

Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year old *Quercus robur*?

Gardner, A; Ellsworth, Ds; Crous, Ky; Pritchard, J; Ar, Mackenzie

DOI:

[10.1093/treephys/tpab090](https://doi.org/10.1093/treephys/tpab090)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Gardner, A, Ellsworth, D, Crous, K, Pritchard, J & Ar, M 2021, 'Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year old *Quercus robur*?', *Tree Physiology*.

<https://doi.org/10.1093/treephys/tpab090>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.



Research paper

Is photosynthetic enhancement sustained through three years of elevated CO₂ exposure in 175-year-old *Quercus robur*?

A. Gardner^{1,2}, D.S. Ellsworth³, K.Y. Crous³, J. Pritchard^{1,2} and A.R. MacKenzie^{1,4,5}

¹Birmingham Institute of Forest Research, University of Birmingham, Edgbaston, B15 2TT, UK; ²School of Biological Sciences, University of Birmingham, Edgbaston, B15 2TT, UK; ³Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith NSW 2751, Australia; ⁴School of Geography, Earth & Environmental Sciences, University of Birmingham, Edgbaston, B15 2TT, UK; ⁵Corresponding author (a.r.mackenzie@bham.ac.uk)

Received March 5, 2021; accepted July 7, 2021; handling Editor David Whitehead

Current carbon cycle models attribute rising atmospheric CO₂ as the major driver of the increased terrestrial carbon sink, but with substantial uncertainties. The photosynthetic response of trees to elevated atmospheric CO₂ is a necessary step, but not the only one, for sustaining the terrestrial carbon uptake, but can vary diurnally, seasonally and with duration of CO₂ exposure. Hence, we sought to quantify the photosynthetic response of the canopy-dominant species, *Quercus robur*, in a mature deciduous forest to elevated CO₂ (eCO₂) (+150 µmol mol⁻¹ CO₂) over the first 3 years of a long-term free air CO₂ enrichment facility at the Birmingham Institute of Forest Research in central England (BIFoR FACE). Over 3000 measurements of leaf gas exchange and related biochemical parameters were conducted in the upper canopy to assess the diurnal and seasonal responses of photosynthesis during the 2nd and 3rd year of eCO₂ exposure. Measurements of photosynthetic capacity via biochemical parameters, derived from CO₂ response curves, (V_{cmax} and J_{max}) together with leaf nitrogen concentrations from the pre-treatment year to the 3rd year of eCO₂ exposure, were examined. We hypothesized an initial enhancement in light-saturated net photosynthetic rates (A_{sat}) with CO₂ enrichment of $\approx 37\%$ based on theory but also expected photosynthetic capacity would fall over the duration of the study. Over the 3-year period, A_{sat} of upper-canopy leaves was $33 \pm 8\%$ higher (mean and standard error) in trees grown in eCO₂ compared with ambient CO₂ (aCO₂), and photosynthetic enhancement decreased with decreasing light. There were no significant effects of CO₂ treatment on V_{cmax} or J_{max} , nor leaf nitrogen. Our results suggest that mature *Q. robur* may exhibit a sustained, positive response to eCO₂ without photosynthetic downregulation, suggesting that, with adequate nutrients, there will be sustained enhancement in C assimilated by these mature trees. Further research will be required to understand the location and role of the additionally assimilated carbon.

Keywords: CO₂ fertilization of photosynthesis, Deciduous forest, downregulation, enriched CO₂, Free-air CO₂ enrichment (FACE), photosynthetic capacity.

Introduction

Forest ecosystems cover $\sim 30\%$ of the Earth's land surface, representing $\sim 50\%$ of terrestrially stored carbon and account for close to 60% of total terrestrial CO₂ fluxes in the global carbon cycle (Luyssaert et al. 2008, Pan et al. 2011). The continual rise in atmospheric CO₂, overwhelmingly due to anthropogenic activity (Friedlingstein et al. 2019), increases the need to understand the terrestrial carbon feedbacks of forests in the global

carbon cycle. As the foundational driver of the carbon cycle of forests (e.g., Bonan 2008), the photosynthetic response to changing atmospheric CO₂ is a necessary process for forests to act as long-standing carbon stores with relatively long-lived carbon (C) pools such as wood (Körner 2017) and soil (Ostle et al. 2009). The amount of forest C-uptake in the future, and subsequent C sequestration, will be crucial determinants of future atmospheric CO₂ concentrations. So, quantifying the

photosynthetic response under elevated CO_2 (eCO_2), especially for mature trees, is critical to understanding the carbon uptake of forests under changing atmospheric composition.

It has been widely observed that eCO_2 can have a stimulatory effect on plant photosynthesis, known as photosynthetic enhancement, at least in the short-term (weeks–months) with adequate nutrient and water availability permitting (Brodribb et al. 2020). Long-term (years to decades) photosynthetic responses to eCO_2 are less well understood and lower-than-expected responses have been observed (Ainsworth and Long 2005, Ellsworth et al. 2017). Note that, even in studies that report sustained and/or strong stimulation of photosynthesis under eCO_2 , the additionally assimilated C does not necessarily translate into increased growth stimulation (Bader et al. 2013, Sigurdsson et al. 2013).

The photosynthetic process and photosynthetic response to eCO_2 is sensitive to changes in environmental variables such as temperature, light, water and availability of nutrients. For example, net photosynthesis (A_{net}) is expected to increase with exposure to eCO_2 , with greatest photosynthetic enhancement expected at maximum photon flux density (Q) if Rubisco carboxylation is limiting (Sage et al. 2008). Decreases in A_{net} have been commonly associated with limitations in water and nutrient availability (Nowak et al. 2004, Ainsworth and Rogers 2007). For example, water availability has been found to increase the magnitude of eCO_2 -induced photosynthetic enhancement in drier years (Nowak et al. 2004, Ellsworth et al. 2012). Thus, interannual differences in eCO_2 -induced photosynthetic enhancement are to be expected as environmental conditions vary. Understanding the photosynthetic response to eCO_2 under different, real-world, environmental conditions provides information essential, but not in itself sufficient, for modeling forest productivity (Jiang et al. 2020), and predicting carbon-climate feedbacks (e.g., Cox et al. 2013, Jones et al. 2016).

Despite a significant body of research on the photosynthetic response to eCO_2 in tree seedlings and saplings (as reviewed in Medlyn et al. 1999, Ainsworth and Long 2005), fewer studies address the long-term (>1 year) photosynthetic responses in mature plantation trees (Liberloo et al. 2007, Crous et al. 2008, Uddling et al. 2009, Warren et al. 2015) and very few in mature forest-grown trees (Bader et al. 2010, Klein et al. 2016, Ellsworth et al. 2017). Currently, the dynamic vegetation components of Earth System models, which diagnose vegetation responses to environmental change, have commonly been constructed using data from eCO_2 experiments on young and/or plantation grown trees (Piao et al. 2013). Yet, it is difficult to compare, generalize, and scale results from young trees in their exponential growth phase to the response of closed-canopy mature forests (Norby et al. 2016). For example, previous work from a long-term natural experiment found CO_2 stimulation declined with tree age in *Quercus ilex* (Hättenschwiler et al. 1997). Therefore, it is plausible that model projections

are currently overestimating the photosynthetic responses of mature forests and, thence, the ' CO_2 fertilization' effect (Zhu et al. 2016). Consequently, uncertainty remains as to the magnitude of, and environmental constraints on, photosynthetic enhancement under eCO_2 in large, long-standing carbon stores such as mature forests (Norby et al. 2016, Jiang et al. 2020).

Free-air CO_2 enrichment (FACE) facilities are valuable to understand system-level responses to eCO_2 (Ainsworth and Long 2005, Terrer et al. 2019) particularly in forests (Medlyn et al. 2015, Norby et al. 2016). The development of second generation forest FACE experiments focuses on tall, mature trees grown in their own forest soil (Hart et al. 2020). To date, forest FACE experiments have observed photosynthetic enhancements ranging from 30 to 60%, depending on tree species and environmental factors (as reviewed in Nowak et al. 2004, Ainsworth and Rogers 2007). Of the few studies on closed-canopy-dominant tree species, smaller photosynthetic enhancement to eCO_2 have been observed (19–49%) than in studies conducted on younger trees (Sholtis et al. 2004, Liberloo et al. 2007, Crous et al. 2008), but the reasons behind this smaller response remain unclear.

There is evidence of a reduction in photosynthetic activity after long-term eCO_2 exposure, known as photosynthetic downregulation (Ainsworth et al. 2004, Crous and Ellsworth 2004), but downregulation is not always observed (Curtis and Wang 1998, Herrick and Thomas 2001). Commonly photosynthetic downregulation under eCO_2 exposure is the result of decreases, either directly or indirectly, in Rubisco carboxylation (V_{cmax} ; Feng et al. 2015, Wujeska-Klaue et al. 2019b). However, the stimulatory effect of photosynthesis under eCO_2 may be reduced but is usually not completely removed (Leakey et al. 2009, Wujeska-Klaue et al. 2019b). Photosynthetic downregulation has largely been observed in young plants (Leakey et al. 2009), with some downregulation observed in two aggrading plantation forests (Crous et al. 2008, Warren et al. 2015), commonly as a result of insufficient soil nitrogen supply (Luo et al. 2004). However, photosynthetic downregulation has largely not been observed in mature forests (Bader et al. 2010) and therefore open questions remain concerning the frequency and magnitude of photosynthetic downregulation under eCO_2 exposure in mature forests.

To understand the photosynthetic responses in mature temperate deciduous forests, we evaluated the photosynthetic enhancement and potential downregulation in ca. 175-year-old canopy-dominant trees of *Q. robur* L. exposed to eCO_2 for 3 years. Considering that forest FACE experiments aim to operate for 10 years or more, we refer to these CO_2 responses as 'early' (Griffin et al. 2000). This study is among the oldest trees that have ever been examined under eCO_2 . To assess the photosynthetic enhancement of the trees on daily and interannual timeframes, measurements of gas exchange and leaf biochemistry were measured in the upper oak canopy over

four growing seasons, that included one pre-treatment year (2015) and 3-treatment years (2016–2019). Our aims were to quantify the photosynthetic response to eCO₂ (i.e., ambient +150 µmol mol⁻¹) for mature *Q. robur* and how light level influences this response, to determine whether photosynthetic downregulation under eCO₂ occurred and to establish whether the relationship between leaf N and photosynthetic capacity changed in eCO₂. We hypothesized that net photosynthetic gas exchange, A_{net} , will significantly increase with eCO₂ and light levels (Q). The greatest enhancement was expected with the highest light levels, as a result of reduced limitations in the light dependent reaction of photosynthesis, and that photosynthetic enhancement would be ≈37% following theory and reasoning in Nowak et al. (2004) (see also Supplemental Appendix 1 available as Supplementary Data at *Tree Physiology Online*). We also hypothesized that leaf nitrogen (N) will be reduced under eCO₂ and that photosynthetic downregulation will be observed under eCO₂ as a result of reduced leaf N and/or a decline in either the maximum rate of photosynthetic Rubisco carboxylation (V_{cmax} , µmol m⁻² s⁻¹); and the maximum rate of photosynthetic electron transport (J_{max} , µmol m⁻² s⁻¹), or both (Griffin et al. 2000).

