



Carbon dioxide enrichment affected flower numbers transiently and increased successful post-pollination development stably but without altering final acorn production in mature pedunculate oak (*Quercus robur* L.)

Ryan McClory¹ · Richard H. Ellis¹ · Martin Lukac^{1,2} · Jo Clark³ · Carolina Mayoral^{4,5,6} · Kris M. Hart⁴ · Andrew R. G. Plackett^{4,5} · A. Rob MacKenzie^{4,6}

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Abstract Acorn production in oak (*Quercus* spp.) shows considerable inter-annual variation, known as masting, which provides a natural defence against seed predators but a highly-variable supply of acorns for uses such as in commercial tree planting each year. Anthropogenic emissions of greenhouse gases have been very widely reported to influence plant growth and seed or fruit size and quantity via the ‘fertilisation effect’ that leads to enhanced photosynthesis. To examine if acorn production in mature woodland communities will be affected by further increase

in CO₂, the contents of litter traps from a Free Air Carbon Enrichment (FACE) experiment in deciduous woodland in central England were analysed for numbers of flowers and acorns of pedunculate oak (*Quercus robur* L.) at different stages of development and their predation levels under ambient and elevated CO₂ concentrations. Inter-annual variation in acorn numbers was considerable and cyclical between 2015 and 2021, with the greatest numbers of mature acorns in 2015, 2017 and 2020 but almost none in 2018. The numbers of flowers, enlarged cups, immature acorns, empty acorn cups, and galls in the litter traps also varied amongst years; comparatively high numbers of enlarged cups were recorded in 2018, suggesting *Q. robur* at this site is a fruit maturation masting species (i.e., the extent of abortion of pollinated flowers during acorn development affects mature acorn numbers greatly). Raising the atmospheric CO₂ concentration by 150 μL L⁻¹, from early 2017, increased the numbers of immature acorns, and all acorn evidence (empty cups + immature acorns + mature acorns) detected in the litter traps compared to ambient controls by 2021, but did not consistently affect the numbers of flowers, enlarged cups, empty cups, or mature acorns. The number of flowers in the elevated CO₂ plots’ litter traps was greater in 2018 than 2017, one year after CO₂ enrichment began, whereas numbers declined in ambient plots. Enrichment with CO₂ also increased the number of oak knopper galls (*Andricus quercuscalicis* Burgsdorf). We conclude that elevated CO₂ increased the occurrence of acorns developing from flowers, but the putative benefit to mature acorn numbers may have been hidden by excessive pre- and/or post-dispersal predation. There was no evidence that elevated CO₂ altered masting behaviour.

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✉ Richard H. Ellis
r.h.ellis@reading.ac.uk

- ¹ School of Agriculture, Policy and Development, University of Reading, Agriculture, Earley Gate, Whiteknights Road, PO Box 237, Reading RG6 6EU, UK
- ² Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague 165 000, Czechia
- ³ Future Trees Trust, Harwell Innovation Centre, Building 173, Curie Avenue, Harwell, Oxford OX11 0QG, UK
- ⁴ Birmingham Institute of Forest Research, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
- ⁵ School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
- ⁶ School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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Introduction

