

Impact of warmer and drier conditions on tree photosynthetic properties and the role of species interactions

Margaux Didion-Gency¹ , Arthur Gessler^{1,2} , Nina Buchmann³ , Jonas Gisler¹, Marcus Schaub¹  and Charlotte Grossiord^{4,5} 

¹Forest Dynamics Research Unit, Swiss Federal Institute for Forest, Snow and Landscape WSL, CH-8903 Birmensdorf, Switzerland; ²Institute of Terrestrial Ecosystems, ETH Zurich, CH-8092 Zurich, Switzerland; ³Institute of Agricultural Sciences, ETH Zurich, CH-8092 Zurich, Switzerland; ⁴Plant Ecology Research Laboratory PERL, School of Architecture, Civil and Environmental Engineering, EPFL, CH-1015 Lausanne, Switzerland; ⁵Community Ecology Unit, Swiss Federal Institute for Forest, Snow and Landscape WSL, CH-1015 Lausanne, Switzerland

Summary

Author for correspondence:
Margaux Didion-Gency
Email: margaux.didion@wsl.ch

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- Increased temperature and prolonged soil moisture reduction have distinct impacts on tree photosynthetic properties. Yet, our knowledge of their combined effect is limited. Moreover, how species interactions alter photosynthetic responses to warming and drought remains unclear.
- Using mesocosms, we studied how photosynthetic properties of European beech and downy oak were impacted by multi-year warming and soil moisture reduction alone or combined, and how species interactions (intra- vs inter-specific interactions) modulated these effects.
- Warming of +5°C enhanced photosynthetic properties in oak but not beech, while moisture reduction decreased them in both species. Combined warming and moisture reduction reduced photosynthetic properties for both species, but no exacerbated effects were observed. Oak was less impacted by combined warming and limited moisture when interacting with beech than in intra-specific stands. For beech, species interactions had no impact on the photosynthetic responses to warming and moisture reduction, alone or combined.
- Warming had either no or beneficial effects on the photosynthetic properties, while moisture reduction and their combined effects strongly reduced photosynthetic responses. However, inter-specific interactions mitigated the adverse impacts of combined warming and drought in oak, thereby highlighting the need to deepen our understanding of the role of species interactions under climate change.

Introduction

Global warming and more frequent and extreme droughts are critical environmental drivers for forest dynamics because of their impacts on atmospheric carbon dioxide (CO₂) acquisition through photosynthesis and tree carbon dynamics (Ciais *et al.*, 2005; Rödenbeck *et al.*, 2020). However, although nearly all studies caution that higher temperatures and droughts are increasingly co-occurring (Overpeck, 2013; Sun *et al.*, 2019), little is known about their combined impacts on tree photosynthetic properties.

Exposure to elevated air temperature but sufficient soil moisture supply should increase leaf-level photosynthetic properties in the long term because of enhanced photochemical reactions, as long as temperatures do not exceed the photosynthesis optimal temperature (Dreyer *et al.*, 2001; Way *et al.*, 2015; Dusenge *et al.*, 2019). Hence, warming can translate into higher light-saturated assimilation (A_{sat}) (e.g. Prieto *et al.*, 2009) and more

efficient leaf-level photochemistry. For instance, warming has been associated with higher maximum rate of carboxylation by Rubisco ($V_{c\max}$) and maximum photosynthetic electron transport rate (J_{\max}), especially when plants are limited by cold temperatures (e.g. Way & Oren, 2010). However, once the optimal temperature threshold of photosynthesis is exceeded, exposure to heat stress could cause the separation of the light-harvesting complexes from the photosystem II (PSII) reaction center or the denaturation of proteins (Gounaris *et al.*, 1984; Yamane *et al.*, 1997). The consequences are reduced A_{sat} , $V_{c\max}$, and J_{\max} with high temperatures (Medlyn *et al.*, 2002; Dai *et al.*, 2021; Dusenge *et al.*, 2021), a reduction of the maximum photochemical efficiency (F_v/F_m) and decreasing chlorophyll (Chl) concentrations. Variation in these responses largely depends on the species' temperature range. Húdoková *et al.* (2022) observed that heat waves of +5°C above daily average during the summer reduce A_{sat} , PSII, and increase the degradation of Chl in temperate European beech trees (*Fagus sylvatica* L.), while sessile oak, a

Mediterranean tree species growing in warmer and drier environments, showed no effect. Thus, species adapted to higher temperature may better resist future conditions, while more sensitive ones may be seriously threatened ecologically.

Low soil moisture can impact the leaf-level photosynthesis through stomatal regulation and nonstomatal processes (Flexas & Medrano, 2002). Reduced moisture decreases stomatal conductance, and thus leaf photosynthetic CO₂ assimilation due to a reduction of CO₂ diffusion and thus lower intracellular CO₂ concentration in the leaf (C_i) (Gallé & Feller, 2007; Zhou *et al.*, 2014), leading to a possible photo-damage to PSII (Powles, 1984; Epron & Dreyer, 1993). Independently of stomatal closure, biochemical processes will be affected during prolonged soil moisture reduction, thereby limiting leaf-level photosynthesis properties via a downregulation of Rubisco activity and content (Parry, 2002). Hence, impacts of low soil moisture could include a reduction of A_{sat} , V_{cmax} , J_{max} , and F_v/F_m (Zhou *et al.*, 2014; Santos *et al.*, 2018). For instance, Arend *et al.* (2013, 2016) showed reduced A_{sat} and impaired PSII photochemistry in drought-exposed beech and downy oak trees (*Quercus pubescens* Willd.), including stronger impacts in beech because of its higher vulnerability to low moisture. Indeed, beech is considered rather isohydric compared to oak as this species will close its stomates at less negative leaf water potentials (Klein, 2014) and is generally assumed to be negatively affected by drought (Gessler *et al.*, 2006).

How species respond to the combined effects of rising temperature and low soil moisture depends on the species tolerance, the range of environmental conditions, and how extreme temperature and precipitation changes are. Some studies have reported strong exacerbated effects of combined warming and high soil moisture stress (Contran *et al.*, 2013; Arend *et al.*, 2016). However, combined effects are not always occurring as plants can acclimate leaf-level photosynthetic properties in response to the environment (e.g. Arend *et al.*, 2011). For example, Grossiord *et al.* (2017) observed that piñon pine and juniper trees growing in semi-arid environments where drought is a recurrent stress have stronger leaf-level responses to soil moisture than to warming with no exacerbation of their combined effects. Moreover, prolonged exposure to warming and low soil water availability can lead to whole-plant structural acclimation (i.e. shift in biomass allocation), allowing plants to maintain leaf-level gas exchange under more stressful conditions (Thomas, 2000; Bréda *et al.*, 2006; Schönbeck *et al.*, 2022). Thus, drought and warming can have distinct or similar effects on the photosynthetic properties depending on species-specific responses to temperature and soil moisture, making projections about their combined effect a big challenge for plant ecologists and land managers (Mittler, 2006; Rennenberg *et al.*, 2006).