Methods and materials

Site description

This study was conducted at the Birmingham Institute of Forest Research (BIFoR) Free Air CO₂ Enrichment (FACE) facility located in Staffordshire (52.801°N, 2.301°W), UK. The BIFoR FACE facility is a 'second generation' Forest FACE facility, extending the scope of first generation facilities; (see Norby et al. 2016), situated within 19 ha of mature northern temperate broadleaf deciduous woodland having a canopy height of 24–26 m. The woodland consists of an overstorey canopy dominated by English oak (*Q. robur* L.) and a dense understorey comprising mostly of hazel coppice (*Corylus avellana* L.), sycamore (*Acer pseudoplatanus* L.), and hawthorn (*Crataegus monogyna* Jacq.). *Quercus robur* (commonly known as pendunculate oak, European oak or English oak) is a common broadleaf species geographically widespread across Europe where it is both economically important and ecologically significant for many biota (Eaton et al. 2016, Mölder et al. 2019). The site was planted with the existing oak standards in the late 1840s and has been largely unmanaged for the past 30–40 years. Like most established forest of the temperate zone, the BIFoR FACE forest is under-managed.

The study site is situated within the temperature-rainfall climate space occupied by temperate forest (Sommerfeld et al. 2018, Jiang et al. 2020) and is characterized by cool wet winters and warm dry summers with a frost-free growing season from April to October. The mean January and July temperatures

were 4 and 17 °C, respectively, and the average annual precipitation for the region is 720 mm (650, 669, 646 and 818 mm, in 2015, 2017, 2018 and 2019, respectively, when the study was conducted; see Figure 1). The total N deposition load at the BIFoR FACE site is ~22 Kg N/ha/year (estimate provided by S. Tomlinson at the Centre for Ecology and Hydrology, Edinburgh, UK; MacKenzie et al. 2021), representing ~15% of the total nitrogen nutrition of temperate deciduous trees (Rennenberg and Dannenmann 2015).

BIFoR FACE consists of nine approximately circular experimental plots of woodland 30 m in diameter (Hart et al. 2020). Only the six plots with infrastructure were considered in the present study. Each 'infrastructure plot' is encircled by steel towers constructed individually to reach 2-m above the local canopy-top height. The facility uses a paired-plot design (Hart et al. 2020): three replicate plots at either ambient CO₂ (aCO₂; ca. 405 µmol mol⁻¹) and three plots supplied with CO₂ enriched air, termed elevated CO₂ plots (eCO₂). The latter plots were operated such that they achieved a target of +150 µmol mol⁻¹ above the minimum measured in the ambient plots (i.e., concentrations in the elevated plots ca. 555 µmol mol⁻¹) as 5-min rolling averages (Hart et al. 2020; see Supplementary Figure 1 available as Supplementary Data at *Tree Physiology Online*). Elevated CO₂ is added from dawn (solar zenith angle, $\text{sza} = -6.5^\circ$) to dusk ($\text{sza} = -6.5^\circ$) throughout the growing season. Daytime exposure to eCO₂ was almost continuous throughout the growing season (Hart et al. 2020), with exceptions if the 15-min average wind speed was >8 m s⁻¹, or when canopy-top, 1-min average, air temperature was <4 °C. In the latter case, gas release was resumed when the air temperature was ≥5 °C. The CO₂ fumigation thresholds for wind speed and temperature were selected because of the high cost of maintaining eCO₂ and the insignificant uptake of carbon under these conditions, respectively. The operation of the FACE system and statistical performance in terms of meeting the target CO₂ concentration in time and space have been described in Hart et al. (2020).

In each plot, canopy access was gained through a custom-built canopy access system (CAS) (Total Access Ltd, UK) that was installed from the central towers with canopy measurements made from a rigged rope access system (see Supplementary Figure 2 available as Supplementary Data at *Tree Physiology Online*). This facilitated in situ gas exchange measurements by allowing access to the upper oak canopy. The hoisting system comprises of an electric winch (Harken Power Seat Compact) that lifts a harnessed (Petzl AVAO BOD 5 point harness) user vertically through the air at a predetermined fixed point to a maximum canopy height of 25 m. The system required operation from the ground by trained staff and the user is seated in a Boatswain's chair. One oak tree per plot was accessible using the CAS system as set up during this study, and all gas

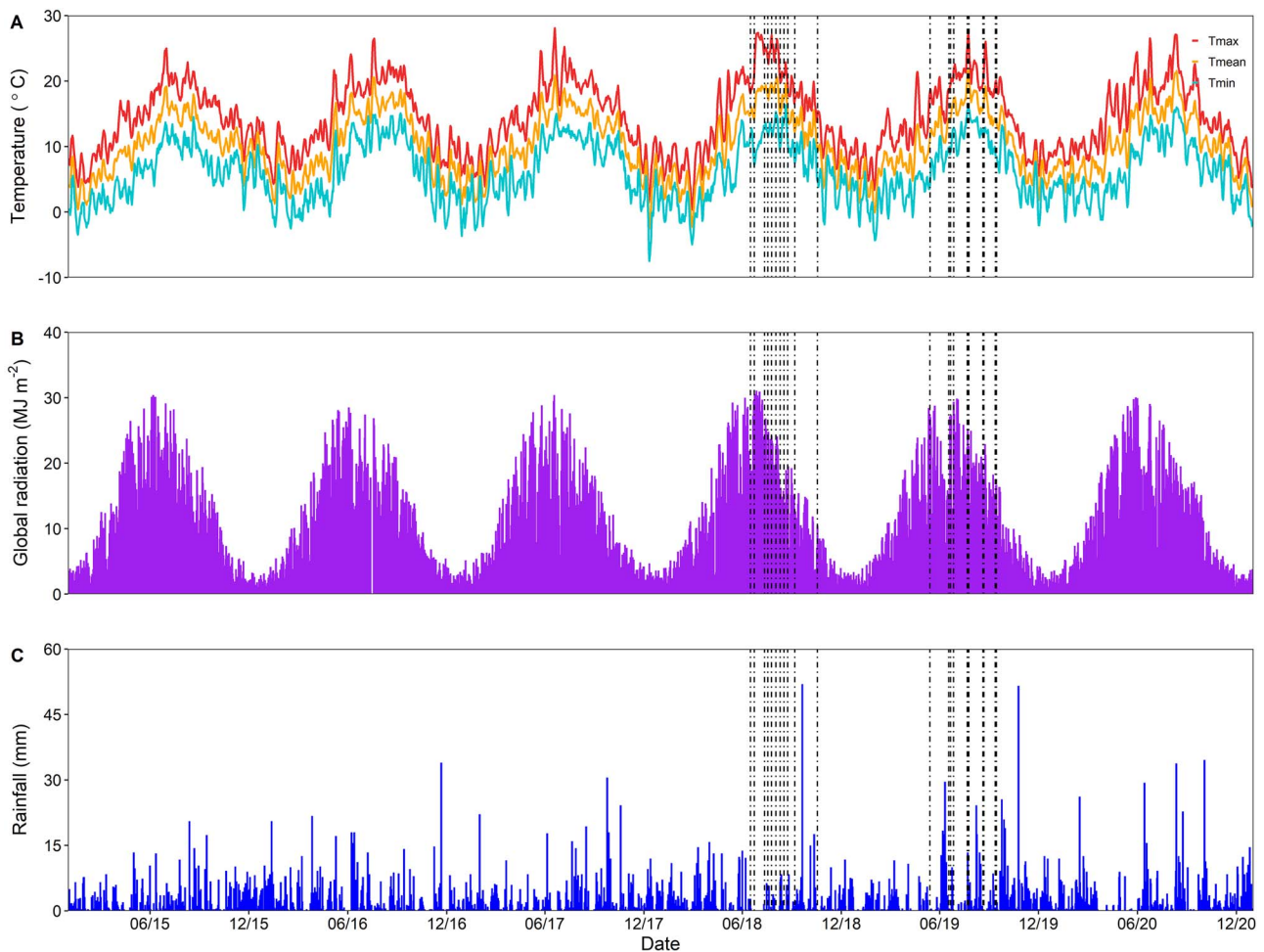


Figure 1. Time series showing the daily meteorological data at the BIFoR FACE facility covering the period of 1 January 2015–1 January 2021. Subplots are: (A) maximum, (red), mean (orange) and minimum (blue) daily air temperatures ($^{\circ}\text{C}$), (B) global downwelling solar radiation (MJ m^{-2}) and (C) total daily precipitation (mm). Vertical dashed lines indicate diurnal sampling days. Clusters of sampling days occurred because different plots were sampled on different days in the same seasonal timeframe. Meteorological data are from RAF Shawbury, located 20 miles west of the BIFoR FACE facility, retrieved from the UK Met Office (<https://www.metoffice.gov.uk/research/climate/maps-and-data/historic-station-data>).

exchange measurements were made on unshaded leaves within the top 2 m of each tree canopy on dominant trees.

For this study, the sample size used throughout the study ($n = 3$) represents the number of replicate experimental plots at BIFoR FACE and includes within-tree replicates that were averaged per plot before analysis. All the three replicates were sampled for the majority of campaigns, except for September 2018 and June 2019 where replicates were reduced to two due to logistic constraints, weather and safe tree access.

Gas exchange measurements

All gas exchange measurements were conducted in situ on upper canopy oak leaves using either a Li-6400XT or Li-6800 portable photosynthesis system (LiCOR, Lincoln, NE, USA) to quantify photosynthetic performance at BIFoR FACE. Measurement campaigns focused on two different types of measurements: (i) instantaneous diurnal measurements, at prevailing

environmental conditions (2018 and 2019), and (ii) net assimilation rate-intercellular CO_2 concentration ($A-C_i$) measurements (includes pre-treatment, 2015; 1st year, 2017; and 3rd year, 2019, of CO_2 fumigation). Measurements were conducted in all six experimental plots with infrastructure, on one chosen candidate tree per plot. The target tree remained the same for all treatment years (2017, 2018 and 2019) but a different tree was measured during the pre-treatment period in 2015. This change was because the plot infrastructure, which determined the CAS system, was not constructed until 2016.

