Heritable Changes in Physiological Gas Exchange Traits in Response to Long-Term, Moderate Free-Air Carbon Dioxide Enrichment

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Atmospheric carbon dioxide ([CO₂]) concentrations significantly alter developmental plant traits with potentially far-reaching consequences for ecosystem function and productivity. However, contemporary evolutionary responses among extant plant species that coincide with modern, anthropogenically driven [CO₂] rise have rarely been demonstrated among field-grown plant populations. Here we present findings from a long-term, free-air carbon dioxide enrichment (FACE) study in a seminatural European grassland ecosystem in which we observe a differential capacity among plant species to acclimate intrinsic water-use efficiencies (WUEs) in response to prolonged multigenerational exposure to elevated [CO₂] concentrations. In a reciprocal swap trial, using controlled environment growth chambers, we germinated seeds from six of the most dominant plant species at the FACE site [Arrhenatherum elatius (L.), Trisetum flavescens (L.), Holcus lanatus (L.), Geranium pratense (L.), Sanguisorba officinalis (L.), and Plantago lanceolata (L.)]. We found that long-term exposure to elevated [CO₂] strongly influenced the dynamic control of WUE in the first filial generations (F₁) of all species as well as an unequal ability to adapt to changes in the [CO₂] of the growth environment among those species. Furthermore, despite trait–environment relationships of this nature often being considered evidence for local adaptation in plants, we demonstrate that the ability to increase WUE does not necessarily translate to an ecological advantage in diverse species mixtures.

Keywords: elevated CO₂, acclimation, intrinsic water-use efficiency, FACE, grasslands, leaf gas exchange

INTRODUCTION

Processes that govern guard cell responses to environmental stimuli and the anatomical, morphological, and physiological responses that are driven by both biotic and abiotic pressures have significant implications for interpreting plant–atmosphere interactions (Franks et al., 2012; Franks et al., 2017). Of particular relevance is how plants will adapt gas exchange rates in response to rapidly increasing atmospheric carbon dioxide [CO₂] concentrations as alterations in this particular atmospheric gas have had profound effects on plant adaptation and evolution in the past (Brodribb
et al., 2009; Haworth et al., 2011; McElwain et al., 2009; Leakey and Lau, 2012; Raven et al., 2008).

However, despite the influence of [CO$_2$] as a driver of plant evolution historically, there remains little evidence that modern-day plant taxa have or are developing evolutionary responses or adaptations to contemporary [CO$_2$] increases (Leakey and Lau, 2012). Yet, growth at elevated [CO$_2$] concentrations has often been shown to provoke morphological (McElwain and Chaloner, 1995; Royer, 2001; Woodward and Kelly, 1995) and physiological (Drake et al., 1997; Ainsworth and Long, 2005) stomatal acclimation responses, which limit water loss, maximize carbon acquisition, increase photosynthetic rate, and increase water-use efficiencies (WUEs) through increasing diffusional resistance (Ainsworth and Rogers, 2007).

Acclimation responses to increased [CO$_2$], specifically those associated with leaf gas exchange rates, have been demonstrated in numerous studies and are typically the result of long-term exposure to elevated [CO$_2$], often resulting in alterations to resource allocation patterns that directly influence leaf photosynthetic and gas exchange rates (Anderson et al., 2001; Chen, 2005; Lee et al., 2011; Rogers and Ellsworth, 2002). Despite the large number of studies that have described acclimation responses to elevated [CO$_2$], there are also some studies that demonstrate no such response (Bader et al., 2010; Crous et al., 2011; Herrick and Thomas, 2001; Leakey et al., 2006; Usuda, 2006), and consequently, there may exist a differential acclimation capacity to increasing [CO$_2$] among modern plant species.

Notwithstanding the species-specific variation in responses, meta-analytical studies of carbon dioxide enrichment experiments demonstrate a reduction in stomatal conductance rates among modern plant species of approximately 20% when exposed to elevated [CO$_2$] concentrations of up to 600 ppm (Ainsworth and Rogers, 2007; Drake et al., 1997; Long et al., 2004; Wullschleger, 1993). However, the lack of stomatal density and/or pore size responses in FACE studies (Ainsworth and Rogers, 2007) argues for a decoupling of morphological and physiological traits over the typical range of [CO$_2$] enrichment concentrations. It has been argued that more derived plant groups, specifically angiosperm species that are the focus of most FACE studies, may be uniquely equipped to respond to [CO$_2$] enrichment via active physiological control as they possess unique mechanisms for detecting and responding to increases in [CO$_2$] that are absent from earlier diverging lineages (Brodribb et al., 2009). Opposingly, more recent studies suggest that a diversity of stomatal regulation and environmental sensitivity exists that does not always group simply along phylogenetic lines (Elliott-Kingston et al., 2016; Mcausland et al., 2016). Whatever the mechanisms might be, highly dynamic stomatal control may confer an important WUE advantage to taxa in terms of their resilience to the increasingly variable climatic conditions predicted in the near future (Hetherington and Woodward, 2003).

It may be the case due to the observed differential capacities of individual species to increase WUE that some species will be better suited to future environments than others as increased WUEs will alter the ecological fitness of some taxa in comparison to neighboring competitors (Blumenthal et al., 2013; Grossman and Rice, 2014; Haus et al., 2018; Huxman and Smith, 2001).

