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Elevated CO$_2$ Did Not Stimulate Stem Growth in 11 Provenances of a Globally Important Hardwood Plantation Species

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Elevated atmospheric carbon dioxide (eCO$_2$) often enhances rates of photosynthesis leading to increased productivity in trees. In their native habitats in Australia, eucalypts display considerable phenotypic plasticity in response to changes in environmental conditions. Little is known whether this plasticity can be harnessed effectively under future atmospheric eCO$_2$ conditions and be used to identify provenances with superior growth. Here, we report two experiments that assessed the physiological and growth responses of *Eucalyptus grandis*—one of the world's most important hardwood plantation species—to eCO$_2$. We used 11 provenances from contrasting climates. Our selection was based on site-specific information of long-term temperature and water availability. In Experiment 1, four provenances exhibited significant variation in light-saturated photosynthetic rates (A_{sat}), stomatal conductance (g_s), and concentrations of non-structural carbohydrates in leaves, stems and roots when grown under ambient CO$_2$ (aCO$_2$). Biomass of leaves, stems and roots varied significantly and were negatively correlated with mean annual temperature (MAT) at seed origin, indicating that provenances from cooler, wetter climates generally produced greater biomass. Yet, stem growth of these provenances was not stimulated by eCO$_2$. Given the vast environmental gradient covered by provenances of *E. grandis*, we expanded the selection from four to nine provenances in Experiment 2. This allowed us to validate results from Experiment 1 with its small selection and detailed measurements of various physiological parameters by focusing on growth responses to eCO$_2$ across a wider environmental gradient in Experiment 2. In Experiment 2, nine provenances also exhibited intraspecific differences in growth, but these were not related to climate of origin, and eCO$_2$ had little effect on growth traits. Growth responses under eCO$_2$ varied widely across provenances in both experiments, confirming phenotypic plasticity in *E. grandis*, though responses were not systematically correlated with climate of origin. These results indicate that selection of provenances for improved stem growth of *E. grandis* under future eCO$_2$ cannot be based solely on climate of origin, as is common practice for other planted tree species.

Keywords: forestry, tree growth, climate change, gas exchange, carbohydrates, phenotypic plasticity, interspecific variation

INTRODUCTION

Elevated atmospheric CO_2 (eCO$_2$) may affect tree growth by increasing photosynthesis (Way et al., 2015). Empirical and theoretical work for a range of eucalypt species has shown that eCO$_2$ will increase biomass production if access to water and nutrients is not limited (e.g., Wong et al., 1992; Ghannoum et al., 2010; Battaglia and Bruce, 2017; Ellsworth et al., 2017). This response is commonly observed due to the expansion of leaf area (LA), improved photosynthesis (A) and water use efficiency (WUE) under eCO$_2$ (McCarthy et al., 2007; Drake et al., 2011; Norby and Zak, 2011; Resco de Dios et al., 2016). However, it is also well-known that genotypes and provenances of a single tree species may differ in their response to eCO$_2$ (Aspinwall et al., 2015). High genetic variability across (and within) populations may result in intraspecific differences of phenotypic responses to environmental cues, commonly termed "genotype-by-environment interaction," or G × E (Matheson and Raymond, 1986; Josephs, 2018). This intraspecific variation may be due to heritability of specific trait characteristics and due to phenotypic plasticity (Moran et al., 2016).

For decades tree breeding programs have been used to identify species and provenances that display fast stem growth (e.g., Melville, 1940; Lavigne, 1996; Pan et al., 2020) and increase productivity of commercial plantations (e.g., Cornelius, 1994; Erskine et al., 2006; Calderia et al., 2020). However, provenances selected for high rates of stem growth today may not necessarily display these favorable traits under future environmental conditions. Higher rates of growth under eCO$_2$ can lead to higher respiratory C loss (Dusenge et al., 2019) and metabolic activity due to higher rates of photosynthetic C assimilation and subsequent increases in non-structural carbohydrates in leaves (e.g., Moore et al., 1999; Poorter et al., 2009; Smith et al., 2012; Schmidt et al., 2017). Under such conditions, the carbon use efficiency (CUE, ratio of net primary production to gross primary production, *sensu* Gifford, 2003) may result in zero growth stimulation under eCO$_2$. If CUE is improved under eCO$_2$, carbon allocation patterns may change such that increased rates of C assimilation stimulate growth of roots rather than stems (Finzi et al., 2007; De Kauwe et al., 2014; Zaehle et al., 2014), which would be undesirable for the timber growing industry, yet potentially improve resilience during dry conditions (Grote et al., 2016). However, identifying provenances that show net-positive stem growth under eCO$_2$ is critical to meeting a rising global demand for wood and wood products (Wang et al., 2010; Booth et al., 2015; Loik et al., 2017).

Nine *Eucalyptus* species dominate hardwood plantations around the world due to their fast growth under a wide range of environmental conditions (Harwood, 2011; Müller da Silva et al., 2019), providing considerable capacity as sinks for atmospheric C (e.g., Binkley et al., 2017; Viera and Rodríguez-Saolliero, 2019). One of the most important eucalypt species used in plantations is *E. grandis* Hill ex Maid (comm. Flooded Gum or Rose Gum). Across its natural habitat this tall, fast-growing species displays considerable plasticity in tolerating a range of climates, from temperate to tropical along the coast of eastern Australia (**Figure 1**). Mean annual temperature (MAT) can vary
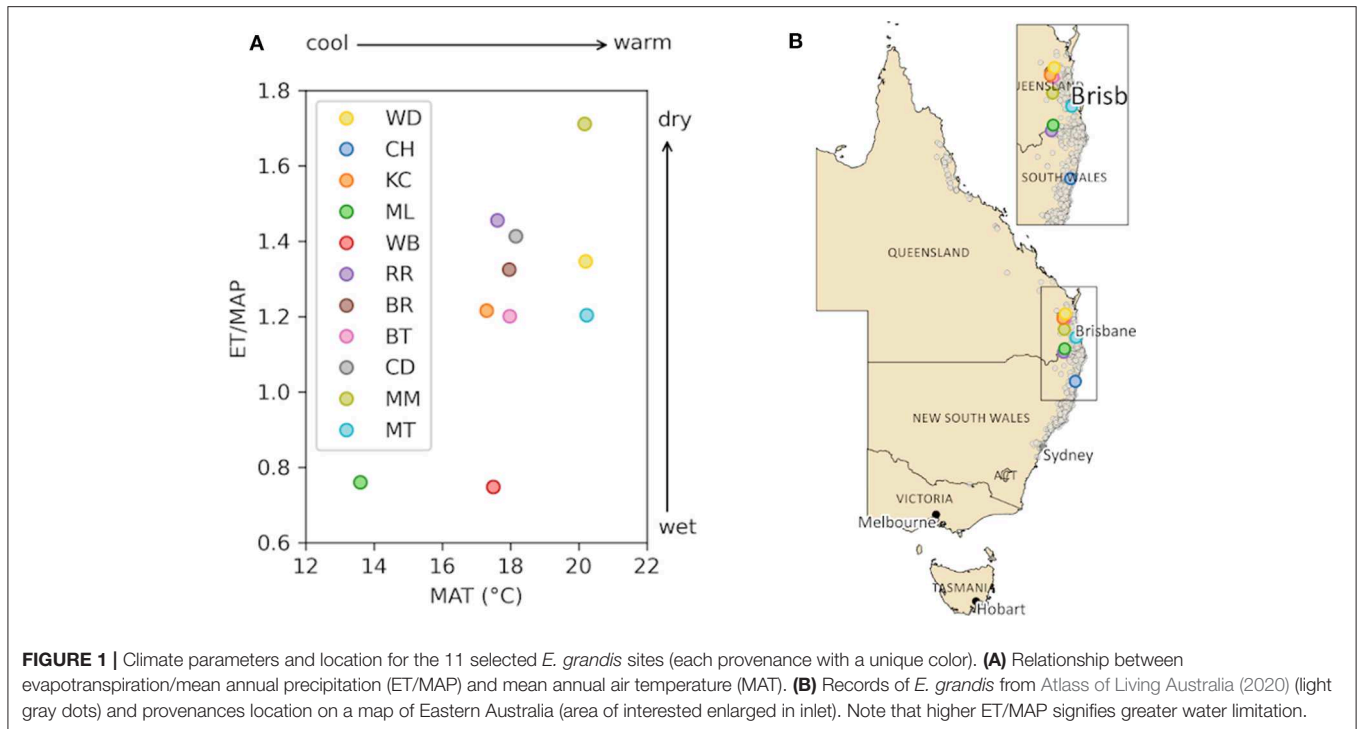
from 12 to 25°C and mean annual precipitation (MAP) from 750 to 3,500 mm across its distributional range (Aspinwall et al., 2017). At least 40 seed provenances are listed for *E. grandis* by the Australian Tree Seed Centre and progeny trials have been operated for some time to identify seed sources of *E. grandis* and other eucalypt species that produce trees with favorable timber properties (Bamber and Humphreys, 1963; Wang et al., 1984; Booth, 2013).

High levels of plasticity in *E. grandis* have been demonstrated in response to nutrient addition, and water deficit (Grassi et al., 2002; Battie-Laclau et al., 2013, 2014). Drake et al. (2015) demonstrated high growth plasticity in *E. grandis* in response to increasing temperatures. Another recent study showed that eCO$_2$ increased leaf dry mass per unit area, but not respiration, in 15 provenances of *E. grandis* (Aspinwall et al., 2017). Yet, these studies did not report how stem growth was affected by eCO$_2$. In absence of this information, valuable insight can be drawn from another study that used a widely distributed eucalypt species. *Eucalyptus camaldulensis* was exposed to eCO$_2$, which stimulated biomass production, although stem biomass increased the least and root biomass the most (Aspinwall et al., 2018). Moreover, the study found no clear trend indicating that the positive growth response to eCO$_2$ was related to climate of origin of the different provenances tested.

Here we report two experiments that assessed phenotypic plasticity in response to growth under eCO$_2$ of *E. grandis* provenances originating from different climates. Our selection was informed by results from Drake et al. (2015) who showed that productivity of *E. grandis* provenances generally increased from cool to warm temperate climates when grown under aCO$_2$. Based on this observation, we tested if superior growth performance in provenances from warmer climates would persist under eCO$_2$, using two individual experiments. In Experiment 1, we examined the response to eCO$_2$ for a range of physiological and growth traits in four provenances of *E. grandis*. The provenances originated from the southern distribution cluster of the species (**Figure 1**) and varied in climate of origin (precipitation and temperature). Given the vast environmental gradient covered by provenances of *E. grandis*, we expanded the geographical range of provenances in Experiment 2, where we used nine provenances to exclusively assess the effect of eCO$_2$ on growth traits. Two provenances of Experiment 1 were included in Experiment 2 to allow cross-validation of results in both experiments and their relevance across wider environmental gradients.

MATERIALS AND METHODS

This study reports two experiments on *Eucalyptus grandis* seedlings, grown in pots in a glasshouse, originating from a variety of climates at seed origin. Experiment 1 included four provenances (four climates) with five individuals per provenance in order to examine the intraspecific response of leaf physiology traits, non-structural carbohydrates and growth traits to aCO$_2$ and eCO$_2$. Following observation of intraspecific variation, we extended the selection to nine provenances in Experiment 2 to assess phenotypic plasticity in growth

FIGURE 1 | Climate parameters and location for the 11 selected *E. grandis* sites (each provenance with a unique color). **(A)** Relationship between evapotranspiration/mean annual precipitation (ET/MAP) and mean annual air temperature (MAT). **(B)** Records of *E. grandis* from Atlas of Living Australia (2020) (light gray dots) and provenances location on a map of Eastern Australia (area of interested enlarged in inlet). Note that higher ET/MAP signifies greater water limitation.

enhancement and correlations with climate of origin using 104 seedlings. Experiment 2 allowed validation of results from Experiment 1 by focusing on growth responses to eCO$_2$ across wider environmental gradients compared to more detailed physiological measurements conducted in Experiment 1. For both experiments, seed from *E. grandis* mother trees were collected at the 11 locations by personnel of the Australian Tree Seed Centre (Commonwealth Scientific and Industrial Research Organization, Government of Australia).

