1
2
3
4
5
6
7
8
9
10
11 **Regional-scale variability in the spread of grasslands in the late**
12 **Miocene**

13 Stephanie T. Chen¹, Selenia Y. Smith^{1,2}, Nathan D. Sheldon¹, Caroline A.E. Strömberg³

14 *¹Department of Earth & Environmental Sciences, University of Michigan, Ann Arbor, MI 48109*
15 *USA*

16 *²Museum of Paleontology, University of Michigan, Ann Arbor, MI 48109 USA*

17 *³Department of Biology and Burke Museum, University of Washington*

18 corresponding author: email, sysmith@umich.edu
19
20
21
22
23
24
25

Abstract

A new paleovegetation reconstruction based on both phytolith and paleosol carbon isotopic data is presented for the middle–late Miocene (11.2–9.5 Ma) of southwestern Montana. Both sources of data indicate an open-habitat, C₃ grassland ecosystem with a small C₄ component (<10%). However, while a penecontemporaneous record from further south within the same region indicated significant spatial and temporal variability in C₄ abundance, the new record indicates relatively little change either in space or in time. Because paleoclimatic conditions reconstructed at these two sites are the same, we hypothesize that other ecological factors controlled C₄ abundance across the region. The relative abundance of C₃ and C₄ plants in the Miocene is the same as in modern environments at both sites, suggesting that their distribution was likely established by the middle-late Miocene. This is consistent with the idea that paleoelevation was also similar, which agrees with independent constraints on the regional tectonic history. The Montana records indicate a more limited distribution of C₄ vegetation than is observed in the Great Plains and elsewhere globally, as well as variable timing in the shift to C₄ dominance in ecosystems. Taken together, this local, regional, and global variability during a time of little or no climate change suggests that C₄ distribution and expansion was driven by local, rather than global, environmental factors.

Keywords: Montana; Neogene; paleoecology; paleosols; paleovegetation; phytoliths

Introduction

Grass-dominated habitats today cover half of the Earth's land surface and are home to 1.8 billion people, providing key ecosystem services such as carbon burial and habitat, and are major range and croplands for agricultural production (Asner et al., 2004). The macrofossil record of grasses is poor (e.g., Smith et al., 2010; Strömberg, 2002, 2011), but other lines of evidence including functional morphology of large mammalian herbivores (e.g., Janis et al., 2000), phytoliths (e.g., Strömberg, 2004, 2005), and stable isotope analyses of paleosols (e.g., Fox and Koch, 2003) and mammal teeth (e.g., Cerling et al., 1997) have been used to examine the global and regional evolutionary history of grasses. Of these, phytoliths and stable isotope analyses can also be used to differentiate between photosynthetic pathways used by grasses (e.g., Fredlund and Tieszen, 1997, McInerney et al., 2011).

The first grasses using the ancestral C_3 photosynthetic pathway had evolved no later than 67 Ma (Prasad et al., 2005). Following the first instance of evolution of C_4 photosynthesis within the tropical PACMAD clade of grasses, by at least the early Oligocene (Edwards et al., 2010, but see Urban et al., 2010), C_4 grasslands spread globally at low-mid latitudes in the late Miocene to Pliocene (8 to 3 Ma) through the replacement of C_3 vegetation (Cerling et al., 1997; Strömberg, 2011). The spread of C_4 grasses was initially linked to low atmospheric CO_2 levels (Cerling et al., 1993), but subsequent work has found that a CO_2 -only trigger is unlikely (Pagani et al., 1999; Bolton and Stoll, 2013; Zhang et al., 2013) and that additional local to regional scale drivers, including changes in fire regime, aridity, and/or evolutionary niche tolerances, were likely to have been important (Osborne, 2008; Edwards et al., 2010; Strömberg and McInerney, 2011). In addition, different factors may have played a larger role in different regions (e.g., aridity and/or rainfall seasonality in South Asia, Huang et al., 2007; Quade et al., 1989; temperature seasonality

and/or aridity in the Great Plains, Fox et al., 2012; Strömberg and McInerney, 2011). Therefore, to understand the potential range of processes responsible for the spread of C₄ grasslands, detailed region-specific studies are imperative.

The global expansion of C₄ grasslands in the late Miocene has been well-documented at several sites (Edwards et al., 2010), but in many regions, C₄ grasses were present for millions of years prior to becoming dominant and replaced C₃ grasses rather than forest vegetation. For example, Miller et al. (2012) identified C₃ grass phytoliths in Montana by ~39 Ma (at THA in Fig. 1) but found no evidence of C₄ vegetation based on either phytoliths or paleosol morphology. In contrast, Cotton et al. (2012) studied a nearby late Miocene site (TFF in Fig. 1; 10.2–8.9 Ma) and found patchy but measurable amounts (up to 25%) of C₄ vegetation based on organic carbon isotopic ratios from paleosol and phytolith evidence, as well as a different C₃ grass phytolith assemblage than at the older site pointing to a shift in C₃ species composition. Taken together, these sites bracket the local evolution or spread of C₄ vegetation into southwestern Montana, and support previous ideas of a more complicated regional history for the spread of grasslands in general (Strömberg, 2005, 2011), and the presence of significant amounts of C₄ plants prior to their global expansion to dominance in some regions (Fox and Koch, 2004; Edwards et al., 2010; Cotton et al., 2012). Here we present a new combined isotope geochemistry and phytolith record dated to 11.2–9.5 Ma from the same region to fill in some of the gaps in the history of C₄ grasses in the Rocky Mountain region and to examine local to regional scale variation in ecosystem composition.

Reconstructing Paleovegetation

C₃ and C₄ photosynthesis fractionate ¹²C and ¹³C isotopes to different degrees, which forms the basis for calculating the vegetation contribution of each pathway towards the δ¹³C ratio

91 of a soil or paleosol. Values for modern C₃ plants range from -22‰ to -34‰, with a mean of -
92 27‰, whereas values for C₄ plants range from -9‰ to -16‰, with a mean of -13‰ (Cerling and
93 Harris, 1999). These differences can be used to derive a two-member mixing model, with mean
94 $\delta^{13}\text{C}$ values as pure C₃ and C₄ end members to calculate the percentage of C₃ and C₄ vegetation
95 abundance. Soil organic matter from A horizons record plant values quantitatively, with
96 significant enrichment in ^{13}C from oxidation occurring deeper in the profile in B and C horizons
97 (Garten et al., 2000; Wynn, 2007; Wynn and Bird, 2007) and as a function of grain size changes
98 (Wynn et al., 2005), but with minimal diagenetic alteration (Sheldon and Tabor, 2009). For
99 example, fine-textured soils may exhibit $\delta^{13}\text{C}_{\text{org}}$ values at depth up to +5‰ relative to their A
100 horizons or overlying vegetation, whereas coarser-grained soils often exhibit less than +1.5‰ at
101 depth relative to their A horizons (Wynn et al., 2005; Wynn, 2007). Thus, paleosol A horizon
102 $\delta^{13}\text{C}_{\text{org}}$ values can be used to reconstruct past relative abundance of C₄ vegetation most
103 accurately, with the upper parts of B horizons of sandy-textured soils also recording the relative
104 abundance of C₄ vegetation fairly accurately; deeper profile depths or finer-grained soils
105 generally require some adjustment to measured value to be useful. The isotopic composition
106 range within a photosynthetic pathway has been linked to moisture availability and ecology
107 (Diefendorf et al., 2010; Kohn, 2010), with the highest C₃ plant isotope values associated with
108 water-stressed regions (typically +1–3‰ for the same plant relative to wetter climates; e.g.,
109 Cotton, 2013) and the lowest C₃ plant isotope values associated with closed canopy settings.
110 Thus, whereas modern $\delta^{13}\text{C}$ values much lower than -25‰ in soil A horizons would likely record
111 closed canopy forest vegetation, values near -25‰ could be reflecting a small proportion of C₄
112 photosynthesis or water-stressed C₃ vegetation.

Phytoliths are microscopic plant silica bodies, produced by the deposition of monosilicic acid (H_4SiO_4) between and within plant cells, which may take on the shape of its originating cell or other characteristic morphology (Piperno, 2006). As a result, phytolith morphology can often be used to identify the original plant taxon or habitat it was produced in, providing a means for vegetation reconstructions in deep time (e.g., Strömberg, 2004, 2005; Miller et al., 2012). Studies of phytolith production in modern plants and soils have helped identify characteristic morphotypes distinctive of plant taxa and functional groups (e.g., Piperno, 1985; Strömberg, 2003; Prychid et al., 2004; Chen and Smith, 2013), which can then be used in the identification of fossil phytoliths. However, representational biases may exist when considering relative abundance preserved in soils in certain environments (e.g., Fredlund and Tieszen, 1994; Barboni et al., 2007; Hyland et al., 2013) and should be accounted for in paleovegetation reconstructions.