In addition to the direct impacts of chronic warming and reduced soil water on individual trees, plant performance in natural ecosystems is driven by the dynamics of the whole forest, including the interactions between trees (Grossiord, 2019). Depending on the species identity, climate, and site conditions, these interactions can have either positive, negative, or no effects on plant functioning (Hooper *et al.*, 2005; Grossiord

et al., 2014a,b,c; Jucker *et al.*, 2016). More specifically, interactions between tree species with complementary structural (e.g. different rooting depths and canopy heights) and functional traits (e.g. phenology, hydraulic traits) have often been associated with mitigating effects during extreme events like droughts (e.g. Grossiord *et al.*, 2014a,b,c; Anderegg *et al.*, 2018; Grossiord, 2019). The potential drivers of such positive effects are associated with niche complementarity and the local environmental conditions created by tree neighbors (Paquette & Messier, 2011; Liang *et al.*, 2015). Local neighbors can increase canopy stratification, and thus decrease the risk of light-damage (Mensah *et al.*, 2018; Kothari *et al.*, 2021), potentially leading to higher A_{sat} during warmer and drier climate. Similarly, having a more diverse litter input (i.e. litter provided by numerous species) can increase soil nutrient content (e.g. Zak *et al.*, 2003). Higher soil fertility can enhance tree-level photosynthetic properties (Gessler *et al.*, 2017; Gillespie *et al.*, 2020). However, to our knowledge, no study has experimentally tested how tree species interactions mitigate leaf-level photosynthetic responses to warming and reduced soil moisture acting alone or combined over multiple years.

We, thus, aimed to understand how the interactions of two co-existing, broadly distributed but contrasting tree species, i.e. European beech and downy oak, influence their leaf-level photosynthetic responses to air warming and reduced soil moisture. We exposed young trees in intra- or inter-specific interactions to air warming and reduced soil moisture alone or combined. The tree species were selected because of their distinct functional strategies to deal with warmer and drier climate. European beech is a temperate species growing in moist environments, with mean annual temperatures varying between 2 and 14°C (Durrant *et al.*, 2016), which tolerates shady conditions. Downy oak is growing in direct light in warmer sub-Mediterranean regions (mean annual air temperatures varying between 5 to 17°C; Pasta *et al.*, 2016). Previous work showed that the interaction between beech and oak can lead to belowground water source partitioning (Zapater *et al.*, 2011; Grossiord *et al.*, 2014a,b,c) and higher productivity (Jourdan *et al.*, 2020) at the tree-level, suggesting a mitigating effect of their interaction. However, when environmental conditions become too stressful, an adverse effect of the interaction between these species on leaf-level gas exchange has also been observed (e.g. Didion-Gency *et al.*, 2021).

Our objectives were to (1) determine how warming and reduced soil moisture acting alone or combined influenced the leaf-level photosynthetic properties (i.e. A_{sat} , J_{max} , V_{cmax} , F_v/F_m and Chl) in beech and oak, and (2) assess how the interactions among these two species impact their leaf-level photosynthetic properties to warming and soil moisture reduction. If grown in intra-specific composition, we expect (1) oak to have higher leaf-level photosynthetic properties with warming compared to ambient conditions while beech will have lower leaf-level photosynthetic properties because of its stronger vulnerability to high temperatures. Reduction in soil moisture will decrease the photosynthetic properties of both species but with larger reductions expected in beech. Combined warming and low soil moisture will exacerbate photosynthetic responses observed under reduced soil

moisture acting alone because of enhanced water stress. If grown in inter-specific interactions, we expect (2) warming to enhance the photosynthetic properties of oak and mitigate the reduction in beech compared to those under intra-specific interactions. Tree responses to soil moisture reduction in inter-specific interactions (i.e. acting alone or combined with warming) will be less impacted than in intra-specific interactions.

Materials and Methods

Experimental design

The study was conducted at the Model Ecosystem Facility of the Swiss Federal Research Institute for Forest, Snow and Landscape WSL, Birmensdorf, Switzerland (47°21'48"N, 8°27'23"E, 545 m above sea level (asl)). The experimental set-up consisted of 16 hexagonal glass-walled open-top chambers (OTCs) of 3 m height and 6 m² area (Fig. 1; Schönbeck *et al.* (2021), Grossiord *et al.* (2022)). The OTCs were covered by mobile glass roofs kept closed during the whole experiment to exclude natural precipitation. The glass walls and roofs reduced photosynthetic active radiation (PAR) inside the OTCs by about 50% compared to the outside. Each chamber has a belowground volume of 9 m³ divided with concrete walls into two semicircular lysimeters (1.5 m deep, 2.5 m²). Each of the two lysimeters is separated into four compartments with plexiglass walls, leading to a total of eight soil compartments per OTC, of which 72 were used in this study. In October 2018, the lysimeters were filled with a 1 m deep layer of gravel, allowing fast drainage, and covered with a fleece layer to avoid root proliferation past the soil layer. On top of the fleece layer, each compartment was filled with 50 cm of an artificial forest soil provided by the company Ökohum (Herbertingen, Germany; pH 6.3, 40% quartz sand, 20% white peat, 20% expanded shale, 16% pumice stone, and 4% clay). Soil fertilization was conducted every year in spring using granules (Unikorn I; Hauert, Grossaffoltern, Switzerland) with an amount of 30 g per compartment (20% potash, 14% nitrogen, 12% sulfur, 4% phosphate, 3% magnesium). Air temperature and humidity at 50 cm and 2 m above the ground were monitored inside each OTC every 10 s, and an average value of 10 min was stored (Atmos 14; Meter Group Inc., Pullman, WA, USA). Soil temperature and moisture were monitored at 25 cm depth every minute, and an average value of 10 min was stored (5TM; Decagon Devices, Pullman, WA, USA). To obtain homogeneous irrigation, six sprinklers (10 cm high) per lysimeter were equally distributed among the four compartments. Compartments were irrigated automatically every 2 d from April to November, and manually twice a month from December to March to avoid frost damage to the pipes. Leaf litter was left in each compartment during the experiment.

In October 2018, 288 trees of 3-yr-old seedlings of European beech (64 ± 12 cm height) and Mediterranean downy oak (63 ± 11 cm height) were planted in two species interaction treatments: intra-specific interactions (four trees of the same species per compartment, $n = 4$ trees) and inter-specific interactions (two trees from each species per compartment, $n = 4$ trees).

In June 2019, after the first campaign of measurements (see detailed list later), a fully crossed combination of two air temperature and soil moisture regimes was started. This resulted in four climatic treatments: a control with ambient air temperature and irrigation at field capacity (C), a warming treatment with an increase of air temperature of around 5°C (i.e. +4.98°C ± 0.6°C) above ambient temperature of the control (W), a soil moisture reduction treatment with a reduction of soil moisture of around 50% (i.e. -46.23% ± 9.9%) relative to the control (MR), and a combined warming + soil moisture reduction treatment where combined warming and reduced soil moisture were applied simultaneously (WMR) (i.e. +4.81°C ± 0.6°C and -47.4% ± 10.3%; Fig. 2). To maintain -50% soil moisture in the MR and WMR treatments, irrigation levels were adjusted throughout the year in each treatment. Each combination of climatic treatment ($n = 4$), species ($n = 2$) and species interactions ($n = 2$) was repeated six times. One tree per species was randomly selected for the measurements in each compartment per OTC, leading to 96 trees in total ($n = 6$ trees per species, climatic treatment, and species interactions). While the objectives of this study was not to assess and predict plant responses under real-world conditions but to better understand the physiological mechanisms under prolonged heat and drought stress, an increase in air temperature of about 5°C and a decrease of 50% in irrigation was selected at our facility to match values observed during extreme events in the study region (e.g. the hot droughts of 2003 and 2018; Rebetez *et al.* (2006); Schuldt *et al.* (2020)).

Five physiological traits related to the photosynthetic properties were measured on all selected trees three times during the growing season (i.e. early, middle and late growing season) for 3 yr (i.e. from 2019 to 2021).