When reporting treatment effects from the present study, we report the *mean enhancement* or *treatment effect*:

$$100 \cdot \frac{\Delta A_i}{A_{i,a}} = 100 \left(\frac{A_{i,e} - A_{i,a}}{A_{i,a}} \right) \quad (1)$$

where $A_{i,x}$ is a measure of gas exchange ($i = \text{'net' or 'sat'}$, see below) at ambient (a) or elevated (e) CO_2 mixing ratios. When

comparing our results with other studies using different eCO₂ treatments, we report the sensitivity to eCO₂, following Keenan et al. (2016):

$$\text{Sensitivity} = \frac{c_a}{\text{GPP}} \frac{\partial \text{GPP}}{\partial c_a} \approx \frac{\Delta A_i}{A_{i,a}} \cdot \frac{c_a}{\Delta c_a} \quad (2)$$

where c_a is the aCO₂ mixing ratio and Δc_a is the treatment size (e.g., +150 $\mu\text{mol mol}^{-1}$ as in our case). For the conditions of the present study (see 'Diurnal measurements' section, below), $c_a/\Delta c_a = 392/150 = 2.61$, and we use net photosynthesis instead of GPP. Hence, our theoretical predicted photosynthetic enhancement (Nowak et al. 2004; see Supplemental Appendix 1 available as Supplementary Data at *Tree Physiology Online*) for the +150 $\mu\text{mol mol}^{-1}$ increase in CO₂ (i.e., $\approx 37\%$; Hart et al. 2020), is equivalent to expecting a sensitivity to eCO₂ of unity.

Diurnal measurements

Near the canopy-top, in situ diurnal measurements of gas exchange were conducted on upper canopy oak leaves on 11 and 12 separate summer days of 2018 and 2019, respectively. Measurements of gas exchange (e.g., net CO₂ photosynthetic assimilation rates, A_{net}) were made using a Li-6800 equipped with the default clear Propafilm (Innovia Films Inc., Atlanta, GA) window chamber head, which allowed for natural sunlight to illuminate the leaf. Measurements were conducted in one pair of plots (i.e., one eCO₂ plot and its paired aCO₂ plot) on each sampling day. Therefore, each full campaign ($n = 3$) took 3 days to complete, with the exception of September 2018 and June 2019 where only two replicate plots could be measured. A total of four diurnal campaigns were conducted in both 2018 and 2019, providing a total of 3426 data points. Five to six healthy leaves were randomly selected in the same oak tree per plot, every 30–40 min across the time course of the day for gas exchange measurements, swapping between aCO₂ and eCO₂ plots.

Measurements were made at the respective growth CO₂ of aCO₂ ($\sim 405 \mu\text{mol mol}^{-1}$) or +150 $\mu\text{mol mol}^{-1}$ aCO₂ ($\sim 555 \mu\text{mol mol}^{-1}$) for eCO₂ plots, along with other environmental variables such as relative humidity (RH); air temperature (T_{air}) and quanta of photosynthetically active radiation (PAR). Measurements were confined to the youngest fully expanded leaves of the leader branch within reaching distance of the CAS system. Measurements were confined to the first flush of leaves across the season for consistency in leaf age. Expanding leaves, judged from color and texture, were avoided for measurements, as they had not matured in terms of chlorophyll and formation of the photosynthetic apparatus. Once a leaf was inside the chamber, the Li-6800 head was gently positioned and held constant at an angle towards the sun. This was to ensure sun exposure on the leaf, to minimize shading of the chamber

head on the measured leaf and to reduce variation across the leaf measurements. Measurements were recorded after an initial stabilization period (typically ~ 40 seconds to 1 minute), to meet programmed stability parameters. This allowed for instantaneous steady-state photosynthesis to be captured, yet avoided chamber-related increases in leaf temperature (Parsons et al. 1998). Care was taken to ensure conditions matched those outside the chamber before each measurement was taken. The daily mean RH inside the leaf chamber was between 50 and 77% for all measurements. The mean C_a values in the LiCOR chamber head were 390 ± 0.9 and $538 \pm 2.7 \mu\text{mol mol}^{-1}$, in 2018, and 393 ± 1.0 and $545 \pm 4.8 \mu\text{mol mol}^{-1}$, in 2019, for aCO₂ and eCO₂, respectively. The mean CO₂ treatments were, therefore, $+148 \pm 2.8 \mu\text{mol mol}^{-1}$ in 2018 and $+152 \pm 4.9$ in 2019, and were not statistically different. The gas exchange systems were calibrated before each growing season.

A–C_i curves

A–C_i curves were conducted in three growing seasons: pre-treatment year (2015), in the 1st year of CO₂ fumigation (2017) and third year of CO₂ fumigation (2019). Measurements were either conducted on attached branches in situ (2015 and 2019) or on detached branches harvested by climbers (2017) using a portable open gas exchange system that incorporated a controlled environment leaf chamber (Li-6400XT and Li-6800, LICOR, Inc., Lincoln, NE, USA). Detached branches were transferred to researchers on the ground immediately after excision, where they were placed in a bucket of water to minimize desiccation. Branches were re-cut under water and allowed to stabilize, before starting measurements. Measurement on detached branches was conducted no longer than 45 min after collection. Previous studies investigating measurements of gas exchange on severed or attached branches found no significant differences between the two methods (Bader et al. 2016, Verryckt et al. 2020). A–C_i curves were measured at a Q of $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (in 2015 and 2019) or $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (in 2017) and at a leaf temperature of 25 °C. Before each curve, a stabilization period of between 5 and 10 min was used depending on the prevailing environmental conditions and each curve took an average of 40 min. Light-saturated net photosynthesis (A_{sat}) was estimated from A–C_i curves at growth [CO₂]. The CO₂ concentrations were changed in 12–14 steps starting at the respective growth [CO₂]; every 100 $\mu\text{mol mol}^{-1}$ down to 50 $\mu\text{mol mol}^{-1}$ (near the photosynthetic CO₂ compensation point), then increasing to 1800 $\mu\text{mol mol}^{-1}$ in roughly 200 $\mu\text{mol mol}^{-1}$ increment steps. Five to six replicate A–C_i curves on different leaves per CO₂ treatment were measured per day. Measurements were taken between 09:00–11:00 and 14:00–17:00 to avoid potential midday stomatal closure (Valentini et al. 1995). Measurements were made using the treatment pair arrangement of one aCO₂ and one eCO₂ plot per day ($n = 3$).

Leaf carbon and nitrogen

Oak leaves were collected from the top of the canopy in each month, May–November in 2015 and 2019, by arborist climbers, and stored immediately at -25°C . Two upper canopy leaves, from one tree per plot, were selected for elemental analyses, these trees corresponded to the measurement tree for leaf gas exchange. Each leaf was photographed on white graph paper, with a ruler for reference. Leaf area analysis was conducted using imaging software Image J (IMAGE J v1.53, National Institutes of Health, Bethesda, MD, USA) and the fresh weight was recorded. Each leaf was oven dried at 70°C for at least 72 h, re-weighed for dry weight and the leaf mass per unit area was calculated. Dried leaf fragments were ground and each sample (~ 2 mg) was enclosed in a tin capsule. Samples were analyzed for $\delta^{13}\text{C}$, total C and total N using an elemental analyzer interfaced with an isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK).

Statistical analysis

All statistical analyses were performed in R version 4.0.3 (R Core Team, 2020). Before statistical analysis, all data were checked for normality by inspection of the Q–Q plots and Levene's test, and residuals from model fitting were checked for evidence of heteroscedasticity. Hourly averages of diurnal measurements were analyzed using a linear mixed effects model ('lmer' package). Fixed categorical factors in this model were CO_2 treatment (i.e., aCO_2 or eCO_2), sampling month and sampling year (i.e., 2018 or 2019), in addition to their interactions. In addition, 'time of day' and 'plot' were represented as random factors, the latter as individual trees were nested within each experimental plot. Type III F -statistics associated with the mixed model analysis (repeated-measures analysis of variance, ANOVA) were reported. Statistically significant CO_2 treatment differences among groups were further tested with Tukey's post hoc test using the R package 'emmeans' ($P < 0.05$ reported as significant). To investigate the dependence of photosynthetic enhancement with variation of light, the diurnal gas exchange data, with leaf temperature, $T_{\text{leaf}} > 18^{\circ}\text{C}$, and vapor pressure deficit (D), $D < 2.2$ kPa, were sub-divided into four light (Q) categories, each sampled about equally. The Q classes were chosen based on the characteristic response of A_{net} to light as follows: $Q < 250$; $250 \leq Q < 500$; $500 \leq Q < 1000$ and $Q \geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. CO_2 treatment, year and Q category were then used as parameters in the ANOVA.

The photosynthetic CO_2 response (A – C_i) curves were fit with the model of Farquhar et al. (1980) to estimate the apparent maximum rate of photosynthetic Rubisco carboxylation (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the apparent maximum rate of photosynthetic electron transport (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) using 'Plantecophys' package in R (Duursma 2015). The model-fitting was undertaken to provide insight into photosynthetic capacity and its response to long-term exposure to elevated

$[\text{CO}_2]$ (Rogers and Ellsworth 2002). We tested for outliers by examining the $J_{\text{max}}/V_{\text{cmax}}$ ratio, RMSE values and standard errors (SE) for fits of J_{max} and V_{cmax} , all of which indicate violations to the theory for fitting these curves (Sharkey et al. 2007). Visual inspection of each A – C_i curve with outliers allowed us to identify any incomplete curves and/or mechanical failures and those curves were subsequently removed. This accounted for $< 10\%$ of the data, leaving a total of 86 A – C_i curves across the 3-sampling years in the analysis.

Results

Measurement conditions

Overall, diurnal measurements were conducted on dry, sunny days (Figure 1), and environmental conditions (Q and T_{leaf}) were consistent between aCO_2 and eCO_2 across the two growing seasons of diurnal measurements (Figures 2A, B and 3A, B). Q levels were largely comparable between CO_2 treatments although cloud and temperature conditions were more variable among sampling days and campaigns in 2018 than in 2019.

Leaf temperature was more stable than Q with lower variability across the diurnal sampling, high similarity between sampling days, and high consistency between CO_2 treatments. There were differences of up to 15°C in midday measurements of T_{leaf} , between months, suggesting a seasonal influence as would be expected from the site's mid-latitude location, with differences more prominent in 2019 than 2018. The highest T_{leaf} values were observed in July with a common seasonal decline after this campaign.

