



# Morphological, physiological and carbon balance response of *Eucalyptus* genotypes under water stress

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## Abstract

Water stress is considered one of the main environmental factors that limit survival and productivity of *Eucalyptus* plantations. Identifying genotypes traits that provide evidence of tolerance to water stress may allow sustained productivity and secure better resilience of forest plantations under climate change in Mediterranean environments. Our study analyzed morphological, physiological, and carbon (C) balance responses of ten *Eucalyptus* genotypes under contrasting water regimes under nursery conditions. One-year-old cuttings of *Eucalyptus nitens* (En1 and En2), *E. smithii* (Es), *E. badjensis* (Eb), *E. nitens x globulus* (Eng1, Eng2, Eng3, and Eng4), *E. globulus* (Eg), and *E. camaldulensis x globulus* (Ecg) were evaluated under progressive drought from well-watered (soil water potential close to 0 MPa) to severe water stress (soil water potential close to  $-2.5$  MPa) conditions. Absolute growth rate (root collar diameter, height, shoot: root biomass ratio), net photosynthesis, stomatal conductance, transpiration, intrinsic water use efficiency (iWUE), predawn water potential ( $\Psi_{pd}$ ) and C balance (flux and partitioning) were evaluated. As expected, water stress significantly reduced growth and physiological activity for all genotypes. Of all evaluated parameters iWUE and  $\Psi_{pd}$  were the key grouping physiological and growth response variables among genotypes. Genotypes En1, Eng3, and Eng4 were considered tolerant genotypes, with the smallest physiological change and larger morphological growth. Genotypes En2, Es, Eng1 and Eng2 were moderately tolerant showing intermediate responses compared to other groups. In contrast, Eg, Eb, and Ecg were considered highly sensitive, with major changes in morphology and physiological variables over time. Our results suggest that nursery stage physiological evaluation of genotypes may allow selection for sites with water resource availability risks and may allow to reduce mortality and early establishment productivity losses under drier climate change scenarios.

**Keywords** Productivity · Early selection · Tree physiology · Carbon balance · Climate change

## Introduction

*Eucalyptus* is one of the most planted hardwood genus worldwide with more than 25 million hectares (Martins et al. 2022). The wide natural distribution of *Eucalyptus* species under a broad range of contrasting environmental conditions (e.g., soil, temperature, and precipitation) have supported the selection of valuable species planted for production of wood, paper, bioenergy, charcoal, and biomaterials for forest industry (Hubbard et al. 2010; Zhang et al. 2022). In Chile, commercial plantations of *Eucalyptus* species have become an important source of fiber and timber resources with more than 850,000 ha (INFOR 2020) and intensive genetic and silvicultural management programs that have reduced rotation cycles, increased yields and reduced pests susceptibility (Gonçalves et al. 2013; Rubilar et al. 2018). In the last decade, *Eucalyptus globulus* (selected for its high pulp yield and adaptability to dry sites), *E. nitens* (selected for its high growth in cold environments) and their hybrid (*E. nitens* × *globulus*) are the dominant species being planted in commercial reforestation (Silva et al. 2017).

Despite advances in intensive forest management, climatic variability still strongly influences *Eucalyptus* productivity (Forrester et al. 2010; Whitehead and Beadle 2004). Water scarcity is considered the main factor limiting trees survival and growth (El-Sharkawy 2014; Martins et al. 2022). In fact, climate simulation models have projected an increase of 1 to 4 °C in the global mean temperatures, with a reduction in rainfall patterns that will lead to water deficits and large scale regional droughts in many regions planted with *Eucalyptus* (Bahuguna and Jagadish 2015; Booth 2013; Elli et al. 2020; Fagundes et al. 2021).

In order to reduce plantations risks, several authors have recommended an acclimation process through changes in early silvicultural management and genetic selection (Corrêa et al. 2017; Pita-Barbosa et al. 2023; Saadaoui et al. 2017). Evidence has shown that, some taxa/genotypes have greater tolerance to water stress conditions from early ages by adjusting morphological, physiological, biochemical, and hormonal response mechanisms (Mueller et al. 2020). Identifying these water stress tolerance physiological or morphological traits in young trees may help to reduce uncertainty of *Eucalyptus* survival and productivity in many regions where climate change has increased the frequency or intensity of drought periods (Saadaoui et al. 2017). Water stress tolerance has been linked to morphological adaptations. For example, Susiluoto & Berninger (2007), found that the root:shoot ratio of juvenile trees of *E. microtheca*, was the key trait that differentiated sensitive to tolerant clones to drought (tolerant clones showed greater survival by increasing roots at the expense of shoot growth). Similarly, Corrêa et al. (2017), comparing 13 commercially planted *Eucalyptus* genotypes, evaluated 34 morphological and nutritional variables and obtained a tolerance ranking explained by 70% only based on morphological traits that included diameter, height, and leaf area. Physiological traits, such as the ability to regulate stomatal and mesophyll conductivity ( $g_s$  and  $g_m$ , respectively) (Chaves et al. 2009; Flexas et al. 2008; Warren et al. 2011), control of water loss by transpiration (Flexas et al. 2008), and photosynthetic adaptation, that allows maintaining metabolic processes and in some cases growth (Erice et al. 2010; Ma et al. 2014), have become relevant to determine tolerance thresholds of genotypes to water stress environmental restrictions. To illustrate, Aspinwall et al. (2016) found that photosynthesis and respiration were key traits for survival and acclimation of *E. tereticornis* genotypes under different stress conditions. Similarly, Nória Júnior et al. (2020) differentiated tolerant clones of *Eucalyptus grandis* × *Eucalyptus urophylla* through changes in C balance, a decrease in stomatal conductance, size, and the number of stomata and variation in water use efficiency. Muller et al. (2020)

classified the response of ten juvenile genotypes (*E. urophylla* and *E. grandis x urophylla*) to water deficit conditions based on changes in net photosynthesis, transpiration, and root growth. Finally, Duan et al. (2013) suggested that water stress adaptability is driven by carbon (C) allocation, with significant reductions in aboveground (leaves>branch>stem) compared to belowground production.