Here we test the hypothesis that species endemic to a seminatural grassland community will show an enhanced capacity to improve intrinsic WUE WUE$_i$ under elevated [CO$_2$] concentrations as a consequence of long-term growth at a marginal FACE CO$_2$ enrichment level (480 ppm). We examine if a 17-year enrichment period under FACE conditions (Jäger et al., 2003) led to heritable adaptations of some grassland species rather than all and test if the detected responses persisted when returned to growth under ambient [CO$_2$]. We use plant growth chambers to perform a reciprocal swap experiment in which the offspring of plants established under elevated FACE conditions were grown at ambient [CO$_2$] concentrations while the offspring (F$_1$ generations) of those grown under ambient field conditions were grown under elevated [CO$_2$] concentrations. We therefore aim to determine the heritability of particular physiological traits indicating the potential for genetic or epigenetic adaptations. We uniquely assess the potential of modern-day plant evolutionary responses to contemporary [CO$_2$] rise.

**MATERIALS AND METHODS**

**FACE Site and CO$_2$ Enrichment System**

The study site (°32’N and 8°41.3’E at an elevation of 172 m above mean sea level) is located on the outskirts of Leihgestern, close to the city of Giessen in the federal state of Hesse, Germany. Situated on a flood plain of the Lücknbach rivulet, the site covers an area of 4.5 ha. As of 1997, six of the most ecologically similar plots from an original set of 16 previously monitored 100-m$^2$ plots were selected as the locations for three ring pairs (three control rings and three CO$_2$-enriched rings), with each treatment being assigned to one ring per block at random. CO$_2$ enrichment is carried out during daylight hours year-round to +20% above ambient.

Vegetation is classified as an *Arrhenatheretum elatioris* (L.) (Br.-Bl.) *Filipendula ulmaria* (L.) subcommunity (Kammann et al., 2005) and is dominated by the grass species *Arrhenatherum elatius* (L.) and *Holorus lanatus* (L.), with *Sanguisorba officinalis* (L.) and *Plantago lanceolata* (L.) being among the most dominant forbs. The soil is a Fluvis Gleysol with a texture of sandy clay loam over a clay layer at varying depths (FAO classification). A full description of the Giessen FACE site is provided by Jäger et al. (2003).

**Gas Exchange Measurements and Species Selection**

Leaf gas exchange measurements were conducted using a CIRAS-2 portable photosynthesis system and PLC (6) cuvette attachment (PP-Systems, Amesbury, MA, USA). A combination of cuvette head plate attachments (4.5 cm$^2$, 2.5 cm$^2$, and 1.29 cm$^2$) was used to maximize the leaf area available for measurements while reducing the amount of uncovered window space in the cuvette head. All gas exchange measurements were taken between 09:00 and 12:00 in the field, and in all cases, conditions in the cuvette head were set to maintain vapor pressure deficit (VPD) below 12 mb (1.2 kPa), leaf temp at 22°C, CO$_2$ concentration at either 400 or 480 ppm, and air flow through the cuvette at 200 ml min$^{-1}$.

Initially, photosynthetic irradiance curves (Pn/I) were run to establish the saturating light for photosynthesis. In this case,
Pn (photosynthetic rate) was allowed to settle at maximum PAR (photosynthetically active radiation) of 2,000 μmol (photon) m\(^{-2}\) s\(^{-1}\) before applying a sequence of light settings (1,600; 1,200; 1,000; 800; 600; 400; 200; 100; 50; 0) with an imposed minimum time step of 120 s at each set point. Light-saturated photosynthetic rate (\(A_{sat}\)) was then calculated using the methods of Norman et al. (1991). Pn/I curves were carried out on two plants for every species in every treatment (giving a total of eight light curves per species), and the maximum \(A_{sat}\) value recorded for each species was then used as the set PAR value for all other gas exchange measurements. In application of the above cuvette conditions, both \(A_{sat}\) and stomatal conductance (\(g_{s[opp]}\)) were recorded as spot measurements under optimal conditions from three plants per species in all treatments, with measurements taken from at least three leaves per plant. For each species, recordings where taken after both \(A_{sat}\) and \(g_{s[opp]}\) had reached steady state under cuvette conditions (approximately 30 min).

Changes in WUE, values were measured by imposing a series of stepped increases in \([\text{CO}_2]\) concentrations. Leaves clamped by the cuvette were allowed to settle until stomatal conductance rates stabilized at 400 ppm \([\text{CO}_2]\), and once measurements were recorded at this initial concentration, a series of step changes was imposed (200; 400; 750; 1,000; 2,000 ppm). At each step, \(A_{sat}\) and \(g_{s[opp]}\) were allowed to reach steady state before physiological measurements were recorded. WUE, was then calculated as the ratio of \(A_{sat}g_{s[opp]}\) (Von Caemmerer, 2000). All measurements were carried out on the youngest, fully expanded leaves of herbaceous forbs and the flag leaf of grass tillers.

Species examined in this study include Arrhenatherum elatius (L.), Trisetum flavescens (L.), Holcus lanatus (L.), Geranium pratense (L.), Sanguisorba officinalis (L.), and Plantago lanceolata (L.), all of which are among the most dominant grass and herb species found at the Giessen FACE site.

**Laboratory Processing and Stomatal Morphological Measurements**

Plant specimens were randomly harvested from the six FACE rings on the May 21, 2010. Once harvested, plants were dried and pressed before being stored in paper envelopes in a fume hood (extraction rate of 450 m³/h) at room temperature until samples could be processed. Maximum stomatal conductance (\(g_{max}\)) measurements are derived from \(n = 5\) stomatal density counts, \(n = 5\) counts of stomatal pore length, and \(n = 5\) counts of guard cell width of the abaxial leaf surface, in the case of hypo-stomatus species, and the summed values of both leaf sides in the case of amphi-stomatus species.