Cumulative net anthropogenic carbon dioxide (CO₂) emissions have amounted to 2400 ± 240 Gt since 1850, 42% of this between 1990 and 2019, raising atmospheric CO₂ concentrations to a global annual average of 410 parts per million in 2019 (IPCC 2023). These emissions together with those of other greenhouse gases increased global surface temperatures by 1.1 °C above 1850–1900 values in the period 2011–2020, with anthropogenic climate change affecting every region of our planet (IPCC 2023). Anthropogenic-induced climate change has well-reported effects and will also have future impacts on forest survival and composition (Flannigan et al. 2000; Ibáñez et al. 2006; Sturrock et al. 2011; Khaine and Woo 2015). It is also established that increased CO₂ will have a direct fertilization effect (Zhu et al. 2016; Ruehr et al. 2023). Under elevated CO₂, plants show increased photosynthetic rates and decreased CO₂ loss via photorespiration, along with a reduced stomatal conductance that results in increased water use efficiency (Drake et al. 1997; Long et al. 2004; Gardner et al. 2022a). Plants may acclimate to greater CO₂ to some extent over time, the degree of acclimation varying with temperature and leaf nitrogen content, but nonetheless a benefit to assimilate production is retained (Wheeler et al. 2004). This CO₂ fertilisation effect leads to increased plant growth (Ainsworth and Long 2005, 2021; De Graaff et al. 2006), induces alterations in plant structure (Pritchard et al. 1999), and affects reproductive outcomes by increasing flowers and seed production, although the strength of these responses vary amongst species (Jablonski et al. 2002). The literature on the effects of differences in CO₂ concentration on plants tends to rely greatly on small-scale studies of individual plants or closely-confined experiments (Wand et al. 1999; Poorter and Navas 2003). This has begun to change since the advent of Free Air Carbon Enrichment (FACE) experiments (Hendrey et al. 1999; De Graaff et al. 2006; DOE 2020), in which CO₂ levels are elevated via a method of open-air CO₂ enrichment (Ainsworth and Long 2021) within the field environment. Whilst FACE experiments have mostly agreed with prior findings of elevated CO₂ under experimental conditions in many species (Kimball et al. 2002; Long et al. 2004), the advantage of FACE is that it subjects intact ecosystem patches to elevated CO₂, so that it is testing a community response rather than the response of (often potted) plants. Hence, FACE experiments are important extensions to work on potted plants not just because of the size of trees but also because all the biotic and abiotic drivers are in play simultaneously.

However, such studies with elevated CO₂ are difficult to perform on mature trees due to their large size, and so there is a need for further research on the topic.

Fruit and seed production by plants is a resource-intensive process that uses large amounts of carbohydrates derived from photosynthesis, often competing with vegetative growth (Obeso 2002). Many long-lived perennial plants employ a reproductive strategy known as "masting" defined, in part, by large inter-annual variation in flowers or seeds/fruits produced (Kelly 1994). One of several mechanistic controls of masting in oak (*Quercus* spp.) has previously been attributed to variations in the rates of fruit maturation or abortion rather than the quantity of flowers produced (Pearse et al. 2016; Hackett-Pain 2021). Recent research, however, suggests that masting species may practice different adaptive strategies of either "fruit maturation" or "flower masting", with the prevalence of one strategy over the other dependent on the environmental conditions of the site (Fleuret et al. 2023). Masting is hypothesized to have evolved as a predator satiation strategy and may be mechanistically controlled by resource dynamics (Isagi et al. 1997; Koenig and Knops 2005). The significance of fruit production in mature communities for tree demography and the maintenance of food webs makes it important to determine the effects of elevated CO₂ on seed production in masting species empirically. An increase in baseline seed production could ultimately result in lower plant fitness if seed supply is surplus to requirement for successful propagation, preventing the herbivore limiting effects of masting's seed predator satiation—starving cycle (Bogdziewicz et al. 2020). On the other hand, a decline in seed production may damage natural regeneration or reduce seed supply for human-managed regeneration projects (Bole 2022).

Isotope studies with several masting tree species have concluded that 100% of the carbon resources for fruiting are provided by fresh photosynthate—rather than from stored carbon resources—and so elevated CO₂ may influence tree fruiting promptly and directly (Hoch et al. 2013). Experimental evidence supports this suggestion. One of the few studies reported on this topic was performed on a plantation of 13-year-old Loblolly pine (*Pinus taeda* L.) at the Duke Forest FACE site, North Carolina, USA, in which the numbers of cones increased up to three fold in individual trees under elevated CO₂ (+200 µL L⁻¹) compared to ambient CO₂ (LaDeau and Clark 2001; Way et al. 2010). Similarly, in the Aspen FACE site in Rhineland, Wisconsin, USA, flower numbers in 10-year-old paper birch (*Betula papyrifera* Marshall) trees grown from seedlings under elevated CO₂ (+200 µL L⁻¹) were increased by up to 100% and 260% in each of two years compared to the control group, while seed mass was only increased in the first year of the experiment (Darbah et al. 2008). In agreement with the above, modelling studies have linked elevated CO₂ to a stronger

