

Transgenerational effects of elevated CO₂ : Downregulation of photosynthetic efficiency and stomatal sensitivity to drought

Andreas Schweiger¹, Fikeremariam Tegegn Awdamet¹, Wiebke Drenckhan¹, and Bernd J. Berauer¹

¹Universität Hohenheim Fakultät Agrarwissenschaften

September 30, 2022

Abstract

Increasing atmospheric CO₂ and drought are major symptoms of anthropogenic climate change with profound effects on plant growth. Transgenerational memory (i.e. influence of the parental environment on offspring phenotype and performance) has been suggested as a relevant mechanism for plants to build-up adaptive capacity for rapid environmental changes. However, this mechanism of pre-adaptation remains poorly investigated so far. We investigated intra- and transgenerational effects of elevated CO₂ on drought response of wheat. We used seeds from a FACE (Free Air Carbon Dioxide Enrichment) experiment with ambient and elevated CO₂ to grow plants in climate chambers in which we varied CO₂, atmospheric water demand and soil moisture. We quantified photosynthetic efficiency, stomatal sensitivity and biomass production. We observed intragenerational upregulation of photosynthetic efficiency but transgenerational downregulation of photosynthetic efficiency, stomatal sensitivity and water use efficiency as response to maternally elevated CO₂. Plant biomass was affected by drought and experimental CO₂ but not by maternal CO₂. Our study showcases the importance of transgenerational memory effects when studying climate change response of plants and could have major implications for our understanding of global dynamics of carbon sequestration. It highlights the pressing need for multi-generational experiments accounting for transgenerational memory effects of elevated CO₂.

Category: Original Article

Transgenerational effects of elevated CO₂: Downregulation of photosynthetic efficiency and stomatal sensitivity to drought

Short title: Transgenerational effects of elevated CO₂

Authors: Andreas H. Schweiger^{*1}, Fikeremariam Tegegn Awdamet¹, Wiebke Drenckhan¹, Bernd Berauer¹

¹ Institute of Landscape and Plant Ecology, Department of Plant Ecology, University of Hohenheim, Otilie-Zeller-Weg 2, 70599 Stuttgart, Germany

* corresponding author: e-mail: andreas.schweiger@uni-hohenheim.de, tel: +49 711 459 22189

Total word count for the main body: 5759 words

Introduction: 1336 words

Materials and Methods: 1566 words

Results: 576 words

Discussion: 2281 words

Number of figures: 2 (all in colour)

Tables: no tables

Supporting information: Tables S1 to S3; Figures S1 to S5

Summary

Increasing atmospheric CO₂ and drought are major symptoms of anthropogenic climate change with profound effects on plant growth. Transgenerational memory (i.e. influence of the parental environment on offspring phenotype and performance) has been suggested as a relevant mechanism for plants to build-up adaptive capacity for rapid environmental changes. However, this mechanism of pre-adaptation remains poorly investigated so far.

We investigated intra- and transgenerational effects of elevated CO₂ on drought response of wheat. We used seeds from a FACE (Free Air Carbon Dioxide Enrichment) experiment with ambient and elevated CO₂ to grow plants in climate chambers in which we varied CO₂, atmospheric water demand and soil moisture. We quantified photosynthetic efficiency, stomatal sensitivity and biomass production.

We observed intragenerational upregulation of photosynthetic efficiency but transgenerational downregulation of photosynthetic efficiency, stomatal sensitivity and water use efficiency as response to maternally elevated CO₂. Plant biomass was affected by drought and experimental CO₂ but not by maternal CO₂.

Our study showcases the importance of transgenerational memory effects when studying climate change response of plants and could have major implications for our understanding of global dynamics of carbon sequestration. It highlights the pressing need for multi-generational experiments accounting for transgenerational memory effects of elevated CO₂.

Keywords : crops, drought, epigenetic, Free Air Carbon Dioxide Enrichment (FACE) experiments, maternal effects, photosynthesis, stomata regulation, water use efficiency, wheat

Introduction

The human-induced increase in atmospheric CO₂ concentrations has profound effects on global climate and ecosystems (Körner, 2006; Li *et al.*, 2017; Liu *et al.*, 2019). An increasing severity and frequency of drought events is thereby one of the main symptoms of anthropogenic climate change (Trenberth *et al.*, 2014). Both, increasing CO₂ concentrations and drought have and will continue to have strong effects on plant growth with significant consequences for ecosystem functioning and agricultural production (Kaur *et al.*, 2016; Böhnisch *et al.*, 2021). Due to the speed in which these environmental changes happen, it becomes inevitable to analyse and understand the adaptive mechanisms of plants to cope with these rapid changes (Pascual *et al.*, 2014). Knowledge about the adaptive capacity of plants to increasing CO₂ concentrations and decreasing water availability as well as their interactions is therefore imperative to develop agendas for efficient and sustainable mitigation and adaptation strategies with the aim to maintain global ecosystem functioning and food security (Sniderhan *et al.*, 2018).

Water deficit is known to limit plant growth. Drought is consequently one of the main drivers of worldwide crop yield reduction with even stronger effects on global agricultural production being expected for the near future (Ng *et al.*, 2016; Zargar *et al.*, 2017). Photosynthesis – the key primary process of plant carbon assimilation and thereby growth – plays a fundamental role for the response of plants to water deficit (Kaur *et al.*, 2016). Water use efficiency (i.e. the amount of carbon assimilated as biomass per unit of water used by a plant) is closely controlled by stomata regulation (Haworth *et al.*, 2011). Mechanisms of plants to prevent drought stress, such as stomata closure, directly interfere with photosynthetic carbon acquisition. However, increasing atmospheric CO₂ concentrations can reduce these limitations linked to water shortage. Higher atmospheric CO₂ concentrations increase photosynthetic carbon acquisition by increasing intercellular CO₂ concentrations and carboxylation efficiency as well as by reducing photorespiration (Bowes, 1993; Ainsworth & Long, 2005; Stiling *et al.*, 2013). All this facilitates plant growth under elevated CO₂ with some variation between different species and photosynthetic pathways (C₃ vs. C₄) (Luo & Reynolds, 1999; Nowak *et al.*, 2004; Prior *et al.*, 2011). Elevated CO₂ can furthermore increase water use efficiency of plants by reducing

stomatal conductance and, thus, transpirational water loss (Curtis & Wang, 1998; Prior *et al.* , 2011; Varga *et al.* , 2017). In other words, plants can reduce stomata opening under elevated CO₂ concentrations to receive the required amounts of CO₂ and, thus, can save water which otherwise is lost with increasingly open stomata. This increase in water use efficiency has been shown to be able to alleviate drought effects on photosynthetic carbon acquisition and plant growth (Bazzaz, 1990; Prior *et al.* , 2011; Medina *et al.* , 2016). Consequently, strong interactive effects on plant productivity have to be expected between increasing atmospheric CO₂ concentrations and the spatio-temporal variability of water availability. However, results obtained so far from studies regarding the short-term plant responses to elevated CO₂ within one generation are increasingly realized to be insufficient to predict long-term, transgenerational responses of plants to a future environment enriched in CO₂ but depleted in water (Luo & Reynolds, 1999; Liu *et al.* , 2019).

The strength and direction of individual and interactive effects of environmental drivers on plant performance are rarely static but do change over time due to the continuous adaptation of plants and the environment they inhabit. Being sessile organisms, plants are constantly forced to adapt to changing environmental conditions during their life-time, across generations or over evolutionary time scales (Pascual *et al.* , 2014). Evolutionary adaptation has been suggested to provide an important way for natural populations to counteract environmental changes (Hoffmann & Sgrò, 2011). However, climate change at its current and predicted pace increases the difficulties of plants to adapt (Mishra *et al.* , 2022). Evolutionary adaptation usually happens over multiple of generations and, thus, can hinder rapid adaptation which depends on fast generation times and high selection pressure (Bradshaw & Holzapfel, 2006; Bell & Collins, 2008; Shoo *et al.* , 2013). Short-term responses to environmental stress, such as acclimation, provides another important strategy for crops and wild plant species to cope with reoccurring harsh environmental conditions (Irigoyen *et al.* , 2014). Such kind of intragenerational memory is active within the lifespan of individual plants but does not transmit the information into the next generation, thus, lacks lasting impacts on the fitness of plant species. However, environmental changes can furthermore induce epigenetic imprints which can be transmitted into the next generation. Epigenetic imprints are here defined as mechanisms that enable the developmentally appropriate expression of genes (cf. House & Lukens, 2014). This mechanism provides a kind of pre-adaptation for subsequent generations as a form of maternal effect or transgenerational memory (Pascual *et al.* , 2014).

