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Effects of climate change on water use efficiency (WUE) and its components, measured at different temporal and spatial scales in *Fagus sylvatica*.

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INDEX

Abstract	1
Introduction	2
Materials and methods	5
1. Plant material and experimental design.....	5
2. Morphological measurements and aboveground biomass estimates.....	7
3. Morphological measurements and aboveground biomass estimates.....	8
4. Gas-exchange parameters.....	9
5. Statistical analyses.....	9
Results	10
1. Soil water content (SWC).....	10
2. Relationships between morphological and biomass measurements.....	10
3. Effects of the experimental treatments on whole-plant measurements.....	11
3.1. Effects of the experimental treatments on plant growth.....	11
3.2. Effects of the experimental treatments on daily transpiration.....	12
3.3. Effects of the experimental treatments on whole-plant water use efficiency (WUE_b).....	13
4. Effects of the experimental treatments on instantaneous measurements at the leaf scale.....	13
4.1. Effects of the experimental treatments on net rates of photosynthetic assimilation.....	14
4.2. Effects of the experimental treatments on instantaneous transpiration rates.....	14
4.3. Effects of the experimental treatments on instantaneous water use efficiency (WUE_i).....	15
5. Effects of the experimental treatments on net rates of photosynthetic assimilation.....	15
Discussion	15
Conclusion	18
Acknowledgments.....	19
References	19
Supplementary material	26

ABSTRACT

The expected dryer climate and higher atmospheric CO₂ concentrations due to climate change, are prone to alter water and carbon cycles in temperate forests. In this study, the ratio between carbon gain and water loss, i.e. water use efficiency (WUE) was characterized by scaling up from single-leaf to whole-tree: instantaneous WUE (WUE_i) and WUE based on biomass production (WUE_b). Potted juveniles (3-year-old) of *Fagus sylvatica* were grown in a controlled experiment under ambient (390 ppm) or elevated CO₂ (614 ppm) concentration, and under well-watered or drought conditions. We studied the effects of these conditions on both WUE estimates (WUE_i and WUE_b) and their components, with the aim of reconciling the two temporal and spatial scales. The components of WUE_i consist of net rate of photosynthetic assimilation (A_{net}) and instantaneous transpiration (E_{inst}), which were significantly correlated, respectively, with tree woody biomass gain and total water loss, i.e., the components of WUE_b. Both WUE_b and WUE_i, as well as their components, were affected by the drought treatment. Trees showed the ability to lower their transpiration rates to overcome water-stress by physiological mechanisms, most likely by partial stomatal closure. Also, in order to calculate WUE_b, we obtained proxies of woody biomass by assessing their relationship with easy-to-determine morphological tree measurements. Finally, we did not detect a good correlation between WUE_b and WUE_i which could suggest that more frequently repeated measurements of the instantaneous gas-exchange parameters over time, and a bigger sample size are required to reconcile WUE estimates measured at different scales.

Key words: Climate change, Elevated CO₂, *Fagus Sylvatica*, Photosynthesis, Transpiration, Water-stress, Water use efficiency.

LABURPENA

Basoetako ur- eta karbono-zikloek aldaketak jasan ditzakete gaur eguneko eta etorkizuneko baldintza klimatikoek eragingo dituzten klima lehorrago eta atmosferako CO₂ kontzentrazio altuagoengatik. Ikerketa honetan, irabazitako karbonoaren eta galdutako uraren arteko proportzioa (uraren erabileraren eraginkortasuna, WUE ingelesez) aztertu da, hosto mailatik hasita, zuhaitz mailara arte: berehalako WUE (WUE_i) eta biomasa ekoizpenean oinarritutako WUE (WUE_b). 3 urteko *Fagus sylvatica* zuhaixkak esperimantu kontrolatu batean hazi ziren, 390 ppm edo 614 ppm CO₂-rekin eta ondo ureztatuta edo ur-eskasiarekin. Ingurune baldintza hauen eragina aztertu zen bi WUE-tan eta baita hauen osagaietan ere, bi espazio- eta denbora-eskalak bateratzeko helburuarekin. WUE_i-ren osagaiak hauek dira: asimilazio fotosintetikoaren tasa garbia (A_{net}) eta berehalako transpirazioa (E_{inst}). Gure ikerketan, bi osagai hauek korrelazioa zuten WUE_b-ren osagaiekin, hau da, egur-biomasa irabazia eta ur-galerara totalarekin hurrenez-hurren. Lehortearen tratamenduak WUE_i eta WUE_b-ri, eta baita hauen osagaiei ere, eragin zien. Zuhaitzek ur-eskasiari aurre egiteko ahalmena erakutsi zuten mekanismo fisiologikoen bidez transpirazioa gutxituz, zihurrenik estomak hein batean itxiz. Bestalde, WUE_b kalkulatzeko beharrezkoak diren egur-biomasaren adierazleak lortu genituen, zuhaitzetan neurtzeko errazagoak diren determinazio morfologikoekin zuten erlazioa aztertuz. Azkenik, ez zen korrelaziorik aurkitu WUE_b-ren eta WUE_i-ren artean. Hori dela eta, ondorioztatzen dugu gas trukeko parametro gehiago neurtu behar direla denboran zehar, eta lagin tamaina handiagoa behar dela, bi WUE-ak bateratu ahal izateko.

Hitz gakoak: Aldaketa limatikoa, CO₂ kontzentrazio altua, *Fagus sylvatica*, Fotosintesia, Transpirazioa, Uraren erabileraren eraginkortasuna, Ur-estresa.

INTRODUCTION

Plant water-use efficiency (WUE) is defined as the amount of carbon (biomass) gained per unit of water loss, a concept that was first coined by Briggs and Shantz more than a century ago (Briggs & Shantz, 1913). This ratio between carbon gain and water loss is essential for understanding the fundamental trade-off regulating the potential carbon gain given a certain amount of water transpired, either by a single plant or at the scale of a whole ecosystem. Indeed, quantifying and determining WUE is crucial for predicting carbon and water cycling, both at the ecosystem level and at the plant level, under the ongoing climate change scenario (Song et al., 2017; van der Molen et al., 2011). Reliable estimates of WUE are necessary to predict changes in carbon and water cycling in response to the environmental changes at different temporal and spatial scales (from single plants and trees, to whole ecosystems), to define and predict patterns of ecosystem functioning and to design adaptation strategies to the effects of climate change (Keenan et al., 2013). In turn, climate change impacts on WUE estimates and its components (carbon gain and water use). Individual responses of WUE, and its components, to climate change have been characterised in depth at the leaf scale (Medlyn et al., 2017; Pérez-López et al., 2012), but there is more uncertainty regarding whole-tree (Duursma et al., 2014; Gimeno et al., 2021) and whole-ecosystem responses (Keenan et al., 2013; Leuzinger & Körner, 2007; Schäfer et al., 2002). Besides, the response of carbon gain and of water loss to climate change might differ from one scale to another, therefore, studying WUE at contrasting spatial and temporal scales is needed to fully understand and predict the impact of climate change on vegetation functioning as a whole.

An important issue with WUE is that uncertainties can arise when comparing estimates across studies, because WUE is often measured at different spatial and temporal scales as aforementioned. Two of the most common estimates of WUE are instantaneous WUE (WUE_i) and integrated WUE based on biomass production (WUE_b). WUE_i is based on instantaneous gas-exchange measurements most often collected at the leaf level and it is defined as the ratio of assimilated CO_2 per unit of transpired water (Tambussi et al., 2007; Yoo et al., 2009). Whereas, WUE_b constitutes an integrated measurement at the whole-plant level, as it is the ratio of plant biomass gained per unit of water used by the plant, over a given time period, usually from several days to months or years (Tambussi et al., 2007; Yoo et al., 2009). Conceptually, both WUE estimates express the ratio between a given product (the numerator) and the energy (or resource use) invested on the process carried out to obtain that product (the denominator) (Bhattacharya, 2019).

