Early-growth results within a *Eucalyptus globulus* breeding population suggest limited scope for selection focused on CO2 responsiveness

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**Abstract**

Increasing [CO2] may influence commercial crop and timber yield. While selection of genotypes sensitive to elevated [CO2] (e[CO2]) appears possible in agricultural crops, there is limited evidence for genotype-by-CO2 (G × CO2) interactions in commercial tree species. We examined [CO2] responsiveness in 124 open-pollinated *Eucalyptus globulus* ssp. *globulus* (*E. globulus*) families with the aim of assessing whether G × CO2 interactions are detectable in seedlings for early-age screening. Plants were grown in ambient (a[CO2]; ~ 405 μmol mol⁻¹) and e[CO2] (640 μmol mol⁻¹) and harvested 25 days after germination. Total, shoot, and root dry weights were determined for each plant. Carbon isotopic discrimination against 13C (Δ13C) was determined at the family level. We observed highly significant (*p* < 0.0001) increases in mean total, shoot, and root dry weights. Mixed-model equations were used to estimate the main and interaction effects of the G × CO2 for each mass trait. The main effects from the mixed-model output ([CO2] and individual-tree effects) were significant for all traits. However, [CO2]-by-individual tree interactions were non-significant for all traits, indicating little G × CO2 interaction. A secondary aim was to examine the correlation between greenhouse and mature-age growth from breeding trials that use common families conducted under ambient [CO2]. These correlations were non-significant, suggesting early growth is not necessarily indicative of later-age responses. Our results suggest that while early growth of *E. globulus* is enhanced under e[CO2], genotypes respond relatively uniformly to e[CO2] and little opportunity exists for seedling-based selection at the population level based upon the response of plants during the first weeks of growth.

**Keywords** G × CO2 · *Eucalyptus* · REML · Elevated CO2 · Carbon-isotope discrimination

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**Introduction**

Atmospheric CO2 concentration ([CO2]) currently exceeds 405 μmol mol⁻¹—45% greater than the pre-industrial (< 1750) average of 280 μmol mol⁻¹ (Dlugokencky and Tans 2017)—and projections suggest a doubling by the end of this century (Pachauri et al. 2014). Elevated [CO2] (e[CO2]) directly enhances the rate of photosynthetic assimilation (A) and inhibits stomatal conductance (gₛ), leading to enhanced plant growth and greater water-use efficiency (WUE) (Drake et al. 1997; Ainsworth and Rogers 2007). If translated to entire production forests, the effects of e[CO2] may increase commercial fibre and timber yield (Easterling et al. 2007).

Experimental observations indicate that the growth response of woody plants to e[CO2] varies widely both between and within species (e.g. Amthor 1995; Curtis and Wang 1998; Saxe et al. 1998; Barnaby and Ziska 2012; Baig et al. 2015; O’Leary et al. 2015). The latter—intraspecific
variability of CO₂ responsiveness—presents an opportunity to enhance e[CO₂]-related crops gains through selection of highly responsive genotypes. Identifying significant genotype-by-CO₂ (G×CO₂) interactions is, therefore, relevant to tree breeders.

Although G×CO₂ interactions are known to exist for yield-related traits in agricultural crops (Shimono et al. 2019), evidence for similar interactions in trees is mixed. In a meta-analysis of e[CO₂] studies of juvenile trees in an array of deciduous and evergreen species from both temperate and tropical regions, Resco de Dios et al. (2016) found no evidence of significant G×CO₂ interactions in growth-based traits. While these results reflect those reported elsewhere (see Radoglou and Jarvis 1990; Ceulemans et al. 1995; Johnsen and Seiler 1996; Johnsen and Major 1998; Wang et al. 2000), significant G×CO₂ interactions have been reported in three instances. Mycroft et al. (2009), for example, reported (1) significant size-related G×CO₂ interactions in 29 genotypes of Picea glauca (Moench) Voss from a widely sourced breeding population; (2) using seed from three distinct physiographic provenances, Mohan et al. (2004) identified significant G×CO₂ interactions for size- as well as germination- and mortality-related traits in Acer rubrum L.; and (3) Lindroth et al. (2001) identified significant G×CO₂ in total biomass of Populus tremuloides Michx. The mixed results of G×CO₂ interactions suggest that while intra-specific variation in CO₂ responsiveness cannot be assumed, opportunities do exist for selection of highly responsive genotypes within breeding and natural populations.

The expense and practical challenges of conducting e[CO₂] experiments (Shimono et al. 2019) mean that much of the e[CO₂] literature focuses upon only a small fraction of the total lifespan of forestry crops, often extending to only a few weeks or months. The use of such short-term studies is predicated on the assumption that the CO₂ stimulation reflects an intrinsic physiological, rather than long-term ontological and developmental, process and is expressed by juvenile plants. Nevertheless, the combination of maternal effects in studies of seedling performance or propagation effects in cutting-based experiments, combined with the accumulation of ontogenetic and site-level environmental influences in field-grown plantations, means that short-term studies may not represent later-age growth outcomes. Reconciling the need to cost-effectively identify traits indicative of both response to e[CO₂] and long-term growth presents a considerable challenge for forestry studies. Part of this challenge lies in selecting [CO₂]-responsive traits that are relevant to both juvenile and mature trees.

Although comprising > 20 million ha of plantation forests worldwide (Booth 2013), eucalypts are relatively poorly represented in the e[CO₂] literature. To date, investigation of intra-specific G×CO₂ interactions has focused solely upon Eucalyptus camaldulensis. Although E. camaldulensis exhibits considerable intraspecific variability in phenotypic plasticity (e.g. Gibson and Bachelard 1989, 1994; Gibson et al. 1994), no significant G×CO₂ interactions have been reported for growth-related traits. This apparent lack of G×CO₂ interaction must be interpreted cautiously; two of the three E. camaldulensis studies that tested responses to e[CO₂] (Blackman et al. 2016; Aspinwall et al. 2018) were based upon a narrow window of the species’ natural physiographic range without considering underlying trait plasticity during selection, while replication in the third study (Dillon et al. 2018) was not designed to adequately represent provenance-level effects. Thus, while the wider literature broadly indicates stimulatory eCO₂ effects on eucalypt growth (Wong et al. 1992; Eamus et al. 1995; Roden and Ball 1996; Roden et al. 1999; Ghannoum et al. 2010; Noviyanti et al. 2012; Smith et al. 2012; Crous et al. 2013; Quentin et al. 2015), evidence of G×CO₂ interactions within the genus remains ambiguous.

Eucalyptus globulus Labill. ssp. globulus (hereafter E. globulus) is a globally important species with a total planted area exceeding 2.5 million ha worldwide (Bataglia and Bruce 2017). In Australia, the species constitutes 52% (~500,000 ha) of the national hardwood plantation estate (Downham and Gavran 2018). Eucalyptus globulus exhibits significant adaptive intraspecific plasticity, evidenced by genotype-by-environment (G×E) interactions, for a suite of attributes (Muneri and Raymond 2000; Pita and Pardos 2001; Raymond et al. 2001; O’Reilly-Wapstra et al. 2005) including growth and related traits (Volker and Orme 1988; Dutkowski and Potts 1999; Costa e Silva et al. 2005; Costa e Silva et al. 2006; Callister et al. 2011).

