

# C<sub>3</sub> plant carbon isotope discrimination does not respond to CO<sub>2</sub> concentration on decadal to centennial timescales

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## Summary

- Plant carbon isotope discrimination is complex, and could be driven by climate, evolution and/or edaphic factors. We tested the climate drivers of carbon isotope discrimination in modern and historical plant chemistry, and focus in particular on the relationship between rising [CO<sub>2</sub>] over Industrialization and carbon isotope discrimination.
- We generated temporal records of plant carbon isotopes from museum specimens collected over a climo-sequence to test plant responses to climate and atmospheric change over the past 200 yr (including *Pinus strobus*, *Platyclusus orientalis*, *Populus tremuloides*, *Thuja koraiensis*, *Thuja occidentalis*, *Thuja plicata*, *Thuja standishii* and *Thuja sutchuenensis*). We aggregated our results with a meta-analysis of a wide range of C<sub>3</sub> plants to make a comprehensive study of the distribution of carbon isotope discrimination and values among different plant types.
- We show that climate variables (e.g. mean annual precipitation, temperature and, key to this study, CO<sub>2</sub> in the atmosphere) do not drive carbon isotope discrimination.
- Plant isotope discrimination is intrinsic to each taxon, and could link phylogenetic relationships and adaptation to climate quantitatively and over ecological to geological time scales.

## Introduction

Plants grow in direct contact with the changing atmosphere and surrounding environment, so they have the potential to record changes in environmental conditions and related stress (Farquhar *et al.*, 1989; Arens *et al.*, 2000). The carbon isotope chemistry of plants has been used by both the geological and the ecological scientific communities to monitor climate change, plant biochemistry and plant productivity (e.g. Feng, 1999; Diefendorf *et al.*, 2010; Kohn, 2010). Carbon isotope discrimination in plants (represented by  $\Delta^{13}\text{C}_{\text{plant}}$  values) results from the combined effects of fractionation selecting for light carbon due to diffusion through the leaf surface (<sup>12</sup>C; 4.4‰; noted as ‘a’ in Eqn 1), fractionation due to Rubisco’s selective preference for light carbon (27–30‰; noted as ‘b’ in Eqn 1), and an array of biochemical and environmental factors that are sometimes collectively termed ‘water use efficiency’ ( $c_i/c_a$  is the ratio of internal to atmospheric CO<sub>2</sub> concentration; Eqn 1; e.g. Farquhar *et al.*, 1989).

$$\Delta^{13}\text{C}_{\text{plant}} = a + (b - a) \frac{C_i}{C_a} \quad \text{Eqn 1}$$

Plant carbon isotope discrimination ( $\Delta^{13}\text{C}_{\text{plant}}$ ) represents the difference between the isotopic composition of the atmosphere ( $\delta^{13}\text{C}_{\text{CO}_2}$ ) and plants ( $\delta^{13}\text{C}_{\text{plant}}$ ; Farquhar *et al.*, 1989; Feng, 1999; Eqn 2).

$$\Delta^{13}\text{C}_{\text{plant}} = \frac{\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{plant}}}{1 + \frac{\delta^{13}\text{C}_{\text{plant}}}{1000}} \quad \text{Eqn 2}$$

Scientists typically interpret carbon isotope values ( $\delta^{13}\text{C}_{\text{plant}}$ ) and  $\Delta^{13}\text{C}_{\text{plant}}$  values as related to and affected by environmental drivers such as mean annual precipitation (Diefendorf *et al.*, 2010; Kohn, 2010; Kohn, 2016) or the amount of carbon dioxide in the atmosphere ([CO<sub>2</sub>]; Schubert & Jahren, 2012; Cui & Schubert, 2016; Cui *et al.*, 2020), or to reflect a fundamental plant trait with variability due to local effects such as edaphic factors (Araus *et al.*, 2002; Bonal *et al.*, 2007). Our ability to use carbon isotope discrimination as recorded in plants to think about either past or future problems is dependent on understanding which combination of those factors drives discrimination.

## Background

The direct interaction that plants have with the environment around them means that leaf (and other plant part) tissues record environmental conditions (Schlanser *et al.*, 2020). Global change biologists and geologists aim to use this fact to look at past ecosystems and to understand biotic responses to elevated carbon dioxide concentration of the atmosphere ([CO<sub>2</sub>]) as well as related changes in seasonal and annual temperature, evapotranspiration and precipitation (Jones *et al.*, 1998). Previous studies

have suggested that  $\Delta^{13}\text{C}_{\text{plant}}$  values are sensitive to changes in  $[\text{CO}_2]$  (Ehleringer & Cerling, 1995; Schubert & Jahren, 2012) and workers have proposed a  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer to reconstruct  $[\text{CO}_2]$  in the fossil record based upon those empirical relationships (Schubert & Jahren, 2012; Cui & Schubert, 2017). In this scenario,  $\Delta^{13}\text{C}_{\text{plant}}$  values increase as the pool of available  $\text{CO}_2$  increases, indicating that the mechanism of  $\text{CO}_2$  uptake is altered in response to elevated  $[\text{CO}_2]$  (Cornwell *et al.*, 2018). Other studies have argued that water use efficiency,  $c_i/c_a$  (Eqn 1) is not constant and that plants modify their leaf gas exchange properties in response to  $\text{CO}_2$ , meaning that discrimination and  $\text{CO}_2$  cannot be directly linked (Ehleringer & Cerling, 1995; Beerling & Royer, 2002).

$\Delta^{13}\text{C}_{\text{plant}}$  values also are cited as representative of intrinsic water use efficiency (iWUE; Farquhar & Richards, 1984; Farquhar *et al.*, 1989; Arous *et al.*, 2002; Bonal *et al.*, 2007); this is probably related to evolutionary components. If  $\Delta^{13}\text{C}_{\text{plant}}$  values are genetically influenced, members of the same species should have constant  $\Delta^{13}\text{C}_{\text{plant}}$  values (with minor variability within a species related to genotypic diversity and local environmental factors regardless of global drivers). Because genetic and edaphic drivers are measurable in the present, relationships between  $\Delta^{13}\text{C}_{\text{plant}}$  and those drivers can be used directly to predict future ecosystem response to local and regional environmental change (Nowak *et al.*, 2004; Ainsworth & Long, 2005; Mueller *et al.*, 2016; Yan *et al.*, 2017). An additional implication of using  $\Delta^{13}\text{C}_{\text{plant}}$  values as intrinsic traits is that other variables used to link physiologically driven fractionation to isotopic values (Eqn 1) become solvable. These assumptions still have geological implications: if climate variables are not driving  $\Delta^{13}\text{C}_{\text{plant}}$  values, variability in  $\Delta^{13}\text{C}_{\text{plant}}$  values is still representative of water use efficiency (Eqn 1), which can be reconstructed in deep time to reconstruct water stress of a plant. However, due to the difficulty of measuring genetic diversity in the fossil record and the infrequency of coeval fossil soil and plant preservation due to different taphonomic filters (Looy *et al.*, 2014), the scope of  $\Delta^{13}\text{C}_{\text{plant}}$  applications would be more limited for geological problems or for attempts to use the geological record to project future change. Furthermore, most ecological validation studies of this concept have been based on single site or single taxon data collection or on short-term free-air concentration experiments (FACE experiments) where environmental variability has either not been considered or has only been examined over short (< 10 yr) periods. As a result, questions remain about whether relationships observed in short-term records (e.g. on an annual scale) would persist over the longer time periods necessary to project the impacts of future climate change.