Reconciling WUE estimates obtained at different temporal and spatial scales can be problematic and challenging (Tomás et al., 2014). WUE_i provides information about instantaneous leaf-scale processes (photosynthesis and transpiration), whereas WUE_b takes into account a longer time span (from days to years), therefore fluctuations affecting CO_2 assimilation and transpiration in

the course of a day or several days are portrayed in WUE_b but not in WUE_i . Also, WUE_b might be influenced by ontogenetic (Gimeno et al., 2019) and/or acclimation processes affecting plant physiological performance over time (Turnbull et al., 2017), whereas the impact of these processes on WUE_i can only be captured by measuring on a recurring basis over time (Anyia & Herzog, 2004). In addition, for large and long-lived plants, such as trees, obtaining representative and accurate measurements of biomass gain and water loss via transpiration is even more challenging (Leuzinger & Körner, 2007; Tor-ngern et al., 2015) and errors in this process can further hinder the reconciliation of WUE_b and WUE_i estimates.

Greenhouse-gas (GHG) emissions from human activity have resulted in a continuous increase in their concentration in the atmosphere. The atmospheric concentration of carbon dioxide (CO_2) is the GHG that has increased the most since the industrial revolution. The global average atmospheric [CO_2] in 2020 was 412.5 parts per million (ppm), this is the highest atmospheric [CO_2] ever experienced in the past 800,000 years, and it is predicted to continue to rise (IPCC, 2021). As a consequence of the rise in atmospheric [CO_2], during the last decade global surface temperature has already increased by 1.1°C, with respect to pre-industrial levels (IPCC, 2021). This temperature increase and global warming entail further climatic changes including changes in precipitation patterns worldwide. In many temperate, arid and semi-arid regions, these changes imply decreased water availability and increased drought risk, particularly for regions that were already water-limited (IPCC, 2021). It is known climate change alters gas-exchange and growth in trees (Peñuelas et al., 2008), hence components from both WUE_i and WUE_b are expected to change, changing these ratios themselves too, although the nature of these changes is not yet fully understood. This makes the reconciliation of both WUE estimates more difficult.

Plants respond to reduced water availability by decreasing transpiration, which is achieved via partial stomatal closure in the leaves (Basu et al., 2016). Plant leaves are protected by an epidermis that limits water loss through their cuticles and hence the bulk of transpirational water loss occurs via the stomata pores. Thus, plant transpiration is conditioned by the stomatal conductance to water vapour (Yoo et al., 2009), which depends on stomata size and density, which can only change over evolutionary time-scales or ontogenetic stages, and stomatal aperture, which can be dynamically regulated to prevent excessive water-loss under drought (Lake & Woodward, 2008). Hence, reducing stomatal conductance to endure drought is a common strategy (Cowan & Farquhar, 1977; Gimeno et al., 2009).

Rising atmospheric CO_2 affects vegetation functioning indirectly by causing climate change, but also directly as CO_2 is the substrate of photosynthesis. As explained by Katul et al. in their stomatal optimization theory (2010), elevated [CO_2] increases the photosynthetic carbon assimilation (A), and this has been widely established in various experimental studies (Bernacchi et al., 2006;

Ellsworth, 1999). Bernacchi et al. experimentally found that A was %20 higher in plants which had been in an elevated $[CO_2]$ treatment for 15 days. The increase in A is often accompanied by an increase in biomass production (Ainsworth & Long, 2005; Uddling et al., 2008). That is because around 50% of total plant biomass is acquired via photosynthesis (Walker et al., 2021), therefore it is generally assumed that enhanced A increases biomass production. Furthermore, tree-ring studies describe a positive effect of elevated atmospheric $[CO_2]$ on tree growth in the last decades (Battipaglia et al., 2013; Gedalof & Berg, 2010).

Furthermore, elevated atmospheric $[CO_2]$ has been reported to lower both stomatal conductance and transpiration in trees (Gunderson et al., 2002; Herrick et al., 2004), and this results in reduced water loss in the whole-tree scale (Battipaglia et al., 2013). Experiments conducted during longer periods of time, such as the 5-year period experiment with poplar trees carried out by Penny J. Tricker et al. (2005), support these observations; they report that both stomatal conductance and transpiration were reduced at elevated atmospheric $[CO_2]$. However, it has been seen that this reduction does not always happen (Ellsworth, 1999), and there is still much ambiguity regarding the effect of elevated $[CO_2]$ in stomatal conductance. Reducing stomatal conductance negatively affects CO_2 uptake, and thus photosynthesis and biomass gain (Ainsworth & Long, 2005). In fact, it has been reported that partial stomatal closure reduced the photosynthetic capacity in young olive trees (*Olea europaea* L.), with the subsequent reduction in biomass production (Bacelar et al., 2007). Nonetheless, under elevated $[CO_2]$, the negative effect of decreased water availability on photosynthesis, caused by stomatal closure, could be partially compensated (Pérez-López et al., 2009).

For slow-growing organisms, such as trees, predicting the impacts of climate and global change on WUE and its components is further complicated by the difficulty of obtaining reliable estimates of both carbon gain and water-use. To obtain parameters such as total tree biomass and its increment over time, destructive harvests from field studies are necessary (Barton & Montagu, 2006). Fortunately, proxies of standing biomass for trees can be obtained from studies assessing their relationship with simple morphological tree measurements (plant diameter, plant height, plant volume etc.). For example, Jucker et al. (2022) recently compiled a database containing morphological measurements of taxonomically standardised and georeferenced tree individuals with this very objective. Establishing significant correlations between these simple morphological parameters and standing biomass would be the first step towards obtaining reliable estimates of WUE_b in trees. Here, we conducted a study under controlled conditions on juvenile (3 year-old) trees of European beech (*Fagus sylvatica* L.) to assess the impacts of two global change drivers, elevated $[CO_2]$ and decreased water availability, on WUE estimates and its components, measured at different temporal and spatial scales. We chose this temperate forest tree, first, because it is one of the most ecologically and economically relevant tree species in Europe due to its wide distribution. Furthermore, this species

reaches its southernmost distribution limit in Spain, where some populations have already shown significant declines due to the current climate change scenario (Peñuelas et al., 2007). In addition, *F. sylvatica* is particularly sensitive to drought stress and it has been shown to respond to elevated [CO₂] too (Aranda et al., 2015; Nalevanková et al., 2018).

In this study we aimed to: (1) obtain meaningful correlations between morphological measurements and plant biomass parameters to calculate WUE_b in juveniles of *F. Sylvatica*; (2) to assess the individual and combined impacts of elevated [CO₂] and decreased water availability on WUE_b and WUE_i and their components, and (3) to assess correlations between WUE measurements, and its components, obtained at different spatial and temporal scales. We expected elevated [CO₂] to increase both estimates of WUE, due to partial stomatal closure reducing transpiration and enhanced photosynthesis growth. Reduced water availability would also be expected to cause an increase in WUE initially, but for our drought-sensitive species, large drops in photosynthesis and growth are also to be expected under drought, hence we might not observe an increase in WUE, despite reduced transpiration. Finally, we expected elevated [CO₂] to mitigate the negative impacts of drought on photosynthesis and biomass gain.