influence on multi-decadal increases in flowering rates in tropical species (Pau et al. 2018). Similarly, an in situ growth chamber study conducted on a mature scrub oak community (*Quercus myrtifolia* Willd., *Q. chapmanii* Sarg., and *Q. geminata* Small) saw increases in acorn production with elevated CO₂ (+ 325 μL L⁻¹) but only for the dominant species in the environment (Stiling et al. 2004). On the other hand, it has also been hypothesised that trees may acclimatise to elevated CO₂ over time, presenting down-regulation of photosynthesis (Ainsworth and Long 2005); mineral nutrients might also become limiting over the longer term (Hoch et al. 2013; Palacio et al. 2014). To date, the few FACE studies examining the effect of elevated CO₂ upon the reproductive behaviour of masting trees have been performed on comparatively young trees; < 19 years old (LaDeau and Clark 2001; Darbah et al. 2008; Way et al. 2010), but not mature communities. Exposing an established tree community to a step-change in atmospheric CO₂ is unlikely to have the same effect on propagule production as in trees grown under elevated CO₂ as juveniles. Whether the masting behaviour of mature trees—typically slower-growing and with greater internal nutrient reserves—will respond differently to elevated CO₂ or not remains unknown.

To address this question we investigated the influence of increased atmospheric CO₂ concentration on flower and acorn production of the masting species (Askeyev et al. 2005; Wesolowski et al. 2015) pedunculate oak (*Quercus robur* L.). Reproductive material was recorded from litter traps over seven years within the Birmingham Institute of Forest Research (BIFoR) Free-Air Carbon Enrichment (FACE) facility, in which mature trees were exposed to elevated CO₂. We tested the null hypotheses that counts of each of the number of flowers, enlarged cups, immature acorns, mature acorns, empty cups, and galls within the litter traps were unaffected by year, by CO₂ treatment, or by the interaction of these factors.

Materials and methods

Study site

The Birmingham Institute of Forest Research (BIFoR) has maintained a Free-Air Carbon Enrichment (FACE) facility at Mill Haft, Staffordshire, UK (52°48'3.6" N, 2°18'0" W) since 2015, with CO₂ treatments beginning in April 2017. Mill Haft is a 19.1 ha deciduous woodland in a temperate maritime climate; the woodland is dominated by *Q. robur* L., planted around 1850, in the upper canopy and *Corylus avellana* L. in the understorey (Hart et al. 2019; MacKenzie et al. 2021) alongside self-seeded *Acer pseudoplatanus* L. and *Crataegus monogyna* Jacq. of varying ages.

The site contains three experimental treatments divided equally across nine experimental areas, each approximately 30 m in diameter. These comprise of three 'elevated CO₂' arrays maintained at + 150 μL L⁻¹ above ambient CO₂ (eCO₂), three control arrays at ambient CO₂ (aCO₂) which blow ambient air collected and redistributed from the site, and three undisturbed woodland areas (i.e., no CO₂ array infrastructure, uCO₂). The FACE arrays have operated during daylight hours throughout the growing season from initial budburst to leaf fall (early April to late October) and the CO₂ treatments since early April 2017 to the present.

There is significant spatial variability in soil volumetric water content at the site, with the undisturbed patches (uCO₂) considerably wetter than the other two treatment groups (MacKenzie et al. 2021). Soil pH and phosphate contents for the treatment arrays aCO₂ and eCO₂ (only), recorded once in 2021, were broadly similar with the aCO₂ array having slightly more phosphate but not significantly so (aCO₂: phosphate = 2.63 ± 0.96 μg PO₄ P/g, pH = 4.41 ± 0.05. eCO₂: phosphate = 1.36 ± 0.48 μg PO₄ P/g, pH = 4.26 ± 0.5). For further details of the site and the long-term experiment see Hart et al. (2019).

Data collection

Three litter traps each 1 m² were placed within each of the nine arrays from 2015. This was changed to six litter traps of 0.25 m² per array from 2020 with no detectable impact on amounts per unit area (see below). The litter traps were in place all year round and their contents were collected at least once a month during acorn fall between August and October each year. The *Q. robur* reproductive material collected from litter traps from 2015 to 2021 was separated, classified, counted, and totalled within each year. The reproductive material was classified into six categories encompassing acorn development and predation: female flowers (unpollinated or aborted flowers with no visible acorn development); enlarged cups (swollen cups and visible premature acorns); immature acorns (immature acorns with length < 14 mm and diameter < 7 mm); mature acorns (fully mature acorns); empty cups (large empty acorn cups with acorns missing); and galls (acorn development prevented by insect attack). A seventh category was calculated for all evidence of acorns. This combined observations for immature and mature acorns with an estimate of seed predation (i.e., empty cups) and provided a best estimate of total acorn numbers because empty cups are often all that remains after post-dispersal seed predation (Martínez-Baroja et al. 2019). To allow comparison between years in which trap number and size differed all data was standardised to the amount of reproductive material per 1 m² of litter trap area. To do this, counts from litter traps of 0.25 m² were multiplied by four (comparable to traps of 1 m²), and these and those of 1 m² were averaged by