Maintaining forest-sustained production and plantation sustainability in the face of climate change requires an early selection of taxa/genotypes with morphological and physiological tolerance to water stress traits (Hodecker et al. 2018; Pita-Barbosa et al. 2023). Therefore, our study objective was to evaluate morphological, physiological, and C balance responses of ten *Eucalyptus* genotypes under contrasting water regimes to understand better the potential response of these selected materials under challenging climate change scenarios.

## Materials and methods

### Plant material and experimental design

The study evaluated 10 selected genotypes of *Eucalyptus nitens* (En1 and En2), *E. nitens x globulus* (Eng1, Eng2, Eng3, and Eng4), *E. globulus* (Eg), *E. badjensis* (Eb), *E. camaldulensis x globulus* (Ecg) and *E. smithii* (Es). Taxa and genotype selection was based on mid-rotation survival, productivity and growth response until eight years of age of the selected materials growing under a large-scale experiment named EucaHydro evaluating their performance under contrasting water stress treatments (details in Rubilar et al. (2020). The plants were produced at Forestal Mininco SpA Forest nursery (Yumbel, Bio-Bio, Chile; 37° 8'0.01" S, 72° 27'34.70" W); and at 11 months old, 420 plants (42 per genotype) were selected considering plants of similar height and root collar diameter showing no signs of mechanical damage or disease (Table 1).

Selected plants were transported to the nursery of the University of Concepción, Concepción, Bio-Bio, Chile (36° 50'3.35" S, 73° 1'55.74" W), transferred to 7.5 L plastic pots filled with homogenized (<2 mm) pine bark compost, and acclimated during 90 days before initiation of the experiment by maintaining soil water potential ( $\Psi_{\text{soil}}$ ) close to field capacity that was monitored daily using SMP 229-L psychrometers (Campbell Scientific, USA). In order

**Table 1** Mean root collar diameter (RCD, mm) and height (H, cm) of all selected *Eucalyptus* genotypes at the beginning of the experiment

Genotype	RCD (mm)	H (cm)
En1	9.57 ± 0.85	79.63 ± 4.25
En2	10.06 ± 0.73	84.13 ± 7.60
Eng1	8.62 ± 0.79	67.32 ± 6.72
Eng2	9.46 ± 0.40	77.50 ± 3.91
Eng3	8.79 ± 0.43	83.50 ± 4.09
Eng4	9.96 ± 0.86	88.13 ± 5.22
Eg	8.09 ± 0.50	73.88 ± 4.04
Eb	8.45 ± 0.42	89.88 ± 7.79
Ecg	8.04 ± 0.41	58.57 ± 8.37
Es	8.43 ± 0.58	103.88 ± 6.81

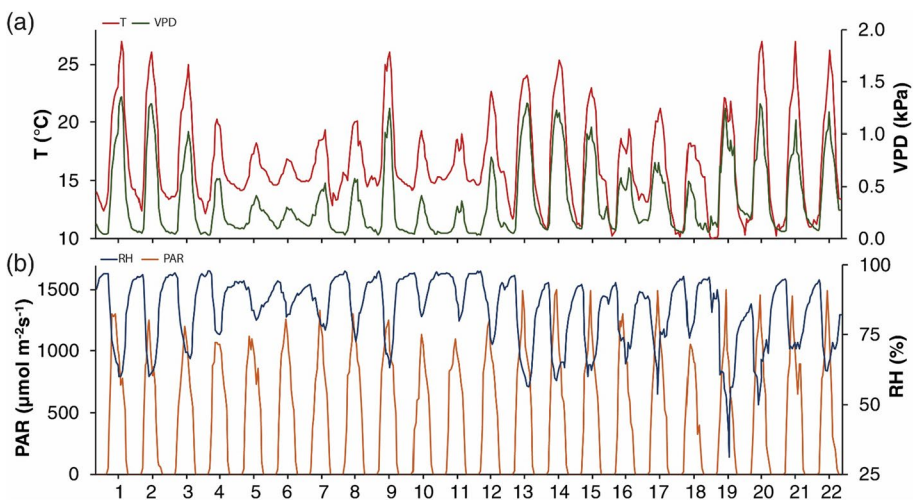
Data shown are the mean ± standard error

to remove potential nutrient limitations and maintain standard plant nutrition, fertilization was applied every 15 days with a standard growth media solution of Phostrogen® (NPK 13–10–27, S, Mg, Ca, Fe, B, Mn, Cu, Mo, Bayer Garden, UK) at  $1 \text{ g L}^{-1}$ . From this point, the experiment was carried out under natural light, temperature, and relative humidity conditions that were monitored using an automated Vantage Pro 2 weather station (Davis Instruments, USA) placed at the nursery site.

The experiment started in February 2021 (summer) for 22 days. Ambient temperature during this period ranged from 10.9 to 24.2 °C (Fig. 1a), vapor pressure deficit (VPD) ranged from 0.24 to 1.72 kPa (Fig. 1a), relative humidity ranged from 25.1 to 93.6% (Fig. 1b); ambient light was homogeneous for all treatments, with an average daily exposure of 12.3 h with maximum photosynthetically active radiation (PAR) ranging from 1020 to 1520  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  (Fig. 1b). A single rainfall event of 0.75 mm was recorded, but a polypropylene insulation plastic film was used to cover plants to avoid undesired input of water in the experiment during the event.