Transgenerational memory (i.e. the influence of the parental environment on offspring phenotype and performance beyond the effects of transmitted genes; Walter *et al.* , 2016), remains longer active than acclimation and enables plants to respond faster and more adequate to subsequent stress events (Walter *et al.* , 2013; House & Lukens, 2014). It furthermore allows plants to prepare subsequent generations comparably fast for future environmental changes (Latzel *et al.* , 2016). Consequently, transgenerational memory such as epigenetic effects are increasingly realised as one of the main sources of quick phenotypic variation and evolutionary change and, thus, are considered a relevant mechanism of plants to build-up adaptive capacity with regard to accelerating global climate change (Pascual *et al.* , 2014; McCaw *et al.* , 2020). Furthermore, epigenetic effects are increasingly recognized as a very promising approach in plant breeding to obtain progeny with pre-adaptations to different environmental conditions (Alvarez-Venegas *et al.* , 2014). However, transgenerational memory remains poorly investigated for climate change responses of plants (Li *et al.* , 2017; Saban *et al.* , 2019; Liu *et al.* , 2019). Therefore, nature conservationists and plant breeders rarely know the underlying causes of the observed phenotypic and genetic variation in populations of wild plants or varieties of crops (House & Lukens, 2014). This hinders effective conservation planning and breeding programs aiming to maximize the adaptive potential of ecological and agricultural systems for future climate change.

In this study we investigated the intra- and transgenerational effects of elevated CO₂ on stress response of wheat to atmospheric water demand and soil water availability – the two major environmental drivers of plant drought stress (Philip, 1966; Passioura, 1982). We focus on wheat as one of the three most important crops globally (Li *et al.* , 2019; Grote *et al.* , 2021). Wheat is furthermore assumed to be strongly susceptible to increasing drought (Farooq *et al.* , 2012) with major efforts being taken e.g. in breeding programs to foster its adaptive capacity to drought (Liet *et al.* , 2021).

Intragenerational effects of elevated CO₂ on wheat biomass production, grain yield and quality have been

intensively studied during the last decades (e.g. Asseng *et al.* , 2004; Högy & Fangmeier, 2008; Myers *et al.* , 2014). Transgenerational effects, however, remain sparsely investigated (but see e.g. Li *et al.* , 2019). Based on results from existing intragenerational studies, we assume elevated atmospheric CO₂ concentrations experienced by mother plants to affect the stress response of the offspring generation to drought, i.e. changes in atmospheric water demand and soil water availability. More specifically we hypothesize that maternally elevated CO₂ will trigger transgenerational pre-adaptations in carboxylation capacity and stomatal control with positive effects on water use efficiency under elevated CO₂ conditions. We tested this assumption by growing plants from seeds that originate from a FACE experiment with ambient and elevated CO₂ concentration. Plants from these seed sources were grown in climate chambers in which we varied atmospheric CO₂ as well as atmospheric water demand (i.e. Vapour Pressure Deficit, VPD) and soil moisture. In this climate chamber experiment we investigated plant ecophysiological characteristics related to photosynthetic efficiency and drought response as well as parameters related to biomass production. Besides photosynthetic efficiency, we specifically focused on stomatal response of plants to changes in atmospheric water demand, as this important driver of drought stress emergence in plants and its interaction with soil water availability and atmospheric CO₂ concentration is chronically understudied.

Material and Methods

For our climate chamber experiment we used seeds of summer wheat (*Triticum durum* L. var Miradoux) grown at ambient (400 ppm) and enriched CO₂ concentration (550 ppm) in a FACE experiment for one generation in 2018 (see Fangmeier *et al.* , 2016 for details on the FACE experiment). For our experiment, the seeds from this FACE experiment were sown in cylinders of 30 cm diameter and a total height of 40 cm at seven equidistant locations on the circumsphere of 7.5 cm radius, with three seeds aligned radial at each segment with 1 cm distance between them. Seeds were sown uniform at 2 cm depth (February 18th 2022, Day 1 of experiment). After germination, seedlings were removed to a density of one seedling per equidistant segment (corresponding to 6.7 cm distance between nearest individuals). At the bottom end of each cylinder a perforated plate with a wick system was installed 5 cm above the bottom to prevent water logging but allow the substrate to keep hydraulic contact to excess water collected at the bottom. Pots were filled with a 50:50 (volume-%) mixture of LD80 and sand to a bulk density of 1 g cm⁻³. Substrate was filled up to 2 cm under the rim of each pot leaving a total of 33 cm depth for rooting and an available rooting volume of 23 L. Each cylinder contained pure stand of plants with the same maternal CO₂ treatment (ambient vs. elevated, see above). In total we had 16 pots, with each 7 individuals.

We used four climate chambers, two with ambient CO₂ concentration (450 ppm) and two with elevated CO₂ concentration (800 ppm). Atmospheric CO₂ concentrations below 600 ppm are generally known to be insufficient to saturate carboxylation in photosynthesis (Nowak *et al.* , 2004). To minimize CO₂ limitation of Rubisco and maximize suppression of competitive oxygenation we decided for a concentration of 800 ppm for our elevated CO₂ treatment which is also in line with previous experiments on transgenerational effects of elevated CO₂ (see Li *et al.* , 2017). In each chamber we placed 4 pots, two pots with seed material from ambient maternal CO₂ and two with enriched maternal CO₂ seed material.

In addition to the experimental application of different levels of CO₂ we also applied different, edaphic drought treatments. 31 days after sowing, we randomly assigned one pot per maternal CO₂ condition in each chamber to a drought treatment while the other one remained well-watered throughout the experiment. The environmental setting of all chambers was similar besides the CO₂ concentration, which means day conditions at 24 °C air temperature, 64 % relative humidity and full light (800 μmol m⁻² s⁻¹) and night conditions at 18 °C, 46 % relative humidity and no light. Thus, Vapour Pressure Deficit (VPD) within the chambers was held mild and similar during day (1.08 kPa) and night (1.12 kPa). To change between day and night we conducted a linear ramp of one-hour duration, for sunrise between 5:00 and 6:00 and for sunset between 20:00 and 21:00, resulting in a day length of 14 hours.

We conducted ACi curves to determine key ecophysiological parameters describing photosynthetic efficiency, namely the maximum rubisco carboxylation rate (V_{cmax}), the maximum electron transport rate (J_{max}), the dark respiration (R_d) and the CO₂ compensation point (Γ) (see Farquhar *et al.* , 1980). With

V_{cmax} indicating the efficiency of rubisco carboxylation and J_{max} indicating ribulose 1,5 biphosphat (RuBP) regeneration, both processes represent key limitations within the photosynthetic pathway. Additionally, Rd and Γ are important parameters to describe both limitation processes. Under ambient atmospheric CO_2 concentrations V_{cmax} is usually limiting light saturated photosynthesis. Consequently, J_{max} , which is driving the regeneration of ribulose 1,5 biphosphat, is becoming increasingly limiting under raising CO_2 concentrations (Pignon & Long, 2020). Based on the parameters derived from ACi curves we are therefore able to quantify and compare changes in photosynthetic efficiency and limitations based on maternal imprints. Net assimilation rate for fitting ACi curves was measured at reference CO_2 of 400 ranging to 0 ppm [400, 300, 200, 100, 50, 0] directly followed by measurements between 400 to 1200 ppm [400, 400, 600, 800, 1000, 1200] with minimum 90 and maximum 180 seconds at each CO_2 concentration allowing for early measurement based on stability criteria. V_{cmax} , J_{max} , Rd and Γ were estimated from ACi curves (based on the model of Farquhar *et al.*, 1980) fitted to the measured assimilation rates using the *plantecophys* -R-package (v.1.4-6; Duursma, 2015).

