early regional spread of C_4 grasses (e.g. Tippie and Pagani, 2007; Edwards et al., 2010; Feranec and Pagnac, 2013). The abundance of fluvial and lacustrine rocks in the DSF, however, suggests the presence of a persistent lake in the center of the basin fed by ephemeral braided streams (Loomis and Burbank, 1988; Whistler et al., 2009). The reconstructed $\delta^{13}\text{C}$ values of plants consumed by the DSF herbivores and the rock record, as well as the presence of fossil taxa such as castorids (beavers) and fish, all indicate that the El Paso Basin ecosystem was not particularly arid in the middle and late Miocene, in stark contrast to its present-day desert environment. The relatively large range of the $\delta^{13}\text{C}$ values also suggests that the basin had a diverse range of habitats including riparian forests and open habitats such as grasslands and/or wooded grasslands.

5.2. Pieces of the puzzle—the Dove Spring and Barstow Formations

Feranec and Pagnac (2013) reported enamel- $\delta^{13}\text{C}$ data from the Barstow Formation, which they interpreted as indicating that C_4 grasses had spread into southern California in the middle Miocene and supporting the hypothesis of an early regional spread of C_4 grasses prior to the late Miocene global C_4 expansion. The study used -8‰ as an enamel- $\delta^{13}\text{C}$ cutoff value for a pure C_3 diet and $+2\text{‰}$ as the cutoff value for a pure C_4 diet, with values in between representing a spectrum of partial C_4 consumption (Feranec and Pagnac, 2013). As a result of this selection of end-member enamel- $\delta^{13}\text{C}$ values for pure C_3 and C_4 diets, Feranec and Pagnac (2013) find that up to 18% C_4 grasses were being consumed by ungulates in the paleoecosystem of the Barstow Formation of southern California. They conclude that additional evidence from other localities with C_4 grasses earlier than late Miocene would provide support for local factors (e.g. aridity, fire) rather than a global factor (e.g. CO_2) as the driving mechanisms for the spread of C_4 grasses (Tippie and Pagani, 2007; Edwards et al., 2010; Feranec and Pagnac, 2013).

The Barstow Formation is middle Miocene in age (Tedford et al., 2004). The fossils analyzed by Feranec and Pagnac (2013) have an age range from ~ 14 to 13 Ma, and are several million years older than the late Miocene global C_4 expansion (Cerling et al., 1993, 1997a, 1997b). According to Feranec and Pagnac (2013), “paleoecosystems from the Clarendonian land mammal age seem particularly important” because they encompass the time period just prior to the global rise in C_4 and could support either an abrupt spread of C_4 biomass or a more gradual increase in abundance. As stated previously, the DSF is circa Clarendonian age and has yielded no evidence of a measurable amount of C_4 biomass from the enamel of the seven herbivore families sampled in this study or in previous studies (Cerling et al., 1997a, 1997b). This lends support to an abrupt spread of C_4 biomass in the late Miocene rather than a gradual increase in abundance. If there were indeed C_4 plants in the paleoecosystem of the Barstow Formation, as suggested by Feranec and Pagnac (2013), it is interesting that we can find no evidence in the DSF. The DSF is both geographically and temporally near the Barstow Formation in the Mojave Desert, only separated by the El Paso Mountains and a few 10's of kilometers (Fig. 1).

As discussed in the previous section, the $\delta^{13}\text{C}$ value of atmospheric CO_2 has changed over time and was higher in the past than today (Tippie et al., 2010). The reconstructed $\delta^{13}\text{C}$ values of atmospheric CO_2 in the middle Miocene were even higher than those in the late Miocene (Passey et al., 2002; Tippie et al., 2010), suggesting that the maximum enamel- $\delta^{13}\text{C}$ value for a pure C_3 diet could be as high as -5‰ (Fig. 5). If the changes in the $\delta^{13}\text{C}$ value of atmospheric CO_2 over time are taken into account so that an enamel- $\delta^{13}\text{C}$ cutoff value for C_3 diets appropriate for the middle Miocene is used, the enamel- $\delta^{13}\text{C}$ values of the ungulates of the Barstow Formation would be well within the