the number of litter traps in each array ($n=3$ or 6) to give the average count per 1 m^2 . The means for each collection date were then added to give the total amount of reproductive material produced by each array per 1 m^2 across the whole year, with three arrays in each treatment ($n=3$).

Data analyses

All analyses were performed using R statistical software (R Core Team 2021). Model assumptions were tested with the *ggrplot* and *rootogram* functions in the package ‘coun-*tre*g’ and by examining diagnostic plots from the functions *plotQQunif* and *plotResiduals* in the package ‘DHARMA’. The best-fitting models were decided by comparing models’ Akaike Information Criterion (AIC) scores (Akaike 1998) and by comparing models via the *vuong* function and package. After the most appropriate family of model had been determined, the selection of explanatory variables to include were decided by AIC scores. The best fits were provided by Negative Binomial Distribution models in all cases, with the explanatory variables Treatment (i.e., array type) and Year, and these are presented here.

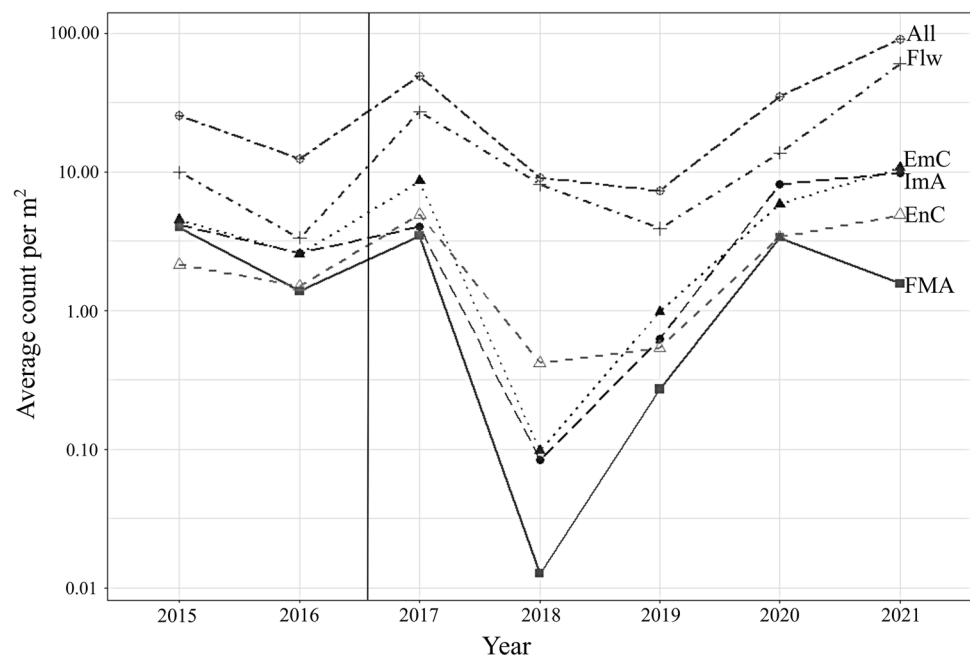
Negative Binomial Models were built with the *glm.nb* function in the package ‘MASS’. Initial models, with the factors Treatment and Year and the Treatment \times Year interaction, were built separately for each of the seven categories of acorn development and predation as the response variables. The function *Anova* from the ‘car’ package was used as an omnibus test to see if there was a significant difference in reproductive material amongst treatments. If the default Anova (type 2) provided a significant interaction, then the model was run again as a type 3. The latter is preferred in

the presence of a significant interaction (Langsrud 2003). Where significant differences were detected, post-hoc Tukey pairwise comparisons were conducted via the *emmeans* model function of the ‘emmeans’ package. To generate a baseline comparison, analyses of observations for the two years before the treatments began (2015 and 2016) were made. Further, as site conditions were found to be quite heterogeneous, especially of the ‘undisturbed’ plots with high soil volumetric water content, statistical analyses were also re-run with only the two treatments eCO₂ and aCO₂ (Supplementary Table 1).