Besides photosynthetic efficiency, we quantified stomatal conductance (gs) and net assimilation rate (A) to increasing atmospheric water demand (increasing VPD) by measuring VPD-response curves. Stomatal opening is regulated by edaphic water supply and atmospheric water demand (see Buckley, 2005). Stomatal opening itself regulates CO_2 uptake as well as H_2O loss at the leaf surface, thus stomatal conductance ultimately regulates the trade-off between net carbon assimilation and transpirational water loss. The stomatal response to drought (reduced opening to avoid water loss) is similar to the stomatal response to elevated CO_2 (reduced opening, as with higher CO_2 concentration less air is needed to achieve a sufficient intercellular CO_2 concentration). By conducting VPD-response curves for plants which experience different levels of maternal and experimental CO_2 as well as different drought treatments, we are able to quantify maternal effects of elevated CO_2 on stomatal regulation under atmospheric and edaphic drought. For fitting VPD-response curves for gs and A we measured both parameters at VPD_{leaf} of 1 to 3 kPa by equidistant steps of 0.5 kPa with each step lasting 15 minutes and measurements obtained every minute. We calculated water use efficiency as the ratio between A and gs under a VPD of 1. We fitted the corresponding VPD-response curves of stomatal conductance and net assimilation rate by using generalised additive models with integrated smoothness estimation using the `gam()`-command from the *mgcv* -R-package (v.1.8-34; Wood, 2004). The optimal degrees of freedoms used for the smoothing spline interpolation were selected based on penalized regression splines. Additionally, we limited the maximum possible degrees of freedom (i.e. limited number of maximum possible knots, $k_{max}=5$) to omit overfitting.

ACi and VPD curves were measured for one (the same) plant individual in each pot using a LiCor 6800 [LiCor, USA] with standardized settings: flow rate $500 \mu\text{mol s}^{-1}$, Temperature $26 \text{ }^\circ\text{C}$, PAR $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, 10.000 rpm ventilation. For the ACi curves VPD_{leaf} was fixed at 1 kPa. For the VPD-response curves, reference CO_2 was fixed to chamber CO_2 , meaning 800 ppm for plants in elevated chambers and 450 ppm for chambers in ambient conditions. Both, ACi- and VPD curves have been measured right before the start of the drought treatment (between 24 to 27 days after sowing) and VPD curves in addition at the end of the drought treatment prior to harvesting (between 38 and 41 days after sowing).

To monitor plant water stress during the experimentally imposed drought, we measured pre-dawn leaf water potential $\Psi_{\lambda\epsilon\alpha\phi}$ during the leaf gas-exchange measurements using a scholander pressure chamber [Model 1000, PMS Instrument Company, USA]. Measurements were conducted on the youngest, unfolded and undamaged leaves 26, 31, 33, 35, 38-41 days after sowing, every time before 5:00 AM.

We harvested aboveground biomass of all individuals at 3 cm aboveground at the end of the drought treatment 42 days after sowing (April 1st 2022). We decided to separate biomass by plant organ for 4 out of 7 individuals. Those 4 individuals were never used for water potential measurements, thereby we avoid any bias by pre-harvest removal of leaves as it had occurred for the individuals used for water potential measurements. For these 4 individuals per pot, aboveground biomass was separated into stem, leaves and senesced leaves, with the latter being separated if the leaf showed more than 50 % senesced tissue. We measured number of leaves, leaf area and fresh weight of all three separated organs per individual. After drying to constant

weight at 60 °C, we measured dry weight. For the remaining three individuals we only measured aboveground biomass without separation of plant organs after drying to constant weight at 60 °C. The calculated ratio of total aboveground biomass of all seven individuals per pot divided by the available 23 L of soil rooting volume spanned a range between 0.38 and 1.58 gL⁻¹ (10 out of 16 pots being < 1 g L⁻¹), which is within the recommended range reported by Porter et al (2012) to omit limitation of root growth in pot experiments.

To account for pseudoreplication in our study, we performed linear mixed models with a random effect for planting pot nested in chamber to properly account for both zero inflation and the correlation structure among the ecophysiological and biomass-related parameters which we measured for the different plant individuals growing in the same pot and chamber (cf. Zimmerman *et al.* , 2021). For this we applied the `lmer()`-command of the `lmerTest` -R-package (v.3.1-3; Kuznetsova *et al.* , 2017). For each model we calculated marginal and conditional R² using the `r.squaredGLMM()`-command of the `MuMIn` -R-package (v. 1.43.17; Barton, 2020). Similarly, we used robust linear mixed effect models to estimate the effects of changing atmospheric drought (VPD) on carbon assimilation and stomatal control by accounting for chamber effects in the random term of the models. We subsequently used the residuals of these models to analyse the effect of maternal CO₂ and edaphic drought on these VPD-responses. All analyses were performed in R (v.4.0.5; R Core Team, 2021) with a level of significance of alpha = 0.05.

Results

Maximum rate of carboxylation V_{cmax} was significantly reduced for plants with maternally elevated CO₂ environments but increased with maturation of experimental plants and increasing experimental CO₂ concentrations with no significant interaction between the different predictors (Fig. 1A and B; R²_{marginal} and R²_{conditional} = 0.70 for a linear mixed effect model with chamber as random effect, see Supplementary Material Table S1 for more details).

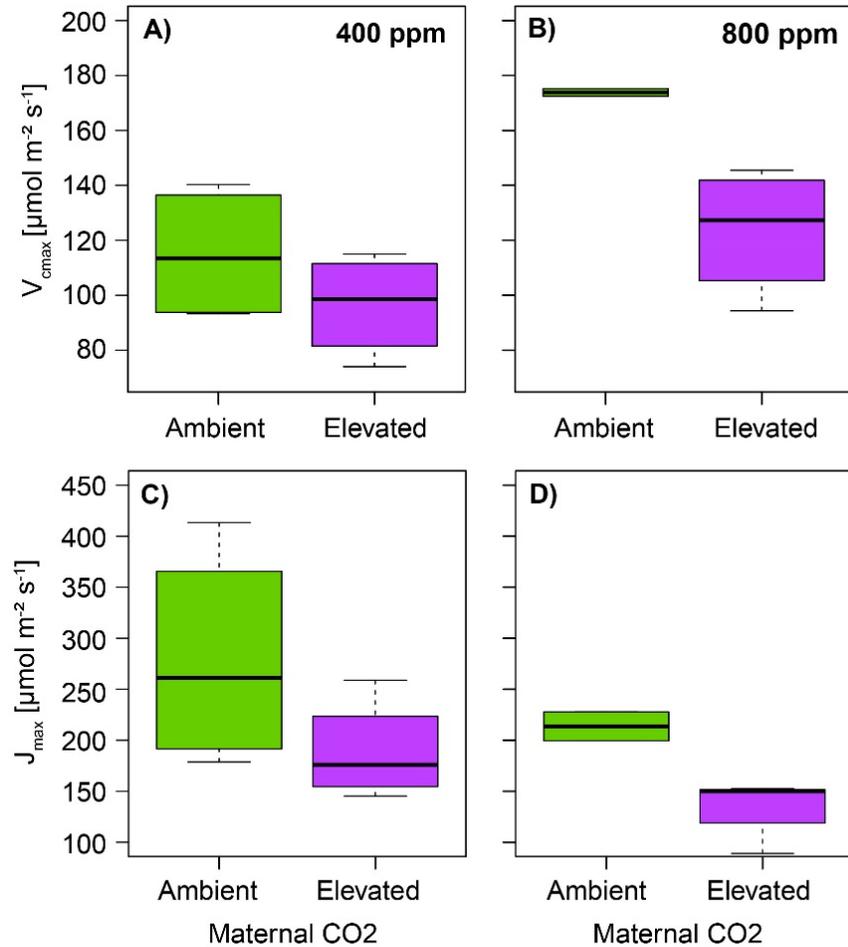


Figure 1. Differences in the maximum rate of carboxylation V_{cmax} and maximum rate of electron transport J_{max} between wheat plants with maternally ambient (400 ppm; green) and elevated (550 ppm; purple) CO_2 environments grown in climate chambers under ambient (400 ppm, left column) and elevated (800 ppm, right column) CO_2 concentrations.