Intrinsic water-use efficiency (iWUE) expressed in terms of discrimination against carbon-13 (Δ₁³C) may be a key trait that captures both growth and physiological responses to e[CO₂] in E. globulus. Under water stress, intraspecific variation in E. globulus growth is negatively correlated to Δ₁³C (Osório and Pereira 1994; Osório et al. 1998; Pita and Pardos 2001). As an integrated measure of the ratio between chloroplastic and atmospheric [CO₂], Δ₁³C reflects both A and gs (Farquhar et al. 1982). Although not distinguishable from Δ¹³C data alone, the impact of relative variation in A and gs on Δ₁³C may be inferred in the context of productivity (Fardusi et al. 2016). For example, positive correlation between Δ¹³C and growth is likely to reflect increased gₛ relative to A. Conversely, negative growth–Δ¹³C correlations likely reflect increased A relative to gₛ.

In E. globulus, a strong positive intraspecific correlation between growth and Δ¹³C, particularly for juvenile plants (Pita et al. 2005), suggests that variation in gₛ plays the principal role in affecting iWUE. Experimental evidence from gas-exchange and growth studies supports this inference and indicates that genotypes expressing the greatest Δ₁³C (lowest iWUE) in well-watered conditions...
exhibit the greatest stomatal and growth responses to drought (Osório and Pereira 1994; Osório et al. 1998; Pita et al. 2001, 2005). That e[CO₂] directly impacts upon gₛ and A suggests Δ¹³C may also be correlated with CO₂ response. If true, in addition to previously stated potential as an early screening tool for E. globulus growth (Bond and Stock 1990; Pita et al. 2005), Δ¹³C may also offer early-age insights to genotypic variation in CO₂ response. This potential has not been tested. Similarly, it is unclear whether the prevalence of G×E interactions for E. globulus growth and related traits translates to population-wide G×CO₂ interactions. Both issues warrant attention as responses to e[CO₂] remain the leading source of uncertainty in modelling productivity of E. globulus in response to projected emissions scenarios (Battaglia and Bruce 2017).

In the present study, we explore the responsiveness of early growth of E. globulus seedlings from 124 open-pollinated families to e[CO₂]. Our primary aim was to rapidly assess potential G×CO₂ interactions within a breeding population. Seedlings were grown in ambient [CO₂] (a[CO₂]) as well as e[CO₂] environments and harvested at a common post-germination age. Total and plant-component weights were determined at the plant level and Δ¹³C at the family level. We anticipated significant intraspecific variation in growth and hypothesised that this variation would be linearly related to Δ¹³C at the family level. Also consistent with the broader literature, we hypothesised that if the relation between plant mass—represented by dry weight—and Δ¹³C differed between [CO₂], that change would be reflected in significant intraspecific G×CO₂ interaction. To test whether the early-growth results are indicative of longer-term field growth, we also compared our greenhouse-based total dry weights to stem-volume data from field trials of the same families used in this study.

**Method**

**Experimental design**

Potted seedlings from 131 families were exposed to two CO₂ concentrations within a split-plot design, comprising two replicates of each {[CO₂]} treatment in separate greenhouse bays. Within treatments, plants were distributed randomly at the family level within 60 rows and 20 columns across two benches in each greenhouse bay (four plots in 2×[CO₂]×2 bays). Plants were destructively harvested at the conclusion of the experimental period. Total and above- and below-ground dry weights were determined for each plant. Stable carbon-isotope discrimination was determined at the family level within each treatment.

**Material and greenhouse methods**

The study population comprised a sample of 131 open-pollinated families of E. globulus. Seeds were sourced from two separate seed orchards managed by commercial-plantation growers FORICO and WAPRES, members of the Tree Breeding Australia (TBA) tree breeding cooperative. Families were selected based on seed availability from later-generation families in TBA members’ production populations. TBA member production populations typically comprise selections from the breeding population managed as open-pollinated seed orchards. As the TBA breeding population is managed using a ‘rolling-front’ strategy rather than discrete generations, the mother trees were of mixed generations, but were predominantly third generation with respect to their wild origins. The TBA breeding strategy initially made use of open-pollinated and, more latterly, control-pollinated mating. The families in this study have ancestral origins in a wide range of provenances, or ‘races’ (sensu Dutkowski and Potts 1999), in Tasmania (including the Bass Strait islands) and Victoria, with known maternal lineages and paternal origins within selected material in the seed orchards (see Kerr et al. 2015 for a detailed description of the TBA breeding population). The pedigree for the families was generated using TBA’s Dataplan software (Dutkowski et al. 2012).

In addition to the pedigree data, later-age growth data were supplied by TBA. These data comprise breeding values (best linear unbiased predictions, BLUPs) for per-hectare volume production at harvest age. Maternal breeding values were supplied for southern Australian regions including Gippsland, Victoria; the Green Triangle region of Victoria and South Australia; Tasmania; Western Australia and an aggregate for the whole of southern Australia. These estimates were obtained using the TBA’s TREEPLAN software and were calculated based on a large amount of information from realised-gain trials aged 3–8 years throughout southern Australia using correlated breeding values for diameter-at-breast-height and height traits. The trials involved both ancestors and descendants of the mothers whose open-pollinated progeny were used in the greenhouse trials.

The early-growth experiment was conducted in four adjacent 16.1-m² (5.35 m × 3.00 m) temperature- and CO₂-controlled greenhouse bays at the Australian National University, Canberra. Plants were grown in 0.6-l pots filled with a medium composted pine bark, coarse river sand and coir (7:2:1) to which slow-release fertiliser (Osmocote Exact Mini) was added at a rate of 1.5 g l⁻¹. After each pot was filled, the growing medium was lightly compressed and topped with a 2-cm layer of seed-raising medium comprising fine composted pine bark and washed river sand (1:1). Seeds from each seedlot were direct sown to nine randomly selected pots in each bay on November 2 and 3, 2017. Pots
were labelled with their respective seedlot code and topped with vermiculite.

Air temperatures within the greenhouse bays were maintained at 18/26 °C night/day cycles consistent with local diurnal cycles. Bays experienced either ambient (a[CO₂]: ~405 µmol mol⁻¹) or elevated [CO₂] (e[CO₂]: 640 µmol mol⁻¹). The latter concentration reflected the mean of CO₂ emissions scenarios at the time of the study’s inception. Leaf samples were taken from each plant at the conclusion of the experiment. Seeds remained for 91 of the 24 (IQR = 11–37) seeds.