Materials and Methods

Field Site

Sampling took place at the Madison Buffalo Jump (MBJ) locality in southwestern Montana, which preserves part of the Sixmile Creek Formation of the Bozeman Supergroup (Fig. 1; Hanneman and Wideman, 1991). MBJ was dated to 11.2–9.5 Ma by Retallack (2007) based on an overlaying caprock recognized by regional sequence stratigraphy (Hanneman and Wideman 2006) and two dated ash layers (Fritz et al., 2007; Retallack, 2007). Samples discussed herein were collected directly above an ash dated to 10.42 Ma, which is at the 71 m level in Retallack's (2007) section. Retallack (2007) and Hamer (2009) previously described both weakly developed (Entisol and Inceptisol-like) and moderately developed (Mollisol-like) paleosols on the basis of horizonation, color changes, grain size changes, burrows, and root traces (both

organic and rhizoliths). Modern Mollisols are associated with grassland ecosystems, whereas Entisols and Inceptisols may be found in a variety of ecosystem types (e.g., Sheldon and Tabor, 2009). All of the selected paleosols have fine-coarse sand grain sizes, meaning that little isotopic difference is expected as a function of depth between the A horizon and upper B horizon. Based on the depth to paleosol Bk horizons, Retallack (2007) reconstructed a mean annual precipitation (MAP) of $389 \pm 85 \text{ mm yr}^{-1}$ for the duration of deposition at MBJ; sub-sampling that data to include the five paleosols preserved above and below the sampling interval described herein yields a statistically indistinguishable MAP of $369 \pm 73 \text{ mm yr}^{-1}$, suggesting long-term, stable paleoprecipitation regime. Retallack (2007) reconstructed a mean annual temperature (MAT) of $10 \pm 4^\circ\text{C}$ for the site based on paleosol geochemistry (Sheldon et al., 2002) and Hamer (2009) reconstructed a MAT of $9 \pm 4^\circ\text{C}$ using the same proxy, and $11 \pm 2^\circ\text{C}$ using a paleosol order-specific proxy for Inceptisols. As with MAP, reconstructed MAT suggests long-term stability.

Paleosol samples were collected both vertically within a short stratigraphic section (Fig. 2), and laterally to measure localized spatial heterogeneity and vegetation change through time and space, respectively. Lateral section samples were taken at 0, 12, and 22 m intervals in the A horizon of the upper and lower paleosols. Vertical section samples were taken from the upper and lower parts of the caprock, and the A and B horizons of the two paleosols.

Isotopic Analyses

Samples were prepared for $\delta^{13}\text{C}_{\text{org}}$ analysis at the University of Michigan following the method outlined in Cotton et al. (2012). Briefly, samples were ultrasonicated in methanol to remove modern organic material, and treated with a 7% HCl solution on a 50°C hotplate to remove calcium carbonate (CaCO_3). The remaining material was rinsed in deionized water and dried, before being crushed and loaded in tin capsules and analyzed using a Costech Elemental

Analyzer coupled to a Delta V+ isotope ratio mass spectrometer. Results are reported in delta notation relative to the VPDB scale. External precision was maintained at <0.1 ‰.

The burning of fossil fuels makes the modern atmospheric $\delta^{13}\text{C}$ value ($\sim 8\text{‰}$) non-analogous with the geologic past, so we adjusted our mixing line by $+2\text{‰}$ based on a reconstructed $\delta^{13}\text{C}_{\text{atm}}$ value of -6‰ for 11–9 Ma from Tipple et al. (2010). Thus, the C_3 end-member is defined as -25‰ and the C_4 end-member as -11‰ for both vegetation and paleosol A horizon organic matter. Analytical uncertainty in isotopic measurements of 0.1‰ equates to 0.7% uncertainty in reconstructed $\% \text{C}_4$ and replicate uncertainty in isotopic measurements of 0.24‰ equates to 1.7% uncertainty in reconstructed $\% \text{C}_4$; uncertainty in $\delta^{13}\text{C}_{\text{atm}}$ is much larger than measurement uncertainty and has been plotted throughout to be conservative.

Phytoliths

Phytoliths were extracted from $\sim 1\text{g}$ of sediment at the University of Michigan following Strömberg (2003, 2005), using heavy liquid floatation with zinc bromide (ZnBr). Phytoliths were mounted onto slides using Cargille Meltmount (refraction index 1.534) mounting medium and immersion oil (Cargille Non-Drying Immersion Oil Type A) for rotation to characterize the 3D and surface morphology of, in particular, grass phytoliths, and viewed under a light microscope at $1000\times$ magnification. Morphotypes were then identified following the scheme established in Strömberg (2003), and using a modern reference collection compiled by S.Y. Smith at University of Michigan for comparison. Diagnostic morphotypes were grouped broadly into two functional groups, so-called forest indicators (FI; phytolith morphotypes which are commonly produced by trees and other plants associated with forests; see Strömberg, 2004) and grasses (i.e., grass silica short cells, GSSCs), to determine vegetation composition; morphotypes with compound variables of CONI/MONO, GRASS/MONO-ND, GRASS-D, and OTH (from Strömberg (2003),

see also Miller et al. (2012) for details; Supplemental Table 2, 3) were excluded from analysis due to, among other things, uncertainty about the functional plant groups they represent (discussed in Strömberg, 2004; McInerney et al., 2011). Non-plant biogenic silica, including diatoms, sponge spicules, and chrysophyte cysts (DSCs), was also counted to provide information on hydrologic conditions (Piperno, 1988; Clarke, 2003). Approximately 200 diagnostic phytoliths were counted and classified per sample (Supplemental Table 2).

Grass silica short cells (GSSCs) were classified according to Strömberg (2003, 2005) and combined into broad taxonomic and ecological groups: closed-habitat grasses (BAMB/B), cool-temperate open-habitat grasses in the Pooideae (POOID-D, POOID-ND), tropical open-habitat grasses in the PACMAD clade, including the mainly C₄ Panicoideae (PANI), the exclusively C₄ (Ingram et al., 2011) Chloridoideae (CHLOR), and other C₃/C₄ PACMADs (PACMAD-GEN), as well as unknown or unclassified GSSCs (OTHG). Current GSSC classification is limited to identifying members of panicoids and chloridoids; in addition, it remains unclear how GSSC production varies between C₃ and C₄ grasses (discussion in McInerney et al., 2011). Nevertheless, modern phytolith studies have shown that grass silica short cell assemblages reflect C₃ vs. C₄ dominance in many ecosystems (e.g., Fredlund and Tieszen, 1994, 1997; Barboni et al., 2007; Bremond et al., 2008; Hyland et al., 2013). To account for some of these uncertainties, we follow Strömberg and McInerney (2011) in calculating a minimum and maximum estimate of C₄ grasses using, respectively the number of panicoid and chloridoid GSSCs (PANI + CHLOR) and the total number of PACMAD GSSCs divided by the sample's total number of diagnostic phytoliths (forest indicators + grass silica short cells). Ninety-five percent confidence intervals (unconditional case; see Strömberg, 2009) for these estimates were

calculated using bootstrapping in R software (script available from authors upon request; R Development Core Team, 2013).

Results

Stratigraphy and Paleosols

Detailed paleosol descriptions can be found in Retallack (2007) and Hamer (2009), and are summarized here. Retallack (2007) previously identified four types of paleosols at MBJ, his Aikkwi, Aisen, Antsi, and Tatsah pedotypes. Each of the four pedotypes is characterized by preserved horizonation (i.e., A and B horizons), including Bk horizons of various depths, and would key out at Entisol- or Inceptisol-like using USDA terminology. Hamer (2009) also identified an additional paleosol type – an Inceptisol-like paleosol with granular peds in the A horizon as in a Mollisol, and either a weakly developed or non-existent Bk horizon. The caprock paleosols (Fig. 3) represent Retallack's (2007) Antsi pedotype (A-Bk-C; Bk <50 cm below surface), and “lower” and “upper” paleosols represent the mollic Inceptisol-like paleosol (A-Bw, no Bk horizon) identified by Hamer (2009). The cap rock was previously identified by Hanneman and Wideman (2006) as a calcic pedocomplex (composite paleosols) that represents a regional sequence stratigraphic marker bed in their Sequence 4. Composite paleosols are typically preserved in rapidly aggrading depositional successions and Mollisol-like paleosols on quiescent floodplains, thus, the identified paleosols represent both periods of both rapid and slow basin filling regimes. Some type of soils/paleosols, such as Alfisols, exhibit the potential for representational bias for grass phytoliths (i.e., overestimate the proportion of grasses). However, neither Mollisols nor Entisols exhibit any biasing, so phytolith results from the paleosols at MBJ do not require a taphonomic correction (Hyland et al., 2013).

Trace Fossils

Back-filled burrows (Fig. 2A) and organic root traces (Fig. 2B) were most abundant in the caprock, but also present in both the lower and upper paleosols. Root traces occasionally preserved whole corticoid cells (Fig. 2C), but were generally present as kerogenized organic rootlets. A large ant nest trace fossil (e.g., Hasiotis, 2004; Hamer et al., 2007) was also found in the caprock (Fig. 2D; Hamer, 2009) and in a low bluff stratigraphically below the main section.

Isotopic ratios

$\delta^{13}\text{C}$ values of paleosol soil organic matter ranged from -25.8‰ to -23.2‰ with a mean of $-24.3 \pm 0.8\text{‰}$ (1σ), corresponding to 0–12.8% C_4 with a mean of $5.6 \pm 4.6\%$ (Supplementary Table 1). Figure 3 plots $\delta^{13}\text{C}$ values by stratigraphic position, as well as the corresponding percentage C_4 composition calculated using the two-component mixing line described above. One sample yielded a value that would imply negative percent C_4 (i.e., $\delta^{13}\text{C} < -25\text{‰}$), which was readjusted to zero.