MATERIALS AND METHODS

1. Plant material and experimental design

The experiment was conducted on 3-year-old European beech trees (*Fagus sylvatica* L.). *F. sylvatica* trees were grown in an open-air commercial nursery (Viveros Talaia, Liendo, Cantabria, Spain) from seeds collected in local beech forests. In February 2021, 120 trees were transplanted into 7 L pots filled with a volume mix of soil: peat: sand (13:5:2). Substrates consisted of sandy forest soil, collected from a native beech forest in Iturrieta (Álava, Spain); commercial peat substrate (Floagard, Oldenburg, Germany) and fine-washed sand. From February 2021 to April 2021, trees were kept in a glasshouse located within the campus of the University of the Basque Country (Leioa, Spain), with no active temperature control. During this period, all plants were watered weekly with 500 mL. Leaf unfolding and expansion occurred between the 24th of March and the 7th of April 2021.

On the 12th of April 2021, all trees were transferred to two growth chambers (Phytotron, Advanced Research Facilities, SGIker of the University of the Basque Country) (Fig. 1). Half of the trees (60) were randomly allocated to a growth chamber where the mean CO₂ concentration was kept at 390 parts per million (ppm) and the other half (60 trees) were allocated to an elevated [CO₂] chamber, where the mean CO₂ concentration was kept at 614 ppm, by injecting pure CO₂. Inside the growth chamber, the trees were exposed to artificial light provided by halogen lamps (Osram T8 30watt Lumilux 840, Licht AG, Munich, Germany and Philips Master T8 18W/965 G13, Philips, Amsterdam, The Netherlands), with a 12-hour photoperiod. The height of the halogen lamps was

regulated so that photosynthetic photon flux density (PPFD) that reached the top of the trees was $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Temperature and relative humidity were set to $22 \text{ }^{\circ}\text{C}$ and 65%, when the lights were on, and to $14 \text{ }^{\circ}\text{C}$ and 85% when the lights were off, in both chambers. The position of all trees was rotated weekly within each chamber to avoid potential differences due to small spatial inhomogeneities in the light environment, air temperature and/or relative humidity. The trees were allowed to acclimate to the conditions inside the growth chambers for three weeks, prior to the first round of measurements. During this acclimation period, all plants received 400 mL of tap water twice a week. Some signs of aphid infestation were detected on several trees on the 24th of April 2021, and all trees were sprayed with biodegradable insecticide (Compo Huerto Urbano[®]). Signs of infestation persisted so all trees were fumigated with 1 mL/L solution of emulsifiable concentrate (EC) of azadiractin (3.2%, m/v) (Zenith[®] JED Natural insecticide from the Neem tree), plus 8 mL/L of orthophosphoric cleaning soap (flower orchard) and 8 mL/L of oil of paraffin 83% pv (Super oleotion), on April 30, May 7, May 14, May 21 and May 28.



Fig. 1. 3-year-old *F. sylvatica* trees in a growth chamber (Phytotron, Advanced Research Facilities, SGIker of the University of the Basque Country).

After the acclimation period, trees were randomly allocated to one of the two watering treatments: control and drought. Trees in the control treatment (25 trees in the ambient [CO_2] chamber and 27 trees in the elevated [CO_2] chamber) continued to receive 400 mL of tap water twice a week, whereas watering was withheld for plants allocated to the drought treatment (35 trees in the ambient [CO_2] chamber and 33 trees in the elevated [CO_2] chamber), from the 12th and the 13th of May 2021 onwards, for the ambient and the elevated [CO_2] chambers, respectively.

We conducted five campaigns of morphological and ecophysiological measurements, together with destructive harvests of selected plants over the course of the experiment from the 4th of May to the 10th of June 2021 (Table 1). The campaigns were conducted 1, 7, 14, 21 and 28 days after the last watering event for trees allocated to the drought treatment (Table 1). Final morphological measurements for those trees that were not harvested were conducted on the 15th and 16th of May 2021 and the experiment finished on 16 June 2021.

Table 1. Sampling dates in 2021 for each one of the five campaigns, mean soil water content (SWC, in %) and number of plants sampled within each treatment combination per campaign, and number of days since the last watering event (N days) for plants allocated to the drought treatment. Trees within the control watering treatment were measured always the day after having been watered. On the first campaign, all measured and harvested trees had been watered the previous day. The calculation of SWC is described in section 3.

Cam- paign	Dates	Ambient CO ₂				Elevated CO ₂				N days
		Control		Drought		Control		Drought		
		N plants	SWC	N plants	SWC	N plants	SWC	N plants	SWC	
1	4 & 5 May	5	34.61±3.3	0	-	5	39.82±3.3	0	-	1
2	18, 19, 20 May	3	39.13±2.1	5	28.01±1.7	3	37.86±2.8	5	35.54±3.3	7
3	25, 26, 27 May	3	39.83±1.8	5	20.09±3.1	3	40.63±1.3	5	22.60±2.5	14
4	1, 2, 3 June	3	32.48±4.9	3	15.83±0.9	3	44.77±4.9	3	15.82±1.8	21
5	7, 8, 9 June	7	32.01±4.3	2	14.16±3.7	7	40.43±4.4	2	18.04±5.7	28

2. Morphological measurements and aboveground biomass estimates

Morphological measurements (tree height, stem diameter at the base of the tree and number of branches) were recorded at the beginning (20 and 22 April 2021) and at the time of the destructive harvest of each tree during the sampling campaigns (Table 1) or at the end of the experiment (15 and 16 May 2021), for remaining trees that were not harvested. Tree height was measured to the nearest 0.5 cm with a measuring tape, from the base of the stem (considered always as the upper limit of the pot, for standardisation purposes) to the terminal tip of the highest branch. Stem base diameter was measured (also at the same height as the upper limit of the pot) to the nearest 0.1 mm using a Vernier calliper. Two perpendicular diameter measurements were taken each time and the average of these two measurements was then recorded. Finally, we also counted the number of branches with visible live leaves.

We use height (H) and diameter measurements to calculate a series of derivate variables. First, we calculated tree basal area (BA) according to:

$$(Eq. 1) BA = \pi r^2,$$

where r is the tree radius. Wood volume (V) for each tree was calculated from H and r and approximating the tree stem to a cone according to:

$$(Eq. 3) V = \frac{BA \times H}{3}$$

Aboveground biomass was estimated following destructive harvests in each campaign. Aboveground was harvested, separated into leaves and stem, dried at 70°C for 48h and weighted to the nearest 0.001 g to determine the dry weight (DW), of the different fractions(leaves and stem). Initial aboveground woody biomass was estimated from initial tree diameter measurements and allometric relationships measured at the time of destructive harvest (see below). Increment of aboveground woody biomass (ΔB_{wood}) was calculated from the difference between initial and final aboveground woody biomass. During the destructive harvest, a subset of 10 leaves was randomly collected from each tree to calculate specific leaf area (SLA) according to:

$$(Eq. 4) SLA = \frac{A_{L-subset}}{DW_{L-subset}},$$

where $A_{L\text{-subset}}$ is the total leaf area of the 10 selected leaves, calculated from a scaled photograph with ImageJ (Schneider et al., 2012), and $DW_{L\text{-subset}}$ is the dry weight for these same leaves after 48 h at 70°C. Next, we used SLA to calculate total tree leaf area (A_L) from total leaf dry weight (DW_L):

$$\text{(Eq. 5)} \quad A_L = SLA \times DW_L$$

Leaf unfolding and expansion was completed by the start of the experiment and no significant leaf shedding occurred over the course of the experiment, therefore we assumed that initial tree leaf area was similar to final tree leaf area measured at the time of destructive harvest.