The maximum rate of electron transport J_{max} was significantly reduced for plants with maternally elevated CO_2 environments with no significant effects of maturation of experimental plants and elevated, experimental CO_2 (Fig. 1C and D; R^2_{marginal} and $R^2_{\text{conditional}} = 0.42$; Supplementary Material Table S1). This effect of maternal CO_2 exposure became marginally insignificant when accounting for interactions between the predictors, with the effects of experimental CO_2 and maturation as well as all interactions being insignificant (R^2_{marginal} and $R^2_{\text{conditional}} = 0.43$; Supplementary Material Table S1). For dark respiration, none of the predictors nor their interactions showed significant effects (Supplementary Material Fig. S 1 a and b and Table S1). Dark respiration as well as CO_2 compensation points significantly decreased with increasing maturation of experimental plants with no effects of experimental and maternal CO_2 and their interactions ($R^2_{\text{marginal}} = 0.35$ and $R^2_{\text{conditional}} = 0.65$ for dark respiration; $R^2_{\text{marginal}} = 0.39$ and $R^2_{\text{conditional}} = 0.68$ for compensation point; Supplementary Material Fig. S1C and D and Table S1).

In contrast to the ecophysiological variables, maternal CO_2 exposure did not show significant effects on any of the considered biomass-related variables (number of leaves, leaf area, leaf fresh and dry weight, total aboveground fresh and dry weight; see Supplementary Material Fig. S2 and Table S2 for more details).

Elevated experimental CO₂ showed significant positive effects on leaf area, number of leaves, leaf fresh and dry weight as well as on total aboveground fresh and dry weight per plant (Supplementary Material Fig. S2 and Table S2). Edaphic drought had significant negative effects on all considered biomass-related variables (see Supplementary Material Table S2). No significant interactions between maternal CO₂, experimental CO₂ and edaphic drought was observable for any of the biomass variables.

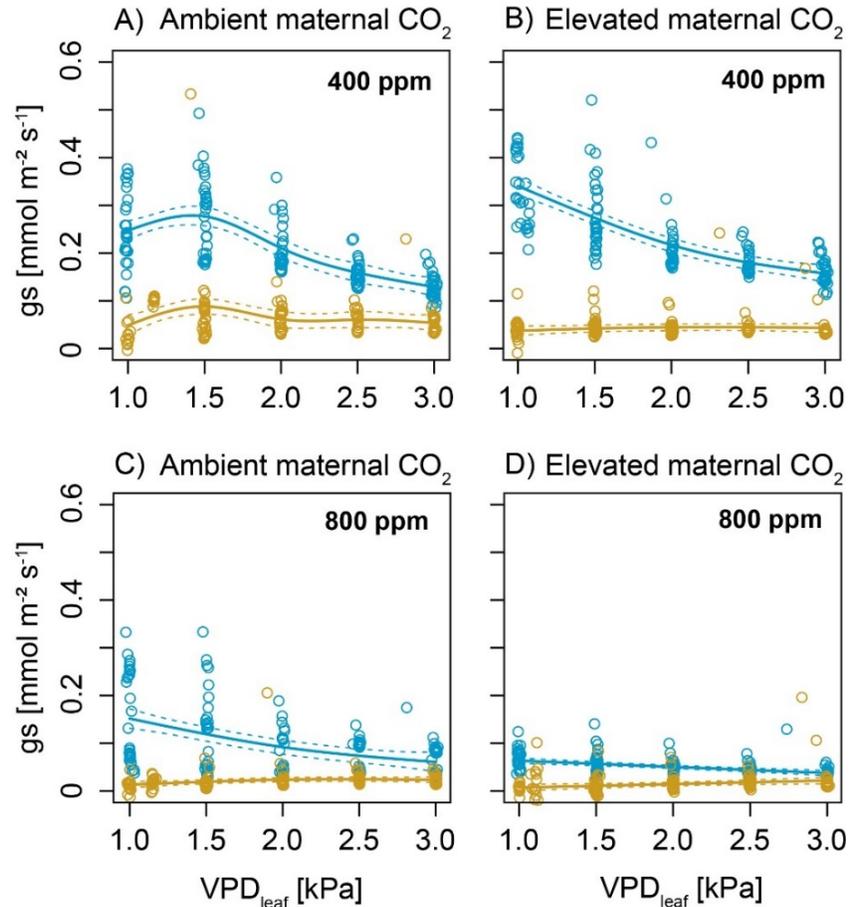


Figure 2. Response of stomatal conductance g_s to changing vapor pressure deficit at leaf surface (VPD_{leaf}) for wheat plants with maternally ambient (400 ppm; A and C) and elevated (550 ppm; B and D) CO₂ environments grown in climate chambers under ambient (400 ppm, A and B) and elevated (800 ppm, C and D) CO₂ concentrations. Blue points and lines reflect plants sufficiently supplied with water whereas orange points reflect plants which experienced edaphic drought. Lines represent predictions of generalised additive models (means indicated by solid lines as well as the 5% and 95% percentile as dashed lines).

Stomatal conductance g_s and net assimilation rate A decreased with increasing atmospheric water demand (increasing Vapor Pressure Deficit, VPD) for well-watered plants. Plants with experienced edaphic drought showed significantly lower g_s and A which slightly increased with increasing VPD (Fig. 2). Water use efficiency increased for plants exposed to edaphic drought (Supplementary material Table S3). All plants exposed to drought treatment experienced significant drought stress during measurements (see Supplementary Material Fig. S3). Net assimilation rate A was significantly lower for plants which experienced edaphic drought and further decreased with increasing atmospheric drought (VPD , Supplementary Material Fig. S4). Stomatal conductance was not affected by material CO₂ under ambient chamber CO₂ but was signifi-

cantly lower for plants with maternally elevated CO₂ with a significant positive interaction between maternal CO₂ and VPD (Fig. 2, $R^2_{\text{adjusted}} = 0.10$ for a linear model in which the effect of edaphic drought and the pots/chambers is accounted for, see Supplementary Material Table S3 for more details). Elevated maternal CO₂ showed negative effects on assimilation rate under ambient chamber CO₂ with a negative interaction between maternal CO₂ and VPD ($R^2_{\text{adjusted}} = 0.34$). This effect of maternal CO₂ turned negative under elevated chamber CO₂ with a positive interaction between maternal CO₂ and VPD ($R^2_{\text{adjusted}} = 0.38$, Supplementary Material Table S3). Water use efficiency was not affected by maternal CO₂ or VPD but was significantly increased for elevated, experimental CO₂ ($R^2_{\text{adjusted}} = 0.43$, $p < 0.001$).

Discussion

Plants are sessile organisms which are rooted in the environment they inhabit and, thus, are constantly forced to adapt to changing environmental conditions to counteract abiotic stress (Pereira, 2016). Populations of plants are therefore generally believed to be primed for the environments they inhabit due to past adaptive and selective forces (Bell & Collins, 2008). However, rapid increases in CO₂ concentrations and drought events – two major facets of contemporary climate change with contrasting effects on plant carbon acquisition and water status – pose new challenges to plants by disrupting these evolutionary dynamics and make it more difficult for plants to sustain productivity under these novel conditions (Duarte-Aké *et al.*, 2019; Hamann *et al.*, 2021). Such rapid climatic changes are assumed to create a range of new selection pressures on plant populations (Hoffmann & Sgrò, 2011). Rapid ways of pre-adaptation such as intra- and transgenerational memory might become increasingly important under the novel settings of accelerating, climatic changes.

