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Do Uncharred Plants Preserve Original Carbon and Nitrogen Isotope Compositions?

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Abstract

The isotopic compositions of plants can provide significant insights into paleodiets, ancient agricultural activities, and past environments. Isotopic compositions of charred (aka carbonized) ancient plant remains are typically preferred over those of uncharred/uncarbonized plants, both because charred plants are more commonly preserved and because early research suggested they experience less post-depositional isotopic alteration. In this paper, we re-explore the question of whether uncharred plants experience large-magnitude post-depositional changes in carbon and nitrogen isotope compositions by analyzing Terminal Pleistocene–Early Holocene plant specimens from rockshelters in the Escalante River Basin (Colorado Plateau, southeastern Utah). Several lines of evidence, including C_3-CAM differences, plant-part comparisons, and dietary estimates from ancient herbivore collagen, suggest that the original carbon isotope compositions of these plants have not been significantly altered. The preservation status of plant nitrogen isotope compositions is equivocal. The direction of temporal shifts in plant $\delta^{15}N$ matches global trends and the magnitude of the shift may have been exacerbated by the extinction of megafauna in an arid environment. However, the Pleistocene plant $\delta^{15}N$ values are higher than would be expected based on herbivore bone collagen $\delta^{15}N$. Nevertheless, in contrast to previous research, the ancient uncharred plants in this study did not have exceptionally high $\delta^{15}N$ values ($> +25‰$). Overall, our research suggests that uncharred plants could be useful substrates for isotopic paleodietary and/or paleoenvironmental studies.

Keywords C_3 plant · CAM plant · Carbon isotope · Nitrogen isotope · Diagenesis · Colorado Plateau

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Introduction

The isotopic compositions of ancient plants can provide crucial baselines for the interpretation of human and animal diets and direct evidence for paleoclimatic conditions and agricultural practices (Blinnikov *et al.* 2011; Bogaard *et al.* 2013; Casey and Post 2011; Fiorentino *et al.* 2015; Kohn 2010; Makarewicz and Sealy 2015; Szpak *et al.* 2013; Vaiglova *et al.* 2014a; Warinner *et al.* 2013; Wooller *et al.* 2011). Isotopic studies of ancient plants typically rely on charred/carbonized remains, in part because they are more often preserved in archaeological contexts (Charles *et al.* 2015; Fiorentino *et al.* 2015), but also because a pioneering study by DeNiro and Hastorf (1985) concluded that uncharred plants are much more susceptible to diagenetic alteration. DeNiro and Hastorf (1985) argued that charred plant remains from Peruvian archaeological sites were reasonably well preserved since their range of $\delta^{13}C$ and $\delta^{15}N$ values and magnitude of intra-plant variations ($\leq 2‰$) were similar to those of the modern plants they studied. Subsequent experimental studies supported this conclusion, demonstrating that isotopic shifts due to charring were relatively small (typically non-systematic shifts of $< 1‰$ for $\delta^{13}C$ and increases in $\delta^{15}N$ of up to $\sim 2‰$) (Aguilera *et al.* 2008; Araus *et al.* 1997; Bogaard *et al.* 2007; DeNiro and Hastorf 1985; Fiorentino *et al.* 2012; Fraser *et al.* 2013; Heaton *et al.* 2009; Kanstrup *et al.* 2012; Marino and DeNiro 1987; Nitsch *et al.* 2015; Poole *et al.* 2002; Styring *et al.* 2013; Tieszen and Fagre 1993; Yang *et al.* 2011). In contrast to charred plants, uncarbonized archaeological plant isotopic compositions were thought to be significantly altered, since (1) the $\delta^{15}N$ values of uncharred plants were considerably (10–35‰) higher than those of modern plants, and (2) intra-plant variations in uncharred remains were much larger than those of the modern plants (up to 8‰ differences for $\delta^{13}C$ and 21‰ for $\delta^{15}N$) (DeNiro and Hastorf 1985).

In this study, we utilize macroscopically well-preserved ancient plant samples from the Escalante River Basin (ERB) to re-examine DeNiro and Hastorf's (1985) conclusion that uncharred plant C- and N-isotope values are significantly diagenetically altered. We do not seek to prove that absolutely no alteration has occurred, but rather to determine whether or not there has been *significant alteration* of original plant isotope compositions (*i.e.*, shifts of > 1–$2‰$). We begin with the assumption that uncharred plants retain the original isotopic compositions of the plant while it was alive and seek to disprove this assumption. We consider four lines of evidence. First, we discuss whether or not ancient plant δ-values and intra-plant variations are outside the range expected for modern plants, taking into account paleoenvironmental changes. These were the original criteria used by DeNiro and Hastorf (1985) to assess preservation of their ancient plants, though the isotopic effects of paleoenvironmental change were not well understood when that paper was published. Second, we examine temporal changes in ancient Escalante River Basin plant $\delta^{13}C$ and $\delta^{15}N$ values to determine whether they match expectations based on local circumstances and global trends. Third, we compare mean plant δ-values with diet estimates derived from local herbivore bone collagen. Fourth, we report the %C, %N, and C/N of the plant samples and their Fourier-transform infrared (FTIR) spectra for comparison with those of modern plants.

Modern Plants

Carbon Isotopes

Since the first reports that photosynthetic pathways affect carbon isotope discrimination, the typical ranges of $\delta^{13}C$ for C_3, C_4, and CAM plants have been well documented (Cerling *et al.* 1999; Kohn 2010; O'Leary 1988; Sternberg *et al.* 1984). Most plants utilize the C_3 photosynthetic pathway (Calvin cycle), which results in low $\delta^{13}C$ values (range = − 37 to − 20‰, mean = − 27‰ for modern plants) (Kohn 2010). C_3 plants with $\delta^{13}C$ values > − 25.5‰ are typically only found in areas with mean annual precipitation of < 500 mm/year (Kohn 2010). Very low $\delta^{13}C$ values in C_3 plants (< − 31.5‰) are found only in closed-canopy forest areas with low light levels and substantial recycling of ^{13}C-depleted CO_2 (Kohn 2010; Medina *et al.* 1991; Van Der Merwe and Medina 1991). C_4 plants have much higher $\delta^{13}C$ values, mostly within the range of − 16 to − 10‰ (Cerling *et al.* 1999; O'Leary 1988). Obligate/constitutive CAM plants have $\delta^{13}C$ values similar to C_4 plants or a little higher, typically ranging between about − 14 and − 10‰ (Sternberg *et al.* 1984). Facultative (inducible) CAM plants can switch between CAM and C_3 photosynthesis and can have values that can fall within or between the C_3 and C_4 plant ranges (about − 30 to − 10‰), depending on the degree to which each pathway is used during tissue formation (Kluge *et al.* 1991; Osmond *et al.* 1973; Teeri and Gurevitch 1984). Recent research suggests that obligate versus facultative CAM are not truly discrete categories, but rather, that some degree of facultative control might be present in all species capable of CAM photosynthesis (Winter *et al.* 2008). In CAM plants, higher values are produced when photosynthesis occurs in the dark, which tends to occur in areas with hot, dry days and relatively cool nights (Osmond *et al.* 1973). It should be noted that any comparison of the $\delta^{13}C$ values of modern and ancient plants must take into account the depletion of ^{13}C in atmospheric CO_2—and hence, in modern plants—that has occurred since humans began burning fossil fuels in industrial processes (*i.e.*, the Suess Effect). This led to an approximately 1‰ decrease from AD 1880 to 1980 and a further 1‰ decrease from 1980 to 2009 (Bocherens *et al.* 2014; Francey *et al.* 1999; Friedli *et al.* 1986; Long *et al.* 2005).

Different parts of the same plant can have different $\delta^{13}C$ values (O'Leary 1981; Tieszen 1991; Tieszen and Fagre 1993). The most consistently observed difference is a *ca.* 1–3‰ lower $\delta^{13}C$ value in leaves relative to non-photosynthetic tissues such as stems and roots (Badeck *et al.* 2005; Ghashghaie and Badeck 2014; Hobbie and Werner 2004). These differences are produced by several factors, including (but not limited to) different biochemical compositions (*e.g.*, low-^{13}C lipids and lignin vs. high-^{13}C cellulose, sugar, and starch), different formation times, and isotopic fractionations during transportation of biomolecules (Badeck *et al.* 2005; Cernusak *et al.* 2009; Ghashghaie and Badeck 2014).

Nitrogen Isotopes

It is more difficult to define a "typical" range of plant $\delta^{15}N$ values since these values are not dependent on fixed processes like photosynthetic pathways but rather on complexities such as nitrogen sources, nitrogen availability, nitrogen losses, symbiotic associations with mycorrhizae, biochemical fractionations within plants,

and environmental factors such as temperature and precipitation (see Szpak 2014 for review). In a survey of modern Peruvian plants from a variety of ecozones, Szpak *et al.* (2013) found that aboveground plant tissues ranged from − 5.3 to + 17.3‰. In a study of Kansas plants derived from herbarium samples dating to the past 130 years, McLauchlan *et al.* (2010) measured foliar $\delta^{13}C$ values between − 7.5 and + 14.5‰. Within single genera of African plants that associate with nitrogen-fixing bacteria, Cerling *et al.* (2009) found $\delta^{15}N$ values that ranged from about 0 to + 11‰ (*Indogofera* spp.) and + 3.5 to + 15.9‰ (*Acacia* spp.). In a study of modern Yukon plants (including various plant parts), Tahmasebi *et al.* (2017) measured $\delta^{15}N$ values between about − 10 to + 27‰, with the majority of measurements falling between about − 5 and + 5‰. Experimental studies have resulted in plant $\delta^{15}N$ values as high as + 25‰ when grown with pig manure and up to + 45‰ when fertilized with seabird guano (Szpak 2014; Szpak *et al.* 2012a). One field study demonstrated plant $\delta^{15}N$ variations in a gradient from about + 20‰ near the nesting sites of seabirds such as penguins and albatross, to − 10‰ at more distant locations (Erskine *et al.* 1998). The highest values resulted from large fractionations that occur when seabird excrement is mineralized: the ammonia gas has a very low $\delta^{15}N$ value and therefore the remaining guano becomes enriched in ^{15}N, which increases the $\delta^{15}N$ values of plants growing near the nests. Low $\delta^{15}N$ values at inland sites downwind of the nesting sites suggested that inland plants utilized some of the ^{15}N-depleted ammonia derived from the guano.