3. Soil water content and estimates of plant water use

Soil water content (SWC) and daily water loss via transpiration (E_{day}) were calculated from the change in weight of a subset of trees for each treatment combination (control and ambient [CO_2], drought and ambient [CO_2], control and elevated [CO_2], and drought and elevated [CO_2]). We weighed ten and five plants for the control and drought treatments, respectively, from each growth chamber from the 3rd and 5th of May to the 8th and 10th of June 2021, for the ambient [CO_2] chamber and elevated [CO_2] chamber respectively. Control trees were weighed before and one hour after being watered. All selected trees (15 plants per chamber) were weighted to the nearest 0.005 kg twice a week until they were destructively harvested. SWC for each tree and measurement date (SWC_t) was calculated according to:

$$\text{(Eq. 6)} \quad \text{SWC}_t = \frac{\text{SFW}_t - \text{SDW}}{\text{SDW}},$$

where SFW_t is soil fresh weight for a given date (t) and SDW is soil dry weight. SFW_t was determined by subtracting to total tree weight measured on a given date the weight of the empty pot and total tree fresh weight (measured at the time of the destructive harvest, including belowground biomass weight, i.e. roots). SDW was the soil dry weight measured at the end of the experiment after being oven-dried for 100°C for 48 h.

Daily transpiration per unit of leaf area (E_{day} in $\text{mol m}^{-2} \text{day}^{-1}$, Fig. S1) was calculated for individual trees and measurement periods as:

$$\text{(Eq. 7)} \quad E_{\text{day}} = \frac{\text{TW}_t - \text{TW}_{t-1}}{A_L \times N_{\text{days}} \times M_{\text{H}_2\text{O}}},$$

where TW_t and TW_{t-1} are the tree (and pot) weights for a given date (t) and for the previous measurements date ($t-1$), A_L is individual total tree leaf area (Eq. 5), N_{days} is the number of days between measurement dates and $M_{\text{H}_2\text{O}}$ is water molecular weight (g mol^{-1}). For control plants that continued to be watered twice a week, TW_t is the weight measured on a given date before watering and TW_{t-1} is the weight measured on a given date after watering. Next, we calculated total tree water loss via transpiration (E_{total}) over the whole experiment (from day 1 to day n) as the cumulative sum of water loss for each plant:

$$(Eq. 8) E_{total} = \sum_{i=1}^{i=n} E_{day-i}.$$

Finally, we calculated WUE_b according to:

$$(Eq. 9) WUE_i = \frac{\Delta B_{wood}}{E_{total}}$$

4. Gas-exchange parameters

Net rates of photosynthetic assimilation per unit of leaf area (A_{net} , in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and instantaneous transpiration rate per unit of leaf area (E_{inst} , in $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were determined from gas-exchange measurements on three plants per treatment combination in the fourth measurement campaign (Table 1). Measurements of gas-exchange were performed on a fully expanded leaf from the upper third of each plant using a Li-Cor 6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA). The CO_2 concentration inside the 6400 leaf chamber was set to either 640 ppm or 450 ppm, to match the corresponding CO_2 concentration inside the elevated or ambient CO_2 growth chamber. Inside the chamber, relative humidity was kept to 65% and the temperature to 25°C . A red/blue LED light source (model LI 6400-02B, LI-COR Inc.) was used to provide a photosynthetic photon flux density of $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ inside the chamber. All measurements were collected during the central part of the day (10-13h). Next, we calculated instantaneous water use efficiency (WUE_i) according to:

$$(Eq. 10) WUE_i = \frac{A_{net}}{E_{inst}}$$

5. Statistical analyses

Data processing and all statistical analyses were performed using the statistical software R (RStudio team, 2021). Linear regression was used to test for significant relationships between aboveground woody biomass and morphological measurements (height, stem diameter and number of branches) and derivate variables (basal area and plant volume), combining measurements from all sampling campaigns and treatment combinations. The effects of the experimental treatments (CO_2 concentration and watering regime) and their interaction on increment of aboveground woody biomass (ΔB_{wood}), daily transpiration rate (E_{day}), WUE_b , photosynthesis (A_{net}), instantaneous transpiration (E_{inst}) and WUE_i were evaluated using two-way analysis of variance (ANOVAs). To evaluate the effects of the experimental treatments and their interaction on daily transpiration we performed separate ANOVAs for two measurement dates were chosen: the day before the drought treatment was implemented (campaign 1, all the plants had been watered regularly up to this point), and 21 days after the last watering event for plants allocated to the drought treatment (4th campaign).

Finally, we used linear regression to test for significant correlations obtained at the whole-plant level over a long time-scale (ΔB_{wood} , E_{day} , WUE_b) and the corresponding instantaneous gas-exchange parameters measured at the leaf level (A_{net} , E_{inst} and WUE_i).

Assumptions of homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test) were checked prior to testing and some variables were log-transformed to meet the normality criteria. We considered that the experimental treatments and their interaction had significant effects when the estimated P-values were less or equal to 0.05.

RESULTS

1. Soil water content (SWC)

SWC of well-watered trees (in the control water treatment) ranged from 34.61 ± 3.3 mL/g to 32.01 ± 4.3 mL/g in ambient [CO_2], and from 39.82 ± 3.3 mL/g to 40.43 ± 4.4 mL/g in elevated [CO_2], across campaigns over the course of the experiment. In contrast, for plants in the drought treatment, initial and final SWC were, respectively, 28.01 ± 1.7 mL/g and 14.16 ± 3.7 mL/g in the ambient [CO_2] treatment, and 35.54 ± 3.3 mL/g and 18.04 ± 5.7 mL/g in the elevated [CO_2] treatment, evidencing a clear decrease of SWC in the drought treatment (Table 1). Mean SWC decreased progressively from one campaign to the next, showing that the drought treatment reduced plant soil water as expected.

2. Relationships between morphological and biomass measurements.

There were significant ($p < 0.05$) positive correlations between all morphological variables (stem diameter, height, number of branches and their derived variables, namely, tree basal area and wood volume) and final measurements of aboveground biomass and leaf area (Table 2). According to estimated coefficients of determination (R^2), the strongest correlations were found for the correlations between aboveground woody (or total) biomass and plant diameter, and its derived variables (basal area, and wood volume, $R^2 = 0.64$, $p < 0.001$). Plant total leaf area was significantly correlated with plant diameter ($p < 0.001$), basal area ($p < 0.001$) and wood volume ($p < 0.001$). All morphological measurements, as well as destructive harvests were collected after all leaves were fully expanded and hence it was assumed that there was no change in leaf area throughout the experiment. In addition, leaf biomass contributed with $21.36 \pm 0.4\%$ (mean \pm se) of the total aboveground biomass, thus, we used aboveground woody biomass (B_{wood}) and its increment (ΔB_{wood}) as the most appropriate proxy to represent plant growth and biomass accumulation. We found similar correlations (in terms of R^2 and P-value) between B_{wood} and stem diameter and its derived variables (tree basal area and wood volume). Thus, we chose the direct measurement (stem diameter) as the most adequate predictor to estimate aboveground woody biomass ($R^2 = 0.64$, $p < 0.001$, Fig. 2). We used the regression coefficients (intercept and slope, Table 2) obtained from the linear correlation between aboveground

woody biomass and diameter measured at the time of harvest to estimate initial aboveground woody biomass from initial measurements of stem diameter. Next, we calculated the increment in aboveground woody biomass over the course of the experiment as the differences between final and initial estimated aboveground woody biomass.

Table 2. Linear regression parameters obtained from the correlation between morphological measurements (predictor variables) and biomass measurements (response variables). Intercept and its standard error (SE interc.), slope and its standard error (SE slope), the coefficient of determination (R^2) and the p -value are shown in the table. The predictor variables are height, diameter, branching, basal area and wood volume (wood vol.), while the response variables are the leaf area, the abg biomass and abg wood biomass.