Analysis of the diurnal dataset showed the range of mean daily A_{net} was similar between years, however the highest mean daily A_{net} ($12.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) was reported in 2018. Contrasting seasonal patterns were observed between the sampling years of 2018 and 2019, with decreases in mean daily A_{net} across the growing season observed in 2018 compared with increases in A_{net} in 2019. In both sampling years, we observed a significant enhancement of A_{net} when exposed to eCO_2 ($P < 0.05$, Table 1 and Figures 2 and 3). Here, we did not observe any significant effect of either season or sampling year on A_{net} (Table 1). Therefore, from measurements of A_{net} collected from the diurnal dataset, a mean eCO_2 -driven photosynthetic enhancement (i.e., $100 \cdot \Delta A_i / A_{i,d}$) of $23 \pm 4\%$ was observed across the 2-year period of this study.

Photosynthesis and variation in photon flux density (Q)

This study analyzed the role of measurement Q affecting A_{net} and its response to eCO_2 in separate growing seasons to investigate photosynthetic enhancement values at different light conditions. In each light category (see section Methods, above), the light conditions between the CO_2 treatments were statistically comparable (Figure 4, see Supplementary Table S1

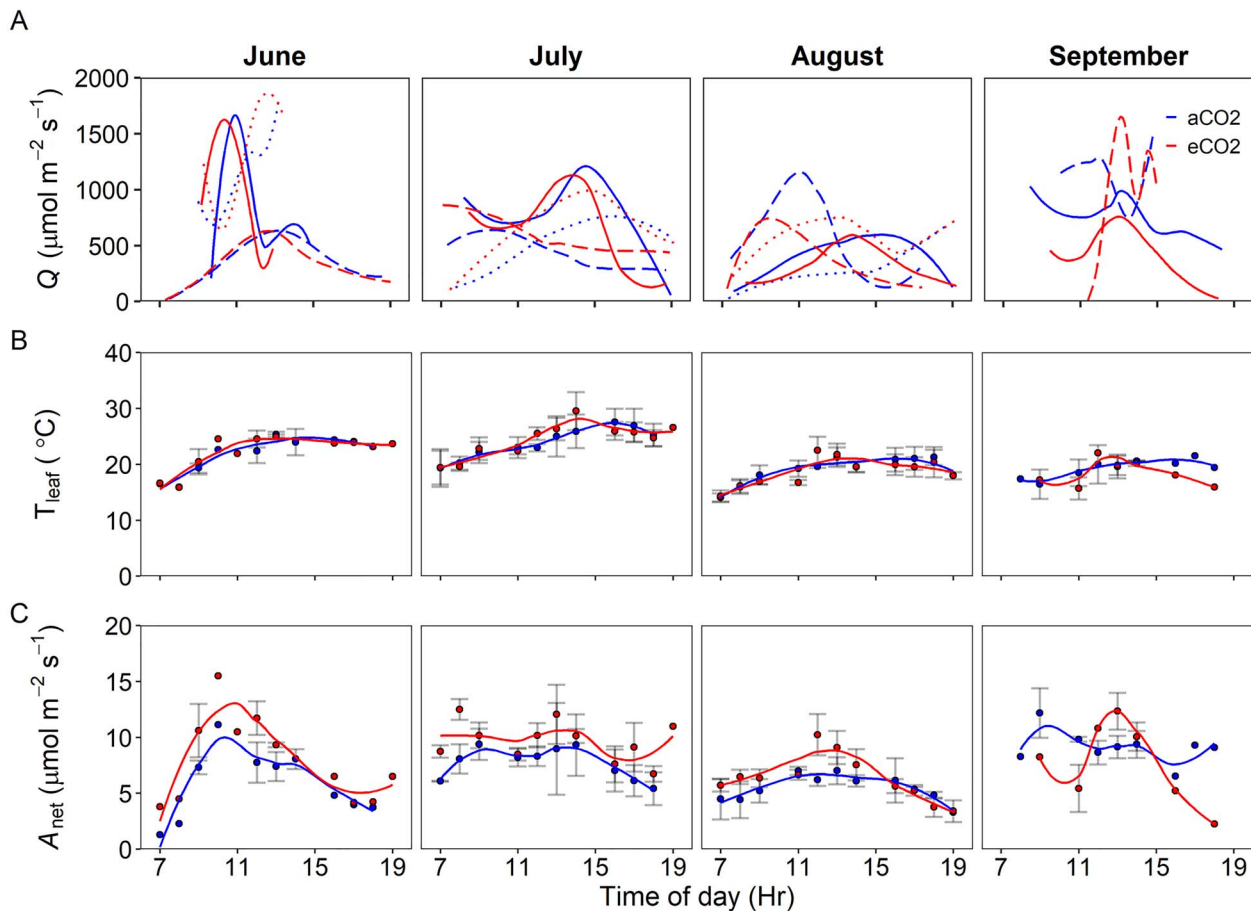


Figure 2. In situ diurnal measurements of (A) Q ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (B) hourly mean T_{leaf} ($^{\circ}\text{C}$) and (C) hourly mean A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$); each fitted with an LOESS regression, at BIFoR FACE in 2018 from the upper *Q. robur* canopy. Error bars indicate $n = 3$, with the exception of September where only two replicate plots were measured and not all time points were replicated. The line types in (A) represent replicate plot pairings of; plots 1 and 3 (dotted), plots 2 and 4 (solid) and plots 5 and 6 (long-dash) and the two colors represent the CO₂ treatments of aCO₂ (blue) and eCO₂ (red).

available as Supplementary Data at *Tree Physiology Online*). Mean, median and interquartile range of A_{net} increased with increasing Q class for both sampling years and CO₂ treatments (Figure 4A and Table 2). We observed no significant effect of year for A_{net} in this study, but we did observe a larger variation in A_{net} in 2019, when compared with 2018 (Table 2 and Figure 4A). Values of mean A_{net} ranged from $4.6 \pm 0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$, at the lowest Q level with a mean of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, to $11.5 \pm 0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ at highest Q (mean Q of $1360 \mu\text{mol m}^{-2} \text{s}^{-1}$). In addition, in both sampling years A_{net} was significantly higher under eCO₂ conditions when compared with aCO₂ ($P < 0.05$, Table 2 and Figure 4A).

Consistent with our hypothesis, we observed mean eCO₂-driven photosynthetic enhancement to increase with increasing Q , with the largest enhancement observed at highest Q in both sampling years, $30 \pm 9\%$ and $35 \pm 13\%$, for 2018 and 2019, respectively (Figure 4B). In 2018, eCO₂-driven photosynthetic enhancement ranged from $7 \pm 10\%$, in the lowest Q class, to $30 \pm 9\%$, in the highest Q class (Figure 4B). A similar positive relationship between eCO₂-driven photosynthetic enhancement

and Q was present in 2019 with enhancement ranging from $11 \pm 6\%$, in the lowest Q class, to $35 \pm 13\%$, in the highest Q class (Figure 4B). There was no significant effect of year (Table 2) and therefore the mean eCO₂-driven photosynthetic enhancement at light saturation (i.e., in the highest Q class) was on average $33 \pm 8\%$ across the 2-sampling years. Our results report that the mean eCO₂-driven photosynthetic enhancement of light-saturated A_{net} (A_{sat}) in both sampling years was consistent, within error (using 95% confidence intervals), of the theoretical predicted enhancement based on proportion of CO₂ increase ($\approx 37 \pm 6\%$), indicating a sensitivity to eCO₂ (Eq. (2), above) of close to unity for A_{sat} .

Photosynthetic capacity and foliar nitrogen

The seasonal and interannual biochemical changes in *Q. robur* were assessed via differences in leaf apparent maximum CO₂ carboxylation capacity (V_{cmax}) and apparent maximum electron transport capacity for RuBP regeneration (J_{max} ; Figure 5) to assess the photosynthetic capacity in the initial years of the long-term experiment. Initially, we tested for differences between