Phytoliths

All sampled stratigraphic levels yielded phytoliths, which ranged from poorly to well preserved. Five of 12 samples were too poorly preserved for a quantitative count; these were instead analyzed qualitatively for absence or presence of key indicator phytoliths (Supplemental Table 2, 3). The remaining seven samples yielded, on average, 31.3 ± 3.3 (1σ) morphotypes (range: 26–36), significantly more than at older southwestern Montana sites (Miller et al., 2012, Middle Eocene: 19.6 ± 3.3 (1σ) morphotypes, range 14–25). MBJ has a lower diversity of total FI morphotypes and higher diversity of total GSSC compared to THA (total 26 FI morphotypes and 9 GSSC morphotypes vs. total 11 FI morphotypes and 23 GSSC morphotypes). There was little lateral variation between samples from the same horizon (Fig. 3), and no charcoal was observed.

Non-phytolith biogenic silica, most commonly diatoms, sponge spicules, and chrysophytes (Fig. 4A-F), were present in all samples and contributed on average $5.3 \pm 2.9\%$ (range: 1.6–10.8%) of total counted biosilica (Supplemental Table 2). All seven quantitatively counted samples were dominated by GSSCs, potential grass (GRASS/MONO-ND), and non-diagnostic OTH morphotypes, suggesting that many of the non-diagnostic forms were produced by grasses. Common non-diagnostic phytoliths included elongates (Elo-1; Fig. 4G), epidermal cells typical of grasses (Epi-9; Fig. 4H), parenchyma cells (M-8; Fig. 4H, M), trichome fillings (Tri-8, Fig. 4I), and rectangular plates (Blo-2; Fig. 4J).

Considering only diagnostic forest indicator (FI) and GSSC (grass) phytoliths, all samples were strongly indicative of grass-dominated ecosystems, with a ground cover made up almost exclusively of C_3 open-habitat grasses (mean: $95.2 \pm 1.7\%$, range: 92.5–97.5%). Phytoliths from potential C_4 grasses contributed only minimally, yielding on average a minimum percent C_4 of $2.6 \pm 0.9\%$ (range: 1.6–4.1%) and a maximum percent C_4 of $3.3 \pm 0.9\%$ (range: 2.1–4.7%) when including all PACMAD forms in the estimate. Potential C_4 morphotypes were limited to panicoid-type bilobates and crosses (BI-8, not pictured; crosses: Fig. 4W–Y) and PACMAD-GEN-type bilobates (BI-5, not pictured; BI-7, Fig. 4V). C_3 GSSCs were dominated by pooid-type conical and keeled rondels, *Stipa* and rounded/rectangular-top bilobates, and crenates (Fig. 4P–U). FI morphotypes consisted mainly of small rugulose spheres (CI-7, Fig. 4L) and rare palm phytoliths (CI-m-2a, Fig. 4N), which were present in most samples. Qualitatively analyzed samples included pooid-type GSSCs, potential grasses (CONI/MONO and GRASS-MONO-ND), and non-diagnostic OTH forms, but not identifiable grass phytoliths from potential C_4 grasses (Supplemental Table 2).

Discussion

Comparison of isotope and phytolith estimates of C₄ in the Miocene of Montana

Results from both carbon isotopic ratios and phytolith assemblages suggest limited amounts of C₄ vegetation (Figs. 3, 5) at the MBJ locality. Isotopic ratios yielded a slightly higher average C₄ composition compared to phytolith assemblage analysis ($5.6 \pm 4.6\%$ and 2.6–3.3%, respectively), but both types of estimates are within error range of each other for most samples (Fig. 5). Phytolith assemblage values also show much less variation (range: 1.6–4.7%) compared to values calculated based on isotopic data (range: 0–13%). As a result, there is almost no statistical correlation between the two estimates of C₄ abundance on the landscape. The nearby TFF site (Cotton et al., 2012) yielded a similar result, with isotopic results indicating a slightly higher proportion of C₄ vegetation than phytoliths for one sample, a close match for another, and one sample where the phytoliths indicated a higher proportion of C₄ (Figs. 5, 6). However, the fact that both methods result in overall similar reconstructions of relative abundance of C₄ biomass (i.e., <15%), and at two different geographic localities, suggests that the inferred overall proportion of C₄ vegetation is robust. In addition, although C₄ plants are known from 19 angiosperm families, grasses are the most widespread C₄ taxa (Sage, 2004; Sage et al., 2011). The close correspondence between inferred C₄ from GSSC phytoliths and isotopic data supports the interpretation that grasses were the only C₄ plants in Montana at this time.

In their study aimed at understanding Rocky Mountain uplift history using $\delta^{18}\text{O}$ values of pedogenic carbonates, Chamberlain et al. (2012) also published isotopic analyses of paleosol carbonates and carbonate cements from MBJ where the $\delta^{13}\text{C}_{\text{carb}}$ values range from -3.4 to -4.5‰ (n = 16). Using the same assumptions as were used to reconstruct the proportion of C₄ plants from the $\delta^{13}\text{C}_{\text{org}}$ data, it is possible to reconstruct the proportion of C₄ plants that would be indicated by the $\delta^{13}\text{C}_{\text{carb}}$ values. Doing this, one would infer that C₄ plants constituted 46–53% of

vegetation, which is clearly at odds with both the phytolith and $\delta^{13}\text{C}_{\text{org}}$ data. Cotton et al. (2012) previously noted a similar discrepancy for the TFF site, where $\delta^{13}\text{C}_{\text{carb}}$ values apparently indicated a much higher proportion of C_4 plants than either phytoliths or $\delta^{13}\text{C}_{\text{org}}$ data. The fact that samples from this study and from Chamberlain et al. (2012) were not all from exactly the same paleosols within the MBJ section (i.e., some data in Chamberlain et al. 2012 are also from paleosols stratigraphically above and below the paleosols in this study) could account for some of the discrepancy. However, some of their isotopic measurements come from the caprock paleosols (Fig. 3), and because of the relatively rapid depositional rate (Retallack, 2007) none of these data are more than a few hundred thousand years younger or older, meaning that it is still possible to evaluate how reasonable the constraints derived from the $\delta^{13}\text{C}_{\text{carb}}$ values are on the proportion of C_4 plants at this site. Pedogenic carbonates that formed in equilibrium with soil-respired CO_2 should be +14–17 ‰ (typically +15.5‰) relative to organic matter from the same soil or paleosol (given as $\Delta^{13}\text{C}$; Cerling and Quade, 1993). Using the mean $\delta^{13}\text{C}_{\text{org}}$ value of -24.3‰, none of the carbonates preserved at MBJ were formed in isotopic equilibrium, with $\Delta^{13}\text{C}$ values ranging from +19.8 to +20.9. Even if one assumes instead that the highest $\delta^{13}\text{C}_{\text{org}}$ value of -23.2‰ was the most representative, the minimum $\Delta^{13}\text{C}$ value would be +18.7, which is still substantially out of equilibrium. The degree of disequilibrium is consistent with groundwater affected or diagenetically altered carbonates (Tabor et al., 2013). Thus, the carbonates at MBJ, like at TFF, can be considered unreliable for reconstructing past vegetation. Because paleoelevation studies rely on the relative changes in $\delta^{18}\text{O}_{\text{carb}}$ rather than the absolute value, this result does not call into question Chamberlain et al.'s (2012) results, but instead suggests that workers should be careful when applying $\delta^{13}\text{C}_{\text{carb}}$ values to understand the proportion of C_4 plants in the absence of other proxies that can rule out pedogenic or diagenetic alteration.

Regional vegetation heterogeneity in Montana

At MBJ (11.2–9.5 Ma) there is evidence for low levels (<15%) of C₄ vegetation in an otherwise C₃ open-habitat ecosystem. In contrast, Cotton et al. (2012) found higher and more variable C₄ presence (0–35%) at a penecontemporaneous (10.2–8.9 Ma), nearby (Fig. 1) site further south (Fig. 6). Neither site demonstrates a secular (i.e., temporal) trend. These differences suggest that there was significant regional heterogeneity in C₄ distribution—as has been noted in other late Miocene ecosystems (Behrensmeyer et al., 2007)—but also that C₄ vegetation had become more abundant on parts of the landscape in the late Miocene compared to earlier times, pointing to expansion of C₄ vegetation during this time. One hypothesis might be that these changes were driven by a changing climate. However, estimates of MAT and MAP from TFF and this site (385 ± 147 mm yr⁻¹ vs. 369 ± 73 mm yr⁻¹, and 9.2 ± 0.9 °C vs. 9–11 °C, respectively; Retallack, 2007; Hamer, 2009; Cotton et al., 2012), are overlapping, and global CO₂ was constant at the time (Pagani et al., 1999; Bolton and Stoll, 2013; Zhang et al., 2013). Therefore, it seems unlikely that changes in overall aridity, temperature, or atmospheric CO₂ were responsible for this increase in variability.

The presence but consistently very low abundance of GSSCs highly diagnostic of chloridoids among potential C₄ grasses in the middle and late Miocene of Montana (Strömberg, 2005; Cotton et al., 2012) supports the idea that changes in aridity were not important for higher C₄ values at TFF compared to MBJ. Chloridoid grasses tend to be associated with more arid and open environments compared to other C₄ grasses (in the Panicoideae; Taub, 2000; Liu et al., 2012), and Strömberg and McInerney (2011) used observed increases in chloridoid relative frequency (coupled with decreasing palm abundance) to argue for late Miocene drying in the central Great Plains. The most abundant GSSCs reflective of potential C₄ grasses at MBJ are

indicative of panicoids, which show an overall preference for wetter habitats (Taub, 2000; Liu et al., 2012).

