1 **A multi-century $\delta^{13}\text{C}$ record of the C₄ grass *Setaria macrostachya* in the US Southwest:**

2 **Identifying environmental causes of variability**

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Abstract Annual and 5-year averages of $\delta^{13}\text{C}$ measured on florets of the C_4 grass *Setaria macrostachya* decreased from -10 to -13 ‰ between 1849 and 2016. Atmosphere-plant $\delta^{13}\text{C}$ discrimination (Δ) changed from 3-4 early in this interval, to 4-5 since 1970. These century-scale changes result from decrease in atmospheric $\delta^{13}\text{C}$ and, prior to 1970, from changes in Δ related to increase in the partial pressure of CO_2 in the atmosphere, both largely caused by the burning of fossil fuels. Short-term fluctuations (interannual to decadal) in slope of the time series of averaged $\delta^{13}\text{C}$ do not result from natural causes, but in certain cases appear to coincide with human economic activities. Annual $\delta^{13}\text{C}$ ranges in years with sufficient measurements are consistently about 2 ‰. It is unlikely that useful information on past atmospheric $\delta^{13}\text{C}$ will be obtained from *S. macrostachya* seeds preserved in packrat middens or other archives.

Keywords: carbon isotopes; time series; discrimination; atmospheric evolution; partial pressure

1. Introduction

Archives of stable carbon isotope data exist in datable plant material. The use of $\delta^{13}\text{C}$ data from sequential growth rings of trees in determining water availability in past climate regimes is well established (Cernusak et al., 2013 and references therein). Almost all tree taxa use the C_3 photosynthetic pathway, in which water availability modulates isotope fractionating processes, leading to isotope data archives that can be interpreted in terms of environmental change under conditions of constant, or near-constant $\delta^{13}\text{C}$, in ambient air (see section 3.2). Much research has also been undertaken to evaluate the use of $\delta^{13}\text{C}$ archives in fossil organic

material produced by C_3 plants for interpreting $\delta^{13}C$ and CO_2 content of past atmospheres, potentially over hundreds of millions of years (Porter et al., 2017, and references therein).

Isotope effects arising from the C_4 photosynthetic pathway were originally considered to be independent of environmental factors such as water availability and ambient concentration of CO_2 (e.g. O'Leary, 1988). Therefore C_4 plant material appeared more likely than C_3 material to preserve evidence of change of $\delta^{13}C$ in past atmospheres, but only in archives of Miocene or younger age, when abundant C_4 plant matter was preserved (Sage, 2003). This advantage was offset by the difficulty of finding archives of material suitable for study. With rare exceptions, C_4 taxa are small in stature (grasses, forbs and shrubs), lacking the growth rings of trees, and in general constitute poor material for long-term preservation. Available dated archives in herbarium and seed collections extend over at most 160 years. Packrat middens, constructed by *Neotoma* sp. (Betancourt et al., 1990) promised a greatly extended archive that could be radiocarbon-dated. Our previous work (Toolin and Eastoe, 1993) focused on fertile florets of the C_4 grass species *Setaria macrostachya* preserved in packrat middens from the American Southwest (i.e. southern Arizona and New Mexico, southwest Texas and the northern Mexican states of Sonora and Chihuahua); other florets were gathered from herbarium specimens and living plants. That material covered changes in atmospheric $\delta^{13}C$ over the last 12 Ka. While 20th-century specimens demonstrated the changes in $\delta^{13}C$ due to burning of fossil fuels, the results for older dated samples were disappointing in showing a broad range but no discernible trend. Our study indicated the need for a more detailed examination of the behavior of $\delta^{13}C$ in *Setaria* florets of recent age.

Subsequent work on C₄ photosynthesis (see section 3.2) has revealed (1) that environmental factors in addition to $\delta^{13}\text{C}$ of ambient air lead to small but consistent isotope effects, at least in certain C₄ genera; (2) that subtypes of C₄ photosynthesis exist, and that isotope effects in the NADP-ME subtype are the least affected by such factors. Furthermore, the intrinsic range of $\delta^{13}\text{C}$ in a single species sampled over a broad area can be large relative to likely changes in $\delta^{13}\text{C}$ of air (Pedecino et al., 2002).

The genera *Setaria*, *Zea*, *Saccharum* and *Sorghum* share the NADP-ME photosynthetic mechanism (Brutnell et al., 2010). All four genera include species important to agriculture, and have therefore been studied intensively under controlled conditions. Consequently, a substantial body of work is available to aid in the interpretation of the new data that we present for *Setaria* in this article. Our aims in this research were (1) to gather a dataset spanning as much as possible of the 19th to 21st centuries, with sufficient measurements to allow for statistical treatment of short time intervals, 1-5 years; (2) to evaluate any isotope effects against the changing $\delta^{13}\text{C}$ of the atmosphere, and other environmental changes; and (3) to re-evaluate the usefulness of such data in paleoenvironmental studies.