Results

All categories of *Q. robur* reproductive material counted from the litter traps showed inter-annual variation, with high synchrony among the five reproductive material groupings (Fig. 1). Flower counts were consistently the most abundant category, whereas the other reproductive material varied in their rank order between groupings (Fig. 1). The lowest year for acorn production at Mill Haft was 2018 (Fig. 1). This was the case for all categories of acorn development (enlarged cups, immature acorns, mature acorns), and also for empty cups. Nevertheless, large numbers of flowers (aborted flowers with no visible acorn development) were collected in that low acorn production year, and post-hoc pairwise comparisons showed that flower numbers in 2018 were not significantly different from those during the past years in 2015 ($P=0.99$) nor 2020 ($P=0.54$). These results demonstrate that masting behaviour was captured at the experimental site over the study period.

Fig. 1 Variation in counts (logarithmic scale) of oak reproductive material from litter traps (mean of all three CO₂ treatments) at the BIFoR FACE facility, Mill Haft, across seven years (CO₂ treatments were provided from early April 2017 [vertical solid black line]): fully mature acorns (solid line, ■, FMA), immature acorns (long-dashed line, ●, ImA), empty cups (dotted line, ▲, EmC), enlarged cups (short-dashed line, △, EnC), flowers (dot-dash line, +, Flw), and all the reproductive material combined (two-dash line, □, All)



To test whether greater CO₂ might either enhance, or mitigate the phenomenon of masting, the relative difference in mature acorn counts under eCO₂ and aCO₂ each year was regressed against the number in aCO₂, viz. ($\frac{eCO_2 - aCO_2}{aCO_2} \sim aCO_2$); the argument being that if CO₂ concentration affected masting then a trend would be detected. There was no trend in this relation across these five years ($P=0.87$); that is the relative difference between the CO₂ treatments did not vary with inter-annual variation in acorn production. Hence, masting was not affected by the differences in CO₂ concentration. Similar analyses also showed no such relation for the relative effect of eCO₂ on numbers of immature acorns ($P=0.74$), the combined category of all evidence of acorns ($P=0.20$), galls ($P=0.24$), or of flowers ($P=0.29$).

The counts of mature acorns, immature acorns, all evidence of acorns, and galls were affected significantly by the main effect of the CO₂ treatments, whereas those of empty cups, enlarged cups and flowers were not (Table 1). The main effect of year (2017–2021) was significant for all seven variables, with a significant treatment × year interaction for immature acorns, empty cups, all evidence of acorns, and flowers: but not any other category. From this we conclude that CO₂ treatment had a significant effect on *Q. robur* reproduction but for four of the seven categories of reproductive material this effect varied depending upon year.

To understand the effect of elevated CO₂ on *Q. robur* reproductive behaviour and check if there was a baseline difference affecting the apparent treatment results found in Table 1, the mean counts of reproductive material were compared within and between each treatment for the periods before (2015–2016) and after the start of CO₂ enrichment (2017–2021). For every category of reproductive material with a significant main effect of treatment and/or interaction of treatment × year except immature acorns (Table 1), the ‘undisturbed’ (uCO₂) arrays provided the greatest number of counts throughout 2015–2021. Post hoc pairwise comparisons show the mean counts of all acorn evidence and galls to be significantly less ($P < 0.05$) in the aCO₂ treatment compared with eCO₂ and uCO₂ (Fig. 2). This significantly

greater quantity of all acorn evidence and galls (but no difference for mature acorns and empty cups) for elevated cf. aCO₂ (Fig. 2) was confirmed by a re-analysis which omitted the undisturbed treatment (Supplementary Table 1). Furthermore, a significant increase ($P < 0.05$) in immature acorns and in all acorn evidence was detected between the periods before and after enrichment began specifically in the eCO₂ treatment (Fig. 2). A significant increase ($P < 0.05$) in flower counts was also detected after the seasonal enrichment period but in both the ambient and elevated CO₂ treatments (Fig. 2). A significant increase ($P < 0.05$) in the number of empty cups was also detected after enrichment began, specifically under the eCO₂ treatment, but before the seasonal enrichment period began there were significantly fewer empty cups in the eCO₂ plots than in the uCO₂ and aCO₂ treatments (Supplementary Table 2, Fig. 2). After enrichment began, however, the numbers of empty cups did not differ significantly amongst treatments ($P > 0.05$). Thus, these results suggest that enrichment with elevated CO₂ resulted in an increase in the mean number of acorns developing compared to the pre-enrichment baseline period, whereas increases in the number of flowers could not be ascribed specifically to eCO₂.

