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Main Manuscript for

C_3 plant carbon isotope discrimination does not respond to CO_2 concentration on decadal to centennial timescales

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Summary

(1) 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 $[CO_2]$ over Industrialization and carbon isotope discrimination.

(2) We generated temporal records of plant carbon isotopes from museum specimens collected over a climo-sequence to test plant response to climate and atmospheric change over the past 200 years (including *Pinus strobus, Platycladus orientalis, Populus tremuloides, Thuja koraiensis, Thuja occidentalis, Thuja plicata, Thuja standishii, Thuja sutchuenensis*). We aggregated our results with a meta-analysis of a wide range of C_3 plants to do a comprehensive study of the distribution of carbon isotope discrimination and values among different plant types.

(3) We show that climate variables (e.g. mean annual precipitation, temperature, and *key to this study,* CO_2 in the atmosphere) do not drive carbon isotope discrimination.

(4) 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.

Keywords

Atmosphere, biogeochemistry, carbon, climate, evolution, isotopes, paleoclimate, plants

Main Text
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 ecological scientific communities to monitor climate change, plant biochemistry, and plant productivity (e.g. Feng et al. 1999; Diefendorf et al. 2010; Kohn 2010). Carbon isotope discrimination in plants (represented by $\Delta^{13}C_{plant}$ values) is the combined effects of fractionation selecting for light carbon due to diffusion through the leaf surface (^{12}C; 4.4‰; noted as "a" in Equation 1), fractionation due to Rubisco's selective preference for light carbon (27–30‰; noted as "b" in Equation 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_2 concentration; Equation 1; e.g. Farquhar et al. 1989).

$$Equation\ (1) \qquad \Delta^{13}C_{plant} = a + (b - a)\frac{C_i}{C_a}$$

Plant carbon isotope discrimination ($\Delta^{13}C_{plant}$) represents the difference between the isotopic composition of the atmosphere ($\delta^{13}C_{CO2}$) and plants ($\delta^{13}C_{plant}$; Farquhar et al. 1989; Feng et al. 1999; Equation 2).

$$Equation\ (2) \qquad \Delta^{13}C_{plant} = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{plant}}{1 + \dfrac{\delta^{13}C_{plant}}{1000}}$$

Scientists typically interpret carbon isotope values ($\delta^{13}C_{plant}$) and $\Delta^{13}C_{plant}$ values as related to and affected by environmental drivers like mean annual precipitation (Diefendorf et al. 2010; Kohn 2010; Kohn 2016) or the amount of carbon dioxide in the atmosphere ([CO_2]; Schubert and Jahren, 2012; Cui and Schubert, 2016; Cui & Schubert 2020), or to reflect a fundamental plant trait with variability due to local effects like 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 drive discrimination.

Background

The direct interaction that plants have with the environment around them makes it such that leaf (and other plant part) tissues record environmental conditions (Schlanser et

al. 2020). Global change biologists and geologists aim to leverage this fact in order to look at past ecosystems and to understand biotic responses to elevated carbon dioxide concentration of the atmosphere ($[CO_2]$) as well as related changes in seasonal and annual temperature, evapotranspiration, and precipitation (Jones et al. 1998). Previous studies have suggested that $\Delta^{13}C_{plant}$ values are sensitive to changes in $[CO_2]$ (Ehleringer & Cerling 1995; Schubert & Jahren 2012) and workers have proposed a $\Delta^{13}C_{plant}$-paleobarometer to reconstruct $[CO_2]$ in the fossil record based upon those empirical relationships (Schubert & Jahren 2012; Cui & Schubert 2017). In this scenario, $\Delta^{13}C_{plant}$ values increase as the pool of available CO_2 increases, indicating that the mechanism of $[CO_2]$ uptake is altered in response to elevated $[CO_2]$ (Cornwell et al. 2018). Other studies have argued that water use efficiency, c_i/c_a (Equation 1), is not constant and that plants modify their leaf gas exchange properties in response to CO_2, meaning that discrimination and CO_2 cannot be directly linked (Ehleringer & Cerling 1995; Beerling & Royer 2002).

$\Delta^{13}C_{plant}$ values also are cited as representative of intrinsic water use efficiency (iWUE; Farquhar & Richards 1984; Farquhar et al. 1989; Araus et al. 2002; Bonal et al. 2007); this is likely related to evolutionary components. If $\Delta^{13}C_{plant}$ values are genetically influenced, members of the same species should have constant $\Delta^{13}C_{plant}$ 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 $\Delta^{13}C_{plant}$ and those drivers can be used directly to predict future ecosystem response to local and regional environmental change (Ainsworth & Long 2005; Nowak et al. 2004; Mueller et al. 2016; Yan et al. 2017). An additional implication of using $\Delta^{13}C_{plant}$ values as intrinsic traits is that other variables used to link physiologically-driven fractionation to isotopic values (Equation 1) become solvable. These assumptions still have geological implications: if climate variables are not driving $\Delta^{13}C_{plant}$ values, variability in $\Delta^{13}C_{plant}$ values is still representative of water use efficiency (Equation 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 2014), the scope of $\Delta^{13}C_{plant}$ applications would be more limited for geologic problems or attempts to use the geologic 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) where environmental variability has either not been considered or has only been examined over short (< 10 years) time periods. As a result, questions remain about whether relationships observed in short-term records (e.g. on annual scale) would persist over the longer time periods necessary to project the impacts of future climate change.