Experiment 1
Plant Material
The objective for Experiment 1 was to (a) identify if temperature or water availability at seed origin affected plant physiology and growth in aCO$_2$ and (b) if the observed trends remain under eCO$_2$. Experiment 1 lasted from March to June in 2015. Four provenances of *E. grandis* were selected (**Table 1**, **Figure 1**). Detailed climate-of-origin data were obtained from eMAST (www.emast.org.au) to calculate MAT, MAP and evapotranspiration (ET) between 1971 and 2010 for each location (see Aspinwall et al., 2017). Final selection for Experiment 1 was based on forming three pairs of MAT and water availability regimes (ratio of ET and MAP). Pair 1: Mt Lindsay and Coffs Harbour had the same low ET/MAP (wet) but different MAT (cool/warm). Pair 2: Coffs Harbour and Kilcoy Creek had similar MAT (warm) but different ET/MAP (wet/dry). Pair 3: Kilcoy Creek and Woondum had similar ET/MAP (dry) but different MAT (warm/hot). Seed was obtained from five mother trees (family) within each provenance of Experiment 1. Seeds were germinated under ambient atmospheric conditions and individuals grown at a plant nursery to enable collection

of vegetative cuttings. Randomly selected seedlings from each provenance were coppiced to produce hedges for rooted cutting propagation. After development of sufficiently strong roots, cuttings were transported into temperature and CO$_2$ controlled glasshouse rooms (12 March 2015) at the Hawkesbury campus of Western Sydney University (Richmond, NSW, Australia). There, seedlings were transplanted into pots made of PVC pipe (15 cm in diameter, 40 cm high; 7 L volume) and filled with a customized soil, which consisted of 90% coarse washed river sand, 10% diatomaceous earth (intermediate grade: 0.9–2 mm from Mt Sylvia, QLD) and mineral soil (Zeogreen, premium turf grade 0.5–1.6 mm from ZeoGreen, VIC, Australia). Pots were watered daily to saturation. Liquid fertilizer (500 mL Aquasol, at 1.6 g l^{-1}; 23% N, 4% P, 18% K, 0.05% Zn, 0.06% Cu, 0.013% Mo, 0.15% Mn, 0.06% Fe, 0.011% B; Yates Australia, NSW, Australia) was added to pots every 14 days to minimize potential nutrient limitation.

Seedlings were grown for 14 weeks (12 March to 15 Jun 2015) under natural light in four adjacent temperature and CO$_2$ controlled glasshouse rooms. Temperature (T) in all glasshouse rooms was programmed to represent the 30 year average of November to May conditions in Richmond NSW and diel variation (0600–1,000 h: 22°C; 1,000–1,600 h: 26°C; 1,600–2,000 h: 24°C; 2,000–0600 h: 15°C). Ambient and elevated CO$_2$ concentrations were set at 400 and 640 μmol mol^{-1} in two rooms per treatment, respectively, and controlled automatically (Lambda T, ADC BioScientific Ltd., Herts, UK). Environmental conditions inside the glasshouse bays were logged every 15 min. Ten plants of each provenance (40 seedlings total) were randomly assigned to the four glasshouse rooms and rotated between and within rooms every 14 days. Initially, 20 seedlings were grown in aCO$_2$ and 20 seedlings in eCO$_2$. At the end of the

TABLE 1 | Location and climate variables characterizing the origin of *E. grandis* seed provenance for Experiment 1 ($n = 40$) and Experiment 2 ($n = 104$); see superscript numbers.

Site name	Site coordinates	MAT	MAP	EL	ET	ET/MAP	[1]n	[2]n
[1,2]Mt Lindsay (ML)	28.21° S 152.45° E	13.6	1,314	1,022	999	0.76	10	12
[1,2]Kilcoy Creek (KC)	26.45° S 152.35° E	17.3	974	603	1,184	1.22	10	14
[1]Coffs Harbour (CH)	30.10° S 153.07° E	17.5	1,549	345	1,160	0.75	10	–
[1]Woondum (WD)	26.18° S 152.49° E	20.2	1,021	216	1,375	1.34	10	–
[2]Wedding Bells SF (WB)	30.10° S 153.07° E	17.5	1,549	100	1,160	0.75	–	12
[2]Richmond Range (RR)	28.41° S 152.39° E	17.6	872	520	1,269	1.45	–	7
[2]Borumba Range (BR)	26.35° S 152.36° E	18.0	929	500	1,232	1.33	–	12
[2]Bellthorpe (BT)	26.52° S 152.45° E	18.0	1,016	200	1,220	1.20	–	12
[2]Connondale (CD)	26.40° S 152.36° E	18.2	882	560	1,247	1.41	–	12
[2]Mt Mee (MM)	27.08° S 152.43° E	20.2	817	200	1,399	1.71	–	12
[2]Mt Tamborine (MT)	27.55° S 153.11° E	20.2	1,129	500	1,360	1.20	–	11

Mean annual air temperatures (MAT, °C), mean annual precipitation (MAP, mm), elevation (EL, m), evapotranspiration (ET, mm) and the ratio of evapotranspiration/MAP (ET/MAP), total number of replicates for Experiment 1 ([1]n) and Experiment 2 ([2]n) are shown. Note that higher ET/MAP represents greater water stress.

experiment, seedlings were destructively harvested. Leaf number was determined for each plant and leaf area (LA) of the canopy of each seedling was measured by placing all leaves into a leaf area meter (LI-3100C, Li-Cor Inc., NE, USA). Roots were carefully washed free of soil medium. Leaves, stems and roots were dried at 70°C to constant mass to determine dry weight.

Leaf Gas Exchange

Light-saturated photosynthetic rates of CO_2 assimilation (A_{sat}, μmol m^{-2} s^{-1}) and stomatal conductance of water vapor (g_s, mol m^{-2} s^{-1}) of fully expanded leaves were measured during the day (0930–1,530 h, sunny days) using an infrared gas analyzer (LI-6400XT, Li-Cor Inc., NE, USA). Measurement conditions for A_{sat} were set as: saturating light = 1600 μmol m^{-2} s^{-1}; block T = 26°C; CO_2 concentration = 400 or 640 μmol mol^{-1}; and flow rate = 500 μmol s^{-1}. Leaf vapor pressure deficit (VPD) was regulated by manipulating relative humidity inside the cuvette to maintain VPD between 1.2–1.6 kPa. Leaves were allowed to equilibrate to environmental conditions inside the cuvette for at least 5 min prior to measurement. Gas analysers were always matched before logging data. A minimum of five measurements per seedling were logged at 5 s intervals and later averaged. Care was taken that leaves covered the full 2 × 3 cm measurement chamber.

Leaf δ¹³C

Relative abundance of ^{13}C to ^{12}C in leaf material can be used as an integrated measurement of water use efficiency (WUE) over a longer timespan (Farquhar et al., 1989). For this purpose, one fully developed leaf per seedling was collected on days of leaf gas exchange measurements during Experiment 1. Leaves were snap-frozen in liquid nitrogen, dried at 60°C for 3 days and ground to a fine powder. Only leaves that were used for gas exchange measurements were collected. Analyses of leaf bulk tissue ($\delta^{13}C_{leaf}$) were completed at the Australian National University using a continuous-flow stable isotope ratio mass spectrometer (Fison Isochrom CF-IRMS, former Fisons Instruments, UK). The photosynthetic discrimination rate ($\Delta^{13}C$), which relates

to WUE for the hours of 0900–1600, was calculated from leaf material ($\delta^{13}C_{leaf}$) and known atmospheric values ($\delta^{13}C_{air}$), expressed in ‰ (Farquhar et al., 1982):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{leaf}}{1 + \frac{\delta^{13}C_{leaf}}{1000}} \qquad (1)$$

Since glasshouse air was artificially enriched with ^{13}C depleted CO_2, the $\delta^{13}C$ fraction of glasshouse room air ($\delta^{13}C_{air}$) was also determined to calculate $\Delta^{13}C$. This was achieved by collecting measurements of air of all four glasshouse rooms separately using a Tuneable Diode Laser (TGA100; Campbell Scientific, UT, USA), repeated for each room three times during a diel cycle to account for diurnal variation of CO_2. Mean $\Delta^{13}C$ was calculated for each glasshouse room. After determining $\Delta^{13}C$, WUE was calculated as:

$$\text{WUE} = \frac{A}{g_s} = \frac{c_a}{1.6}\left(\frac{b' - \Delta^{13}C}{b' - a}\right) \qquad (2)$$

where g_s is stomatal conductance of water vapor, a is the average fractionation constant (4.4‰, O'Leary, 1981) for the diffusion of gases through stomata and b' represents the carboxylation fractionation by Rubisco and PEP carboxylase (27‰, Farquhar and Richards, 1984). The factor 1.6 accounts for the greater diffusivity of water vapor vs. CO_2 in air and c_a represents the partial pressure of CO_2 in the outer atmosphere.

Mean response ratios (RR) for A_{sat}, g_s, and $\Delta^{13}C$ between eCO₂ and aCO₂ were calculated by dividing the mean value of the parameter in eCO₂ by the mean value in aCO₂. Variance of these RR was calculated according to Hedges et al. (1999).

Non-structural Carbohydrates

Plant tissues (leaves, stem, and roots) were sampled during morning hours of the final harvest of Experiment 1 to assess concentration of non-structural carbohydrates (NSC). Tissues were microwaved for 10 s to stop metabolic activity and then