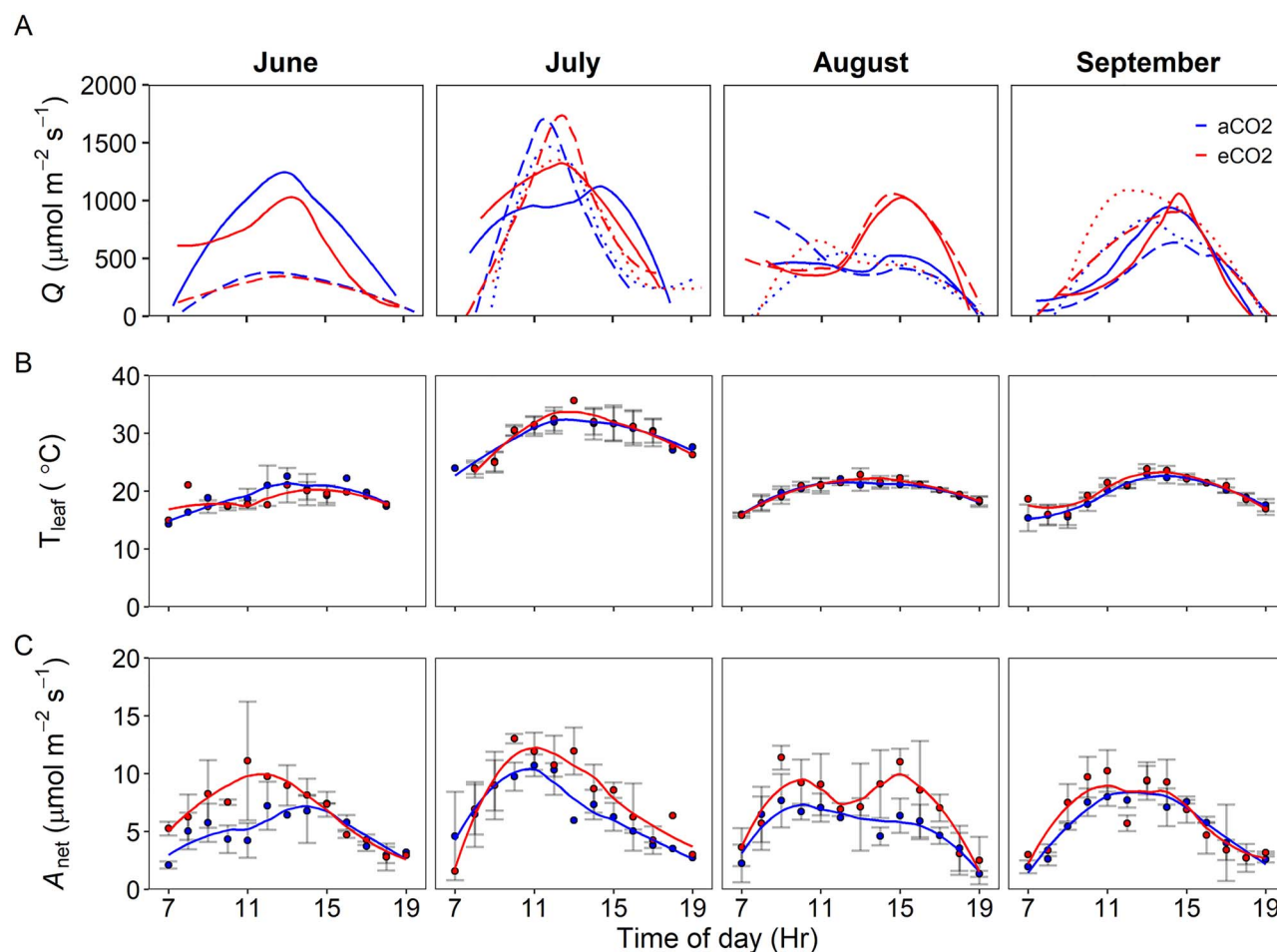


Figure 3. In situ diurnal measurements of (A) Q ($\mu\text{mol m}^{-2} \text{s}^{-1}$), (B) hourly mean T_{leaf} ($^{\circ}\text{C}$) and (C) hourly mean A_{net} ($\mu\text{mol m}^{-2} \text{s}^{-1}$); each fitted with an LOESS regression, at BIFoR FACE in 2019 from the upper *Q. robur* canopy. Error bars indicate $n = 3$, the exception of June where only two replicate plots were measured and not all time points were replicated. The line types in (A) represent replicate plot pairings of; plots 1 and 3 (dotted), plots 2 and 4 (solid) and plots 5 and 6 (long-dash) and the two colors represent the CO_2 treatments of $a\text{CO}_2$ (blue) and $e\text{CO}_2$ (red).

the year of sampling and found no statistical difference of either V_{cmax} or J_{max} between the 3-sampling years (2015, 2017 and 2019; Figure 5, see Supplementary Table S2 available as Supplementary Data at *Tree Physiology Online*). This study found no significant effects of CO_2 enrichment on V_{cmax} or J_{max} across the 2 years of CO_2 enrichment, i.e., the 1st and 3rd years, and no significant effect of season between the 3 measurement years (Figure 5 and Table 3). However, this study did observe a significant effect of month for the variable V_{cmax} in 2019, whereby an increase in V_{cmax} was observed with progression of the growing season (Figure 5A and Table 3). Thus, this study observed no statistical evidence to suggest photosynthetic downregulation of either V_{cmax} or J_{max} under $e\text{CO}_2$ across the 3 years of $e\text{CO}_2$ exposure in *Q. robur*.

Consistent with previous research, this study observed a strong positive linear relationship between J_{max} and V_{cmax} , which remained unchanged across CO_2 treatments and growing season ($r^2 = 0.75$ ambient; $r^2 = 0.71$ elevated; see Supplementary Figure 3 available as Supplementary Data at *Tree*

Table 1. Linear mixed-effects model analysis for photosynthesis with CO_2 treatment (CO_2) using the diurnal dataset, sampling month (Month) and sampling year (Year) as fixed factors and random effects of 'plot' and 'time'. Type III sums of squares computed using restricted maximum likelihood estimates for F -tests. The numerator degrees of freedom (df) for each F -test are shown. A post-hoc Tukey test was used to determine the significance relationships. Significance of CO_2 treatment is noted in the rightmost column as (* = $P < 0.05$).

Parameter	df	P -value
CO_2	1	0.044*
Month	3	0.14
Year	1	0.31
$\text{CO}_2 * \text{Month}$	3	0.18
$\text{CO}_2 * \text{Year}$	1	0.18
Month * Year	3	0.43
$\text{CO}_2 * \text{Month} * \text{Year}$	3	0.079

Physiology Online). In addition, no $e\text{CO}_2$ -induced decreases in either area-based foliar nitrogen (N_a) or mass-based foliar nitrogen (N_m) were observed (Figure 5C and D and Table 3) across

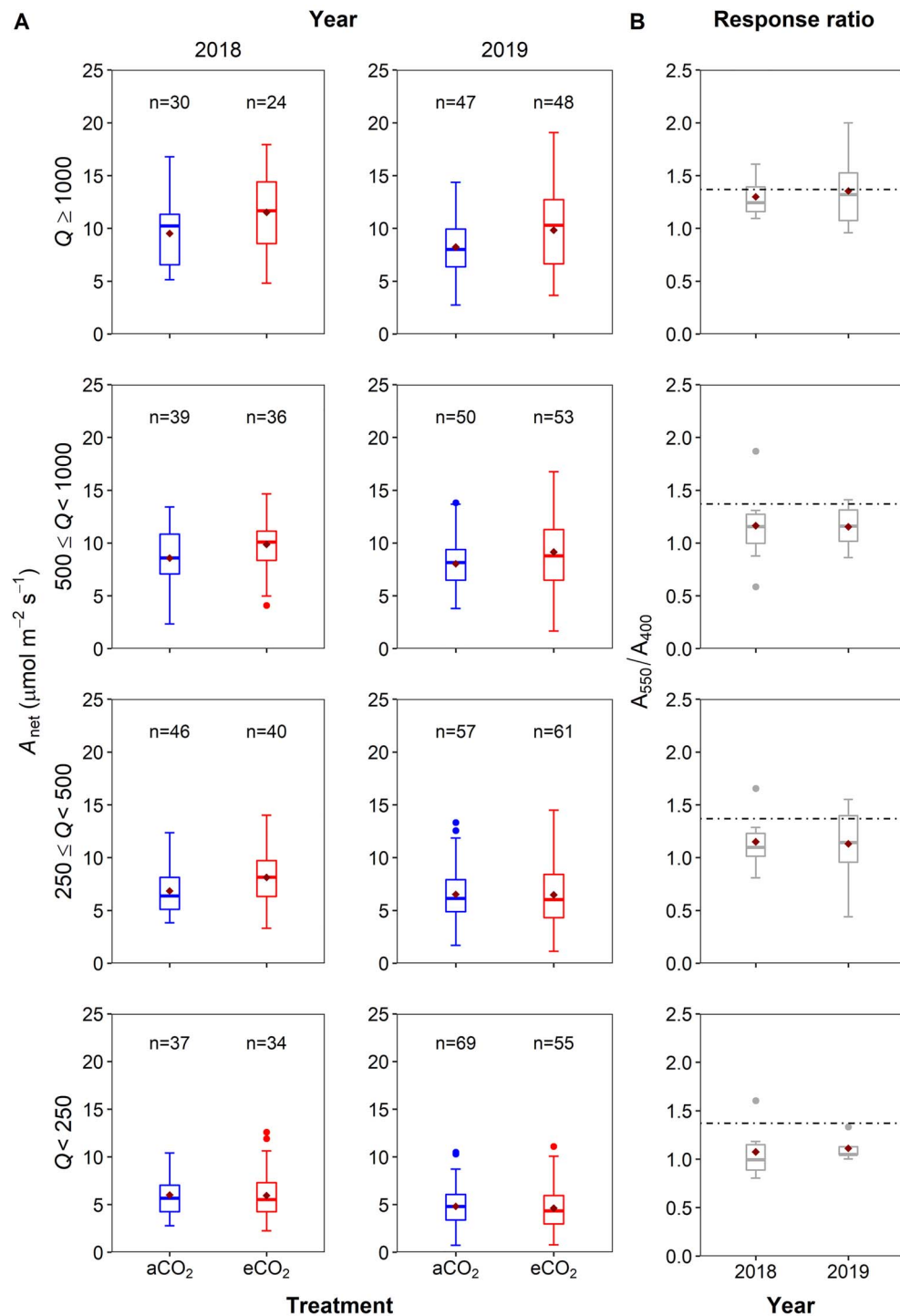


Figure 4. (A) The distribution of net photosynthesis (A_{net} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) in each of the four photon flux density (Q) categories ($Q < 250$; $250 \leq Q < 500$; $500 \leq Q < 1000$ and $Q \geq 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) for years 2018 (left) and 2019 (right). Whiskers denote the 5%ile and 95%ile; outliers are plotted as individual points (filled circles). The box denotes the interquartile range and the bar denotes the median with the number of data points above each boxplot. The mean is also plotted as a diamond symbol. Data use diurnal gas exchange measurements in the upper canopy oak trees at the BIFoR FACE facility with $T_{\text{leaf}} > 18^\circ\text{C}$ and $D < 2.2 \text{ kPa}$, in $e\text{CO}_2$ (red) or $a\text{CO}_2$ (blue) treatments. Red diamonds indicate the mean A_{net} values. (B) Boxplots of the enhancement response ratio (A_{550}/A_{400}) (gray) for each year, and predicted enhancement ratio (dashed line) (1.37) following Nowak et al. (2004).

the study period. No change in foliar nitrogen is corroborative of the results in Figure 5 and also suggests the absence of

photosynthetic downregulation under $e\text{CO}_2$ in mature *Q. robur* in the first 3 years of the long-term experiment.

Table 2. Linear mixed-effects model parameters for prediction of A_{net} with variation in photo flux density (Q). Type III sums of squares computed using restricted maximum likelihood estimates for F -tests. The numerator df for each F -test are shown. A post-hoc Tukey's test was used to determine the significance relationships. Significance is noted in bold in the rightmost column as (** = $P < 0.001$; * = $P < 0.01$ and * = $P < 0.05$).

Parameter	df	P -value
CO_2	1	0.016*
Year	1	0.062
Q	3	<0.001***
$\text{CO}_2 * \text{Year}$	1	0.97
$\text{CO}_2 * Q$	3	0.011*
$\text{Year} * Q$	3	0.0078**
$\text{CO}_2 * \text{Year} * Q$	3	0.13

The instantaneous response ratio (2015) and the longer-term response ratio (2017 and 2019) were calculated using the light-saturated A_{net} (i.e., A_{sat}) values at growth CO_2 from the A - C_i datasets (Figure 6B). There was no significant difference between the measurement years in either A_{sat} or the response ratio suggesting comparability between the instantaneous response ratio and the longer-term response ratio (see Supplementary Table S3 available as Supplementary Data at *Tree Physiology Online*). A significant treatment effect was observed for A_{sat} (Figure 6A and Table 3) in all 3-sampling years, with a mean eCO_2 -driven photosynthetic enhancement of $24 \pm 2\%$, $31 \pm 7\%$ and $32 \pm 11\%$ in 2015, 2017 and 2019, respectively, under eCO_2 when compared with aCO_2 . A significant effect of month on A_{sat} was observed in 2019, with A_{sat} increasing with the progression of the growing season (Table 3 and Figure 6A). The photosynthetic enhancement observed from our A - C_i curve datasets are consistent with the values obtained in the diurnal dataset ($33 \pm 8\%$, Figure 5) but is lower than the theoretical predicted enhancement calculated via CO_2 increase (37%) (see Supplemental Appendix 1 available as Supplementary Data at *Tree Physiology Online*). In summary, the consistency in the two separate measurements (i.e., diurnal and A - C_i curves) support the finding of sustained eCO_2 -driven photosynthetic enhancement in mature *Q. robur* across the first 3 years of the BIFoR FACE experiment.