The effects of terrestrial herbivore dung and urine on plant $\delta^{15}N$ in natural environments is less straightforward. In Yellowstone National Park, areas frequented by grazing herbivores had higher soil $\delta^{15}N$ but lower aboveground plant $\delta^{15}N$ than areas from which herbivores were excluded (Frank and Evans 1997; Frank *et al.* 2004). Herbivores increase N losses from soils through processes like ammonia (NH_3) volatilization from urine/feces, which results in ^{15}N enrichment of soils (Frank and Zhang 1997). However, plants grown in these soils may then rely more on NO_3^- (produced by bacteria and depleted in ^{15}N) than NH_4^+ (enriched in ^{15}N), leading to lower plant $\delta^{15}N$ values (Frank and Zhang 1997). Lower aboveground plant $\delta^{15}N$ can also be caused by absorption of volatile ammonia by shoots and leaves through their stomata and cuticle, as was the case in the seabird study described above (Erskine *et al.* 1998; Frank *et al.* 2004). It should be noted that the studies of herbivore effects on plant $\delta^{15}N$ values cited above were conducted in temperate grasslands, and nitrogen dynamics in arid environments can be very different. For example, abiotic (rather than microbial) gas formation plays a key role in nitrogen loss in desert environments, particularly when soils become wet (McCalley and Sparks 2009). In combination with herbivore nitrogen inputs to soils, this could theoretically cause very large nitrogen fractionations, leading to ^{15}N-enriched soils and plants. Herbivores can also affect nitrogen cycling in arid ecosystems by damaging/destroying (layers of cyanobacteria, lichens, and mosses) that fix atmospheric nitrogen and provide the primary nitrogen input to some arid ecosystems (Evans and Ehleringer 1993).

Environmental variables such as soil nitrogen availability and climate (temperature, aridity) can also affect plant $\delta^{15}N$. Numerous studies have noted a negative correlation between foliar $\delta^{15}N$ and mean annual precipitation (Amundson *et al.* 2003; Austin and Vitousek 1998; Craine *et al.* 2009; Handley *et al.* 1999; Hofmeister *et al.* 2012). Very generally, plants growing in more arid environments tend to have higher $\delta^{15}N$ values than those growing in wet environments. Some studies have found a "hump-shaped

pattern" between soil/plant $\delta^{15}N$ and aridity, where $\delta^{15}N$ increases with aridity up to a threshold value, after which increases in aridity are associated with decreases in soil/plant $\delta^{15}N$ (Díaz *et al.* 2016; Wang *et al.* 2014). Some studies have also found a positive correlation between $\delta^{15}N$ and local temperature (Amundson *et al.* 2003; Craine *et al.* 2009; Martinelli *et al.* 1999), but this is generally less robust than the relationship with aridity.

Significant variations in $\delta^{15}N$ can occur within a single plant. In many plants (especially annuals), stored proteins are mobilized to newly grown plant parts (*i.e.*, those with reproductive functions), resulting in intra-plant nitrogen sources (*e.g.*, leaf/stem/bark) having higher $\delta^{15}N$ values than intra-plant nitrogen sinks (*e.g.*, fruits, grains, and/or flowers) or whole plants (Choi *et al.* 2002; Crawford *et al.* 1982; Szpak 2014; Szpak *et al.* 2012a, 2013). However, some studies have found no difference between these tissues (*e.g.*, Szpak *et al.* 2013; Tahmasebi *et al.* 2017), and others provided seemingly contradictory results—for example, that cereal grains have higher $\delta^{15}N$ values than the rachis (Fraser *et al.* 2011).

Isotopic Effects of Plant Decomposition

Plant decomposition is a continuum that begins with the accumulation of plant litter and ends with the formation and stabilization of soil organic matter (Melillo *et al.* 1989). Numerous studies have examined changes in soil $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C/N ratios with depth that are related in part to decomposition processes (but also to fractionations associated with transfer of N from soil to aboveground portions of growing plants). Carbon isotope fractionation during plant tissue degradation and soil formation is typically relatively small, and most studies have found either no change in $\delta^{13}C$ with depth, or increases of up to a few permil (Boström *et al.* 2007; Brueggemann *et al.* 2011; Ehleringer *et al.* 2000; Kramer *et al.* 2003; Krull *et al.* 2016; Tiunov 2007). In contrast, biochemical reactions of the nitrogen cycle (*e.g.*, nitrification, ammonification, uptake, and assimilation of N into plants) are associated with very large fractionations and hence much greater changes in plant/soil $\delta^{15}N$ (Robinson 2001). Soil organic matter $\delta^{15}N$ can increase by up to 20‰ with depth (Hogberg *et al.* 1996; Kramer *et al.* 2003; Ponsard and Arditi 2000; Scheu and Falca 2000; Steele *et al.* 1981; Tiunov 2007; Turner *et al.* 1983). The magnitude of ^{15}N enrichment with depth seems to depend little on mean annual temperature, precipitation, or nitrification rates, but depends strongly on the dominant type of mycorrhizal fungus associated with plant roots (Hobbie and Ouimette 2009). The largest increases (~9.6‰, on average) occur in ectomycorrhizal systems and smaller increases (~4.6‰) in arbuscular mycorrhizal systems (Hobbie and Ouimette 2009; Martinelli *et al.* 1999). The former are more typically found in temperate regions, including forests, whereas the latter are more common in tropical areas. Typically, the total carbon, total nitrogen, and C/N ratios of soil organic matter all decrease with depth (Boström *et al.* 2007; Krull *et al.* 2016; Melillo *et al.* 1989; Pardo *et al.* 1997; Ponsard and Arditi 2000; Salazar *et al.* 2012; Scheu and Falca 2000; Steele *et al.* 1981; Vervaet *et al.* 2002).

Experimental studies have shown that plant decomposition is associated with mass loss and overall decreases in C/N ratios, though increases in C/N ratios can also occur (Benner *et al.* 1991; Connin *et al.* 2001; Salazar *et al.* 2012). The effects of decomposition on %N and $\delta^{15}N$ are less straightforward. Benner *et al.* (1991) found that

decomposing plant %N decreased over a period of about 120 days (with no significant change in $\delta^{15}N$), then increased substantially (along with a $\delta^{15}N$ decrease of ~2‰). Connin *et al.* (2001) found no consistent relationship between changes in $\delta^{15}N$ and %N over time in decomposing plants in an arid environment, and the magnitude of changes in $\delta^{15}N$ were small (≤2.6‰). In a colder, temperate context, Tahmasebi *et al.* (2018) measured much larger $\delta^{15}N$ increases of 2.5 to 10‰ in decomposing buried plant samples. Decomposing plant $\delta^{15}N$ values were negatively correlated with C/N and %C, but no relationship between $\delta^{15}N$ and %N was reported (Tahmasebi *et al.* 2018). The studies cited above suggest that decomposition processes may occur differently—and be associated with smaller isotopic shifts—in arid relative to temperate environments. For example, photodegradation of plant material (abiotic breakdown by solar radiation) accounts for a significant portion of plant decomposition and gas release in hot, dry ecosystems (King *et al.* 2012; Lee *et al.* 2011), whereas microbial degradation is the dominant process in most other ecosystems. In hot, dry, shaded areas (such as Escalante River Basin alcoves), plant litter would be protected from both photodegradation (due to lack of sunlight) and microbial alteration (due to lack of water), which would account for the exceptional preservation of samples from these locations.

Evershed *et al.* (1997) demonstrated that extraordinarily well-preserved archaeological plant remains from ancient Egypt had undergone Maillard reactions between proteins and sugars, and that the volatiles produced during these reactions were trapped inside the desiccated plant remains. They suggested that Maillard reactions, which form stable compounds called melanoidins, are typical of plant decay. In normal circumstances, volatiles produced by the Maillard reaction are released when plants are broken down, but ancient plants preserved in hot, dry environments retain structural and storage macromolecules that trap the volatiles within the plant (Evershed *et al.* 1997). Bland *et al.* (1998) demonstrated that desiccated archaeological seeds had undergone Maillard reactions but that the composition of proteins and polysaccharides in the samples had undergone minimal changes. Isotopic shifts associated with Maillard reactions in experimentally charred samples have been studied and are relatively minimal (< 1‰ for $\delta^{13}C$ and up to ~2‰ for $\delta^{15}N$) (Aguilera *et al.* 2008; Araus *et al.* 1997; Bogaard *et al.* 2007; DeNiro and Hastorf 1985; Fiorentino *et al.* 2012; Fraser *et al.* 2013; Heaton *et al.* 2009; Kanstrup *et al.* 2012; Marino and DeNiro 1987; Nitsch *et al.* 2015; Poole *et al.* 2002; Styring *et al.* 2013; Tieszen and Fagre 1993; Yang *et al.* 2011). The magnitude of fractionations that might occur when Maillard reactions proceed at lower temperatures and/or in association with ancient desiccated plants has not been explored.

Can %C, %N, or C/N Be Used to Assess Post-Depositional Isotopic Alteration of Plants?