dried at 70°C for 3 days. A detailed protocol for NSC extraction can be found in Tissue and Wright (1995). In short, subsamples of plant material were dried and ground to a fine powder before extracting NSC. Starch was separated from soluble sugars, lipids and amino acids using a methanol/chloroform/water solution. Water soluble sugars were partitioned from remaining cell components by phase separation. Starch was digested into ss with perchloric acid. Sugar content of both fractions was quantified colorimetrically in a spectrophotometer (DU800, Beckman Coulter Australia Pty Ltd, NSW, Australia) via a phenol-sulfuric acid reaction (Tissue and Wright, 1995).
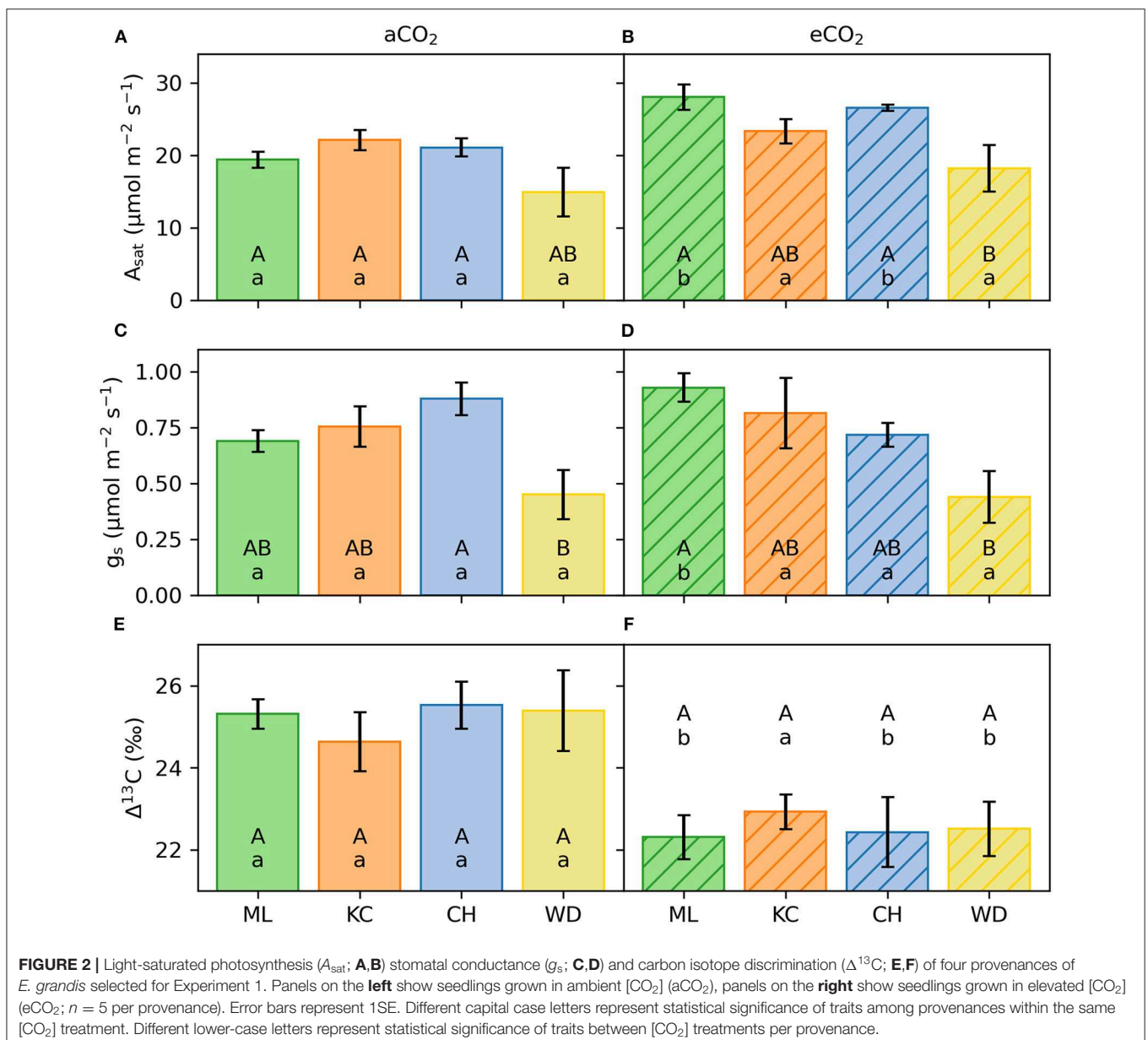
Biomass

At harvest (15–18 June 2015, 14 weeks since repotting), leaf area (LA) of the whole canopy was measured by placing all leaves into a leaf area meter (LI-3100C, Li-Cor Inc., NE, USA); leaf size was estimated by dividing LA by leaf number. Leaf mass per area (LMA) was calculated by dividing dry weight of its leaves (DW_{leaf}) by LA. Measurements of height (h, cm) and diameter (d, mm; measured 2 cm above soil surface) commenced at week 2 since repotting into the glasshouse and were subsequently conducted on a weekly basis. Stem volume (v_{stem}, cm³) was estimated using h and d, assuming the shape of a cone using the following formula:

$$v_{stem} = \frac{1}{3}\,\pi\,r^2\,h \qquad (3)$$

Total plant dry mass (DW_{plant}) was calculated by summing DW_{leaf}, DW_{stem}, and DW_{root}. Biomass fraction was calculated

FIGURE 2 | Light-saturated photosynthesis (A_{sat}; **A,B**) stomatal conductance (g_s; **C,D**) and carbon isotope discrimination (Δ^{13}C; **E,F**) of four provenances of *E. grandis* selected for Experiment 1. Panels on the **left** show seedlings grown in ambient [CO₂] (aCO₂), panels on the **right** show seedlings grown in elevated [CO₂] (eCO₂; $n = 5$ per provenance). Error bars represent 1SE. Different capital case letters represent statistical significance of traits among provenances within the same [CO₂] treatment. Different lower-case letters represent statistical significance of traits between [CO₂] treatments per provenance.

by dividing DW of plant organs by DW$_{plant}$ as leaf-mass-fraction (LMF), stem-mass-fraction (SMF) and root-mass-fraction (RMF).

Data Analyses

Data were analyzed using R-3.6.1 software (R Core Team, 2019). Analysis of variance (ANOVA) was used to test if the [CO$_2$] treatment as well as the origin of the four provenances had a significant effect on leaf gas exchange, growth-related traits (DW, v_{stem}, LMF, SMF, RMF) leaf specific traits (leaf number, leaf size, LA, LMA) and NSC. Statistical models were tested with all interactions between factors ([CO$_2$] and provenance). Assumptions of data normality and equal variance were tested using Shapiro-Wilk and Levene's test, as well as a quantile-quantile plot for visual assessment. The "car" package (Fox and Weisberg, 2019) was used for Anova() and leveneTest() functions with Type III sums of squares and Levene's test for equal variance among test groups, respectively. *Post-hoc* tests were conducted when plant traits were significantly different across provenances. To establish whether A_{sat} or g$_s$ was the driver for changes in Δ^{13}C correlations were analyzed with Pearson's correlation coefficient using the cor.test() function of the base package. All means are presented with one standard error (SE).

Experiment 2

Plant Material

The objective of Experiment 2 was to validate results of Experiment 1 recorded for a narrow climate of seed origin gradient and be able to identify a generalized growth response strategy of *E. grandis* provenances. Experiment 2 lasted from February to May 2017. The range of climate at seed origin was extended to nine provenances in Experiment 2 including two provenances from Experiment 1 (**Table 1**, **Figure 1**). Detailed selection criteria for the provenances used in Experiment 2 can be found in Aspinwall et al. (2017). Seed was obtained from 2 to 5 mother trees within each provenance. Seeds were germinated as described for Experiment 1 and were transported into temperature and CO$_2$ controlled glasshouse rooms on 27 February 2017 using similar pots and soil as in Experiment 1.

Seedlings of Experiment 2 were grown at the same location as Experiment 1 for 10 weeks (27 February to 11 May 2017). Glasshouse environmental conditions were comparable to Experiment 1. Experiment 2 included 3–7 plants per provenance, of which 52 were randomly assigned and grown in aCO$_2$ and 52 plants in eCO$_2$. Seedlings were destructively harvested in the same way as Experiment 1.

Biomass

Harvest was conducted on 10 and 11 May 2017, 10 weeks since repotting. The same measurements were taken as in Experiment 1. Measurements of *h* and *d* commenced during the first week following replanting into the glasshouse.

Data Analyses

Data were analyzed using R-3.6.1 software (R Core Team, 2019). Analysis of variance (ANOVA) was used to test if the [CO$_2$] treatment as well as climate origin of the nine provenances had a significant effect on growth-related traits (DW, v_{stem}) and leaf
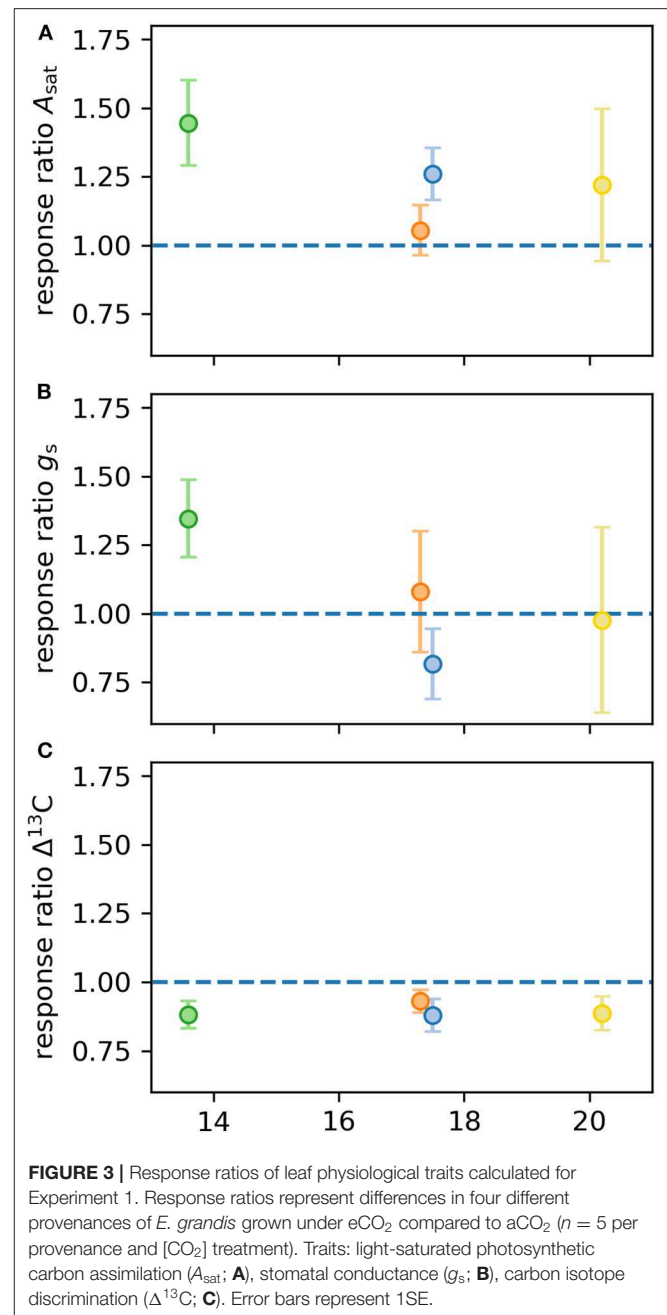
FIGURE 3 | Response ratios of leaf physiological traits calculated for Experiment 1. Response ratios represent differences in four different provenances of *E. grandis* grown under eCO$_2$ compared to aCO$_2$ (*n* = 5 per provenance and [CO$_2$] treatment). Traits: light-saturated photosynthetic carbon assimilation (A_{sat}; **A**), stomatal conductance (g_s; **B**), carbon isotope discrimination (Δ^{13}C; **C**). Error bars represent 1SE.

specific traits (LA, LMA). See Experiment 1 for further details. Correlations between DW$_{shoot}$ and DW$_{stem}$, respectively, and climate variables (MAT, MAP, ET/MAP) were analyzed with Pearson's correlation coefficient using the cor.test() function of the base package. The same approach was used for determining the correlation between DW$_{stem}$ and LMA. All figures were drawn using Python (Phyton Software Foundation)[1].

All figures were plotted using Python (Phyton Software Foundation).