Plants of each genotype were randomly split in two water regime treatments (21 plants per genotype). Water regimes considered: (i) well-watered (WW) treatment, where soil water potential ( $\Psi_{\text{soil}}$ ) was maintained near 0 MPa (control treatment); and (ii) water-stressed (WS) treatment, in which no irrigation was applied until  $\Psi_{\text{soil}}$  reached  $-2.5 \text{ MPa}$  (considered a severe plant water deficit).  $\Psi_{\text{soil}}$  was estimated indirectly using volumetric moisture content (VWC) values estimated using a TDR sensor (CS-615 Campbell Scientific Inc.). A volumetric moisture content relationship with  $\Psi_{\text{soil}}$  was adjusted by fitting a generalized cubic polynomial equation (Eq. 1,  $R^2=0.94$ ,  $p\text{-value}<0.001$ ) after taking repeated soil samples at a range of  $\Psi_{\text{soil}}$  values following the methodology described by Silva et al. (2014a) using a Richards pressure plate model 1500 (Soilmoisture Equipment Corp.).

$$\Psi_{\text{soil}} = -1 \times 10^5 (\text{VWC})^3 + 0.003 (\text{VWC})^2 - 0.1802 \text{VWC} \quad (1)$$



**Fig. 1** Daily air temperature (T), vapor pressure deficit (VPD), relative humidity (RH) and photosynthetic active radiation (PAR) values measured during the experiment in February 2021

where  $\Psi_{\text{soil}}$  is soil water potential in kPa; and VWC is volumetric moisture content in percentage.

## Morphological variables

Plant height (H) and root collar diameter (RCD) were measured at the beginning and end of the experiment ( $n=6$  plants per genotype  $\times$  water regime). Also, shoot (SB) and root (RB) dry biomass was determined using destructive sampling ( $n=4$  plants per genotype  $\times$  water regime). Stem, leaves, branches, and root biomass components were weighed green, and dried at 65 °C for 48 h to estimate dry weight. Measured biomass dry weights, H, and RCD, were used to develop allometric equations for each genotype and water regime (supplementary information S1) to obtain biomass dry weight estimates for all plants at each measurement instance. Morphological variables were used to estimate the absolute growth rate using the equation reported by Pommerening and Muszta (2015) (Eq. 2).

$$V_{AGR} = \frac{V_i + 1 - V_i}{t_i + 1 - t_i} \quad (2)$$

where  $V_{AGR}$  is the absolute growth rate of morphological variable  $V$  (RCD in mm each 10 days<sup>-1</sup>, H in mm day<sup>-1</sup>, and dry biomass in g day<sup>-1</sup>);  $V_i$  is the morphological measurement at time  $i$ ;  $V_{i+1}$  at time  $i$ ,  $t$  and  $t_{i+1}$  are starting and final times of measurement (day).

## Physiological variables

Individual plant physiological assessments considered net photosynthetic assimilation rate ( $A_N$ ), stomatal conductance ( $g_s$ ), transpiration ( $E$ ), intrinsic water-use efficiency was estimated as the ratio of  $A_N/g_s$  ( $iWUE$ ), and predawn leaf water potential ( $\Psi_{pd}$ ). Measurements were taken at the beginning of the experiment when  $\Psi_{\text{soil}}$  was close to 0 MPa, and when plants reached  $-2.5$  MPa, on six leaves of six independent plants for each genotype and water regime.  $A_N$ ,  $E$  and  $g_s$  were measured using an infrared gas analyzer (IRGA) model LI-6400XT (LI-COR, Inc. USA) under a photosynthetic photon flux density (PPFD) of 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , a leaf temperature of 20 °C, a constant flow rate of 500  $\mu\text{mol s}^{-1}$ , a  $\text{CO}_2$  concentration of 400  $\mu\text{mol mol}^{-1} \text{CO}_2$ , and maintaining a relative humidity range of 40 to 70%. Measurements were performed between 10:00 to 12:00 (UTC – 3:00 Chile summer time) under sunny weather conditions.  $\Psi_{pd}$  was evaluated between 4:00 to 6:00 (UTC – 3:00 Chile summer time) using a Scholander pressure pump model 1505D (PMS Instruments Co.) by sampling tree plants per genotype for each water regime.

## Carbon balance

C flux (expressed on  $\text{g C m}^{-2} \text{month}^{-1}$ ) and C partition (% of each biomass components) were estimated for the study period using the mass balance approach proposed by Ryan (1991), Giardina and Ryan (2002) and Giardina et al. (2003). Gross primary production (GPP) was determined as the sum of aboveground net primary production (ANPP), aboveground autotrophic respiration ( $R_{ag}$ ) and total belowground C flux (TBCF). ANPP was calculated as the sum of foliage net primary production (FNPP) and wood net primary production (WNPP); both variables were estimated using biomass allometric equations developed and determining C concentration ( $n=9$  for each biomass component of each

genotype and water regime) using a CHONS elemental analyzer (SERCON. Co.) (supplementary information S2). In the case of  $R_{ag}$ , it was calculated using Eq. 3 and considering a CUE (carbon use efficiency) value of 0.53 as suggested from previous studies of *Eucalyptus* spp. plantations (Campoe et al. 2012; Epron et al. 2012; Stape et al. 2008).