If the previously proposed relationship between $\Delta^{13}C_{plant}$ and $[CO_2]$ is consistent across plants, it indicates that overall, plants are responsive and adaptive to $[CO_2]$ in real time. This paleobarometer potentially circumvents common problems of other deep-time paleo-barometric tools, like high error at high CO_2 concentrations 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 $\Delta^{13}C_{plant}$ and $[CO_2]$ has been tested previously in growth chamber experiments (Cui & Schubert 2017; Lomax et al. 2019) and fossil plants directly with an independent proxy constraint on CO_2 (Schlanser et al. 2020). Growth chamber experiments showed increased $\Delta^{13}C_{plant}$ values with increasing $[CO_2]$ 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 $[CO_2]$ peaked ~200 ppm with S = 0.03‰ ppm^{-1} increase in discrimination and began to flatten around 1000 ppm with values closer to S = 0.0025‰ ppm^{-1} (Schubert & Jahren 2012). The highest sensitivity values were collected from literature, but Shubert and Jahren's (2012) meta-analysis specifically only sampled studies that saw increases in discrimination with $[CO_2]$, 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, these species are both 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 ($\delta^{13}C_{fossil}$) to reconstruct $[CO_2]$ during geologic warm periods (Cui & Schubert, 2017; Cui et al. 2020), and has been used as evidence to support a future plant response to anthropogenically-driven rise in $[CO_2]$. When Lomax et al. (2019) attempted to use $\Delta^{13}C_{plant}$ as a paleobarometer in growth chamber experiments with varied water regimes, they found that low water availability led to under-predicting $[CO_2]$ values for high $[CO_2]$ treatments designed to simulate most of the geologic past. This suggests that $[CO_2]$ 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 $\Delta^{13}C_{plant}$ -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 $[CO_2]$; therefore, there are questions about the reliability of a *Raphanus sativus- Arabidopsis thaliana*-focused model as a paleobarometer for a high CO_2 world or for non-angiosperm plants. This approach to study the relationship between $[CO_2]$ and $\Delta^{13}C_{plant}$ is high resolution and well-controlled, and assumes that $[CO_2]$ 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 $[CO_2]$ and $\Delta^{13}C_{plant}$ values in deep time have mixed results. Paleogene plants collected from the Paleocene Fort Union Formation and the Eocene Willwood Formation demonstrate no observable response to large changes in $[CO_2]$ (Diefendorf et al. 2015). Kohn (2016), however, found a small gradual increase in $\Delta^{13}C_{plant}$ values in Pleistocene-Holocene sediments, that, when corrected for mean annual precipitation, could be linked to increased $[CO_2]$. However, this same study found a slight decrease in $\Delta^{13}C_{plant}$ values in Pleistocene and Tertiary herbivore data (Kohn 2016). Recent work using leaf waxes

collected from sediments of both the Cretaceous and Oligocene shows that $\Delta^{13}C_{plant}$ values in these sediments had no clear positive $[CO_2]$-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 prior geologic studies and growth chamber experiments.

To test to what extent $\Delta^{13}C_{plant}$ values of plants are responsive to changes in $[CO_2]$ 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 $\Delta^{13}C_{plant}$ (Giguère-Croteau, et al. 2019). In this study, we measured $\Delta^{13}C_{plant}$ response to the Industrialization-driven rise in $[CO_2]$ in several plant species; non-barometric climate variables are not controlled but are constrained and considered. Industrialization (1850–present) provides a unique, natural $[CO_2]$ enrichment "experiment" wherein $[CO_2]$ values range from 280 ppm to nearly 420 ppm (i.e., an increase of ~50%), allowing us to track actual plant $\Delta^{13}C_{plant}$ responses to rising $[CO_2]$. Previous works have tested the relationship of $\Delta^{13}C_{plant}$ 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 CO_2 levels and only rarely have tracked the long-term (> 100 years) response of individual species (e.g. Stein et al. 2019). Based upon growth chamber experiments, Industrialization provides a range of $[CO_2]$ wherein plant $\Delta^{13}C_{plant}$ responses are thought to be extremely sensitive (Schubert & Jahren 2012). In tandem with changes in $[CO_2]$, the isotopic composition of atmospheric CO_2 has changed significantly over this time due to the burning of fossil fuels (from a value of -6.7 to -8.5‰; Keeling et al. 2001), allowing us to examine changes in $\Delta^{13}C_{plant}$ versus changes in $\delta^{13}C_{plant}$ 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 $\Delta^{13}C_{plant}$ values (Sheldon et al. 2020). In addition, we

have compiled $\delta^{13}C_{plant}$ values and calculated $\Delta^{13}C_{plant}$ values for a wide range of herbaceous and woody C_3 plants to compare between plant groups and growth strategies. Compiled literature data include herbaceous and woody angiosperms, and bryophytes (Fig. 3, Supp. Figs. S1–S2; Supp. 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 ($\Delta^{13}C_{plant}$ and $\delta^{13}C_{plant}$) 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 species from another angiosperm, *Populus tremuloides* species (n = 1264 total specimens for modern and historical specimens, combined) from between 1806 and 2019, spanning a wide range of climate conditions (Supp. Table S2). Specimens were washed in deionized (DI) water within an ultrasonic bath for thirty minutes to remove herbarium glue and other sediments, then dried in a 50°C oven for 48 hours, and finally ground to homogeneity with an agate mortar and pestle. Ground specimens were stored in air-tight glass vials within sealed chambers with desiccant to absorb water vapor. Specimen aliquots were weighed into tin capsules from 0.600 mg to 0.800 mg and run on a Picarro Cavity Ring Down Spectroscope (CRDS) for $\delta^{13}C$ values, with official IAEA standards (IAEA-CH6: sucrose, $\delta^{13}C$ = -10.45‰; IAEA-600: caffeine, $\delta^{13}C$ = -27.77‰) and laboratory internal standards (C_3 sugar: $\delta^{13}C$ = -26.14‰, C_4 sugar: $\delta^{13}C$ = -12.71‰, acetanilide: $\delta^{13}C$ = -28.17‰). CRDS machine specifications indicate reproducibility of ± 0.3‰, but our standard reproducibility was ± 0.12‰. These 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 University of Michigan's Earth System Science Laboratory.