2. Background

C₄ grasses are distributed widely, and dominate the grass flora in many areas, particularly in regions with hot, wet summers (Hattersley, 1983; Collatz et al., 1998). In the American Southwest, C₄ grasses are adapted to arid climates with high minimum temperature during the growing season, and to low levels of shade (Teeri and Stowe, 1976; Ehleringer et al., 1997). Collatz et al. (1998) determined that C₄ grasses are favored by warm, humid climates in

which the normal temperature does not fall below 22°C during the warmest month, at a partial pressure of CO₂ (p_{CO2}) of 35 Pa (the prevailing atmospheric value at the time of their research), and by precipitation during the growing (warm) season. The threshold temperature depends on p_{CO2}, which is increasing owing to burning of fossil fuels. Higher p_{CO2} might favor a contraction of the range of C₄ grasses.

In southern Arizona, 82% of grass species are of C₄ type (Teeri and Stowe, 1976). Our research focuses on the C₄ grass *Setaria macrostachya*, an important element of the present grass flora in the American southwest (Toolin and Reeder, 2000) and also in past floras, represented by fossils in packrat middens (Toolin and Eastoe, 1993). The northern limits of distribution of *S. macrostachya* are shown in Figure 1. In the American southwestern region, perennial C₄ grasses are dormant during the winter, and respond to summer rainfall to initiate growth and reproduction. In the warm growing season, the region around Tucson, Arizona receives about 66% of annual precipitation from the North American monsoon (National Oceanic and Atmospheric Administration, 2016).

3. Previous work

3.1 Photosynthesis and carbon isotope discrimination.

Plant tissues have stable carbon isotope ratios (¹³C/¹²C) lower than those of CO₂ in ambient air. The isotope discrimination between a plant and air is

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 + \delta^{13}C_p} \quad (1)$$

where $\delta^{13}\text{C}_a$ denotes the isotope composition of atmospheric CO_2 ($\delta^{13}\text{C}_a$ in the following text) and $\delta^{13}\text{C}_p$ denotes the isotope composition of plant material ($\delta^{13}\text{C}_p$). Δ has the advantage of being independent of varying $\delta^{13}\text{C}_a$ and is useful in plant physiology studies in which $\delta^{13}\text{C}_a$ may vary as a result of plant growth. Researchers in paleoclimate applications of plant isotope compositions favor data expressed as $\delta^{13}\text{C}$, which has the advantage of being directly measured on combustion products of plant matter, and of requiring no independent knowledge of $\delta^{13}\text{C}$ of the ancient atmosphere.

Cernusak et al. (2013), O'Leary (1988) and Farquhar et al. (1989; 1983) have reviewed the factors that control isotope discrimination due to photosynthesis; this summary is drawn from their work. Discrimination arises from multiple fractionating processes, including kinetic processes (e.g. diffusion of CO_2 through leaf stomata, through boundary air, and through liquid water) and equilibrium processes (e.g. enzymatic fixation of CO_2 and HCO_3^- , and hydration or dissolution of CO_2). Photosynthetic discrimination in C_3 and C_4 plants differs mainly because: (1) diffusion is the dominant (limiting) fractionating process in C_4 plants whereas enzymatic carboxylation is dominant in C_3 plants; (2) enzymatic fixation and decarboxylation lead to larger discrimination in C_3 plants than in C_4 plants; and (3) C_4 plants, but not C_3 plants, are subject to partial leakage of CO_2 and HCO_3^- from the bundle sheath cells in which Rubisco decarboxylation occurs, the leaked carbon being recycled through enzymatic fixation. Discrimination due to leakage depends on ϕ , the proportion of carbon lost to leakage (Henderson et al., 1992; 1998). Discrimination also differs among subtypes of C_4 plants distinguished by different carboxylating enzymes (Henderson et al., 1992). Summed overall operating processes, Δ in C_3 plants has large

values ($>15\text{‰}$ in Figure 1 of Farquhar et al., 1989), while values in C_4 plants are smaller ($<4\text{‰}$). Genetic selection may modify Δ (Cernusak et al., 2013).

3.2 Influence of environmental factors.

Photosynthetic discrimination may be modified by environmental factors such as light intensity, water availability, salinity, and atmospheric composition (Cernusak et al., 2013, and references therein). Other authors have documented modifications due to nutrient supply (e.g. Ranjith et al., 1995; Fravolini et al., 2002).