To investigate the significant interaction between CO₂ treatment and year for several of the reproductive groups (Table 1), the significant differences of group means before and after enrichment began (Fig. 2), and how any effect of CO₂ enrichment changed, the counts for each treatment per year were analysed. Analysis showed that before enrichment began (2015 and 2016), those woodland patches destined to be subjected to eCO₂ had the lowest production of several reproductive material groups (Fig. 3), although the only significant pairwise comparison found was of empty cups in 2015 (aCO₂ cf. eCO₂, $P < 0.001$; eCO₂ cf. undisturbed, $P < 0.001$). By the fifth year of CO₂ enrichment (the last of the seven-year period studied, 2021), however, the elevated CO₂ treatment provided the greatest numbers for immature acorns, empty cups, and all evidence of acorns (Fig. 3). Although this difference was not statistically significant between any treatment with post hoc pairwise comparison within 2021

Table 1 Results of ANOVA tests of a series of negative binomial regressions with category of *Q. robur* reproductive material as the response variable and the explanatory variables treatment (elevated CO₂, ambient CO₂, or ‘undisturbed’) and year (2017–2021); significant effects are shown in bold

Category	Treatment			Year			Treatment × Year		
	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>	χ^2	df	<i>P</i>
Mature acorns	9.38	2	0.009	70.13	4	<0.0001	9.93	8	0.270
Immature acorns	11.64	2	0.002	145.86	4	<0.0001	17.9	8	0.022
Empty cups	5.12	2	0.070	159.71	4	<0.0001	17.21	8	0.028
All evidence of acorns	13.20	2	0.001	200.32	4	<0.0001	17.05	8	0.030
Enlarged cups	4.67	2	0.090	48.22	4	<0.0001	12.31	8	0.140
Flowers	2.40	2	0.300	152.63	4	<0.0001	29.34	8	0.0003
Galls	10.56	2	0.005	44.49	4	<0.0001	8.62	8	0.380