Climate variables (MAP, MAT, Maximum Summer Temperature) associated with each specimen were obtained from PRISM Climate Group (Prism Climate Group 2004) for samples obtained in the contiguous United States and WorldClim (version 2 at 2.5km resolution; Fick & Hijmans 2017) and Vostok Ice Core ([CO_2], $\delta^{13}C_{CO2}$; White et al. 2015). Altitude and latitude were also gathered for samples and compared to isotope analysis results. $\Delta^{13}C_{plant}$ values were calculated using $\delta^{13}C_{CO2}$ as collected from the Vostok Ice Core and Mauna Loa Observatory (White et al. 2015) using Equation 2 (e.g. Feng et al. 1999; Diefendorf et al. 2010).

We compared our historical findings regarding the relationship between $\delta^{13}C_{plant}$ values and $\delta^{13}C_{CO2}$ with Arens et al.'s (2000) generalized empirical relationship between $\delta^{13}C_{plant}$ values and $\delta^{13}C_{CO2}$ values in C_3 plants (Equation 3).

$$Equation\ (3) \quad \delta^{13}C_{plant} = 1.05(\delta^{13}C_{CO2}) - 18.72\ (Arens\ et\ al.\ 2000)$$

Additional carbon isotopic values were obtained from previously published studies with coincident [CO_2] and $\delta^{13}C_{CO2}$ values to compare this study's focal plant results with a wider range of plant 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 $\Delta^{13}C_{plant}$ values had the same mean and variances as the $\Delta^{13}C_{plant}$ 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 (Supp. Table S3). We included analysis of plant specimens grown under [CO_2] values from 280–1000 ppm, because although atmospheric [CO_2] is ~410 ppm at present, concentrations of 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 $[CO_2]$ levels up to 1000 ppm. We excluded values from the literature from plants that were grown at >1000 ppm.

Equation 4 (Cui & Schubert 2016) was used to reconstruct values of $[CO_2]$ for each isotope value we collected, then compared reconstructed values with our known values collected from Mauna Loa Observatory and ice cores (Prism Climate Group 2004; White et al. 2015).

$$Equation\ (4)\ \Delta^{13}C = [(28.26)(0.21)(pCO_2 + 25)]/[28.26 + (0.21)(pCO_2 + 25)]$$
$$(\text{Schubert \& Jahren 2012; Cui \& Schubert 2016})$$

where 28.26 represents A, the maximum fractionation value, while 0.21 and 25 represent B and C, constants derived iteratively to find the best fit curve, and $pCO_2 = [CO_2]$ (Cui & Schubert 2016).

Results

Six of the eight focal species showed significant shifts in $\delta^{13}C_{plant}$ in tandem with $\delta^{13}C_{CO2}$ values. These plants (*Pinus strobus, Platycladus orientalis, Populus tremuloides, Thuja occidentalis, Thuja plicata, Thuja standishii*), when corrected for each species' average $\Delta^{13}C_{plant}$ values, demonstrated changes in $\delta^{13}C_{CO2}$ over Industrialization 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), though the gymnosperms tested had different responses in $\delta^{13}C_{plant}$ values to changing $\delta^{13}C_{CO2}$ (Supp. Table S4). The other focal species (*Thuja koraiensis, Thuja sutchuenensis*) did not span a sufficient period of time to examine the direction; *Thuja 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 $\Delta^{13}C_{plant}$ nor significant relationship with $[CO_2]$ (Fig. 2 shows the four largest datasets; others are plotted in Supp. 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 $[CO_2]$ increase due to more rapid industrialization of developing nations (Keeling et al. 2001; MacFarling et al. 2006) (Supp. Table S5). Of the seven species with pre-1960 data points (<320 ppm $[CO_2]$) and post-1960 values (>320 ppm $[CO_2]$), only two (*Thuja standishii* and *Thuja occidentalis;* Cupressaceae) showed a change in sensitivity to $[CO_2]$, and both declined rather than increasing as would have been predicted by growth chamber experiments. The sensitivity to $[CO_2]$ (*S*; change in ‰ of $\Delta^{13}C_{plant}$ per ppm of $[CO_2]$) exhibited by *Arabidopsis thaliana* and *Raphanus sativus* in growth chamber experiments was not observed in any of the Industrialization-spanning historical records (Supp. Table S5). In fact, we found negative shifts in *S* for *Populus tremuloides* (Salicaceae) and *Thuja koraiensis;* none of the modern/historical species exhibited sensitivity to $[CO_2]$ similar to the growth chamber experiments of Schubert & Jahren (2012).