In C_3 plants, factors that govern p_i/p_a (the ratio of intercellular to ambient partial pressures of CO_2) notably water availability and atmospheric CO_2 levels, cause large changes in discrimination.). Note that in the text to follow, p_a will be used to denote ambient partial pressure of CO_2 (which may be an experimentally controlled value), and p_{CO_2} to denote the partial pressure in the earth's atmosphere. Diefendorf et al. (2010) documented a global relationship between discrimination in C_3 woody plants and mean annual precipitation, and Lomax et al (2012) demonstrated the dependence of discrimination on water availability in *Arabidopsis*. Variations in controlled atmospheric CO_2 levels, expressed as p_a or as p_{CO_2}/p_{O_2} , also control discrimination (Lomax et al. 2010; Schubert and Jahren, 2015; Porter et al., 2017). The precision of p_{CO_2} and $\delta^{13}C_a$ determined from C_3 organic matter archives is limited by discrimination differences among plant taxa and by the dependence of discrimination on p_{CO_2}/p_{O_2} (Porter et al., 2017). Nonetheless, Schubert and Jahren (2015), in a statistical treatment of a large, noisy dataset for plant material from the last 30,000 years, showed a 2‰ increase in discrimination related to increasing p_{CO_2} .

Because Δ variability in C_4 plants is attenuated and appeared not to be strongly related to water-use efficiency (e.g. O'Leary, 1988), early work suggested that the $\delta^{13}C$ values of preserved C_4 plant material might record past variations in $\delta^{13}C$ of atmospheric CO_2 . Subsequent work (summarized in Cernusak et al., 2013) has documented small dependences of Δ in C_4 plants on water availability and light intensity, the latter observed only at low light levels. Several species of C_4 grasses develop higher values of Δ when subjected to low availability of water and light, but the NADP-ME subtype of C_4 photosynthesis is less influenced by environmental change than other subtypes (Buchmann et al., 1996). Small changes in $\delta^{13}C_p$ of C_4 plants with altitude (Wang et al., 2008; Van de Water et al., 2003; Kelly et al., 1997) indicate a relationship between Δ and either temperature or p_{CO_2} . Gale (1972), however, suggested that effects of decreasing p_{CO_2} with increasing altitude should be small because they are offset by increased diffusivity of CO_2 at low temperatures. In controlled experiments, Fravolini et al. (2002) investigated factors controlling Δ of *Aristida glabrata*, belonging to the NADP-ME subtype of C_4 plants. Δ was higher under drought conditions than under wet conditions, the change being about 1 ‰ at 370 ppm ambient CO_2 , and less at 690 ppm. Under wet conditions, increasing p_a from 370 to 690 ppm resulted in an increase of about 1 ‰ in Δ . Ghannoum et al. (2002) found that drought caused an increase of about 0.5 ‰ in Δ for several NADP-ME species. In *Sorghum bicolor*, a NADP-ME C_4 grass, values of ϕ are typically 0.2 to 0.3 (Henderson et al., 1992), for which values Δ is expected to decrease with increasing p_i/p_a , and increase with decreasing water availability (Cernusak et al., 2013).

3.3 Past studies of $\delta^{13}C$ archives in C_4 plants.

Marino and McElroy (1991) studied archived kernels of *Zea mays* dating from 1948 to 1987, and found no change in Δ . Pedecino et al. (2002) measured $\delta^{13}\text{C}$ on herbarium samples of leaves from *Atriplex canescens* ($n = 29$) and *A. confertifolia* ($n = 35$) spanning the 1880s to 1990s. Δ for *A. canescens* changed from about 8 to 12 ‰ between the 1940s and the 1990s, but no change was detected for *A. confertifolia*. Kelome et al. (2006) reported that $\delta^{13}\text{C}_p$ of the C_4 grass *Eleusine indica* correlated with $\delta^{13}\text{C}_a$ of fossil-fuel polluted air in an urban CO_2 dome over the city of Cotonou, Benin. Toolin and Eastoe (1993) compared $\delta^{13}\text{C}_p$ of *Setaria* florets from herbaria (1940-1990) and packrat (*Neotoma* sp.) middens, the latter ^{14}C -dated and spanning a time interval of about 12 Ka to the present. While a decrease of about 1.5 ‰ was recorded between the 1940s and 1990s, no temporal trend was found for the midden samples, which had a $\delta^{13}\text{C}_p$ range of about 2.5 ‰.