Physiological measurements

Foliar gas exchange Measurements of the light-saturated assimilation (A_{sat}), maximum carboxylation rate (V_{cmax}), and the maximum photosynthetic electron transport rate (J_{max}) were carried out in the form of CO₂ response curves (A/C_i curves). Measurements were done using a Li-Cor 6800 infrared gas analyzer system (Li-Cor, Lincoln, NE, USA), except in September 2020, where a Li-Cor 6400 infrared gas analyzer system was used. One mature and fully sun-exposed leaf from the top third of the canopy was selected from each tree. The measurements were carried out between 09:00 and 16:00 h when the trees were the most active, excluding periods of midday stomatal closure if they occurred (mostly between 00:00 and 14:00 h in the middle of the growing season). The measurements were conducted with a reference CO₂ concentration in sequence steps of 400, 300, 200, 100, 50, 0, 400, 400, 600, 800, 1000, 1200, 1500, 2000 ppm, with the following settings: 1500 μmol m⁻² s⁻¹ light-saturating photosynthetic photon flux density, block temperature approximately. Matching mean daytime (between 09:00 and 16:00 h) ambient air temperature during each campaign (i.e. 20°C: early 2019, 2020, middle 2021, late 2019, 2020, 2021; 25°C: early 2021, middle 2019, 2020) with an increase of +5°C in W and WMR compared to the control, except for the first campaign



Fig. 1 (a) Aerial pictures of the 16 open-top chambers with the four treatments: control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) ($n = 4$ chambers per treatment). (b) Picture showing the side of the open-top chambers during the measurement campaign of September 2021. (c) Aerial picture from a control chamber including a central fan and eight compartments with different species interactions between beech and oak trees. (d) Aerial picture from a warming + soil moisture reduction chamber including a central heating system and eight compartments with different species interactions between beech and oak trees.

when the treatments were not applied yet. Mean daytime conditions over the full 5–7 d campaigns were selected instead of instantaneous air temperature during the measurements to compare results among treatments within each campaign without including temperature effects driven by daytime variability. Relative air humidity (RH) was set at 50% to reflect ambient conditions, except for 2019, when the vapor pressure deficit (VPD) was set to 1.5 kPa. In summer 2020, we performed consecutive A_{sat} measurements on a subsample of trees in all climatic treatments to evaluate the impact of changing RH vs VPD in the LI-6800: RH was either set at 50%, or VPD at 1.5 kPa on the same trees. We found no significant differences in A_{sat} (data not shown). Thus, we assume that the setting differences among 2019, 2020 and 2021 had no impact on our measurements. Leaf temperature was estimated using the instrument's energy balance calculations. Measurements were recorded after steady-state gas exchange rates had been maintained for at least 5 min. After each measurement, the

part of the leaf enclosed in the cuvette was harvested if it was smaller than the chamber (i.e. 6 cm² in 2019 and 2020, and 2 cm² in 2021), and the projected leaf area, which was used to correct recorded values, was measured using a flatbed scanner (Epson Perfection V800 Photo; Epson, Amsterdam, the Netherlands).

A_{sat} was extracted from the first point of the A/C_i curves at 400 ppm of CO₂ concentration. $V_{\text{c,max}}$ and J_{max} were determined by fitting the A/C_i curves using the Farquhar model for photosynthesis (Sharkey *et al.*, 2007) and the PLANTECOPHYS package (Duursma, 2015). Before fitting and after removing obvious outliers, curves were selected if they reached at least 600 ppm of C_i and had a minimum of five data points. The default fitting methods of the PLANTECOPHYS package were used to determine $V_{\text{c,max}}$ and J_{max} . The model used the temperature conditions applied in the cuvette. Nonfitting curves were removed from the data, leading to a total of 787 out of the 864 measured curves over the 3 yr with an average of 12 points per curve.

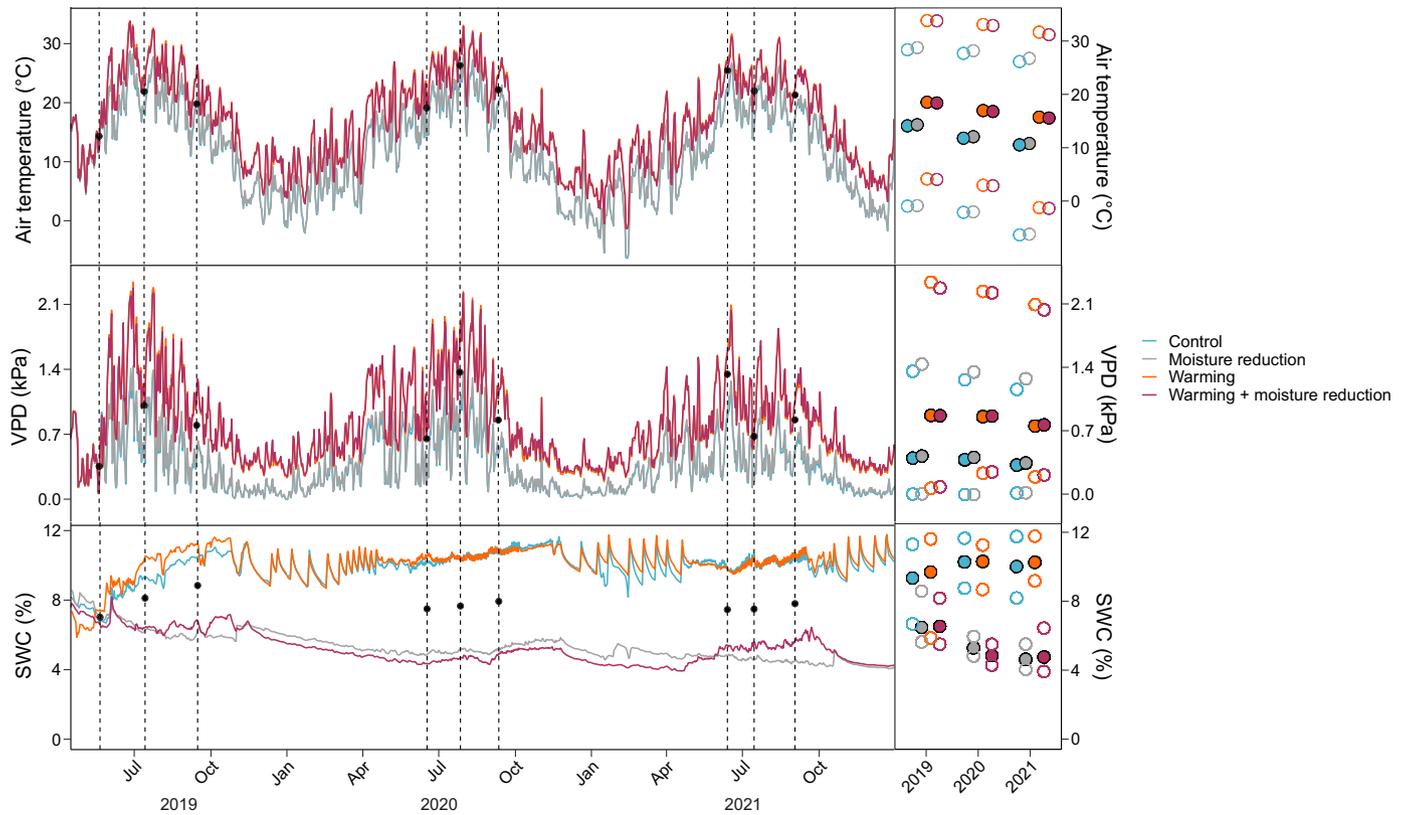


Fig. 2 Air temperature, vapor pressure deficit (VPD), and soil water content (SWC) under control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) measured in the open-top chambers. Solid lines show the daily averages ($n = 4$ chambers per treatment), dashed vertical lines indicate the measurements' campaigns, black filled dots stand for the averages during the campaigns (i.e. average of all 16 open-top chambers), color-filled dots show the yearly averages (mean \pm SE), and open color dots show the daily maximum and minimum over the entire year in each climatic treatment.