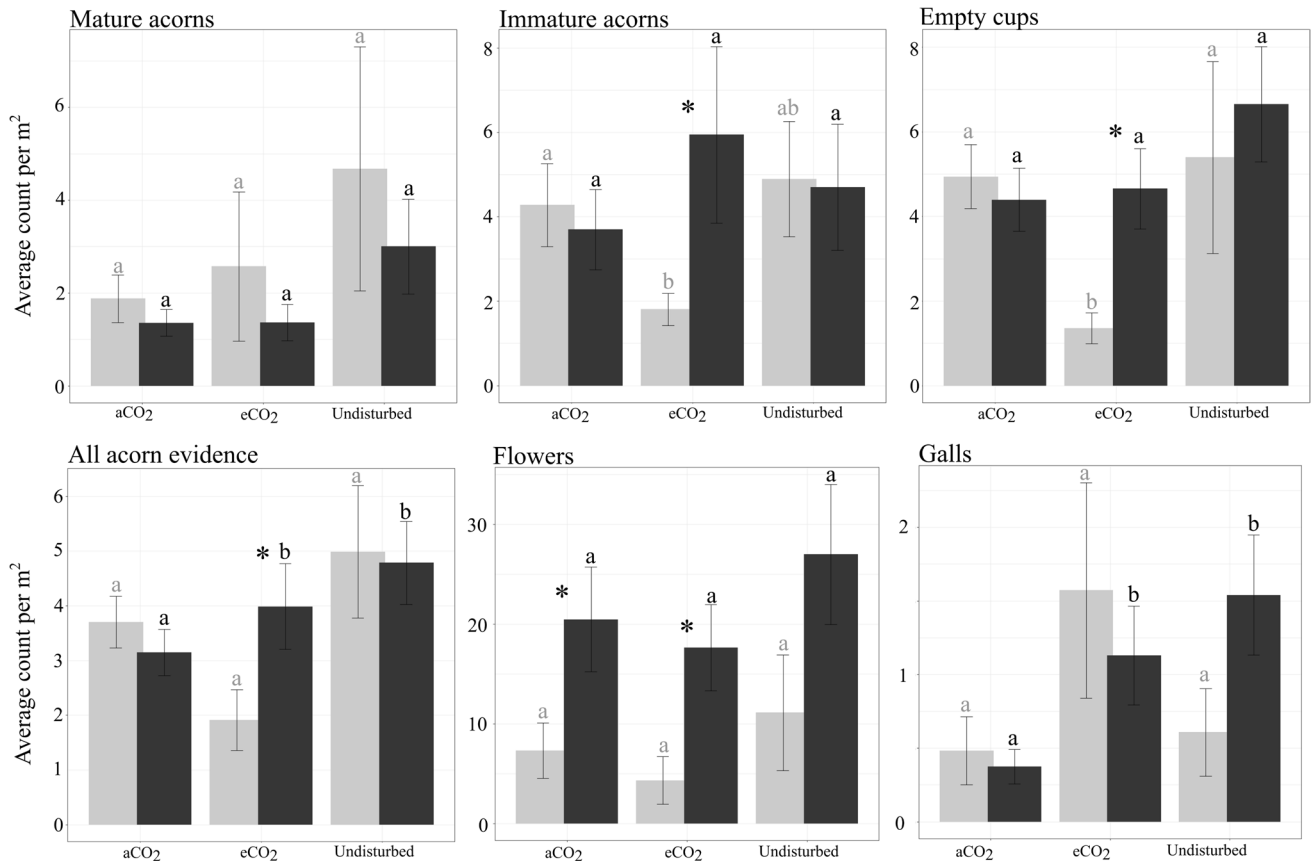


Fig. 2 Variation in counts of *Q. robur* reproductive material from litter traps (mean of two years pre-treatment [2015–2016/light grey] and five years post-treatment [2017–2021/dark grey]) at the BIFoR FACE study at Mill Haft for the aCO₂, eCO₂ or uCO₂ treatments. The vertical bars represent the mean \pm standard error. Asterisks denote significant differences ($P < 0.05$) of a post hoc pairwise comparison between the periods before and after seasonal enrichment started

(i.e. 2015–2016 cf. 2017–2021) within a treatment (aCO₂, eCO₂ or uCO₂). Bars labelled with different letters (a, b) denotes significant differences ($P < 0.05$) between treatment groups during the two years before seasonal enrichment (2015–2016, light grey letters) and within the period after seasonal enrichment started (2017–2021, dark grey letters)

($P = 0.64$ to 0.99), a clear trend is nevertheless visible where counts in the elevated CO₂ treatment transition from being the lowest of the three treatments (2017) to the highest (2021). Flower counts oscillated mostly in step between the treatment groups each year other than in 2018, one year after the CO₂ treatments started. In that year, flower numbers in the elevated CO₂ treatment were much greater than in 2017, whereas they decreased substantially between 2017 and 2018 in the ambient and undisturbed treatments (Fig. 3). Post hoc pairwise comparisons for flower numbers in 2018 showed a difference between eCO₂ and undisturbed treatments ($P < 0.05$), but not between eCO₂ and aCO₂ ($P = 0.86$). These results suggest that the significant increase in developing acorns identified across the entire period of elevated CO₂ enrichment (Fig. 2) is the result of a progressive effect over multiple years since enrichment began. In contrast, *Q. robur* flower production

apparently demonstrated an immediate-but-transient response to elevated CO₂ enrichment in 2017–2018.