3.4 Atmospheric evolution.

Because of the current high priority given to climate change research, much information on changes in $\delta^{13}\text{C}_a$ and p_{CO_2} is available. For this study, we cite records obtained by Rubino et al. (2013) at the Law Ice Dome, Antarctica, and by Dlugokencky et al. (2016) and White et al. (2015) for Mauna Loa, Hawaii (Fig. 2). Direct measurements of p_{CO_2} and $\delta^{13}\text{C}_a$ are available since 1970 and 1990, respectively, at Mauna Loa. The Law Ice Dome data are presented as 20-year running averages; averaging over multiple years is unavoidable because of diffusion of trapped air in firn. Small differences in $\delta^{13}\text{C}_a$ exist between the Mauna Loa record and shorter records from other sites (Oak Ridge National Laboratory, 2016). For example, data from Sinton, Texas (28.038°N, 97.509°W) are consistently a little lower ($0.10 \pm 0.04\text{‰}$) than those at Mauna Loa (Fig. 2). For the purposes of this study, it is assumed that the averages of p_{CO_2} and $\delta^{13}\text{C}_a$

from Mauna Loa and Law Ice Dome correspond to averages in the study area. Small differences such as those between Sinton and Mauna Loa make no difference to the determinations of isotope discrimination reported here.

4. Methods

The indurated fertile floral bracts (lemmas and paleas), which enclose the reproductive parts of flowers of *S. macrostachya* and form a seed-like structure (see photos in Toolin and Reeder, 2000) are the organs found preserved in packrat middens. These florets were used throughout our research in order to avoid any isotope variations in different plant tissues. Samples were dried at air temperature, treated with 1N HCl to ensure removal of any carbonate dust, then 1% NaOH solution to remove any alkali-soluble organic matter, and finally very dilute HCl prior to rinsing and drying.

Data are reported as

$$\delta^{13}\text{C}_p, \text{‰} = \left(\frac{R(x)}{R(s)} - 1 \right) \times 1000 \quad (2)$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$, x denotes a sample, and s denotes a standard, currently V-PDB, but PDB for earlier measurements.

Whole, processed florets were combusted for each measurement. Prior to 2005, CO_2 was prepared by reaction of 4 to 8 florets with CuO in evacuated Vycor® tubes, followed by cryogenic separation of CO_2 from H_2O in vacuum lines. Early measurements of $\delta^{13}\text{C}$ were made using dual-inlet gas-source mass spectrometers (VG Micromass 602C prior to 1990; Finnigan

MAT Delta S between 1990 and 2004), with standardization relative to NBS 19 (+1.95 ‰) and an analytical precision (1σ) of 0.15 ‰ or better. From 2005 to the present, we used a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL) coupled to an elemental analyzer (Costech). Two to four florets were combusted in the elemental analyzer. Standardization is based on NBS-22 (−30.031 ‰) and USGS-24 (−16.049 ‰), and analytical precision (1σ) is better than ± 0.10 ‰.

Samples from prior to 1990 were obtained from the University of Arizona (ARIZ), and U.S. National (US) herbaria, and span the area shown in Figure 1. Since 1990, we have collected samples from living plants in southeastern Arizona. Most of these are from the flanks of the Santa Catalina, Santa Rita, Sierrita and Rincon Mountains near Tucson, at altitudes ranging from 740 to 1285 m above sea level. We sampled mostly between July and November, taking up to three flower heads, each from a different plant, at each site. Samples were collected throughout the flowering season, the onset of which may vary by a month or more, depending on the timing of summer monsoon rains. In total, 610 samples were measured, including 339 (average 13/year) for the period of more intensive sampling between 1990 and 2016.

5. Results

Individual $\delta^{13}\text{C}$ measurements are presented in Supplementary Table 1, and in Figure 3. In Figures 4 A, B and C, the data are presented as annual and five-year running means. The following observations emerge.

1. Individual data for single years have a broad range of $\delta^{13}\text{C}_p$ (Fig. 3). Since 1996, when we began collecting at least 12 samples each year, the annual range of $\delta^{13}\text{C}_p$ is consistently

about 2 ‰. The year 2006 was exceptional, having a range of 3.6 ‰. Values of $\delta^{13}\text{C}_p$ tend to increase through the flowering season (for examples, see Table S1), consistent with the sense of the annual cyclic change in $\delta^{13}\text{C}_a$ at Mauna Loa (White et al., 2015). Minimum $\delta^{13}\text{C}_p$ values from around 1880 have been occasionally observed as maximum values since 2000.

2. Annual mean $\delta^{13}\text{C}_p$ has changed from about -10 ‰ in 1849-1880 to about -13 ‰ since 2010 (Figures 3 and 4A). A fitted fourth-order polynomial curve indicates general long-term change towards lower average $\delta^{13}\text{C}_p$ values, but change at decadal and shorter time scale has not been monotonic in detail. The difference between the two curves in Fig. 4A is the isotope discrimination Δ , which has increased since 1850.