Dark-adapted fluorescence Directly after the A/C_i measurements, the maximum photochemical efficiency (F_v/F_m) was measured on an adjacent leaf using the LI-COR 6800 infrared gas analyzer system. Leaves were maintained in darkness using aluminum foil for at least 30 min before the measurements. Parameter settings were the same as for the A/C_i curves described earlier, except for the CO_2 concentration, which was kept at 400 ppm. The initial fluorescence (F_0) and maximum fluorescence value (F_m) were measured using a saturating pulse of bright red light ($8000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ PAR, 1000 ms pulse width). The variable fluorescence (F_v) was calculated by subtracting F_0 from F_m .

Chlorophyll concentration Chlorophyll concentration (Chl, in $\mu\text{mol m}^{-2}$) was measured on the same leaves as those used for A/C_i measurements with a chlorophyll concentration meter (MC-100; Apogee Instruments Inc., Logan, UT, USA).

Statistical analysis

The response of A_{sat} , $V_{C_{\text{max}}}$, J_{max} , F_v/F_m , and Chl to the climatic treatments and species interactions was determined through linear mixed-effects models for each species. The interactive effects of warming (yes/no), soil moisture reduction (yes/no), year (2019, 2020, 2021), and growing season stages (early, middle,

late) were used as fixed effects. The test was performed first only on trees in intra-specific interactions, and an additional interactive effect of the interaction (intra- vs inter-specific) was added to the fixed effect to determine the impact of species interactions on the full dataset. The individual chambers were treated as a random effect. Tukey type *post hoc* tests were used to reveal significant differences between treatments for each measurement campaign (multcomp function).

To determine the impact of air temperature and soil moisture reduction on photosynthetic responses, the relationships between A_{sat} , $V_{C_{\text{max}}}$, J_{max} , F_v/F_m , and Chl in the intra-specific interaction treatment and the daily air temperature or soil moisture content (i.e. during the same day as the measurements) were determined through Pearson's correlation test (cor.test function) across all climatic treatments and for each species.

All analyses were performed using the R v.3.6.1 statistical platform (R Development Core Team, Vienna, Austria, 2019).

Results

Warming and soil moisture reduction impacts in intra-specific treatments

We found no effect of warming (W) on most photosynthetic properties of beech, besides that W significantly reduce the light-

saturated assimilation (A_{sat}) in the last year of measurements and increased the maximum carboxylation rate ($V_{\text{C}_{\text{max}}}$), but only at one time in the middle of the growing season in 2019 (Supporting Information Table S1; Fig. 3). For oak, W had a positive impact on photosynthetic properties, resulting in increased $V_{\text{C}_{\text{max}}}$ and maximum photosynthetic electron transport rate (J_{max}) in 2020 (Table S2; Fig. 3).

We found a significant reduction of A_{sat} , $V_{\text{C}_{\text{max}}}$, J_{max} and the chlorophyll concentration (Chl) in 2021 for beech in response to soil moisture reduction (MR). We also observed a significant decrease in the maximum photochemical efficiency (F_v/F_m) in 2020 for beech (Table S1; Fig. 3). For oak, we found a significant decline of A_{sat} in 2020 and 2021, $V_{\text{C}_{\text{max}}}$ and J_{max} in 2020, F_v/F_m in 2019, and Chl in 2019 and 2020 (Table S2; Fig. 3) in response to MR.

Under combined warming and soil moisture reduction (WMR), we found similar reduction in A_{sat} compared to trees exposed to MR alone for beech (in 2020 and 2021). We also observed a significant decrease in $V_{\text{C}_{\text{max}}}$ and J_{max} in 2019 and 2021, F_v/F_m in 2021, and Chl in 2020 depending on the campaign for beech (Table S1; Fig. 3). For oak, WMR had similar impacts as MR only. Indeed, we found a reduction of A_{sat} and

J_{max} in 2020 and 2021, and Chl in 2020 in response to combined impacts (Table S2; Fig. 3) but no exacerbated effects were found.

Responses of photosynthetic properties to air temperature and soil water content in intra-specific treatments

With an increase in daily air temperature, beech had significantly lower F_v/F_m ($r^2 = 0.21$ and $P = 0.002$) and Chl ($r^2 = 0.30$ and $P < 0.001$) (Fig. 4). For beech, no other relationship was found between the photosynthetic properties and daily air temperature. Oak showed an increase in A_{sat} ($r^2 = 0.16$ and $P = 0.016$), $V_{\text{C}_{\text{max}}}$ ($r^2 = 0.36$ and $P < 0.001$), and F_v/F_m ($r^2 = 0.20$ and $P = 0.003$) when daily air temperatures increased (Fig. 4). No relationship between Chl and the daily air temperature was found for oak.

With a decrease in daily soil water content, beech had a significantly lower A_{sat} ($r^2 = 0.43$ and $P < 0.001$), $V_{\text{C}_{\text{max}}}$ ($r^2 = 0.25$ and $P < 0.001$), J_{max} ($r^2 = 0.28$ and $P < 0.001$), and F_v/F_m ($r^2 = 0.23$ and $P = 0.009$) (Fig. 5). No relationship between Chl and soil water content was found in beech. Oak showed a significant decrease in all photosynthetic properties, including A_{sat} ($r^2 = 0.50$ and $P < 0.001$), $V_{\text{C}_{\text{max}}}$ ($r^2 = 0.43$ and $P < 0.001$), J_{max} ($r^2 = 0.47$ and $P < 0.001$), F_v/F_m ($r^2 = 0.24$ and $P = 0.003$), and Chl