Calculation of theoretical \(g_{max}\) was carried out according to the protocol of McElwain, Yiots, and Lawson (2016). For all species, the following formula was applied to stomatal data recorded on the adaxial and/or abaxial leaf surface:

\[
g_{max} = \frac{dw \cdot SD \cdot pa_{max}}{pd + \frac{\pi}{2} \sqrt{\frac{pa_{max}}{\pi}}} \tag{1}
\]

Where \(dw\) = diffusivity of water vapor at 25°C (0.0000249 m² s⁻¹), \(v\) = molar volume of air (0.0224 m³ mol⁻¹), \(SD =\) stomatal density (m⁻²). As it was not possible to determine the precise pore depth for the species assessed in this study, pore depth is considered equivalent to half the width of an inflated, fully turgid guard cell (Franks and Beerling, 2009a, 2009b).

Leaf morphological measurements (stomatal density, stomatal pore length, and guard cell width) were assessed for each leaf from the exact position used for leaf physiological measurements using either clear nail varnish impressions or epifluorescent microscopy. In the case of epifluorescent microscopy, five photomicrographs were recorded at x200 magnification using a Leica (DMLB, Wetzlar, Germany) epifluorescent microscope and auto-Montage (v.5.03). The SD was estimated by placing a 0.09-mm² grid on the image for each photomicrograph using AcQuis (v.4.0.1.10; Syncroscopy Ltd., Cambridge, UK). In the case of nail varnish impressions, the same number of photo-micrographs was recorded at x200 magnification as before but under standard light microscopy.

**Growth Chamber Conditions**

Seeds for all six species were collected in August 2014. For each species, seeds were harvested at random from a minimum of five maternal plants from each of the three ambient and three elevated FACE rings that had been under continuous treatment for 18 years. To capture as much inherent within-species diversity as possible, seeds were taken from widely spaced maternal plants to account for the potential clustering of interrelated species. Harvested seeds for each of the five species where mixed thoroughly and stored in aluminum foil before being transferred to growth chambers for germination and experimental trials.

Seeds harvested from the six rings (three ambient and three elevated) at the University of Giessen’s FACE site were then germinated and grown in two Conviron BDW-40 (Winnipeg, MB, Canada) walk-in growth chambers at University College Dublin's Program for Experimental Atmospheres and Climate (PEAC). In a reciprocal swap experiment, seeds collected from both ambient and elevated rings were grown at 400 ppm (ambient) and 480 (+20%) ppm [CO₂] to determine whether observations of plants grown under elevated [CO₂] in the field would persist when returned to growth under ambient conditions.

Environmental conditions within chambers (Supplementary Table 10) were programmed to match the mean [CO₂] concentrations of the Giessen field site and, in so far as possible, the climate data for the Hessen region over the course of July/August 2014 when field measurements were carried out and leaf samples were harvested (Supplementary Table 11).

Six plants per species (three each from both ambient and elevated FACE conditions) were allowed to establish in 3-liter pots containing a 3:1 potting mixture of multipurpose potting compost (Scotts Horticulture Ltd., Newbridge Co., Kildare, Ireland) and perlite (William Sinclair Horticulture LTD, Chester, UK) and irrigated manually to field capacity every 48 h over the course of the experimental trial (April–December 2014). Plants were positioned randomly within both chambers and rotated on a weekly basis to ensure uniformity of exposure to chamber conditions.
[CO₂] concentrations were controlled in each chamber using a WMA-4 infra-red gas analyzer (PP Systems, Amesbury, MA, USA). Chamber conditions were consistently maintained for the duration of the experiment in a simulated diurnal program over a 16/8-h light–dark photoperiod (5.00–6.00 incandescent light only of 0–300 μmol m⁻² s⁻¹; 6.00–9.00 light intensity rises from 300 to 600 μmol m⁻² s⁻¹; 9.00–17.00 midday light intensity of 600 μmol m⁻² s⁻¹; 17.00–20.00 light intensity decreases 600 to 300 μmol m⁻² s⁻¹; 20.00–21.00 incandescent light only of 0–300 μmol m⁻² s⁻¹). Ambient atmospheric O₂ concentrations were monitored using a PP-systems OP-1 O₂ sensor, and relative humidity was held constant at 70%.

**Data Analysis**

Statistical analysis was performed using R (R Core Team, 2012). A generalized linear model was applied to test the effects of stepped increases in [CO₂] (200; 400; 750; 1,000; 2,000 ppm) on WUEi values, and the influence of either field population source or growth chamber CO₂ was then added individually to the model as interactive terms. Improvements to model fit due to the inclusion of interacting factors were assessed using AIC scores and calculated R² values [R² = 1 - the residual deviance (model deviance)/null deviance].

**RESULTS**

Long-term exposure to elevated [CO₂] at the Giessen FACE site directly influenced plant dynamic WUEi responses (response to CO₂ step changes) when grown in reciprocal swap chamber trials. Stepped increases in [CO₂] from 200 to 2,000 ppm (Figure 1) revealed significant differences (Table 1) between plants grown at either ambient (400 ppm) or elevated (480 ppm) [CO₂] at the Giessen FACE site.