If the previously proposed relationship between  $\Delta^{13}\text{C}_{\text{plant}}$  and  $[\text{CO}_2]$  is consistent across plants, it indicates that, overall, plants are responsive and adaptive to  $[\text{CO}_2]$  in real time. This paleobarometer potentially circumvents common problems of other deep-time paleo-barometric tools, for example the high error at high  $[\text{CO}_2]$  or issues with statistical robustness, taphonomic bias, effects specific to certain taxonomy and convolved environmental effects (e.g. oceanographic; Royer *et al.*, 2004). The relationship between  $\Delta^{13}\text{C}_{\text{plant}}$  and  $[\text{CO}_2]$  has

been tested previously in growth chamber experiments (Cui & Schubert, 2017; Lomax *et al.*, 2019) and for fossil plants directly with an independent proxy constraint on  $\text{CO}_2$  (Schlanser *et al.*, 2020). Growth chamber experiments showed increased  $\Delta^{13}\text{C}_{\text{plant}}$  values with increasing  $[\text{CO}_2]$  in *Arabidopsis thaliana* (thale cress) and *Raphanus sativus* (wild radish), two weedy herbaceous angiosperms. This relationship was substantiated with selectively sampled literature values to demonstrate that sensitivity ( $S$ ) of plant isotopic response to  $[\text{CO}_2]$  peaked at *c.* 200 ppm with  $S = 0.03\text{‰ ppm}^{-1}$  increase in discrimination and began to flatten around 1000 ppm with values closer to  $S = 0.0025\text{‰ ppm}^{-1}$  (Schubert & Jahren, 2012). The highest sensitivity values were collected from the literature, although Schubert & Jahren's (2012) meta-analysis specifically only sampled studies that saw increases in discrimination with  $[\text{CO}_2]$ , excluding all studies that did not. Each of the species used to create this model have completely sequenced genomes and have been widely studied as model organisms; in addition to well-constrained biochemistry, both these species are easy to grow quickly for real-time experiments. Results from experiments using *A. thaliana* and *R. sativus* have been extrapolated to other groups of plants (Schubert & Jahren, 2012; Shen *et al.*, 2013; Cui *et al.*, 2020). This empirically derived relationship has been used with carbon isotope measurements in fossils ( $\delta^{13}\text{C}_{\text{fossil}}$ ) to reconstruct  $[\text{CO}_2]$  during geological warm periods (Cui & Schubert, 2017; Cui *et al.*, 2020), and has been used as evidence to support a future plant response to anthropogenically driven increases in  $[\text{CO}_2]$ . When Lomax *et al.* (2019) attempted to use  $\Delta^{13}\text{C}_{\text{plant}}$  as a paleobarometer in growth chamber experiments with varied water regimes, they found that low water availability led to under-predicting  $[\text{CO}_2]$  values for high  $[\text{CO}_2]$  treatments designed to simulate most of the geological past. This suggests that  $[\text{CO}_2]$  cannot be implicated as the main driver for carbon isotope discrimination without considering water availability, but even this may be further complicated by evolution. The studies used to formulate a  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer model have not distinguished plant growth rate nor plant growth habit, and have assumed that there is no difference between angiosperms and any other type of plant. *Raphanus* and *Arabidopsis* have limited fossil records (Miocene–present; e.g. Beilstein *et al.*, 2010) and first evolved during periods of low  $[\text{CO}_2]$ ; therefore, there are questions about the reliability of an *R. sativus* – *A. thaliana*-focused model as a paleobarometer for a high  $\text{CO}_2$  world or for nonangiosperm plants. This approach to study the relationship between  $[\text{CO}_2]$  and  $\Delta^{13}\text{C}_{\text{plant}}$  is high resolution and well controlled, and assumes that  $[\text{CO}_2]$  is the main driving factor and does not address other climate and edaphic factors (e.g. soil moisture, nitrogen and nutrient availability, salinity; Bowman *et al.*, 1989; Condon *et al.*, 1992; Högberg *et al.*, 1993; Guehl *et al.*, 1995; Dawson *et al.*, 2002).

Additional geological studies to examine the relationship between  $[\text{CO}_2]$  and  $\Delta^{13}\text{C}_{\text{plant}}$  values in deep time have had mixed results. Paleogene plants collected from the Paleocene Fort Union Formation and the Eocene Willwood Formation

demonstrate no observable response to large changes in  $[\text{CO}_2]$  (Diefendorf *et al.*, 2015). Kohn (2016), however, found a small gradual increase in  $\Delta^{13}\text{C}_{\text{plant}}$  values in Pleistocene–Holocene sediments that, when corrected for mean annual precipitation, could be linked to increased  $[\text{CO}_2]$ . However, this same study found a slight decrease in  $\Delta^{13}\text{C}_{\text{plant}}$  values in Pleistocene and Tertiary herbivore data (Kohn, 2016). Recent work using leaf waxes collected from sediments of both the Cretaceous and the Oligocene shows that  $\Delta^{13}\text{C}_{\text{plant}}$  values in these sediments had no clear positive  $[\text{CO}_2]$  dependence and in fact responded negatively, perhaps related to adaptation to minimize water loss (Schlanser *et al.*, 2020). Testing in geological sediments is important and relevant to the utility of this paleobarometer, but would be strengthened by adding an additional timescale between previous geological studies and growth chamber experiments.