Discussion

There are ample data on the short-term enhancement of photosynthesis by eCO_2 in young trees using a variety of experimental set-ups from tree chambers to FACE experiments (e.g., Ainsworth and Rogers 2007, Crous et al. 2008), but few data for mature forest-grown trees with multi-year CO_2 exposure in a FACE setting. For mature trees, available evidence suggests that there are significant increases in light-saturated A_{net} (Körner et al. 2005, Ellsworth et al. 2017) but there have

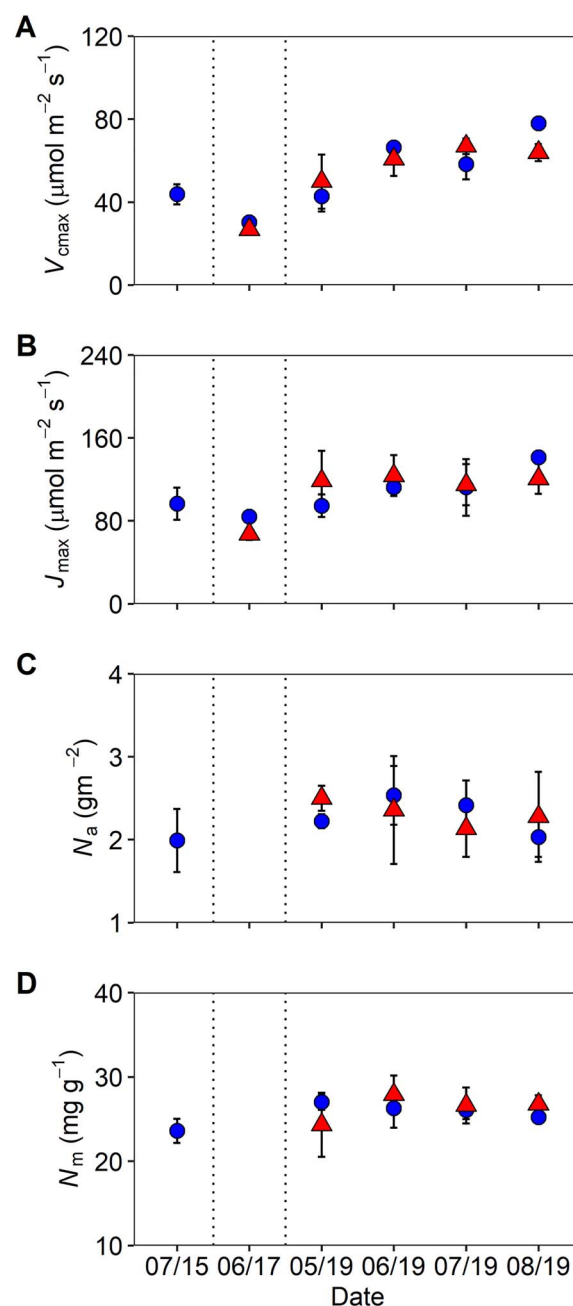


Figure 5. Maximum rates of (A) carboxylation (V_{cmax}) and (B) electron transport (J_{max}), in addition to (C) area-based (N_a) and (D) mass based (N_m) leaf nitrogen of upper canopy *Q. robur* from 2015 to 2019 at BIFoR FACE. Means (\pm SE) of whole-plot averages ($n = 3$) for ambient (blue circles) and elevated (red triangles) CO_2 treatments. Dashed line indicates the separation of sampling years with campaigns labelled 'month/year', as follows: Pre-treatment ('07/15'); 1st year ('06/17') and the 3rd year ('05/19'–'08/19') of CO_2 fumigation. Data points may obscure error bars.

been mixed results regarding the magnitude of photosynthetic enhancement (range 13–49% per 100 ppm of CO_2 increase) and occurrence of photosynthetic downregulation in mature forest-grown trees (Crous et al. 2008, Bader et al. 2010,

Table 3. Linear mixed-effects model analysis for V_{cmax} , J_{max} , net photosynthesis (A_{net}), area-based leaf nitrogen (N_a) and mass-based leaf nitrogen (N_m) with CO₂ treatment (CO₂) and sampling month (month) as fixed factors and random effects of 'plot' and 'time'. Type III sums of squares computed using restricted maximum likelihood estimates for F -tests. The numerator df for each F -test are shown. Significance is noted in boldface as (* $P < 0.05$).

Parameter	df	V_{cmax} P -value	J_{max} P -value	A_{net} P -value	N_m P -value	N_a P -value
CO ₂	1	0.70	0.37	0.042*	0.42	0.64
Month	3	0.02*	0.15	0.034*	0.93	0.052
CO ₂ * Month	3	0.20	0.57	0.33	0.69	0.11

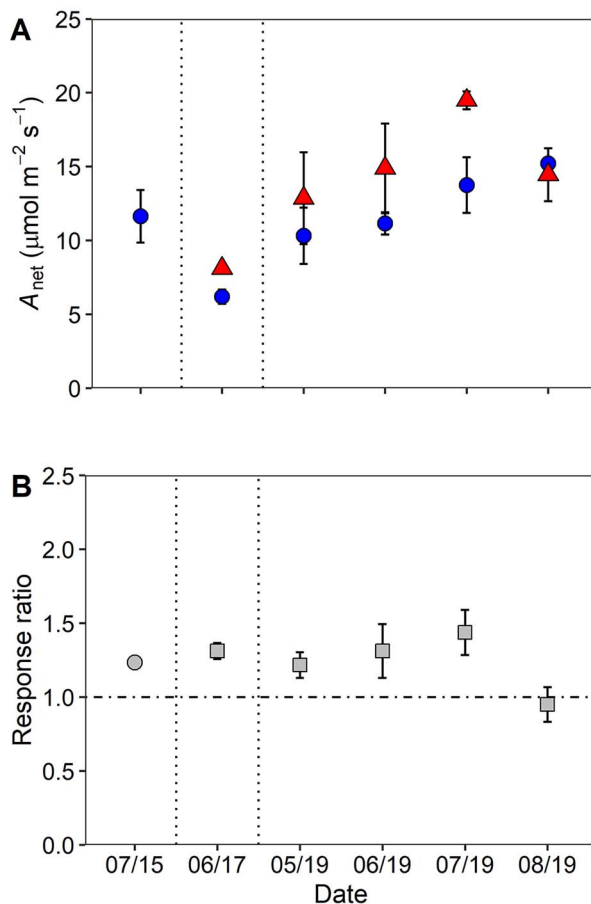


Figure 6. (A) Net photosynthesis (A_{net}) at growth CO₂ and (B) instantaneous (2015) and longer-term (2017 and 2019) response ratios in the upper oak canopy using the A - C_i curve data. Means (\pm SD) of the plots per treatment are shown across six sampling campaigns for aCO₂ (blue circles), eCO₂ (red triangles) and either the instantaneous (gray squares) or longer-term response ratio (grey circles). Dashed line indicate the separation of sampling years with campaigns labelled as follows; pre-treatment ('07/15'), 1st year ('06/17') and the 3rd year ('05/19'–'08/19') of CO₂ fumigation.

2016, Warren et al. 2015, Ellsworth et al. 2017). In this study, we predicted a theoretical A_{net} enhancement of 37% for the 150 $\mu\text{mol mol}^{-1}$ increase in CO₂ at BIFoR FACE following reasoning in Nowak et al. (2004; see Supplemental Appendix 1 available as Supplementary Data at *Tree Physiology Online*).

After 3 years of eCO₂ exposure in mature temperate oak forest, net photosynthetic rates of upper canopy foliage from *Q. robur* were on average $23 \pm 4\%$ higher, based on the diurnal dataset, in the trees exposed to eCO₂ when compared with control plots (Figures 2–4; Tables 1 and 3). The eCO₂-driven photosynthetic enhancement observed is substantially lower than the theoretical expected enhancement of 37%, likely due to diurnal and seasonal variation in prevailing environmental conditions such as lower air temperatures, lower light conditions and varying vapor pressure deficits. Only considering light-saturated A_{net} (A_{sat}) from the diurnal dataset, our mean photosynthetic enhancement is greater than the average diurnal enhancement, at $33 \pm 8\%$ rather than 23%. Furthermore, our independent estimate of A_{sat} enhancement based on the A - C_i curve data is $32 \pm 11\%$, which is comparable within error (using 95% confidence intervals) to both the A_{sat} value from the diurnal measurements and the hypothesized enhancement of 37%. A slight stomatal closure in eCO₂ could have contributed to the slightly lower photosynthetic enhancement than the hypothesized enhancement of 37% (see Supplemental Appendix 1 available as Supplementary Data at *Tree Physiology Online*). However, our average light-saturated photosynthetic enhancement is generally lower than previously reported values in canopy-dominant trees from other forest FACE experiments (Bader et al. 2010, 42–48%; Crous et al. 2008, 40–68%; Liberloo et al. 2007, 49% and Sholtis et al. 2004, 44%), but is somewhat higher than the value of 19% from the EucFACE experiment on mature *Eucalyptus* trees (Ellsworth et al. 2017). The lower photosynthetic enhancement observed at EucFACE was likely due to lower nutrient availability compared with BIFoR (Crous et al. 2015), although there were other differences such as the tree species and prevailing temperatures that would also affect the magnitude of the photosynthetic enhancement.

The role of environmental conditions for photosynthetic enhancement

Consistent with our initial hypothesis, we observed significantly higher A_{net} and a 24% higher photosynthetic enhancement under the highest light conditions at BIFoR FACE (i.e., $Q > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared with the lowest light category. Thus, a negative linear relationship was observed for

both A_{net} and $e\text{CO}_2$ -induced photosynthetic enhancement with decreasing light levels. Our results are consistent with previous research on mature trees that observed an effect of light on the magnitude of CO_2 -driven stimulation of photosynthesis (Bader et al. 2016), suggesting variation in light should be considered when assessing the response to $e\text{CO}_2$. Consequently, the relationship of A_{net} and CO_2 treatment effect with light intensity is important when scaling upper canopy data both across diurnal periods of light limitation and extending to the whole canopy, of shaded and sunlit leaves, to avoid overestimating canopy-scale photosynthesis by temperate forests.