Response variable	Predictor variable	Intercept	SE (interc.)	Slope	SE (slope)	R^2	p -value
Leaf area	Height	-547.18	1103.11	23.10	9.03	0.08	0.013
	Diameter	-300.80	406.60	1795.40	280.60	0.36	<0.001
	No. Branches	661.26	462.40	73.10	20.77	0.15	<0.001
	Basal area	999.90	217.70	768.90	125.90	0.34	<0.001
	Wood vol.	1121.04	198.13	16.97	2.77	0.34	<0.001
Aboveground total biomass	Height	-29.23	20.63	0.62	0.17	0.16	<0.001
	Diameter	-21.25	6.01	47.44	4.15	0.64	<0.001
	No. Branches	23.74	9.11	1.05	0.41	0.08	0.013
	Basal area	12.15	3.17	20.91	1.84	0.64	<0.001
	Wood vol.	15.53	2.88	0.46	0.04	0.64	<0.001
Aboveground woody biomass	Height	-25.72	16.80	0.51	0.14	0.16	<0.001
	Diameter	-18.82	4.87	38.82	3.36	0.64	<0.001
	No. Branches	21.80	7.55	0.68	0.34	0.05	0.049
	Basal area	8.47	2.57	17.14	1.48	0.64	<0.001
	Wood vol.	11.24	2.33	0.38	0.03	0.64	<0.001

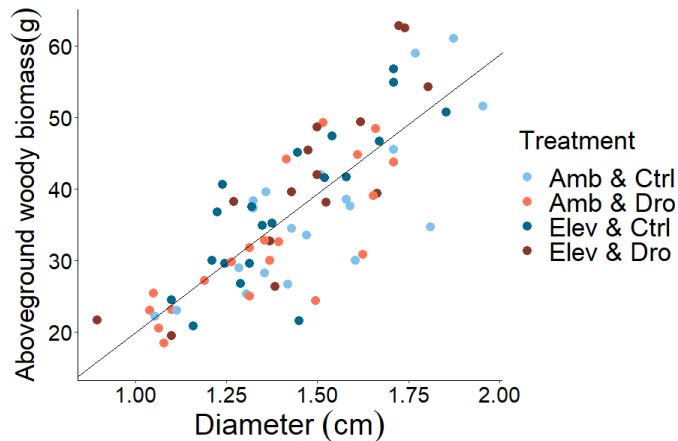


Fig. 2. Significant positive correlation ($R^2= 0.64$, $p < 0.001$) between aboveground woody biomass (g) and stem diameter (cm). Each dot represents one plant, colours depict the four treatment combinations: control and ambient [CO_2] (Amb & Ctrl), drought and ambient [CO_2] (Amb & Dro), control and elevated [CO_2] (Elev & Ctrl), and drought and elevated [CO_2] (Elev & Dro).

3. Effects of the experimental treatments on whole-plant measurements

3.1. Effects of the experimental treatments on plant growth

According to the two-way ANOVA performed on trees harvested on the 4th campaign, the drought treatment had a significant negative effect on stem diameter growth (Fig. 3) and on biomass gain calculated after diameter (ΔB_{wood} , Fig. S2) ($p < 0.05$). The elevated [CO_2] did not exert any significant

effect on diameter growth nor on biomass gain ($p > 0.05$), and there was not any significant interaction between the water and CO_2 treatments ($p > 0.05$). Mean diameter growth for trees under the drought treatment was 0.7 ± 0.02 mm and 0.9 ± 0.02 mm and in the control treatment: 1.4 ± 0.03 mm and 1.6 ± 0.03 mm for well-watered trees, for the ambient and elevated $[\text{CO}_2]$ treatments respectively (Fig. 3).

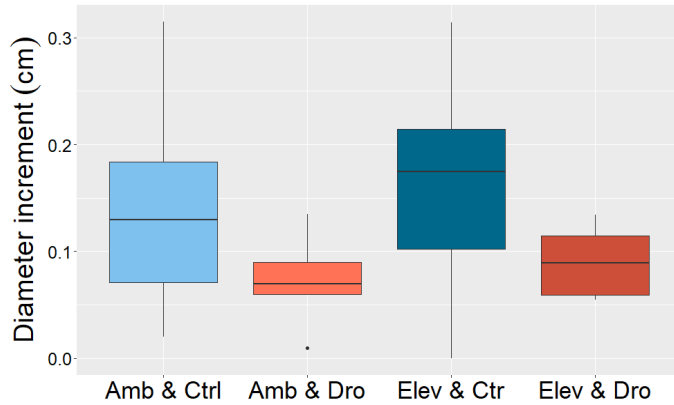


Fig. 3. Boxplot displaying the distribution of diameter growth (cm) in each treatment combination: control and ambient $[\text{CO}_2]$ (Amb & Ctrl), drought and ambient $[\text{CO}_2]$ (Amb & Dro), control and elevated $[\text{CO}_2]$ (Elev & Ctrl), and drought and elevated $[\text{CO}_2]$ (Elev & Dro). Each box represents the interquartile range (IQR, from the 25th to the 75th percentile), and the median is indicated by a line across the box. The vertical lines coming out of the boxes extend from the 25th and the 75th percentile to the most extreme data points.

3.2. Effects of the experimental treatments on daily transpiration

Results of the two-way ANOVA for estimates of whole-plant daily transpiration (E_{day} calculated after measurements of pot weights) from the first campaign (hence before the drought treatment was imposed), evidenced that the CO_2 treatment had a significant effect on E_{day} ($p < 0.05$): elevated $[\text{CO}_2]$ significantly decreased E_{day} (Fig. 4). As expected, at this point in time (before the onset of the drought treatment), we did not find any significant difference in E_{day} between groups of plants allocated to either one of the water treatments ($p > 0.05$, Fig. 4), similarly, we did not find any significant effect of the interaction between experimental treatments. In contrast, according to the two-way ANOVA for E_{day} estimates from the same plants measured three weeks later (4th campaign, 21 days since the onset of the drought treatment, Table 1), we found that E_{day} significantly decreased under the drought treatment ($p < 0.05$, Fig. 5). In contrast, at this point in time, we did not find any significant effect neither of the CO_2 treatment ($p > 0.05$), nor of its interaction with the drought treatment ($p > 0.05$). Mean E_{day} values for trees under the drought treatment in the 4th campaign were 13.22 ± 3.7 mol $\text{m}^{-2}\text{day}^{-1}$ and 11.59 ± 5.3 mol $\text{m}^{-2}\text{day}^{-1}$ for the ambient and elevated $[\text{CO}_2]$ treatments respectively, and 33.90 ± 2.6 mol $\text{m}^{-2}\text{day}^{-1}$ and 25.60 ± 1.4 mol $\text{m}^{-2}\text{day}^{-1}$ for well-watered trees (Fig. 5).

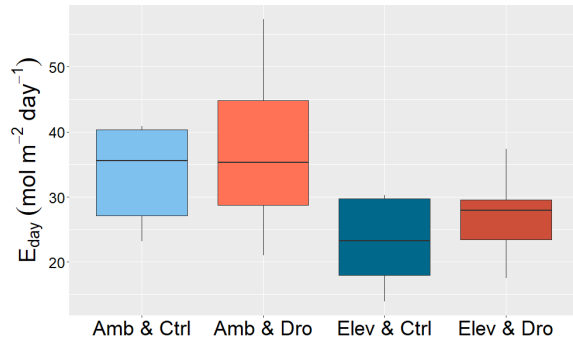


Fig. 4. Boxplots of daily transpiration per unit of leaf area ($\text{mol m}^{-2} \text{day}^{-1}$), in each treatment combination before imposing the drought treatment. Explanation of boxplot and treatments as in Fig. 3.