To test to what extent  $\Delta^{13}\text{C}_{\text{plant}}$  values of plants are responsive to changes in  $[\text{CO}_2]$  and how taxon-specific is this response, we turned to Industrialization as a natural experiment. This historical approach has the benefits of high-resolution, semi-annual data while also accounting for natural influence from environmental, edaphic and other variables that would be seen in the fossil record and can offset changes in water use efficiency and  $\Delta^{13}\text{C}_{\text{plant}}$  (Giguère-Croteau, *et al.*, 2019). In this study, we measured  $\Delta^{13}\text{C}_{\text{plant}}$  responses to the Industrialization-driven rise in  $[\text{CO}_2]$  in several plant species; nonbarometric climate variables are not for controlled but are constrained and considered. Industrialization (1850–present) provides a unique, natural  $[\text{CO}_2]$  enrichment ‘experiment’ wherein  $[\text{CO}_2]$  values range from 280 ppm to nearly 420 ppm (i.e. an increase of *c.* 50%), allowing us to track actual plant  $\Delta^{13}\text{C}_{\text{plant}}$  responses to rising  $[\text{CO}_2]$ . Previous works have tested the relationship of  $\Delta^{13}\text{C}_{\text{plant}}$  values to environmental, edaphic and genetic factors among modern plants (e.g. Cornwell *et al.*, 2018). However, most of those studies reflect only present-day or near present-day  $\text{CO}_2$  levels and only rarely have tracked the long-term (> 100 yr) response of individual species (e.g. Stein *et al.*, 2019). Based upon growth chamber experiments, Industrialization provides a range of  $[\text{CO}_2]$  wherein plant  $\Delta^{13}\text{C}_{\text{plant}}$  responses are thought to be extremely sensitive (Schubert & Jahren, 2012). In tandem with changes in  $[\text{CO}_2]$ , the isotopic composition of atmospheric  $\text{CO}_2$  has changed significantly over this time due to the burning of fossil fuels (from  $-6.7$  to  $-8.5\text{‰}$ ; Keeling *et al.*, 2001), allowing us to examine changes in  $\Delta^{13}\text{C}_{\text{plant}}$  vs changes in  $\delta^{13}\text{C}_{\text{plant}}$  values (Fig. 1a–c) during this interval.

The high-resolution temporal datasets tracking several species are supplemented by modern samples of the same species, to examine whether there are relationships between other climate variables and  $\Delta^{13}\text{C}_{\text{plant}}$  values (Sheldon *et al.*, 2020). In addition, we have compiled  $\delta^{13}\text{C}_{\text{plant}}$  values and calculated  $\Delta^{13}\text{C}_{\text{plant}}$  values for a wide range of herbaceous and woody  $\text{C}_3$  plants to compare between plant groups and growth strategies. Compiled literature data include herbaceous and woody angiosperms, and bryophytes (Fig. 3; Supporting Information Figs S1, S2; Table S1). The total dataset includes 2585 isotope analyses, with data collected for this study accompanied with measured climate variables.

## Materials and Methods

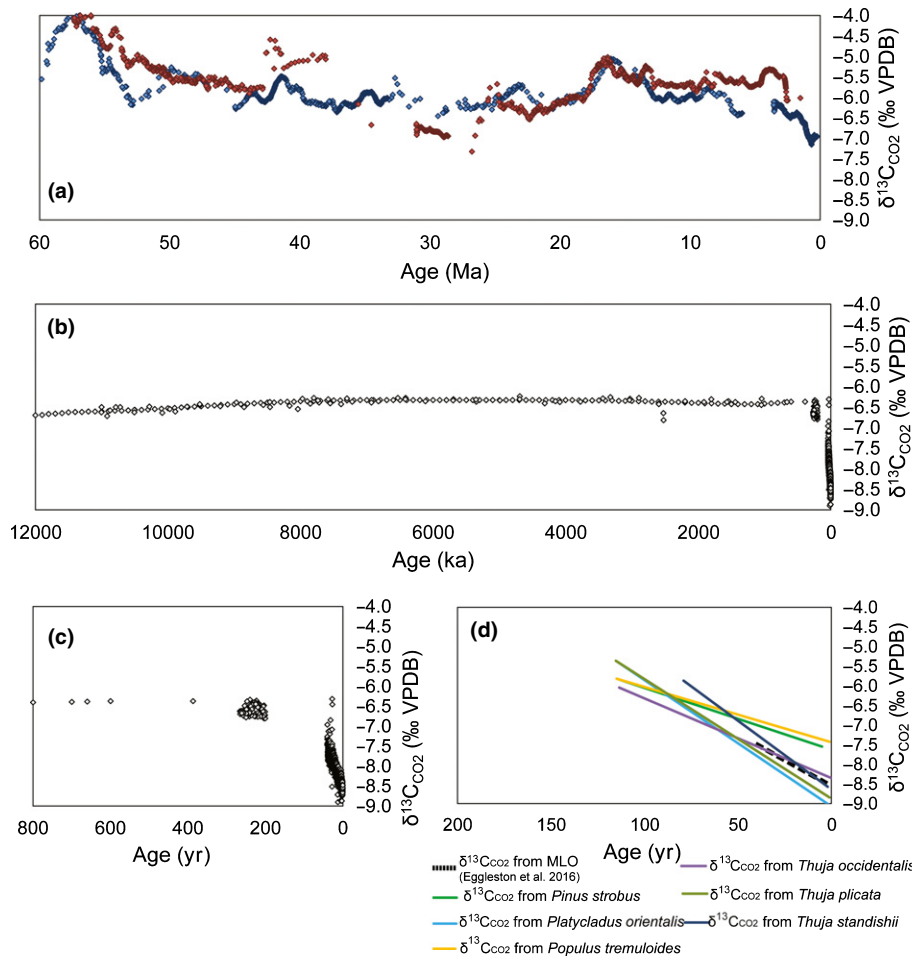
We used records from 11 herbaria and museum collections facilities (COLO, CS, F, HMAS, KHD, KUN, MICH, MSC, SG, WTU, YU) to evaluate the relationship between carbon isotope chemistry ( $\Delta^{13}\text{C}_{\text{plant}}$  and  $\delta^{13}\text{C}_{\text{plant}}$ ) and changing environmental drivers of a number of woody gymnosperms species and one woody angiosperm over the period of Industrialization. We collected modern gymnosperm (2015–2019) leaf material from across the Northern Hemisphere ( $n = 469$ ) of *Pinus strobus*, *Platycladus orientalis*, *Thuja koraiensis*, *Thuja occidentalis*, *Thuja plicata*, *Thuja standishii* and *Thuja sutchuenensis* as well as additional modern material from an angiosperm, *Populus tremuloides* ( $n = 1264$  total specimens for modern and historical specimens, combined), from between 1806 and 2019, spanning a wide range of climate conditions (Table S2). Specimens were washed in deionized (DI) water within an ultrasonic bath for 30 min to remove herbarium glue and other sediments, then dried in a  $50^\circ\text{C}$  oven for 48 h, and finally ground to homogeneity with an agate mortar and pestle. Ground specimens were stored in airtight glass vials within sealed chambers with desiccant to absorb water vapor. Specimen aliquots were weighed in tin capsules from 0.600 to 0.800 mg and run on a Picarro Cavity Ring Down Spectroscopy (CRDS) for  $\delta^{13}\text{C}$  values, with official IAEA standards (IAEA-CH6: sucrose,  $\delta^{13}\text{C} = -10.45\text{‰}$ ; IAEA-600: caffeine,  $\delta^{13}\text{C} = -27.77\text{‰}$ ) and laboratory internal standards ( $\text{C}_3$  sugar:  $\delta^{13}\text{C} = -26.14\text{‰}$ ,  $\text{C}_4$  sugar:  $\delta^{13}\text{C} = -12.71\text{‰}$ , acetanilide:  $\delta^{13}\text{C} = -28.17\text{‰}$ ). CRDS machine specifications indicate reproducibility of  $\pm 0.3\text{‰}$ , but our standard reproducibility was  $\pm 0.12\text{‰}$ . The same specimens were also run on a Costech Elemental Analyzer for %C and %N and C : N ratio using laboratory standards (acetanilide: 71.09%C, 10.34%N; and atropine: 70.56%C, 4.84%N) in the University of Michigan’s Earth System Science Laboratory.