Instead of broad-scale changes in climate (i.e., global climate change), we suggest that differential expansion of C₄ grasses was driven by one or more local factors, such as disturbance (fire regime, herbivory, sediment deposition), topography/elevation (see below), potentially in combination with climatic seasonality (see below). Previous work has pointed to the importance of disturbance, in particular fire, in maintaining today's savanna vegetation in many regions (e.g., Lehmann et al., 2011). A simulation of fire regimes by Scheiter et al. (2012) for Africa (with vast areas of generally warmer and more arid conditions than in Montana) found that fire contributed mainly to the invasion of grasses (C₄ and C₃) into (C₃) forests, whereas the expansion of C₄ grasses at the expense of C₃ grasses was driven by the physiological advantages of C₄ grasses in hot, arid, low-CO₂ environments. Therefore, if fire was an important driver of local C₄ distribution, it should be reflected principally in the abundance of trees over grasses (C₃/C₄), with the younger site (TFF) having a lower frequency of forest indicators. Data from TFF shows that high relative C₄ biomass, reconstructed based on $\delta^{13}\text{C}_{\text{org}}$ values, only occurs in samples with low relative abundance of forest indicator phytoliths (i.e., in more open habitats; Strömberg et al., 2012). However, overall, TFF has a higher inferred tree cover than MBJ, which is not consistent with the hypothesis that fire influenced the expansion of C₄ grasses in southwestern Montana. The charcoal record needed to further test this idea has yet to be collected.

The degree of within-site "patchiness" also differed between MBJ and TFF. When heterogeneity in paleovegetation was reconstructed through lateral sampling at the scale of tens of meters, MBJ (Fig. 3) shows consistently low C₄ abundance across the landscape, whereas there seems to have existed considerable spatial variability at TFF (Cotton et al., 2012; their Figs.

5, 6). Although it is possible that more lateral sampling at MBJ would have revealed similar patchiness, the fact that the TFF site exhibits a wide range of C₄ abundance values laterally as well as vertically (i.e., temporally) suggests that the site overall records substantially more pronounced variation (“patchiness”). Previous work in Montana has indicated that such landscape patchiness may be linked to disturbance through stream avulsion (Miller et al., 2012). Under this scenario, the more variable TFF site would reflect a more highly disturbed ecosystem. The MBJ section is very short, complicating an in-depth study of depositional mode (e.g., stream avulsion rate). However, the only major change in depositional mode identified by Retallack’s (2007) study of a longer section is the cap rock within the short MBJ section, which suggests that depositional environment changes did not impact C₄ abundance there.

Paleoelevation implications

Local reconstructed paleoclimate conditions are cool and dry (Retallack, 2007; Hamer, 2009), consistent with a dry scrubland (Gulbranson et al., 2011). At both TFF and MBJ, very few FI phytoliths were identified, and relatively low levels of C₄ are indicated by combined isotope and phytolith records, with less than 10% C₄ indicated for most of the two records, and lower reconstructed C₄ at MBJ than at TFF. Thus, both the paleovegetation and paleoclimatic results are consistent with a relatively open, relatively sparse vegetative cover. This paleo-C₄ distribution closely matches that modern C₄ distribution in Montana, where present-day TFF has less than 20% C₄ plants and modern day MBJ has less than 10% C₄ plants (Epstein et al., 2002). This suggests that a distribution of C₄ plants similar to the modern may have been established by at least the middle Miocene. Today the distribution of C₃ and C₄ grasses along elevational gradients is highly predictable, and linked to temperature (e.g., in South America, C₄ plants are absent at elevations >2500 meters; Cavagnaro, 1988; Cabido et al., 1997). Although the exact

elevation of C₄ absence appears to differ among continents and regions (e.g., at 2100 m in China; Li et al., 2009), a similar relationship with elevation has been observed in China (Li et al., 2009), Central Asia (Taft et al., 2011), on the Arabian Peninsula (Masrahi et al., 2012), and in the southwestern part of the United States (Van de Water et al., 2002). The similarities in C₄ biomass between the late Miocene and today of Montana therefore suggest that the paleo-elevation of Montana was also similar. Cotton et al. (2012) reported a mean modern (Preindustrial) soil $\delta^{13}\text{C}_{\text{org}}$ value of -24.3‰ for soils at a similar elevation and nearby to the TFF site, which compared favorably with their Miocene mean value of -23.9‰, further supporting the idea that the Miocene and modern C₄ distributions were analogous and were probably controlled by similar ecological factors. This hypothesis finds independent support in a study by Chamberlain et al. (2012). They compiled a variety of isotopic records both in space and in time and found that there was little evidence for uplift after 15 Ma ago in Montana, and that regional paleoelevation was most likely over 2 km at the time of MBJ's deposition.

North American spread of grasslands

A body of paleobotanical and isotopic evidence now indicates that, during the Eocene, Montana was covered in paratropical forests with C₃, potentially open-habitat grasses growing at disturbed sites (Strömberg, 2003, 2005; Miller et al., 2012). Macrofossil evidence from the latest Eocene and early Oligocene Ruby and Beaverhead basin floras shows these forests persisted, becoming dominated by coniferous and temperate angiosperm elements throughout the Paleogene (Becker, 1960, 1961, 1966, 1969, 1972, 1973; Lielke et al., 2012). By the late early-middle Miocene, these forests had been replaced by open-habitat woodlands dominated by C₃ grasses with a minor C₄ component (Strömberg, 2003, 2005; Cotton et al., 2012; this study). Paleobotanical and isotopic data from other parts of the continental interior – the central and

southern Great Plains (see Table 1) – indicate a similar pattern of vegetation change. However, $\delta^{13}\text{C}$ of ungulate tooth enamel has classically been interpreted as showing a latitudinal gradient in the emergence of C_4 dominated habitats (e.g., Passey et al., 2002). Whereas phytolith assemblage data show the presence of PACMAD grasses in general and recent work on leaf waxes indicates that C_4 grasses in particular were present in the early Oligocene of the Great Plains (Strömberg, 2005; Tipple and Pagani, 2010), there is no clear evidence for PACMADs in the Paleogene of Montana (Strömberg, 2005; Miller et al., 2012). This contrast in PACMAD abundance supports the notion that C_4 grasses reached more southern areas in the Great Plains first and, hence, that a latitudinal gradient in vegetation existed within the continental interior during the Cenozoic (Leopold and MacGinitie, 1972; Strömberg, 2005). In contrast, a recent comparison of ungulate functional morphology, isotopic and phytolith data found no such gradient in the late Miocene (~7 Ma; Fraser and Theodor, 2013). Other late Miocene Great Plains sites (Fox and Koch, 2003; Strömberg and McInerney, 2011; Fox et al., 2012) indicate 10–30% and 0–20% C_4 composition (Table 1), respectively, compared to modern values of 75% and 30–40% C_4 (Epstein et al., 2002), respectively, indicating significant change in regional vegetation composition since the late Miocene. This shift was accompanied by, or possibly driven by, substantial cooling and aridification (Strömberg and McInerney, 2011; Fox et al., 2012). In contrast, Montana saw little change in C_4 abundance over the same period (late Miocene: 0–15% C_4 , present: 10–20% C_4), as noted above. This pattern suggests that the lower elevation Great Plains sites have experienced substantially greater shifts in vegetation community composition than the higher elevation Montana sites since the late Miocene.

Regional Montana record in a global context

Paleovegetation records at TFF and MBJ (Fig. 1) highlight the importance of local ecological drivers rather than a global climatic trigger for changing C₄ abundance, which is also observed in Pakistan (Behrensmeyer et al., 2007). In Montana, measurable amounts (5–30%) of C₄ grasses appear to have existed millions of years prior to the late Miocene-early Pliocene global spread of C₄ grasslands, usually as minor components of open, C₃ grass-dominated habitats. The earliest widespread (C₃) grasslands were previously thought to be in Argentina by the middle Eocene (e.g., Jacobs et al., 1999; Strömberg, 2011), and the earliest widespread C₄ vegetation in at low latitudes across several continents by late Miocene (8–5 Ma; Edwards et al., 2010). Edwards et al.'s (2010) global compilation of estimates of the timing of C₄ origin and expansion based on pedogenic carbonates, ungulate tooth C isotopes, and phytoliths, revealed major difference in the tempo and mode of C₄ expansion even at similar latitudes. More recent work (post-2010 literature summarized in Table 1) has further developed our understanding of the geologic history of grassland ecosystems, highlighting the regional nuance pointed out by Edwards et al. (2010), in particular, depending on which proxy is used, emphasizing the need for multiproxy reconstructions like the one presented herein. For example, Cotton et al. (2014) found significant differences in regional C₄ abundance between late Miocene–mid-Pliocene sites at different elevations in northern Argentina. In Asia, Jia et al. (2012) and Singh et al. (2013) found evidence for ~20% C₄ 3–6 Ma prior to previous estimates of C₄ expansion in the Tibetan Plateau region, although studies for the same region by Leier et al. (2009), Martin et al. (2011), Charreau et al. (2012), and Zhang et al. (2012) found no such evidence. In Inner Mongolia, Zhang et al. (2009) found little evidence for C₄ pre-8 Ma, and mixed C₃-C₄ post-8 Ma. In both Eastern and Western Africa, studies of mammalian tooth enamel indicated C₄ expansion in the range of 7–5 Ma as opposed to the 10 Ma found by earlier studies of in Kenya (Uno et al., 2011). Taken

together, these studies emphasize both local and regional variation in C₄ vegetation prior to the global late Miocene-Pliocene expansion event (Table 1). In particular, prior to the latest Miocene, the presence and abundance of C₄ vegetation, and the composition of coexisting C₃ vegetation (trees/shrubs vs. grasses) seems to have been spatially and temporally very variable. Generally speaking, areas of low paleoelevation and drier climates appear to have maintained a low abundance of C₄ plants first, but in some regions their subsequent rise to dominance can be linked to climatic changes (e.g., Kansas; Fox et al., 2012), whereas in other areas such as Montana (*this study*; see Retallack, 2007 for long-term climate changes) climatic and tectonic conditions were stable over millions of years. These findings underscore the importance of local factors such as seasonality, topography/elevation, fire regime, and biological community composition on C₄ distribution (or combinations thereof), as opposed to singular global or regional controls.