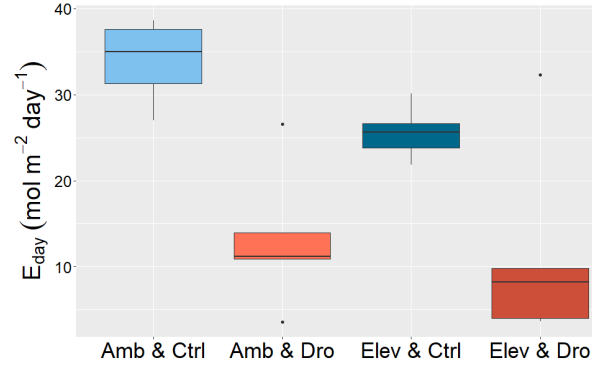


Fig. 5. Boxplots of daily transpiration per unit of leaf area unit ($\text{mol m}^{-2} \text{day}^{-1}$), in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.

3.3. Effects of the experimental treatments on whole-plant water use efficiency (WUE_b)

We performed a two-way ANOVA for trees harvested in the 4th campaign, to test for the effects of the elevated CO_2 and drought treatments on whole-plant estimates of WUE_b calculated from estimates of increment of aboveground woody biomass and whole-plant water use (WUE_b Eq. 9). We found a significant effect of the drought treatment ($p < 0.05$): plants in the drought treatment (either at ambient or elevated [CO_2]) had significantly lower WUE_b values (Fig. 6). Mean WUE_b values for trees under the drought treatment were $1.11 \pm 0.3 \text{ g L}^{-1}$ and $1.36 \pm 0.2 \text{ g L}^{-1}$ for the ambient and elevated [CO_2] treatments respectively, and $1.64 \pm 0.4 \text{ g L}^{-1}$ and $2.32 \pm 0.2 \text{ g L}^{-1}$ for well-watered trees (Fig. 6). There were no significant effects of the CO_2 treatment ($p > 0.05$), nor of its interaction with the water treatment ($p > 0.05$).

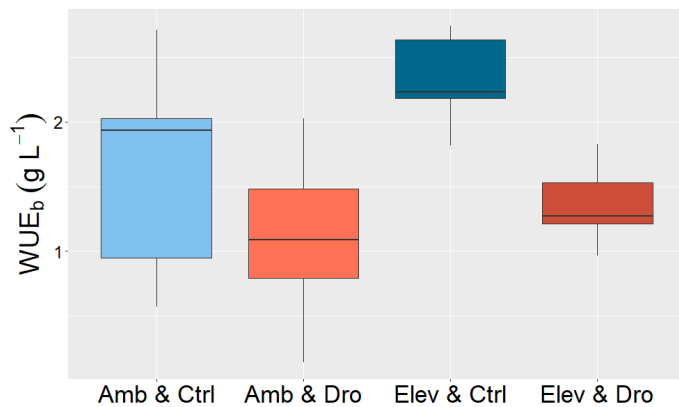


Fig. 6. Boxplots of whole-plant water use efficiency (WUE_b , g L^{-1}) values in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.

4. Effects of the experimental treatments on instantaneous measurements at the leaf scale

We performed two-way ANOVAs to test for the effects of the CO_2 and the watering treatment on gas-exchange parameters measured on the 4th campaign (21 days after the last watering event for plants in the drought treatment).

4.1. Effects of the experimental treatments on net rates of photosynthetic assimilation

Net rates of photosynthetic assimilation per unit of leaf area (A_{net}) rates were significantly different between the two water treatments (control and drought, $p < 0.05$): the drought treatment significantly decreased A_{net} (Fig. 7). We did not find any significant effects of the CO_2 treatment ($p > 0.05$), nor of its interaction with the water treatment ($p > 0.05$). Mean A_{net} values for trees under the drought treatment were $5.03 \pm 1.9 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $3.76 \pm 1.3 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the ambient and elevated $[\text{CO}_2]$ treatments respectively, and $11.62 \pm 1.4 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $12.49 \pm 0.06 \mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for well-watered trees (Fig. 7).

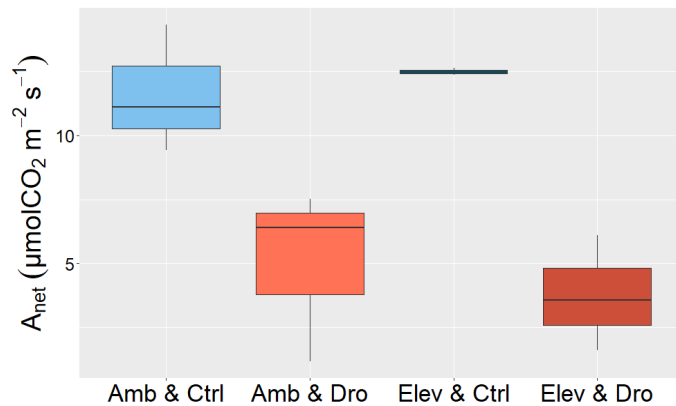


Fig. 7. Boxplot of net rates of photosynthetic assimilation per unit of leaf area (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.

4.2. Effects of the experimental treatments on instantaneous transpiration rates

Instantaneous rates of transpiration per unit of leaf area (E_{inst}) were significantly affected by the water treatment ($p < 0.05$) and by the interaction between the water treatment and the CO_2 treatment, although the CO_2 treatment on its own did not have any significant effect ($p > 0.05$, Fig. 8). Mean WUE_i values for trees under the drought treatment were lower ($0.64 \pm 0.3 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $0.07 \pm 0.06 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for the ambient and elevated $[\text{CO}_2]$ treatments respectively) than in the well-watered treatment ($0.77 \pm 0.2 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $1.11 \pm 0.2 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for ambient and elevated $[\text{CO}_2]$, respectively) (Fig. 8), and the effect of the drought treatment was larger under elevated $[\text{CO}_2]$.

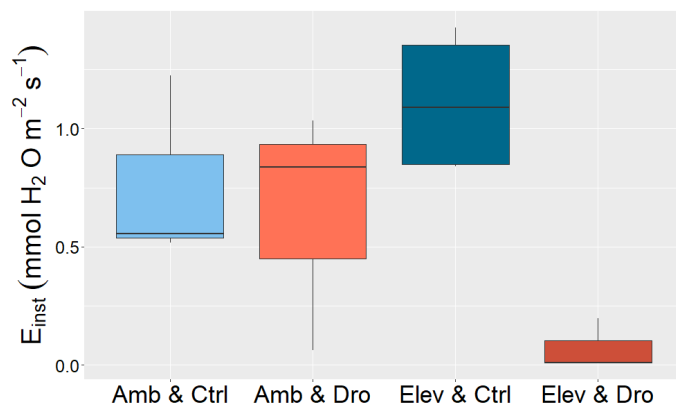


Fig. 8. Boxplots of instantaneous transpiration rates (E_{inst}) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.

4.3. Effects of the experimental treatments on instantaneous water use efficiency (WUE_i)

Estimates of instantaneous water use efficiency (WUE_i , Eq. 10) were logarithmically transformed prior to statistical analyses. We found a significant effect of the water treatment ($p < 0.05$), as well as a significant effect of the interaction between the water treatment and the CO_2 treatment ($p < 0.05$), although the CO_2 treatment itself did not have a significant effect ($p > 0.05$). The drought treatment increased the instantaneous WUE_i and this increase was larger under elevated $[\text{CO}_2]$ (Fig. 9). Mean WUE_i values for trees under the drought treatment were $2.32 \pm 0.3 \mu\text{molCO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$ and $4.80 \pm 1.0 \mu\text{molCO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$ for the ambient and elevated $[\text{CO}_2]$ treatments respectively, and $2.79 \pm 0.2 \mu\text{molCO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$ and $2.45 \pm 0.1 \mu\text{molCO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$ for well-watered trees (Fig. 9).