Climate variables (mean annual precipitation (MAP), mean annual temperature (MAT), maximum summer temperature) associated with each specimen were obtained from the PRISM Climate Group (Prism Climate Group, 2004) for samples obtained in the contiguous United States and WorldClim (v.2 at 2.5-km resolution; Fick & Hijmans, 2017) and Vostok Ice Core ( $[\text{CO}_2]$ ,  $\delta^{13}\text{C}_{\text{CO}_2}$ ; White *et al.*, 2015). Altitude and latitude were also gathered for samples and compared to isotope analysis results.  $\Delta^{13}\text{C}_{\text{plant}}$  values were calculated using  $\delta^{13}\text{C}_{\text{CO}_2}$  as collected from the Vostok Ice Core and Mauna Loa Observatory (White *et al.*, 2015) using Eqn 2 (e.g. Feng, 1999; Diefendorf *et al.*, 2010).

We compared our historical findings regarding the relationship between  $\delta^{13}\text{C}_{\text{plant}}$  values and  $\delta^{13}\text{C}_{\text{CO}_2}$  with Arens *et al.*’s (2000) generalized empirical relationship between  $\delta^{13}\text{C}_{\text{plant}}$  values and  $\delta^{13}\text{C}_{\text{CO}_2}$  values in  $\text{C}_3$  plants (Eqn 3) (Arens *et al.*, 2000).

$$\delta^{13}\text{C}_{\text{plant}} = 1.05(\delta^{13}\text{C}_{\text{CO}_2}) - 18.72 \quad \text{Eqn 3}$$

Additional carbon isotopic values were obtained from previously published studies with coincident  $[\text{CO}_2]$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  values to compare this study’s focal plant results with a wider range of plant



**Fig. 1** Evolution of  $\delta^{13}\text{C}_{\text{CO}_2}$  (‰ VPDB), as compared to the Vienna PDB standard for calibration) (a) over the Cenozoic, 60 million yr ago (Ma) to the present, with red diamonds representing values reconstructed using planktonic foraminifera and blue diamonds representing values reconstructed using benthic foraminifera (Tippie *et al.*, 2010), (b) over the last 12 000 yr with open diamonds representing  $\delta^{13}\text{C}_{\text{CO}_2}$  from ice cores (Elsig *et al.*, 2009; Bauska *et al.*, 2018), (c) over the past 800 yr with open diamonds representing  $\delta^{13}\text{C}_{\text{CO}_2}$  from ice cores (Elsig *et al.*, 2009; Bauska *et al.*, 2018), (d) over the period of Industrialization, 200 yr ago to the present, including  $\delta^{13}\text{C}_{\text{CO}_2}$  values as measured from Mauna Loa Observatory (MLO; Keeling *et al.*, 2001; Eggleston *et al.*, 2016) and  $\delta^{13}\text{C}_{\text{CO}_2}$  values reconstructed using  $\Delta^{13}\text{C}_{\text{plant}}$  values from six species we collected ( $n \geq 10$  specimens).

functional types ( $n = 2585$ ; Table S1). We excluded all genera and families with fewer than 20 and 25 isotopic measurements (respectively) when comparing taxa and isotope discrimination values, but included these in plant habit or plant reproductive group comparisons. To test whether these genera, families and/or plant habits/reproductive strategies  $\Delta^{13}\text{C}_{\text{plant}}$  values had the same mean and variances as the  $\Delta^{13}\text{C}_{\text{plant}}$  values from the Schubert & Jahren (2012) study, we conducted  $F$ -tests where the null hypothesis was equal variance, then conducted two-sample  $t$ -tests assuming unequal variances wherein the null hypothesis was equal mean values (Table S3). We included analysis of plant specimens grown under  $[\text{CO}_2]$  values from 280 to 1000 ppm because although atmospheric  $[\text{CO}_2]$  is *c.* 410 ppm at present, concentrations of  $\text{CO}_2$  as high as 1800 ppm have been observed at ground level within very dense canopy on a very nutrient-rich Mollisol (Bazzaz & Williams, 1991) – to account for this potential fluctuation, of our sites, we included values for plants grown at  $[\text{CO}_2]$  levels up to 1000 ppm. We excluded values from the literature from plants that were grown at  $> 1000$  ppm.

Eqn 4 (Cui & Schubert, 2016) was used to reconstruct values of  $[\text{CO}_2]$  for each isotope value we collected, and we then compared reconstructed values with our known values collected from Mauna Loa Observatory and ice cores (Prism Climate Group, 2004; White *et al.*, 2015).

$$\Delta^{13}\text{C} = [(28.26)(0.21)(p\text{CO}_2 + 25)] / [28.26 + (0.21)(p\text{CO}_2 + 25)] \quad \text{Eqn 4}$$

where 28.26 represents  $A$  (Schubert & Jahren, 2012; Cui & Schubert, 2016), the maximum fractionation value, while 0.21 and 25 represent  $B$  and  $C$ , constants derived iteratively to find the best fit curve, and  $p\text{CO}_2 = [\text{CO}_2]$  (Cui & Schubert, 2016).