3. Averaged data at annual time scale are clearly noisy (Fig. 4A). Filtering by application of five-year running means of $\delta^{13}\text{C}_p$ suggests the presence of a series of short-term (5-20 year) perturbations superimposed on the trend of decreasing $\delta^{13}\text{C}_p$ (Fig. 4 B). Abrupt (2-3 year) changes are considered significant if they result in non-overlapping ranges of mean ± 2 standard errors (2se). For example, increases of magnitude about 0.5 ‰, occurred around 1980 and 1993. The abrupt increase of 1 ‰ between 1897 and 1900 is based on fewer data, and has a small overlap in the ranges of mean $\pm 2\text{se}$ (standard errors); however, it is corroborated by a distinct change in slope of the $\delta^{13}\text{C}_p$ time series. Changes in slope and statistically-significant changes in mean $\delta^{13}\text{C}_p$ mark the well-defined perturbation beginning in 1927. Mean $\delta^{13}\text{C}_p$ increases by about 1 ‰, peaking in 1932, and subsequently declines by about 1 ‰.

4. Average discrimination (Δ) has increased from 3 - 4 ‰ between 1860 and 1880 to 4 - 5 ‰ since 1990 (Figs. 4A and 5). Values of Δ increase as p_{CO_2} increases from 290 to about 320 ppm, between 1860 and 1970 (Fig. 5). Since 1970, there is no evidence, considering the scatter of data, for systematic change in Δ with time or with p_{CO_2} . Mean Δ since 1990 has a value of 4.5 ± 0.3 ‰ (Fig. 5).

5. Data for *Zea mays* (Marino and McElroy, 1991), filtered by applying a five-year running mean, show a trend generally similar to that of *S. macrostachya*, but with values of Δ that are about 1 ‰ less (Fig. 4B). Small differences in the two curves are probably insignificant, because the dataset for *Zea mays* is relatively small ($n = 51$), leading to poorer statistics.

6. Discussion.

6.1 Hierarchy of $\delta^{13}\text{C}_p$ changes.

Our data show temporal variations of $\delta^{13}\text{C}_p$ differing in time-scale by two orders of magnitude. At century scale, we observe two phenomena: a 3 ‰ decrease in $\delta^{13}\text{C}_p$, and a 1-2 ‰ increase in Δ . At a time-scale of a few decades, we detect 0.5 to 1 ‰ fluctuations in $\delta^{13}\text{C}_p$, and changes in slope of the $\delta^{13}\text{C}_p$ curve. At a time-scale of a few years, we see suggestions of fluctuations of 0.5 to 1 ‰ (e.g., compare 1902 and 1903, 1975 and 1976, 2008 and 2009 in Figure 3), but with poorer statistical significance.

6.2 Time-scale of changes in atmospheric composition.

Measurements of annual average $\delta^{13}\text{C}_a$ since 1990 and p_{CO_2} since 1970 show monotonic, accelerating change. Fluctuations at annual scale are small, and close to measurement error. For this reason, the possibility that changes in *Setaria* $\delta^{13}\text{C}_p$ as large as 0.5 to 1 ‰ reflect changes in the atmosphere as a result of human activities (burning of forest or peat, reforestation), and of natural processes (oceanic upwelling associated with El Niño phases, volcanic eruptions) can be discounted for decadal or shorter time scales. Annual net fluxes of carbon to the atmosphere are small relative to the size of the atmospheric reservoir (Denman et al., 2007), and their fluctuations are therefore unable to bring about large, abrupt changes.

Ice-core records of $\delta^{13}\text{C}_a$ and p_{CO_2} also appear smooth (Fig. 2), but are subject to decadal-scale averaging (Rubino et al., 2013). For this reason, they cannot be interpreted as excluding measurable short-term changes to the atmosphere prior to 1970 (p_{CO_2}) or 1990 ($\delta^{13}\text{C}_a$). The argument arising from the size of natural carbon fluxes still applies, but it remains possible that a large human-caused flux before 1970 might have shifted $\delta^{13}\text{C}_a$ measurably. A case in point is the pioneer effect, a decrease of about 1 ‰ in $\delta^{13}\text{C}_a$ that occurred largely between 1870 and 1890, proposed by Wilson (1978).

Annual cyclic fluctuation in $\delta^{13}\text{C}_a$ in the northern hemisphere mid-latitudes has an amplitude of about 0.5 ‰, with highest values in early May and lowest values in late September to early October (White et al., 2015). A corresponding variation occurs in p_{CO_2} (Keeling, 1976). *S. macrostachya*, with growing season May-October, samples the entire range of atmospheric $\delta^{13}\text{C}_a$ each year. To the extent that $\delta^{13}\text{C}_p$ of *S. macrostachya* reflects $\delta^{13}\text{C}_a$, it should therefore reflect approximately the annual average of $\delta^{13}\text{C}_a$.