The importance of intra- and transgenerational memory for plant fitness and survival has previously been investigated on certain organisational levels. These include investigations on the carbon and nutrient acquisition and allocation of single plants (Bloom *et al.*, 1985) or with regard to demographic dynamics of plant populations and as a potential mechanism ensuring species coexistence (Warner & Chesson, 1985). Empirical evidence exists that plants are able to adapt e.g. their metabolism in response to previous environmental stress (Bruce *et al.*, 2007). This ecological stress memory can play out within the lifespan of an individual plant (intragenerational; Walter *et al.*, 2011) or in the subsequent generations (transgenerational; Walter *et al.*, 2016). Transgenerational memory such as epigenetic regulation is increasingly recognized as an important process in plants' response to environmental changes in e.g. temperature, light and water availability (Duarte-Aké *et al.*, 2019). Intra- and transgenerational memory is furthermore discussed as an important component of single organisms or population resilience to climatic extreme events such as drought (Walter *et al.*, 2013, 2016).

Transgenerational effects of elevated CO₂ have been reported by a number of studies including effects on flowering phenology (Springer & Ward, 2007; Johnston & Reekie, 2008), reproductive traits (i.e. number of flowers, number and mass of fruits and seed, nitrogen and carbon concentrations of seeds; Jablonski *et al.*, 2002; Frenck *et al.*, 2013) and enhanced seedling growth in the subsequent generation (Derner *et al.*, 2004; Lau *et al.*, 2008). In our study, we observed for the first time intra- and transgenerational effects of elevated, atmospheric CO₂ on photosynthetic efficiency and stomatal sensitivity to atmospheric water demand and soil water availability – the two main environmental drivers of plant drought stress. We observed carboxylation efficiency to decrease for plants with maternally elevated CO₂ and to increase with maturation of the experimental plants. Carboxylation capacity under Rubisco-limited conditions (V_{cmax}) and RuBP regeneration (J_{max}) – the two major components of the photosynthetic dark reaction (Farquhar *et al.*, 1980) – are generally assumed to scale with one another (Leuning, 1997; Medlyn *et al.*, 1999). This is partly true for our study. V_{cmax} was significantly reduced for plants with maternally elevated CO₂ environments but increased under elevated chamber CO₂ and with maturation of experimental plants. Also J_{max} was significantly lower for plants with maternally elevated CO₂ environments but showed no response to the maturation of experimental plants and elevated chamber CO₂. Stomatal conductance was significantly lower for plants with maternally elevated CO₂ growing under elevated, experimental CO₂ with a significant positive interaction between maternal CO₂ and VPD. This suggests plants to become more risky with regard to transpirational water loss under high atmospheric water demand. Such kind of down regulation of

stomatal sensitivity to drought was also observed by Li *et al.* (2017) for plants with maternally elevated CO₂. However, water use efficiency was observed by Li and colleagues to be higher for plants with an maternal exposure to elevated CO₂. In our study, we observed no effects of maternal CO₂ or VPD on WUE. We, furthermore, observed a significant positive effect of increased maternal CO₂ on assimilation rate for plant grown under ambient, experimental CO₂. This positive effect reversed into a negative effect under elevated, experimental CO₂. The interactive effects between maternal CO₂ and VPD changed from negative under ambient experimental CO₂ into positive for elevated, experimental CO₂. All these observations hint towards a transgenerational memory of maternally elevated, atmospheric CO₂ which modulate carboxylation efficiency and stomatal sensitivity of the offspring generation under drought. Generally, plants seem to become riskier with regard to carbon assimilation under drought when the previous generation has experienced elevated CO₂ concentrations.

Photosynthetic capacity is closely associated with stomatal regulation (Körner *et al.*, 1979; Hetherington & Woodward, 2003). Stomatal control is furthermore a critical component of the adaptation of plants to the environment (Haworth *et al.*, 2011). Elevated CO₂ has been shown for numerous species to reduce stomatal conductance by 77%–86% as compared to ambient CO₂ conditions (Bunce, 1995). Contrary, lower diffusion of CO₂ from the atmosphere through drought regulated stomata is considered to be the major reason behind decreased photosynthetic rate during mild to moderate drought stress (Pinheiro & Chaves, 2011). Generally, reduced availability of CO₂ is predicted to be compensated by increasing carboxylation efficiency of RubisCO and/or increasing stomatal conductance (Woodward, 1998; Franks & Beerling, 2009). In our study, this effect seems to be downregulated by the transgenerational memory of elevated CO₂ concentrations. Transgenerational downregulation of stomatal sensitivity seems furthermore to be different from the downregulation of carboxylation efficiency. Whereas the downregulating effect of maternally elevated CO₂ just appeared for stomatal conductance under experimentally elevated CO₂, it changed from upregulation to downregulation for assimilation rate. Thus, experimentally elevated CO₂ seem to be able to modulate the maternal effects on stomatal control and even more on assimilation rate. All this hint at differences in the strength of transgenerational memory effects between stomatal control and photosynthetic carboxylation capacity.

Our results furthermore suggest that the intragenerational response of carboxylation capacity to elevated, atmospheric CO₂ is contrasting to the transgenerational response with an intragenerational upregulation (by increased experimental CO₂ in the chambers) but a transgenerational down-regulation of carboxylation capacity. These findings – if verified in future studies – could have major implication for the general validity and transferability of Free Air Carbon Enrichment (FACE) experiments as a major tool in plant-focussed climate change impact research. The strong interest in FACE experiments originates from the potential of such investigations to foster our understanding on potential future trajectories in the function and structure of our agroecosystems in a CO₂-enriched world (Luo & Reynolds, 1999). A major challenge for these experiments comes with the nature of FACE experiments being usually implemented as step experiments with an abrupt increase in atmospheric CO₂ concentration in contrast to the gradual increase in CO₂ concentrations we observe to happen as the consequences of rising global greenhouse gas emissions. Modelling studies concluded that the structural and functional responses yielded from these step experiments might most often be different to those that would be observed by exposing the same study system to a gradual increase of the same magnitude over several decades as currently happening under climate change (e.g. Luo & Reynolds, 1999). In fact, numerous studies have been reported intragenerational downregulation (cf. Sage, 1994), i.e. a biochemical adjustments in photosynthetic capacity to increase CO₂ concentrations (Huxman *et al.*, 1998b; Bernacchi *et al.*, 2003). Another challenge comes with the fact, that FACE experiments are predominately designed as single-generation experiments investigating intragenerational responses to elevated CO₂ but rarely investigate effects across multiple generations and in interaction with other major symptoms of change such as drought or eutrophication (but see e.g. Ainsworth *et al.*, 2003). Results from long-term FACE experiments are furthermore reported to differ from short-term responses with water and nutrients availability and demand being discussed as main abiotic drivers (Schneider *et al.*, 2004). Based on the existing literature and our observations we postulate, that transgenerational memory effects of elevated CO₂ on carboxylation capacity and stomatal sensitivity to drought modulated by water and nutrients availability and demand

could be a main biotic mechanism causing inconsistency in observed responses between single- and multi-generational FACE experiments.

Transgenerational memory effects of elevated CO₂ might be furthermore a key component to understand global dynamics of carbon sequestration. Increasing CO₂ concentrations has been identified to be a global driver for enhanced vegetation productivity. This CO₂ fertilisation effect, which is reported to result from the ecophysiological effects of elevated CO₂ on photosynthetic carbon gain and water use efficiency as discussed above – has been postulated to strongly affect ecosystem functioning, global food security and biosphere-atmosphere feedbacks in the near future (Saban *et al.*, 2019). However, the fertilizing effect of increasing CO₂ concentrations has been recently reported to have substantially declined in most terrestrial regions of the globe during the last decades implying substantial reductions of the positive effects rising CO₂ concentrations on terrestrial carbon sequestration (Wang *et al.*, 2020). Changes in nutrient concentrations and water availability have been proposed as abiotic drivers of this process. Based on our results we argue, that transgenerational memory effects of elevated CO₂ in combination with memory effects of nutrient and water availability and demand are key evo-evolutionary mechanisms behind the changing dynamics of global carbon sequestration. Such transgenerational memory effects will furthermore have profound effects on stress response, grain yield and quality in crops which will be not predictable from single-generation investigations (Bottley, 2014; Li *et al.*, 2019). However, all this is still rather speculative and in-depth investigations are urgently needed to test these assumptions.