## Results

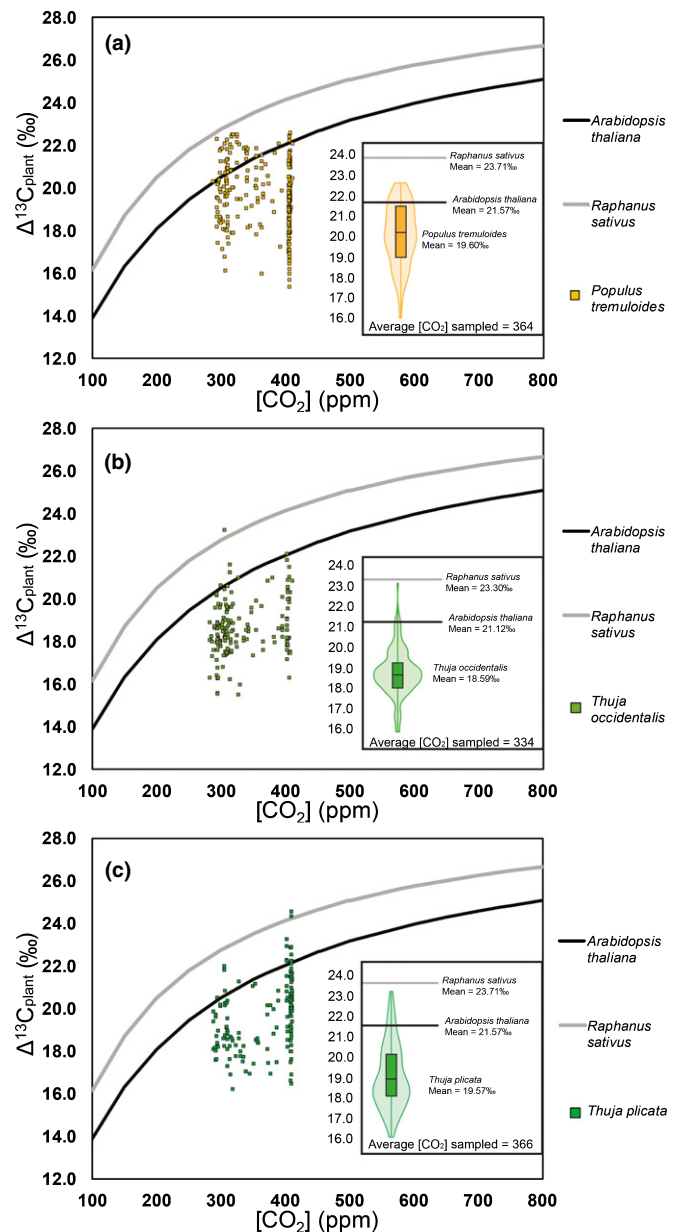
Six of the eight focal species showed significant shifts in  $\delta^{13}\text{C}_{\text{plant}}$  in tandem with  $\delta^{13}\text{C}_{\text{CO}_2}$  values. These plants (*P. strobus*, *P. orientalis*, *P. tremuloides*, *T. occidentalis*, *T. plicata*, *T. standishii*),



when corrected for each species' average  $\Delta^{13}\text{C}_{\text{plant}}$  values, demonstrated changes in  $\delta^{13}\text{C}_{\text{CO}_2}$  over the Industrialization period comparable to those recorded in ice and at Mauna Loa Observatory (Fig. 1c,d). *Populus tremuloides* displayed a slope and intercept similar to the generalized relationship found in the Arens *et al.* (2000) study (1.08 and  $-18.77$ , respectively), although the gymnosperms tested had different responses in  $\delta^{13}\text{C}_{\text{plant}}$  values to changing  $\delta^{13}\text{C}_{\text{CO}_2}$  (Table S4). The other focal species (*T. koraiensis*, *T. sutchuenensis*) did not span a sufficient period of time to examine the direction; *T. sutchuenensis* was only rediscovered recently (Qiaoping *et al.*, 2002) after over a century of being listed as extinct in the wild (EW) by IUCN standards.

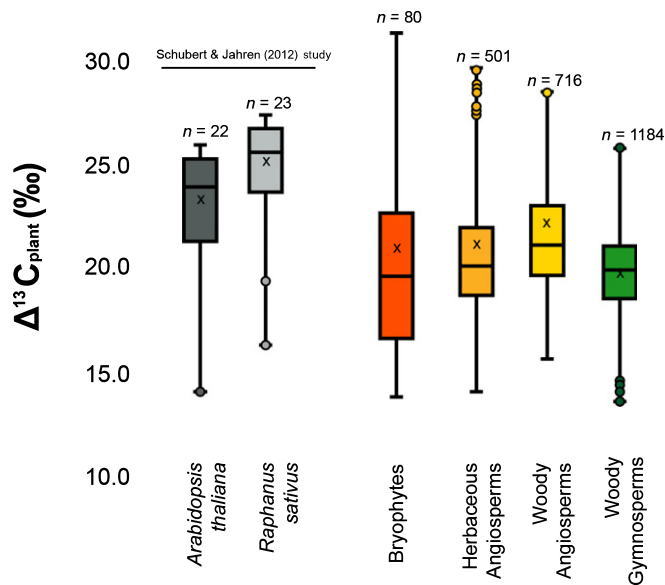
Over the span of Industrialization, none of the eight species studied showed any significant changes in  $\Delta^{13}\text{C}_{\text{plant}}$  nor significant relationship with  $[\text{CO}_2]$  (Fig. 2 shows the four largest datasets; others are plotted in Fig. S1). We examined changes in sensitivities for all data, and for pre-1960 values (280–320 ppm) and post-1960 values (320–410 ppm), with 1960 representing where slope changes in  $[\text{CO}_2]$  increase due to more rapid industrialization of developing nations (Keeling *et al.*, 2001; Macfarling Meure *et al.*, 2006) (Table S5). Of the seven species with pre-1960 data points ( $< 320$  ppm  $[\text{CO}_2]$ ) and post-1960 values ( $> 320$  ppm  $[\text{CO}_2]$ ), only two (*T. standishii* and *T. occidentalis*, Cupressaceae) showed a change in sensitivity to  $[\text{CO}_2]$ , and both declined rather than increasing as would have been predicted by growth chamber experiments. The sensitivity to  $[\text{CO}_2]$  ( $S$ ; change in ‰ of  $\Delta^{13}\text{C}_{\text{plant}}$  per ppm of  $[\text{CO}_2]$ ) exhibited by *A. thaliana* and *R. sativus* in growth chamber experiments was not observed in any of the Industrialization-spanning historical records (Table S5). In fact, we found negative shifts in  $S$  for *P. tremuloides* (Salicaceae) and *T. koraiensis*; none of the modern/historical species exhibited sensitivity to  $[\text{CO}_2]$  similar to the growth chamber experiments of Schubert & Jahren (2012).