281 6.3 Origins of century-scale $\delta^{13}\text{C}_p$ change.

282 As expected in the context of earlier studies, long-term changes of $\delta^{13}\text{C}_p$ are mainly
 283 driven by change in $\delta^{13}\text{C}_a$. However, the discrimination Δ has also increased since the mid-19th
 284 century (Fig. 4B). During this period, the CO_2 concentration in the atmosphere has steadily
 285 increased from 280 to 400 ppm. Other environmental factors such as water availability and
 286 temperature vary greatly at annual to decadal time scales (see below), and do not match the
 287 uniform increase in Δ . The controlled-condition research of Fravolini et al. (2002) indicates that
 288 Δ can increase with increasing p_{CO_2} ; in their experiments, when water supply was adequate, a
 289 doubling of p_{CO_2} led to an increase of 1 ‰ in Δ . A similar dependence of Δ on p_{CO_2} is present in
 290 C_3 tree rings (McCarroll et al., 2009).

291 6.4 Short-term change: Water availability.

292 The study area is prone to multi-decadal cycles of drought (e.g. Griffin et al., 2013).
 293 Figure 4C shows extended periods of drier-than-average growing (warm) seasons. There is no
 294 clear relationship to the $\delta^{13}\text{C}_p$ record. At shorter time scale (a few years), the El Niño Southern
 295 Oscillation correlates, albeit imperfectly in the study area, with precipitation amount. There is
 296 no discernible relationship between ENSO and changes in $\delta^{13}\text{C}_p$ (Fig. 4B).

297 It is unlikely that a set of samples from a broad area – including the more limited area
 298 near Tucson in which we have sampled live plants since 1990 – can provide information about
 299 dependence of $\delta^{13}\text{C}_p$ on water availability for the following reasons. First, monsoon rains in
 300 semi-arid southwestern North America are variable at a scale of a few kilometers. It does not
 301 follow that regional above-average monsoon rainfall results in wet conditions in all areas.

Second, the extent to which water supply is adequate for individual small plants such as *S. macrostachya* may be governed by microtopography in addition to the local intensity of monsoon rains. Small, shallow depressions in the surface may favor germination and long-term survival of individuals. It would be necessary to sample a single cluster of plants over many years to evaluate the effects of water availability.

6.5 Short-term change: Volcanic eruptions.

While volcanic eruptions are incapable of producing sufficient CO_2 to shift $\delta^{13}\text{C}_a$ measurably (Denman et al., 2007), injections of dust particles into the stratosphere may lead to cooling of global climate for a few years after very large eruptions. Figure 4B shows the timing of such large eruptions (Self et al., 1987) plus the 1992 Pinatubo eruption. Each eruption appears to be followed by 3 to 14 years of decrease in $\delta^{13}\text{C}_p$. In several cases, the timing of an eruption coincides with the termination of an interval of positive slope in the averaged $\delta^{13}\text{C}_p$ time series. This indication is problematic for two reasons. First, a weak but significant relationship between $\delta^{13}\text{C}_p$ and summer temperatures (Fig. 6) suggests that climatic cooling should result in an increase in $\delta^{13}\text{C}_p$, rather than the decreases observed after the large eruptions. Second, intervals of negative slope are not anomalous in a record with a general negative slope; rather it is the initiation of intervals of positive slope that requires explanation.

6.6 Short-term change: Human-caused effects.

Wilson (1978) proposed the “pioneer effect,” a rapid decrease in $\delta^{13}\text{C}_a$ and consequent decrease in $\delta^{13}\text{C}_p$ of contemporaneous plant material. The proposed decrease in $\delta^{13}\text{C}_a$ would have resulted from the burning of forest-derived C_3 organic matter ($\delta^{13}\text{C} < -23\text{‰}$) as land was

cleared for agricultural expansion at several locations around the world between 1860 and 1890. Between 1875 and 1895, $\delta^{13}\text{C}_p$ in a bristlecone pine changed by 0.9 ‰ per decade (Wilson, 1978). In our record for *S. macrostachya*, the slope is comparable between 1880 and 1900, 1.0 ‰ per decade. For comparison, the average rate of change in our record between 1900 and 2015 is 0.2 ‰ per decade. The record for *S. macrostachya* is therefore consistent with the presence of a pioneer effect. We note that the proposed pioneer effect is absent in southern hemisphere ice records of $\delta^{13}\text{C}_a$ (McCarroll and Loader, 2004), and that in our region, the effect coincided with an extended period (Fig. 4C) of below-average warm-season rainfall (Griffin et al., 2013).