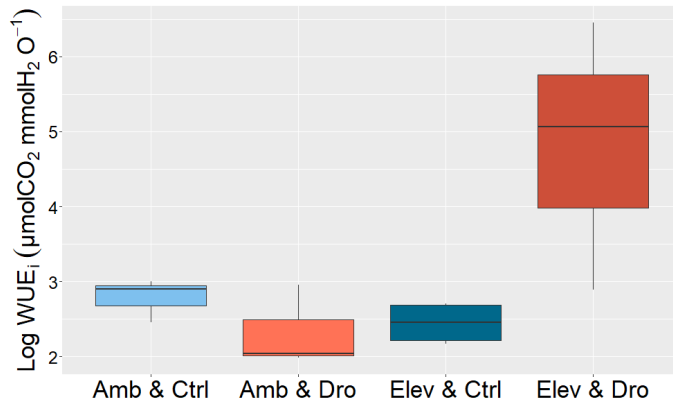


Fig. 9. Boxplots of log-transformed instantaneous water use efficiency (WUE_i , $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{H}_2\text{O}$) in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.

5. Relationships between whole-plant scale variables and leaf-scale instantaneous variables

There was a significant positive correlation between increment of aboveground woody biomass gain (ΔB_{wood} , measured at the whole plant level) and instantaneous net rates of photosynthetic assimilation (A_{net} , $R^2=0.57$, $p < 0.05$, Fig. 10), measured on the same plants. Similarly, there was a significant positive correlation between daily transpiration measured at the whole-plant scale (E_{day}) and instantaneous transpiration (E_{inst} , $R^2=0.80$, $p < 0.05$, Fig. 11). We did not find a statistically significant correlation between estimates of water use efficiency (WUE) measured at the whole-plant (WUE_b) and at the leaf level (WUE_i).

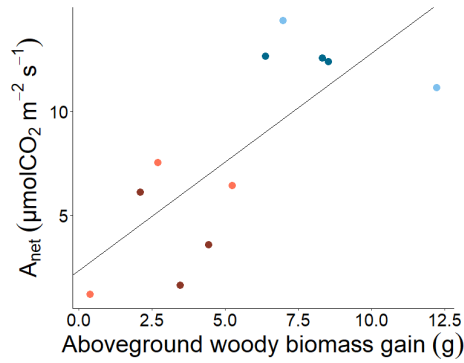


Fig. 10. Significant positive correlation ($R^2=0.57$, $p < 0.01$) between aboveground woody biomass gain (g) and photosynthesis (A_{net}) ($\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$). Each dot represents one plant, colours depict the four treatment combinations (ambient $[\text{CO}_2]$ and control, ambient $[\text{CO}_2]$ and drought, elevated $[\text{CO}_2]$ and control, and elevated $[\text{CO}_2]$ and drought). Explanation treatments as in Fig. 3.

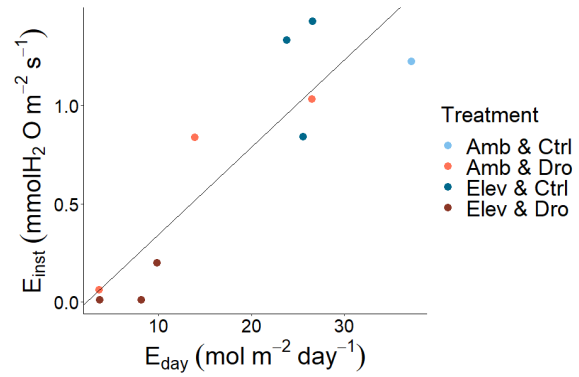


Fig. 11. Significant positive correlation ($R^2=0.80$, $p < 0.01$) between daily whole-plant transpiration (E_{day}) ($\text{mol m}^{-2} \text{day}^{-1}$) and instantaneous transpiration (E_{inst}) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$). Each dot represents one plant, colours depict the four treatment combinations (ambient $[\text{CO}_2]$ and control, ambient $[\text{CO}_2]$ and drought, elevated $[\text{CO}_2]$ and control, and elevated $[\text{CO}_2]$ and drought). Explanation of treatments as in Fig. 3.

DISCUSSION

In this study we characterized the water use efficiency (WUE), and its components, at different temporal and spatial scales in a controlled experiment with potted juveniles (3 year-old) of *F. sylvatica*. According to our predictions, daily transpiration (E_{day}), instantaneous transpiration (E_{inst}), diameter growth and net rate of photosynthetic assimilation (A_{net}) decreased under the drought treatment. Contrary to our predictions, WUE_b and WUE_i showed lower values under the drought treatment, and the elevated $[\text{CO}_2]$ did not mitigate the impacts of drought. Overall, our results highlight that global change drivers have complex effects on the different components of WUE that vary over time and depending on the spatial scale considered.

Our first objective was to explore allometric relationships between morphological variables (mainly stem diameter and tree height) and estimates of aboveground biomass. We found significant positive correlations between various morphological measurements and biomass estimates, the strongest one being between tree diameter and aboveground woody biomass (Table 2, Fig. 2). This solid correlation allowed us to estimate the initial tree biomass from measurements of stem diameter to then calculate tree biomass gain over the course of the experiment, which was needed to calculate WUE_b . Furthermore, our results also suggest that stem diameter can serve as a useful proxy to estimate biomass increments, also in juvenile trees in field studies (Zianis & Mencuccini, 2005). As expected, our results showed a significant negative effect of the drought treatment on tree diameter growth (Fig. 3). Similar to previous studies, we found that beech growth rate was impeded under water scarcity (Nalevanková et al., 2018; Scharnweber et al., 2011). This effect has also been observed in the field and on a larger temporal scale, as reviewed recently by Leuschner (2020), who

highlighted that intensified summer droughts due to climate change would be the main cause of reduced growth in European beech. Contrary to our expectations, we did not find a significant enhancement of radial growth under elevated $[\text{CO}_2]$. We attribute this latter result to the limited duration of our experiment, as it has been shown that a longer exposure is often needed to see CO_2 -induced enhanced tree growth (Asshoff et al., 2006; Epron et al., 1996).

The observed decrease in diameter growth agreed with the results from leaf-scale gas-exchange measurements, since we found that A_{net} also decreased significantly under the drought treatment (Fig. 7), presumably due mainly to stomatal closure, as seen in other studies (Singh & Raja Reddy, 2011). In fact, our data revealed a significant correlation between diameter growth and A_{net} values. The decline in A_{net} under the drought treatment was not compensated nor alleviated by the elevated $[\text{CO}_2]$. We did not find any significant effects of the CO_2 treatment, nor of its interaction with the drought treatment, in contrast to studies that had found that elevated $[\text{CO}_2]$ can alleviate the impact of drought on photosynthesis (Allen et al., 2011; Robredo et al., 2007). Our results might suggest that the metabolic limitation exerted by drought was greater than stomatal limitation to carbon assimilation, as seen in sunflower plants by Tezara et al. (2002). However, our study encompassed only a limited sample size for the gas-exchange measurements. It is also worth mentioning that after ca. 2 months of exposure to elevated CO_2 , lack of sensitivity of photosynthesis to elevated CO_2 could also be partially due to acclimation to high $[\text{CO}_2]$, as explained by Sicher & Bunce (1997). Under elevated $[\text{CO}_2]$, if other resources (e.g. water or nutrients) are limiting, plants could reduce RubisCo content or/and activity to maintain the photosynthetic metabolic rate found in ambient $[\text{CO}_2]$ (Bowes, 1991).