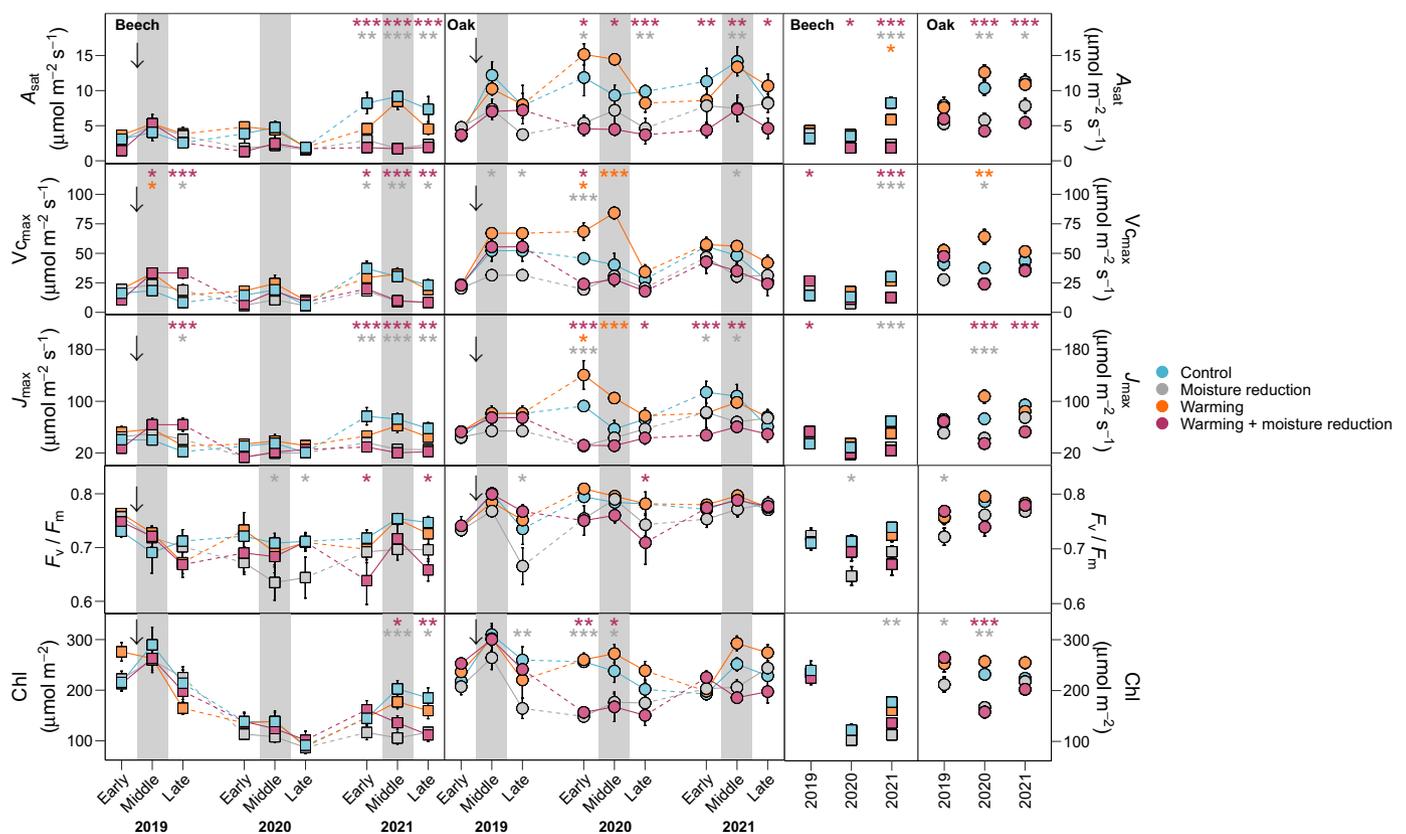


Fig. 3 Light-saturated assimilation (A_{sat}), maximum carboxylation rate ($V_{\text{C}_{\text{max}}}$), maximum photosynthetic electron transport rate (J_{max}), maximum photochemical efficiency (F_v/F_m), and chlorophyll concentration (Chl) for each measurement campaign (i.e. early, middle, and late growing season of 2019, 2020, and 2021, mean \pm SE, $n = 6$ trees) and year (i.e. 2019, 2020, and 2021, mean \pm SE, $n = 18$ trees) for beech (squares) and oak (circles) trees growing under control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) in the intra-specific treatments. The black arrows indicate the onset of the climatic treatment. Significant differences between climatic treatments and the control are highlighted per campaign and species, and per year using a linear mixed-effects models followed by a Tukey type *post hoc* test (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

($r^2 = 0.42$ and $P < 0.001$), when the daily soil water content decreased (Fig. 5).

Warming and soil moisture reduction impacts in intra- vs inter-specific interactions

In W conditions, we found no impact of species interactions on the photosynthetic properties of beech (Table S3; Fig. 6). For oak, trees in inter-specific compositions had a lower J_{\max} compared to trees in intra-specific compositions in early 2020 (Table S4; Fig. 6).

In MR conditions, we found no impact of species interactions on the photosynthetic properties of both species (Tables S3, S4; Fig. 6).

In WMR conditions, we found that species interactions altered the photosynthetic properties of oak but not beech (Tables S3,

S4; Fig. 6). For oak, we found that A_{sat} , V_{Cmax} , J_{\max} , and Chl were consistently higher in inter- vs intra-specific compositions in 2020 and 2021 (only in 2021 for V_{Cmax}), suggesting a mitigating effect of the interaction with beech under the most extreme climatic conditions.

Discussion

Warming and soil moisture reduction impacts in intra-specific composition

As we initially expected, warming had opposite effects depending on the species. Beech leaf-level light-saturated assimilation (A_{sat}) was negatively impacted by a chronic warming of +5°C during the last year of treatment exposure (Figs 3, 4). Biochemical

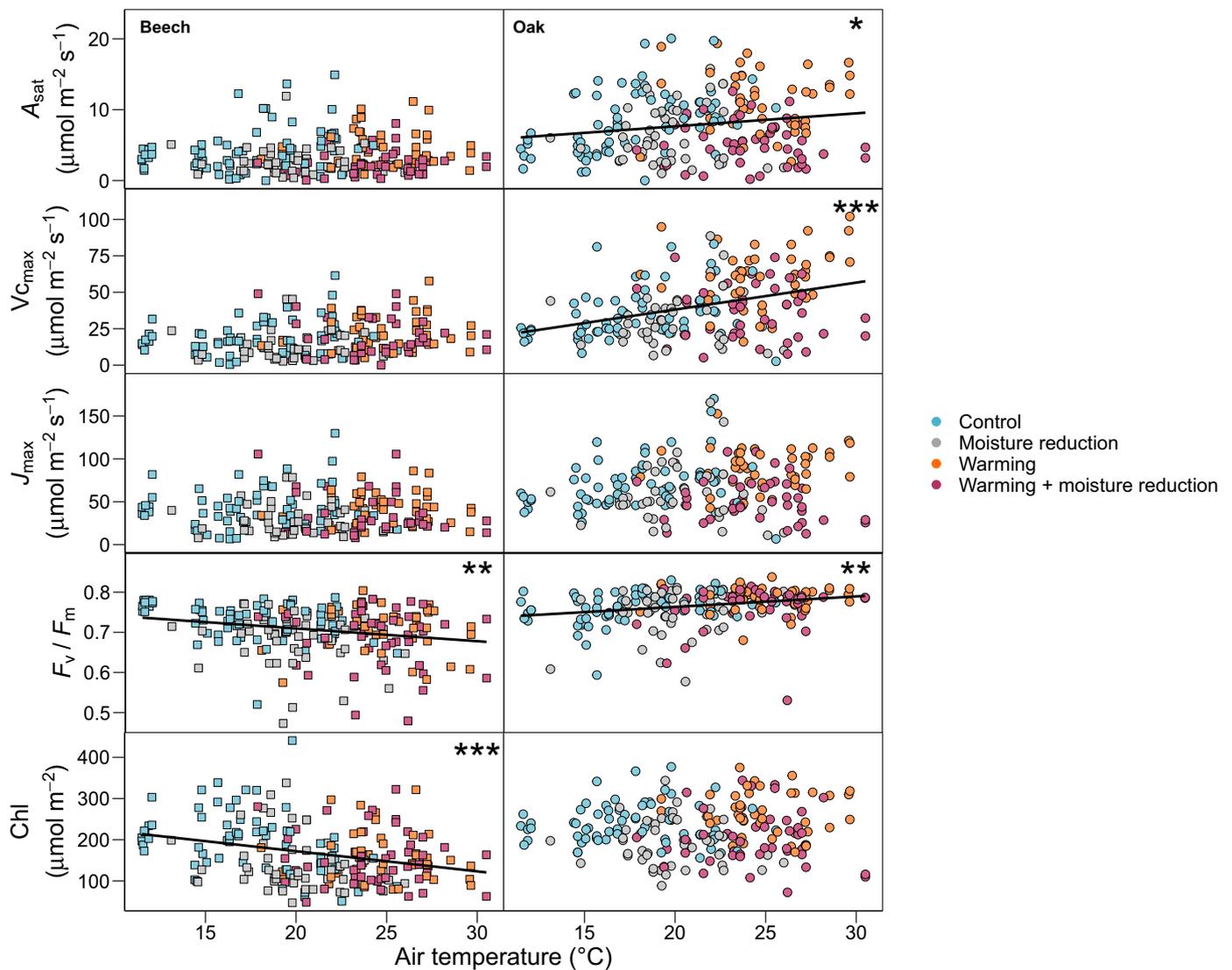


Fig. 4 Relationships between the light-saturated assimilation (A_{sat}), maximum carboxylation rate (V_{Cmax}), maximum photosynthetic electron transport rate (J_{\max}), maximum photochemical efficiency (F_v/F_m), and chlorophyll concentration (Chl) as a function of the daily air temperature (°C) for each measurement for beech (squares) and oak (circles) growing under control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) in the intra-specific treatments. Significant regression lines across all treatments for each species are shown using a Pearson's correlation test (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