$$R_{ag} = ANPP \left( \frac{1 - CUE}{CUE} \right) \quad (3)$$

TBCF was estimated using Eq. 4, where Soil CO<sub>2</sub> efflux ( $F_S$ ) was measured using a gas analyzer system model LI-850 (Lincoln, NE, USA) on days where genotypes reached each target  $\Psi_{soil}$  ( $n=4$  plants per genotype  $\times$  water regime). Measurements were conducted on daytime (6:00 to 20:00) and nighttime hours (21:00 to 24:00) to evaluate diurnal genotype belowground respiration ( $n=4$  trees per genotype  $\times$  water regimes).  $F_E$  was not considered of significance given that different of other studies there was no C loss by erosion or leaching in the substrate of the pots at these nursery conditions (Campoe et al. 2020; Rodrigues et al. 2021; Ryan et al. 2010). Also,  $F_A$  was discarded, given that litterfall was not significant (no liter fall during the duration of the study). Similarly, changes in substrate-soil C ( $\Delta C_S$ ) and the organic horizon ( $\Delta C_L$ ) were considered negligible in our study given the short period of the study (22 days). Finally,  $\Delta C_R$  was estimated as the change in coarse root biomass C content using biomass allometric equations and C concentration.

$$TBCF = F_S + F_E - F_A + \Delta(C_R + C_L + C_S) \quad (4)$$

## Statistical analysis

The experiment considered a complete factorial randomized design with genotype (10 genotypes) and irrigation (control vs suppressed irrigation regimes) as main factors and individual plants as replicates. Morphological (RCD, H, SB, RB, total dry mass TDM), physiological ( $A_N$ ,  $g_s$ , E, iWUE and  $\Psi_{pd}$ ), and C balance (GPP, ANPP,  $R_{ag}$  and TBCF) data were analyzed using a two-way ANOVA mixed model (Eq. 5). ANOVA analyses considered genotype ( $G_i$ ) and genotype  $\times$  water regime interaction ( $I_{ij}$ ) as random and water regime ( $\tau_j$ ) as a fixed effect. Differences among treatment means were determined using a Tukey test. Principal Component Analysis (PCA) and a dendrogram analysis using Ward's linkage with Bray–Curtis dissimilitude index were used to identify variables that contributed more significantly to explain experimental variation and were used to define similar genotype groups response for each measured variable.

$$y_{ij} = \mu + G_i + \tau_j + I_{ij} + \varepsilon_{ij} \quad (5)$$

All data were analyzed with R version 4.2.1 (Team, 2021) and analyses were considered statistically significant with a level of  $p < 0.05$ .

## Results

### Morphological and physiological responses

Differences in all morphological variables were found among genotypes, water regimes, and their interaction (Table 2). The RCD, H, and SB absolute growth rate were

**Table 2** ANOVA *p*-value results of the analyses evaluating the effects of water regime (WR), genotype (Gen), and the genotype x water regime interaction (Gen x WR) for each morphological and physiological variable evaluated for all evaluated *Eucalyptus* genotypes

Variable	Effects		
	Gen	WR	Gen x WR
<i>Morphological</i>			
RCD	<0.001	0.002	0.002
H	<0.001	0.003	<0.001
SB	<0.001	0.002	0.002
RB	<0.001	0.002	0.002
<i>Physiological</i>			
$A_N$	0.002	0.002	<0.001
$g_s$	0.133	0.003	0.110
iWUE	<0.001	<0.001	<0.001
$\Psi_{pd}$	0.002	0.002	<0.001

RCD root collar diameter, H height, RB root biomass, SB shoot biomass,  $A_N$  net photosynthesis rate,  $g_s$  stomatal conductance, E transpiration rate,  $\Psi_{pd}$  predawn leaf water potential and iWUE, and intrinsic water use efficiency. Values in bold indicate significant values ( $p < 0.05$ )

significantly reduced for all genotypes under water stress conditions compared to well-watered conditions (Table 3). Interestingly, RCD, showing almost no growth for several genotypes, was the most affected variable under water stress compared to H, SB, and RB. The RCD absolute growth rate of Eng1, Eng2, Eb, Ecg, and Es did not show any changes under WS regime; in contrast, En1, En2, Eng3, and Eng4 showed an average increment of  $0.85 \text{ mm} \times 10 \text{ day}^{-1}$ . In the case of En1 and Eng3 genotypes, both reported the lowest reduction in absolute growth rate under water stress conditions with average values of 7.5% for RCD, 46.2% for H, and 29.0% for SB. Contrastingly, Eg, Eb, and Ecg genotypes obtained the most significant reduction in H (average 78.2%) and SB (average 65.5%). An interesting result was observed for the absolute growth rate of RB comparing both water regimes that under water stress showed an average increment of 23.1% for En1, En2, Eng1, Eng3, and Eb, while for Eng2, Eng4, Eb, Ecg, and Es showed an average reduction of 43.0%. Finally, for the root-to-shoot ratio (R:S) differences were observed between WW and WS water regimes where only Ecg reported a decrease of 33.1% underwater stress, compared to the rest of the genotypes that showed average increases from 3.0 to 69.2%.