RESULTS

Experiment 1
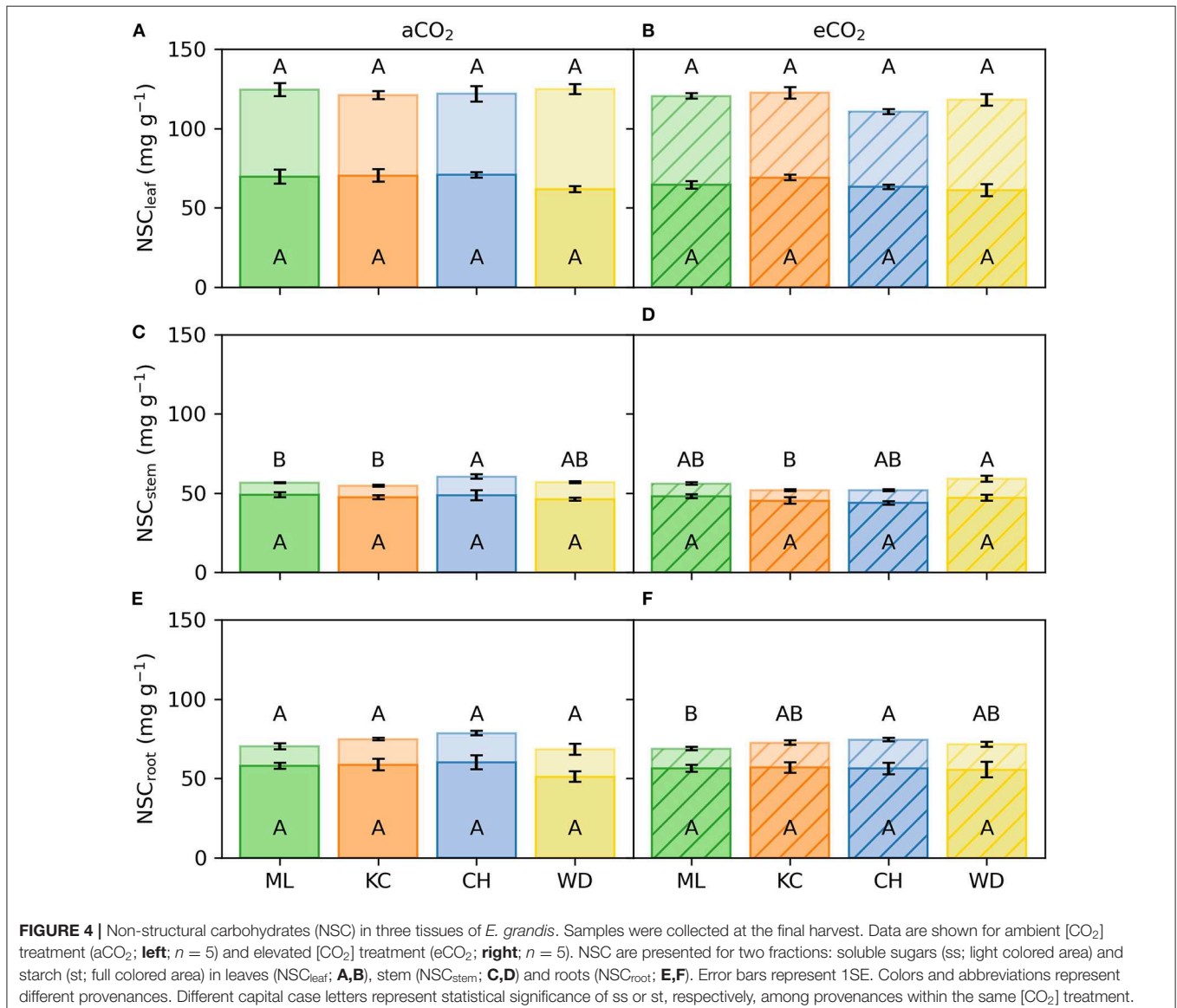
Environmental Conditions

Throughout Experiment 1, relative humidity ranged from 57–85%, T averaged $26.9 \pm 0.1 (\pm 1SE)$ °C during the photoperiod, and excellent [CO$_2$] control was achieved (aCO$_2$: $401 \pm 0.2 \, \mu$mol mol^{-1}; eCO$_2$: $645 \pm 0.1 \, \mu$mol mol^{-1}). From March to June 2015, mean photon flux density during mid-day was 950 μmol m^{-2} s^{-1}, ranging from 115 μmol m^{-2} s^{-1} on a cloudy day to 1,900 μmol m^{-2} s^{-1} on a sunny day.

Physiological Responses to eCO$_2$

A_{sat} differed significantly by provenance and [CO$_2$] treatment. However, there were no provenance by [CO$_2$] interactions statistically confirming these observed trends. In aCO$_2$, A_{sat} of seedlings originating from the warmest climate (provenance Woondum) was significantly lower ($18.2 \pm 1.4 \, \mu$mol m^{-2} s^{-1}, $p = 0.002$; **Figure 2**) than A_{sat} of seedlings in the other three provenances, which were similar to each other ($22.1 \pm 0.6 \, \mu$mol m^{-2} s^{-1}). Across all provenances, A_{sat} was on average 23% greater in eCO$_2$ ($23.3 \pm 0.9 \, \mu$mol m^{-2} s^{-1}) compared to those grown in aCO$_2$ ($19.0 \pm 0.6 \, \mu$mol m^{-2} s^{-1}, $p = 0.003$). In eCO$_2$, A_{sat} of seedlings from Woondum was 26% lower than seedlings originating from cooler climates (**Figure 2**). Notably, seedlings from the coolest climate (Mt Lindsay) showed on average the largest response to eCO$_2$, increasing A_{sat} by 40% in eCO$_2$ compared to aCO$_2$, yet also displayed high variability within this provenance (**Figure 3**).

[CO$_2$] treatment did not affect g_s, but values differed across provenances. In May 2015, seedlings growing in aCO$_2$ and originating from provenance Woondum had 49% lower g_s (0.45 ± 0.11 mol m^{-2} s^{-1}) than seedlings from provenance Coffs Harbour, averaging 0.88 ± 0.07 mol m^{-2} s^{-1} ($p = 0.001$;

FIGURE 4 | Non-structural carbohydrates (NSC) in three tissues of *E. grandis*. Samples were collected at the final harvest. Data are shown for ambient [CO$_2$] treatment (aCO$_2$; **left**; $n = 5$) and elevated [CO$_2$] treatment (eCO$_2$; **right**; $n = 5$). NSC are presented for two fractions: soluble sugars (ss; light colored area) and starch (st; full colored area) in leaves (NSC$_{leaf}$; **A,B**), stem (NSC$_{stem}$; **C,D**) and roots (NSC$_{root}$; **E,F**). Error bars represent 1SE. Colors and abbreviations represent different provenances. Different capital case letters represent statistical significance of ss or st, respectively, among provenances within the same [CO$_2$] treatment.

Figures 2, **3**). Seedlings from provenances Mt Lindsay and Kilcoy Creek had intermediate mean values of 0.69 ± 0.05 mol m^{-2} s^{-1} and 0.75 ± 0.09 mol m^{-2} s^{-1}, respectively. In eCO$_2$, seedlings from provenance Woondum had similar low g_s averaging 0.44 ± 0.12 mol m^{-2} s^{-1} while seedlings from provenance Mt Lindsay had the highest values with 0.93 ± 0.06 mol m^{-2} s^{-1} (35% increase from aCO$_2$ to eCO$_2$).

Provenances did not differ in Δ^{13}C when grown in aCO$_2$. Seedlings grown in eCO$_2$ had an improved WUE, indicated by Δ^{13}C being significantly lower compared to seedlings grown in aCO$_2$ (22.5 ± 0.3‰ vs. 25.2 ± 0.3‰, $p < 0.001$; **Figures 2**, **3**). A_{sat} was negatively correlated with Δ^{13}C (Pearson's $r = -0.59$, $p > 0.001$) whereas g_s was not ($p = 0.99$).
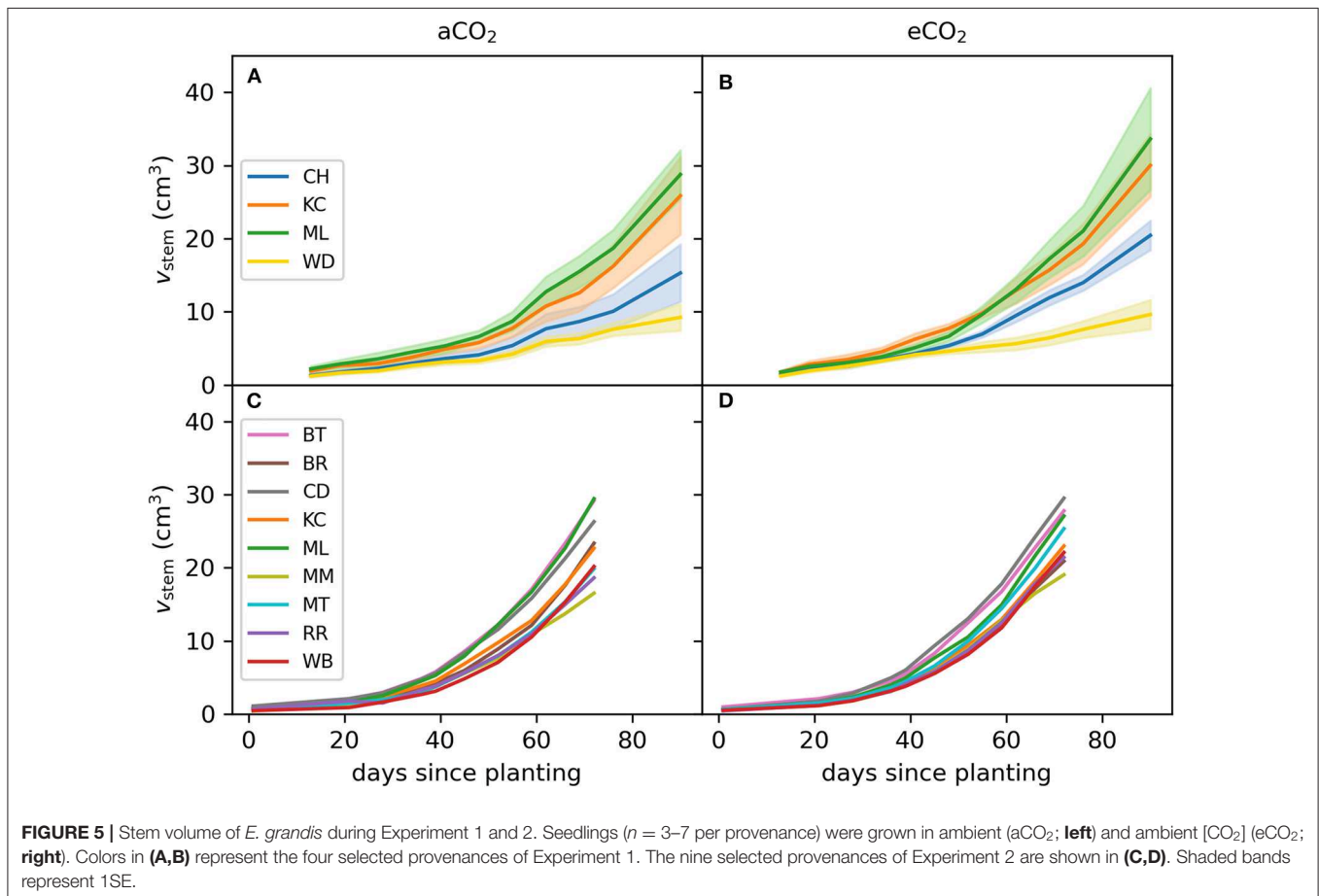
Non-structural Carbohydrates

eCO$_2$ did not change concentrations of soluble sugars or starch in any provenance or tissue. For instance, soluble sugars in leaves averaged 55.0 ± 2.1 mg g^{-1} dry weight in aCO$_2$ and 53.8 ± 1.6 mg g^{-1} dry weight in eCO$_2$. Leaf starch averaged 68.0 ± 1.7 mg g^{-1} dry weight in aCO$_2$ and 64.5 ± 1.4 mg g^{-1} dry weight in eCO$_2$. Data were pooled for [CO$_2$] treatments to assess variation of NSC concentration in plant organs among provenances. Concentrations of soluble sugars at harvest differed significantly in leaves, stem and roots among

provenances. Seedlings originating from Woondum had the greatest concentrations of soluble sugars in leaves (60.1 ± 2.5 mg g^{-1} dry weight, $p = 0.02$) and stem tissue (11.4 ± 1.0 mg g^{-1} dry weight, $p < 0.001$), respectively. Concentrations in leaves were 21% greater than soluble sugars in seedlings from Coffs Harbour and 64% greater than soluble sugars in stem tissue of Kilcoy Creek. In roots, concentration of soluble sugars differed significantly among provenances ($p = 0.02$), where seedlings originating from Coffs Harbour contained the greatest amount of soluble sugars (18.3 ± 0.9 mg g^{-1} dry weight) followed by those from Woondum (16.7 ± 1.8 mg g^{-1} dry weight). Concentrations of starch at harvest only differed among provenances in leaves ($p = 0.05$; **Figure 4**). Seedlings from Kilcoy Creek had the greatest starch concentrations in leaves (69.7 ± 2.0 mg g^{-1} dry weight), whereas those from Woondum had the lowest (61.3 ± 2.0 mg g^{-1} dry weight; **Figure 4**).