This study compares  $\Delta^{13}\text{C}_{\text{plant}}$  values of *Arabidopsis* and *Raphanus* with a breadth of  $\text{C}_3$  plant functional types, representing taxa with different reproduction styles (angiosperms, gymnosperms and bryophytes), growth habits (woody and herbaceous) and vascular systems (vascular and nonvascular). When comparing the long historical records of  $\Delta^{13}\text{C}_{\text{plant}}$  with modern data from a variety of plant types from the literature, we found a distinct difference between gymnosperms, woody angiosperms, herbaceous angiosperms and bryophytes, and *A. thaliana* and *R. sativus* for any  $\text{CO}_2$  level  $< 1000$  ppm (Fig. 3). This was true even for other herbaceous, fast-growing members of the same family as the  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer model organisms (Brassicaceae: *A. thaliana*, *R. sativus*). Values above 1000 ppm are unlikely to be found at woody tree sampling height (*c.* 1–2 m), despite the source of soil-respired  $\text{CO}_2$  rising from the ground (Bazzaz & Williams, 1991). When performing a *t*-test for the mean values of each of these plant functional types as compared to the two tested weeds, we found that all of the tested differences were statistically significant (Figs 3, S2, S3; Table S3), indicating that none of the wild-grown species were behaving like the growth chamber experiments. When further analyzed at higher taxonomic levels, all genera and families showed statistically different  $\Delta^{13}\text{C}_{\text{plant}}$  values compared with *A. thaliana* and *R. sativus*.



**Fig. 2**  $[\text{CO}_2]$  values plotted against  $\Delta^{13}\text{C}_{\text{plant}}$  for species with high-resolution records of the period of Industrialization. Data are for: (a) *Populus tremuloides* (quaking aspen), (b) *Thuja occidentalis* (northern white cedar) and (c) *Thuja plicata* (western red cedar). The outer panel shows change in  $\Delta^{13}\text{C}_{\text{plant}}$  vs  $[\text{CO}_2]$  over the period of Industrialization, while the inner panel shows the range and distribution of  $\Delta^{13}\text{C}_{\text{plant}}$  values for this species. Each of the species occupies different geographic ranges and different ranges of climatic variability, but none shows a significant  $\Delta^{13}\text{C}_{\text{plant}}$  response to rising  $[\text{CO}_2]$  over the period of Industrialization.

To test the idea that  $\Delta^{13}\text{C}_{\text{plant}}$  should be related to  $[\text{CO}_2]$ , we compared the measured  $[\text{CO}_2]$  with  $[\text{CO}_2]$  reconstructed using an empirical relationship derived from growth chamber experiments (see Methods, Eqn 4). The actual  $[\text{CO}_2]$  values showed no significant relationship with reconstructed  $[\text{CO}_2]$  values (Fig. 4;  $P = 0.63$ ); often the model-reconstructed  $[\text{CO}_2]$  values underestimated measured  $[\text{CO}_2]$  (Fig. 4), which is consistent with other recent growth chamber experiments that included water stress as



**Fig. 3**  $\Delta^{13}\text{C}_{\text{plant}}$  values of plants divided by growth form, as collected from the literature and this study. Meta-analysis plants are shown in color, compared to *Arabidopsis* and *Raphanus* values in grey. Xs denote mean values and circles denote outlier  $\Delta^{13}\text{C}_{\text{plant}}$  values. Boxes show the 75<sup>th</sup> percentile of the data, while whiskers show the remaining 25<sup>th</sup> percentile of the data.

a variable (Lomax *et al.*, 2019). Thus, we find no evidence that  $\Delta^{13}\text{C}_{\text{plant}}$  is related to  $[\text{CO}_2]$ . Instead, the historical data showed consistent linear relationships between  $\delta^{13}\text{C}_{\text{plant}}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  for all tested species, with each having a slope close to 1 : 1 (Stein *et al.*, 2019; Sheldon *et al.*, 2020), and where the observed  $\delta^{13}\text{C}_{\text{atm}}$  values correspond closely to  $\delta^{13}\text{C}_{\text{atm}}$  values reconstructed using  $\delta^{13}\text{C}_{\text{plant}}$  measurements (Fig. 1d). In addition, most individual measurements of  $\Delta^{13}\text{C}_{\text{plant}}$  of a given species are within  $\pm 1.5\%$  of the mean  $\Delta^{13}\text{C}_{\text{plant}}$  value found for that species, independent of other climate variables (Figs 3, S2, S3; Sheldon *et al.*, 2020).

## Discussion

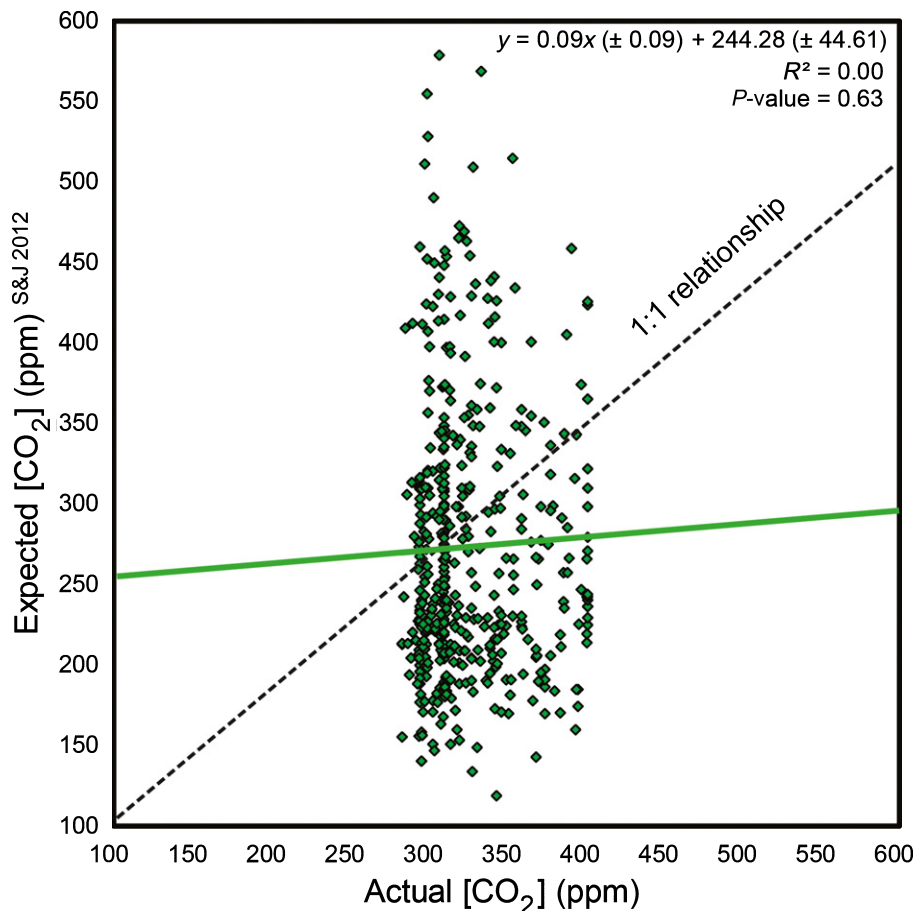
### Carbon isotope discrimination and $[\text{CO}_2]$