range for a pure C_3 diet (Fig. 5b). Thus, available enamel carbon isotope data, after accounting for changes in the $\delta^{13}\text{C}$ of atmospheric CO_2 , show no clear evidence of an early expansion of the C_4 photosynthetic pathway in southern California as suggested previously (Feranec and Pagnac, 2013). The next step would be to look for evidence of C_4 in paleoenvironments of Hemphillian age or younger in southern California to determine if C_4 plants were present in this area during the ‘global’ rapid increase in C_4 ecosystems. Presently, most plants in the Mojave desert are either C_3 or CAM plants due to a combination of the regional precipitation regime and the aridity (Teeri and Stowe, 1976; Mulroy and Rundel, 1977; Tieszen et al., 1979, 1997; Ehleringer and Monson, 1993; Ehleringer et al., 1997; Edwards et al., 2010). The question now becomes: have C_4 plants ever represented a significant portion of the biomass in southern California?

Tentative evidence of C_4 biomass has been found in the DSF, in the form of petrified grasses with Kranz anatomy (Nambudiri et al., 1978; Tidwell and Nambudiri, 1989), but its reliability is questionable. The original publication claimed that petrified C_4 grasses evidenced by both stable carbon isotope analysis and the presence of Kranz anatomy had been found in the Sperry Hills and Last Chance Canyon of the Ricardo Formation, now the DSF (Nambudiri et al., 1978). The Sperry Hills, and related Sperry Wash, are actually south of Death Valley near the San Bernardino County line, not in Kern County, California where the DSF is located (Fig. 1). Additionally, the Sperry Hills and Sperry Wash are latest Miocene to Pliocene or Pleistocene in age, not middle to late Miocene in age as is the DSF (Tidwell and Nambudiri, 1990). Also problematic is the fact that ‘Last Chance Canyon’ is not a locality name in the DSF but rather is a local place name and encompasses a large stratigraphic range from the base of the DSF to the bottom of the lower basalt flow sequences, the top of Member 3. Just above what is considered to be Last Chance Canyon are steep slopes representing even younger strata within the Dove Spring Formation that these grasses also could have come from (Nambudiri et al., 1978). At best these findings represent the possibility of C_4 biomass in the El Paso Basin ecosystem even if no C_4 grasses were being consumed by the herbivores of that time, but the results are likely misleading due to the imprecise locality information available for the collection site of these petrified grasses.

6. Conclusions

In this study, the stable carbon isotopic composition of fossil herbivore tooth enamel from seven mammalian herbivore families was analyzed to determine if C_4 plants were present in the El Paso Basin of southern California in the middle to late Miocene. The enamel- $\delta^{13}\text{C}$ values of herbivores from the Miocene DSF are $-10.1 \pm 1.1\text{‰}$ ($n = 89$), ranging from -13.3 to -6.7‰ . Calculations based on the reconstructed- $\delta^{13}\text{C}$ values of paleo-atmospheric CO_2 and the modern cutoff enamel- $\delta^{13}\text{C}$ value of -8‰ for pure C_3 diets give an average $\delta^{13}\text{C}$ cutoff value for pure C_3 diets of $-5.8 \pm 0.2\text{‰}$ for the time interval 8–12.5 Ma. This implies that previous studies using the modern cutoff enamel- $\delta^{13}\text{C}$ value of -8‰ as the end member value for a pure C_3 diet for Miocene herbivores most likely have overestimated the amount of C_4 in diets or yielded a false signal of C_4 presence. After accounting for the changes in the $\delta^{13}\text{C}$ value of atmospheric CO_2 , enamel- $\delta^{13}\text{C}$ values from the DSF indicate that herbivores in the El Paso Basin in the middle and late Miocene had primarily C_3 diets and lived in an environment dominated by C_3 plants. The present-day El Paso Basin is a desert with sparse vegetation dominated by C_3 and CAM plants. The results of the stable carbon isotope analysis, along with the evidence from the rock record, and the life habits of the fossil taxa present—both grazers and browsers—indicate a very different environment in this area during the middle and late Miocene. The carbon isotope data suggest that the environment in the El Paso Basin in the middle to late Miocene likely had a diversity of habitats ranging from C_3 grasslands or wooded C_3 grasslands to riparian forests.