Daily transpiration rates per unit of leaf area (E_{day}) in the 4th campaign significantly decreased under the drought treatment (Fig. 5). Daily transpiration dropped to a similar extent in the study conducted by Hájíčková et al. (2017) with European beech seedlings under drought for 21 days. Given that vapour pressure deficit did not vary along the experiment (temperature and relative humidity were kept constant inside the growth chambers), the reduction in E_{day} must have been due to stomatal closure, a mechanism which has been observed in various similar experiments (Anyia & Herzog, 2004; Bacelar et al., 2007; Nalevanková et al., 2018). Indeed, stomatal conductance to water vapour is very sensitive to drought in *F.sylvatica* trees (Aranda et al., 2005), acting as an effective mechanism to cope with water stress in this species. This effectiveness was well demonstrated in the intense heatwave and drought episode of 2003, when European beech trees were able to endure the harsh climatic conditions thanks to stomatal closure (Hentschel et al., 2016). Our data also showed that before implementing the drought treatment, the $[\text{CO}_2]$ treatment exerted a significant effect on daily transpiration (Fig. 4): trees transpired significantly less under elevated $[\text{CO}_2]$, all along the experiment (Fig. S1), again most likely due to partial stomatal closure (Ellsworth, 1999).

At the leaf scale, instantaneous transpiration (E_{inst}) was also significantly affected by the water treatment, and plants under the combined effects of elevated $[CO_2]$ and drought also showed the lowest (E_{inst}) rates (Fig. 8), thus reinforcing the results obtained at the whole-plant scale. Moreover, our data revealed a significant correlation between E_{day} and E_{inst} (Fig. 11), hence, water loss measurements based on transpiration at the leaf and at the whole plant scales, both coincided in capturing the effects of climate change. Overdieck & Forstreuter found (1994) had previously reported lower instantaneous transpiration in *F. sylvatica* under elevated $[CO_2]$. We did not find a direct effect of elevated $[CO_2]$ on E_{inst} , but we did find a significant interaction between the CO_2 treatment and the water treatment. Also, the extend of our results is probably limited by the small sample size utilised for the gas-exchange measurements.

Our data indicate that WUE_b decreased significantly under drought, but we did not find significant effects of the elevated $[CO_2]$ treatment. Increased WUE in response to drought is often expected (Bhattacharya, 2019), but results from other studies on trees present mixed responses of WUE_b under water stress, showing that WUE_b can decrease in response to drought or remain unaltered (Bacelar et al., 2007; Tomás et al., 2012). WUE_i was also significantly affected by the water treatment, but no significant correlation was found between both estimates (WUE_b and WUE_i), again this result is likely limited by the number of trees measured for gas-exchange. Trees under the combined effects of elevated $[CO_2]$ and drought showed extremely high WUE_i values driven by the extremely low values of E_{inst} recorded for two (out of three) tree individuals allocated to this treatment. Besides limited sample-size, there are some other possible factors that could have blurred the expected correlation. First, leaf-scale gas-exchange measurements do not consider water loss that might have happened over the non-assimilatory periods (Tomás et al., 2012) (when the chamber lights were off at night), as well as water loss that happens from plant tissues other than leaf epidermis (Martín-Gómez et al., 2017). As Anyia and Herzog (Anyia & Herzog, 2004) propose, repeated measurements of the instantaneous gas-exchange parameters over time, would help reconcile WUE_i and WUE_b , as it would help match the temporal scales in both estimations. Also, in order to match the spatial scales (i.e., leaf scale and whole-tree scale) when working with adult trees in the field, it has been proposed to first study the canopy structure (Tomás et al., 2014), as it is strongly related to WUE regulation.

CONCLUSION

In conclusion, both WUE_b and WUE_i , as well as their components, were affected by the drought treatment. The effect of the CO_2 treatment was not perceived in tree growth due to the short duration of the experiment and the slow-growing rate of *F. sylvatica*. Also, contrary to expectations, the net rate of photosynthetic assimilation (A_{net}) was not significantly affected by the CO_2 treatment, but it should be noted that the limited sample size in this study may have prevented direct inference of the

effect of elevated [CO₂] in gas-exchange parameters. Reconciliation of leaf and whole-tree scales was possible when studying WUE's components separately, since daily transpiration (E_{day}) and instantaneous transpiration (E_{inst}) were significantly correlated, as well as diameter increase and the net rate of photosynthetic assimilation (A). However, the lack of correlation between WUE_b and WUE_i suggests that more frequent repeated measurements of the instantaneous gas-exchange parameters over time, and a bigger sample size are required to reconcile WUE estimates.

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SUPPLEMENTARY MATERIAL

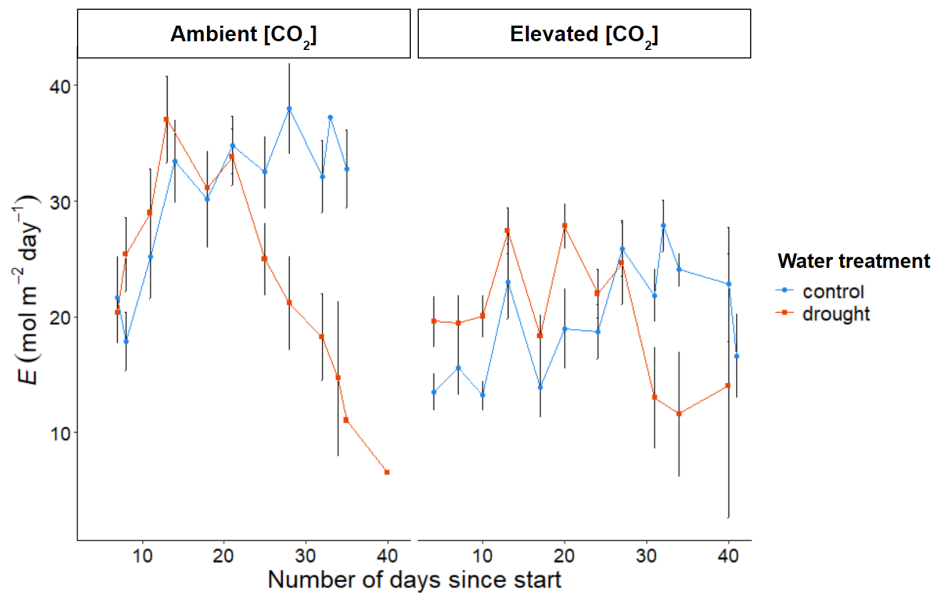


Fig. S1. Mean (\pm SE, n varies across treatment combinations, see main text) daily transpiration per unit of leaf area (E_{day}) over time since the start of the experiment (20 April 2021) for trees in the ambient (left panel) and the elevated [CO₂] (right panel) chambers. Symbol lines and colours depict the two watering treatments: control (blue) and drought (red). Watering was withheld for trees in the drought treatment from day 20 since the start of the experiment.

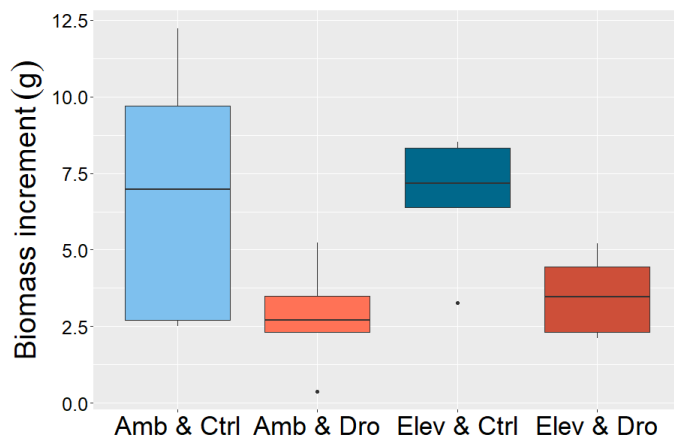


Fig. S2. Biomass gain of plants in each treatment combination in the 4th campaign. Explanation of boxplot and treatments as in Fig. 3.