Generalized linear models (Table 1) demonstrated that elevated [CO₂] at the FACE site (WUEi ~ CO₂ x FACE Treatment) was a significant factor in predicting dynamic WUEi responses for all species (p < 0.05). Chamber treatment (WUEi ~ CO₂ x Chamber Treatment) was also a significant factor in the step change responses of H. lanatus, P. lanceolata, S. officinalis, and G. pratense (p < 0.05) but not a significant factor for either A. elatius or T. flavescens. Model fit parameters (AIC and R² values) demonstrated that FACE treatment was a better predictor of dynamic WUEi response than chamber treatment for both A. elatius and T. flavescens, whereas for all other species, chamber treatment was the more significant factor. Thus, results demonstrate a strong link between the WUEi values of F₁ generations in growth chambers and the [CO₂]
TABLE 1 | Generalized linear model outputs predicting the intrinsic water-use efficiency (WUE) response to increasingly elevated atmospheric carbon dioxide ([CO₂]) concentrations (200, 400, 750, 1,000, 2,000 ppm).

| Species               | Coefficients | WUE, CO₂R x chamber treatment | WUE, CO₂R x FACE treatment |
|-----------------------|--------------|--------------------------------|-----------------------------|
|                       | Estimate     | Std. Error | t value | Pr (>|t|) | Estimate     | Std. Error | t value | Pr (>|t|) |
| Anthrenatherum elatius| (Intercept)  | -1.03E-02 | 4.00E-02 | -0.258 | 0.797 | -9.88E-02 | 7.17E-02 | -1.378 | 0.171 |
|                       | CO₂R         | 2.55E-04 | 2.83E-05 | 9.01  | 4.65E-15 | 3.66E-04 | 9.65E-06 | 37.902 | <2e-16 |
| Elevated chamber      | -1.02E-02 | 5.69E-02 | -0.179  | 0.859 | | | |
| treatment             | -1.28E-05 | 4.02E-05 | -0.318  | 0.751 | | | |
| Ambient FACE treatment|             |           |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated chamber treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| AIC                   | -77.605, R² = 0.57, n = 100 | | | | | | | |
| Trisetum flavescens   | (Intercept)  | -4.59E-02 | 5.55E-02 | -0.827 | 0.41  | 5.07E-03 | 2.89E-02 | 0.175 | 0.8611 |
|                       | CO₂R         | 2.88E-04 | 3.91E-05 | 7.351 | 3.00E-11 | 1.23E-04 | 2.04E-05 | 6.059 | 1.74E-08 |
| Elevated chamber      | 2.36E-02 | 7.85E-02 | 0.301  | 0.764 | | | |
| treatment             | -3.74E-06 | 5.53E-05 | -0.675  | 0.501 | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| AIC                   | -1.2432, R² = 0.45, n = 100 | | | | | | | |
| Holcus lanatus        | (Intercept)  | 3.78E-02 | 1.73E-02 | 2.185 | 0.030895 | 1.45E-02 | 3.38E-02 | 0.429 | 0.669 |
|                       | CO₂R         | 1.75E-04 | 1.22E-05 | 14.34 | <2e-16 | 2.42E-04 | 2.38E-05 | 10.175 | <2e-16 |
| Elevated chamber      | -9.52E-02 | 2.45E-02 | -3.894  | 0.000165 | | | |
| treatment             | 2.13E-04 | 1.72E-05 | 12.34  | <2e-16 | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| AIC                   | -2.81, R² = 0.93, n = 100 | | | | | | | |
| Plantago lanceolata   | (Intercept)  | -6.68E-03 | 2.53E-02 | -2.64 | 0.792 | 4.20E-02 | 2.60E-02 | 1.614 | 0.108615 |
|                       | CO₂R         | 2.09E-04 | 1.78E-05 | 11.726 | <2e-16 | 6.20E-05 | 1.83E-05 | 3.384 | 0.000918 |
| Elevated chamber      | 4.49E-02 | 3.26E-02 | 1.378  | 0.17  | | | |
| treatment             | -1.63E-04 | 2.30E-05 | -7.095  | 5.18E-11 | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| AIC                   | -239.09, R² = 0.63, n = 100 | | | | | | | |
| Sanguisorba officinalis| (Intercept)  | -9.90E-03 | 9.82E-03 | 1.008 | 0.3146 | 1.01E-02 | 9.96E-03 | 1.018 | 0.310105 |
|                       | CO₂R         | 1.30E-04 | 6.92E-06 | 18.796 | <2e-16 | 5.81E-05 | 7.02E-06 | 8.273 | 3.13E-14 |
| Elevated chamber      | 3.38E-02 | 1.29E-02 | 1.386  | 0.0017 | | | |
| treatment             | -8.18E-05 | 8.48E-06 | -9.652  | <2e-16 | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| AIC                   | -628.31, R² = 0.76, n = 100 | | | | | | | |
| Geranium pratense     | (Intercept)  | 1.74E-04 | 4.65E-03 | 3.747 | 0.000225 | 2.87E-04 | 4.61E-03 | 5.968 | 8.72E-09 |
|                       | CO₂R         | 5.48E-05 | 3.28E-06 | 16.731 | <2e-16 | 4.14E-05 | 7.59E-06 | 8.182 | 5.05E-10 |
| Elevated chamber      | 1.49E-02 | 6.57E-03 | 2.259  | 0.00478 | | | |
| treatment             | -1.81E-05 | 4.63E-06 | -3.909  | 0.000121 | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| CO₂R x Elevated FACE treatment| |         |         |       | | | |
| Elevated FACE treatment|            |           |         |       | | | |
| AIC                   | -1032.4, R² = 0.63, n = 100 | | | | | | | |