Conclusions

Organic carbon isotopes and phytolith assemblages are independent and complementary techniques for reconstructing past vegetation. In this study of the late Miocene (11.2–9.5 Ma) MBJ locality, the techniques combined suggested an open-habitat, C₃ dominated grassland with a small C₄ component (<15%) during a period of arid climate, similar to the present ecosystem. When combined with other records from the region, these results support the idea that C₃ closed forests were replaced by C₃ grasslands with a small C₄ component in Montana as well as in the Great Plains (Strömberg, 2005); similar patterns have been suggested for other parts of the world as well (Edwards et al., 2010; Strömberg, 2011). Furthermore, in most areas around the world, C₄ vegetation was present at low to moderate levels for millions of years prior to global expansion with both variable timing of the onset of and ultimate scale of C₄ expansion. Similar

479 heterogeneity is evident both at the local and regional scale when comparing
480 penecontemporaneous sites in Montana. Patchiness in C₄ distribution and expansion even during
481 times of climatic quiescence during the Miocene suggests that local and regional factors such as
482 fire regime, biodiversity, topography, and seasonality, which affect modern grassland
483 distribution, were also important factors in historic grassland expansion and distribution.

484 **Acknowledgements**

485 The authors would like to thank Tess Nugent and Tim Gallagher (University of Michigan) for
486 assistance with isotopic analyses. SYS would like to acknowledge the support of the Michigan
487 Society of Fellows. This research was supported by NSF Award #1024535 to NDS and SYS;
488 NSF Award #1024681 to CAES.

References Cited

- Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E., Harris, T., 2004. Grazing systems, ecosystem responses, and global change. *Ann. Rev. Environ. Res.* 29, 261–299.
- Barboni, D., Bremond, L., Bonnefille, R., 2007. Comparative study of modern phytolith assemblages from inter-tropical Africa. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 246, 454–470.
- Becker, H.F., 1960. Additions to the Tertiary Ruby paper shale flora of southwestern Montana. *Bull. Torrey. Bot. Club* 87, 386–396.
- Becker, H.F., 1961. Succulent display in Israel and Sweden. *Cactus and Succ. Jour.* 33, 71–73.
- Becker, H.F., 1966. Additions to and revision of the Oligocene Ruby Paper Shale flora of southwestern Montana. *Univ. Mich. Mus. Paleontol. Contrib. Mus. Paleontol.* 20, 89–119.
- Becker, H.F., 1969. Fossil plants of the Tertiary Beaverhead Basins in southwestern Montana. *Palaeontographica B* 127:1–142 + 44 pls.
- Becker, H.F., 1972. The Metzel Ranch Flora of the Upper Ruby River Basin, southwestern Montana. *Palaeontographica B* 141:1–61 + 16 pls.
- Becker, H.F., 1973. The York Ranch Flora of the Upper Ruby River Basin, southwestern Montana. *Palaeontographica B* 143:18–93 + 40 pls.
- Bedaso, Z.K., Wynn, J.G., Alemseged, Z., Geraads, D. 2013. Dietary and paleoenvironmental reconstruction using stable isotopes of herbivore tooth enamel from middle Pliocene Dikika, Ethiopia: Implication for *Australopithecus afarensis* habitat and food resources.

509 Behrensmeyer, A.K., Quade, J., Cerling, T.E., Kappelman, J., Khan, I.A., Copeland, P., Roe, L.,
510 Hicks, J., Stubblefield, P., Willis, P., Latorre, C., 2007. The structure and rate of late
511 Miocene expansion of C₄ plants: evidence from lateral variation in stable isotopes in
512 paleosols of the Siwalik Group, northern Pakistan. *Bull. Geol. Soc. Am.* 119, 1486–1505.

513 Bolton, C.T., Stoll, H.M., 2013. Late Miocene threshold response of marine algae to carbon
514 dioxide limitation. *Nature* 500, 558–562.

515 Bonnefille, R. 2010. Cenozoic vegetation, climate changes and hominid evolution in tropical
516 Africa. *Global and Planetary Change* 72, 390–411.

517 Bremond, L., Alexandre, A., Wooller, M.J., Hely, C., Williamson, D., Schafer, P.A., Majule, A.,
518 Guiot, J., 2008. Phytolith indices as proxies of grass subfamilies on East African tropical
519 mountains. *Glob. Plan. Ch.* 61, 209–224.

520 Cabido, M., Ateca, N., Astegiano, M.E., Anton, A.M., 1997. Distribution of C₃ and C₄ grasses
521 along an altitudinal gradient in Central Argentina. *J. Biogeog.* 24, 197–204.

522 Cavagnaro, J.B., 1988. Distribution of C₃ and C₄ grasses at different altitudes in a temperate arid
523 region of Argentina. *Oecologia* 76, 273–277.

524 Cerling, T. E., Harris, J. M., 1999. Carbon isotope fractionation between diet and bioaplite in
525 ungulate mammals and implications for ecological and paleoecological studies.
526 *Oecologia* 120, 347–363.

527 Cerling, T.E., Quade, J., 1993. Stable carbon and oxygen isotopes in soil carbonates, in Swart,
528 P.K., Lohmann, G.C., McKenzie, J.A., Savin, S. (Eds.), *Climate Change in Continental*
529 *Isotopic Records*. American Geophysical Union, Washington, D.C., pp. 217–231.

530 Cerling, T.E., Wang, Y., Quade, J., 1993, Expansion of C₄ ecosystems as an indicator of global
531 ecological change in the late Miocene. *Nature* 361, 344–345.

532 Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V.,
533 Ehleringer, J. R., 1997. Global vegetation change through the Miocene/Pliocene
534 boundary. *Nature* 389, 153–158.

535 Chamberlain, C.P., Mix, H.T., Mulch, A., Hren, M.T., Kent-Corson, M.L., Davis, S.J., Horton,
536 T.W., Graham, S.A., 2012. The Cenozoic climatic and topographic evolution of the
537 western North American Cordillera. *Am. J. Sci.* 312, 212–262.

538 Charreau J., Kent-Corson, M.L., Barrier, L., Augier, R., Ritts, B. D., Chen, Y., France-Lannord,
539 C., Fuilmette, C., 2012. A high-resolution stable isotopic record from the Junggar Basin
540 (NW China): Implications for the paleotopographic evolution of the Tianshan Mountains.
541 *Earth Planet Sc. Lett.* 341-344, 158–169.

542 Chen, S. T., and Smith, S.Y., 2013. Phytolith Variability in Zingiberales: A Tool for
543 Reconstruction of Past Tropical Vegetation. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*
544 370, 1–12.

545 Clarke, J., 2003. The occurrence and significance of biogenic opal in the regolith. *Earth-Science*
546 *Rev.* 60, 175–194.

547 Cotton, J.M., 2013. Using soils to constrain past and future terrestrial climate change. PhD
548 Dissertation, University of Michigan, 278 p.

549 Cotton, J.M., Hyland, E.G., Sheldon, N.D., 2014. Multi-proxy evidence for tectonic control on
550 the expansion of C₄ grasses in northwest Argentina. *Earth and Plan. Sci. Lett.* 395, 41–50.

551 Cotton, J. M., Sheldon, N. D., and Strömberg, C.A.E., 2012. High-resolution isotopic record of
 552 C₄ photosynthesis in a Miocene grassland. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 337–
 553 338, 88–98.

554 Diefendorf, A.F., Mueller, K.E., Wing, S.L., Koch, P.L., and Freeman, K.H., 2010. Global
 555 patterns in leaf¹³C discrimination and implications for studies of past and future climate.
 556 *PNAS* 107, 5738–5743.

557 Dupont, L.M., Rommerskirchen, F., Mollenhauer, G., Schefuß, E. 2013. Miocene to Pliocene
 558 changes in South African hydrology and vegetation in relation to the expansion of C₄
 559 plants. *Earth Planet. Sci. Lett.* 375, 408–417.

560 Edwards, E.J., Osborne, C.P., Strömberg, C.A.E., Smith, S.A., C₄ Grasses Consortium, 2010.
 561 The Origins of C₄ Grasslands: Integrating Evolutionary and Ecosystem Science. 328,
 562 587–591.

563 Epstein, H.E., Gill, R.A., Paruelo, J.M., Lauenroth, W.K., Jia, G.J., Burke, I.C., 2002. The
 564 relative abundance of three plant functional types in temperate grasslands and shrublands
 565 of North and South America: effects of projected climate change. *J. Biogeogr.* 29, 875–
 566 888.

567 Feakins, S.J., Levin, N.E., Liddy, H.M., Sieracki, A., Eglinton, T.I., Bonnefille, R. 2013.
 568 Northeast African vegetation change over 12 m.y. *Geology* 41, 295–298.