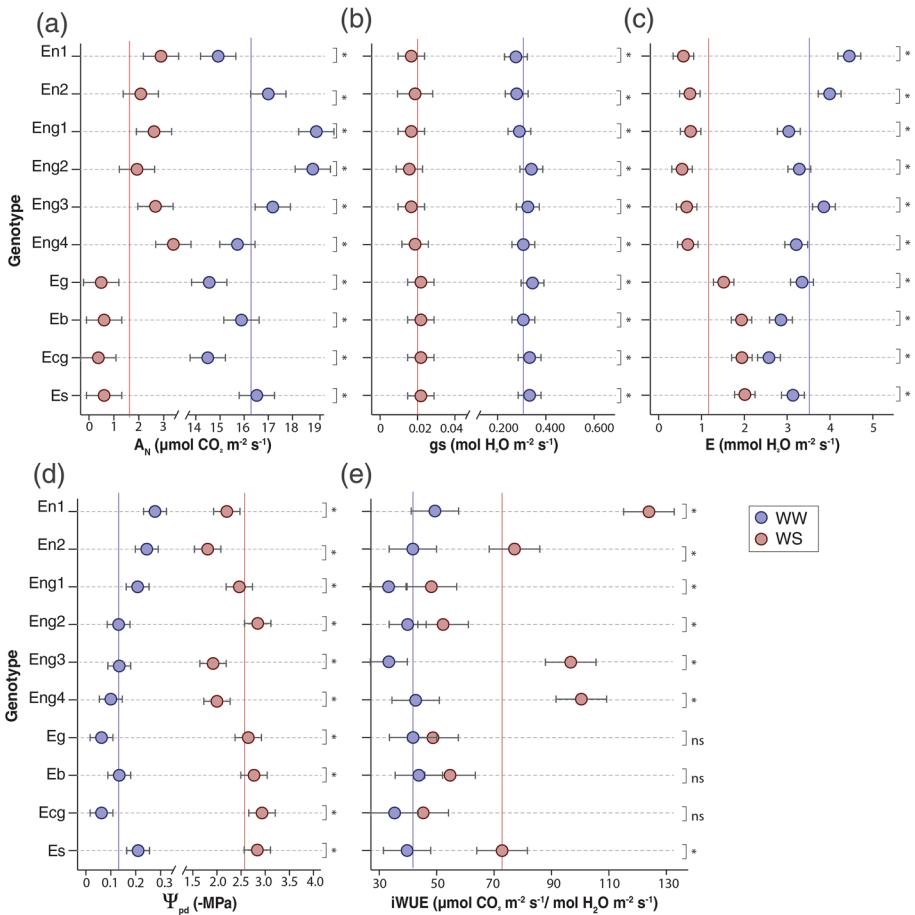
Regarding all evaluated physiological variables, only  $g_s$  did not show differences for the effect of genotype and genotype x water regime interaction (Table 2). In the case of  $A_N$ , genotypes under WW showed average values ranging from 13.2 to  $19.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; contrastingly, under water stress conditions,  $A_N$  presented values ranging from 0.01 to  $3.10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Fig. 2a). Genotypes Eng, Eg, and Eb showed the largest  $A_N$  reductions under water stress (average 95.4%), followed by En2, Eng1, Eng2, Eng4, and Es with an average reduction of 91.2% and En1 and Eng3 showing the smallest reduction (84.0%). In the case of  $g_s$  an average reduction of 95.1% was observed for all genotypes under WS (Fig. 2b). For E (Fig. 2c), genotypes under WW showed values ranging from 2.51 to  $4.42 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , and 0.73 to  $2.02 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$  under WS. Of all evaluated genotypes, Eb, Ecg, and Es showed the lowest declines in E (average 37.0%). In the case of  $\Psi_{pd}$ , plants showed a drop ranging from  $-1.12$  to  $-3.02$  (MPa) under water stress (Fig. 2d), where Eng1, Eb, and Ecg presented the largest reductions, and En1 and En2 the

**Table 3** Morphological variables absolute growth rate increment estimated for all *Eucalyptus* genotypes under well watered (WW) and water stress (WS) water regimes

Genotype	Water regimes		Absolute growth rate				
	WW	WS	RCD (mmx10 day <sup>-1</sup> )	H (mm day <sup>-1</sup> )	SB (g day <sup>-1</sup> )	RB (g day <sup>-1</sup> )	RB:SB
En1	WW	WS	1.56 (0.32) A**	8.98 (1.33) C**	2.00 (0.46) B*	0.44 (0.08) C*	0.22 (0.05) C*
	WW	WS	0.10 (0.02) a	3.98 (0.56) b	1.63 (0.16) a	0.47 (0.09) b	0.26 (0.03) b
En2	WW	WS	1.73 (0.28) A**	12.73 (2.26) B**	2.55 (0.36) B**	0.42 (0.05) C*	0.17 (0.06) D**
	WW	WS	0.07 (0.01) a	7.50 (0.32) a	1.03 (0.09) b	0.44 (0.06) c	0.23 (0.07) c
Eng1	WW	WS	1.31 (0.25) B**	10.63 (1.56) B**	2.53(0.50) B**	0.45 (0.06) C*	0.18 (0.06) D*
	WW	WS	0 (0) b	6.02 (0.21) a	0.63 (0.05) c	0.50 (0.05) b	0.22 (0.05) c
Eng2	WW	WS	1.34 (0.30) B**	12.95 (2.44) B**	2.74 (0.55) B**	0.57 (0.07) C**	0.21 (0.07) C**
	WW	WS	0 (0) b	2.27 (0.15) c	0.97 (0.08) b	0.50 (0.09) b	0.26 (0.06) b
Eng3	WW	WS	1.48 (0.25) B**	12.61 (2.48) B**	2.02 (0.41) B**	0.33 (0.08) D**	0.16 (0.05) D**
	WW	WS	0.09 (0.03) a	8.07 (0.16) a	1.23 (0.12) a	0.49 (0.07) b	0.27 (0.03) a
Eng4	WW	WS	1.67 (0.16) A**	15.86 (2.96) A**	3.42 (0.33) A**	0.74 (0.05) A**	0.22 (0.05) C**
	WW	WS	0.07 (0.01) a	4.89 (0.11) b	0.53 (0.07) c	0.43 (0.06) c	0.28 (0.03) a
Eg	WW	WS	1.61 (0.11) A**	11.82 (1.89) B**	2.48 (0.42) B**	0.59 (0.05) B**	0.24 (0.05) C**
	WW	WS	0 (0) b	2.16 (0.12) c	0.50 (0.06) c	0.46 (0.07) c	0.31 (0.04) a
Eb	WW	WS	1.17 (0.12) C**	18.89 (2.36) A**	2.55 (0.44) B**	0.26 (0.07) E**	0.10 (0.03) E**
	WW	WS	0 (0) b	8.41 (0.23) a	0.92 (0.07) b	0.63 (0.06) a	0.33 (0.04) a
Ecg	WW	WS	0.81 (0.07) C**	9.94 (2.99) C**	1.49 (0.29) C**	0.67 (0.05) A**	0.45 (0.05) A**
	WW	WS	0 (0) b	0.45 (0.16) d	0.93 (0.09) b	0.46 (0.06) c	0.24 (0.04) c
Es	WW	WS	1.13 (0.09) C*	16.48 (1.90) A**	2.08 (0.26) B**	0.60 (0.07) B*	0.29 (0.06) B*
	WW	WS	0 (0) b	7.27 (0.15) a	0.94 (0.05) b	0.57 (0.05) a	0.30 (0.04) a