In isotopic studies of bone collagen, well-established ranges for carbon and nitrogen contents (%C, %N) and atomic C/N ratios are used to identify well-preserved versus diagenetically altered samples (Ambrose 1990; DeNiro 1985; van Klinken 1999). As noted by Szpak *et al.* (2013) and Szpak (2014), no such quality control indicators exist for ancient plant remains, in part because of the chemical heterogeneity of plant samples. For modern (uncharred) plants, mean carbon contents tend to be between ~40 and 47%, mean nitrogen contents are generally between ~1 and 3%, and mean atomic C/N ratios are typically between ~20 and 60 (Cerling *et al.* 2003; Codron *et al.* 2007; Fraser *et al.*

2011; Kristensen *et al*. 2011; Martinelli *et al*. 1999; Styring *et al*. 2016; Tahmasebi *et al*. 2017; Wooller *et al*. 2003). However, the full ranges of values for individual modern plant samples in any given study can be much larger. For example, Tahmasebi *et al*. (2017) measured atomic C/N values from 13 to 157 in modern Yukon plants. Furthermore, several studies have shown that large-magnitude changes in %C and %N associated with senescence (age-related remobilization of nutrients to other parts of the plant) do not necessarily correspond to any changes in $\delta^{13}C$ or $\delta^{15}N$ (Kolb and Evans 2002; Szpak *et al*. 2012a; Wooller *et al*. 2003). Thus, %C, %N, and C/N ranges cannot be used as quality control indicators for $\delta^{13}C$ and $\delta^{15}N$ of ancient plants.

Correlations between plant %C, %N, or C/N and isotope values can occur as a result of decomposition processes and might be used as evidence for alteration; however, similar correlations can also occur as a result of variations that occur when plants are alive. Thus, such correlations are not acceptable quality-control indicators. For example, experimental studies have demonstrated that increases in leaf litter $\delta^{15}N$ values during decomposition may be accompanied by increases in %N, decreases in %C, and decreases in C/N (Connin *et al*. 2001; Salazar *et al*. 2012; Tahmasebi *et al*. 2018). However, similar correlations have been reported in freshly collected modern plants. Correlations between modern plant $\delta^{15}N$ and %N have been reported in such diverse environments as African savanna (Codron *et al*. 2013), Hawaiian rainforest (Vitousek *et al*. 1989), recently deglaciated Alaska (Hobbie *et al*. 2000), southern Yukon grasslands (Tahmasebi *et al*. 2017), and in global compilations (Craine *et al*. 2009; Martinelli *et al*. 1999). In modern C_3 plants from Elk Island National Park (Alberta, Canada), a positive correlation between $\delta^{15}N$ and %N ($r = 0.27$, $p < 0.01$) and between $\delta^{15}N$ and C/N ($r = 0.34$, $p < 0.001$) were observed (Metcalfe, unpublished data). These examples show that these relationships do not provide robust evidence for post-depositional alteration of ancient plant isotopic compositions. Thus, we report %C, %N, and C/N ratios for the plants examined in the current study, but do not discuss them further as quality-control indicators.

Study Area

In the mid-1980s, ancient megafaunal dung and plant samples were collected from rockshelters/alcoves in the Forty-Mile Canyon and Willow Gulch areas of the Escalante River Basin (Glen Canyon National Recreation Area). The dung samples were used to constrain the timing of Late Pleistocene megafaunal extinctions/extirpations (Mead and Agenbroad 1992) and to reconstruct the dietary habits of extinct taxa (Davis *et al*. 1984; Kropf *et al*. 2007). The plant samples were used to reconstruct changes in vegetation in response to Late Pleistocene–Early Holocene climate change (Withers and Mead 1993). Since their collection, the remaining materials have been curated at the Museum of Northern Arizona (Flagstaff) on behalf of the Glen Canyon National Recreation Area. In this study, we examine the isotopic compositions of some of these morphologically well-preserved uncharred plant samples (Fig. 1) to explore their potential for reconstructing paleoenvironments and as baselines for isotopic studies of ancient animal diets.

The Escalante River Basin is located in southern Utah, on the central Colorado Plateau. With elevations ranging from ~ 1100 m at the Escalante River to ~ 2300 m at the top of the Kaiparowits Plateau, the canyons in this area support diverse environments and plant communities (Anderson *et al*. 2000; Withers and Mead 1993). Pinyon–

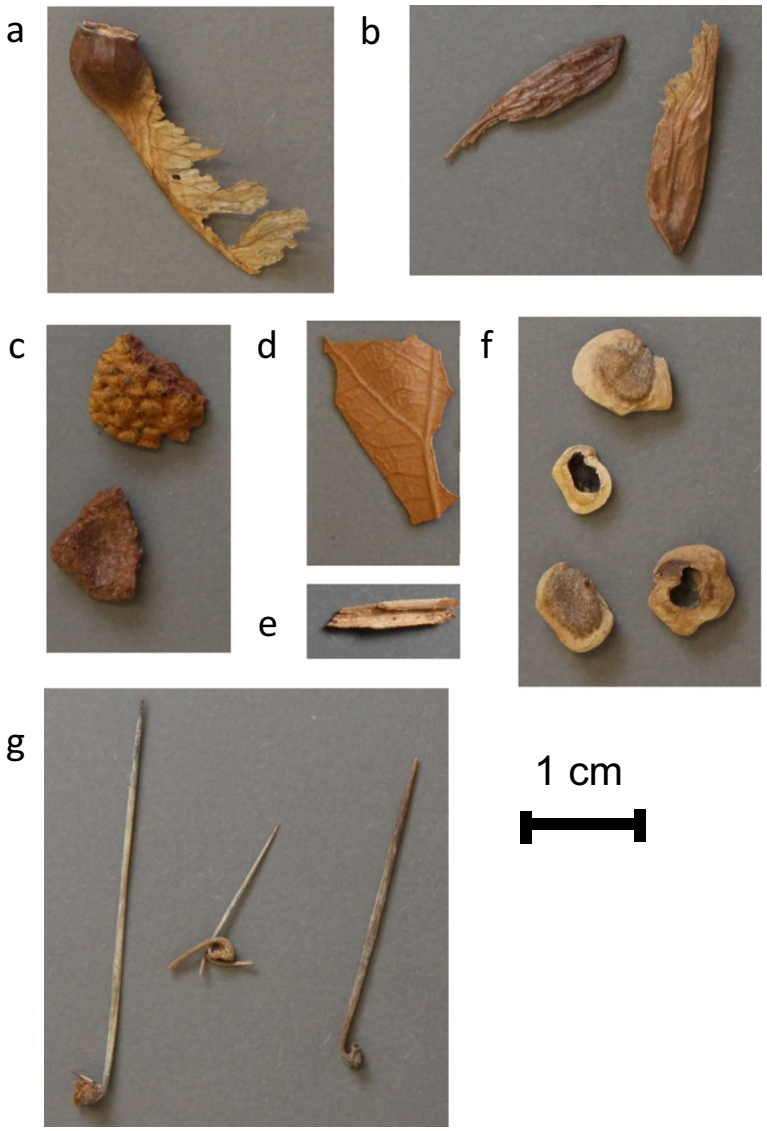

Fig. 1 Representative samples of Late Pleistocene–Early Holocene uncharred plants from the Escalante River Basin on the Colorado Plateau. **a** Seed of Bigtooth maple (*Acer grandidentatum*). **b** Seeds of box elder (*Acer negundo*). **c** Seeds of Gambel oak (*Quercus gambelii*). **d** Leaf of cottonwood (*Populus cf. fremontii*). **e** Unidentified twig. **f** Seeds of *Opuntia* cactus. **g** Needles of *Opuntia* cactus

juniper woodlands dominate the upper elevations. The middle and lower elevations host a wide range of habitats, including riparian woodlands, hanging gardens, active sand dunes, and mixed shrublands/grasslands (Agenbroad *et al*. 1989; Withers and Mead 1993). Temperature and precipitation also vary with altitude: along the river, mean annual temperature (MAT) is 12 °C and mean annual precipitation (MAP) is ≤ 250 mm, whereas on the Aquarius Plateau MAT is 2 °C and MAP is 575 mm (Webb 1985). The Forty-Mile Canyon alcoves (Hooper's Hollow, Grobot Grotto, BF Alcove)

are located between 1100 and 1300 m elevation, about 10 m above a steep drop to the deeply entrenched modern streambed (Withers and Mead 1993). Hooper's Hollow and Grobot Grotto are large (100–200 m wide) southwest-facing alcoves whereas BF alcove is narrower and faces northeast. The modern vegetation around these alcoves is predominantly desert grasses with a few scattered shrubs. The Willow Gulch alcoves (Shrub-Ox Alcove, Oak Haven) face northwest and west (respectively) and host a greater number and diversity of arboreal and shrub species, as well as grasses and cacti. Withers and Mead (1993) collected plant macrofossils from "vegetation mats" buried under eolian and/or colluvial sands within these alcoves. Some of these vegetation mats included dung with perfectly preserved morphology, indicating a lack of physical or chemical breakdown of material within the mats. Withers and Mead argued that the plants grew in the immediate vicinity of the alcoves and were essentially deposited *in situ*. Radiocarbon dates ($n = 14$) directly on the plant remains indicate that most of the samples were deposited between the terminal Pleistocene (*ca.* 13,000 ^{14}C yr. BP) and early Holocene (*ca.* 7500 ^{14}C yr. BP) (Withers and Mead 1993). Xerophytic grasses, shrubs, and cacti (*Opuntia* sp.) occurred throughout the temporal sequence. Terminal Pleistocene vegetation also included conifers and mesophytic species such as rose and water birch. Early Holocene vegetation lacked conifers and included reduced abundances of mesophytic species and increased reticulated hackberry. Terminal Early Holocene vegetation included abundant Gambel oak and prickly pear, and little else. The plant assemblage data suggest that there was a significant decrease in water availability over time, likely related to decreased stream levels rather than increased local precipitation (Withers and Mead 1993). The plant assemblage data also suggests that summers were cooler and drier, and winters were warmer and wetter, during these early time periods relative to the present (Withers and Mead 1993).