Stomatal regulation under drought has been recently described to be closely coupled to a *de novo* synthesis of abscisic acid (ABA) within the leaf as a response to the exposure to dry air (high VPD; Buckley, 2016; McAdam *et al.*, 2016). ABA has been documented to play an important role in guard cell movement (Tardieu & Davies, 1993; McAdam & Brodribb, 2018) and, by this, allows plants to control the transpirational water loss under increasing edaphic and atmospheric drought (Liu *et al.*, 2005). This process of ABA biosynthesis is described to respond very fast to increasing atmospheric drought, i.e. within 20 min resulting in stomatal closure (Buckley, 2016). Nevertheless, ABA concentration has been reported to be affected across generations by maternally elevated CO₂ with increased concentrations of leaf ABA for plants with maternally elevated CO₂ concentrations (Li *et al.*, 2017). Also photosynthetic enzymes responsible for carboxylation efficiency have been documented to be modulated by epigenetic mechanisms (Duarte-Ake *et al.*, 2019). In our study we covered different aspects of carboxylation capacity but did not investigate ABA concentrations which opens room for speculation about the importance of ABA for transgenerational memory effects of elevated CO₂. Further studies are urgently needed to disentangle the molecular mechanisms behind the observed, transgenerational effects of elevated, atmospheric CO₂ on the photosynthetic efficiency and stomatal sensitivity of plants to drought.

In our study we did not observe any effects of maternal CO₂ on any of the considered, biomass-related parameters. This might be due to the relatively short duration of the experiment or the overriding effect of experimental CO₂ and drought. However, transgenerational effects of elevated CO₂ on biomass-related parameters have been reported e.g. for the annual grass *Bromus madritensis* L. where growth rate of seedling was increased by elevated experimental CO₂ but the increase was less pronounced when parent plants also experienced elevated CO₂ (Huxman *et al.*, 1998a, 2001). Furthermore, growth of spring wheat (*Triticum aestivum* L.) was reported to respond more to elevated CO₂ when the previous generations also already experienced elevated CO₂ (reported for second and third generation; Derner *et al.*, 2004). Generally, there is no one-to-one translation of photosynthetic CO₂ response into growth response, but the latter depends on allocation dynamics modulated by additional, environmental factor such as water and nutrient demand and availability (Korner, 2006). Elevated CO₂ has been documented to change source-sink-dynamics in crops with effects of carbon and nutrient allocation from vegetative organs into grain (Bourgault *et al.*, 2013; Biswas *et al.*, 2013). Furthermore, reduced transpiration via reduced stomatal conductance can be considered to indirectly limit nutrition uptake under elevated CO₂ with effects in the plant growth and yield quality of crops (Hogy *et al.*, 2009; Myers *et al.*, 2014). We investigated the effects of edaphic and atmospheric water availability but did not account for nutrient availability. However, future studies should definitely account for the interrelations between transgenerational effects of elevated CO₂ and allocation

dynamics modulated by water and nutrient availability and demand to get a more complete picture about historical contingencies in plant responses under changing environmental conditions.

Conclusions

Memory effects are a major feature of ecosystem dynamics which influence present and future response trajectories (Schweiger, 2017). Memory effects become especially important when environmental changes happen fast and single organisms, populations or entire ecosystems have to adapt to these novel environmental conditions (Schweiger *et al.*, 2019). In our study, we observed transgenerational memory effects of elevated CO₂ on the photosynthetic efficiency and drought sensitivity of plants. Such kind of memory effects can have major implications for global carbon sequestration and crop breeding under accelerating, global climate change and, thus, demonstrate the necessity to re-visit plant–environment interactions (Pereira, 2016). More in-depth knowledge on intra- and transgenerational effects on allocation dynamics modulated by water and nutrient availability and demand will be needed to implement a mechanistic understanding about memory effects of elevated CO₂ into modelling – one major tool for climate change impact assessment and future prediction. To achieve this, we urgently need a stronger focus on multi-generational greenhouse and FACE experiments accounting for transgenerational memory effects of elevated CO₂ to disentangle the molecular, cellular and organismal components of epigenetic pre-adaptation and its importance for the fitness of single organisms, viability of populations and functioning/productivity of agricultural and ecological systems.

Acknowledgements

We would like to thank Petra Hogy for providing the seed material for the study as well as Gina Gensheimer and Jurgen Franzaring for their support during the experiment.

Author Contribution

AHS and BB developed the study idea and planned the experiment, FTA, WD and WD conducted the experiment, AHS and BB performed the analyses, AHS led the writing with significant contribution by all authors.

Data Availability

All data and script for data analyses will be made freely available on zenodo upon publication.

References

- Ainsworth EA, Davey PA, Hymus GJ, Osborne CP, Rogers A, Blum H, Nosberger J, Long SP . 2003 .** Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO₂Enrichment (FACE). *Plant, Cell & Environment* **26** : 705–714.
- Ainsworth EA, Long SP . 2005 .** What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytologist* **165** : 351–372.
- Alvarez-Venegas R, De la Pena C, Casas-Mollano JA (Eds.) .2014 .** *Epigenetics in plants of agronomic importance: Fundamentals and applications* . Heidelberg: Springer.
- Asseng S, Jamieson PD, Kimball B, Pinter P, Sayre K, Bowden JW, Howden SM . 2004 .** Simulated wheat growth affected by rising temperature, increased water deficit and elevated atmospheric CO₂. *Field Crops Research* **85** : 85–102.
- Barton K . 2020 .** MuMIn: Multi-Model Inference. R package.
- Bazzaz FA . 1990 .** The Response of Natural Ecosystems to the Rising Global CO₂ Levels. *Annu. Rev. Ecol. Syst* **21** : 167–196.
- Bell G, Collins S . 2008 .** Adaptation, extinction and global change. *Evolutionary Applications* **1** : 3–16.

- Bernacchi CJ, Calfapietra C, Davey PA, Wittig VE, Scarascia-Mugnozza GE, Raines CA, Long SP . 2003 .** Photosynthesis and stomatal conductance responses of poplars to free-air CO₂ enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytologist* **159** : 609–621.
- Biswas DK, Xu H, Li YG, Ma BL, Jiang GM . 2013 .** Modification of photosynthesis and growth responses to elevated CO₂ by ozone in two cultivars of winter wheat with different years of release. *Journal of Experimental Botany* **64** : 1485–1496.
- Bloom AJ, Chapin FS, Mooney HA . 1985 .** Resource Limitation in Plants-An Economic Analogy. *Annual Review of Ecology and Systematics* **16** : 363–392.
- Böhnisch A, Mittermeier M, Leduc M, Ludwig R . 2021 .** Hot Spots and Climate Trends of Meteorological Droughts in Europe—Assessing the Percent of Normal Index in a Single-Model Initial-Condition Large Ensemble. *Frontiers in Water* **3** .
- Bottley A . 2014 .** Epigenetic variation amongst polyploidy crop species. In: Alvarez-Venegas R, De la Pena C, Casas-Mollano JA, eds. Epigenetics in plants of agronomic importance: Fundamentals and applications. Heidelberg: Springer, 33–47.
- Bourgault M, Dreccer MF, James AT, Chapman SC . 2013 .** Genotypic variability in the response to elevated CO₂ of wheat lines differing in adaptive traits. *Functional plant biology: FPB***40** : 172–184.
- Bowes G . 1993 .** Facing the Inevitable: Plants and Increasing Atmospheric CO₂. *Annual Review of Plant Physiology and Plant Molecular Biology* **44** : 309–332.
- Bradshaw WE, Holzapfel CM . 2006 .** Evolutionary Response to Rapid Climate Change. *Science* **312** : 1477–1478.
- Bruce TJA, Matthes MC, Napier JA, Pickett JA . 2007 .** Stressful “memories” of plants: Evidence and possible mechanisms. *Plant Science* **173** : 603–608.
- Buckley TN . 2005 .** The control of stomata by water balance. *New Phytologist* **168** : 275–292.
- Buckley TN . 2016 .** Stomatal responses to humidity: has the ‘black box’ finally been opened?: Stomatal responses to humidity. *Plant, Cell & Environment* **39** : 482–484.
- Bunce JA . 1995 .** The effect of carbon dioxide concentration on respiration of growing and mature soybean leaves. *Plant, Cell & Environment* **18** : 575–581.
- Curtis PS, Wang X . 1998 .** A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia***113** : 299–313.
- Derner JD, Tischler CR, Polley HW, Johnson HB . 2004 .** Intergenerational above- and belowground responses of spring wheat (*Triticum aestivum* L.) to elevated CO₂. *Basic and Applied Ecology***5** : 145–152.
- Duarte-Aké F, Us-Camas R, Cancino-García VJ, De-la-Peña C .2019 .** Epigenetic changes and photosynthetic plasticity in response to environment. *Environmental and Experimental Botany***159** : 108–120.
- Duursma RA . 2015 .** Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data. *PLOS ONE***10** : e0143346.
- Fangmeier A, Torres-Toledo V, Franzaring J, Damsohn W .2016 .** Design and performance of a new FACE (free air carbon dioxide enrichment) system for crop and short vegetation exposure. *Environmental and Experimental Botany* **130** : 151–161.
- Farooq M, Hussain M, Wahid A, Siddique KHM . 2012 .** Drought Stress in Plants: An Overview. In: Aroca R, ed. Plant Responses to Drought Stress: From Morphological to Molecular Features. Berlin, Heidelberg: Springer, 1–33.