RCD, root collar diameter; H, height; SB, shoot biomass; RC, root biomass and RB:SB, root:shoot ratio. Capital letters compare different genotypes under the well-watered (WW) regime, and lower letters compare different genotypes under the water stress (WS) regime using a Tukey test ( $p < 0.05$ ). \* indicates significance differences ( $p < 0.05$ ) between water regimes means for each genotype



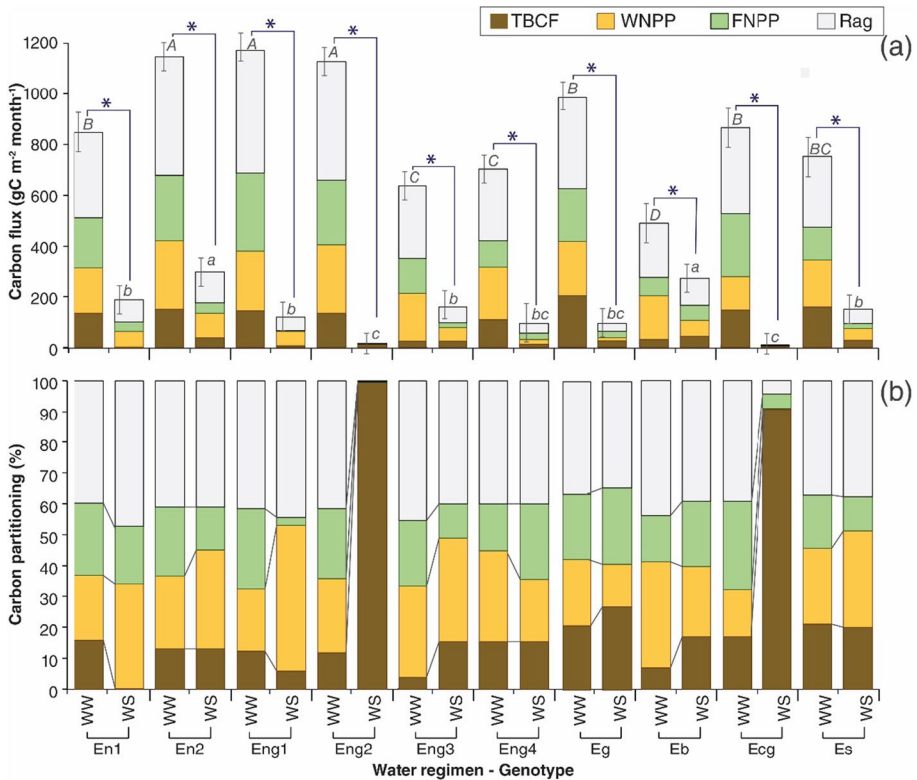


**Fig. 2** Changes in net photosynthesis rate ( $A_N$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), predawn water potential ( $\Psi_{pd}$ ), and intrinsic water use efficiency ( $iWUE$ ) for all the *Eucalyptus* genotypes evaluated under well-watered (WW) and water stress (WS) regimes. Points and bars represent the mean  $\pm$  standard error of individual genotypes, and the corresponding blue and red lines are the general average value of all genotypes under WW and WS, respectively

smallest change in  $\Psi_{pd}$ . Finally, all genotypes under water stress reported higher  $iWUE$  values (Fig. 2e), where En1, Eng3, and Eng4 showed the highest increase with an average of 65.20% followed by En2 and Es with an average of 35.42%. Contrastingly, Eng1, Eng2, Eg, Eb, and Ecg showed the smallest change in  $iWUE$  with an average of 15.21%.

### Carbon flux and partitioning

Anova analyses of C flux and partitioning showed significant effects of genotype, water regime and the genotype x water regime interaction (Table 2). Under WW regime, C flux (Fig. 3a) ranged from 200 to 1170 g C m<sup>-2</sup> month<sup>-1</sup>; where En 2, Eng 1, and Eng2 genotypes showed the highest C flow (average 1140 g C m<sup>-2</sup> month<sup>-1</sup>) and Eb showed the lowest flow with 460 g C m<sup>-2</sup> month<sup>-1</sup>. Considering C partitioning (Fig. 3b) under WW



**Fig. 3** Carbon flux and partition for all *Eucalyptus* genotypes evaluated under well-watered (WW) and water stress (WS) regimes. Measurements include wood net primary production (WNPP), foliage net primary production (FNPP), aboveground autotrophic respiration (Rag), and total belowground carbon flux (TBCF). Capital letters compare different genotypes under WW regime, and lower letters compare different genotypes under WS regime using the Tukey test ( $p < 0.05$ ). \* denotes an statistical significance ( $p < 0.05$ ) difference between means of each genotype under different water regimes

regime values ranged from 32 to 46% for Rag, less than 25% for TBCF, and from 20 to 36% for WNPP. In contrast, under WS regime C flux showed significant reductions ranging from 60 to 235 g C m<sup>-2</sup> month<sup>-1</sup>; where En1, En2, and Eb genotypes in average reduced C flux by less than 78%, while Ecg and Eng2 showed the largest falls with an average of 96%. Genotypes under water stress conditions revealed three types of response: (i) En1, Eng1 and Es showed a significant reduction in TBCF; (ii) En2 and Eng4 maintained TBCF and ANPP partition; and (iii) Eng2, Eng3, Eg, Eb end Ecg raised TBCF (Table 4).