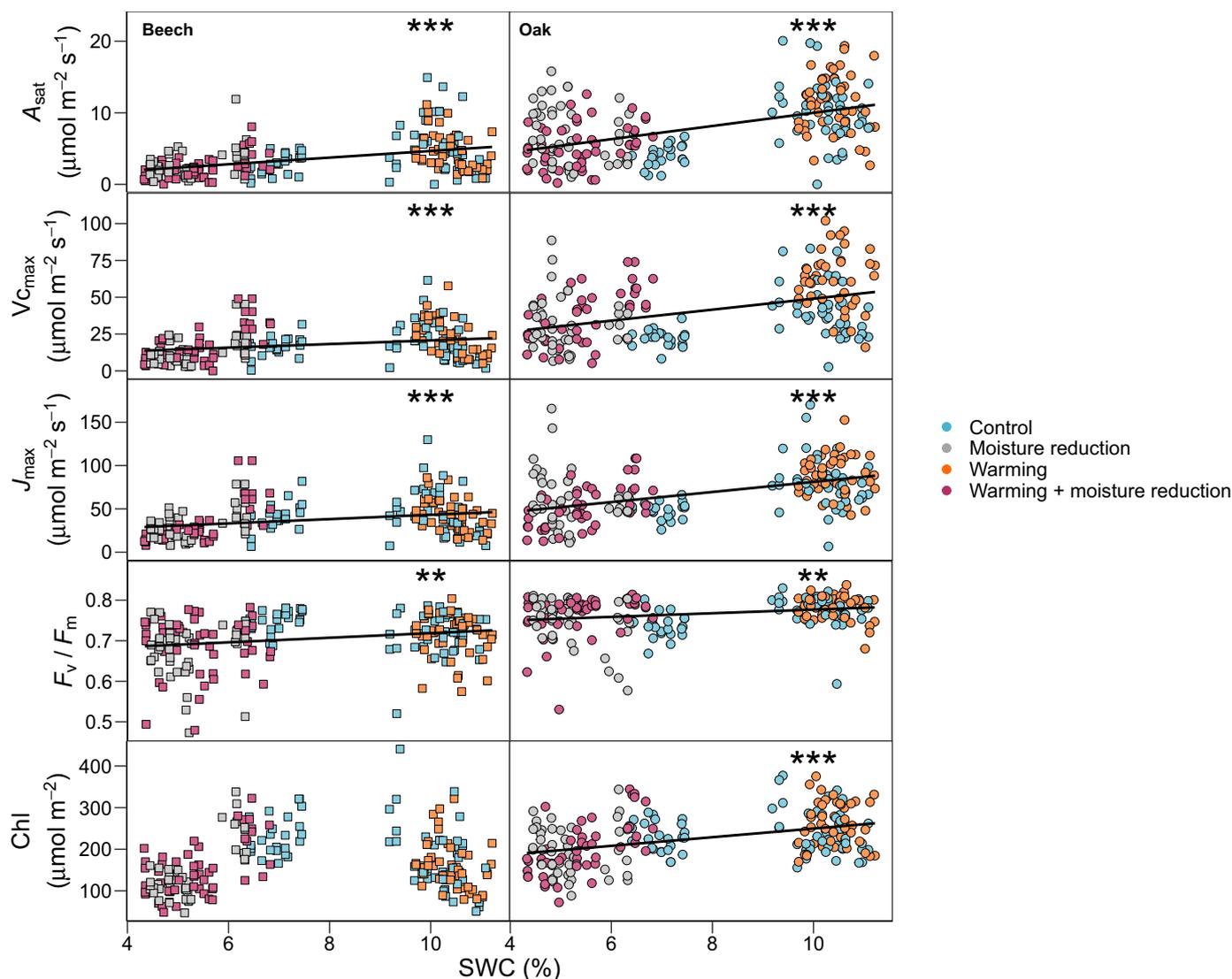


Fig. 5 Relationships between the light-saturated assimilation (A_{sat}), maximum carboxylation rate ($V_{\text{c,max}}$), maximum photosynthetic electron transport rate (J_{max}), maximum photochemical efficiency (F_v/F_m), and chlorophyll concentration (Chl) as a function of the daily soil water content (SWC, %) for each measurement for beech (squares) and oak (circles) growing under control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) in the intra-specific treatments. Significant regression lines across all treatments for each species are shown using a Pearson's correlation test (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

limitations may have partially driven this effect as we observed a negative relationship between maximum photochemical efficiency (F_v/F_m), chlorophyll concentration (Chl) and air temperature (Fig. 4). However, no severe warming impact was observed on other leaf-level photosynthetic properties, suggesting that the response of CO_2 uptake to temperature must be driven primarily by stomatal limitation. Increased air temperature leads to a rise in VPD, which restricts stomatal conductance during warm days (Lendzion & Leuschner, 2008; Ambrose *et al.*, 2016), thereby resulting in a lower assimilation. In our study, VPD frequently reached values above 3 kPa during the day (Fig. 2), especially in the warming treatment, which should lead to important stomatal restrictions (Grossiord *et al.*, 2020). Furthermore, we found that stomatal conductance (g_s) was severely reduced in the warming treatment compared to the control (Fig. S1), supporting our

hypothesis about stomatal limitation. Moreover, Holišová *et al.* (2013) showed that beech has an optimal foliar temperature of about 24.5°C for A_{sat} . We observed a mean air temperature of around 29°C between 09:00 and 16:00 h in the middle of the growing season (i.e. July and August) in the warming treatment, suggesting that heat stress could have contributed to reduced A_{sat} .

On the contrary, oak trees benefited from warming already during the second year, where an increase in the leaf-level maximum carboxylation rate ($V_{\text{c,max}}$) and maximum photosynthetic electron transport rate (J_{max}) was observed (Figs 3, 4). Furthermore, a positive relationship was found between A_{sat} , $V_{\text{c,max}}$, F_v/F_m and air temperature (Fig. 4). However, although these photosynthetic properties increased, enhanced temperature-induced photosynthetic responses did not result in higher A_{sat} in the warming treatment. We also observed that g_s was not reduced by