A 5-mg leaf sample was taken from each plant and bulked at the plant level for isotopic analyses. Average seed weight was estimated for seedlots using all remaining seeds at the seedlot level for isotopic analyses. Average seed weight of fine roots. Harvested plants were dried at 60 °C for a minimum of 5 days. Root and shoot dry weights were then recorded for each pot when the upper surfaces of the emergent cotyledons were clearly visible. The date of germination and respective north–south row (60 rows) and east–west column (10 columns) position were recorded for each seedling. Twice-daily watering was maintained throughout the growing period. To avoid nutrient limitations, seedlings received twice-weekly applications of liquid macronutrients (Debco Plant Food) and fortnightly applications of micronutrients (Everris Micromax Premium TE).

Zea mays seeds were sown into two 3-l pots within each greenhouse bay for subsequent isotopic analyses following the same cultural methods applied to E. globulus seeds.

Each E. globulus plant was harvested 25 days after germination. During harvesting, plants were immersed in water and then gently freed from the growing medium. Roots had not fully penetrated the available pot space and were easily separated from the growing medium without obvious loss of fine roots. Harvested plants were dried at 60 °C for a minimum of 5 days. Root and shoot dry weights were then estimated separately for each plant to 1-mg precision. A 5-mg leaf sample was taken from each plant and bulked at the seedlot level for isotopic analyses. Average seed weight was estimated for seedlots using all remaining seeds at the conclusion of the experiment. Seeds remained for 91 of the original 131 seedlots. Those seedlots retained a median of 24 (IQR = 11–37) seeds.

Zea mays plants were harvested at the conclusion of the growth experiment. Leaf samples were taken from each Z. mays leaf and bulked at the plant level for isotopic analyses.

**Isotopic measurement**

Bulked E. globulus and Z. mays leaf samples were individually ground to a fine powder within a Retsch ball mill and 5 mg of the resulting powder was then transferred to individual tin cups. The tin cups containing plant matter were combusted in an elemental analyser (Carlo Erba 1110, Milan) and introduced into a mass spectrometer (Isoprime Micromass) as N₂ and CO₂ via a gas chromatograph. Beet (δ¹³C = −24.63‰) and cane (δ¹³C = −10.45‰) sugars were used to calibrate δ¹³C of leaf matter.

The contribution of source CO₂ substrates to the isotopic composition of sample materials (δ_sample) was corrected by calculating Δ¹³C as

$$
\Delta^{13}C = \frac{\delta_{\text{source}} - \delta_{\text{sample}}}{1 + \frac{\delta_{\text{sample}}}{1000}}
$$

We used mean δ¹³C of Z. mays leaves plus 4‰—assuming discrimination by Z. mays of exactly 4‰ in both a[CO₂] and e[CO₂] environments—as δ_source.

**Statistical analyses**

**Data exploration**

Shoot, root, and total dry weights and root-weight-ratio (RWR; root dry weight/total dry weight) data were averaged at the family level to examine the overall effect of [CO₂]. Treatment family-level means were first checked for normality and then compared using paired-means tests in JMP 13 (Sall et al. 2012).

**Mixed modelling of heritability and G×CO₂**

Mixed-model equations (Henderson 1953; Searle 1968) were implemented for prediction of breeding values and genetic parameters. These models were defined as

$$
\begin{bmatrix}
X^{-1}X & X^{-1}Z \\
Z^{-1}X & X^{-1}Z + G^{-1}
\end{bmatrix}
\begin{bmatrix}
b \\
u
\end{bmatrix}
= 
\begin{bmatrix}
X^{-1}y \\
Z^{-1}y
\end{bmatrix}
(2)
$$

where y is the vector of trait observations for each trait, b and u are vectors of fixed- and random-effect estimates, respectively, and X and Z are incidence matrices for fixed and random model terms. Also included are terms R for the variance/covariance matrix of the residuals and, if the random-effect factors are assumed to be independent, G is the direct sum of the variance/covariance matrices of each of the random effect factors. We assumed that the levels of the random effects are not independent. For breeding values, the additive (or numerator) relationship matrix (A) was then used.

Equation 2 was first used to generate spatially corrected phenotypic values for individual trees, correcting for within-greenhouse spatial effects. In this model, room and [CO₂] were included as fixed effects in vector b, while u contained sub-vectors for the random effects of family and spatial terms row, row-within-room, and column-within-room. The best linear unbiased predictor (BLUP) results of this analysis were then used as an input to PPG-Map software (Dutkowski 2009). This software was used to partition the spatial effects...
of rooms, rows, and columns away from the remainder, leaving spatially corrected individual-tree phenotypic values from which family means were then derived. By accounting for effects in columns that ran parallel with the axis of the benches, partitioning of spatial effects also addressed differences between benches within greenhouse bays. Family-level means were used to examine linear association between \( \Delta^{13}C \) and seed weight within JMP 13.

Equation 2 was then used to estimate the main and interaction effects of the [CO\(_2\)] by genotype experiment for each trait. This implementation was an individual-tree model, a forestry analogue of the animal model (Mrode 2014). Both [CO\(_2\)] and greenhouse-room-within-[CO\(_2\)] were included as fixed effects in vector \( \mathbf{b} \), while \( \mathbf{u} \) contained sub-vectors for the random effects of individual-tree, [CO\(_2\)]-by-individual-tree interaction, row-within-room, and column-within-room. Residual variance was estimated separately for each [CO\(_2\)] environment. The recorded pedigree of families was used to construct the inverse of the additive relationship matrix \( \mathbf{A} \). The relationship matrix was modified to include genetic groups (Westell et al. 1988) (corresponding to ancestral provenance and seed orchard origins of the included families) using the restricted maximum likelihood (REML) functionality built into ASReml (Gilmour et al. 2015) software.

The narrow-sense heritability of each trait was estimated as

\[
\hat{h}^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_R}
\]

where \( \sigma^2_A \) is the individual-tree (additive) genetic variance and \( \sigma^2_R \) is the residual variance. The standard errors of \( \hat{h}^2 \) were calculated using a first-order Taylor series expansion to approximate the variance of a ratio of variances implemented in ASReml (Lynch and Walsh 1998; Gilmour et al. 2002). Although \( E. globulus \) has a mixed mating system, we did not adjust \( A \) to account for inbreeding or full-sib offspring. Studies of outcrossing rates in \( E. globulus \) seed orchards have given widely variable estimates between 0.5 and 0.9 (Moncur et al. 1995; Russell et al. 2001; Patterson et al. 2004; Rao et al. 2008; Bush et al. 2011) making choice of a suitable adjustment strategy rather arbitrary and not necessarily superior to assuming that progeny within families are the result of outcrosses, on average. As a result, estimates of additive variance and narrow-sense heritability derived from the open-pollinated progeny may be inflated if inbreeding and/or correlated paternity in the two orchards was significant (see Bush et al. (2011) for discussion on how inbreeding in eucalypt open-pollinated families can impact genetic parameter estimates; also see Costa e Silva et al. (2010) and Hodge et al. (1996) for discussion of the relationship between parental effects estimated from open-pollinated families and comparable full- and half-sib families in \( E. globulus \)).