Full tables are presented for comparison where either chamber treatment or free-air carbon dioxide enrichment (FACE) treatment is included as interactive terms. Akaike’s information criterion (AIC) and R² values are presented as indicators of model fit, and parameter estimates of model coefficients are presented to evaluate the direction and relative strength of each predictor. Significant predictors for each species are in bold.

concentrations parent plants were exposed to at the FACE site. It is also apparent that there is variability among the F₁ generations of these six species in their ability to acclimate to changes in [CO₂]. Despite observed physiological responses to moderate [CO₂] enrichment, chamber trials revealed no significant differences in the gₘᵤₓ values for the F₁ generations of H. lanatus, A. elatius, G. pratense, P. lanceolata, or S. officinalis. The only significant difference in terms of gₘᵤₓ was found for T. flavescens. In the case of this species, gₘᵤₓ was significantly reduced for the F₁ generation of plants grown under ambient [CO₂] at the FACE site in response to elevated [CO₂] in growth chambers. In addition, significant differences were found between the F₁ generations of plants grown under ambient or elevated [CO₂] at the FACE when grown under elevated [CO₂] in growth chambers (Table 2, Figure 2). For a complete breakdown of morphological and physiological measurements see Supplementary Material (Tables 1 – 10).

**DISCUSSION**

Of the six species included in this study, growth under elevated [CO₂] at the Giessen FACE site resulted in an enhanced capacity to increase WUE as [CO₂] increased from 200 to 2,000 ppm (Figure 1).
TABLE 2 | Statistical outputs of theoretical maximum stomatal conductance ($g_{\text{max}}$) responses (of combined abaxial and adaxial surfaces) to chamber treatments ([400 ppm]/[480 ppm]) for the generations of plants grown at either ambient or elevated atmospheric carbon dioxide ([CO$_2$]) concentrations in the Giessen free-air carbon dioxide enrichment (FACE) site.

| Species                  | Mean (400 ppm) | Mean (480 ppm) | Relative change in mean within chamber (%) | Relative change in mean between chambers [Ambient populations (%)] | Relative change in mean between chambers [Elevated populations (%)] | Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value | Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value | Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |
|--------------------------|----------------|----------------|-------------------------------------------|-------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| **Arrhenatherum elatius**|                |                |                                           |                                                                   |                                                                     |                                                                                   |                                                                                  |                                                                                  |
| Mean                     | 1659           | 1739           | 4.6                                      | -11.17812626                                                     | -24.39198856                                                       | 0.5345, 0.4647                                                                   | 0.0982, 0.754                                                                    |                                                                                  |
| Standard deviation       | 333.74         | 297.48         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Standard error           | 149.25         | 133.04         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean within chamber (%) |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Ambient populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Elevated populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| **Trisetum flavescens**  |                |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Mean                     | 1938           | 2100           | 7.7                                      | -51.52462862                                                    | -27.32989434                                                       | 0.2727, 0.6015                                                                   | 4.8109, 0.02828                                                                   | 4.8109, 0.02828                                                                  |
| Standard deviation       | 653.05         | 623.73         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Standard error           | 292.05         | 278.94         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean within chamber (%) |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Ambient populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Elevated populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| **Holcus lanatus**       |                |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Mean                     | 1220           | 1268           | 3.8                                      | -19.3                                                             | 24.00174422                                                        | 0.5345, 0.4647                                                                   | 0.8836, 0.3472                                                                    |                                                                                  |
| Standard deviation       | 145.87         | 163.83         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Standard error           | 65.23          | 73.26          |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean within chamber (%) |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Ambient populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Elevated populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| **Plantago lanceolata**  |                |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Mean                     | 1559           | 1741           | 10.5                                     | 2.257053292                                                      | 5.225911813                                                        | 4.8109, 0.02828                                                                   | 2.4545, 0.1172                                                                    | 0.0982, 0.754                                                                    |
| Standard deviation       | 126.51         | 338.13         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Standard error           | 56.57          | 151.21         |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean within chamber (%) |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Ambient populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Relative change in mean between chambers [Elevated populations (%)] |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |
| Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |                  |                |                                           |                                                                   |                                                                     |                                                                                  |                                                                                  |                                                                                  |

(Continued)
This unique physiological response was persistent among offspring and was nonreversible in at least a single generation. However, four of the six species \( (H. \text{lanatus}, \ P. \text{lanceolata}, \ S. \text{officinalis}, \ \text{and G. pratense}) \) also exhibited significant effects of chamber treatment on the \( F_1 \) generation of field-grown plants in terms of this response, and consequently, we find there exists a differential capacity among these species to adapt to changes in growth environment.

As fluctuations in \([\text{CO}_2]\) concentration directly determine photosynthetic rate and, indirectly, affect plant productivity and fitness, it may be considered to act as a selective pressure driving adaptation and evolution \((\text{Bunce}, 2018; \text{Watson-lazowski} \text{et al., 2016})\). However, it is not commonly demonstrated in practice, and how it might influence different plant species and/or functional groups is relatively poorly understood, particularly in the context of whole plant communities and naturally fluctuating environments.