This study compares $\Delta^{13}C_{plant}$ values of *Arabidopsis* and *Raphanus* with a breadth of 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 non-vascular). When comparing the long historical records of $\Delta^{13}C_{plant}$ 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 CO_2 level <1000 ppm (Fig. 3). This was true even for other herbaceous, fast-growing members of the same family as the $\Delta^{13}C_{plant}$-paleobarometer model organisms (Brassicaceae; *A. thaliana, R. sativus*). Values above 1000 ppm are unlikely to be found at woody tree sampling height (~1–2m), despite the source of soil-respired 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 (Fig. 3; Fig. S2; Fig. S3; Supp. 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 $\Delta^{13}C_{plant}$ values than *A. thaliana* and *R. sativus*.

To test the idea that $\Delta^{13}C_{plant}$ should be related to $[CO_2]$, we compared the measured $[CO_2]$ with $[CO_2]$ reconstructed using an empirical relationship derived from growth chamber experiments (see *Methods*, Equation 4). The actual $[CO_2]$ values showed no significant relationship with reconstructed $[CO_2]$ values (Fig. 4; p-value = 0.63); often the model-reconstructed $[CO_2]$ values underestimated measured $[CO_2]$ (Fig. 4), which is consistent with other recent growth chamber experiments that included water stress as a variable (Lomax et al. 2019). Thus, we find no evidence that $\Delta^{13}C_{plant}$ is related to $[CO_2]$. Instead, the historical data showed consistent linear relationships between $\delta^{13}C_{plant}$ and $\delta^{13}C_{CO2}$ 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 $\delta^{13}C_{atm}$ values correspond closely to $\delta^{13}C_{atm}$ values reconstructed using $\delta^{13}C_{plant}$ measurements (Fig. 1d). In addition, most individual measurements of a given species' $\Delta^{13}C_{plant}$ value are within ± 1.5 ‰ of the mean $\Delta^{13}C_{plant}$ value found for that species, independent of other climate variables (Fig. 3; Fig. S2; Fig. S3; Sheldon et al. 2020).

Discussion

Carbon isotope discrimination and [CO2]

Our results indicate that $\Delta^{13}C_{plant}$ values are not driven by $[CO_2]$ for any of the studied plants. $\Delta^{13}C_{plant}$ values remained flat for a given genus, plant functional type, and species as $[CO_2]$ increased over Industrialization (Fig. 2; Fig. S1). $\delta^{13}C_{plant}$ values, in contrast, closely tracked observed changes in $\delta^{13}C_{CO2}$ driven by human-combusted fossil fuels, which are isotopically more negative than natural CO_2 sources (i.e. volcanic emissions; Keeling et al. 2001). $\delta^{13}C_{plant}$ values tracked $\delta^{13}C_{CO2}$ in multiple species, though individual species exhibited stronger or weaker carbon isotope discrimination. Our study confirms the previously evaluated connection between $\delta^{13}C_{plant}$ values and atmospheric CO_2 sources in the geologic record (Arens et al. 2000).

Regardless of growth habit or reproductive mode (i.e., angiosperm versus gymnosperm; seed vs. spore), the newly collected data and collated literature values for

our aggregate meta-analysis exhibited $\Delta^{13}C_{plant}$ values significantly lower than expected under current and historic $[CO_2]$ ranges based upon growth-chamber derived values. These results indicate $\Delta^{13}C_{plant}$ values of all of our tested plants, including herbaceous, fast-growing plants and bryophytes, which are known for their high discrimination against ^{13}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 the atmospheric $[CO_2]$. Thus, previously observed $\Delta^{13}C_{plant}$-$[CO_2]$ dependence may instead represent an intrinsic trait of a limited range of highly water-use efficient, weedy plants, may reflect plant response under unnaturally consistent conditions in growth chambers, or may be related to the range of $[CO_2]$ studied or length of study. This affirms previous findings that though short-term $[CO_2]$ enhancement experiments can result in increased $\Delta^{13}C_{plant}$ values, decadal-scale plant responses, like 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.

While our historical records confirm that the $\Delta^{13}C_{plant}$-paleobarometer typically under-predicted $[CO_2]$ in the natural world (Fig. 4), in the context of previous findings (Stein et al. 2019; Sheldon et al. 2020), we interpret this as due to $\Delta^{13}C_{plant}$ 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 $\Delta^{13}C_{plant}$–paleobarometer, climate factors related to water availability (e.g. mean annual precipitation, humidity) and/or nutrient availability (Giguère-Croteau et al. 2019) could confound the relationship between $[CO_2]$ and $\Delta^{13}C_{plant}$ 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 the $\Delta^{13}C_{plant}$ at the species to family level, nor did a combination of $[CO_2]$ and precipitation (Stein et al. 2019; Sheldon et al. 2020; Supp. Table S6). The most parsimonious explanation is that the previous $\Delta^{13}C_{plant}$–paleobarometer predicts a much greater sensitivity (*S;* expressed in ‰ $\Delta^{13}C_{plant}$/ppm $[CO_2]$; Supp. Table S5) than is

observable in nature; this demonstrates that $[CO_2]$ is not the main driver of $\Delta^{13}C_{plant}$ values based on highly-selected, single or few-species experiments over limited ranges in $[CO_2]$ (e.g. Van de Water et al. 1994; Peñuelas & Estiarte 1997; Saurer et al. 2004). By sampling a wide range of taxa, we show that the discrepancy between our $\Delta^{13}C_{plant}$ values and those expected based on a $\Delta^{13}C_{plant}$-paleobarometer are not due to the taxa used and that $\Delta^{13}C_{plant}$ values vary between taxa. In other words, $\Delta^{13}C_{plant}$ 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' $\Delta^{13}C_{plant}$ 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 $[CO_2]$.