Growth

At harvest, seedlings were 62–118 cm tall. Throughout the experiment, stem volume (v_{stem}) was not significant across [CO$_2$] treatments ($p = 0.15$) nor [CO$_2$] x provenance interaction ($p = 0.91$) but varied across provenances ($p < 0.001$; **Figures 5A,B**). Specifically, seedlings originating from the warmest climate

FIGURE 5 | Stem volume of *E. grandis* during Experiment 1 and 2. Seedlings ($n = 3$–7 per provenance) were grown in ambient (aCO$_2$; **left**) and ambient [CO$_2$] (eCO$_2$; **right**). Colors in **(A,B)** represent the four selected provenances of Experiment 1. The nine selected provenances of Experiment 2 are shown in **(C,D)**. Shaded bands represent 1SE.

(Woondum) had continuously lower stem volume than seedlings from the coolest climate (Mt Lindsay). Trends were detected for relative responses of v_{stem} from aCO$_2$ to eCO$_2$ with seedlings from Coffs Harbour indicated the greatest increase (+33%). At harvest, DW$_{plant}$ of individual seedlings varied from 7.3 to

59.5 g. Leaf, stem and root biomass was only significant across provenances, but not [CO$_2$] nor their interactions (**Table 7**). Under aCO$_2$, seedlings from Woondum had the lowest DW$_{leaf}$ ($p = 0.004$), DW$_{stem}$ ($p < 0.001$; **Figure 6A**), and DW$_{root}$ ($p < 0.001$) followed by Coffs Harbour. Seedlings from Mt Lindsay had
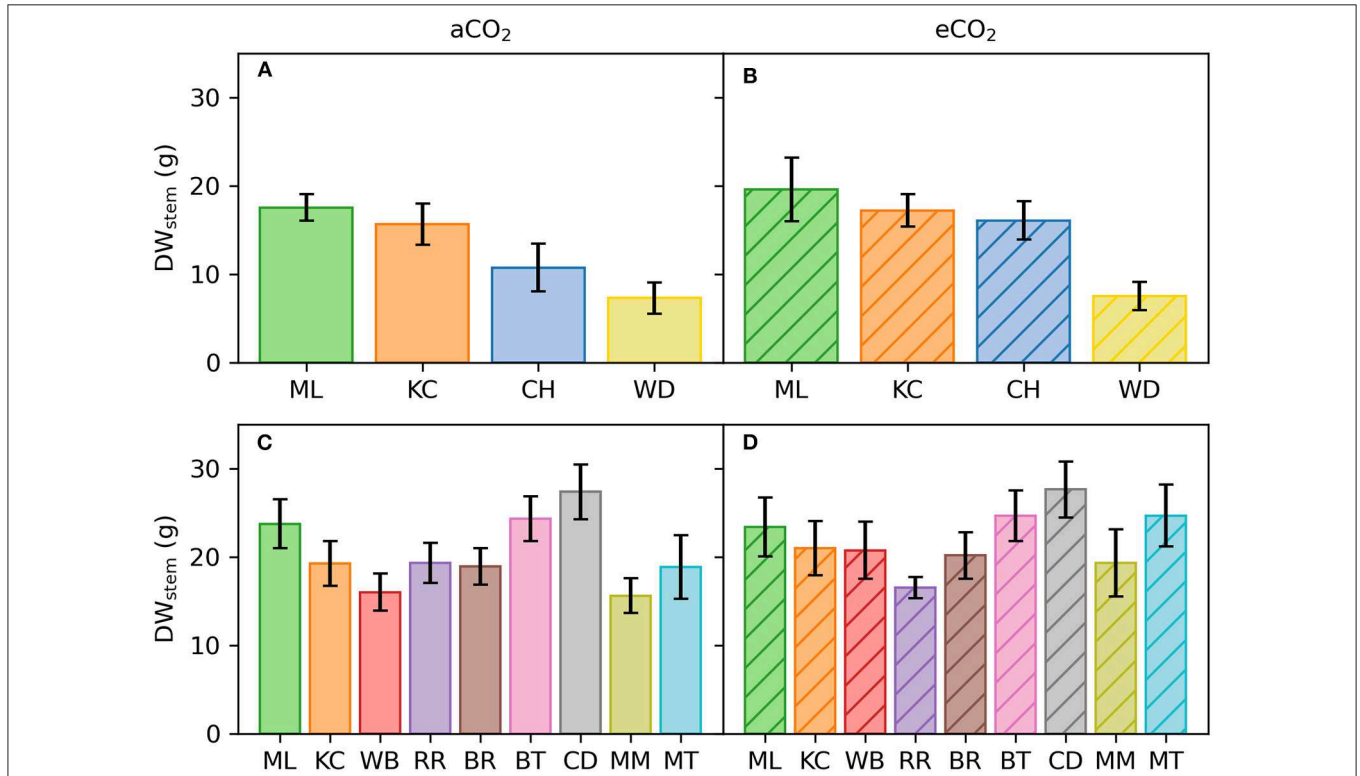
FIGURE 6 | Mean stem dry weight (g) of *E. grandis* at the end of Experiment 1 and 2. Seedlings ($n = 3$–7 per provenance) were grown in ambient (aCO$_2$; **left**) and elevated CO$_2$ (eCO$_2$; **right**). Colors in **(A,B)** represent the four selected provenances of Experiment 1. The nine selected provenances of Experiment 2 are shown in **(C,D)**. Error bars represent 1SE.
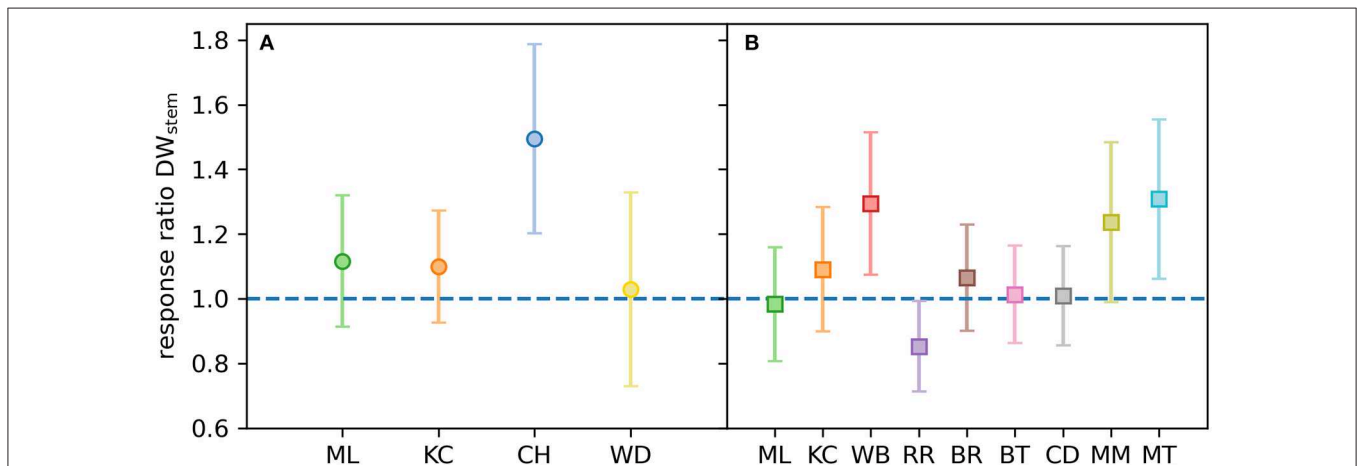
FIGURE 7 | Response ratios of stem dry weight at harvest (DW$_{stem}$) for Experiment 1 and 2. Response ratios represent differences in the different provenances of *E. grandis* grown under elevated [CO$_2$] **(B)** compared to ambient [CO$_2$] **(A**; $n = 3$–7 per provenance and [CO$_2$] treatment). Colors in **(A)** represent the four selected provenances of Experiment 1 and colors in **(B)** represent the nine selected provenances of Experiment 2. Error bars represent variance.

the greatest DW$_{plant}$ (39.8 ± 3 g; **Table 3**; **Figure 6A**). DW$_{root}$ was essentially statistically significant ($p = 0.07$) increasing from 4.9 ± 0.6 g in aCO$_2$ to 6.3 ± 0.8 g in eCO$_2$ (**Table 3**). Responses of DW$_{plant}$ from aCO$_2$ to eCO$_2$ widely differed across provenance for biomass of all plant tissues (DW$_{stem}$ as example shown in **Figures 6B, 7A**). As a trend, seedlings from Coffs Harbour increased mass in all tissues resulting in a mean DW$_{plant}$ of 34.7 ± 4.4 g (+44%). DW$_{stem}$ was correlated to climate parameters MAT, MAP, ET/MAP, mean annual minimum and maximum temperature. The best fit was found for MAT ($r^2 = 0.38$, $p = 0.004$; **Figures 8A,B**) where biomass decreased with greater MAT. In aCO$_2$, this linear fit had a negative slope of −1.54, whereas the slope in eCO$_2$ was slightly steeper with −1.74. Biomass partitioning calculated as LMF, SMF and RMF did not differ among provenances, but LMF was statistically significant and RMF essentially significant, respectively between [CO$_2$] treatments (**Table 7**). In aCO$_2$, LMF averaged 0.40 ± 0.008 compared to 0.37 ± 0.009 in eCO$_2$ ($p = 0.01$; **Table 3**). RMF showed an opposite trend increasing from 0.17 ± 0.006 in aCO$_2$ to 0.19 ± 0.01 in eCO$_2$ ($p = 0.09$; **Table 3**).