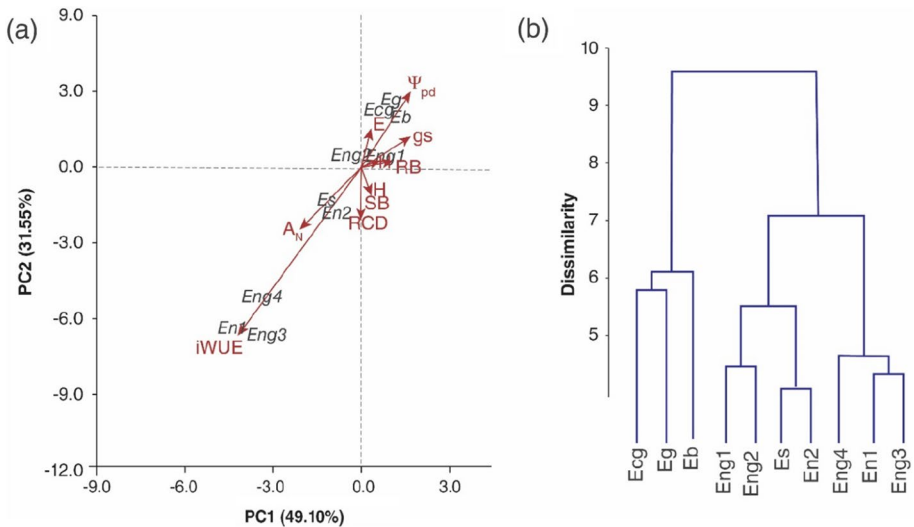
### Genotypes dissimilitude

When analyzing differences among genotypes, the principal component analysis test (PCA) explained 90.65% of the variability (Fig. 4a). The first PCA component was strongly associated with iWUE (59.10%), and  $\Psi_{pd}$  was associated with the second component explaining 28.96% of the variability. The analysis suggested three genotype groups (Fig. 4b) which considered: (i) Ecg, Eg, and Eb that included stress-sensitive or less tolerant genotypes that showed larger or stronger morphological and/or physiological changes under water stress;

**Table 4** ANOVA ( $p$ -value) results of the analyses evaluating the effect of water regime (WR), genotype (Gen), and genotype  $\times$  water regime interaction (Gen  $\times$  WR) for GPP, ANPP, Rag, and TBCF for all evaluated *Eucalyptus* genotypes

Effects	Variable			
	GPP	ANPP	Rag	TBCF
Gen	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
WR	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.004</b>	<b>0.003</b>
Gen $\times$ WR	<b>0.003</b>	<b>0.004</b>	<b>0.003</b>	<b>0.003</b>

GPP, gross primary production; ANPP, aboveground net primary production; Rag, aboveground autotrophic respiration; and TBCF, total belowground carbon flux. Values in bold indicate significant values ( $p < 0.05$ )

**Fig. 4** Principal components biplot analysis (PC1 and PC2) showing the contribution of morphological and physiological variables differentiating genotypes groups and the hierarchical clustering using Ward's linkage with Bray-Curtis dissimilarity index for all *Eucalyptus* genotypes under the water stress (WS) regime

(ii) Es, En2, Eng1, and Eng2 genotypes that showed moderate responses or were moderately tolerant to water stress, and showed the smaller physiological changes but allowing an increase in biomass; (iii) En1 Eng3 and Eng4 showed the most tolerant pattern, showing smaller physiological responses with the highest iwUE values maintaining plant growth under stress.

## Discussion

### Identification of water stress tolerant genotypes by functional traits

Is well known that water stress-tolerant genotypes may survive during long periods of water restriction by maintaining minimal growth (Eilmann and Rigling 2012;

Pita-Barbosa et al. 2023). However, adaptation to shortages in soil water availability has been strongly related to plasticity and is expressed through morphological, physiological, and biochemical mechanisms (Chen et al. 2020; Corrêa et al. 2017). On this regard, morphological traits have been usually considered as a baseline for comparison and pre-selection of genotypes susceptible to water stress (Gonçalves et al. 2017; Lucani et al. 2018). Silva et al. (2014b) found that stress-tolerant *E. urograndis* hybrids showed a higher survival rate by increasing their root:shoot ratio but maintaining height growth. Similarly, Chaves et al. (2003) concluded that drought-sensitive genotypes are characterized by showing an abrupt change in growth with significant increases in belowground biomass and reduction in leaf area as a protection mechanism to prevent dehydration. Our results showed similar morphological responses for stress tolerant genotypes that showed a reduction in the absolute growth rate, maintained growth and showed a notorious increase in the root: shoot ratio.