It has been previously suggested that larger photosynthetic enhancement may be expected in low light environments (Hättenschwiler 2001, Norby and Zak 2011). For example, deep shaded tree seedlings displayed greater photosynthetic gains than those in moderate shade (photosynthetic enhancement of 97% and 47%, respectively) with exposure to $e\text{CO}_2$ (Kitao et al. 2015). In light-limited environments, higher CO_2 concentrations can increase the apparent quantum yield and reduce the light compensation point leading to enhanced carbon uptake (Larcher, 2003, Kitao et al. 2015). Hättenschwiler (2001) found large interspecific variability and, in *Quercus*, that greater photosynthetic responses to CO_2 occurred under higher light when compared with low light. However, both Kitao et al. (2015) and Hättenschwiler (2001) studied tree seedlings in contrast to upper canopy leaves of a canopy-dominant species in the present study. Although shade leaves were not measured here, the results here from the top of the tree canopy provide an important benchmark for the magnitude of photosynthetic enhancement by $e\text{CO}_2$ in a mature oak forest.

In addition to light intensity, the photosynthetic response of *Q. robur* varied across the growing season, as has been observed in many other trees (Tissue et al. 1999, Rogers and Ellsworth 2002, Sholtis et al. 2004). Here, A_{sat} (derived from the $A-C_i$ dataset) in both CO_2 treatments increased about 50% from early in the season (May), to the middle of the season (July); yet, the relative response ratio to $e\text{CO}_2$ was stable throughout this period at 32%. In addition, when assessing the diurnal dataset, we found contrasting seasonal patterns between 2018 and 2019, with decreases in A_{net} across the growing season observed in 2018 compared with increases in A_{net} in 2019, likely due to drier and warmer conditions in 2018. Previous research has identified reductions in photosynthesis across the season is largely associated with drier conditions (Gunderson et al. 2002), which support the results observed in the present study. This suggests that the influence of soil water availability on the seasonal pattern in oak physiology is critical for determining seasonal C-uptake by mature forests and should be further investigated in mature *Q. robur* to improve longer term carbon-climate models (see Limousin et al. 2013).

Previous research has identified $e\text{CO}_2$ -driven photosynthetic responses observed in seedlings and saplings may not reflect

the photosynthetic responses of mature forest-grown trees (Hättenschwiler et al. 1997). The present study provided a unique opportunity to assess the $e\text{CO}_2$ -driven photosynthetic responses in 175-year-old canopy-dominant trees and found lower photosynthetic stimulation than the many previous studies on tree seedlings and younger trees (e.g., Curtis and Wang 1998, Sholtis et al. 2004, Ainsworth and Long 2005, Liberloo et al. 2007, Crous et al. 2008). The age dependency of CO_2 responsiveness to photosynthesis in trees (Turnbull et al. 1998, Wujeska-Klaue et al. 2019a), highlights the importance of long-term experiments, such as the present study and others in understanding potential variable responses across the lifetime of a tree, vital for accurate climate-carbon modeling of forests.

Did changes to photosynthetic capacity or leaf biochemistry occur under $e\text{CO}_2$?

In some studies, a time-dependent decline in the magnitude of $e\text{CO}_2$ -induced photosynthetic enhancement, i.e., photosynthetic downregulation, has been observed (Cure and Acock 1986, Gunderson and Wullschleger 1994). Here, we hypothesized that there may be reductions in V_{cmax} , J_{max} and leaf N, particularly in the 3rd year of $e\text{CO}_2$ exposure (Luo et al. 2004). Our analysis of the 86 $A-C_i$ curves collected in this experiment revealed no decrease in the rate of V_{cmax} or J_{max} , indicating that there were no significant changes in the photosynthetic capacity of *Q. robur* over the first 3 years of exposure to $e\text{CO}_2$. A lack of photosynthetic downregulation has also been found in similar seasonally deciduous species, including the closely related species *Quercus petraea* (Bader et al. 2010), in addition to *Liquidambar styraciflua*, *Populus spp.* and *Betula papyrifera* (Herrick and Thomas 2001, Sholtis et al. 2004, Liberloo et al. 2007, Uddling et al. 2009). An apparent lack of downregulation has also been observed in other mature forest-grown species (Bader et al. 2010, Ellsworth et al. 2017).

As nitrogen is required for the synthesis and maintenance of photosynthetic proteins, $e\text{CO}_2$ -driven photosynthetic downregulation has been associated with declines in foliar N (as reviewed in Medlyn et al. 1999) and soil N-limitations (e.g., Rogers and Ellsworth 2002, Crous et al. 2008, Warren et al. 2015). The current study on *Q. robur* did not find any changes in either mass- or area-based leaf nitrogen across the study period, indicating there are no reductions to photosynthetic capacity (Figure 5). This corroborates the findings from the V_{cmax} and J_{max} parameters, supporting the suggestion for sustained photosynthesis in *Q. robur* over the first 3 years of exposure to $e\text{CO}_2$. Hence, there were no changes to the ratio of J_{max} to V_{cmax} , indicating that the relationship between carboxylation and light-harvesting processes was not affected by CO_2 treatment, as found in previous studies (Medlyn et al. 1999, Crous et al. 2008), including the closely related species, *Q. petraea* (Bader

et al. 2010). These results may point to soil nutrient availability not yet limiting the photosynthetic processes in this forest system. The BIFoR FACE site receives moderately high atmospheric N deposition (~22 Kg N/ha/yr) thought to represent 15% of the total nitrogen nutrition of temperate deciduous trees, likely preventing ecosystem N-limitation at present (Rennenberg and Dannenmann 2015). Therefore, with adequate N deposition in the soil, sustained photosynthetic enhancement was observed in the first 3 years of eCO₂ exposure at BIFoR FACE.

Conclusions

After 3 years of eCO₂ exposure in a temperate deciduous forest at the BIFoR FACE facility, photosynthetic enhancement of mature *Q. robur* leaves at the top of the canopy was sustained across all years and was $33 \pm 8\%$ (mean \pm SE) at light saturation, close to the theoretical expectation. The magnitude of photosynthetic enhancement was significantly affected by light conditions with higher enhancement at higher light. We found no evidence of photosynthetic downregulation under eCO₂ and no declines in leaf nitrogen in the upper canopy. The lack of evidence for downregulation suggest there are sufficient soil nutrients for *Q. robur* to maintain a relatively high photosynthetic enhancement under eCO₂ conditions, at least to this point in the eCO₂ experiment. Much further work remains to determine the movement and allocation of this enhanced C-uptake in the forest. Our results are consistent with a sustained, positive C-uptake response to rising atmospheric CO₂ in a mature deciduous forest tree species, provided adequate nutrients are available.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Acknowledgments

We thank the BIFoR technical team for canopy access operations and Ian Boomer for technical support with leaf elemental analysis. AG gratefully thanks Agnieszka Wujeska-Klaue for guidance with statistical analysis in the early stages of the manuscript. AG gratefully acknowledges a studentship provided by the John Horseman Trust and the University of Birmingham. The BIFoR FACE facility is supported by the JABBS foundation, the University of Birmingham and the John Horseman Trust. ARMK acknowledges support from the Natural Environment Research Council through grant (NE/S015833/1) which also facilitated DSE's participation. We further gratefully acknowledge advice and field measurement collection in the first CO₂ fumigation season from Michael Tausz and Sabine Tausz-Pösch, respectively.

Conflict of interest

None declared.

Author contributions

ARMK, JP and AG designed the study; AG, KYC and DSE collected the data. AG organized the datasets under the supervision of DSE, with input from ARMK; AG and DSE designed and performed the statistical analyses, with input from KYC and ARMK. AG and DSE wrote the first draft of the paper. All authors contributed to the manuscript revision, and read and approved the submitted version.

References

- 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 Phytol* 165:351–372.
- Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ* 30:258–270.
- Ainsworth EA, Rogers A, Nelson R, Long SP (2004) Testing the “source-sink” hypothesis of down-regulation of photosynthesis in elevated [CO₂] in the field with single gene substitutions in *Glycine max*. *Agric For Meteorol* 122:85–94.
- Bader MKF, Leuzinger S, Keel SG, Siegwolf RTW, Hagedorn F, Schleppi P, Körner C (2013) Central european hardwood trees in a high-CO₂ future: synthesis of an 8-year forest canopy CO₂ enrichment project. *J Ecol* 101:1509–1519.
- Bader MKF, Mildner M, Baumann C, Leuzinger S, Körner C (2016) Photosynthetic enhancement and diurnal stem and soil carbon fluxes in a mature Norway spruce stand under elevated CO₂. *Environ Exp Bot* 124:110–119.
- Bader MKF, Siegwolf R, Körner C (2010) Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO₂ enrichment. *Planta* 232:1115–1125.
- Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* 320:1444–1449.
- Brodribb TJ, Powers J, Cochard H, Choat B (2020) Hanging by a thread? Forests and drought. *Science* 368:261–266.
- Cox PM, Pearson D, Booth BB, Friedlingstein P, Huntingford C, Jones CD, Luke CM (2013) Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. *Nature* 494:341–344.
- Crous KY, Ellsworth DS (2004) Canopy position affects photosynthetic adjustments to long-term elevated CO₂ concentration (FACE) in aging needles in a mature *Pinus taeda* forest. *Tree Physiol* 24:961–970.
- Crous KY, Ósváldsson A, Ellsworth DS (2015) Is phosphorus limiting in a mature eucalyptus woodland? Phosphorus fertilisation stimulates stem growth. *Plant and Soil* 391:293–305.
- Crous KY, Reich PB, Hunter MD, Ellsworth DS (2010) Maintenance of leaf N controls the photosynthetic CO₂ response of grassland species exposed to 9 years of free-air CO₂ enrichment. *Glob Chang Biol* 16:2076–2088.
- Crous KY, Walters MB, Ellsworth DS (2008) Elevated CO₂ concentration affects leaf photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE. *Tree Physiol* 28:607–614.
- Cure JD, Acock B (1986) Crop responses to carbon dioxide doubling: a literature survey. *Agric For Meteorol* 38:127–145.