Our results indicate that  $\Delta^{13}\text{C}_{\text{plant}}$  values are not driven by  $[\text{CO}_2]$  for any of the studied plants.  $\Delta^{13}\text{C}_{\text{plant}}$  values remained flat for a given genus, plant functional type and species as  $[\text{CO}_2]$  increased over the Industrialization period (Figs 2, S1).  $\delta^{13}\text{C}_{\text{plant}}$  values, by contrast, closely tracked observed changes in  $\delta^{13}\text{C}_{\text{CO}_2}$  driven by human-combusted fossil fuels, which are isotopically more negative than natural  $\text{CO}_2$  sources (i.e. volcanic emissions; Keeling *et al.*, 2001).  $\delta^{13}\text{C}_{\text{plant}}$  values tracked  $\delta^{13}\text{C}_{\text{CO}_2}$  in multiple species, although individual species exhibited stronger or weaker carbon isotope discrimination. Our study confirms the previously evaluated connection between  $\delta^{13}\text{C}_{\text{plant}}$  values and atmospheric  $\text{CO}_2$  sources in the geological record (Arens *et al.*, 2000).

Regardless of growth habit or reproductive mode (i.e. angiosperm vs gymnosperm; seed vs spore), the newly collected

data and collated literature values for our aggregate meta-analysis exhibited  $\Delta^{13}\text{C}_{\text{plant}}$  values significantly lower than expected under current and historical  $[\text{CO}_2]$  ranges based upon values derived in growth-chamber experiments. These results indicate that  $\Delta^{13}\text{C}_{\text{plant}}$  values of all our tested plants, including herbaceous, fast-growing plants and bryophytes, which are known for their high discrimination against  $^{13}\text{C}$  due to their primitive vascular system (Rundel *et al.*, 1979; Proctor *et al.*, 1992; Royles *et al.*, 2016), are driven by something other than atmospheric  $[\text{CO}_2]$ . Thus, the previously observed  $\Delta^{13}\text{C}_{\text{plant}}-[\text{CO}_2]$  dependence may instead represent an intrinsic trait of a limited range of highly water-use-efficient, weedy plants, may reflect a plant response under unnaturally consistent conditions in growth chambers, or may be related to the range of  $[\text{CO}_2]$  studied or length of study. This affirms previous findings that although short-term  $[\text{CO}_2]$  enhancement experiments can result in increased  $\Delta^{13}\text{C}_{\text{plant}}$  values, decadal-scale plant responses, such as adjustment of stomatal size and density, counteract this effect (Peñuelas & Azcón-Bieto, 1992; Saurer *et al.*, 2004; Diefendorf & Freimuth, 2017). This is important for modern ecological studies that look to extrapolate broad implications from smaller, shorter and/or local experiments. We add a note of caution not to over-generalize results.

Although our historical records confirm that the  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer typically under-predicted  $[\text{CO}_2]$  in the wild (Fig. 4), in the context of previous findings (Stein *et al.*, 2019; Sheldon *et al.*, 2020), we interpret this as due to  $\Delta^{13}\text{C}_{\text{plant}}$  values being an intrinsic plant trait, and not driven by individual climate parameters. Previous workers (e.g. Kohn, 2016; Lomax *et al.*, 2019) have suggested that while there is potential for this  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer, climate factors related to water availability (e.g. MAP, humidity) and/or nutrient availability (Giguère-Croteau *et al.*, 2019) could confound the relationship between  $[\text{CO}_2]$  and  $\Delta^{13}\text{C}_{\text{plant}}$  values because of water's fundamental role in controlling photosynthesis and carbon uptake (Diefendorf *et al.*, 2010; Franks *et al.*, 2013). We found that none of those other climate variables significantly impacted  $\Delta^{13}\text{C}_{\text{plant}}$  at the species to family level, nor did a combination of  $[\text{CO}_2]$  and precipitation (Stein *et al.*, 2019; Sheldon *et al.*, 2020; Table S6). The most parsimonious explanation is that the previous  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer predicts a much greater sensitivity ( $\mathcal{S}$ ; expressed in  $\% \Delta^{13}\text{C}_{\text{plant}}/\text{ppm } [\text{CO}_2]$ ; Table S5) than is observable in nature; this demonstrates that  $[\text{CO}_2]$  is not the main driver of  $\Delta^{13}\text{C}_{\text{plant}}$  values based on highly selected, single- or few-species experiments over limited ranges in  $[\text{CO}_2]$  (e.g. Van de Water *et al.*, 1994; Peñuelas & Azcón-Bieto, 1992; Saurer *et al.*, 2004). By sampling a wide range of taxa, we show that the discrepancy between our  $\Delta^{13}\text{C}_{\text{plant}}$  values and those expected based on a  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer are not due to the taxa used and that  $\Delta^{13}\text{C}_{\text{plant}}$  values vary between taxa. In other words,  $\Delta^{13}\text{C}_{\text{plant}}$  values are intrinsic to a given taxon; the evolutionary implications of intrinsic discrimination could quantitatively link phylogenetic relationships and adaptation to climate. Variability within species'  $\Delta^{13}\text{C}_{\text{plant}}$  values is related to genotypic diversity and/or unmeasured but relevant-to-growth edaphic effects, but not related to the measured climate drivers and not sensitive to  $[\text{CO}_2]$ .



**Fig. 4**  $[\text{CO}_2]$  (ppm) values as measured at Mauna Loa Observatory ( $[\text{CO}_2]_a$ ), compared to reconstructed  $[\text{CO}_2]$  values ( $[\text{CO}_2]_r$ ) based on the proposed  $\Delta^{13}\text{C}_{\text{plant}}$ -paleobarometer (Eqn 4; Schubert & Jahren, 2012; Cui & Schubert, 2016). The solid line shows the trendline for our data ( $[\text{CO}_2]_r = 0.09 (\pm 0.09) \times [\text{CO}_2]_a + 244.28 (\pm 44.61)$ ;  $R^2 = 0.00$ ,  $P = 0.63$ ), while the dashed line shows the expected relationship if reconstructed  $[\text{CO}_2]$  values were equal to measured  $[\text{CO}_2]$  values ( $[\text{CO}_2]_r = [\text{CO}_2]_a$ ).