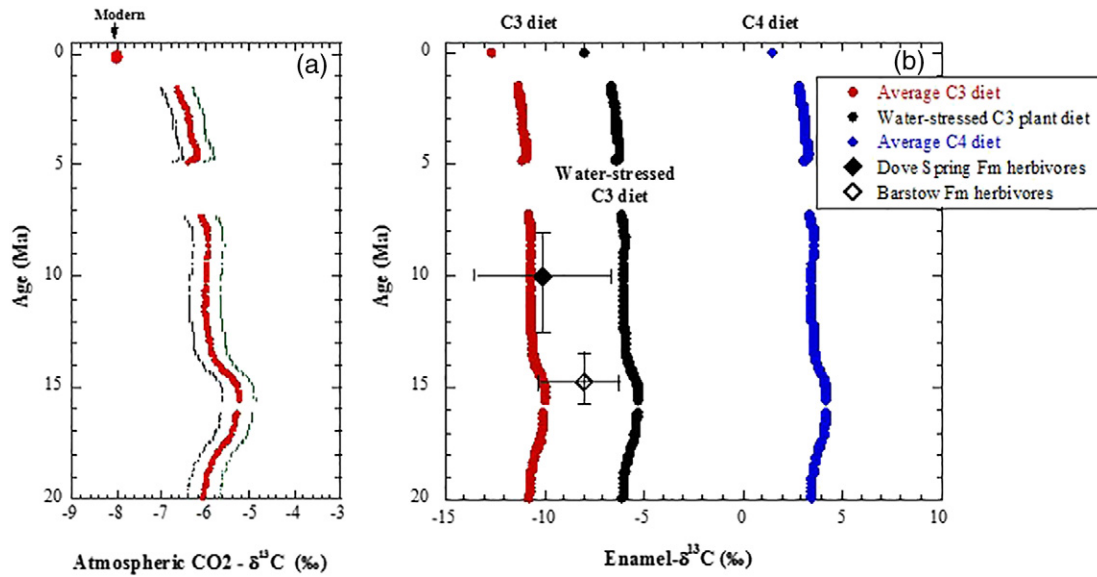


Fig. 5. Reconstructions of (a) $\delta^{13}\text{C}$ of atmospheric CO_2 (red) with 90% confidence intervals (green) from Tipler et al. (2010), and (b) enamel- $\delta^{13}\text{C}$ values for average C₃, water-stressed C₃ and average C₄ diets, with the width of the curve corresponding to the 90% confidence intervals in (a). Also shown are the means and ranges of enamel- $\delta^{13}\text{C}$ values of herbivores from Dove Spring Formation (this study) and Barstow Formation (Feranec and Pagnac, 2013). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The lack of seasonal signal in the carbon isotope data of the serial enamel samples from individual teeth provides further evidence that these herbivores consumed C₃ plants year round and that there were probably little to no C₄ grasses (warm season grasses) available for consumption in their habitats. The results from this study do not support an earlier regional C₄ expansion, prior to the rapid increase in global C₄ biomass in the late Miocene, in southern California. If the C₄ photosynthetic pathway did spread to southern California prior to the rapid increase in C₄ ecosystems in the late Miocene, it was not a significant food source as the various herbivore taxa sampled did not incorporate C₄ grasses into their diets.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.palaeo.2017.03.008>.

Acknowledgements

We thank Bruce Barnett, Janine Giambalvo, Bill Parker, Leroy Odom, Jorge Natal, and Hilary Dennis for their valuable input and assistance with this project. This work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida.

The materials for this study were provided by the Department of Vertebrate Paleontology at the Natural History Museum of Los Angeles County (LACM). Thanks to Samuel A. McLeod and Vanessa R. Rhue of the LACM for providing loans of the materials studied herein. Fossil collecting was conducted under permits from the California State Department of Parks and Recreation and the United States Department of Interior, Bureau of Land Management. Forty-five years of field work was supported by the LACM and its Foundation with supplemental support from various other granting agencies acknowledged in detail in Whistler and Burbank, 1992.

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