569 Ferenac, R.S., Pagnac, D. 2013. Stable carbon isotope evidence for the abundance of C₄ plants in
 570 the middle Miocene of southern California. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 388,
 571 42–47.

572 Fraser, D., Theodor, J.M., 2013. Ungulate diets reveal patterns of grassland evolution in North
 573 America. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 369, 409–421.

574 Fredlund, G.G., Tieszen, L.T., 1994. Modern Phytolith Assemblages from the North American
 575 Great Plains. *J. Biogeogr.* 21, 321–335.

576 Fredlund, G.L., Tieszen, L.L., 1997. Phytolith and carbon isotope evidence for late Quaternary
 577 vegetation and climate change in the southern Black Hills, South Dakota. *Quat. Res.* 47,
 578 206–217.

579 Fritz, W.J., Sears, J.W., McDowell, R.J., Wampler, J.M., 2007. Cenozoic volcanic rocks of
 580 southwestern Montana. *Northwest Geol.* 36, 91–110.

581 Fox, D.L., Koch, P.L., 2003. Tertiary history of C₄ biomass in the Great Plains, USA. *Geology*
 582 31, 809–812.

583 Fox, D.L., Honey, J.G., Martin, R.A., Peláez-Campomanes, P., 2012. Pedogenic carbonate stable
 584 isotope record of environmental change during the Neogene in the southern Great Plains,
 585 southwest Kansas, USA: Carbon isotopes and the evolution of C₄-dominated grasslands.
 586 *Geo. Soc. Am. Bull.* 124, 444–462.

587 Garten Jr., C.T., Cooper, L.W., Post III, W.M., Hanson, P.J., 2000. Climate controls on forest
 588 soil C isotope ratios in the southern Appalachian Mountains. *Ecology* 81, 1108–1119.

589 Gulbranson, E.L., Montañez, I.P., Tabor, N.J., 2011. A proxy for humidity and floral province
 590 from paleosols. *J. Geol.* 119, 559–573.

591 Hanneman, D.L., Wideman, C.J., 1991. Sequence stratigraphy of Cenozoic continental rocks,
 592 southwestern Montana. *Geo. Soc. Am. Bull.* 103, 1335–1345.

593 Hanneman, D.L., Wideman, C.J., 2006. Calcic pedocomplexes – regional sequence boundary
594 indicators in Tertiary deposits of the Great Plains and western United States. GSA Spec.
595 Paper 416, 1–15.

596 Hamer, J.M.M., 2009. Interpreting Cenozoic floodplains: a paleopedological and ichnological
597 perspective. PhD Dissertation, Royal Holloway, 462 p.

598 Hamer, J.M.M., Sheldon, N.D., Nichols, G.J., Collinson, M.E., 2007. Late Oligocene-Early
599 Miocene paleosols of distal fluvial systems, Ebro Basin, Spain. *Palaeogeography,*
600 *Palaeoclimatology, Palaeoecology* 247, 220–235.

601 Hasiotis, S.T., 2004. Reconnaissance of Upper Jurassic Morrison Formation ichnofossils, Rocky
602 Mountain Region, USA: paleoenvironmental, stratigraphic, and paleoclimatic
603 significance of terrestrial and freshwater ichnocoenoses. *Sediment. Geol.* 167, 117–268.

604 Huang, Y., Clemens, S.C., Liu, W., Wang, Y., Prell, W.L. 2007. Large-scaled hydrological
605 change drove the late Miocene C₄ plant expansion in the Himalayan foreland and Arabian
606 Peninsula. *Geology* 35, 531–534.

607 Hui, Z., Li, J., Xu, Q., Song, C., Zhang, J., Wu, F., Zhao, Z. 2011. Miocene vegetation and
608 climatic changes reconstructed from a sporopollen record of the Tianshui Basin, NE
609 Tibetan Plateau. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 308, 373–382.

610 Hyland, E.H., Smith, S.Y., Sheldon, N.D., 2013. Representational bias in phytoliths from
611 modern soils of central North America: Implications for paleovegetation reconstructions.
612 *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 374, 338–348.

613 Hynek, S., Passey, B.H., Prado, J.L., Brown, F.H., Cerling, T.E., Quade, J. 2012. Small mammal
614 carbon isotope ecology across the Miocene-Pliocene boundary, northwestern Argentina.
615 Earth Planet. Sc. Lett. 321-322, 177–188.

616 Ingram, A.L., Christin, P.A., Osborne, C.P., 2011. Molecular phylogenies disprove a
617 hypothesized C₄ reversion in *Eragrostis walteri* (Poaceae). Ann. Botany 107, 321–325.

618 Jacobs, B.F., Kingston, J.D., Jacobs, L.L., 1999. The origin of grass-dominated ecosystems. Ann.
619 Miss. Bot. Gard. 86, 590–643.

620 Janis, C.M., Damuth, J., Theodor, J.M., 2000. Miocene ungulates and terrestrial primary
621 productivity: Where have all the browsers gone?. Proc. Natl. Acad. Sci. 97, 7899–7904.

622 Jia, G., Li, Z., Peng, P., Zhou, L., 2012. Aeolian n-alkane isotopic evidence from North Pacific
623 for a Late Miocene decline of C₄ plant in the arid Asian interior. Earth Planet. Sc. Lett.
624 321-322, 32–40.

625 Kohn, M.J., 2010. Carbon isotope compositions of terrestrial C₃ plants as indicators of
626 (paleo)ecology and (paleo)climate. PNAS 107, 19691–19695.

627 Larsson, L.M., Dybkjaer, K., Rasmussen, E.S., Piasecki, S., Utescher, T., Vajda, V. 2011.
628 Miocene climate evolution of northern Europe: A palynological investigation from
629 Denmark. Palaeogeog. Palaeoclimat. Palaeoecol. 309, 161–175.

630 Lehmann, C.E.R., Archibald, S.A., Hoffmann, W.A., Bond, W.J., 2011. Deciphering the
631 distribution of the savanna biome. New Phyt. 191, 197–209.

- 632 Leier, A., Quade, J., Decelles, P., Kapp, P., 2009. Stable isotopic results from paleosol carbonate
633 in South Asia: Paleoenvironmental reconstructions and selective alteration. *Earth Planet*
634 *Sc. Lett.* 279, 242–254.
- 635 Leopold, E.B., MacGinitie, H.D., 1972. Development and affinities of Tertiary floras in the
636 Rocky Mountains, in: Graham, A. (ed.), *Floristics and Paleofloristics of Asia and Eastern*
637 *Li, J., Wang, G., Liu, X., Han, J., Liu, M., Liu, X., 2009. Variations in carbon isotope*
638 *ratios of C₃ plants and distribution of C₄ plants along an altitudinal transect on the*
639 *eastern slope of Mount Gongga. Sci. China D: Ear. Sci.* 52, 1714–1723.
- 640 Lielke, K., Manchester, S., Meyer, H., 2012. Reconstructing the environment of the northern
641 Rocky Mountains during the Eocene/Oligocene transition: constraints from the
642 palaeobotany and geology of south-western Montana, USA. *Acta Palaeobotanica* 52,
643 317–358.
- 644 Liu, H., Edwards, E.J., Freckleton, R.P., Osborne, C.P. 2012. Phylogenetic niche conservatism in
645 C₄ grasses. *Oecologia* 170:835–845.
- 646 Martin, C., Bentaleb, I., Antoine, P.O., 2011. Pakistan mammal tooth stable isotopes show
647 paleoclimatic and paleoenvironmental changes since the early Oligocene. *Palaeogeogr.*
648 *Palaeoclimatol. Palaeoecol.* 311, 19–29.
- 649 Masrahi, Y.S., Al-Turki, T.A., Sayed, O.H., 2012. Geographic distribution and chorology of
650 grasses in the Arabian Peninsula. *Flora – Morph., Dist. Func. Eco Plants* 207, 250–256.

651 McInerney, F.A., Strömberg, C.A.E., White, J.W.C., 2011. The Neogene transition from C₃ to C₄
652 grasslands in North America: stable carbon isotope ratios of fossil phytoliths.
653 *Paleobiology* 37, 23–49.

654 Miller, L.A., Smith, S.Y., Sheldon, N.D., Strömberg, C.A.E., 2012. Eocene vegetation dynamics
655 inferred from a high-resolution phytolith record. *Geol. Soc. Am. Bull.* 124, 1577–1589.

656 Osborne, C.P., 2008. Atmosphere, ecology and evolution: what drove the Miocene expansion of
657 C₄ grasslands? *J. Ecology* 96, 35–45.

658 Pagani, M., Freeman, K. H., Arthur, M. A. 1999. Late Miocene atmospheric CO₂ concentrations
659 and the expansion of C₄ grasses. *Science* 285, 876–879.

660 Passey, B.H., Cerling, T.E., Perkins, M.E., Voorhies, M.R., Harris, J.M., Tucker, S.T. 2002.
661 Environmental change in the Great Plains: an isotopic record from fossil horses. *J. Geol.*
662 110, 123–140.

663 Piperno, D.R., 1985. *Phytolith Analysis and Tropical Paleo-ecology: Production and Taxonomic*
664 *Significance of Siliceous Forms in New World Plant Domesticates and Wild Species.*
665 *Rev. Paleobot. Palyno.* 45, 185–228.

666 Piperno, D.R., 1988. *Phytolith Analysis, an Archaeological and Geological Perspective.*
667 Academic Press, San Diego.