A number of previous studies have documented shifts in the relative abundances of species at the Giessen FACE site in response to elevated \([\text{CO}_2]\). For example, \( \text{Grüters et al. (2006)} \) demonstrated that both \( A. \text{elatius} \) and \( T. \text{flavescens} \) have shown relative increases in dominance in the past, whereas \( \text{G. pratense}, \ P. \text{lanceolata}, \ \text{and S. officinalis} \) have shown a relative decrease.

However, a more recent study by \( \text{Andresen et al. (2018)} \), examining the effects of elevated \([\text{CO}_2]\) on total aboveground biomass (TAB) at this FACE site, has highlighted a positive response to \([\text{CO}_2]\) enrichment and an overall gain in TAB for the forb species over the full 17-year period. Differences in TAB between species and functional groups (grasses and forbs) were strongly modified by climatic extremes and in particular an unusually late and severe frost episode. This frost event was followed by a reduced abundance of forbs relative to grasses under elevated \([\text{CO}_2]\) for 2 years \( (2001 \text{ and 2002)} \). It is asserted that the damage caused to forb species during a critical phenological stage triggered an initial negative response to elevated \([\text{CO}_2]\), an effect which was not observable for grass species. The implications of the frost event persisted for a further 8 years when forb species began to show a long-term positive response to \([\text{CO}_2]\) enrichment and an overall gain in TAB.

By comparison with the most recent FACE study \((\text{Andresen et al., 2018})\), our findings are contradictory to general assumptions that increasing WUE, should necessarily confer an ecological advantage under future \([\text{CO}_2]\) regimes and, while it does seem to be the case that species preadapted to elevated \([\text{CO}_2]\) show improved WUE, under increasingly elevated \([\text{CO}_2]\), our own study has shown that the ability to adapt to sudden changes in the growth environment is not equal among species. In that sense, the implications of environmental change for diverse plant communities are not clearly understood. Complementary to the Andresen study, an assessment of how a potential \([\text{CO}_2]\) fertilization effect \((\text{CFE})\) at this site might interact with additional climatic variables \((\text{temperature, ground water, vapor pressure deficit, etc.)})\) was done. Results demonstrated that increased atmospheric \([\text{CO}_2]\) increases grassland biomass production under

| TABLE 2 | Continued |
|----------|-------------|-------------|-------------|-------------|-------------|
|          |              | \( g_{\text{max}} \) (TOTAL) mmol m\(^{-2}\) s\(^{-1}\) \((i = 0.5) \) |              |              |              |              |              |
|          | (400 ppm)    | Ambient \([\text{CO}_2]\) | Elevated \([\text{CO}_2]\) | (408 ppm)    | Ambient \([\text{CO}_2]\) | Elevated \([\text{CO}_2]\) |
| Sanguisorba officinalis | Mean       | 1098.2       | 1419       | 1040.5       | 1353       |
|          | Standard deviation | 149.21    | 204.47    | 184.94       | 298.4       |
|          | Standard error       | 66.72    | 91.44    | 82.7         | 133.44      |
|          | Relative change in mean within chamber (%) | 22.6     | 23.1     |              |              |
|          | Relative change in mean between chambers [Ambient populations (%)] |              | -5.5451086 |              |              |
|          | Relative change in mean between chambers [Elevated populations (%)] |              | -4.8780478 |              |              |
|          | Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value | 4.8109, 0.02828 | 2.4545, 0.1172 |              |              |
|          | Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |              | 0.0982, 0.754 |              |              |
|          | Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |              | 0.0109, 0.9168 |              |              |
| Geranium pratense | Mean       | 2160       | 1638       | 1731       | 1755       |
|          | Standard deviation | 536.51    | 240.14    | 277.54       | 643.2       |
|          | Standard error       | 239.93    | 107.39    | 124.12       | 287.65      |
|          | Relative change in mean within chamber (%) | -31.9     | 1.4      |              |              |
|          | Relative change in mean between chambers [Ambient populations (%)] |              | -24.7836222 |              |              |
|          | Relative change in mean between chambers [Elevated populations (%)] |              | 6.666666667 |              |              |
|          | Kruskal-Wallis (within chamber ambient vs elevated populations): chi-squared, P-value | 2.4545, 0.1172 | 0.0109, 0.9168 |              |              |
|          | Kruskal-Wallis (between chambers ambient populations): chi-squared, P-value |              | 1.8436, 0.1745 |              |              |
|          | Kruskal-Wallis (between chambers elevated populations): chi-squared, P-value |              | 0.0109, 0.9168 |              |              |

The beta function \( (i) \) in this case is an estimation of guard cell depth, which in this case is assumed to be half of the guard cell width (0.5). Significant effects for each species are in bold.
average environmental conditions, but the effect is reduced as local conditions become substantially more extreme (wetter, drier, or hotter than average; Obermeier et al., 2016). Here it was suggested that increasing WUE was advantageous in situations where plants may need to cope with additional environmental stressors, such as increased drought stress. However, in situations where water availability is not limiting, the benefit from increased WUE is less obvious. In this study, it was considered that plants adapted to adequate water supply should show improved WUE under elevated \([\text{CO}_2]\) in combination with reduced water availability. This assertion we can confirm with our own results; however, it was also demonstrated that although increases in WUE were greatest in plants exposed to extreme conditions, the CFE in terms of biomass was greater under more moderate environmental conditions where, theoretically, increased WUE is not necessarily an advantage. These results would seem to reinforce our own conclusions and those of Andresen et al. (2018) in that plants may show an enhanced capacity to increase WUE under elevated \([\text{CO}_2]\) in combination with reduced water availability. This assertion we can confirm with our own results; however, it was also demonstrated that although increases in WUE were greatest in plants exposed to extreme conditions, the CFE in terms of biomass was greater under more moderate environmental conditions where, theoretically, increased WUE is not necessarily an advantage. These results would seem to reinforce our own conclusions and those of Andresen et al. (2018) in that plants may show an enhanced capacity to increase WUE under elevated \([\text{CO}_2]\), all other conditions remaining equal, but where moderate environmental conditions prevail, this adaptation is not particularly useful in terms of increasing TAB.