Ancient bio-atmosphere implications

Our results indicate we may be able to use $\delta^{13}C_{plant}$ values to reconstruct paleo-$\delta^{13}C_{CO2}$ values as experimented with in previous studies (i.e. Arens et al. 2000; Fig. 1d) given that the other tested environmental factors were not significant drivers of $\delta^{13}C_{plant}$ (Supp. Table S6). We suggest that using species-specific plant-atmosphere isotope relationships to track $\delta^{13}C_{CO2}$, 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 relation by Arens et al. (2000; Equation 3) does not account for variability in c_i/c_a within or between plant lineages. While this "universal" relationship based on a range of species works, we found that the responses of each species in our historical dataset to changing $\delta^{13}C_{CO2}$ were different (as represented by equations relating the $\delta^{13}C_{plant}$ values of each species to $\delta^{13}C_{CO2}$ values; Supp. Table S4). The woody angiosperm, *Populus tremuloides*, behaved similarly to the generalized relationship found in the Arens et al. (2000) study, but the other species tested, all gymnosperms, had vastly different responses in $\delta^{13}C_{plant}$ values to changing $\delta^{13}C_{CO2}$. This highlights the importance of evolution in response to changing climate and atmospheric variables.

For plants with pre-instrument or fossil records, modern $\Delta^{13}C_{plant}$ 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 $[CO_2]$ sources and fluxes in deep time (Franks et al. 2014). With the depth and breadth of our study, we provide robustly constrained $\Delta^{13}C_{plant}$ values of several species (*Thuja occidentalis, Thuja plicata, Pinus strobus, Populus tremuloides*), and validate that $\Delta^{13}C_{plant}$ values are approximately constant. Although carbon isotope discrimination is complex and influenced by many factors (Diefendorf & Freimuth 2017), in tandem with previous works (Mervenne 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 three months) do not have predictive relationships with $\Delta^{13}C_{plant}$ values (Supp. Table S6). This means that while there are diagenetic and preservation-related factors to take into account before using $\delta^{13}C_{plant}$ to reconstruct paleo-$\delta^{13}C_{CO2}$, it is plausible to look at relative perturbations in $\delta^{13}C_{CO2}$ in time using plants over time scales 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 $\delta^{13}C_{CO2}$ 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 $\delta^{13}C$ values, which assumed no confounding climate factors (e.g. Sheldon et al. 2020; Supp. Table S6). While $\Delta^{13}C_{plant}$ values should not be used to reconstruct environmental drivers like $[CO_2]$, 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, like 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 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. 2011; 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 CO_2.

If $\Delta^{13}C_{plant}$ values are inherent to species, soil chemistry, a catchment of aboveground ecosystem inputs, may demonstrate relationships with environmental drivers related to the biogeography of plant adaptation and distribution (Cornwell et al. 2018). Previous works have demonstrated that $\Delta^{13}C_{plant}$ values provide insight into plant health associated with measurable genotypic and edaphic effects (Reich et al. 2006); to isolate the effects of these variables, additional future studies could examine foliar to critical zone carbon isotope variability over natural experiment of Industrialization 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 $\Delta^{13}C_{plant}$ values to understand plant biochemistry in response to things like water stress, yield, and growth success and strategically manage landscapes to maximize plants as a biological carbon sink.

Author Contributions

NDS and SYS conceptualized the study. RAS and SYS were responsible for data curation. RAS and NDS did the formal analysis. NDS and SYS were responsible for funding acquisition, project administration, provided resources, and supervision to RAS. RAS was primarily responsible for the investigation and did 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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Figures and Tables

Figure 1. Evolution of $\delta^{13}C_{CO2}$ (‰ VPDB, as compared to the Vienna PDB standard for calibration) (a) over the Cenozoic, 60 million years ago (Ma) to present, with red diamonds representing values reconstructed using planktonic foraminifera and blue diamonds representing values reconstructed using benthic foraminifera (Tipple et al. 2010), (b) over the last 12,000 years with white diamonds representing $\delta^{13}C_{CO2}$ from ice cores (Elsig et al. 2009, Bauska et al. 2018), (c) over the past 800 years with white diamonds representing $\delta^{13}C_{CO2}$ from ice cores (Elsig et al. 2009, Bauska et al. 2018), (d) over Industrialization, 200 years ago to present, including $\delta^{13}C_{CO2}$ values as measured from Mauna Loa Observatory (MLO; Keeling et al. 2001, Keeling et al. 2005, Eggleston et al. 2016) and $\delta^{13}C_{CO2}$ values reconstructed using $\Delta^{13}C_{plant}$ values from six species we collected (n $\geq$ 10 specimens).

Figure 2. $[CO_2]$ values plotted against $\Delta^{13}C_{plant}$ 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 $\Delta^{13}C_{plant}$ vs. $[CO_2]$ over Industrialization, while the inner panel shows the range and distribution of $\Delta^{13}C_{plant}$ values for this species. Each of the species occupies different geographic ranges and different ranges of climatic variability, but none shows a significant $\Delta^{13}C_{plant}$ response to rising $[CO_2]$ over the period of Industrialization.

Figure 3. $\Delta^{13}C_{plant}$ values of plants divided by growth form, as collected from 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 $\Delta^{13}C_{plant}$ values. Boxes show 75th percentile of data, while whiskers show remaining 25th percentile of data.