Physiological traits in our experiment also showed interesting responses among genotypes under water stress where differences in  $\Psi_{pd}$  and iWUE were the key physiological variables that explained experimental variation and final grouping of genotypes of similar response to water stress. Previous experiments have shown that soil/substrate water stress produces a reduction in photosynthetic rate followed by stomatal closure as a mechanism to avoid water loss through transpiration (Mueller et al. 2020; Saadaoui et al. 2017), but the magnitude and timing of this response has been shown to depend on taxa and/or genotype for *Eucalyptus* (Chaves et al. 2003; Coopman et al. 2008b; Mokotedi 2010). The  $\Psi_{pd}$  is considered an indicator of the tree water status of the soil prospected by roots (Silva et al. 2014a); it has been strongly correlated with the relative transpiration rate (Madhibha et al. 2013). Previous studies by Hakamada et al. (2017) highlighted that *Eucalyptus* genotypes with greater susceptibility to water stress showed lower  $\Psi_{pd}$  values as soil water limitation negatively affect growth and physiological activity. Our results are similar to those reported by Maseda and Fernández (2016) and Muller et al. (2020) for *Eucalyptus* genotypes at early stages of development where a strong correlation between mortality and  $\Psi_{pd}$  has been observed. This strong correlation has also been suggested to differentiate *Eucalyptus* genotypes response to water stress (Coopman et al. 2008b; Silva et al. 2017), where stress-tolerant genotypes show high stomatal closure sensitivity to changes affecting plant water. Similarly, iWUE has been considered as an indicator associated with resistance and growth of genotypes under water stress (Pita-Barbosa et al. 2023). Florencio et al. (2022) reported that tolerant genotypes showed higher iWUE under water stress conditions by enhancing photosynthetic assimilation metabolism that, in turns, allows to reduce the effect of stress by maintaining above- and below-ground plant growth. Consequently, stress tolerance of different genotypes is reflected in the net plant C balance (Booth 2013). Several authors have suggested that water stress reduces aboveground C by drastically reducing FNPP and eventually leaf fall as a mechanism of plant adaptation to drought. In the case of TBCF, changes in this flux may be observed for genetic materials that increase root biomass, a response that has been usually associated with the strategy of plant survival by increasing its physical capability to capture soil/substrate water resource (Amrutha et al. 2019; Coopman et al. 2008a; Duan et al. 2013; Maseda and Fernández 2016). Interestingly, our C flux and partition results at nursery stage are similar to those reported by Campoe et al. (2020) investigating *Eucalyptus* plantations response to a gradient of tropical and semitropical sites, where ANPP decreased considerably for drought-susceptible genotypes.

## Implications for *Eucalyptus* plantations under climate change scenarios

Our results may require a long-term validation; however, may be considered a potential first step procedure for selecting genotypes at young ages that may adapt better to water stress conditions. It is estimated that given the current climate change scenario, 80% of the areas dedicated to *Eucalyptus* plantations will present moderate to severe water stress in the forthcoming decades (Martins et al. 2022). Therefore, is critical to develop strategies for predicting the match of highly improved genotypes to sites with increasing risk of prolonged droughts under climate change (Gonçalves et al. 2017). Previous studies have suggested that genotype selection should focus on productivity and consider phenotypic plasticity under water stress conditions and focus on efficiency in water use to create productive mosaics in which planted genotypes and silvicultural management also guarantee ecological and social water sustainability needs (Chaves et al. 2003; de Toledo Picoli et al. 2021; Saadaoui et al. 2017).

Early selection of genotypes may provide a strategy to reduce uncertainty in *Eucalyptus*'s survival and early productivity (Nóia Júnior et al. 2020). The first 24 months of development after establishment of new plantations is when individuals are more susceptible to water stress due to high competition for site space and resources (e.g., nutrients and light), and the root systems are not consolidated in depth so that drought events could affect survival and long-term productivity (Chaves et al. 2003; Elli et al. 2020). However, uncertainties exist in selecting genotypes at nursery or young ages (Mueller et al. 2020; Pita-Barbosa et al. 2023), as genotypes may show different long-term responses at different soil environments if unexpected genetic x environment interactions exist (de Toledo Picoli et al. 2021; McDowell et al. 2008), and also when intraspecific competition, before canopy closure, may enhance genetic x environment x silvicultural interactions (Boreham and Pallett 2009). Therefore, selecting genotypes at early stages may reduce some uncertainty in establishing *Eucalyptus* plantations under increasing drought risk climate change scenarios (Beltrame et al. 2012; Matusick et al. 2016). However, long-term experiments linking early genotype selection, based on physiological or morphological traits and the long-term maintenance of such adaptation mechanisms, are required to validate their tolerance to water stress and impacts on forest productivity.

## Conclusions

Our results showed large differential response effects on morphological, physiological, and C balance of ten *Eucalyptus* genotypes under water stress. Reductions water availability showed contrasting effects on C flux and partition, but most genotypes reduced photosynthesis rates, transpiration, and stomatal conductivity in contrast to increasing iWUE. Interestingly, the magnitude of stress response in growth matched physiological adaptation mechanisms allowing to identify tolerant, intermediate, and sensitive genotypes groups characterized by their response pattern in iWUE and  $\Psi_{pd}$ . Identifying physiological traits that differentiate *Eucalyptus* genotypes at nursery or early stages of development may provide opportunities to maintain site productivity of commercial plantations growing at sites with frequent and/or longer drought events associated with climate change.

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**Authors contributions** LG participated in the study design, planning, data analysis and was the main responsible for experimental set-up, measurements, and manuscript writing. RR developed the core research project, design and planning of the experiment, guided data analyses, and revised the manuscript. JCV provided support on manuscript writing, analysis and revision of the manuscript. VE, and AM provided experimental material, funding for trial installation and genotypes information used in the experiment. LB, provided support on experimental measurements and DB participated in initial set-up and development of the experiment.

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## Declarations

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