### Ancient bio-atmosphere implications

Our results indicate that we may be able to use  $\delta^{13}\text{C}_{\text{plant}}$  values to reconstruct paleo- $\delta^{13}\text{C}_{\text{CO}_2}$  values as in previous studies (i.e. Arens *et al.*, 2000; Fig. 1d) given that the other tested environmental factors were not significant drivers of  $\delta^{13}\text{C}_{\text{plant}}$  (Table S6). We suggest that using species-specific plant-atmosphere isotope relationships to track  $\delta^{13}\text{C}_{\text{CO}_2}$ , rather than a generalized relationship, will add certainty, and takes a step to address the critiques raised by Beerling & Royer (2002) that the generalized empirical relationship by Arens *et al.* (2000; Eqn 3) does not account for variability in  $c/c_a$  within or between plant lineages. While this ‘universal’ relationship based on a range of species does work, we found that the responses of each species in our historical dataset to changing  $\delta^{13}\text{C}_{\text{CO}_2}$  were different (as represented by equations relating the  $\delta^{13}\text{C}_{\text{plant}}$  values of each species to  $\delta^{13}\text{C}_{\text{CO}_2}$  values; Table S4). The woody angiosperm, *P. tremuloides*, behaved similarly to the generalized relationship found by Arens *et al.* (2000), but the other species tested, all gymnosperms, had vastly different responses in  $\delta^{13}\text{C}_{\text{plant}}$  values to changing  $\delta^{13}\text{C}_{\text{CO}_2}$ . This highlights the importance of evolution in response to changing climate and atmospheric variables.

For plants with pre-instrument or fossil records, modern  $\Delta^{13}\text{C}_{\text{plant}}$  values are a key input parameter in many other tools (including paleo-barometric techniques) previously applied in the fossil record, and are a key tracer of  $[\text{CO}_2]$  sources and fluxes in deep time (Franks *et al.*, 2014). With the depth and breadth of our study, we provide robustly constrained  $\Delta^{13}\text{C}_{\text{plant}}$  values of several species (*T. occidentalis*, *T. plicata*, *P. strobus*, *P. tremuloides*), and validate that  $\Delta^{13}\text{C}_{\text{plant}}$  values are approximately constant. Although carbon isotope discrimination is complex and influenced by many factors (Diefendorf & Freimuth, 2017), in tandem with previous works (Merveille, 2015; Stein *et al.*, 2019; Sheldon *et al.*, 2020), we support that many measures of temperature (e.g. MAT, growing season temperature) and moisture (e.g. MAP, wettest 3 months) do not have predictive relationships with  $\Delta^{13}\text{C}_{\text{plant}}$  values (Table S6). This means that while there are diagenetic and preservation-related factors to take into account before using  $\delta^{13}\text{C}_{\text{plant}}$  to reconstruct paleo- $\delta^{13}\text{C}_{\text{CO}_2}$ , it is plausible to look at relative perturbations in  $\delta^{13}\text{C}_{\text{CO}_2}$  in time using plants over timescales from hundreds to millions of years to track changes in the carbon cycle. This would be an excellent terrestrial complement to Tipple *et al.*'s (2010) foraminifera-derived  $\delta^{13}\text{C}_{\text{CO}_2}$  record and would provide a way to compare



marine–terrestrial reconstructions and to time-calibrate major paleoclimatic transitions.

Our results affirm previous experiments that calculated paleo-water use efficiency in *Metasequoia* and *Thuja* fossils based on  $\delta^{13}\text{C}$  values, which assumed no confounding climate factors (e.g. Sheldon *et al.*, 2020; Table S6). While  $\Delta^{13}\text{C}_{\text{plant}}$  values should not be used to reconstruct environmental drivers such as  $[\text{CO}_2]$ , they can be used to identify water use efficiency in ancient plants. Ancient plants' water use efficiency provides insight into general adaptation to climate events and evolutionary history – a critical aspect of how the past can inform the future (McElwain, 2018).

### Modern and future climate change

This study's markedly longer duration of the 'natural experiments' considered provides context and validation for important shorter experiments, such as growth chamber (Lomax *et al.*, 2019) and FACE experiments (Ainsworth & Long, 2005; Norby & Zak, 2011). These experiments have been used to show short-term plant adaptation to enhanced  $\text{CO}_2$  in certain plants, but muted response with longer time and a wider breadth of plant types (Long *et al.*, 2006; Hickler *et al.*, 2008; Norby & Zak, 2011). We have demonstrated responses over the entirety of Industrialization, validating the value of those shorter-term experiments for predicting future response. Our results suggest that extending these experiments and incorporating a number of comparative species of different plant functional types grown in the same environment would be useful to determine longer-term and broader plant reactions to elevated  $\text{CO}_2$ .

If  $\Delta^{13}\text{C}_{\text{plant}}$  values are inherent to species, soil chemistry (a catchment of above-ground ecosystem inputs) may demonstrate relationships with environmental drivers related to the biogeography of plant adaptation and distribution (Cornwell *et al.*, 2018). Previous studies have demonstrated that  $\Delta^{13}\text{C}_{\text{plant}}$  values provide insight into plant health associated with measurable genotypic and edaphic effects (Reich *et al.*, 2006); to isolate the effects of these variables, future studies could examine foliar to critical-zone carbon isotope variability over the natural experiment of the Industrialization period with measured soil parameters (e.g. soil moisture, texture, pH, nutrient availability, microbiota; Kaplan *et al.*, 2002; McKee *et al.*, 2002; Cornwell *et al.*, 2018). With deeper understanding of what edaphic, morphological and genetic factors drive variability, we can use  $\Delta^{13}\text{C}_{\text{plant}}$  values to understand plant biochemistry in response to, for example, water stress, yield and growth success and to strategically manage landscapes to maximize plants as a biological carbon sink.

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### Author contributions

NDS and SYS conceptualized the study. RAS and SYS were responsible for data curation. RAS and NDS performed the formal analysis. NDS and SYS were responsible for funding acquisition, project administration, provision of resources and supervision to RAS. RAS was primarily responsible for the investigation and performed all of the visualizations. All three authors were responsible for the writing, including both the original draft and review and editing.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** [CO<sub>2</sub>] vs Δ<sup>13</sup>C<sub>plant</sub> values for historical species with medium-resolution records of Industrialization.

**Fig. S2** Δ<sup>13</sup>C<sub>plant</sub> values of plants by genus.

**Fig. S3** Δ<sup>13</sup>C<sub>plant</sub> values of plants by family.

**Table S1** All data in .xlsx file.

**Table S2** Range of climate variables and atmospheric parameters included in the historical portion of this study.

**Table S3** *T*-test statistics (assuming unequal variances) comparing the means and ranges of Δ<sup>13</sup>C<sub>plant</sub> values for different taxa in this study.

**Table S4** The relationship between δ<sup>13</sup>C<sub>CO2</sub> and δ<sup>13</sup>C<sub>plant</sub> values for the historical specimens spanning Industrialization.

**Table S5** Sensitivity (S given as ‰ ppm<sup>-1</sup>) for eight species with long historical record.

**Table S6** The relationship between Δ<sup>13</sup>C<sub>plant</sub> values and non-barometric climate variables for historical and modern species sampled in this study.

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