668 Piperno, D.R., 1989. The occurrence of phytoliths in the reproductive structures of selected
669 tropical angiosperms and their significance in tropical paleoecology, paleoethnobotany
670 and systematics. *Rev. Pal. Pal.* 61, 147–173.

671 Piperno, D.R., 2006. *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists*.
672 AltaMira Press, Plymouth, 304 p.

673 Prasad, V., Strömberg, C. A. E., Alimohammadian, H., and Sahni, A. 2005. Dinosaur Coprolites
674 and the Early Evolution of Grasses and Grazers. *Science* 310:1177–1180.

675 Prychid, C.J., Rudall, P.J., and Gregory, M., 2004. Systematics and Biology of Silica Bodies in
676 Monocotyledons. *Bot. Rev.* 69, 377–440.

677 Retallack, G.J., 2007. Cenozoic paleoclimate on land in North America. *J. Geol.* 115, 271–294.

678 Sage, R.F., 2004. The evolution of C₄ photosynthesis. *New Phytol.* 161, 341–370.

679 Sage, R.F., Christin, P.-A., Edwards, E.J. 2011. The C₄ plant lineages of planet Earth. *J. Exp. Bot.*
680 62, 3155–3169.

681 Scheiter, S., Higgins, S. I., Osborne, C.P., Bradshaw, C., Lunt, D., Ripley, B.S., Taylor, L.L., and
682 Beerling, D.J., 2012. Fire and fire-adapted vegetation promoted C₄ expansion in the late
683 Miocene. *New Phytol.* 195, 653–666.

684 Sheldon, N.D., Tabor, N.J., 2009. Quantitative paleoenvironmental and paleoclimatic
685 reconstruction using paleosols. *Earth-Science Reviews* 95, 1–52.

686 Sheldon, N. D., Retallack, G. J., Tanaka, S., 2002. Geochemical climofunctions from North
687 American soils and application to paleosols across the Eocene-Oligocene boundary in
688 Oregon. *J. Geol.* 110, 687–696.

689 Singh, S., Awasthi, A.K., Parkash, B., and Kumar, S., 2013. Tectonics or climate: What drove
690 the Miocene global expansion of C₄ grasslands?. *Int. J. Earth Sci.*, DOI 10.1007/s00531-
691 013-0893-5

692 Smith, S.Y., Collinson, M.E., Rudall, P.J., Simpson, D.A., 2010. The Cretaceous and Paleogene
693 fossil record of Poales: review and current research. *In*: Seberg, O., Petersen, G., Barfod,
694 A., Davis, J.I., eds. *Diversity, Phylogeny, and Evolution in Monocotyledons: Proceedings*
695 *of the Fourth International Conference on the Comparative Biology of the*
696 *Monocotyledons & the Fifth International Symposium on Grass Systematics and*
697 *Evolution*. Aarhus University Press, Aarhus, p. 333–356.

698 Strömberg, C A.E., 2002. The origin and spread of grass-dominated ecosystems in the late
699 Tertiary of North America: preliminary results concerning the evolution of hypsodonty.
700 *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 177, 59–75.

701 Strömberg, C.A.E., 2003. The origin and spread of grass-dominated ecosystems during the
702 Tertiary of North America and how it relates to the evolution of hypsodonty in equids.
703 Ph.D. thesis, UC Berkeley 779 p.

704 Strömberg, C.A.E., 2004. Using phytolith assemblages to reconstruct the origin and spread of
705 grass-dominated habitats in the great plains of North America during the late Eocene to
706 early Miocene. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 207, 239–275.

707 Strömberg, C.A.E., 2005. Decoupled taxonomic radiation and ecological expansion of open-
708 habitat grasses in the Cenozoic of North America. *PNAS* 102, 11980–11984.

709 Strömberg, C.A.E., 2009. Methodological concerns for analysis of phytolith assemblages: Does
 710 count size matter? *Quat. Int.* 193, 124–140.

711 Strömberg, C.A.E., 2011. The origin and spread of grass-dominated habitats. *Annu. Rev. Earth*
 712 *Planet Sci.* 39, 517–544.

713 Strömberg, C.A.E., McInerney, F.A., 2011. The Neogene transition from C₃ to C₄ grasslands in
 714 North America: assemblage analysis of fossil phytoliths. *Paleobiology* 37, 50–71.

715 Strömberg, C.A.E., Dunn, R.E., Madden, R.H., Kohn, M.J., Carlini, A., A., 2013. Decoupling the
 716 spread of grasslands from the evolution of grazer-type herbivores in South America.
 717 *Nature Communications* 4, 1–8.

718 Tabor, N.J., Myers, T.S., Gulbranson, E., Rasmussen, C., Sheldon, N.D. 2013. Carbon stable
 719 isotope composition of modern calcareous soil profiles in California: implications for
 720 CO₂ reconstructions from calcareous paleosols, in, Driese, S.G. and Nordt, L. (Eds.),
 721 *New Frontiers in Paleopedology and Terrestrial Paleoclimatology*, SEPM Special Paper
 722 No. 104, p. 17–34.

723 Taft, J.B., Phillippe, L.R., Dietrich, C.H., Robertson, K.R. 2011. Grassland composition,
 724 structure, and diversity patterns along major environmental gradients in the Central Tien
 725 Shan. *Plant Ecol.* 212:, 1349–1361.

726 Taub, D. R., 2000. Climate and the U.S. distribution of C₄ grass subfamilies and decarboxylation
 727 variants of C₄ photosynthesis. *Am. J. Bot.* 87, 1211–1215.

728 Tipple, B. J., Meyers, S. R., Pagani, M., 2010. Carbon isotope ratio of Cenozoic CO₂: A
 729 comparative evaluation of available geochemical proxies. *Paleoceanography* 25,
 730 doi:10.1029/2009PA001851.

731 Tipple, B. J., Pagani, M., 2010. A 35 Myr North American leaf-wax compound-specific carbon
 732 and hydrogen isotope record: Implications for C₄ grasslands and hydrologic cycle
 733 dynamics. *Earth Planet Sc. Lett.* 299, 250–262.

734 Uno, K.T., Cerling, T.E., Harris, J.M., Kunitatsu, Y., Leakey, M.G., Nakatsukasa, M., Nakaya,
 735 H. 2011. Late Miocene to Pliocene carbon isotope record of differential diet change
 736 among East African herbivores. *Proc. Nat. Acad. Sci.* 108, 6509–6514.

737 Urban, M.A., Nelson, D.M., Jiménez-Moreno, G., Châteauneu, J., Pearson, A., Hu, F.S. 2010.
 738 Isotopic evidence of C₄ grasses in southwestern Europe during the Early Oligocene–
 739 Middle Miocene. *Geology* 38, 1091–1094.

740 Van de Water, P.K., Leavitt, S.W., Betancourt, J.L., 2002. Leaf $\delta^{13}\text{C}$ variability with elevation,
 741 slope aspect, and precipitation in the Southwest United States. *Oecologia* 132, 332–343.

742 Wynn, J.G., 2007. Carbon isotope fractionation during decomposition of organic matter in soils
 743 and paleosols: implications for paleoecological interpretations of paleosols.
 744 *Palaeogeography, Palaeoclimatology, Palaeoecology* 251, 437–448.

745 Wynn, J.G., Bird, M.I., 2007. C₄-derived soil organic carbon decomposes faster than its C₃
 746 counterpart in mixed C₃/C₄ soils. *Global Change Biology* 13, 2206–2217.

747 Wynn, J.G., Bird, M.I., Wong, V.N.L., 2005. Rayleigh distillation and the depth profile of
 748 $^{13}\text{C}/^{12}\text{C}$ ratios of soil organic carbon from soils of disparate texture in Iron Range

749 National Park, Far North Queensland, Australia. *Geochim. Cosmochim. Acta* 69, 1961–
750 1973.

751 Zin-Maung-Maung-Thein, Takai, M., Uno, H., Wynn, J.G., Egi, N., Tsubamota, T., Thaung-
752 Htike, Aung-Naing-Soe, Maung-Maung, Nishimura, T., Yoneda, M. 2011. Stable isotope
753 analysis of the tooth enamel of Chaingzauk mammalian fauna (late Neogene, Myanmar)
754 and its implication to paleoenvironment and paleogeography. *Palaeogeog. Palaeoclimatol.*
755 *Palaeoecol.* 300, 11–22.

756 Zhang, C., Wang, Y., Deng, T., Wang, X., Biasatti, D., Xu, Y., Li, Q., 2009. C₄ expansion in the
757 central Inner Mongolia during the latest Miocene and early Pliocene. *Earth Planet Sc.*
758 *Lett.* 287, 311–319.

759 Zhang, C., Wang, Y., Wang, X., Deng, T., Tseng, Z. J., Takeuchi, G. T., Xie, G., et al., 2012.
760 Diets and environments of late Cenozoic mammals in the Qaidam Basin, Tibetan Plateau:
761 Evidence from stable isotopes. *Earth Planet Sc. Lett.* 333–334, 72–80.

762 Zhang, Y.G., Pagani, M., Liu, Z., Bohaty, S.M., DeConto, R., 2013. A 40-million-year history of
763 atmospheric CO₂. *Phil. Trans. Roy. Soc. A*, doi: 10.1098/rsta.2013.0096.

764

Captions

Figure 1. Map showing location of sampling site and of a penecontemporaneous site from Cotton et al. (2012). Modified from Miller et al. (2012).

Figure 2. Examples of trace fossils found on site. A, Back-filled burrows. B, Downward branching burrows, organic root traces (dark). C, Calcified roots, with preserved cortical cells. D, Ant nest trace fossil (e.g., Hasiotis, 2004; Hamer et al., 2007).