Figure 4. $[CO_2]$ (ppm) values as measured at MLO ($[CO_2]_a$), compared to reconstructed $[CO_2]$ values ($[CO_2]_r$) based on the proposed $\Delta^{13}C_{plant}$-paleobarometer (Equation 4; Schubert & Jahren 2012 (S&J2012); Cui & Schubert 2016). Solid line shows trendline for our data ($[CO_2]_r = 0.09 (\pm 0.09)*[CO_2]_a + 244.28 (\pm 44.61)$; $R^2 = 0.00$, p-value = 0.63), while dashed line shows expected relationship if reconstructed $[CO_2]$ values were equal to measured $[CO_2]$ values ($[CO_2]_r = [CO_2]_a$).

Supporting Information

Fig. S1 $[CO_2]$ versus $\Delta^{13}C_{plant}$ values for historical species with medium-resolution records of Industrialization.

Fig. S2 $\Delta^{13}C_{plant}$ values of plants by genus.

Fig. S3 $\Delta^{13}C_{plant}$ values of plants by family.
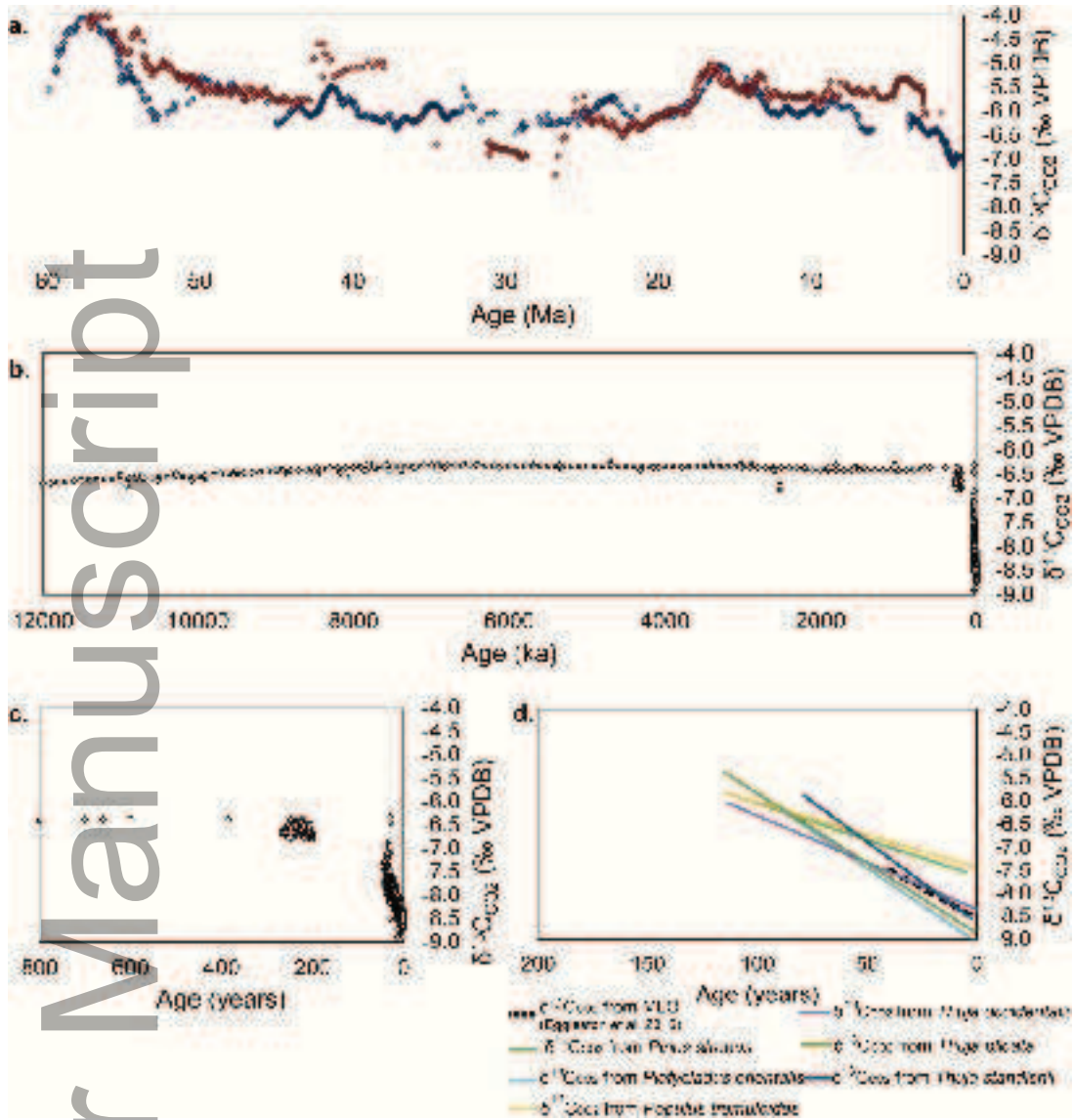

Table S1 All data in .xlsx file.