- Farquhar GD, von Caemmerer S, Berry JA . 1980 .** A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149** : 78–90.
- Franks PJ, Beerling DJ . 2009 .** CO₂-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic. *Geobiology* **7** : 227–236.
- Frenck G, van der Linden L, Mikkelsen TN, Brix H, Jørgensen RB .2013 .** Response to multi-generational selection under elevated [CO₂] in two temperature regimes suggests enhanced carbon assimilation and increased reproductive output in *Brassica napus* L. *Ecology and Evolution* **3** : 1163–1172.
- Grote U, Fasse A, Nguyen TT, Erenstein O . 2021 .** Food Security and the Dynamics of Wheat and Maize Value Chains in Africa and Asia. *Frontiers in Sustainable Food Systems* **4** .
- Hamann E, Denney D, Day S, Lombardi E, Jameel MI, MacTavish R, Anderson JT . 2021 .** Review: Plant eco-evolutionary responses to climate change: Emerging directions. *Plant Science* **304** : 110737.
- Haworth M, Elliott-Kingston C, McElwain JC . 2011 .** Stomatal control as a driver of plant evolution. *Journal of Experimental Botany* **62** : 2419–2423.
- Hetherington AM, Woodward FI . 2003 .** The role of stomata in sensing and driving environmental change. *Nature* **424** : 901–908.
- Hoffmann AA, Sgrò CM . 2011 .** Climate change and evolutionary adaptation. *Nature* **470** : 479–485.
- Högy P, Fangmeier A . 2008 .** Effects of elevated atmospheric CO₂ on grain quality of wheat. *Journal of Cereal Science* **48** : 580–591.
- Högy P, Wieser H, Köhler P, Schwadorf K, Breuer J, Franzaring J, Muntifering R, Fangmeier A . 2009 .** Effects of elevated CO₂ on grain yield and quality of wheat: results from a 3-year free-air CO₂ enrichment experiment. *Plant Biology* **11** : 60–69.
- House M, Lukens L . 2014 .** The role of geminally inherited epialleles in plant breeding. In: Alvarez-Venegas R, De la Pena C, Casas-Mollano JA, eds. Epigenetics in plants of agronomic importance: Fundamentals and applications. Heidelberg: Springer, 1–13.
- Huxman TE, Charlet TN, Grant C, Smith SD . 2001 .** The effects of parental CO₂ and offspring nutrient environment on initial growth and photosynthesis in an annual grass. *International Journal of Plant Sciences* **162** : 617–623.
- Huxman TE, Hamerlynck EP, Jordan DN, Salsman KJ, Smith SD .1998a .** The effects of parental CO₂ environment on seed quality and subsequent seedling performance in *Bromus rubens*. *Oecologia* **114** : 202–208.
- Huxman TE, Hamerlynck EP, Moore BD, Smith SD, Jordan DN, Zitzer SF, Nowak RS, Coleman JS, Seemann JR . 1998b .** Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO₂: interaction with drought under glasshouse and field (FACE) exposure. *Plant, Cell & Environment* **21** : 1153–1161.
- Irigoyen JJ, Goicoechea N, Antolín MC, Pascual I, Sánchez-Díaz M, Aguirreolea J, Morales F . 2014 .** Growth, photosynthetic acclimation and yield quality in legumes under climate change simulations: An updated survey. *Plant Science* **226** : 22–29.
- Jablonski LM, Wang X, Curtis PS . 2002 .** Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytologist* **156** : 9–26.
- Johnston A, Reekie E . 2008 .** Regardless of Whether Rising Atmospheric Carbon Dioxide Levels Increase Air Temperature, Flowering Phenology Will Be Affected. *International Journal of Plant Sciences* **169** : 1210–1218.

- Kaur V, Singh S, Behl RK . 2016 .** Heat and Drought Tolerance in Wheat: Integration of Physiological and Genetic Platforms for Better Performance Under Stress. *Ekin Journal of Crop Breeding and Genetics* **2** : 1–14.
- Körner C . 2006 .** Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist* **172** : 393–411.
- Körner C, J.a S, H B . 1979 .** Maximum Leaf Diffusive Conductance in Vascular Plants. *Photosynthetica* **13** : 45–82.
- Kuzentsova A, Brockhoff PB, Christensen RHB . 2017 .** “lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82** : 1–26.
- Latzel V, Rendina González AP, Rosenthal J . 2016 .** Epigenetic Memory as a Basis for Intelligent Behavior in Clonal Plants. *Frontiers in Plant Science* **7** .
- Lau JA, Peiffer J, Reich PB, Tiffin P . 2008 .** Transgenerational effects of global environmental change: long-term CO₂ and nitrogen treatments influence offspring growth response to elevated CO₂. *Oecologia* **158** : 141.
- Leuning R . 1997 .** Scaling to a common temperature improves the correlation between the photosynthesis parameters J_{max} and V_{cmax}.
- Li Y, Li X, Yu J, Liu F . 2017 .** Effect of the transgenerational exposure to elevated CO₂ on the drought response of winter wheat: Stomatal control and water use efficiency. *Environmental and Experimental Botany* **136** : 78–84.
- Li P, Ma B, Palta JA, Ding T, Cheng Z, Lv G, Xiong Y .2021 .** Wheat breeding highlights drought tolerance while ignores the advantages of drought avoidance: A meta-analysis. *European Journal of Agronomy* **122** : 126196.
- Li X, Ulfat A, Lv Z, Fang L, Jiang D, Liu F . 2019 .** Effect of multigenerational exposure to elevated atmospheric CO₂ concentration on grain quality in wheat. *Environmental and Experimental Botany* **157** : 310–319.
- Liu F, Jensen CR, Shahanzari A, Andersen MN, Jacobsen S-E .2005 .** ABA regulated stomatal control and photosynthetic water use efficiency of potato (*Solanum tuberosum* L.) during progressive soil drying. *Plant Science* **168** : 831–836.
- Liu X, Zhang H, Wang J, Wu X, Ma S, Xu Z, Zhou T, Xu N, Tang X, An B . 2019 .** Increased CO₂ concentrations increasing water use efficiency and improvement PSII function of mulberry seedling leaves under drought stress. *Journal of Plant Interactions* **14** : 213–223.
- Luo Y, Reynolds JF . 1999 .** Validity of Extrapolating Field Co₂ Experiments to Predict Carbon Sequestration in Natural Ecosystems. *Ecology* **80** : 1568–1583.
- McAdam SAM, Brodribb TJ . 2018 .** Mesophyll Cells Are the Main Site of Abscisic Acid Biosynthesis in Water-Stressed Leaves. *Plant Physiology* **177** : 911–917.
- McAdam SAM, Susmilch FC, Brodribb TJ . 2016 .** Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms: Rapid ABA biosynthesis during VPD transition. *Plant, Cell & Environment* **39** : 485–491.
- McCaw BA, Stevenson TJ, Lancaster LT . 2020 .** Epigenetic Responses to Temperature and Climate. *Integrative and Comparative Biology* **60** : 1469–1480.
- Medina S, Vicente R, Amador A, Araus JL . 2016 .** Interactive Effects of Elevated [CO₂] and Water Stress on Physiological Traits and Gene Expression during Vegetative Growth in Four Durum Wheat Genotypes. *Frontiers in Plant Science* **7** .