### Relationship between plant biomass, seed weight, and \( \Delta^{13}C \)

We explored the relation between spatially corrected family-level dry weights in a[CO\(_2\)] and e[CO\(_2\)] using orthogonal regression. This decision reflected error associated with the estimation of both variables and absence of clear dependency, and was aimed at revealing the underlying relation between dry weights in a[CO\(_2\)] and e[CO\(_2\)] environments. We also modelled the relation between [CO\(_2\)] response ratios (\( R_{PW} \)) of spatially corrected family-level dry weights and seed weight using a power term, where

\[
R_{PW} = \frac{e[CO_2]\text{family - levelcorrectedweight}}{a[CO_2]\text{familylevelcorrectedweight}}
\]

We tested for the relation between germination date and the corrected dry weight using linear regression. To test the strength of the relation between iWUE and growth in \( E. globulus \), Pearson’s correlation coefficient was calculated between dry weights and \( \Delta^{13}C \) of experimental plants.

To test the relation between early- and later-age performance, we calculated correlation coefficients between spatially corrected family-level total dry weight of our experimental plants grown in a[CO\(_2\)] and BLUPs for volume per hectare from field trials. Correlation was also tested between family-level a[CO\(_2\)] \( \Delta^{13}C \) of our seedlings and the same plantation-sourced BLUPs for stem volume.

### Results

#### Effects of [CO\(_2\)] on plant mass traits and \( \Delta^{13}C \)

Data from plants that died during the experiment were excluded from analyses. Limited germination in at least one of the growing environments for seven seedlots restricted replication to fewer than 10 plants. These seedlots were excluded from analyses, reducing our final dataset to 124 families.

Mass-related traits within the remaining families were significantly affected by [CO\(_2\)] (Table 1). Mean total dry weight was 29.9% greater for plants grown in the e[CO\(_2\)] environment than in a[CO\(_2\)]. Similar stimulation was evident for shoot (30.1%) and root dry weights (29.5%). Accordingly, RWR was not significantly affected by [CO\(_2\)]. Discrimination against \( ^{13}C \) was significantly lower in e[CO\(_2\)] than a[CO\(_2\)] plants, albeit by only 0.43‰. Family-level means for total, shoot, and root dry weights were strongly and significantly correlated between the a[CO\(_2\)] and e[CO\(_2\)] environments. Weaker, although highly significant, correlation was evident between the growing environments for RWR and \( \Delta^{13}C \).
Effects of position on harvest traits

Mixed models for mass traits (i.e. shoot, root, and total dry weights) revealed a marked degree of heterogeneity within and among the four greenhouse rooms for total dry weight (see Supplementary content S1). Accordingly, both column and row terms included within the mixed models explained significant variance for all traits (Table 2) with addition of terms resulting in a lower Akaike Information Criterion (AIC) in all cases. Outputs from orthogonal regression modelling indicate that while the strength of the relation between total dry weight in \([\text{CO}_2]\) and \([\text{eCO}_2]\) environments was improved by spatial corrections (Fig. 1a) associated with REML modelling, the form of the relation itself was unchanged. With near-zero intercept terms for both raw and spatially corrected data, the orthogonal models indicate a consistent 26% (raw) and 24% (spatially corrected) stimulation mass accumulation across the range of plant dry weights—lower than the mean stimulation across the entire dataset.

A weakly reactive family was apparent in both raw and corrected total dry weight datasets (Fig. 1a). However, a high Spearman rank correlation coefficient between environments indicates that overall family-level rankings of corrected dry weight were relatively unaffected by \([\text{CO}_2]\) (Fig. 1a). A weak, but significant, curvilinear negative trend was evident in \(R_{DW}\) (Fig. 1b) when plotted against corrected family-level dry weight from the \([\text{aCO}_2]\) environment.

Correlation between corrected dry weight and date of germination was non-significant (\(r = 0.010, p = 0.642\)). A significant positive relation was evident between seed weight and corrected dry weights from both \([\text{aCO}_2]\) and \([\text{eCO}_2]\) (Fig. 2a), but not \(R_{DW}\) (Fig. 2b).

Family-level variability in \(\Delta^{13}\text{C}\) was not significantly related to dry weight in either growing environment (Fig. 3).

### Table 1

| Trait                  | \([\text{aCO}_2]\) (se, CV%) | \([\text{eCO}_2]\) (se, CV%) | \([\text{eCO}_2]\) - \([\text{aCO}_2]\) | \(r\)     | \(r\) (95% CI) |
|------------------------|-------------------------------|-------------------------------|------------------------------------------|-----------|---------------|
| Dry weight (g)         |                               |                               |                                          |           |               |
| Shoot                  | 0.116 (0.002, 43.10)          | 0.151 (0.002, 43.64)          | 0.035‡                                   | 0.707‡†   | 0.606–0.785   |
| Root                   | 0.033 (0.001, 47.19)          | 0.043 (0.001, 50.07)          | 0.010‡                                   | 0.686‡†   | 0.580–0.769   |
| Total                  | 0.149 (0.002, 42.08)          | 0.194 (0.003, 43.22)          | 0.045‡                                   | 0.746‡†   | 0.656–0.815   |
| RWR                    | 0.223 (0.002, 24.11)          | 0.224 (0.002, 24.18)          | 0.001 ns                                 | 0.524‡†   | 0.383–0.641   |
| \(\Delta^{13}\text{C}\) (%) | 25.21 (0.03, 1.505)          | 24.84 (0.06, 2.874)          | −0.43‡                                   | 0.434‡†   | 0.279–0.567   |

### Table 2

| Model term             | Total dry weight (g) | Shoot dry weight (g) | Root dry weight (g) | Root weight ratio |
|------------------------|----------------------|----------------------|---------------------|------------------|
| **Random**             |                      |                      |                     |                  |
| Row-within-room        | 9.83                 | 6.93                 | 6.28                | 6.94             | 0.45            | 6.50            | 0.04            | 2.45             |
| Column-within-room     | 1.51                 | 4.21                 | 1.00                | 4.29             | 0.06            | 3.32            | 0.03            | 2.09             |
| Individual tree        | 14.48                | 5.76                 | 8.83                | 5.73             | 0.88            | 5.50            | 0.43            | 4.27             |
| Residual [\(\text{aCO}_2\)] | 13.89               | 7.15                 | 9.19                | 7.67             | 1.22            | 9.48            | 2.31            | 20.54            |
| Residual [\(\text{eCO}_2\)] | 31.50               | 13.84                | 19.44               | 13.91            | 2.69            | 16.77           | 2.22            | 19.69            |
| Narrow-sense heritability (\(h^2\)) | 0.42                | 6.51                 | 0.40                | 6.44             | 0.35            | 6.14            | 0.16            | 3.95             |
| **Fixed**              |                      |                      |                     |                  |                |
| \([\text{CO}_2]\) level | < 0.001              | < 0.001              | < 0.001             |                 | 0.191           |
| Room-within-[\(\text{CO}_2\)] level | 0.004               | 0.023                | < 0.001             | < 0.001          | 0.001           |
| AIC (no interaction)   | −17,914.6            | −19,752.5            | −28,224.8           | −19,500.8        |
| AIC ([\(\text{CO}_2\)] interaction) | −17,825.3         | −19,665.5            | −28,144.3           | −19,499.0        |

Z ratios are the variance component divided by their standard error. AIC based on model variants with and without individual-tree-by-[\(\text{CO}_2\)] interactions were calculated. A lower AIC indicates a better model fit: the variance estimates presented are therefore those from the simpler models without the \(G \times \text{CO}_2\) parameter.
Main and interaction effects ([CO₂] genotype and G × CO₂)

The mixed-model output indicated that the main effects (i.e. [CO₂] and individual-tree additive effects) were significant for all traits, as gauged by reduced AIC for models with the random individual-tree term excluded (result not given) or the Wald-based $F$ probability for the fixed [CO₂] term (Table 2). Individual tree (additive) effects were generally estimated with high precision as gauged by the $Z$ ratio. However, the [CO₂]-by-individual tree interaction was non-significant for all traits as gauged by the AIC (Table 2).