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 $\Delta^{13}C_{plant}$ values for different taxa in this study.
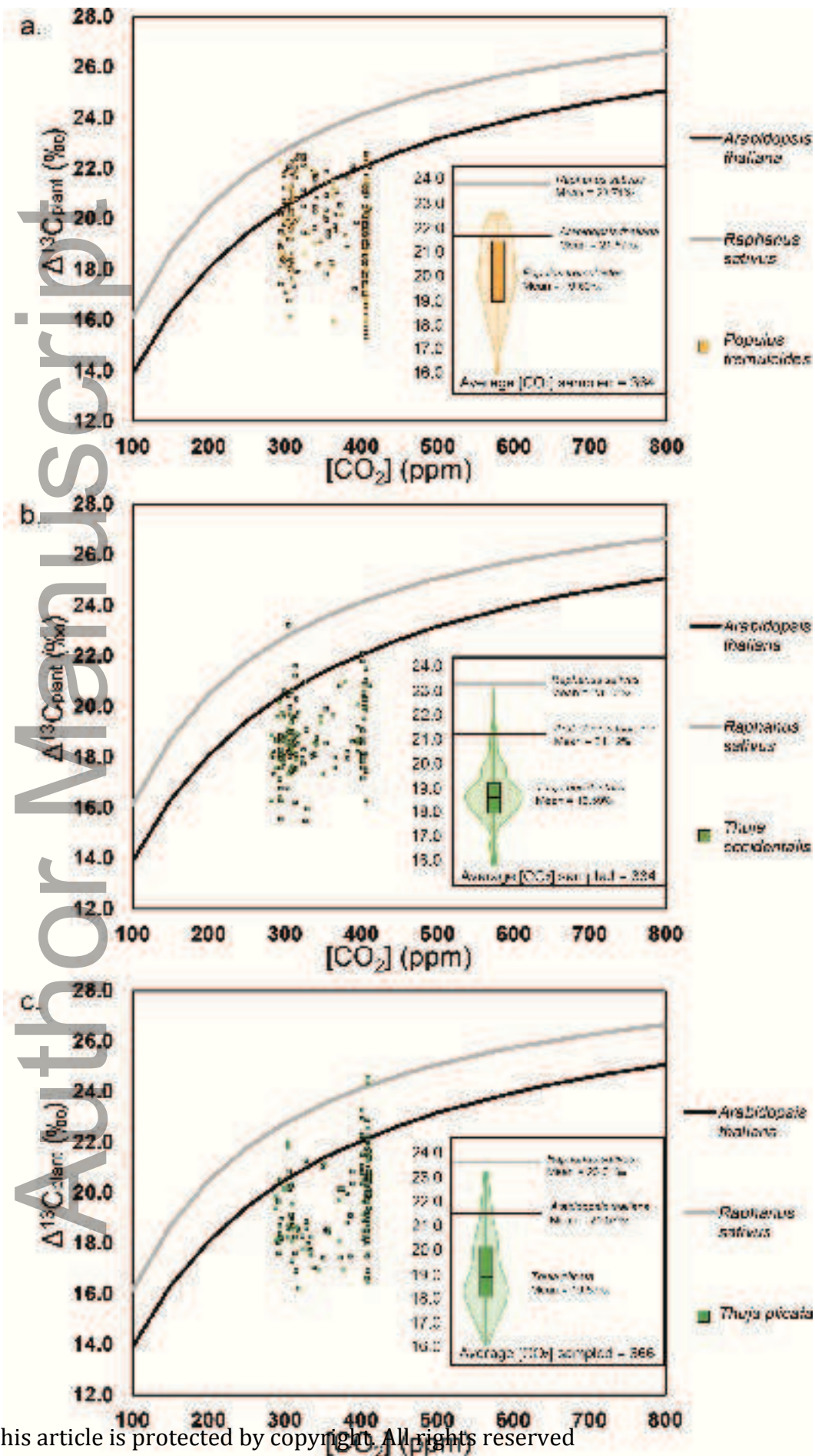
Table S4 The relationship between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{plant}$ values for the historical specimens spanning Industrialization.

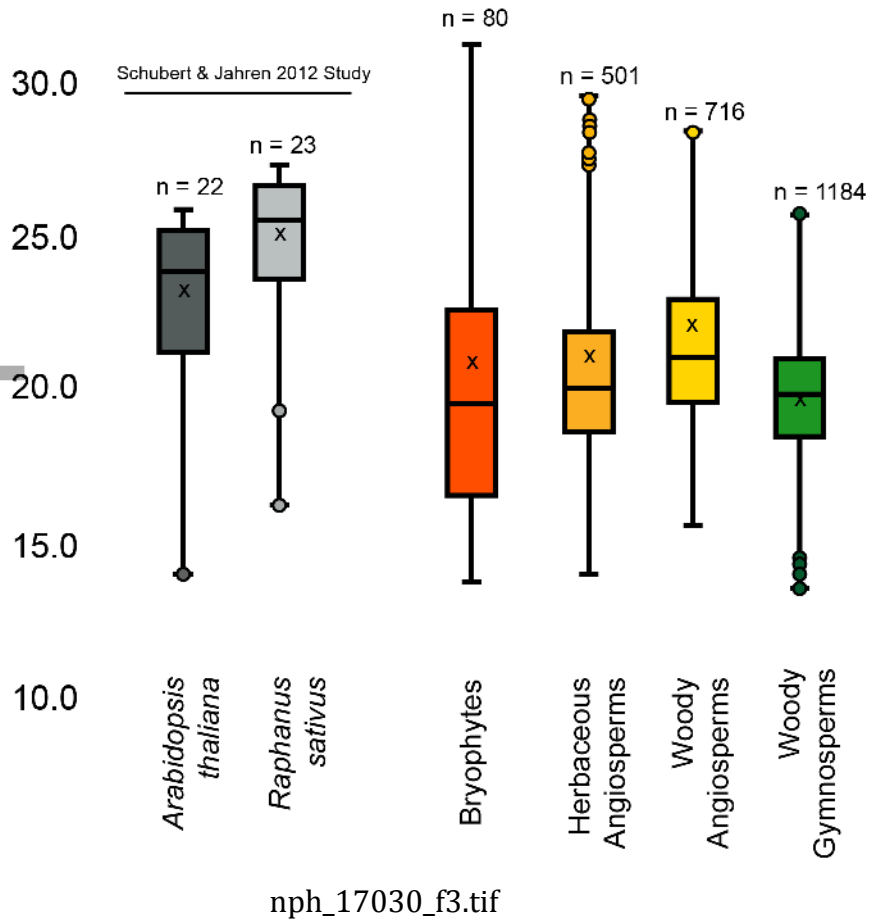**Table S5** Sensitivity (S given as ‰ ppm^{-1}) for eight species with long historical record.