At final harvest, leaf number and LA differed significantly among the four provenances, while leaf size and LMA did not. Leaf traits did not significantly respond to [CO$_2$] nor [CO$_2$] x provenance interaction. Under aCO$_2$, seedlings from Mt Lindsay

had developed the most leaves (236 ± 24 plant^{-1}, $p = 0.01$) and had also the largest leaf area (4249 ± 143 cm^2 plant^{-1}, $p = 0.003$; **Table 2**). Seedlings from provenance Woondum had developed about half of the number and area of leaves compared to those from Mt Lindsay. Seedlings from Kilcoy Creek had the largest leaf size (19.0 ± 3.0 cm^2) and those from Coffs Harbour the smallest (15.1 ± 1.9 cm^2; **Table 2**). LMA under aCO$_2$ was similar among provenances, averaging 36.5 to 40.0 g m^{-2}. Although a trend toward increased leaf size and area was apparent in all seedlings when grown in eCO$_2$, large intra-specific variation resulted in non-significant changes compared to seedlings grown in aCO$_2$.
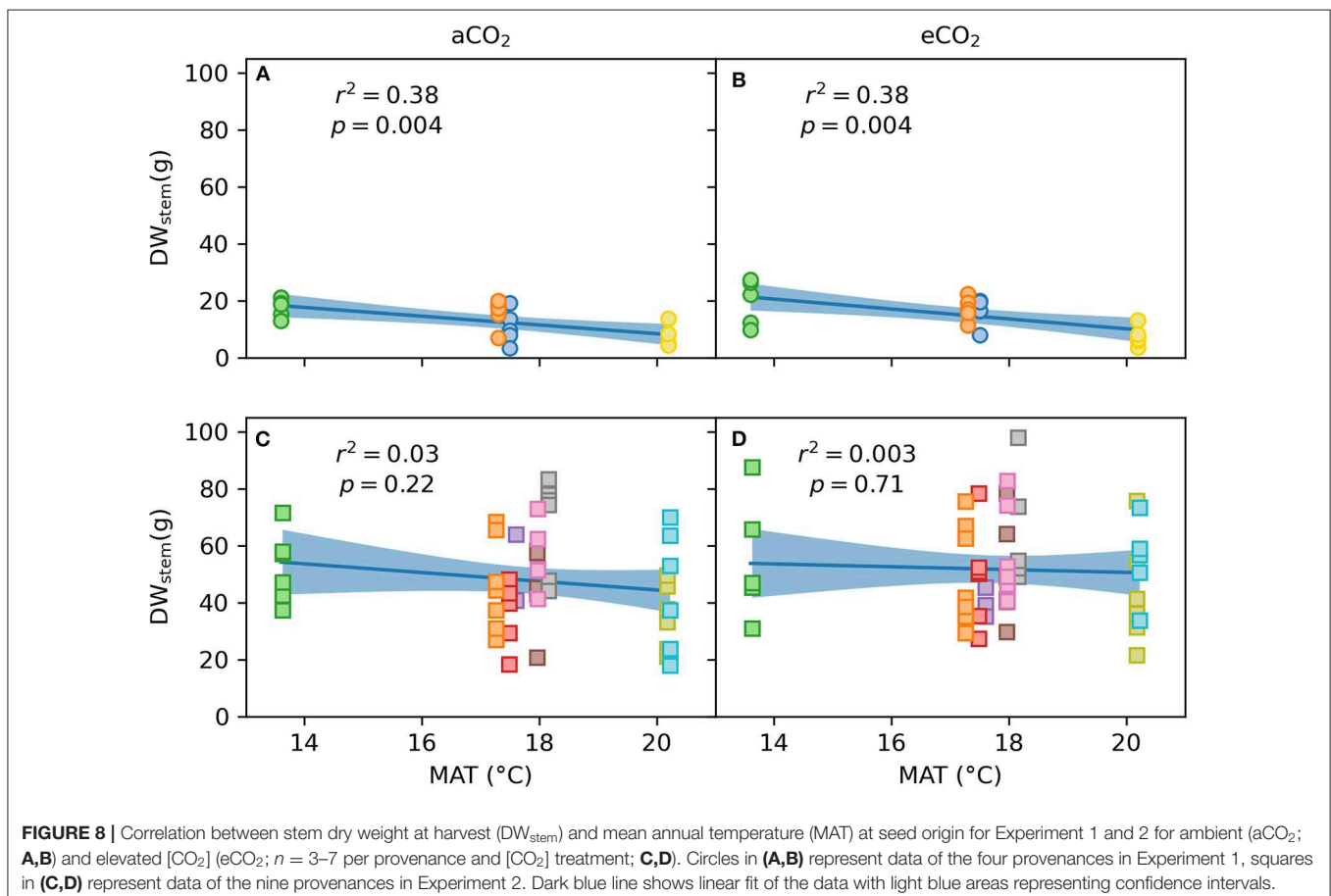
Experiment 2
Environmental Conditions

Relative humidity during Experiment 2 ranged from 62 to 88% between the hours of 10:00 and 16:00. T averaged 27.6 ± 0.2°C and mean [CO$_2$] were for aCO$_2$ 396 ± 0.2 μmol mol^{-1} and for eCO$_2$ 643 ± 0.2 μmol mol^{-1}.

Growth

At harvest, seedlings were 58–109 cm tall. Throughout Experiment 2, v_{stem} was not significant across [CO$_2$] treatments nor [CO$_2$] x provenance interaction yet varied across provenances ($p < 0.001$; **Figures 5C,D**). Seedlings

FIGURE 8 | Correlation between stem dry weight at harvest (DW$_{stem}$) and mean annual temperature (MAT) at seed origin for Experiment 1 and 2 for ambient (aCO$_2$; **A,B**) and elevated [CO$_2$] (eCO$_2$; $n = 3$–7 per provenance and [CO$_2$] treatment; **C,D**). Circles in **(A,B)** represent data of the four provenances in Experiment 1, squares in **(C,D)** represent data of the nine provenances in Experiment 2. Dark blue line shows linear fit of the data with light blue areas representing confidence intervals.

TABLE 2 | Leaf characteristics of *E. grandis* seedlings of Experiment 1 grown in ambient [aCO$_2$; (a)] and elevated [CO$_2$] [eCO$_2$; (b)].

	Provenance			
CO$_2$ treatment	Mt Lindsay	Kilcoy Creek	Coffs Harbour	Woondum
(a) aCO$_2$				
Leaf number	236 (24)	216 (40)	181 (48)	123 (21)
Leaf size	18.7 (1.8)	19.0 (3.0)	15.1 (1.9)	16.3 (3.3)
LA	4249 (143)	3907 (644)	2605 (646)	1975 (582)
LMA	35.7 (1.3)	36.5 (1.2)	37.3 (1.9)	38.3 (4.7)
(b) eCO$_2$				
Leaf number	238 (44)	231 (20)	256 (37)	134 (19)
Leaf size	16.2 (1.7)	16.4 (2.3)	14.4 (0.9)	13.6 (3.0)
LA	3939 (854)	3636 (290)	3678 (532)	1902 (459)
LMA	37.8 (1.7)	37.0 (1.6)	37.0 (1.8)	40.0 (2.1)
(c) Δ (eCO$_2$-aCO$_2$)				
Leaf number	2.0 (0.8%)	15.0 (6.9%)	75.0 (41.4%)	11.0 (8.9%)
Leaf size	−2.5 (−13.3%)	−2.6 (−13.7%)	−0.7 (−4.6%)	−2.7 (−16.5%)
LA	−310 (−7.3%)	−271 (−6.9%)	1073 (41.2%)	−73 (−3.7%)
LMA	2.1 (5.9%)	0.5 (1.3%)	−0.3 (−0.9%)	1.7 (4.5%)

Leaf number and size (cm^2), leaf area (LA, cm^2), and leaf mass area (LMA, g m^{-2}) were averaged per provenance and [CO$_2$] treatment (n = 5). Standard error of mean is shown in parenthesis. Relative changes in leaf characteristics when grown under aCO$_2$ and eCO$_2$ are shown in parenthesis of (c).

TABLE 3 | Dry mass (g) and biomass fractions of *E. grandis* at the end of Experiment 1.

	Provenance			
CO$_2$ treatment	Mt Lindsay	Kilcoy Creek	Coffs Harbour	Woondum
(a) aCO$_2$				
DW$_{leaf}$	15.3 (1.0)	14.1 (2.8)	9.7 (2.4)	7.3 (2.3)
DW$_{stem}$	17.6 (1.5)	15.7 (2.3)	10.8 (2.7)	7.3 (1.8)
DW$_{root}$	7.0 (1.0)	6.1 (1.1)	3.7 (0.9)	2.8 (0.6)
DW$_{plant}$	39.8 (3)	35.9 (5.6)	24.1 (6)	17.4 (4.6)
LMF	0.39 (0.008)	0.39 (0.013)	0.41 (0.012)	0.40 (0.025)
SMF	0.44 (0.016)	0.44 (0.012)	0.44 (0.016)	0.43 (0.013)
RMF	0.17 (0.018)	0.17 (0.007)	0.15 (0.005)	0.17 (0.013)
(b) eCO$_2$				
DW$_{leaf}$	14.6 (2.9)	13.5 (1.3)	13.3 (1.7)	7.7 (1.7)
DW$_{stem}$	19.6 (3.6)	17.2 (1.8)	16.1 (2.2)	7.5 (1.6)
DW$_{root}$	8.0 (1.5)	8.6 (2.1)	5.3 (0.6)	3.3 (0.6)
DW$_{plant}$	42.2 (7.9)	39.4 (4.7)	34.7 (4.4)	18.2 (3.5)
LMF	0.34 (0.005)	0.35 (0.021)	0.38 (0.007)	0.39 (0.027)
SMF	0.47 (0.01)	0.44 (0.008)	0.46 (0.009)	0.42 (0.027)
RMF	0.19 (0.013)	0.21 (0.026)	0.15 (0.008)	0.19 (0.027)
(c) Δ (eCO$_2$-aCO$_2$)				
DW$_{leaf}$	−0.7 (−4.6%)	−0.6 (−4.3%)	3.6 (37.1%)	0.1 (1.4%)
DW$_{stem}$	2.0 (11.4%)	1.5 (9.6%)	5.3 (49.1%)	0.2 (2.7%)
DW$_{root}$	1.0 (14.3%)	2.5 (41.0%)	1.6 (43.2%)	0.8 (17.9%)
DW$_{plant}$	2.3 (5.8%)	4.4 (12.6%)	10.5 (43.4%)	0.8 (4.6%)
LMF	−0.043 (−11.2%)	−0.046 (−11.6%)	−0.022 (−5.5%)	−0.012 (−3.1%)
SMF	0.025 (5.7%)	0.002 (0.4%)	0.021 (4.9%)	−0.010 (−2.2%)
RMF	0.018 (10.4%)	0.044 (26.4%)	0.001 (0.5%)	0.022 (12.9%)

Seedlings were grown in ambient [aCO$_2$; (a)] and elevated [CO$_2$] [eCO$_2$; (b)] atmospheres. Values are separated into dry weight of leaf (DW$_{leaf}$), stem (DW$_{stem}$), and root (DW$_{root}$) tissue, total DW (DW$_{plant}$), leaf-mass-fraction (LMF), stem-mass-fraction (SMF), and root-mass-fraction (RMF) and averaged per treatment group and provenance (n = 5). Standard error of mean for each plant organ is shown in parenthesis. Relative weight difference between plant organs grown under aCO$_2$ and eCO$_2$ is shown in (c).

originating from provenance Bellthorpe had continuously greater stem volume than seedlings from provenance Mt Mee (**Figure 5**). Leaf and stem biomass were not significant across [CO$_2$] treatments ($p =$) nor [CO$_2$] x provenance interaction but differed among the nine provenances (**Table 5**). Under aCO$_2$, seedlings from provenance Mt Mee of the warmest and wettest climate had the lowest DW$_{leaf}$ ($p = 0.008$) and DW$_{stem}$ ($p = 0.01$; **Figure 6C**). Seedlings from provenance Connondale had the greatest DW$_{shoot}$ (67.3 ± 6.9 g; **Table 5**), 91% greater than seedlings from Mt Mee. Similar to Experiment 1, trends for intraspecific variability in growth response to eCO$_2$ were noticeable (**Table 5**; **Figures 6D, 7B**). Seedlings from provenance Wedding Bells SF, similar to seedlings from Coffs Harbour in Experiment 1, had the greatest increase of DW$_{shoot}$ resulting in a mean of 49.1 ± 7.1 g ($+31\%$ from aCO$_2$ to eCO$_2$). Yet, dry weight of shoot and stem was, contrary to Experiment 1, not correlated to any climate parameters of seed origin in aCO$_2$ or eCO$_2$ (**Table 6**; **Figures 8C,D**).