Figure 3. Stratigraphic column showing sampling levels and phytolith assemblage, isotopic value, and corresponding percent C₄ estimate for each method. Stars represent horizons sampled for organic matter $\delta^{13}\text{C}$ analysis and pie charts correspond to phytolith samples with good yields. Sampling horizons with isotopic results but not pie chart correspond to samples with poor phytolith yields. Error bars (for isotopic value): $\pm 1\sigma$ based on $\delta^{13}\text{C}_{\text{atm}}$ uncertainty (Tippie et al., 2010). Ninety-five percent confidence intervals (for phytoliths) derived through bootstrapping in R (R Development Core Team 2013). Small symbols denote error/confidence intervals. Phytolith-isotope sample offsets are for readability purposes; they are derived from the same samples and horizons. For A horizon samples from lower paleosol, the error range is averaged from all three samples.

Figure 4. Microphotographs of selected biosilica. A–C, diatoms. D, sponge spicule. E, F, algal cyst. G, Elo-1: Smooth elongate (OTH). H, Epi-9: Spiny elongate (GRASS/MONO-ND). I, Tri-8: Trichome filling (GRASS/MONO-ND). J, Blo-2: Thickened rectangular plate (OTH). K, Cl-11: Small, grainy sphere (OTH). L, Cl-7: Small rugulose sphere and subsphere (FI-GEN). M, M-8: Solid parenchyma cell (OTH). N, Clm-2a: Echinate (spiky) sphere (PALM-D). O, Kn-1: M-8 with knobs (OTH). P, CE-1: Crenate with symmetry A (POOID-D). Q, CE-1: Crenate with

symmetry A (POOID-D), lateral view. R, BI-2: Bilobate with rounded top (POOID-D). S, BI-1: Regular *Stipa* type bilobate (POOID-D). T, KR-1: Regular keeled rondel (POOID-ND). U, CO-1: Generic (truncated) conical rondel (POOID-ND). V, BI-7: Symmetry D bilobate (PACMAD-GEN). W, CR3-1: 3-lobe cross with cross-shape top (PANI), lateral view. X, CR3-2: 3-lobe cross with near cross-shape top (PANI). Y, CR4-2: 4-lobed cross with near cross-shaped top (PANI). Scale bar: 5 μm .

Figure 5. Sample-to-sample comparison of paleosol organic carbon isotope and phytolith assemblage C_4 percentage estimates, including data from Cotton et al., (2012). Error bars (for isotopic value; in black): $\pm 1\sigma$ based on $\delta^{13}\text{C}_{\text{atm}}$ uncertainty (Tippie et al., 2010) for this study, $\pm 1\sigma$ based on replication for Cotton et al., (2012). Ninety-five percent confidence intervals (for phytoliths; in grey) derived through bootstrapping in R (R Development Core Team 2013).

Figure 6. Comparison of percent grass and maximum percent C_4 estimated from phytolith and C isotope data for Montana and the Great Plains from 16–8 Ma. Montana C isotope data derived from paleosol organic matter (this paper; Cotton et al., 2012; Miller et al., 2012); Great Plains data plotted as running average from paleosol carbonates (two-point running average; Fox and Koch, 2003) and phytolith data (four-point running average; Strömberg, 2005; Strömberg and McInerny, 2011). Note that maximum phytolith estimates for C_4 in the central Great Plains are much lower than paleosol carbonate data estimates, whereas soil organic matter and phytoliths agrees well in Montana.

Table 1. Percent C_4 estimates and vegetation interpretations from other late Miocene sites. Data presented are in addition to those compiled by Edwards et al. (2010). Ecosystems were described as C_3 -dominant unless additional evidence (e.g. phytoliths or pollen) existed about openness.

810 ^aPedogenic carbonates have been found to bias towards C₄ composition, potentially due to
811 diagenesis or diffusion of atmospheric CO₂ (see Cotton et al. (2012) for discussion). ^bIsotopes in
812 phytoliths from grass-dominated habitats measure mainly the isotopic signal of the grass
813 community rather than of overall vegetation, hence these values are higher than isotopic analyses
814 of, for example, bulk organic material (see McInerney et al. (2011) for discussion).

Figure 1

Figure 1

Figure 2

Figure 2 greyscale

Figure 1 illustrates the stratigraphic levels in Retallack (2007) and the corresponding isotopic data ($\delta^{13}\text{C}_{\text{org}}$) for various depths and lateral distances. The stratigraphic column on the left shows units from 0 to 86 m, including silty sst, caprock, upper paleosol, lower paleosol, and volcanic ash. The isotopic data on the right shows $\delta^{13}\text{C}_{\text{org}}$ values ranging from -25 to -23 ‰. The data points are categorized by depth (0 m, 12 m lateral, 22 m lateral) and phytolith type (vertical, lateral, 22 m lateral). A legend at the bottom defines symbols for silty sst, calc sst, volcanic ash, root trace, and isotopes (vertical, lateral, 22 m lateral).

Figure 3 greyscale

Figure 4

Figure 5

Figure 6

Table 1

Location	Time	$\delta^{13}\text{C}$ (‰)	%C ₄	Inferred Ecosystem	Proxy	Source
North America						
Montana, USA	11.2-9.5 Ma	-24.3 (-25.8 to -23.2)	0-15%	C ₃ -grassland	Soil isotopes and phytoliths	<i>This paper</i>
	10.2-8.9 Ma	-23.9 (-26.5 to 19.9)	0-25%	C ₃ -grassland	Soil isotopes and phytoliths	Cotton et al. (2012)
	39.2 Ma	-25.1 to -21.8	0%	C ₃ -forest	Phytoliths	Miller et al. (2012)
	12-2 Ma	-27 to -15	0-100% ^b	C ₃ -grassland	Carbon isotopes from phytoliths	McInerney et al. (2011)
	12-2 Ma	n/a	0-60%	C ₃ -grassland	Phytoliths	Strömberg and McInerney (2011)
Kansas, USA	25 Ma-present	-8.7 to 0.9	10-78% ^a	C ₃ -dominant	Carbonate isotopes	Fox et al. (2012)
Great Plains	35 Ma-present	-30 to -28 (n-C ₂₉) and -30 to -24 (n-C ₃₁)	20-50%	C ₃ -dominant	Leaf wax isotopes	Tipple and Pagani (2010)
California, USA	14-13.4 Ma	-10 to -6.2	0-18%	C ₃ -grassland	Enamel isotopes	Feranac and Pagnac (2013)
South America						
Argentina	40-18 Ma	n/a	0%	C ₃ -forest	Phytoliths	Strömberg et al. (2013)
	9-3.5 Ma	-14 to 4	0-80%	C ₃ -dominant	Enamel isotopes	Hynek et al. (2012)
	6.2-3.3	-24.6 (-27.5 to -22.3)	0%	C ₃ -dominant	Soil isotopes and phytoliths	Cotton et al. (2014)
Asia						
Tibetan Plateau	14 Ma-present	-12 to -4	0-30%	C ₃ -dominant	Enamel isotopes	Zhang et al. (2012)
	16 Ma-present	-25 to -21	10-20%	C ₃ -dominant	Leaf wax isotopes	Jia et al. (2012)
	50-15 Ma	-11 to -2	0%	C ₃ -dominant	Carbonate isotopes	Leier et al. (2009)
	23.6-1 Ma	-12.9 to 3	0%	C ₃ dominant	Carbonate isotopes	Charreau et al. (2012)
	17.1-6.1 Ma	n/a	-	Forest (until 14.7 Ma), Forest-steppe (14.7-11. Ma), Steppe (after 8.5 Ma)	Pollen	Hui et al. (2011)
Pakistan	14 Ma-present	-12 to 3	0-100%	C ₃ -dominant	Carbonate isotopes	Singh et al. (2013)
	30-7.5 Ma	-12 to -8	0-26%	C ₃ -forest to C ₃ -C ₄ grass-forest mosaic	Enamel isotopes	Martin et al. (2011)

Inner Mongolia	25 Ma-present	-13 to -2	0-80%	C ₃ -dominant to C ₃ - C ₄ grass-forest mosaic	Enamel isotopes	Zhang et al. (2009)
Myanmar	6 Ma	-14 to 2.6	0-100%	C ₃ - C ₄ grass-forest mosaic	Enamel isotopes	Zin-Maung-Maung-Thein et al. (2011)
Africa						
East Africa	11.7-8.7 Ma	-31.1 to -25.0	<20%	C ₃ grassland	Leaf wax isotopes	Feakins et al. (2013)
	5-1 Ma	n/a	-	Grass-forest mosaic	Pollen	Bonnefille (2010)
Kenya	10-4 Ma	-10 to 2	0-100%	C ₃ - C ₄ grass-forest mosaic	Enamel isotopes	Uno et al. (2011)
Ethiopia	4-3 Ma	-14 to 2	50-80%	C ₃ - C ₄ grassland	Enamel isotopes	Bedaso et al. (2013)
South Africa	14-3 Ma	-30 to -26	>10%	C ₃ - C ₄ grass-desert mosaic	Pollen and leaf wax isotopes	Dupont et al. (2013)
Europe						
Denmark	19-8 Ma	n/a	-	Forest-swamp	Pollen	Larsson et al. (2011)
Spain	35-15 Ma	n/a	0-60%	C ₃ - C ₄ grass-forest mosaic	Pollen isotopes	Urban et al. (2010)