- Medlyn BE, Badeck F-W, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomäki S, *et al.* 1999 . Effects of elevated [CO₂] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant, Cell & Environment* **22** : 1475–1495.
- Mishra A, Vishwakarma K, Malaviya P, Kumar N, Pavón LR, Shandilya C, Sharma R, Bisht A, Takkar S . 2022 . Chapter 18 - Influence of greenhouse gases on plant epigenomes for food security. In: Thakur IS, Pandey A, Ngo HH, Soccol CR, Larroche C, eds. Biomass, Biofuels, Biochemicals. Elsevier, 421–450.
- Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey ADB, Bloom AJ, Carlisle E, Dietterich LH, Fitzgerald G, Hasegawa T, *et al.* 2014 . Increasing CO₂ threatens human nutrition. *Nature* **510** : 139–142.
- Ng LM, Shanker A, Shanker C . 2016 . Abscisic acid signalling as a target for enhancing drought tolerance. In: Abiotic and biotic stress in plants-recent advances and future perspectives. London, UK: IntechOpen.
- Nowak RS, Ellsworth DS, Smith SD . 2004 . Functional responses of plants to elevated atmospheric CO₂— do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162** : 253–280.
- Pascual J, Canal MJ, Correia B, Escandon M, Hasbun R, Meijon M, Pinto G, Valledor L . 2014 . Can epigenetics help forest plants to adapt to climate change? In: Alvarez-Venegas R, De la Pena C, Casas-Mollano JA, eds. Epigenetics in plants of agricultural importance: Fundamentals and applications. Heidelberg: Springer, 125–147.
- Passioura JB . 1982 . Water in the Soil-Plant-Atmosphere Continuum. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. Encyclopedia of Plant Physiology. Physiological Plant Ecology II: Water Relations and Carbon Assimilation. Berlin, Heidelberg: Springer, 5–33.
- Pereira A . 2016 . Plant Abiotic Stress Challenges from the Changing Environment. *Frontiers in Plant Science* **7** .
- Philip JR . 1966 . Plant Water Relations: Some Physical Aspects. *Annual Review of Plant Physiology* **17** : 245–268.
- Pignon CP, Long SP . 2020 . Retrospective analysis of biochemical limitations to photosynthesis in 49 species: C₄ crops appear still adapted to pre-industrial atmospheric [CO₂]. *Plant, Cell & Environment* **43** : 2606–2622.
- Pinheiro C, Chaves MM . 2011 . Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany* **62** : 869–882.
- Prior SA, Runion GB, Marble SC, Rogers HH, Gilliam CH, Torbert HA . 2011 . A Review of Elevated Atmospheric CO₂ Effects on Plant Growth and Water Relations: Implications for Horticulture. *HortScience* **46** : 158–162.
- R Core Team . 2021 . R: A Language and Environment for Statistical Computing.
- Saban JM, Chapman MA, Taylor G . 2019 . FACE facts hold for multiple generations; Evidence from natural CO₂ springs. *Global Change Biology* **25** : 1–11.
- Sage RF . 1994 . Acclimation of photosynthesis to increasing atmospheric CO₂: The gas exchange perspective. *Photosynthesis Research* **39** : 351–368.
- Schneider MK, Lüscher A, Richter M, Aeschlimann U, Hartwig UA, Blum H, Frossard E, Nösberger J . 2004 . Ten years of free-air CO₂ enrichment altered the mobilization of N from soil in Lolium perenne L. swards. *Global Change Biology* **10** : 1377–1388.

- Schweiger AH . 2017 .** The complex adaptive character of spring fens as model ecosystems. *Frontiers of Biogeography* **9** .
- Schweiger AH, Boulangeat I, Conradi T, Davis M, Svenning J-C .2019 .** The importance of ecological memory for trophic rewilding as an ecosystem restoration approach. *Biological Reviews* **94** : 1–15.
- Shoo LP, Hoffmann AA, Garnett S, Pressey RL, Williams YM, Taylor M, Falconi L, Yates CJ, Scott JK, Alagador D, et al.2013 .** Making decisions to conserve species under climate change. *Climatic Change* **119** : 239–246.
- Sniderhan AE, McNickle GG, Baltzer JL . 2018 .** Assessing local adaptation vs. plasticity under different resource conditions in seedlings of a dominant boreal tree species. *AOB PLANTS* **10** : ply004.
- Springer CJ, Ward JK . 2007 .** Flowering time and elevated atmospheric CO₂. *New Phytologist* **176** : 243–255.
- Stiling P, Moon D, Rossi A, Forkner R, Hungate BA, Day FP, Schroeder RE, Drake B . 2013 .** Direct and legacy effects of long-term elevated CO₂ on fine root growth and plant–insect interactions. *New Phytologist* **200** : 788–795.
- Tardieu F, Davies WJ . 1993 .** Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. *Plant, Cell & Environment* **16** : 341–349.
- Trenberth KE, Dai A, van der Schrier G, Jones PD, Barichivich J, Briffa KR, Sheffield J . 2014 .** Global warming and changes in drought. *Nature Climate Change* **4** : 17–22.
- Varga B, Vida G, Varga-László E, Hoffmann B, Veisz O .2017 .** Combined Effect of Drought Stress and Elevated Atmospheric CO₂ Concentration on the Yield Parameters and Water Use Properties of Winter Wheat (*Triticum aestivum* L.) Genotypes. *Journal of Agronomy and Crop Science* **203** : 192–205.
- Walter J, Harter DEV, Beierkuhnlein C, Jentsch A .2016 .** Transgenerational effects of extreme weather: perennial plant offspring show modified germination, growth and stoichiometry. *Journal of Ecology* **104** : 1032–1040.
- Walter J, Jentsch A, Beierkuhnlein C, Kreyling J .2013 .** Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. *Environmental and Experimental Botany* **94** : 3–8.
- Walter J, Nagy L, Hein R, Rascher U, Beierkuhnlein C, Willner E, Jentsch A . 2011 .** Do plants remember drought? Hints towards a drought-memory in grasses. *Environmental and Experimental Botany* **71** : 34–40.
- Wang S, Zhang Y, Ju W, JingM.Chen, Ciais P, Cescatti A, Sardans J, Janssens I, Wu M, Berry J, et al. 2020 .** Recent global decline of CO₂ fertilization effects on vegetation photosynthesis. *Science* **370** : 1295–1300.
- Warner RR, Chesson PL . 1985 .** Coexistence mediated by recruitment fluctuations: A field guide to the storage effect. *The American Naturalist* **125** : 769–787.
- Wood SN . 2004 .** Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association* **99** : 673–686.
- Woodward FI . 1998 .** Do plants really need stomata? *Journal of Experimental Botany* **49** : 471–480.
- Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Shikari AB, Salgotra RK . 2017 .** Impact of drought on photosynthesis: Molecular perspective. *Plant Gene* **11** : 154–159.
- Zimmerman KD, Espeland MA, Langefeld CD . 2021 .** A practical solution to pseudoreplication bias in single-cell studies. *Nature Communications* **12** : 738.