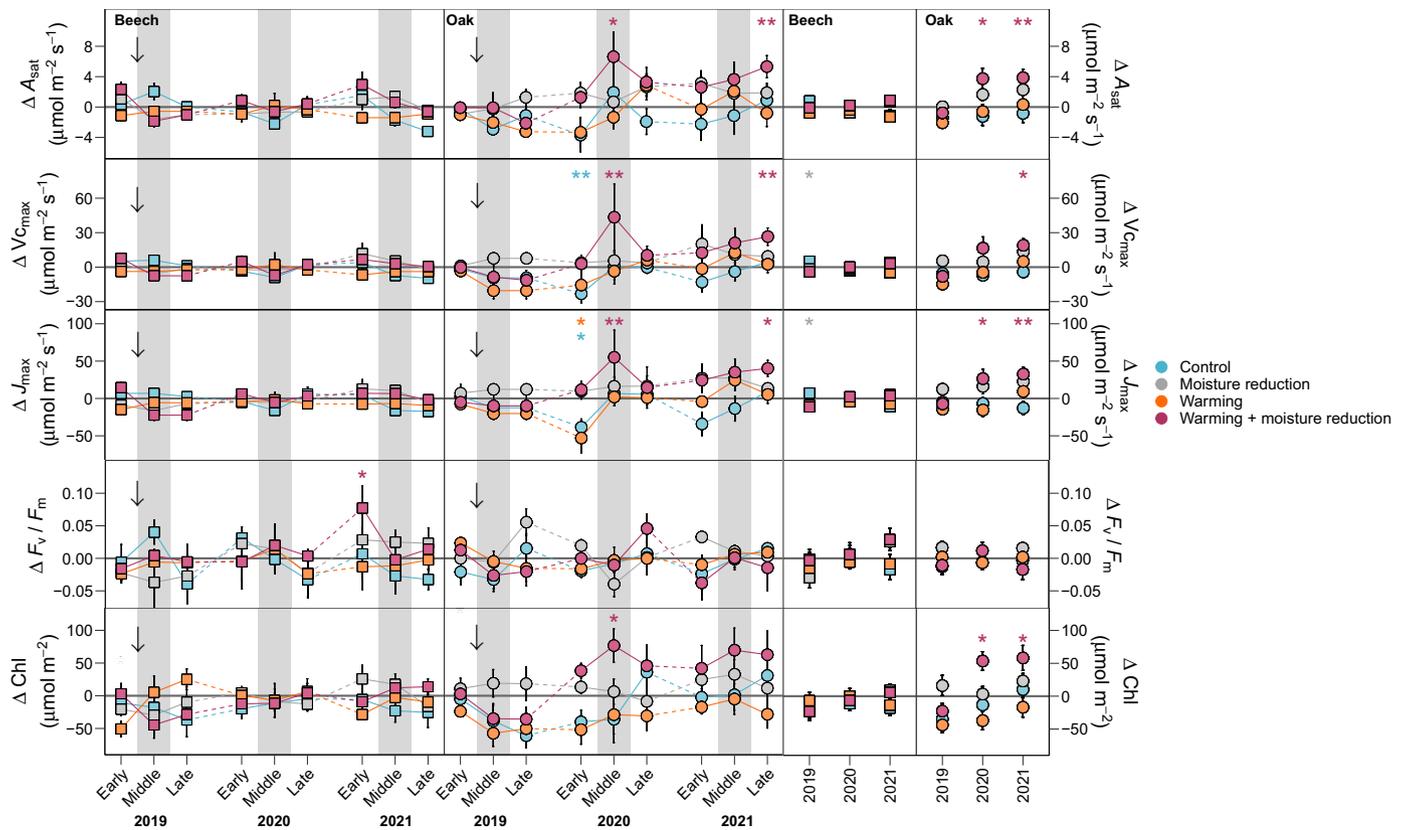


Fig. 6 Differences of light-saturated assimilation (A_{sat}), maximum carboxylation rate (V_{cmax}), maximum photosynthetic electron transport rate (J_{max}), maximum photochemical efficiency (F_v/F_m), and chlorophyll concentration (Chl) for each measurement campaign (i.e. early, middle, and late growing season of 2019, 2020, and 2021, mean \pm SE, $n = 6$ trees) and year (i.e. 2019, 2020, and 2021, mean \pm SE, $n = 18$ trees) for beech (squares) and oak (circles) trees growing under control (blue), soil moisture reduction (gray), warming (orange), and warming + soil moisture reduction (purple) in the inter-specific compositions compare to the intra-specific treatments. Positive values indicate higher physiological rates in the inter-specific treatments compared to the intra-specific treatments. The black arrows indicate the onset of the climatic treatment. Significant differences between inter-specific and intra-specific treatments are highlighted per treatments, campaign and species using a linear mixed-effects models followed by a Tukey type *post hoc* test (*, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \geq 0.001$).

warming (Fig. S1), suggesting that stomatal limitation did not occur in this treatment for oak. Nonstomatal acclimation processes such as a higher CO_2 diffusion through the mesophyll cells may partially explain this response: increased mesophyll conductance has been observed under warmer conditions (Bernacchi *et al.*, 2002), but these responses are strongly variable across species. Von Caemmerer & Evans (2015) showed that Engelmann oak (*Quercus engelmannii* Greene.) shows a strong activation of the membrane diffusion with an increase in temperature. Thus, increased mesophyll conductance with higher temperature facilitates CO_2 diffusion to the chloroplast and might therefore drive our higher carboxylation rates in downy oak. Furthermore, we found that the ratio between J_{max} and V_{cmax} was lower in the warming treatment (Fig. S2), suggesting that trees grown at warmer temperatures tended to acclimate for the optimum temperature of J_{max} and V_{cmax} (Kattge & Knorr, 2007). Overall, our findings suggest that beech will suffer from a warming atmosphere (Šimpraga *et al.*, 2011; Holišová *et al.*, 2013), while oak may benefit from temperature rise in temperate climates where heat stress is not yet limiting this species (Arend *et al.*, 2013; Kuster *et al.*, 2014). As shown in our study, photosynthetic

responses to chronic warming are highly dependent of local climatic conditions and species' origin. The same experiment conducted in warmer or colder climates may lead to contrasting results with either stronger reductions or increases in photosynthesis depending on the temperature tolerance of the studied species (e.g. Stojnić *et al.*, 2015).

Contrary to warming impacts and in accordance to our expectations, we found strong adverse soil moisture reduction effects for both species, particularly during the last year of measurements and most pronounced for beech (Figs 3, 5). This finding confirms many studies showing that drought negatively affects carbon assimilation and that beech is generally more affected than oak (Epron & Dreyer, 1993; Bréda *et al.*, 2006; Gallé & Feller, 2007; Santos *et al.*, 2018). Our results showed that both species had a lower A_{sat} , V_{cmax} , J_{max} , F_v/F_m , and Chl under moisture reduction conditions (Figs 3, 5). This finding suggests that reduced A_{sat} might be driven by a combination of stomatal and nonstomatal limitation, as previously observed in several studies conducted on this species (e.g. Arend *et al.*, 2013). Nonstomatal limitation might be attributed to a reduction in mesophyll conductance (e.g. Hommel *et al.*, 2014) or anatomical changes (e.g.

smaller and higher density of stomata; Schuldt *et al.*, 2016; Tognetti *et al.*, 1995). Further work investigating leaf anatomical, diffusional, and hydraulic changes would be needed to fully understand how prolonged moisture reduction impacted the photosynthetic properties of our trees.