Materials and Methods

Ancient C_3 and CAM plant samples were obtained from National Park Service collections housed at the Museum of Northern Arizona (Fig. 1, Table 1). The samples were derived from five alcoves in the Escalante River Basin of the Colorado Plateau (BF Alcove, Grobot Grotto, Hooper's Hollow, Oak Haven, and Shrub-Ox Alcove). Plant samples from vegetation mats dating between $12{,}690 \pm 180$ and 7510 ± 160 ^{14}C yr. BP (Mead and Agenbroad 1992) were selected for carbon and nitrogen isotope analysis. The plant samples in this study were not directly dated, but dates were assigned based on stratigraphic association with directly dated plant specimens (Table 1) (Withers and Mead 1993). For temporal comparisons, we followed Withers and Mead (1993) in dividing the samples into three phases: Late Pleistocene (> 11,000 ^{14}C yr. BP), Early Holocene (11,000–8000 ^{14}C yr. BP), and Terminal Early Holocene (8000–7000 ^{14}C yr. BP).

Plant samples utilized in this study were dry-screened and separated from sediments but never subjected to chemical cleaning or wet processing (Withers and Mead 1993). Prior to isotopic analysis, plant parts (*e.g.*, seeds, leaves, twigs/branches, needles) were separated, and each sample was ground to a fine powder using a Wig-L-Bug ball mill. A total of 90 samples (81 C_3 and 9 CAM plants) were analyzed, including 11 different identifiable species (Table 1). Stable carbon and nitrogen isotope values of the plant samples were obtained at the University of British Columbia using an Isoprime stable

Table 1 Ancient plant isotopic compositions and elemental contents

Lab no.	Museum no.	Site	Species	Part	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C/N
CAM Plants										
9419	GLCA 2834	BF Alcove	Prickly pear *(Opuntia* sp.*)*	needle	LP	-9.8	+ 10.6	29.9	2.1	16.9
9392	GLCA 2832	BF Alcove	Prickly pear *(Opuntia* sp.*)*	needle	LP	-10.8	+ 10.6	30.6	2.2	16.5
9403	GLCA 2836	BF Alcove	Prickly pear *(Opuntia* sp.*)*	needle	LP	-10.6	+ 8.9	29.7	2.2	15.4
9399	GLCA 2844	BF Alcove	Prickly pear *(Opuntia* sp.*)*	seed	LP	-10.5	+ 9.3	35.0	0.8	70.3
9405	GLCA 2853	BF Alcove	Prickly pear *(Opuntia* sp.*)*	seed	LP	-10.5	+ 11.4	33.0	1.0	40.2
9418	GLCA 2834	BF Alcove	Prickly pear *(Opuntia* sp.*)*	seed	LP	-10.7	+ 13.2	34.7	1.0	41.0
9393	GLCA 2847	BF Alcove	Prickly pear *(Opuntia* sp.*)*	seed	LP	-9.7	+ 9.2	28.9	2.9	11.7
9948	GLCA 2895	Grobot Grotto	Prickly pear *(Opuntia* sp.*)*	needle	EH	-9.6	+ 22.6	36.2	1.6	25.9
9949	GLCA 2895	Grobot Grotto	Prickly pear *(Opuntia* sp.*)*	seed	EH	-10.7	+ 9.6	35.2	1.2	33.5
C_3 Plants										
9398	GLCA 2835	BF Alcove	Ash (*Fraxinus anomala*)	fruit	LP	-25.4	+ 8.4	30.7	3.0	12.0
9404	GLCA 2842	BF Alcove	Ash (*Fraxinus anomala*)	seed	LP	-23.6	+ 9.0	30.0	3.2	10.9
9395	GLCA 2837	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	fruit	LP	-21.8	+ 9.7	30.1	2.5	14.5
9400	GLCA 2856	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-21.4	+ 8.5	28.4	3.2	10.4
9402	GLCA 2833	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-22.9	+ 7.1	28.6	3.3	10.2
9410	GLCA 2859	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-21.7	+ 8.0	27.8	3.2	10.3
9411	GLCA 2857	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-23.3	+ 8.9	28.9	3.0	11.1
9412	GLCA 2850	BF Alcove	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-22.0	+ 6.6	33.0	2.3	16.4
9396	GLCA 2845	BF Alcove	Box-elder (*Acer negundo*)	seed	LP	-23.8	+ 8.3	28.3	3.2	10.4
9397	GLCA 2851	BF Alcove	Box-elder (*Acer negundo*)	seed	LP	-23.5	+ 8.5	32.6	2.5	15.3
9413	GLCA 2854	BF Alcove	Box-elder (*Acer negundo*)	seed	LP	-23.3	+ 9.8	33.3	3.1	12.5
9414	GLCA 2855	BF Alcove	Box-elder (*Acer negundo*)	seed	LP	-23.4	+ 7.7	34.6	1.2	34.1
9408	GLCA 2838	BF Alcove	*Comandra pallida*	seed	LP	-25.1	+ 9.2	27.9	3.7	8.8

Table 1 (continued)

Lab no.	Museum no.	Site	Species	Part	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C/N
9394	GLCA 2858	BF Alcove	Douglas fir (*Psuedotsuga menziesii*)	needle	LP	-23.5	+ 13.6	35.6	1.5	28.5
9401	GLCA 824	BF Alcove	Douglas fir (*Psuedotsuga menziesii*)	needle	LP	-23.8	+ 10.4	37.8	2.0	21.6
9407	GLCA 2841	BF Alcove	Douglas fir (*Psuedotsuga menziesii*)	needle	LP	-23.1	+ 12.5	37.9	1.8	24.1
9391	GLCA 2840	BF Alcove	Gambel oak (*Quercus gambelii*)	leaf	LP	-25.9	+ 8.7	27.5	3.2	10.1
9390	GLCA 2843	BF Alcove	Gambel oak (*Quercus gambelii*)	seed	LP	-24.8	+ 10.2	28.6	3.3	10.4
9406	GLCA 2839	BF Alcove	Gambel oak (*Quercus gambelii*)	seed	LP	-24.3	+ 8.5	26.5	3.5	8.8
9409	GLCA 2846	BF Alcove	Gambel oak (*Quercus gambelii*)	seed	LP	-23.5	+ 8.6	26.9	3.4	9.1
9422	GLCA 2860	BF Alcove	Plant	bark	LP	-20.6	+ 7.7	27.9	3.5	9.4
9426	GLCA 2861	BF Alcove	Plant	bark	LP	-21.3	+ 9.7	25.8	3.3	9.1
9424	GLCA 2861	BF Alcove	Plant	branch	LP	-24.6	+ 8.2	26.3	3.5	8.9
9425	GLCA 2861	BF Alcove	Plant	branch	LP	-23.2	+ 8.4	34.9	2.3	18.1
9415	GLCA 23134	BF Alcove	Plant	needle	LP	-23.7	+ 8.9	43.6	1.4	35.1
9416	GLCA 2852	BF Alcove	Plant	twig	LP	-26.1	+ 7.5	30.1	3.3	10.8
9417	GLCA 2852	BF Alcove	Plant	twig	LP	-24.9	+ 8.7	29.3	2.7	12.8
9421	GLCA 2849	BF Alcove	Plant	twig	LP	-25.5	+ 9.1	32.4	2.0	19.0
9423	GLCA 2860	BF Alcove	Plant	twig	LP	-25.0	+ 8.5	32.0	2.2	16.7
9427	GLCA 2861	BF Alcove	Plant	twig	LP	-22.9	+ 7.9	29.6	2.8	12.2
9420	GLCA 2849	BF Alcove	Plant	wood	LP	-22.8	+ 8.2	35.1	1.6	25.3
9947	GLCA 2894	Grobot Grotto	Gambel oak (*Quercus gambelii*)	leaf	EH	-27.1	+ 12.3	35.1	0.8	53.8
9950	GLCA 2896	Grobot Grotto	Gambel oak (*Quercus gambelii*)	leaf	TEH	-28.3	+ 1.2	37.6	0.8	53.4
9955	GLCA 2899	Grobot Grotto	Gambel oak (*Quercus gambelii*)	leaf	TEH	-28.6	+ 3.3	31.7	0.6	62.9
9951	GLCA 2896	Grobot Grotto	Gambel oak (*Quercus gambelii*)	petiole	TEH	-28.1	+ 3.8	33.3	0.5	85.9
9954	GLCA 2898	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed	TEH	-27.4	+ 4.5	37.1	1.2	35.0
9952	GLCA 2896	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed	TEH	-28.8	+ 1.7	38.6	0.8	55.7

Table 1 (continued)