A review of plant responses to free-air \([\text{CO}_2]\) enrichment indicates significant capacity for acclimation among modern plant taxa (Anderson et al., 2001; Drake et al., 1997; Maherali et al., 2002) and, in some cases, the capacity for those acclimations to be observable among offspring (Grossman and Rice, 2014; Haus et al., 2018; Li et al., 2019; Nakamura et al., 2011; Saban et al., 2019). However, there are a number of studies that specifically describe a lack of any acclamatory responses (Bader et al., 2010; Crous et al., 2011; Herrick and Thomas, 2001; Leakey et al., 2006; Usuda, 2006). Results presented here may go some way toward explaining this discrepancy as we demonstrate that adaptive responses are not uniform among species (Figure 1). Furthermore, there may be poor detection in some cases, and subtle adaptations may go overlooked. This latter point we account for in exposing species to instantaneous increases in \([\text{CO}_2]\) concentrations, as small but definite...
trait responses may not be readily observable in response to moderate changes in environmental factors but may become apparent under climatic extremes. This is significant as even subtle, almost imperceptible changes in plant gas exchange behavior may strongly influence species responses to extreme weather/climate events in the future.

CONCLUSION

The aim of this study has been to determine whether or not any long-term adaptive/acclamatory responses to [CO2] enrichment have occurred under FACE conditions since enrichment began at the Giessen FACE site in 1998. Of the six species included in this study, we observed that long-term exposure to elevated [CO2] strongly influenced the dynamic control of WUE. This response was unique to plants that had been grown under 480 ppm [CO2] at the Giessen FACE site and persisted among the F1 generations of those species even when returned to growth at 400 ppm [CO2] in growth chambers. We conclude that this particular response was potentially inheritable in that it was observable in the F1 generation and irreversible despite the imposition of altered growth [CO2] concentrations as imposed by reciprocal swap trials.

However, we also observed that plants germinated from seed in growth chambers did not respond to the altered growth conditions uniformly as, despite the influence of FACE conditions, some species were also significantly influenced by chamber treatments. This is a key finding, as despite the evidence for local adaptation in WUE, previous studies of fluctuating TAB at this site demonstrate that an ability to increase WUE does not necessarily translate to an ecological advantage in diverse species mixtures.

REFERENCES

Ainsworth, E. A., and Long, S. P. (2005). What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165, 351–372. doi: 10.1111/j.1469–8137.2004.01224.x

Ainsworth, E. A., and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant Cell Environ. 30, 258–270. doi: 10.1111/j.1365-3040.2007.01641.x

Anderson, L. J., Maherali, H., Johnson, H. B., Wayne Polley, H., and Jackson, R. B. (2001). Gas exchange and photosynthetic acclimation over subambient to elevated CO2 in a C3-C4 grassland. Glob. Chang. Biol. 7, 693–707. doi: 10.1046/j.1354–1013.2001.00438.x

Andresen, L. C., Yuan, N., Seibert, R., Moser, G., Kammann, C. I., Lutterbacher, J., et al. (2018). Biomass responses in a temperate European grassland through 17 years of elevated CO2. Glob. Chang. Biol. 24, 3875–3885. doi: 10.1111/gcb.13705

Bader, M. K.-F., Siegwolf, R., and Körner, C. (2010). Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free-air CO2 enrichment. Planta 232, 1115–1125. doi: 10.1007/s00425-009-1240-8

Blumenthal, D. M., Resco, V., Morgan, J., Williams, D. G., Lecain, D. R., Hardy, E. M., et al. (2013). Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO2 and warming. New Phytol. 200, 1156–1165. doi: 10.1111/nph.12459

Brodribb, T. J., McAdam, S. A. M., Jordan, G. J., and Feild, T. S. (2009). Evolution of stomatal responsiveness to CO2 and optimization of water-use efficiency among land plants. New Phytol. 183, 839–847. doi: 10.1111/j.1469-8137.2009.02844.x

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However, we also observed that plants germinated from seed in growth chambers did not respond to the altered growth conditions uniformly as, despite the influence of FACE conditions, some species were also significantly influenced by chamber treatments. This is a key finding, as despite the evidence for local adaptation in WUE, previous studies of fluctuating TAB at this site demonstrate that an ability to increase WUE does not necessarily translate to an ecological advantage in diverse species mixtures.