Interestingly, a similar response in most photosynthetic properties was observed in response to moisture reduction acting alone and under combined warming (Fig. 3), indicating that water resource has a more important role in the photosynthetic response of our species than temperature. These results further reinforce previous work in which combined heat and drought negatively impacted leaf-level photosynthetic properties but without additive effects (e.g. Rennenberg *et al.*, 2006; Arend *et al.*, 2013). Similar results have also been observed in piñon pine where foliar traits responded to drier conditions and where additional warming did not exacerbate these responses (Grossiord *et al.*, 2017). For oak, the absence of additive effects and the overarching impact of soil moisture could be driven by compensation mechanisms between temperature and soil water shortage as warming increased most photosynthetic properties (Fig. 5), while reduced moisture impaired them. Moreover, as downy oak is adapted to drought stress, physiological characteristics may be more responsive to changes in soil water availability. Furthermore, warming in our experiment may not have been experienced as a stressful factor for oak trees as the temperature range during the middle of the growing season (i.e. average of 29°C between 09:00 and 16:00 h for July and August) fell well within the thermal breadth of photosynthesis for this species (Warren, 2006). Further experiments on how warming and reduced soil moisture will impact photosynthesis would be needed to understand the consequences of our findings at the whole tree scale. Indeed, temperature and drought can modify plant allometry (Lines *et al.*, 2012; Eziz *et al.*, 2017), which could further enhance trends found at the leaf-level. We observed that height and diameter increment were severely reduced for both species in the combined warming and soil moisture reduction treatment compared to the control (Fig. S3), suggesting lower leaf area and potentially stronger reductions in gas exchange at the tree level. However, further work including aboveground and belowground biomass assessment would be needed to draw solid conclusions on the carbon sequestration and allocation of these trees. Moreover, it is important to point out that we observed strong year-to-year variability in photosynthetic properties for both species, highlighting that both ontogeny and inter-annual climatic variations are significant drivers of leaf photosynthetic responses to stress (Bréda & Granier, 1996; Verbeeck *et al.*, 2008; Wang *et al.*, 2008). Variability in photosynthesis and in photosynthetic capacity is commonly observed even if it is still rarely included in mechanistic models, which tend to use constant values per species. For instance, Wilson *et al.* (2001) demonstrated that neglecting the inter-annual variations could result in an overestimate of the carbon exchange by around 50%. Hence, these warming and low soil moisture-driven physiological changes are particularly relevant properties to be better incorporated in mechanistic plant physiological models.

Impact of species interactions on photosynthetic responses to warming and soil moisture reduction

Tree species interactions altered the photosynthetic responses to the climatic treatments, but mainly for oak and most strongly under extreme conditions (i.e. combined warming and moisture reduction). We initially expected beech and oak trees to both exhibit beneficial effects of their interaction because of their different functional strategies for resource uptake, such as light and water, which should lead to reduced competition for resources (e.g. Jonard *et al.*, 2011; Zapater *et al.*, 2011; Pretzsch *et al.*, 2013). Yet, for beech, we found no changes between intra- and inter-specific interactions in all climatic treatments, suggesting that the identity of their neighbors did not influence their response to environmental variability. This result contradicts many studies suggesting that species interactions and increased diversity could improve the photosynthetic properties of trees under low moisture conditions (Raftoyannis, 2002; Goisser *et al.*, 2016; Metz *et al.*, 2016) and reduce abiotic stress in combined warming and drought (e.g. de Andrés *et al.*, 2017). The absence of response could be explained by the short time period of the experiment. Indeed, our trees are still at an early development stage (i.e. 5 yr old), and interactions between trees can take multiple years to establish (Domisch *et al.*, 2015; Haase *et al.*, 2015), especially belowground (Grossiord *et al.*, 2014a,b,c) where water resource partitioning could be a primary mechanism driving mitigating effects of species interactions.

Nevertheless, our results highlighted a positive effect of inter-specific interactions for oak, mainly under combined warming and reduced moisture (Fig. 6). This finding reinforces and complements previous studies suggesting that the interaction between the two tree species leads to beneficial carbon uptake processes during extreme events (Grossiord *et al.*, 2015; Pardos *et al.*, 2021). For instance, in a Mediterranean oak forest, Grossiord *et al.* (2014a,b,c) showed that turkey oak benefited from being in mixture with sessile oak by having less strong reductions in sap flux during dry periods compared to monoculture stands. Similarly, Forrester *et al.* (2016) showed that not necessarily all species benefit from species interactions, and that the beneficial effect of mixture strongly depends on the species and climatic conditions. In our case, the presence of beech trees could have reduced soil water competition for oak during hot and dry conditions as beech had lower carbon assimilation and transpiration rates than oak. Moreover, reduced shading in aboveground compartments could have benefited oak trees when mixed with beech as this species tends to grow in full sun light conditions. Indeed, the relatively strong light inside the OTCs may have been detrimental to beech seedlings, which are known to be shade-tolerant and are normally growing under low light conditions in the understory (e.g. Ferrio *et al.*, 2009), and could have contributed to the low photosynthetic properties observed in intra-specific conditions (e.g. F_v/F_m , Fig. 3). However, we can only speculate about these underlying processes and further analyses on soil properties (e.g. nutrient content, decomposition rates), aboveground and belowground biomass and hydraulic traits would be needed to disentangle potential complementarity, facilitation and selection effects taking place in our study.

Conclusion

Our study highlights that European beech will likely suffer from an air temperature rise of +5°C, leading to impaired leaf-level photosynthetic properties, independently of soil moisture. On the contrary, downy oak will probably benefit from prolonged warming by taking advantage of a more efficient photosynthetic responses, which could enhance CO₂ uptake as long as soil moisture is not limiting. However, the beneficial effect of warming on the photosynthetic properties of oak quickly disappeared when warming co-occurred with low soil moisture. Moreover, we show that interactions between beech and oak can further limit the adverse effects of warmer and drier soils for oak, suggesting that this species might be less vulnerable to future conditions in mixed-species stands compared to monocultures.

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Author contributions

MD-G and CG conceived and designed the study; MS and JG provided and managed the OTC facility and implemented measurement and control systems; MD-G and CG collected the data; MD-G analyzed the data and led the writing of the manuscript; MD-G, CG, AG, NB, MS and JG critically contributed to the manuscript and gave final approval for publication.

ORCID

Nina Buchmann  <https://orcid.org/0000-0003-0826-2980>
 Margaux Didion-Gency  <https://orcid.org/0000-0001-8967-3655>
 Arthur Gessler  <https://orcid.org/0000-0002-1910-9589>
 Charlotte Grossiord  <https://orcid.org/0000-0002-9113-3671>
 Marcus Schaub  <https://orcid.org/0000-0002-0158-8892>

Data availability

Data used in this manuscript is available from the Dryad Digital Repository, <https://doi.org/10.5061/dryad.2bvq83bt1> (Didion-Gency *et al.*, 2021).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Light-saturated stomatal conductance for each measurement campaign and year for beech and oak trees growing under control, soil moisture reduction, warming, and warming + soil moisture reduction in the intra-specific treatments.

Fig. S2 Thermal acclimation for beech and oak trees growing under control, soil moisture reduction, warming, and warming + soil moisture reduction in intra-specific treatments.

Fig. S3 Growth traits for beech and oak trees growing under control, soil moisture reduction, warming, and warming + soil moisture reduction in intra-specific treatments.

Table S1 Summary of the ANOVA tests of the linear mixed-effects models with the interactive effects of warming, soil moisture reduction, year, and growing season stage evaluated on the measured traits for beech trees in intra-specific treatments.

Table S2 Summary of the ANOVA tests of the linear mixed-effects models with the interactive effects of warming, soil moisture reduction, year, and growing season stage evaluated on the measured traits for oak trees in intra-specific treatments.

Table S3 Summary of the ANOVA tests of the linear mixed-effects models with the interactive effects of warming, soil moisture reduction, year, growing season stage, and interaction (i.e. intra-specific + inter-specific) evaluated on the measured traits for beech trees.

Table S4 Summary of the ANOVA tests of the linear mixed-effects models with the interactive effects of warming, soil moisture reduction, year, growing season stage, and interaction (i.e. intra-specific + inter-specific) evaluated on the measured traits for oak trees.

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