Lab no.	Museum no.	Site	Species	Part	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C/N
9956	GLCA 2899	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed	TEH	-27.1	+ 3.5	35.6	0.9	47.1
9945	GLCA 2892	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed	TEH	-28.7	+ 3.0	29.4	0.5	62.9
9946	GLCA 2893	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed		-26.1	+ 9.0	34.2	1.6	25.5
9953	GLCA 2897	Grobot Grotto	Gambel oak (*Quercus gambelii*)	seed		-27.9	+ 0.3	35.9	0.6	66.0
9942	GLCA 2890	Hooper's Hollow	Gambel oak (*Quercus gambelii*)	leaf	EH	-27.4	+ 6.4	42.2	1.4	35.3
9943	GLCA 2890	Hooper's Hollow	Gambel oak (*Quercus gambelii*)	seed	EH	-27.2	+ 0.7	34.0	1.0	39.4
9944	GLCA 2891	Hooper's Hollow	Gambel oak (*Quercus gambelii*)	seed		-26.6	+ 0.0	35.6	1.2	35.5
9957	GLCA 2862	Oak Haven	Bigtooth maple (*Acer grandidentatum*)	leaf	EH	-27.1	+ 1.4	30.2	1.2	30.1
9963	GLCA 2868	Oak Haven	Bigtooth maple (*Acer grandidentatum*)	leaf	LP	-25.4	+ 3.6	32.6	1.3	30.4
9974	GLCA 2877	Oak Haven	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-25.6	+ 8.9	33.3	2.5	15.8
9977	GLCA 2879	Oak Haven	Bigtooth maple (*Acer grandidentatum*)	seed	LP	-27.5	+ 7.2	35.0	1.1	37.9
9978	GLCA 2880	Oak Haven	Bigtooth maple (*Acer grandidentatum*)	seed	EH	-27.3	+ 2.5	36.2	4.9	8.6
9971	GLCA 2874	Oak Haven	Plant	branch	LP	-27.9	+ 2.7	32.1	1.5	24.5
9975	GLCA 2878	Oak Haven	Plant	branch	LP	-28.3	+ 7.8	36.1	2.3	18.7
9976	GLCA 2878	Oak Haven	Plant	bark	LP	-28.4	+ 0.2	36.5	1.2	34.4
9959	GLCA 2864	Oak Haven	Rose (*Rosa woodsii*)	branch	LP	-26.8	+ 2.4	37.7	1.8	24.7
9961	GLCA 2866	Oak Haven	Rose (*Rosa woodsii*)	branch	LP	-25.8	+ 4.2	29.2	1.1	29.9
9970	GLCA 2873	Oak Haven	Rose (*Rosa woodsii*)	branch	LP	-25.7	+ 0.3	33.1	1.4	28.0
9966	GLCA 2870	Oak Haven	Rose (*Rosa woodsii*)	fruit	LP	-25.7	+ 3.5	31.8	1.8	20.2
9962	GLCA 2867	Oak Haven	Rose (*Rosa woodsii*)	fruit & stipule	LP	-26.1	+ 7.1	36.3	1.5	27.5
9972	GLCA 2875	Oak Haven	Rose (*Rosa woodsii*)	leaf	LP	-26.7	+ 4.0	33.6	1.2	33.7
9968	GLCA 2871	Oak Haven	Rose (*Rosa woodsii*)	leaf	LP	-27.5	+ 1.0	33.8	2.4	16.7
9958	GLCA 2863	Oak Haven	Rose (*Rosa woodsii*)	seed	LP	-25.7	+ 3.2	32.5	2.5	15.2
9969	GLCA 2872	Oak Haven	Rose (*Rosa woodsii*)	seed	LP	-23.2	+ 6.4	27.3	1.2	26.3

Table 1 (continued)

Lab no.	Museum no.	Site	Species	Part	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C/N
9964	GLCA 2869	Oak Haven	Rose (*Rosa woodsii*)	seed	LP	-25.3	+ 10.5	25.1	1.9	15.3
9965	GLCA 2869	Oak Haven	Rose (*Rosa woodsii*)	stipule	LP	-25.4	+ 5.2	34.1	0.9	42.8
9967	GLCA 2870	Oak Haven	Rose (*Rosa woodsii*)	stipule	LP	-26.5	+ 2.6	32.8	1.0	39.9
9973	GLCA 2875	Oak Haven	Rose (*Rosa woodsii*)	twig/petiole	LP	-26.2	+ 3.6	31.9	1.2	31.1
9960	GLCA 2865	Oak Haven	Spruce (*Picea* sp.)	needle	LP	-24.1	+ 3.4	32.0	1.0	35.6
9993	GLCA 2889	Shrubox Alcove	Cottonwood (*Populus* cf. *fremontii*)	leaf	EH	-25.6	+ 1.9	29.1	0.8	42.8
9992	GLCA 2888	Shrubox Alcove	Hackberry (*Celtis* sp.)	leaf	EH	-25.3	+ 1.4	26.5	0.7	44.6
9979	GLCA 2881	Shrubox Alcove	Box-elder (*Acer negundo*)	seed	EH	-23.5	+ 8.3	35.1	1.1	38.0
9988	GLCA 2886	Shrubox Alcove	Box-elder (*Acer negundo*)	seed	LP	-25.5	+ 7.7	28.8	3.2	10.6
9991	GLCA 2887	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	flower	EH	-27.0	+ 5.3	37.9	2.5	17.9
9981	GLCA 2882	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	fragment	EH	-28.0	+ 1.5	37.2	1.5	29.1
9989	GLCA 2887	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	leaf	EH	-28.1	+ 9.5	35.3	1.7	23.6
9982	GLCA 2883	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	leaf	EH	-28.1	+ 8.8	39.1	1.3	34.9
9980	GLCA 2882	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	seed	EH	-25.7	+ 6.1	30.6	1.2	30.6
9986	GLCA 2885	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	seed	LP	-26.3	+ 12.7	36.3	2.8	15.2
9984	GLCA 2884	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	seed	EH	-25.1	+ 8.3	34.7	1.5	27.0
9990	GLCA 2887	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	seed	EH	-26.2	+ 6.8	37.5	1.5	29.7
9983	GLCA 2883	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	seed	EH	-27.2	+ 3.0	35.8	1.6	25.6
9985	GLCA 2884	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	twig	EH	-27.7	+ 7.2	32.2	1.3	28.2
9987	GLCA 2885	Shrubox Alcove	Gambel oak (*Quercus gambelii*)	twig & flower	LP	-27.2	+ 11.8	34.9	2.5	16.1

Ages are as defined in the text (*LP* Late Pleistocene, *EH* Early Holocene, *TEH* Terminal Early Holocene)

isotope ratio mass spectrometer coupled to an Elementar VarioMicro Cube elemental analyzer. All plant samples were analyzed two or more times (Appendix A). USGS-40 and USGS-41 were used to calibrate the $\delta^{13}C$ and $\delta^{15}N$ to the VPDB and AIR scales, respectively. Analytical uncertainty was monitored using an in-house methionine standard with well-characterized isotopic compositions ($\delta^{13}C = -28.60 \pm 0.09‰$, $\delta^{15}N = -5.04 \pm 0.14‰$), and two international standards (IAEA CH-3 cellulose and IAEA CH-6 sucrose). Following the method presented by Szpak *et al*. (2017), precision ($u(R_w)$) was measured at ± 0.18‰ for $\delta^{13}C$ and ± 0.16‰ for $\delta^{15}N$ based on repeated measurements of calibration standards, check standards, and sample replicates. Accuracy or systematic error, *u(bias)*, was ± 0.09‰ for $\delta^{13}C$ and ± 0.16‰ for $\delta^{15}N$. The total analytical uncertainty (u_c) was estimated to be ± 0.20‰ for $\delta^{13}C$ and ± 0.23‰ for $\delta^{15}N$. Further details are provided in Appendix A.

Carbon and nitrogen contents of plant samples were calculated based on the amplitudes of the major carbon and nitrogen peaks relative to the weight of the sample, calibrated using USGS-40 (40.82 %C, 9.52 %N). Methionine (40.25 %C, 9.39 %N) and USGS-41 (41.90 %C, 9.76 %N) were used as check standards. Accuracy and precision were analyzed using the same approach as for isotope values (described above). For %C, precision ($u(R_w)$) was ± 2.78%, accuracy *u(bias)* was ± 0.83%, and the total analytical uncertainty (u_c) was ± 2.90%. For %N, precision ($u(R_w)$) was ± 0.21%, accuracy *u(bias)* was ± 0.15%, and the total analytical uncertainty (u_c) was ± 0.26% (Appendix A).

Skeletal remains are rare in Glen Canyon deposits, but five faunal samples were obtained for comparison of their $\delta^{13}C$ and $\delta^{15}N$ with those of the plants: a Harrington's mountain goat (*Oreamnos harringtoni*) tooth, a shrub-ox (*Euceratherium collinum*) tooth, and three mammoth (*Mammuthus* sp.) bones (Table 2). Bone/dentin was demineralized using 0.5 M HCl, gelatinized at 70 °C, and ultrafiltered to obtain the > 30 kDa size fraction. Two of the mammoth bone samples did not have sufficient collagen for analysis. The C and N isotope values and element contents of the remaining three samples were measured at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

FTIR was used to examine the molecular structure of selected ancient plant samples (Gambel oak seeds (*Quercus gambelii*), maple seeds (*Acer* spp.), and cactus needles (*Opuntia* sp.)) as well as modern samples from the same categories. The ancient samples were selected to include specimens with disparate $\delta^{13}C$ and $\delta^{15}N$ values. Modern oak and maple seeds were collected from the ground underneath trees growing outdoors in

Table 2 Skeletal samples for collagen carbon and nitrogen isotope analysis

Sample number	Species	Site	Element	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR}$	%C	%N	C/N
GLCA 3498	*Oreamnos harringtoni*	Hooper's Hollow	Tooth dentin	− 19.4	+ 4.9	31.9	10.3	3.6
GLCA 3059 A	*Euceratherium collinum*	Bechan Cave	Tooth dentin	− 8.8	+ 4.7	42.3	15.2	3.2
GLCA 750	*Mammuthus* sp.	Wire Grass	Bone	− 19.2	+ 7.8	35.1	11.6	3.5
GLCA 3149	*Mammuthus* sp.	Disappearing Alcove	Bone	Insufficient collagen for analysis				
GLCA 12513	*Mammuthus* sp.	Mammoth Alcove	Bone (phalange)	Insufficient collagen for analysis				

Vancouver, British Columbia. Cactus needles were collected from plants (one alive, one dead) grown indoors in Vancouver, BC. All samples (2 mg) were mixed with KBr (200 mg) and compressed into 12-mm pellets. Absorbance spectra between 400 and 4000 cm^{-1} were obtained using a Bruker Vector 22 FTIR spectrometer, scanning 16 times with a resolution of 4 cm^{-1}. A baseline correction was applied to the region between about 3800 and 400 cm^{-1}. All spectra were normalized to the amide II peak (~ 1610 to 1650 cm^{-1}), as suggested by Baker *et al.* (2014).