REFERENCES

Ainsworth, E. A., and Long, S. P. (2005). What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165, 351–372. doi: 10.1111/j.1469–8137.2004.01224.x

Ainsworth, E. A., and Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant Cell Environ. 30, 258–270. doi: 10.1111/j.1365-3040.2007.01641.x

Anderson, L. J., Maherali, H., Johnson, H. B., Wayne Polley, H., and Jackson, R. B. (2001). Gas exchange and photosynthetic acclimation over subambient to elevated CO2 in a C3-C4 grassland. Glob. Chang. Biol. 7, 693–707. doi: 10.1046/j.1354–1013.2001.00438.x

Andresen, L. C., Yuan, N., Seibert, R., Moser, G., Kammann, C. I., Lutterbacher, J., et al. (2018). Biomass responses in a temperate European grassland through 17 years of elevated CO2. Glob. Chang. Biol. 24, 3875–3885. doi: 10.1111/gcb.13705

Bader, M. K.-F., Siegwolf, R., and Körner, C. (2010). Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free-air CO2 enrichment. Planta 232, 1115–1125. doi: 10.1007/s00425-009-1240-8

Blumenthal, D. M., Resco, V., Morgan, J., Williams, D. G., Lecain, D. R., Hardy, E. M., et al. (2013). Invasive forb benefits from water savings by native plants and carbon fertilization under elevated CO2 and warming. New Phytol. 200, 1156–1165. doi: 10.1111/nph.12459

Brodribb, T. J., McAdam, S. A. M., Jordan, G. J., and Feild, T. S. (2009). Evolution of stomatal responsiveness to CO2 and optimization of water-use efficiency among land plants. New Phytol. 183, 839–847. doi: 10.1111/j.1469-8137.2009.02844.x

AUTHOR CONTRIBUTIONS

AH led the experimental design, carried out the experiment, analyzed the data, and wrote the manuscript. JM and CM were involved in all aspects of the above and provided critical feedback that helped shape the research, analysis, and manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.01210/full#supplementary-material
Heritable Acclimations in Plant WUEi

McElwain, J. C., and Chaloner, W. G. (1995). Stomatal density and index of fossil plants track atmospheric carbon dioxide in the paleozoic. Ann. Bot. 76, 389–395. doi:10.1006/anbo.1995.1112

McElwain, J. C., Wagner, P. J., and Hesselbo, S. P. (2009). Fossil Plant Relative Abundances Indicate Sudden Loss of Late Triassic Biodiversity in East Greenland. Science (80-.). 1554–1556. doi:10.1126/science.1171706

McElwain, J. C., Yotis, C., and Lawson, T. (2016). Using modern plant trait relationships between observed and theoretical maximum stomatal conductance and vein density to examine patterns of plant macroevolution. New Phytol 209, 94–103. doi:10.1111/nph.13579

Nakamura, I., Onoda, Y., Matsushima, N., Yokoyama, J., Kawata, M., and Hikosaka, K. (2011). Phenotypic and genetic differences in a perennial herb across a natural gradient of CO2 concentration. Oecologia 165, 809–818. doi:10.1007/s00442-010-1900-1

Norman, J. M., Welles, J. M., and McDermitt, D. K. (1991). Estimating canopy light-use and transpiration efficiencies from leaf measurements. LICOR Appl. Note 105, 18.

Obermeier, W. A., Lehnert, L. W., Kammann, C. I., Müller, C., Grünhage, L., Luterbacher, J., et al. (2016). Reduced CO2 fertilization effect in temperate C3 grasslands under more extreme weather conditions. Nat. Clim. Chang. 7, 137. doi:10.1038/nclimate3191

Panio, G., Mottoz, R., Mastrandrello, A.M., Marone, D., Cattivelli, L., Giunta, F., DeVita, P. (2013). Molecular mapping of stomatal-conductance related traits in durum wheat (Triticum turgidum ssp. durum). Ann. Appl. Biol. 162, 258–270. doi:10.1111/aab.12018

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rogers, A., and Ellsworth, D. S. (2002). Photosynthetic acclimation of Pinus taeda (lobolly pine) to long-term growth in elevated p CO2 (FACE). Plant Cell Environ. 815–858. doi:10.1046/j.1365-3040.2002.00868.x

Royer, D. L. (2001). Stomatal density and stomatal index as indicators of paleoatmospheric CO2 concentration. Rev. Palaeobot. Palynol. 114, 1–28. doi:10.1016/S0034-6667(00)00074-9

Saban, J. M., Chapman, M. A., and Taylor, G. (2019). FACE facts hold for multiple generations; Evidence from natural CO2 springs. Glob. Chang Biol. 25 (1), 1–11. doi:10.1111/gcb.14437

Usuda, H. (2006). Effects of Elevated CO2 on the Capacity for Photosynthesis of a Single Leaf and a Whole Plant, and on Growth in a Radish. Plant Cell Physiol. 47, 262–269. doi:10.1093/pcp/pcp244

Von Caemmerer, S. (2000). Biochemical Models of Leaf Photosynthesis. Biochem. Model. Leaf Photosynth. 165, 165. doi:10.1016/s07399314

Watson-lazowski, A., Lin, Y., and Miglietta, F. (2016). Plant adaptation or acclimation to rising CO2? Insight from first multigenerational RNA-Seq transcriptome. Glob. Chang Biol. 22 (11), 3760–3773. doi:10.1111/gcb.13322

Woodward, F. I., and Kelly, C. K. (1995). The Influence of CO2 Concentration on Stomatal Density. New Phytol. 131, 311–327. doi:10.1111/j.1469-8137.1995.tb03607.x

Wullschleger, S. D. (1993). Biochemical Limitations to Carbon Assimilation in C3 Plants—A Retrospective Analysis of the A/Ci Curves from 109 Species. J. Exp. Bot. 44, 907–920. doi:10.1093/jxb/44.5.907

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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