Slowing or reversal of the general increase in $\delta^{13}\text{C}_a$ may have occurred in response to economic slowdown if consumption of low- $\delta^{13}\text{C}$ fossil fuels declined sufficiently. Such effects are more likely to be observed at times of relatively low fossil fuel consumption, e.g. at the beginning of the 20th century rather than at the end. Figure 4B shows the duration of economic slumps considered by *The Economist* (2016) to be the most serious since 1880. Only those of longer duration probably should be considered in the context of changing atmospheric composition. Correlation with positive slope in the $\delta^{13}\text{C}_p$ trend is not evident. Considering this in a different way, the intervals of positive slope and possible correlated events are as follows: 1897-1900 (end of pioneer expansion); 1916-1918 (World War I); 1928-1932 (Great Depression); 1939-1945 (World War II); 1953 (a weak change, no known event); 1972-1981 (no event); 1990-1992 (no event); 1998-2000 (no event); 2007-2008 (Great Recession).

6.7 Variability of discrimination.

For florets sampled in a given year, the characteristic 2 ‰ range in $\delta^{13}\text{C}_p$ implies that Δ is variable at the seasonal time scale of carbon fixation in *S. macrostachya*. The range cannot be explained by cyclic seasonal variations in the atmosphere, e.g. 0.5 ‰ in $\delta^{13}\text{C}_a$, and 6-7 ppm in p_{CO_2} observed at Mauna Loa (White et al., 2015; Dlugokencky et al., 2016). Δ itself appears to vary, possibly among individual plants. Despite the short-term variability of Δ , annual averages of $\delta^{13}\text{C}_p$ and Δ show clear relationships to averaged atmospheric parameters $\delta^{13}\text{C}_a$ and p_{CO_2} (Figs. 4 and 5). The lack of systematic change in averages of Δ at $p_{\text{CO}_2} > 320$ ppm since about 1970 (Fig. 5) seems inconsistent with experimental data (see section 3.2). The reason for this observation remains unclear.

6.8 Summary.

There are no consistent explanations for the short-term changes of $\delta^{13}\text{C}_p$ in our record. The coincidence between large volcanic eruptions and the termination of intervals of positive slope in the $\delta^{13}\text{C}_p$ curve for *S. macrostachya* seems the strongest association, but is problematic for reasons explained above. Economic slumps and widespread war coincide better with inflections in the $\delta^{13}\text{C}_p$ curve than natural environmental fluctuations in the earlier half of our record. Is there any independent evidence for similar changes? Chronologies for the C_4 shrub genus *Atriplex* in Pedecino et al. (2002) lack sufficient detail for comparison. Comparison may also be made with C_3 plants, bearing in mind their greater sensitivity to environmental factors. Neither the pioneer effect event nor the 1930 peak appears in C_3 tree ring chronologies constructed from 5-year samples from the southwestern USA (Leavitt and Long, 1988). A general decline in $\delta^{13}\text{C}_p$ through the 1890s is present in C_3 tree-rings from Scandinavia (Gagen

et al., 2008) but not in C_3 tree-rings from Scotland (Loader et al., 2008), and neither set of data contains an event like the 1930 peak reported here.

6.9 Implications for paleoenvironmental studies.

Despite our efforts to minimize isotope variability by sampling tissue of a single kind, we have found that an annual $\delta^{13}C_p$ range of 2 ‰ or more is characteristic of *S. macrostachya* florets. This range is comparable to the entire range (about 2.4 ‰) of $\delta^{13}C_p$ reported for pre-industrial Holocene specimens of *S. macrostachya* by Toolin and Eastoe (1993), and also comparable to likely changes in $\delta^{13}C_a$ (see Fig. 2: the change in $\delta^{13}C_a$ due to burning of fossil fuels since 1840 is about 2 ‰). The 2 ‰ range is at least twice as large as any demonstrated shift in Δ resulting from experimentally-controlled changes in environmental parameters such as water availability and p_a (see section 3.2). Even though relationships between average annual $\delta^{13}C_a$ and Δ may exist, they cannot be invoked in interpreting small sample sets. We therefore conclude that one of the aims of our previous study (Toolin and Eastoe, 1993), namely precise determination of $\delta^{13}C_a$ in the early Holocene using florets preserved in packrat middens, was unattainable given the small number of florets available in each midden sample.. A set of 12-15 contemporaneous florets might yield a mean $\delta^{13}C_p$ from which atmospheric $\delta^{13}C_a$ could be estimated. However, the possibility of abrupt interannual changes unrelated to long-term trends of $\delta^{13}C_a$, as observed in this study, casts doubt on the meaning of such an estimate. Furthermore, collections of 12 or more demonstrably contemporaneous florets are highly unlikely to be available in packrat middens, because the florets are scarce as fossils and may move within the middens. C_4 fossils from packrat middens are unlikely to support reconstructions of paleoatmospheric conditions.