LA and LMA of the nine selected provenances only differed significantly among provenances at the end of Experiment 2 (**Table 4**). Under aCO$_2$, seedlings from Connondale originating from warmer and wetter climate had the largest LA (8212 ± 779 cm^2, $p = 0.002$; **Table 4**). Seedlings from provenance Mt Mee, originating from the wettest climate, had only half the LA compared to Connondale. LMA under aCO$_2$ differed among the nine provenances. Seedlings from provenance Richmond Range with relatively wet climate had the greatest LMA averaging 55.9 ± 3.0 g m^{-2}. Similar to Experiment 1, seedlings from provenance Mt Lindsay had the lowest LMA (41.9 ± 1.2 g m^{-2}). Trends for intraspecific variation in the

response of leaf traits to eCO$_2$ were noticeable. Six of the nine provenances had increased LA under eCO$_2$ with the largest increase in seedlings from provenance Wedding Bells SF ($+28\%$; **Table 4**), resembling similar climate to seedlings from provenance Coffs Harbour in Experiment 1. In aCO$_2$, LA was negatively correlated to MAT (Pearson's $r = -0.27$, $p = 0.05$) and ET of seed origin (Pearson's $r = -0.27$, $p = 0.05$; **Table 6**). LMA was positively correlated to DW$_{stem}$ (Pearson's $r = 0.29$, $p = 0.04$ in aCO$_2$ and Pearson's $r = 0.44$, $p = 0.001$ in eCO$_2$, respectively).

DISCUSSION

Two experiments on *E. grandis* were used to investigate phenotypic plasticity in this important hardwood plantation species. These experiments revealed significant variability regarding leaf physiology traits, concentrations of non-structural carbohydrates and growth parameters among provenances

TABLE 4 | Leaf characteristics of *E. grandis* seedlings of Experiment 2 grown in ambient [aCO$_2$; (a)] and elevated [CO$_2$] [eCO$_2$; (b)].

					Provenance				
CO$_2$ treatment	Mt Lindsay	Kilcoy Creek	Wedding Bells SF	Richmond Range	Borumba Range	Bellthorpe	Connondale	Mt Mee	Mt Tamborine
(a) aCO$_2$									
LA	6786 (461)	5972 (687)	4833 (558)	5186 (752)	5377 (556)	6796 (644)	8212 (779)	4336 (522)	4999 (968)
LMA	41.9 (1.2)	44.1 (2.1)	44.5 (1.6)	55.9 (3.0)	47.5 (2.4)	45.8 (1.6)	48.8 (1.9)	44.9 (2.4)	50.3 (1.5)
(b) eCO$_2$									
LA	6466 (813)	6356 (639)	6173 (821)	4808 (282)	5886 (631)	7351 (973)	7216 (997)	5290 (598)	5872 (574)
LMA	46.5 (1.7)	44.9 (1.5)	46.0 (1.6)	48.4 (1.4)	50.3 (2.9)	45.5 (2.0)	49.8 (1.6)	44.3 (2.6)	53.1 (3.8)
(c) Δ (eCO$_2$-aCO$_2$)									
LA	−320 (−4.7%)	384 (6.4%)	1340 (27.7%)	−378 (−7.3%)	509 (9.5%)	555 (8.2%)	−996 (−12.1%)	954 (22%)	873 (17.5%)
LMA	4.5 (11%)	0.8 (1.8%)	1.5 (3.4%)	−7.5 (−13.4%)	2.9 (5.9%)	−0.3 (−0.7%)	1.0 (2.1%)	−0.6 (−1.3%)	2.8 (5.6%)

Leaf area (LA, cm^2) and leaf mass area (LMA, g m^{-2}) were averaged per provenance and [CO$_2$] treatment (n = 3–7). Standard error of mean is shown in parenthesis. Relative changes in leaf characteristics when grown under aCO$_2$ and eCO$_2$ are shown in parenthesis of (c).

TABLE 5 | Dry mass (g) of *E. grandis* at the end of Experiment 2.

					Provenance				
CO$_2$ treatment	Mt Lindsay	Kilcoy Creek	Wedding Bells SF	Richmond Range	Borumba Range	Bellthorpe	Connondale	Mt Mee	Mt Tamborine
(a) aCO$_2$									
DW$_{leaf}$	28.6 (2.4)	26.6 (3.6)	21.5 (2.6)	29.2 (5.4)	26.1 (3.5)	30.9 (2.6)	39.9 (3.8)	19.6 (2.8)	25.5 (5.2)
DW$_{stem}$	23.8 (2.8)	19.3 (2.5)	16 (2.1)	19.4 (2.3)	18.9 (2.1)	24.3 (2.5)	27.4 (3.1)	15.6 (2)	18.9 (3.6)
DW$_{shoot}$	52.3 (5.1)	45.9 (6.1)	37.5 (4.7)	48.6 (7.7)	45 (5.5)	56 (5.4)	67.3 (6.9)	35.2 (4.7)	44.3 (8.7)
(b) eCO$_2$									
DW$_{leaf}$	30.5 (4.9)	29 (3.8)	28.4 (3.9)	23.2 (1)	30.3 (4.7)	33 (4.1)	35.7 (4.7)	24.1 (4)	30.1 (3.1)
DW$_{stem}$	23.4 (3.3)	21 (3.1)	20.7 (3.2)	16.5 (1.2)	20.2 (2.6)	24.7 (2.9)	27.6 (3.2)	19.3 (3.8)	24.7 (3.5)
DW$_{shoot}$	53.9 (8.1)	50 (6.8)	49.1 (7.1)	39.7 (2.1)	50.5 (7.3)	57.7 (6.9)	63.3 (7.8)	43.5 (7.8)	54.7 (6.4)
(c) Δ (eCO$_2$-aCO$_2$)									
DW$_{leaf}$	1.9 (6.6%)	2.4 (9%)	6.9 (9%)	−6 (−20.6%)	4.2 (16.1%)	2.1 (6.8%)	−4.2 (−10.5%)	4.5 (23%)	4.6 (18%)
DW$_{stem}$	−0.4 (−1.7%)	1.7 (8.8%)	4.7 (29.4%)	−2.9 (−15%)	1.3 (6.9%)	0.4 (1.6%)	0.2 (0.7%)	3.7 (23.7%)	5.8 (30.7%)
DW$_{shoot}$	1.6 (3.1%)	4.1 (8.3%)	11.6 (30.9%)	−8.9 (−18.3%)	5.5 (12.2%)	1.7 (3%)	−3.7 (−5.9%)	8.3 (23.6%)	10.4 (23.5%)

Seedlings were grown in ambient [aCO$_2$; (a)] and elevated [CO$_2$] [eCO$_2$; (b)] atmospheres. Values are separated into biomass of leaf (DW$_{leaf}$) and stem tissue (DW$_{stem}$) as well as cumulated for shoot DW (DW$_{shoot}$) and averaged per treatment group and provenance (n = 3–7). Standard error of mean for each plant organ is shown in parenthesis. Relative weight difference between plant organs grown under aCO$_2$ and eCO$_2$ is shown in (c).

TABLE 6 | Pearson's correlation coefficient (*Pearson's r*) between leaf area (LA), shoot dry weight (DW$_{shoot}$) and stem dry weight (DW$_{stem}$), and climate variables of seed origin of the nine provenances selected for Experiment 2: mean annual temperature (MAT, °C), difference between mean annual maximum and minimum temperature (ΔT, °C), mean annual precipitation (MAP, mm), mean annual evapotranspiration (ET, mm), and evapotranspiration/MAP (ET/MAP).

		MAT		ΔT		MAP		ET		ET/MAP	
Trait	CO$_2$	r	p	r	p	r	p	r	p	r	p
LA	aCO$_2$	−0.27	**0.05**	−0.06	0.69	−0.08	0.57	−0.27	**0.05**	−0.08	0.59
	eCO$_2$	−0.11	0.42	−0.14	0.32	0.06	0.66	−0.16	0.25	−0.14	0.34
DW$_{shoot}$	aCO$_2$	−0.17	0.22	0.02	0.89	−0.14	0.34	−0.16	0.26	0.002	0.99
	eCO$_2$	−0.05	0.71	−0.12	0.41	0.03	0.84	−0.09	0.52	−0.09	0.55
DW$_{stem}$	aCO$_2$	−0.22	0.12	−0.04	0.80	−0.09	0.53	−0.21	0.15	−0.05	0.75
	eCO$_2$	−0.02	0.87	−0.12	0.40	0.03	0.84	−0.06	0.69	−0.07	0.62

Tests were conducted separately for seedlings grown in ambient (aCO$_2$) and elevated (eCO$_2$) [CO$_2$] conditions. Results for p-values below the significance level of 0.05 are highlighted in bold.

grown in aCO$_2$. Yet, contrary to our expectation, variation in biomass was not systematically related to climate at seed origin in *E. grandis* seedlings.

Although eCO$_2$ increased A_{sat} and WUE (calculated from leaf carbon isotope discrimination and reflecting the lifespan of the leaf), this did not result in a systematic stimulation of growth and

TABLE 7 | Statistical results with degrees of freedom (*df*), *f*-values (*f*), and *p*-values (*p*) for Analysis of Variance (ANOVA) for *E. grandis*.

Plant trait	Treatment								
	P			C			P × C		
	df	f	p	df	f	p	df	f	p
(a)									
A_{sat}	3.32	5.9	**0.002**	1.32	10.6	**0.003**	3.32	1.2	0.31
g_s	3.32	6.8	**0.001**	1.32	0.2	0.64	3.32	1.5	0.23
$\Delta^{13}C$	3.32	0.05	0.99	1.32	32.0	**<0.001**	3.32	0.5	0.71
ss_{leaf}	3.31	3.6	**0.02**	1.31	0.4	0.54	3.31	0.8	0.51
st_{leaf}	3.31	2.9	**0.05**	1.31	3.0	0.09	3.31	0.6	0.61
ss_{stem}	3.32	9.6	**<0.001**	1.32	1.6	0.21	3.32	1.9	0.15
st_{stem}	3.32	0.7	0.59	1.32	1.9	0.17	3.32	0.9	0.44
ss_{roots}	3.32	3.9	**0.02**	1.32	0.1	0.71	3.32	0.1	0.98
st_{roots}	3.32	0.8	0.49	1.32	0.1	0.79	3.32	0.5	0.67
LA	3.32	5.8	**0.003**	1.32	0.1	0.79	3.32	0.7	0.57
LMA	3.32	0.5	0.67	1.32	0.4	0.55	3.32	0.1	0.95
Leaf size	3.32	0.9	0.45	1.32	1.6	0.21	3.32	0.1	0.97
Leaf number	3.32	4.3	**0.01**	1.32	1.2	0.28	3.32	0.5	0.69
LMF	3.32	1.8	0.18	1.32	6.8	**0.01**	3.32	0.5	0.71
SMF	3.32	1.5	0.23	1.32	0.8	0.37	3.32	0.6	0.62
RMF	3.32	1.9	0.16	1.32	3.2	0.09	3.32	0.5	0.68
DW_{leaf}	3.32	5.6	**0.004**	1.32	0.2	0.68	3.32	0.5	0.68
DW_{stem}	3.32	9.1	**<0.001**	1.32	2.0	0.16	3.32	0.5	0.72
DW_{root}	3.32	8.9	**<0.001**	1.32	3.5	0.07	3.32	0.3	0.81
DW_{plant}	3.32	7.8	**<0.001**	1.32	1.4	0.25	3.32	0.4	0.79
(b)									
LA	8.85	3.5	**0.002**	1.85	0.9	0.36	8.85	0.5	0.84
LMA	8.85	4.2	**<0.001**	1.85	0.3	0.56	8.85	1.0	0.44
DW_{leaf}	8.86	2.8	**0.009**	1.86	1.0	0.33	8.86	0.5	0.84
DW_{stem}	8.85	2.7	**0.01**	1.85	1.3	0.26	8.85	0.4	0.94
DW_{shoot}	8.85	2.7	**0.01**	1.85	1.1	0.30	8.85	0.4	0.91