Results

Carbon and nitrogen isotope values, C and N contents, and atomic C/N ratios for ancient uncharred Escalante River Basin plants are presented in Table 1 and Fig. 2. For ancient C_3 plants, the mean ($\pm 1\sigma$) $\delta^{13}C$ was $-25.5 \pm 2.0‰$ (range = -28.8 to $-20.6‰$, $n = 81$) and the mean $\delta^{15}N$ was $+4.7 \pm 5.5‰$ (range = -12.3 to $+13.6‰$). For ancient CAM plants (*Opuntia* sp.), the mean $\delta^{13}C$ was $-10.3 \pm 0.5‰$ (range = -10.8 to $-9.6‰$, $n = 9$) and the mean $\delta^{15}N$ was $+11.7 \pm 4.3‰$ (range = $+8.9$ to $+22.6‰$). There was a statistically significant positive correlation between $\delta^{13}C$ and $\delta^{15}N$ for the entire ancient plant sample set (Pearson's $r = 0.54$, $p < 0.001$, $df = 88$) and for C_3 plants alone

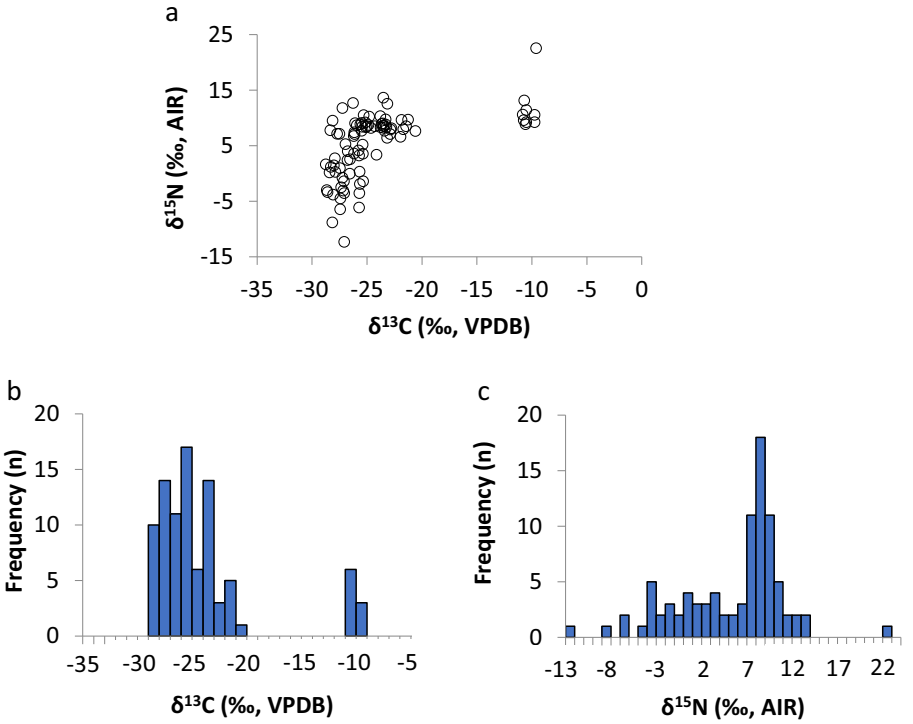

Fig. 2 Carbon and nitrogen isotope values of ancient plants from the Escalante River Basin. **a** Bivariate plot of carbon and nitrogen isotope compositions. **b** Frequency of measured $\delta^{13}C$ values. **c** Frequency of measured $\delta^{15}N$ values

($r = 0.58$, $p < 0.001$, $df = 79$). There was no significant correlation between $\delta^{13}C$ and $\delta^{15}N$ for ancient CAM plants alone.

For ancient C_3 plants (excluding conifers), $\delta^{13}C$ values of leaves ($-27.0 \pm 1.1‰$, $n = 13$) were lower than those of other plant parts ($-25.3 \pm 2.1‰$, $n = 63$), but the difference was not significant ($t = 1.5$, $df = 30$, $p = 0.07$). However, the $\delta^{13}C$ of leaves was significantly lower than that of seeds, both for the ancient plant sample set as a whole ($t = 4.0$, $df = 38$, $p < 0.001$) and for oak alone ($t = 2.0$, $df = 21$, $p < 0.05$) (Table 3). Comparing the seeds of different taxa, oak specimens had $\delta^{13}C$ values that were 2.5‰ lower than those of maple ($t = 3.9$, $df = 25$, $p < 0.001$) (Table 3). There was no significant difference between the $\delta^{15}N$ of plant tissues that act as nitrogen sources (leaf/stem/bark) versus those that act as sinks (fruit/seed/flower) for the whole sample ($+3.2 \pm 5.6‰$ vs. $+5.5 \pm 5.1‰$), oak alone ($-0.9 \pm 8.0‰$ vs. $+3.7 \pm 6.1‰$), or rose alone ($+2.9 \pm 1.7‰$ vs. $+4.8 \pm 5.3‰$).

Early Holocene and Terminal Early Holocene C_3 plants had significantly lower $\delta^{13}C$ ($F_{2,75} = 18.9$, $p < 0.001$) and $\delta^{15}N$ values ($F_{2,75} = 28.7$, $p < 0.001$) than Late Pleistocene C_3 plants (Table 4, Fig. 3). With Gambel oak removed (because the results above indicated that oak had lower $\delta^{13}C$ values), there was no longer a temporal difference between the Late Pleistocene and Early Holocene for $\delta^{13}C$ ($t = 1.3$, $df = 5$, $p = 0.2$), but the significant difference for $\delta^{15}N$ remained ($t = 4.0$, $df = 5$, $p < 0.001$).

The ancient Escalante River Basin plants had %C of $32.8 \pm 3.8\%$, %N of $1.9 \pm 1.0\%$, and atomic C/N of 26.6 ± 15.9. C_3 and CAM plants did not have significantly different %C, %N, or C/N values (Table 4). For the whole ancient plant sample set, $\delta^{15}N$ was positively correlated with %N ($r = 0.42$, $p < 0.001$), negatively correlated with C/N ($r = -0.52$, $p < 0.001$), and not correlated with %C ($p > 0.05$). For ancient C_3 plants alone, $\delta^{15}N$ was positively correlated with %N ($r = 0.52$, $p < 0.001$), and negatively correlated with C/N ($r = -0.62$, $p < 0.001$) and %C ($r = -0.25$, $p < 0.05$). For ancient CAM plants alone, there were no significant correlations between $\delta^{15}N$ and %N, C/N, or %C ($p > 0.05$).

For the whole ancient plant sample set, there were no correlations between $\delta^{13}C$ and %C, %N, or C/N ($p > 0.05$). For ancient C_3 plants alone, $\delta^{13}C$ was positively correlated with %N ($r = 0.49$, $p < 0.001$) and negatively correlated with %C ($r = -0.43$, $p < 0.001$) and C/N ($r = -0.55$, $p < 0.001$). For ancient CAM plants alone, there were no correlations between $\delta^{13}C$ and %C, %N, or C/N ($p > 0.05$).

FTIR spectra of modern and ancient plant samples were broadly similar, with prominent peaks representing amides, CH_2, OH, and C–O, which are characteristic of plant remains (Fig. 4) (Baker *et al.* 2014; Styring *et al.* 2013). CH_2 and amide I peaks

Table 3 Plant part and species comparisons for carbon isotope values obtained from ancient Escalante River Basin samples

	All taxa (all sites)		Oak (all sites)		Maple (all sites)	
	n	$\delta^{13}C$ (‰)	*n*	$\delta^{13}C$ (‰)	*n*	$\delta^{13}C$ (‰)
Leaf	13	-27.0 ± 1.1	7	-27.6 ± 0.9		
Seed	35	-25.1 ± 2.0	16	-26.4 ± 1.5	14	-23.9 ± 1.9
Leaf–seed		-1.9		-1.2		

Table 4 Temporal shifts in the carbon and nitrogen isotope compositions of Escalante River Basin C_3 plants

	All taxa			Gambel oak excluded		
	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Terminal Early Holocene	7	−28.1 ± 0.7	−2.2 ± 2.5	0		
Early Holocene	17	−26.7 ± 1.3	+0.1 ± 6.6	5	−25.8 ± 1.5	+0.2 ± 4.5
Late Pleistocene	24	−24.7 ± 1.9	+7.2 ± 3.4	48	−24.6 ± 1.9	+6.8 ± 3.4

were less pronounced in some ancient plant samples, but loss or diminishment of these peaks was not necessarily greater in samples with higher $\delta^{15}N$ values (Fig. 4). There was no evidence for contamination with carbonates (870 and 720 cm^{-1}), nitrates (3300, 1450, and 1985 cm^{-1}), or humic salts (1080 and 1010 cm^{-1}) in any of the samples, though we note that the detection limits for these compounds are 5–10% of the total sample weight (Vaiglova *et al.* 2014b).

Ancient herbivore bone and tooth collagen samples had acceptable carbon and nitrogen contents (35–42% and 10–15%, respectively) and C/N ratios (3.2 to 3.6). The mammoth, mountain goat, and shrub-ox had similar $\delta^{13}C$ values (−19.2, −19.4, and −18.8‰, respectively). The mammoth had a higher $\delta^{15}N$ value (+7.8‰) than the mountain goat or shrub-ox (+4.9 and +4.7‰, respectively).