Narrow-sense heritability (i.e. the proportion of the genetic variance that can be passed from one generation to the next) was moderate and precisely estimated for each trait (Table 2). These estimates are somewhat above the low-to-moderate estimates normally indicated for growth traits in mature forest trees (e.g. Cornelius 1994).

Fig. 1 Scatterplot of raw and spatially corrected family-level means for total dry weight. A weakly reactive family is shown circled. a Orthogonal regression fits are represented by the overlapping lines for raw (dashed; $y = 0.005 + 1.26x$) and spatially corrected (solid; $y = 0.008 + 1.24x$) datasets. A 1:1 relation is shown (dashed). b $[CO₂]$ response ratios ($R_{DW}$) of spatially corrected family-level dry weights for each of the 124 experimental seedlots included in mixed modelling and power-fitted $[CO₂]$ response-ratio function ($y = 0.8782x - 0.203$). Pearson’s ($r$) and Spearman’s rank ($\rho$) correlation coefficient are reported for comparative $a[CO₂]$ and $e[CO₂]$ spatially corrected dry weights and the coefficient of determination ($r^2$) for $R_{DW}$. Superscripted ‡ denotes significance at $p < 0.0001$

Fig. 2 Scatterplots of spatially corrected family-level mean total and seed weights and $[CO₂]$ response ratio ($R_{DW}$). a Ordinary least squares fits and their respective coefficient of determination ($r^2$) for $a[CO₂]$ (dashed; $y = 0.10 + 0.03x$) and $e[CO₂]$ (solid; $y = 0.11 + 0.05x$) dry weights plotted against seed weight. b Coefficient of determination ($r^2$) is reported for $R_{DW}$.
Pairwise correlations between the field-based regional—Gippsland, Green Triangle, Tasmanian and Western Australian—trials and whole-of-Australia (Fig. 4) breeding values were all significantly different from 0 at the 95% confidence level and moderate to high ($0.76 < r < 0.93$). However, correlations between the field breeding values and family-level BLUPs for our $a[CO_2]$ greenhouse-grown seedlings were very low and non-significant. Correlations between field data and greenhouse-based Δ$^{13}$C were also low and non-significant (Fig. 4).

**Discussion**

**Main [CO$_2$] and seed-weight effects**

In the present study, we aimed to explore potential intraspecific variation in early-growth responses of 124 *E. globulus* families to $e[CO_2]$. We observed a ~30% mean enhancement in total biomass (dry weight) as a consequence of a 60% [CO$_2$] increase (i.e. from ~400 to 640 μmol mol$^{-1}$) (Table 1)—a response similar to the average across a broad spectrum of species (Poorter 1993).

The relation between biomass in $a[CO_2]$ and $e[CO_2]$ environments (Fig. 1a) differed little between family-level raw and spatially corrected data, suggesting that position effects within greenhouse bays were largely eliminated by within-room randomisation. Consistent with the orthogonal regression fits that indicate similar absolute stimulation of dry weight across the range of plant size, curvilinear negative relation between family-level mean dry weight and $R_{DW}$ (Fig. 1b) approximates the mathematical product of a mass-independent absolute response to $e[CO_2]$.

Intraspecific variation in seed weight strongly reflects maternal environmental conditions and can significantly impact upon growth traits in *E. globulus* (Martins-Corder et al. 1998; López et al. 2003). Maternal carryover effects, including seed weight, may also significantly affect phenotypic responsiveness to environmental change (Roach and Wulff 1987; Vivas et al. 2019) and, hence, response to $e[CO_2]$. However, carryover effects may also inflate estimates of genetic variation, particularly during the early growth of seedlings. This may provide some explanation for the heritability estimates made here, which are higher than usual for growth-related traits in forest trees in general (Cornelius 1994) and for *E. globulus* specifically (e.g. Costa e Silva et al. 2009; Hamilton et al. 2015; Mora et al. 2019). It is also important to note that while we modelled ancestral subpopulation effects in the NRM, which effectively removes this potential source of confounding non-additive variance, and the study has explicitly dealt with CO2x genotype interaction, other environmental interactions that the design has not accounted for may contribute to upwardly biased heritability estimates.

Consistent with previous *E. globulus* studies, we report a positive and significant relation between spatially corrected biomass and family-level mean seed weight in both [CO$_2$] environments. While the stronger relation between seed weight and spatially corrected biomass for plants grown at $e[CO_2]$ is suggestive of an interaction between maternal effects and $e[CO_2]$, $R_{DW}$ itself was not related to seed weight. These results indicate that while our estimates of heritability in mass-related traits (Table 2) may have been inflated by maternal carryover effects, no similar maternal effect is evident in [CO$_2$] responsiveness of our sample population.

**Effect of [CO$_2$] on Δ$^{13}$C**

Family-level Δ$^{13}$C means were significantly lower for plants grown in $e[CO_2]$ than those in $a[CO_2]$ (Fig. 2). That result may reflect our assumption of discrimination by *Z. mays* against $^{13}$C. Contrary to our assumption of constant discrimination, evidence (Vogel 1980; Ubierna et al. 2018) suggests a slight increase in discrimination against $^{13}$C in *Z. mays* with $[CO_2]$ that is roughly equivalent to the difference between CO$_2$ environments we report in this study.

Moderate correlation in Δ$^{13}$C between environments suggests a relatively stable response to $e[CO_2]$ between families (Table 1). Contrary to moisture-stress studies of *E. globulus* (e.g. Osório and Pereira 1994; Osório et al. 1998; Pita et al. 2001), our experiment did not yield a strong relation between Δ$^{13}$C and biomass (Fig. 2). That is, our results indicate that while intraspecific variation in $g_s$ drives variable iWUE responsiveness to drought severity...
in *E. globulus*, similar variability in stomatal sensitivity to e[CO$_2$] appears, at least during early growth, from the species.

**G × CO$_2$ interactions**

By including a wide array of genotypes and a relatively high rate of replication within each family, our study overcame limitations associated with narrow genetic representation and imprecise estimation of growth-related phenotypic parameters characteristic of previous G × CO$_2$ studies of eucalypts. Nevertheless, consistent with earlier eucalypt studies, we did not detect significant additive G × CO$_2$ in growth-related traits within our study population.