Plant traits were analyzed for significant effects of [CO$_2$] levels (C) and provenance (P). Results of Experiment 1 are shown in (a), results for Experiment 2 in (b). Traits were: light-saturated photosynthesis rate (A$_{sat}$), stomatal conductance (g$_s$), water use efficiency (Δ^{13}C), concentration of soluble sugars in leaf (ss$_{leaf}$), stem (ss$_{stem}$) and roots (ss$_{roots}$) as well as starch concentrations (st), leaf area (LA), leaf mass area (LMA), leaf-mass-fraction (LMF), stem-mass-fraction (SMF), root-mass-fraction (RMF), dry weight (DW) of leaves, stem, roots, shoots (DW$_{leaf}$ + DW$_{stem}$), or total plant, respectively. Results for p-values below the significance level of 0.05 are highlighted in bold.

increased biomass among provenances. Systematic stimulation of growth by eCO$_2$ was not apparent, possibly due to the limited number of selected provenances and high intraspecific plant-to-plant variation. However, even by increasing the number of tested provenances to improve statistical power in Experiment 2, we did not observe a uniform growth stimulation and biomass (stem, leaves, roots) increase in eCO$_2$ nor a significant provenance by CO$_2$ effect. However, some provenances did show greater responses of physiology (A_{sat} and g_s) or growth in eCO$_2$ than others, potentially related to environmental conditions of seed origin—as seen with provenances Coffs Harbour and Wedding Bells SF with strong positive biomass increases under eCO$_2$. Yet, unidirectional phenotypic plasticity among provenances as result of eCO$_2$ was not observed due to great variability within groups.

Physiological Differences Among Provenances

Greater carbon assimilation, due to greater A_{sat} and a larger canopy, enabled seedlings from regions with low ET/MAP to also produce more stem biomass (i.e., provenance Coffs Harbour) relative to seedlings from drier climates (i.e., provenance Woondum and Mt Mee). Variability in LMA among provenances can also influence plant growth (Poorter et al., 2009) as shown with our LMA data correlating with stem biomass. As LMA has a strong positive relationship with leaf density, our results suggest that provenances MT and RR have greater leaf density than provenances KC and ML. Higher leaf density may be caused by a greater proportion of mesophyll tissue or lignified tissue resulting in greater leaf toughness and consequently plant survival (Poorter et al., 2009). Despite these observed trends, stem biomass of *E. grandis* was not correlated to climate of origin in Experiment 2. We thus had to reject the notion that results from Experiment 1 could be used to extrapolate growth responses to eCO$_2$ across wider environmental gradients. However, intraspecific differences across provenances or families are frequently reported for trees in response to their local climate conditions. European pine and beech species (Correia et al., 2008; Rose et al., 2009; Bachofen et al., 2018) as well as North

American poplars (Kaluthota et al., 2015) as well as eucalypts (Blackman et al., 2016; Aspinwall et al., 2018) have been shown to display considerable intraspecific variation in physiological and growth-related traits when grown as potted plants or in common gardens. These variations were correlated to temperature, water availability and the length of the growing season at seed origin. Seedlings from provenances originating either from areas with wet and cool or wet and warm climate produced the greatest biomass when grown at aCO₂. In support of this trend of adaptation to local climate conditions, biomass production in *E. grandis* was greater in trees from cooler and wetter climates (Drake et al., 2015). Thus, *E. grandis* does show signs of a systematic adaption to environmental conditions at seed origin, yet climate conditions alone are insufficient to explain observed responses for every provenance.

Physiological Responses of Provenances to eCO₂

Intraspecific variation across provenances was more prominent than responses to eCO₂. Hence, ranking among provenances for physiology and growth traits persisted, supporting Hypothesis 2. A_{sat} increased by 24% on average, similar to the stimulation of A_{sat} under eCO₂ in other trees (e.g., Ainsworth and Rogers, 2007; Ghannoum et al., 2010; Leakey et al., 2012; Resco de Dios et al., 2016), which predominantly results from increased rates of carboxylation by Rubisco and reduced rates of photorespiration (Drake et al., 1997; Long et al., 2004). Additionally, eCO₂ increased WUE in all provenances (i.e., $\Delta^{13}C$ declined), an effect that has been attributed to decreases in g_s (Saxe et al., 1998; Medlyn et al., 2001) and subsequent decline in conductance of water vapor by leaves (e.g., Ainsworth and Rogers, 2007; Adair et al., 2011; Keenan et al., 2013; Reich et al., 2014; Frank et al., 2015; Dekker et al., 2016). Yet, in *E. grandis*, improved WUE was due to increasing carbon uptake (increases in A_{sat}) under non-limiting water supply rather than decreases in g_s, a strategy also confirmed for *Fagus sylvatica* (Aranda et al., 2017). Under eCO₂, g_s is commonly reported to decline (e.g., Ainsworth and Long, 2005). However, a lack of downregulation of g_s under eCO₂ was also found in *Larix* and *Pinus* trees (Streit et al., 2014). Furthermore, the strong influence of leaf tissue structure on gas exchange in *E. camaldulensis* (Blackman et al., 2016) may explain why g_s response to eCO₂ was not significant given that LMA was also not affected in *E. grandis*. Regrettably, we did not investigate stomatal traits, which might have helped differentiate anatomical from biochemical constraints on WUE (Blackman et al., 2016). Limited information suggests changes in stomatal density of broadleaved trees may occur as an interactive response to [CO₂], water availability and origin of provenance (Pyakurel and Wang, 2014). In agreement with the current study, most leaf physiological traits in 14 genotypes within six provenances of *E. camaldulensis* showed extensive intraspecific variation, but a limited growth response to eCO₂ (Blackman et al., 2016).

When trees are grown under eCO₂, they often exhibit increased carbon assimilation rates. However, these increases sometimes are not reflected in simultaneous increases in stem biomass, indicating that additional carbon gained is not necessarily allocated to stem growth (Resco de Dios et al., 2016). For example, net carbon assimilation of *E. camaldulensis* was greatly stimulated by eCO₂, yet no increase in stem biomass or LMA (leaf structure) was detected (Blackman et al., 2016). In the present study, concentration of starch in leaves of *E. grandis* was greater in eCO₂ compared to aCO₂, suggesting that leaves may serve as temporary storage organs under eCO₂ rather than building new leaf structure (e.g., larger or thicker leaves).

Another possible fate of additional carbon gained in eCO₂ can be its loss through elevated rates of cell respiration and/or root exudation. We did not examine either of the two processes, but studies have shown that large variability exists in the effects of eCO₂ on plant mitochondrial respiration rates (see Dusenge et al., 2019 for review). Studies have found respiration to remain stable, increase or decline when plants are exposed to eCO₂ (Curtis, 1996; Wang et al., 2001; Tissue et al., 2002; Ayub et al., 2011; Crous et al., 2012; Li et al., 2013; Gauthier et al., 2014). Increased rates of respiration would explain the absence of greater allocation of assimilated carbon into aboveground biomass in *E. grandis*. However, higher respiration rates have also been linked to greater carbohydrate accumulation in pine (Tjoelker et al., 2009; Li et al., 2013), but it was not observed here in *E. grandis*.

Growth is often enhanced in other species of *Eucalyptus* grown under eCO₂ (Ghannoum et al., 2010; Ayub et al., 2011; Smith et al., 2012; Duan et al., 2013; Aspinwall et al., 2018), but was not statistically significant here for stem and leaf biomass of *E. grandis*. However, the lack of significant aboveground growth stimulation in eCO₂ has been observed in other species of *Eucalpytus* (Duff et al., 1994) and *Populus* (Liberloo et al., 2005) despite increased carbon assimilation (Cantin et al., 1997; Atwell et al., 2007; Lawson et al., 2017; Bachofen et al., 2018; Killi et al., 2018). It has also been observed that carbon may be allocated belowground to facilitate increased nutrient uptake when photosynthesis is increased in eCO₂ (Hättenschwiler and Körner, 1997; Iverson et al., 2008; Pritchard et al., 2008; Souza et al., 2016; Aspinwall et al., 2018). Here, 13% more biomass was on average allocated to roots rather than leaves (−8%) in Experiment 1. Thus, biomass responses of *E. grandis* to eCO₂ may be due to carbon allocation to roots rather than aboveground biomass allocation.

Environmental Drivers

Cooler climate and low ET/MAP at seed origin of trees from Mt Linsday may represent contributing factors to the pronounced increase of A_{sat} in the different provenances when grown in eCO₂. A common garden study on European *Picea abies* found that populations from cooler climates at high-altitudes had higher leaf nitrogen concentrations, greater A_{max} and higher concentrations of chlorophyll than those from lower elevations (Oleksyn et al., 1998). In *E. camaldulensis*, genotypes with the largest growth response to eCO₂ had large increases in root mass fraction and photosynthetic nitrogen-use efficiency (Aspinwall et al., 2018). The Coffs Harbour (Experiment 1) and Wedding Bells (Experiment 2) provenances had the largest relative increase of leaf area and biomass when grown in eCO₂. Both locations are in very close proximity to each other and experience a similarly

low ET/MAP compared to Mt Lindsey. Shifting such species from cooler to warmer growth conditions could influence temperature optima for photosynthesis. This physiological optimization due to warming while maintaining optimal access to water is in agreement with previous temperature-shift experiments using *E. grandis* (Drake et al., 2015).

In addition to climate, soil characteristics are known to influence productivity of eucalypt trees (Cavalli et al., 2020). *Eucalyptus grandis*, however, tolerates a wide range of soil conditions, reflected in high growth rates of provenances in their native habitats in Australia as well as plantations around the world. Here we controlled the influence of soil on growth by providing all provenances with the same customized, non-nutrient limited soil. The impact of eCO$_2$ on stem growth of individual provenances, when grown in soil from their native habitat, should be investigated in future glasshouse experiments.

CONCLUSIONS

It now seems well-established that tree genotypes and provenances respond differentially to eCO$_2$ (Ceulemans et al., 1996; Dickson et al., 1998; Isebrands et al., 2001; Mohan et al., 2004). While individual species may show increased stem growth and biomass accumulation under eCO$_2$, this is not a widely observed trend (Resco de Dios et al., 2016). Here we added one of the most important hardwood plantation species to the growing list of tree species that seem to accelerate photosynthetic carbon assimilation under eCO$_2$ yet do not use surplus photosynthates to increase stem growth. Selection of high performing tree provenances for the next generation of

hardwood plantations in a higher CO$_2$ world must account for this effect to remain economically profitable.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AW, SP, and DT designed Experiment. AW, CB, DT, and SP designed Experiment. RS managed the controlled environment facilities, including CO$_2$ treatments. AW analyzed the data and wrote the complete first draft. All authors collected plant physiological measurements, harvested data, and assisted in developing the final version of the manuscript.

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