Discussion

FTIR

The reduction or loss of peaks attributable to CH_2 and C–O groups in ancient plant samples, along with their lower %C relative to modern plants, indicates some loss of organic carbon. Styring *et al.* (2013) attributed reduced C–O peaks in charred cereal grains to conversion of starch into melanoidins during Maillard reactions. It is possible a similar process occurred in the ancient uncharred plant samples from the Escalante River Basin, albeit at lower temperatures (which, theoretically, might cause larger isotopic

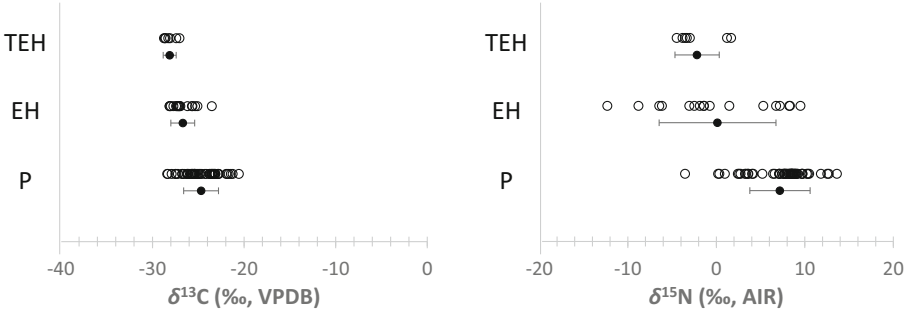

Fig. 3 Temporal changes in the $\delta^{13}C$ and $\delta^{15}N$ of ancient Escalante River Basin C_3 plants, from the Pleistocene (P, > 11,000 ^{14}C yr. BP) to the Early Holocene (EH, 11,000–8000 ^{14}C yr. BP) and Terminal Early Holocene (TEH, 8000–7000 ^{14}C yr. BP). Open circles are results from individual plant samples. Closed circles are means and error bars represent one standard deviation

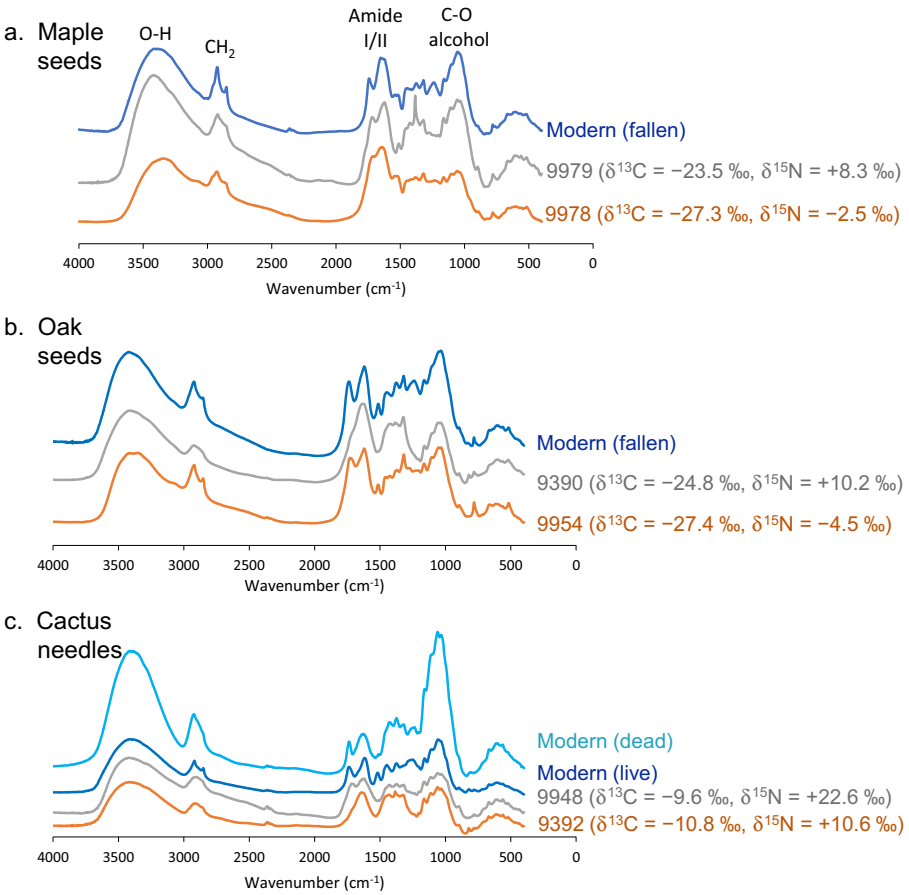

Fig. 4 Baseline-corrected and normalized FTIR spectra for modern and ancient **a** maple seeds, **b** oak seeds, and **c** cactus needles. GLCA catalog numbers and carbon and nitrogen isotope compositions for the ancient specimens are indicated

fractionations). However, as discussed above, mass losses or changes in biochemical composition do not necessarily indicate that isotopic alteration has occurred since isotopic fractionation does not always result from changes in %C, %N, or mass loss. If the high $\delta^{15}N$ values observed in the ancient uncharred plants resulted from diagenetic alteration, we might expect the greatest changes in the FTIR spectra to be in the amide peaks of samples with the highest $\delta^{15}N$ values, but this was not the case. A controlled study of the spectroscopic and isotopic effects of plant decomposition would be useful in further exploring the use of FTIR to identify isotopically altered plant samples.

Have the Isotopic Compositions of the Ancient Plants Been Altered?

Carbon Isotope Compositions

There is no strong evidence that the original plant $\delta^{13}C$ values have been significantly altered. The 15.2‰ separation between C_3 and CAM plants and the range of $\delta^{13}C$ values

for each photosynthetic category (Fig. 2) is similar to what is seen in modern plants. The ERB leaves had lower $\delta^{13}C$ values than other plant parts (*e.g.*, seeds), a phenomenon that has been reported in numerous previous studies of modern plants (Cerling *et al.* 2004; Cernusak *et al.* 2009; Codron *et al.* 2005; Hobbie and Werner 2004; Leavitt and Long 1982; Szpak *et al.* 2013). The lower $\delta^{13}C$ values of oak seeds compared to maple is consistent with the former species occupying wetter areas, and/or relying on winter precipitation and deeper water reserves (Phillips and Ehleringer 1995; Williams and Ehleringer 2000; Withers and Mead 1993). Finally, the mean $\delta^{13}C$ of C_3 plants matches the expectation for local herbivores based on their bone collagen $\delta^{13}C$ values. Large herbivores have a collagen-diet offset of about 5‰ (Drucker *et al.* 2008), so their mean collagen $\delta^{13}C$ (− 19.1‰) suggests a diet $\delta^{13}C$ of − 24.1‰, which is close to the mean $\delta^{13}C$ of Late Pleistocene C_3 plants measured in this study (− 24.7‰). It is interesting to note that despite their different dietary preferences (mammoths are grazers, shrub-ox are browsers, and mountain goats are mixed feeders) all three consumed a C_3-dominated diet, which suggests that C_4 grasses and shrubs were rare or absent in this environment.

When the mean $\delta^{13}C$ for C_3 plants (− 25.5‰) is converted to a "modern equivalent" by subtracting 1.5‰ to account for the depletion of ^{13}C in modern atmospheric CO_2 due to fossil fuel combustion (*i.e.*, the "Suess Effect") (Bocherens *et al.* 2014; Long *et al.* 2005), the result (− 27.0‰) is identical to the modern global mean C_3 plant value (excluding closed-canopy environments) (Kohn 2010). Likewise, the "modern equivalent" for the mean CAM plant $\delta^{13}C$ (− 11.8‰) is consistent with $\delta^{13}C$ values of modern obligate CAM plants (Fleming *et al.* 1993; Sternberg *et al.* 1984; Winter and Holtum 2002) and is within the range of values reported for modern *Opuntia* cacti (Sayed 2001). The ancient C_3 plant $\delta^{13}C$ values are lower than we might expect for an arid environment, but consistent with Withers and Mead's (1993) conclusion that the ancient plants grew in relatively wetter environments than are present at the alcoves today. Flanagan *et al.* (1997) reported shrub and tree $\delta^{13}C$ values from modern Glen Canyon environments that are useful for comparison: -26.7 ± 1.2‰ in the riparian zone, a combined mean of -27.7 ± 1.3‰ in five hanging gardens (plants growing around water seeps in canyon walls), and -25.4 ± 1.6‰ in the ephemeral wash (a nearby arid zone). The $\delta^{13}C$ of the C_3 plants presented in the present study (once corrected for the Suess Effect) are consistent with growth in a riparian environment and with the inference of a higher water table in the Escalante River Basin during the terminal Pleistocene–Early Holocene. The $\delta^{13}C$ values of the CAM plants are consistent with photosynthesis in an environment with hot, dry days and cool nights. Altogether, these results suggest that no large-magnitude diagenetic shifts in ancient plant $\delta^{13}C$ values have occurred.

Nitrogen Isotope Compositions

The comparison between ancient and modern plant isotopic compositions is more complicated for nitrogen than it is for carbon. Nitrogen isotope compositions of plants vary dramatically with environmental differences, and many/most modern plant studies obtained samples from diverse microhabitats. On the other hand, there were highly variable microhabitats in the vicinity of the Escalante River Basin alcoves from which the ancient plants were recovered (Withers and Mead 1993). Furthermore, the ancient plant samples were deposited over a period of more than 4000 years, which included

major climate and ecosystem changes that may have had substantial impacts on the local nitrogen cycle, including (but not limited to) the extinction of megaherbivores, carnivores, and huge predatory birds that would have added nitrogen to soil and promoted N losses through deposition of urine, feces, and decomposing flesh. As a result, it would be difficult—if not impossible—to collect modern plant samples that could be used as reasonable analogues to the Escalante River Basin samples. In this paper, we compare our ancient plant $\delta^{15}N$ values with previously published modern values, recognizing the limitations of this comparison.

The Escalante River Basin ancient plant $\delta^{15}N$ values are within the ranges previously reported for modern plants from individual regions as diverse as southern Yukon and east Africa (Cerling *et al*. 2009; Koch *et al*. 1991; McLauchlan *et al*. 2010; Szpak *et al*. 2013; Tahmasebi *et al*. 2017). For example, within a limited region of southern Yukon, Tahmasebi *et al*. (2017) reported modern plant $\delta^{15}N$ values ranging from about − 10 to + 27‰. The range of ancient C_3 plant $\delta^{15}N$ values in the present study (− 12 to + 14‰) is smaller that this modern range, despite the long temporal span and the diverse environments within the Escalante River Basin. Furthermore, Tahmasebi *et al*. (2017) showed that modern plants can have intra-plant differences (*e.g.*, leaf vs. stem) of up to 12‰, so large ranges might be expected for various plant parts from multiple species over a long period of environmental change. Arid environments such as the Escalante River Basin have highly variable nitrogen dynamics and tend to have high rates of gaseous N losses, which are associated with large fractionations that can enrich soils and plants in ^{15}N (Evans and Ehleringer 1994; Schaeffer and Evans 2005). In and of itself, the range of plant $\delta^{15}N$ values measured for the ERB samples does not indicate post-depositional alteration.