- Curtis PS, Wang X (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia* 113:299–313.
- Duursma RA (2015) Plantecophys - an R package for analysing and modelling leaf gas exchange data. *PLoS One* 10:e0143346. <https://doi.org/10.1371/journal.pone.0143346>.
- Eaton E, Caudullo G, Oliveira S, de Rigo D (2016) *Quercus robur* and *Quercus petraea* in Europe: distribution, habitat, usage and threats. In: European Atlas of Forest Tree Species, Joint Research Centre (European Commission), Brussels, pp. 160–163. ISBN 978-92-79-36740-3, DOI: [10.2788/4251](https://doi.org/10.2788/4251).
- Ellsworth DS, Anderson IC, Crous KY et al. (2017) Elevated CO₂ does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate Change* 7:279–282.
- Ellsworth DS, Thomas R, Crous KY, Palmroth S, Ward E, Maier C, Delucia E, Oren R (2012) Elevated CO₂ affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. *Glob Chang Biol* 18: 223–242.
- Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C3 species. *Planta* 149:78–90.
- Feng Z, Rütting T, Pleijel H, Wallin G, Reich PB, Kammann CI, Newton PCD, Kobayashi K, Luo Y, Uddling J (2015) Constraints to nitrogen acquisition of terrestrial plants under elevated CO₂. *Glob Chang Biol* 21:3152–3168.
- Friedlingstein P, Betts R, Bopp L et al. (2006) Climate –carbon cycle feedback analysis, results from the C4MIP model intercomparison. *J Climate* 19:3337–3353.
- Friedlingstein P, Jones MW, O'Sullivan MA et al. (2019) Global carbon budget 2019. *Earth Syst Sci Data* 11:1783–1838.
- Griffin KL, Tissue DT, Turnbull MH, Whitehead D (2000) The onset of photosynthetic acclimation to elevated CO₂ partial pressure in field-grown *Pinus radiata* D. don. After 4 years. *Plant Cell Environ* 23:1089–1098.
- Gunderson CA, Sholtis JD, Wulschleger SD, Tissue DT, Hanson PJ, Norby RJ (2002) Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO₂ enrichment. *Plant Cell Environ* 25:379–393.
- Gunderson CA, Wulschleger SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO₂: a broader perspective. *Photosynth Res* 39:369–388.
- Hart KM, Curioni G, Blaen P et al. (2020) Characteristics of free air carbon dioxide enrichment of a northern temperate mature forest. *Glob Chang Biol* 26:1023–1037.
- Hättenschwiler S (2001) Tree seedling growth in natural deep shade: functional traits related to interspecific variation in response to elevated CO₂. *Oecologia* 129:31–42.
- Hättenschwiler S, Miglietta F, Raschi A, Körner C (1997) Thirty years of in situ tree growth under elevated CO₂: a model for future forest responses? *Glob Chang Biol* 3:463–471.
- Hendrey G, Ellsworth D, Lewin K, Nagy J (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Glob Chang Biol* 5:293–309.
- Herrick JD, Thomas RB (2001) No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO₂ enrichment at the Duke Forest Face experiment. *Plant Cell Environ* 24:53–64.
- Jiang M, Medlyn BE, Drake JE et al. (2020) The fate of carbon in a mature forest under carbon dioxide enrichment. *Nature* 580:227–231.
- Jones CD, Ciais P, Davis SJ et al. (2016) Simulating the Earth system response to negative emissions. *Environ Res Lett* 11:095012. <https://doi.org/10.1088/1748-9326/11/9/095012>.
- Keenan T, Prentice I, Canadell J et al. (2016) Recent pause in the growth rate of atmospheric CO₂ due to enhanced terrestrial carbon uptake. *Nat Commun* 7:13428. <https://doi.org/10.1038/ncomms13428>.
- Kitao M, Hida T, Eguchi N, Tobita H, Utsugi H, Uemura A, Kitaoka S, Koike T (2015) Light compensation points in shade-grown seedlings of deciduous broadleaf tree species with different successional traits raised under elevated CO₂. *Plant Biology* 18:31–42.
- Klein T, Bader MKF, Leuzinger S, Mildner M, Schleppi P, Siegwolf RTW, Körner C (2016) Growth and carbon relations of mature *Picea abies* trees under 5 years of free-air CO₂ enrichment. *J Ecol* 104:1720–1733.
- Körner C (2017) A matter of tree longevity. *Science* 355:130–131.
- Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zott G (2005) Ecology: carbon flux and growth in mature deciduous forest trees exposed to elevated CO₂. *Science* 309:1360–1362.
- Larcher W (2003) *Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups*. Springer-Verlag, New York. <https://doi.org/10.1007/978-3-662-05214-3>.
- Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J Exp Bot* 60:2859–2876.
- Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R (2007) Photosynthetic stimulation under long-term CO₂ enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). *New Phytol* 173:537–549.
- Limousin JM, Bickford CP, Dickman LT, Pangle RE, Hudson PJ, Boutz AL, Gehres N, Osuna JL, Pockman WT, McDowell NG (2013) Regulation and acclimation of leaf gas exchange in a piñon-juniper woodland exposed to three different precipitation regimes. *Plant Cell Environ* 36:1812–1825.
- Luo Y, Su B, Currie WS et al. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bio Sci* 54:731–739.
- Luyssaert S, Schulze ED, Börner A, Knohl A, Hessenmöller D, Law BE, Ciais P, Grace J (2008) Old-growth forests as global carbon sinks. *Nature* 455:213–215.
- MacKenzie R, Krause S, Hart K et al. (2021) BIFoR FACE: water-soil-vegetation-atmosphere research in a temperate deciduous forest catchment, including under elevated CO₂. *Hydrol Process* 35:e14096. <https://doi.org/10.1002/hyp.14096>.
- Medlyn BE, Badeck FW, De Pury DGG et al. (1999) Effects of elevated [CO₂] on photosynthesis in European forest species: a meta-analysis of model parameters. *Plant Cell Environ* 22:1475–1495.
- Medlyn BE, Zaehle S, De Kauwe MG et al. (2015) Using ecosystem experiments to improve vegetation models. *Nat Clim Change* 5:528–534.
- Mölder A, Meyer P, Nagel RV (2019) Integrative management to sustain biodiversity and ecological continuity in Central European temperate oak (*Quercus robur*, *Q. petraea*) forests: an overview. *For Ecol Manage* 437:324–339.
- Norby RJ, De Kauwe MG, Domingues TF et al. (2016) Model-data synthesis for the next generation of forest free-air CO₂ enrichment (FACE) experiments. *New Phytol* 209:17–28.
- Norby RJ, Zak DR (2011) Ecological and evolutionary lessons from free air carbon enhancement (FACE) experiments. *Annu Rev Ecol Evol Syst* 42:181–203.
- 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 Phytol* 162:253–280.
- Ostle NJ, Levy PE, Evans CD, Smith P (2009) UK land use and soil carbon sequestration. *Land Use Policy* 26:S274–S283. <https://doi.org/10.1016/j.landusepol.2009.08.006>.

- Pan Y, Birdsey RA, Fang J et al. (2011) A large and persistent carbon sink in the World's forests. *Science* 333:4. <http://science.sciencemag.org/content/333/6045/988#BIBL>.
- Parsons R, Weyers JDB, Lawson T, Godber IM (1998) Rapid and straightforward estimates of photosynthetic characteristics using a portable gas exchange system. *Photosynthetica* 34:265–279.
- Piao S, Sitch S, Ciais P et al. (2013) Evaluation of terrestrial carbon cycle models for their response to climate variability and to CO₂ trends. *Glob Chang Biol* 19:2117–2132.
- Rennenberg H, Dannenmann M (2015) Nitrogen nutrition of trees in temperate forests-the significance of nitrogen availability in the pedosphere and atmosphere. *Forests* 6:2820–2835.
- Rogers A, Ellsworth DS (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO₂ (FACE). *Plant Cell Environ* 25:851–858.
- Sage RF, Way DA, Kubien DS (2008) Rubisco, rubisco activase, and global climate change. *J Exp Bot* 59:1581–1595.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant Cell Environ* 30:1035–1040.
- Sholtis JD, Gunderson CA, Norby RJ, Tissue DT (2004) Persistent stimulation of photosynthesis by elevated CO₂ in a sweetgum (*Liquidambar styraciflua*) forest stand. *New Phytol* 162:343–354.
- Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S (2013) Growth of mature boreal Norway spruce was not affected by elevated [CO₂] and/or air temperature unless nutrient availability was improved. *Tree Physiol* 33:1192–1205.
- Sommerfeld A, Senf C, Buma B et al. (2018) Patterns and drivers of recent disturbances across the temperate forest biome. *Nat Commun* 9:4355. <https://doi.org/10.1038/s41467-018-06788-9>.
- Terrer C, Jackson RB, Prentice IC et al. (2019) Nitrogen and phosphorus constrain the CO₂ fertilization of global plant biomass. *Nature Climate Change* 9:684–689.
- Tissue DT, Griffin KL, Ball JT (1999) Photosynthetic adjustment in field-grown ponderosa pine trees after six years of exposure to elevated CO₂. *Tree Physiol* 19:221–228.
- Turnbull MH, Tissue DT, Griffin KL, Rogers GND, Whitehead D (1998) Photosynthetic acclimation to long-term exposure to elevated CO₂ concentration in *Pinus radiata* D. don. Is related to age of needles. *Plant Cell Environ* 21:1019–1028.
- Uddling J, Teclaw RM, Pregitzer KS, Ellsworth DS (2009) Leaf and canopy conductance in aspen and aspen-birch forests under free-air enrichment of carbon dioxide and ozone. *Tree Physiol* 29:1367–1380.
- Valentini R, Epron D, De Angelis P, Matteucci G, Dreyer E (1995) In situ estimation of net CO₂ assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. *Plant Cell Environ* 18: 631–640.
- Verryckt LT, Van Langenhove L, Ciais P et al. (2020) Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest. *Biotropica* 52:608–615.
- Warren JM, Jensen AM, Medlyn BE, Norby RJ, Tissue DT (2015) Carbon dioxide stimulation of photosynthesis in *Liquidambar styraciflua* is not sustained during a 12-year field experiment. *AoB PLANTS* 7:plu074. <https://doi.org/10.1093/aobpla/plu074>.
- Wujeska-Klaus A, Crous KY, Ghannoum O, Ellsworth DS (2019a) Leaf age and eCO₂ both influence photosynthesis by increasing light harvesting in mature *Eucalyptus tereticornis* at EucFACE. *Environ Exp Bot* 167:103857. <https://doi.org/10.1016/j.envexpbot.2019.103857>.
- Wujeska-Klaus A, Crous KY, Ghannoum O, Ellsworth DS (2019b) Lower photorespiration in elevated CO₂ reduces leaf N concentrations in mature eucalyptus trees in the field. *Glob Chang Biol* 25:1282–1295.
- Zhu Z, Piao S, Myneni RB et al. (2016) Greening of the earth and its drivers. *Nature Climate Change* 6:791–795.