Taken at face value, our results suggest that current breeding strategies aimed at maximising current yield will not disadvantage future yield. That is, the fastest growers under a[CO$_2$] are also the fastest growers in the e[CO$_2$] environment. This may mean that the costs and complexities of additional [CO$_2$]-focused selection strategies may be avoided for *E. globulus*. However, this practical implication is predicated on the assumption that the lack of G × CO$_2$ observed

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**Fig. 4** Scatterplot matrix of family-level BLUPs and Δ$^{13}$C of seedlings raised in a[CO$_2$] (this study) and regionally averaged plantation-based stem volumes. Product moment correlation coefficients ($r$) are reported on each scatterplot (ns denotes non-significance at $p=0.05$ and ‡significance at $p<0.0001$)
in very young seedlings is representative of responsiveness throughout the plantation growth cycle. Some evidence suggesting that this may be a reasonable assumption can be drawn from observed consistency between short- and longer-term studies of a small number of poplar clones suggesting that CO₂ responsiveness varies little in the first decade of growth (e.g. Wang et al. 2000; Kubiske et al. 2007). However, it should be acknowledged that direct comparison of seedling-based with clonally based research material may be problematic. Specifically, the physiological status of cuttings differs from that of seedlings. In addition, while cuttings are not affected by the same maternal influences associated with seedlings (López et al. 2003), the performance of cuttings is well known to be affected by the properties of stock plants as well as genetic variation in rooting success (Makouanzi et al. 2014).

A unique aspect of this experiment relative to earlier G×CO studies was that we compared greenhouse growth performance to mature-age (half-rotation or older) performance under ambient growth conditions for each family. We found no correlation between greenhouse and later-age growth performance, highlighting a further difficulty with CO₂-based experimentation in very young trees. That is, maternal and/or nursery effects may have a significant influence on growth performance (López et al. 2003; Rix et al. 2012). These effects include both genetically inherited effects associated with long-lasting maternal contributions to the nuclear DNA and contributions to the endosperm, seed coat, and seed provisioning. These latter contributions significantly affect seed size and seedling growth (Roach and Wulff 1987)—effects that dissipate at later ages in eucalypts and other long-lived forest tree species (Close et al. 2006; Naidu and Jones 2007). Thus, while replicated experiments of mature trees are extremely challenging under controlled CO₂ conditions (Shimono et al. 2019), screening large plants in long-term e[CO₂] appears to remain necessary in the absence of a correlation between juvenile and mature growth stages as demonstrated in this study.

While the implications of our results are difficult to interpret in terms of mature plantation trees, they may hold relevance for natural E. globulus stands. In particular, qualitative and rank-based correlation between families in the ambient and CO₂-enriched growing environments, and absence of significant interaction terms within our modeling, suggests that genetically driven competitive hierarchies may not be affected by increasing [CO₂]. Rather, in the absence of additional environmental stressors, e[CO₂] will uniformly stimulate growth in native regenerating E. globulus stands.

The present study has provided a robust indication that additive G×CO₂ is non-significant at the population level in E. globulus. Nevertheless, some families differed substantially in their response to e[CO₂]. For example, a weakly reactive family (Fig. 1a) was clearly present within our study population. That the responsiveness of this and other weakly reactive families markedly differs from strongly reactive families suggests that genuine G×CO₂ interactions may yet exist at a comparative level despite the lack of significant population-level statistics. Nevertheless, exploiting such individuals or families would be practically difficult in this eucalypt species as selection intensity would need to be very high, and clonal propagation of the species is difficult.

Our experiment tested [CO₂] responsiveness in well-watered, richly fertilized plants that were free of competition, grown in optimal temperature conditions, and did not exceed the critical biomass-to-pot volume ratio advocated for growth experiments (Poorter et al. 2012). That is, in an effort to isolate [CO₂] responsiveness, we eliminated key plant stressors and resource limitations from our experiment. However, stressors may be central to G×CO₂ interactions. In particular, although primary G×CO₂ is rare in woody plants, secondary interactions (i.e. G×CO₂×E) between, for example, [CO₂] and competition (Bazzaz et al. 1995; Wayne and Bazzaz 1997), temperature regimes (Wang et al. 1994), water (Riikonen et al. 2005), and nutrient availability (Lindroth et al. 2001) or O₃ (Isebrands et al. 2001) are far more prevalent. Furthermore, while Mohan et al. (2004) reported significant G×CO₂ in germination and growth traits of Acer rubrum, they concluded that competition for light was a likely cause of significant G×CO₂ interactions.

In our experiment, e[CO₂] increased plant growth, but did not affect the coefficient of variation (Table 1) at the treatment level. Thus, although a novel environment, our e[CO₂] conditions had no effect upon expression of overall genetic variance in mass or Δ¹³C. We posit that rather than acting as an interactive stimulus in itself, e[CO₂] may only enhance responses to abiotic and biotic selective pressures. This makes intuitive sense given CO₂ is relatively well mixed in the environment and may play little role in intraspecific natural selection.

**Broader implications for G×CO₂ experimentation**

The absence of G×CO₂ interactions from this and previous studies, and the possibility that genetic adaptation to e[CO₂] may occur incrementally, begs the question as to whether ongoing testing in plantation tree species remains relevant. We believe so, given the potential long-term yield and sequestration benefits associated with selective breeding for increased growth under e[CO₂] and need to understand the interplay between CO₂ responsiveness and, for example, drought tolerance. From that perspective, it is instructive to consider lessons gained from the present study.

Shimono et al. (2019) emphasised that to generate interpretable outcomes, G×CO₂ studies identifying genetic determinants of [CO₂] responsiveness must include >100
genotypes. Testing for significant population-level variability is more stringent, requiring > 100 families each with as many as 20 genotypes. In the present study, the short 25-day growth period meant that both a large number of families and replicates could be grown. The latter element—high replication—was particularly important in controlling for within- and between-room variability. However, longer-term studies of woody plants—even those spanning only a few months—face practical space and cost constraints if replication within and between experimental units is to be maintained (Shimono et al. 2019). We suggest several options for future experimentation to overcome this impasse. First, given the limitations presented by similarly responding genotypes implied by our data, population-level testing could be considered an initial rapid screening process followed by subsequent detailed longer-term comparative experimentation aimed at drawing out this variability and its underpinning physiological mechanisms. Ideally, the latter would draw upon families that represent the extremes of the response range identified during the screening phase.

As an alternative, we suggest that comparative studies of e[CO$_2$] responsiveness focus explicitly upon a small number of well-replicated genotypes, families, or provenances that represent contrasting intra-specific environmental and/or plasticity extremes. A rich suite of opportunities exists in eucalypts. Considerable variation, for example, exists in plasticity of leaf traits and growth strategies between arid, seasonally dry, and humid tropical provenances of E. camaldulensis (Gibson and Bachelard 1989, 1994; Gibson et al. 1994). However, it is important to emphasise that, as highlighted earlier, intraspecific variation in trait plasticity may only yield significant G × CO$_2$ interactions in the presence of additional environmental stressors. Although the addition of environmental stressors greatly complicates the conduct of experiments, projected climate changes associated with increasing [CO$_2$] suggest that designs integrating, for example, heat and drought stress as primary treatment effects may be necessary.