Ancient CAM plants had significantly higher $\delta^{15}N$ values than ancient C_3 plants, even when the high-^{15}N CAM outlier was excluded ($t = 7.1$, $df = 36$, $p < 0001$). Several studies have reported higher $\delta^{15}N$ in modern CAM versus C_3 plants in the same regions (Codron *et al*. 2005, 2006; Koch *et al*. 1991; Muzuka 1999), though the magnitude of the difference in these studies (*e.g.*, + 13.2 vs. + 9.8‰ in Amboseli National Park, + 7.7 vs. + 3.4‰ in Waterberg National Park) is smaller than that observed for the ancient plants in the present study (+ 11.7 vs. + 4.7‰). Nevertheless, the higher values in ancient succulents could reflect original plant values.

The mean $\delta^{15}N$ values for ancient Escalante River Basin plants were relatively high (+ 4.7‰ for C_3 and + 11.7‰ for CAM) compared to modern leaf $\delta^{15}N$ values of semi-arid woodland plants in south-central Utah, which averaged + 0.9 to + 2.7‰ (Evans and Ehleringer 1994), or to foliar $\delta^{15}N$ values of hanging-garden plants (relatively wet environments) in modern Glen Canyon, which averaged − 2.3‰ (Flanagan *et al*. 1997). However, when the C_3 plants are separated by time period, we see that the high $\delta^{15}N$ values predominantly occurred during the Late Pleistocene (mean = + 7.2‰) (Table 4 and Fig. 3). The largest range for $\delta^{15}N$ occurs in the Early Holocene—perhaps because of the "transitional" nature of this period—and only low $\delta^{15}N$ values occur in the Terminal Early Holocene (Fig. 3). Furthermore, the Early Holocene and Terminal Early Holocene mean $\delta^{15}N$ values (+ 0.1 and − 2.2‰, respectively) are comparable to those of modern plants in south-central Utah (Evans and Ehleringer 1994; Flanagan *et al*. 1997). It seems that either the Late Pleistocene plant $\delta^{15}N$ values systematically increased after deposition whereas the Early Holocene values did not, or that neither were significantly altered and the Late Pleistocene to Holocene shift in plant $\delta^{15}N$ reflects an underlying change in the ecosystems themselves.

Major changes in nitrogen cycling occurred at the end of the Pleistocene. A global study of 86 lakes on 6 continents recently showed a significant decrease (~2‰ on average) in lake-sediment $\delta^{15}N$ values between about 15,000 and 7000 cal yr. BP (McLauchlan *et al.* 2013). The authors suggested that the decrease represented a major change in the global nitrogen cycle associated with increased terrestrial carbon sequestration, which consequently decreased nitrogen availability relative to biotic demand, leading to less open nitrogen cycles and reduced fractionation during processes associated with N loss. In a recent synthesis paper, Rabanus-Wallace *et al.* (2017) similarly showed a large decrease in megaherbivore $\delta^{15}N$ in western North America at the end of the Pleistocene, followed by an increase during the Holocene. Although they interpreted these $\delta^{15}N$ variations as resulting from moisture changes, the megafaunal extinctions themselves likely had significant effects on biogeochemical cycling, though these effects are not fully understood (Doughty *et al.* 2016; Gill 2014). Megaherbivores exert a disproportionate influence on their ecosystem relative to the same biomass of smaller herbivores (du Toit and Owen-Smith 1989; Owen-Smith 1988). Like modern bison, Pleistocene megaherbivores would have promoted nitrogen cycling and increased spatial heterogeneity in nitrogen availability through activities such as selective grazing/browsing, depositing large quantities of urine and feces, and returning nutrients to soils and plants through carcass decomposition (Doughty *et al.* 2013; Knapp *et al.* 1999), which may have led to larger nitrogen isotope fractionations and hence higher $\delta^{15}N$ values during the Pleistocene. The loss of megafauna in the Late Pleistocene may also have allowed for the proliferation of cryptobiotic crusts, layers of cyanobacteria, lichens, and mosses that fix nitrogen (Evans and Ehleringer 1993), leading to lower soil and plant $\delta^{15}N$ values in arid regions throughout the Holocene.

The results above suggest that the ancient plant $\delta^{15}N$ values need not be rejected as altered simply because they seem relatively high. However, there is one line of isotopic evidence that does call these results into question: the plant $\delta^{15}N$ values are higher than what would be expected based on our (small) sample of Pleistocene herbivore collagen $\delta^{15}N$. Given a collagen-diet offset of about 3‰ for these mammals (Caut *et al.* 2009), we would expect our three Glen Canyon Pleistocene herbivores to have been consuming plants with mean $\delta^{15}N$ values of about + 2 to + 5‰. Since no ancient grasses were analyzed in this study, the most reasonable expected mean plant estimate might be derived from the browser (shrub-ox), whose $\delta^{15}N$ value produces the lower plant $\delta^{15}N$ estimate of about + 2‰. This is considerably lower than the measured mean plant $\delta^{15}N$ for the Pleistocene samples (+ 7.2‰), and therefore suggests the possibility that the Late Pleistocene plant $\delta^{15}N$ values may have systematically increased after deposition. On the other hand, this line of reasoning has critical limitations: the small sample size of herbivore bone collagen, the assumption that the ancient plant remains analyzed in this study are representative of the plants that comprised the shrub-ox diet, and the assumed 3‰ diet-tissue fractionation—which in reality can be quite variable (Caut *et al.* 2009).

Why Did DeNiro and Hastorf's (1985) Peruvian Plants Have Such High $\delta^{15}N$ Values?

One thing is clear: the results for the ancient Escalante River Basin plants differ from those of DeNiro and Hastorf (1985), who found incredibly high $\delta^{15}N$ values (many between + 20 and + 46‰) for uncharred plants from coastal regions of Peru. The results

of the present study suggest that these high values cannot necessarily be considered representative of uncharred plants from other contexts. One possible explanation for DeNiro and Hastorf's results is that their uncharred archaeological plants were fertilized with seabird guano, which can produce extremely high plant $\delta^{15}N$ values (Szpak *et al.* 2012b). Indeed, recent studies have suggested that guano fertilization contributed to high $\delta^{15}N$ values in ancient human bone collagen recovered from the Atacama Desert (Santana-Sagredo *et al.* 2015, 2017). It is notable that DeNiro and Hastorf's uncharred plants were obtained from Peruvian coastal desert environments whereas their charred plants were found at wetter inland sites—very different depositional contexts with potentially different ancient land management practices. Guano was likely highly accessible at some of these coastal sites, and its impact on the samples studied by DeNiro and Hastorf remains to be further explored.

Conclusions

Ancient plants from the Escalante River Basin had $\delta^{13}C$ values that were consistent with good preservation based on several lines of evidence: separation between plants utilizing different photosynthetic pathways (C_3 vs. CAM), differences among plant parts (higher values in leaves vs. seeds), and species environmental preferences (oak vs. maple). Furthermore, the $\delta^{13}C$ values of the ancient plants were consistent with expected diet values estimated from local Pleistocene herbivore bone and tooth collagen. This evidence suggests that ancient uncharred plant $\delta^{13}C$ values did not undergo significant post-depositional alteration.

The evidence for preservation of original plant $\delta^{15}N$ values is equivocal. Ancient plant $\delta^{15}N$ values could not be rejected as altered based on their range relative to modern plants. CAM plants had higher $\delta^{15}N$ values than C_3 plants, as expected based on modern studies. Temporal changes in plant $\delta^{15}N$ were in a similar direction to—though of a larger magnitude than—global changes in sedimentary $\delta^{15}N$ related to shifts in the nitrogen cycle. In the Escalante River Basin, the mean $\delta^{15}N$ values of Early Holocene plants were similar to those of modern plants in south-central Utah, whereas the Late Pleistocene plant values were much higher. Thus, it seems that either Late Pleistocene plants experienced systematic isotopic alteration while Early Holocene plants did not, or neither were significantly altered and the temporal change reflects an underlying change in the ecosystem itself. Late Pleistocene plant $\delta^{15}N$ values were higher than expected based on Pleistocene herbivore collagen $\delta^{15}N$, which suggests the possibility that the plants experienced decay-related ^{15}N enrichment. However, this line of reasoning relies on a small sample size and several critical assumptions that remain uncertain.

In contrast to DeNiro and Hastorf (1985), we did not find exceptionally high $\delta^{15}N$ values ($> +25‰$) in our ancient uncharred plants. The possibility that the coastal Peruvian plants had such high $\delta^{15}N$ values because of fertilization with seabird guano should be further explored. The role of megaherbivores on nutrient cycling and variations in soil, plant, and animal $\delta^{15}N$—particularly in arid regions—also requires further investigation.

Plant $\delta^{15}N$ values are important tools for understanding nitrogen fluxes in modern ecosystems, which can help predict the effects of anthropogenic climate change (*e.g.*,

Liu *et al*. 2017; Robinson 2001). Aside from their use in paleodietary research, ancient plant isotopic compositions could provide an important long-term record of nitrogen fluxes, if we can work out the taphonomic processes associated with their deposition and alteration (or lack thereof). %C, %N, and C/N ratios hold little promise for evaluating isotopic alterations of ancient plants, but the use of FTIR or other structural characterization techniques to identify isotopically altered samples and to characterize decay processes should be assessed in larger and more systematic studies. This study suggests that archaeologists and paleontologists should carefully consider the use of ancient uncharred plants for paleoenvironmental and paleodietary research.

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