7. Conclusions

1. Annual and 5-year averages of $\delta^{13}\text{C}_p$ in florets of *Setaria macrostachya* decreased from –10 to –13 ‰ since about 1849.
2. C-isotope discrimination (Δ) between the atmosphere and florets of *S. macrostachya* increased from 3–4 ‰ in the 19th century to 4–5 ‰ at present.
3. The annual range of $\delta^{13}\text{C}_p$ in the florets is consistently near 2 ‰ where sufficient data are available.
4. The time series of averaged $\delta^{13}\text{C}_p$ contains several fluctuations (1–2 decade intervals of positive slope or above-average negative slope; abrupt changes at interannual scale) that are not documented in other available $\delta^{13}\text{C}_p$ archives.
5. The century-scale changes in $\delta^{13}\text{C}_p$ and Δ of *S. macrostachya* result from decreases in $\delta^{13}\text{C}_a$ and increases in p_{CO_2} .
6. Five-year average values of Δ increased prior to 1970, as p_{CO_2} increased from 290 to about 320 ppm. Since 1970, there is no evidence for systematic increase of annual or five-year averages of Δ with increasing p_{CO_2} .
7. No satisfactory explanation of shorter-term $\delta^{13}\text{C}_p$ fluctuations is available. Human economic influences such as the pioneer effect, depressions and major wars may coincide with $\delta^{13}\text{C}_p$ fluctuations in the earlier half of the time series.
8. Measurement of $\delta^{13}\text{C}_p$ in ancient preserved specimens of *S. macrostachya* is unlikely to yield useful information on atmospheric evolution.

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Figure captions

Figure 1 Map of study area, showing the northern boundary of the distribution of *Setaria macrostachya* (SEInet, 2016), the area represented by herbarium samples dating from 1849 to 1990, and the area represented by post-1990 samples.

594 **Figure 2** Changes in p_{CO_2} and $\delta^{13}C_a$ since 1840. Values of p_{CO_2} from 1840 to 1970 and $\delta^{13}C_a$
 595 from 1840 to 1990 are from the Law Ice Dome (Rubino et al., 2013) and more recent data are
 596 from Mauna Loa observatory, Hawaii, and Sinton, Texas (Dlugokencky et al., 2016; White et al.
 597 , 2015).

598 **Figure 3** Time-series of individual measurements of $\delta^{13}C_p$ in *Setaria macrostachya*. The fitted
 599 curve is a 2nd-order polynomial.

600 **Figure 4** A. Time series of annual mean $\delta^{13}C_p$ in *Setaria macrostachya* with 4th-order fitted
 601 curve, and of $\delta^{13}C_a$ (see Fig. 2 for sources. B. Five-year running means of $\delta^{13}C_p$ in *S.*
 602 *macrostachya*, with envelope showing mean $\pm$ 2 standard errors. Also shown are time series of:
 603 volcanic eruptions large enough to inject particulate material into the stratosphere (Self et al.,
 604 1987); major economic slumps (The Economist, 2016); El Niño and La Niña events of strong or
 605 higher intensity (Gergis and Fowler, 2008); 5-year running means of $\delta^{13}C_p$ in *Zea mays* (Marino
 606 and McElroy, 1991). C. Five-year running means of $\delta^{13}C_p$ in *S. macrostachya*, relative to periods
 607 of below-normal precipitation (indicated as shaded areas) during the warm season (Griffin et
 608 al., 2013).

609 **Figure 5** Isotope discrimination (Δ) of *Setaria macrostachya* as a function of p_{CO_2} . For 1881-
 610 1985, Δ is calculated using five-year running means of $\delta^{13}C_p$ from this study, and $\delta^{13}C_a$ data
 611 from the Law Ice Dome (Rubino et al., 2013). For 1990-2015, Δ is shown as both annual and
 612 5-year running means, calculated using annual means of $\delta^{13}C_p$ from this study, and annual
 613 mean $\delta^{13}C_a$ from Mauna Loa (White et al., 2015).

Commented [1]:

614 **Figure 6** Annual mean $\delta^{13}\text{C}_\text{p}$ as a function of mean summer temperature in Tucson (June to
615 October), 1982 to 2012.

616 **Figure S1** Time-series of individual measurements of $\delta^{13}\text{C}_\text{p}$ in *Setaria macrostachya*, classified
617 by geographic origin.

618

Fig. 1