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Declarations

Conflict of interest Seeds for this study were supplied by Tree Breeding Australia; co-author M.I. is employed by Tree Breeding Australia. On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data archiving statement Data used in this study have been prepared for submission to Dryad (https://datadryad.org/). Dryad is a curated repository for scientific data. The data for submission to Dryad comprise plant mass, carbon-isotope discrimination, and seed mass data as well as greenhouse position, treatment room, and treatment type coding. Randomised family-level identifiers have been applied to the dataset to protect commercial intellectual-property rights held by the Tree Breeding Australia. Data to be submitted to Dryad once the manuscript has been accepted for publication and will be accompanied by a README text file with the following details:

Institution: Australian National University.
Project name: Forests for the Future (RP04-122).
Funding agency: Science Infrastructure Endowment Fund.
Citation(s): None. Currently in review for publication.
Date of experiment: October 2017 to December 2017.
Description: Plant mass and isotopic discrimination data arising from elevated [CO$_2$] experiment.
Study species: Eucalyptus globulus ssp. globulus.
De-identification: Family-level seedlot codes for experimental families have been randomly de-identified.
Dataset: BrookhouseMT_SIEF-Eglobulus-CO2_Plan-Mass_May-2019.csv.
Randomised seedlot: randomised number from 1 to 132.
Position code: alphabetical (west–east) column and numeric row (north–south) coding.
Germination_date: calendar date of opening of cotyledons.
CO2_treatment: 400 or 640.
Greenhouse_bay: 2 or 3 for ambient CO$_2$ and 4 or 5 for elevated CO$_2$.
West-East_column: west-to-east experimental level numeric coding for column.
South-North_row: north-to-south experimental level numeric coding for row.
Shoot_dry_weight_(g): dry weight of plant stem and leaves in grams.
Root_dry_weight_(g): dry weight of plant roots in grams.
Total_dry_weight_(g): combined dry weight of plant stems, leaves, and roots in grams.
Seed_mass_(g): mean weight of seeds.
Seed_count_(n): count of seeds weighed at the conclusion of the experiment.
D13C: isotopic discrimination against 13C.
Corrected_total_dry_weight_(g): total dry weight corrected for greenhouse position effects.

Tree Genetics and Genomes is not listed among the journals for which Dryad supports submission of datasets prior to acceptance of a published manuscript. An accession number will be supplied once the manuscript has been accepted and Dryad permits submission.

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References

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant, Cell Environ 30(3):258–270

Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO2] in relation to the global carbon cycle. Glob Change Biol 1(4):243–274

Aspinwall MJ, Blackman CJ, de Dios VR, Busch FA, Rymer PD, Loik ME, Drake JE, Pfautsch S, Smith RA, Tjoelker MG (2018) Photosynthesis and carbon allocation are both important predictors of genotype productivity responses to elevated CO2 in Eucalyptus camaldulensis. Tree Physiol 46(1):1–14

Baig S, Medlyn BE, Mercado LM, Zaehe S (2015) Does the growth response of woody plants to elevated CO2 increase with temperature? A Model-Oriented Meta-Analysis Global Change Biology 21(12):4303–4319

Barnaby JY, Ziska LH (2012) Plant responses to elevated CO2. Encyclopedia of Life Sciences

Battaglia M, Bruce J (2017) Direct climate change impacts on growth and drought risk in blue gum (Eucalyptus globulus) plantations in Australia. Aust for 80(4):216–227. https://doi.org/10.1080/00382167.1990.1016/0269-7491(94)00088-U

Booth TH (2013) Eucalypt plantations and climate change. For Ecol Manage 301:28–34. https://doi.org/10.1016/j.foreco.2012.04.004

Bush D, Kain D, Matheson C, Kanowski P (2011) Marker-based adjustment of the additive relationship matrix for estimation of genetic parameters—an example using Eucalyptus cladocalyx. Tree Genet Genomes 7(1):23–35. https://doi.org/10.1007/s11295-010-0312-z

Callister A, England N, Collins S (2011) Genetic analysis of Eucalyptus globulus diameter, straightness, branch size, and forking in Western Australia. Can J for Res 41(6):1333–1343

Ceulemans R, Jiang X, Shao B (1995) Growth and physiology of Eucalyptus globulus: effects of seedling size and container type on early afterplanting performance. Ann Bot 75(6):609–617

Close DC, Batl I, Hunter S, Beadle CL (2006) Defining seedling specifications for Eucalyptus globulus: effects of seedling size and container type on early afterplanting performance. Ann for 69(1):2–8

Cornelius J (1994) Heritabilities and additive genetic coefficients of variation in forest trees. Can J for Res 24(2):372–379

Costa e Silva J, Borralho NMG, Araújo JA, Vaillancourt RE, Potts BM (2009) Genetic parameters for growth, wood density and pulp yield in Eucalyptus globulus. Tree Genetics & Genomes 5(2):291–305. https://doi.org/10.1007/s11295-008-0174-9

Costa e Silva J, Dukkowski GW, Borralho NM (2005) Across-site heterogeneity of genetic and environmental variances in the genetic evaluation of Eucalyptus globulus trials for height growth. Annals of Forest Science 62(2):183–191

Costa e Silva J, Hardner C, Potts BM (2010) Genetic variation and parental performance under inbreeding for growth in Eucalyptus globulus. Annals of Forest Science 67(6):606–606. https://doi.org/10.1051/forest/2010019

Costa e Silva J, Potts BM, Dukkowski GW (2006) Genotype by environment interaction for growth of Eucalyptus globulus in Australia. Tree Genetics & Genomes 2(2):61–75. https://doi.org/10.1007/s11295-005-0025-x

Crous KY, Quentin AG, Lin YS, Medlyn BE, Williams DG, Barton CVM, Ellsworth DS (2013) Photosynthesis of temperate Eucalyptus globulus trees outside their native range has limited adjustment to elevated CO2 and climate warming. Glob Change Biol 19(12):3790–3807. https://doi.org/10.1111/gcb.12314

Curtis PS, Wang X (1998) A meta-analysis of elevated CO2 effects on woody plant mass, form, and physiology. Oecologia 113(3):299–313

Dillon S, Quentin A, Ivković M, Furbank RT, Pinkard E (2018) Photosynthetic variation and responsiveness to CO2 in a widespread riparian tree. PloS one 13 (1):e0189635

Dlugokencky E, Tans P (2017) Trends in atmospheric carbon dioxide. NOAA Earth System Research Laboratory. https://www.esrl.noaa.gov/gmd/ccgg/trends/. Accessed April 4 2019 2019

Downham R, Gavran M (2018) Australian plantation statistics 2018 update. ABARES, Canberra