Table S6 The relationship between $\Delta^{13}C_{plant}$ values and non-barometric climate variables for historical and modern species sampled in this study.

nph_17030_f1.tif
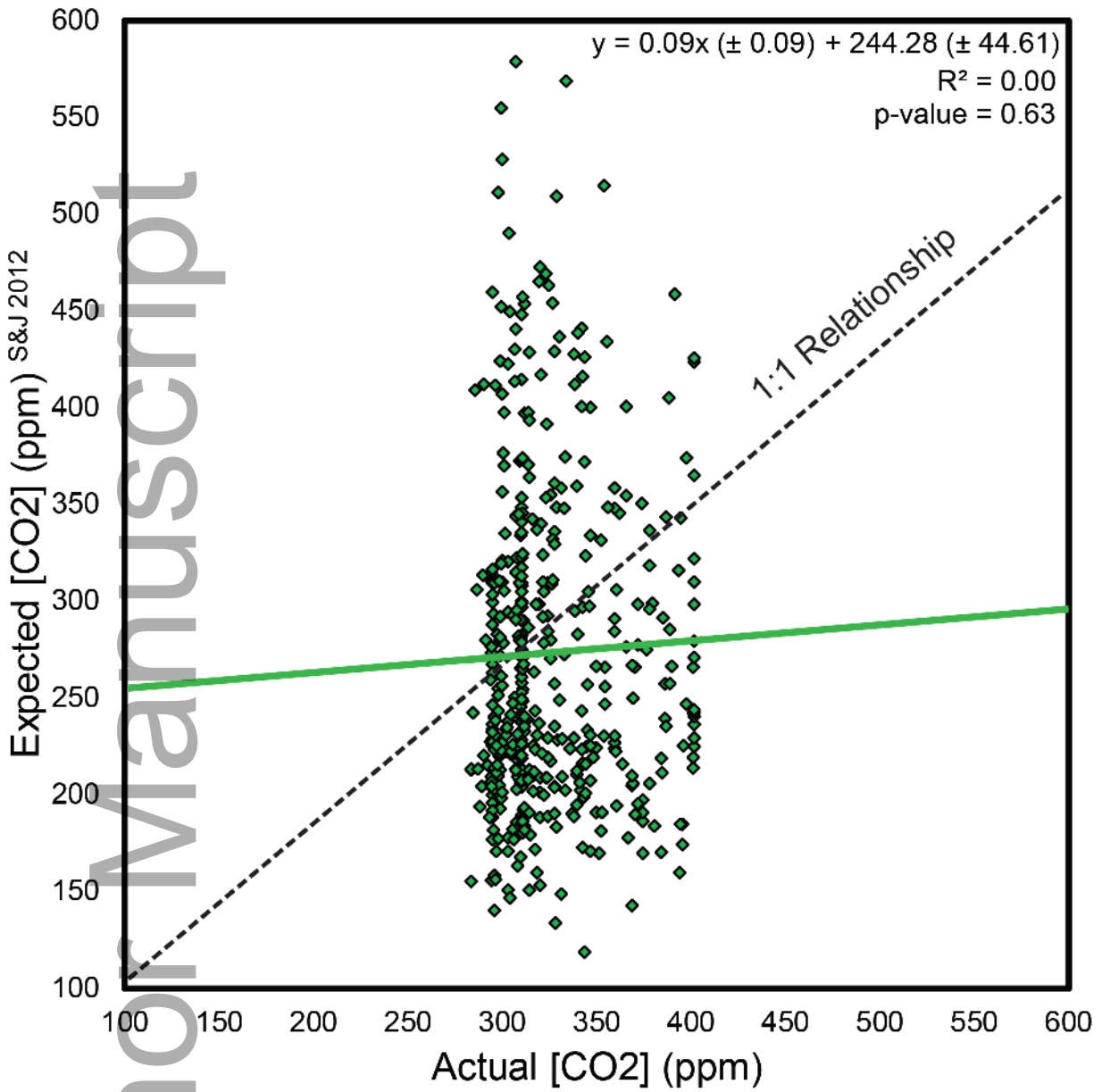
nph_17030_f2.tif

nph_17030_f3.tif

y = 0.09x (± 0.09) + 244.28 (± 44.61)
R² = 0.00
p-value = 0.63

1:1 Relationship

Expected [CO2] (ppm) S&J 2012

Actual [CO2] (ppm)

nph_17030_f4.tif