Drake BG, González-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO2? Annu Rev Plant Biol 48(1):609–639

Dutkowski G, Kerr R, Tier B, Li L, Costa e Silva J, Ivković M, Potts B, McRae T Next generation breeding values. In: Genetic aspects of adaptation and mitigation: forest health, wood quality and biomass production. Riga, Latvia, 2012. pp 3–5

Dutkowski G, Potts B (1999) Geographic patterns of genetic variation in Eucalyptus globulus ssp. globulus and a revised racial classification. Australian Journal of Botany 47 (2):237–263

Dutkowski GW (2009) PPG Map software. PlantGenetics LTD, Mount Gambier

Eamus D, Duff GA, Berryman CA (1995) Photosynthetic responses to temperature, light flux-density, CO2 concentration and vapour pressure deficit in Eucalyptus tetrodonta grown under CO2 enrichment. Environ Pollut 90(1):41–49. https://doi.org/10.1016/0269-7491(94)00088-U

Easterling WE, Aggarwal PK, Batima P, Brander KM, Erda L, Huber J, Tubiello FN (2007) Food, fiber and forest products. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) Climate Change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge UK, pp 273–313

Fardusi MJ, Ferrio JP, Comas C, Voltas J, Resco de Dios V, Serrano L (2016) Intra-specific association between carbon isotope composition and productivity in woody plants: a meta-analysis. Plant Sci 251:110–118. https://doi.org/10.1016/j.plantsci.2016.04.005

Farquhar GD, O’Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Funct Plant Biol 9(2):121–137

Ghanonou O, Phillips NG, Sears MA, Logan BA, Lewis JD, Conroy JP, Tissue DT (2010) Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric CO2 and temperature. Plant, Cell Environ 33(10):1671–1681. https://doi.org/10.1111/j.1365-3040.2010.02172.x
temperatures for warmed and non-warmed *Eucalyptus globulus* trees. Tree Physiol 35(11):1249–1263. https://doi.org/10.1093/ treephys/tpv110

Radoglou K, Jarvis P (1990) Effects of CO\(_2\) enrichment on four popular clones. I. Growth and leaf anatomy. Annals of Botany 65 (6):617–626

Rao H-x, Patterson B, Potts B, Vaillancourt R (2008) A microsatellite study on outcrossing rates and contamination in an Eucalyptus globulus breeding arboretum. Journal of Forestry Research 19(2):136–140. https://doi.org/10.1007/s11676-008-0023-6

Raymond CA, Schimleck LR, Muneri A, Michel A (2001) Genetic parameters and genotype-by-environment interactions for pulp-yield predicted using near infrared reflectance analysis and pulp productivity in *Eucalyptus globulus*. For Genet 8:213–224

Resco de Dios V, Mereed TE, Ferrio JP, Tissue DT, Voltas J (2016) Intraspecific variation in juvenile tree growth under elevated CO\(_2\) alone and with O\(_3\): a meta-analysis. Tree Physiol 36(6):682–693

Riikonen J, Holopainen T, Oksanen E, Vapaavuori E (2005) Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO\(_2\) and O\(_3\) in the field. Tree Physiol 25(5):621–632

Rix KD, Gracie AJ, Potts BM, Brown PH, Spurr CJ, Gore PL (2012) Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus*. Ann for Sci 69(6):673–679. https://doi.org/10.1007/s13595-012-0190-7

Roach DA, Wulff RD (1987) Maternal effects in plants. Annu Rev Ecol Syst 18(1):209–235

Roden JS, Ball MC (1996) Growth and photosynthesis of two eucalypt species during high temperature stress under ambient and elevated [CO\(_2\)]. Glob Change Biol 2(2):115–128. https://doi.org/10.1111/j.1365-2486.1996.tb00056.x

Roden JS, Egerton JIG, Ball MC (1999) Effect of elevated CO\(_2\) on photosynthesis and growth of snow gum (*Eucalyptus pauciflora*) seedlings during winter and spring. Aust J Plant Physiol 26(1):37–46. https://doi.org/10.1071/pp98134

Russell J, Marshall D, Griffin R, Harbard J, Powell W Gene flow in South American *Eucalyptus grandis* and *Eucalyptus globulus* seed orchards. In: IUFRO Symposium—Developing The Eucalypt Of The Future, Valdivia, Chile, 2001. pp 149–150

Sall J, Lehman A, Stephens ML, Creighton L (2012) JMP start statistics: a guide to statistics and data analysis using JMP. SAS Institute

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO\(_2\) atmosphere. New Phyto 139(3):395–436

Searle SR (1968) Another look at Henderson’s methods of estimating variance components. Biometrics:749–787

Shimono H, Farquhar G, Brookhouse M, Busch FA, O’Grady A, Tausz M, Pinkard EA (2019) Prescreening in large populations as a tool for identifying elevated CO\(_2\)-responsive genotypes in plants. Funct Plant Biol 46(1):1–14

Smith RA, Lewis JD, Ghannoun O, Tissue DT (2012) Leaf structural responses to pre-industrial, current and elevated atmospheric [CO\(_2\)] and temperature affect leaf function in *Eucalyptus sideroxylon*. Funct Plant Biol 39(4):285–296. https://doi.org/10.1071/FP11238

Ubierna N, Gandin A, Cousins AB (2018) The response of mesophyll conductance to short-term variation in CO\(_2\) in the C4 plants *Setaria viridis* and *Zea mays*. J Exp Bot 69(5):1159–1170

Vivas M, Rolo V, Wingfield MJ, Slippers B (2019) Maternal environment regulates morphological and physiological traits in *Eucalyptus grandis*. For Ecol Manage 432:631–636

Vogel JC (1980) Fractionation of the carbon isotopes during photosynthesis. Fractionation of the carbon isotopes during photosynthesis. Springer, Berlin, pp 5–29

Volker PW, Orme RK (1988) Provenance trials of *Eucalyptus globulus* and related species in Tasmania. Aust for 51(4):257–265. https://doi.org/10.1080/00049158.1988.10676051

Wang X, Curtis PS, Pregitzer KS, Zak DR (2000) Genotypic variation in physiological and growth responses of *Populus tremuloides* to elevated atmospheric CO\(_2\) concentration. Tree Physiol 20(15):1019–1028

Wang ZM, Lechowicz MJ, Potvin C (1994) Early selection of black spruce seedlings and global change: which genotypes should we favor? Ecol Appl 4(3):604–616

Wayne P, Bazzaz F (1997) Light acquisition and growth by competing individuals in CO\(_2\)-enriched atmospheres: consequences for size structure in regenerating birch stands. Journal of Ecology:29–42

Westell R, Quaas R, Van Vleck LD (1988) Genetic groups in an animal model. J Dairy Sci 71(5):1310–1318

Wong S, Kriedemann P, Farquhar G (1992) CO\(_2\)×nitrogen interaction on seedling growth of four species of eucalypt. Aust J Bot 40(5):457–472. https://doi.org/10.1071/BT9920457

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