Discussion

Masting

Masting is an important feature in the ecology of oak woodlands, which affects the ability of oak to regenerate naturally, and the supply of seed for tree nurseries. The counts of mature acorns and empty cups differed considerably amongst years (Table 1 and Fig. 1); mature acorns were most numerous in 2015, 2017, and 2020 but almost none were collected in 2018. The inter-annual variation at Mill Haft coincided with that for acorn production at other sites across the UK, in which 2015 and 2020 gave above-average numbers of acorns and 2018 very few. This

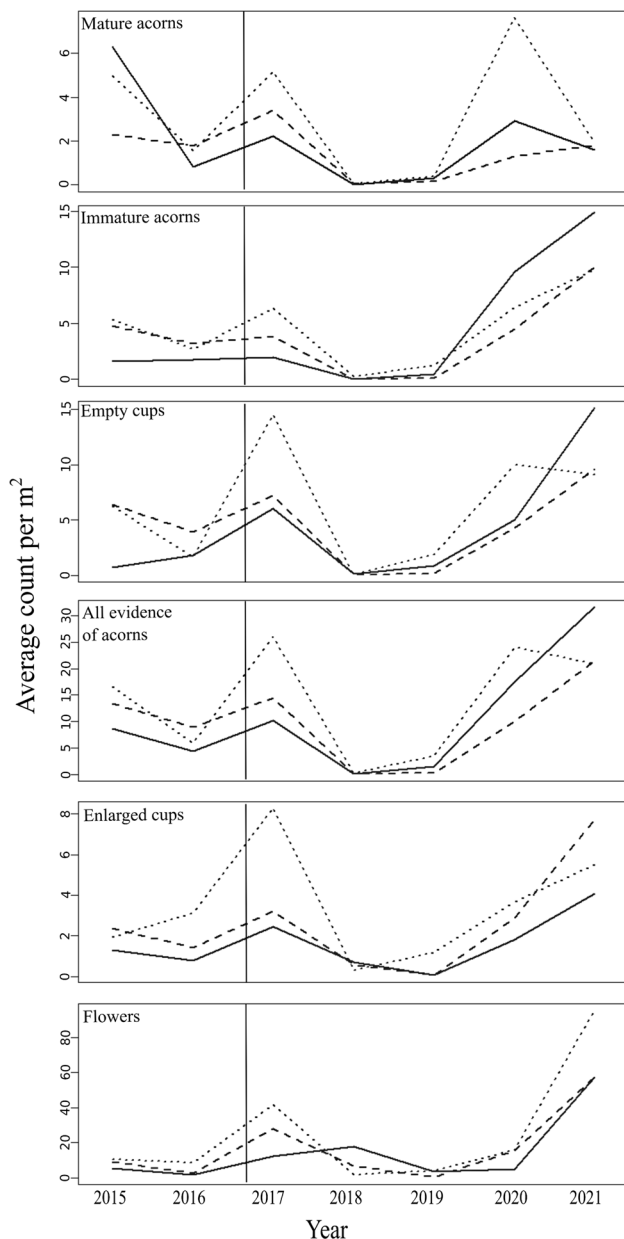


Fig. 3 Variation in counts of *Q. robur* reproductive material for mature acorns, immature acorns, empty cups, all evidence of acorns, enlarged cups, and flowers from litter traps at the BIFoR FACE study at Mill Haft across seven years for elevated CO₂ (solid line), ambient CO₂ (dashed line) and undisturbed plots (dotted line). The CO₂ treatments were provided from early April 2017, shown by the vertical solid black line

is further evidence for the masting behaviour of high inter-annual variation in acorn output that is geographically synchronous, already well reported within oak species (Kelly 1994).

Oaks are said to be a “fruit maturation” species whereby it is the abortion of flowers and underdeveloped acorns

that determines the level of acorn production each year rather than the number of flowers produced (Pearse et al. 2016; Bogdziewicz et al. 2019). This has been questioned recently; whether a population practices “fruit maturation” or “flower masting” may depend upon the climatic conditions experienced (Fleuret et al. 2023). All the reproductive material counted within the litter traps represents a flower that has either aborted or developed into an acorn. This total, along with the flower numbers, remained high each year, and consistently so compared to variation in the much lower counts of other reproductive material (Fig. 1). Thus, non-masting years were not caused by too few flowers; indeed, the failed acorn crop of 2018 followed an average number of flowers (Fig. 1) which was not significantly different from flower numbers during the mast years of 2015 ($P=0.99$) and 2020 ($P=0.54$). On average flowers were 53% of the count of reproductive material found each year, but 89% in 2018 highlighting the influence of early flower abortion during a failed year (Fig. 1). Hence, inter-annual variation in flowering (Table 1) may set an upper limit to acorn production in any one year but not markedly affect the numbers of acorns actually produced. We conclude that *Q. robur* masting is affected primarily by the success of reproductive processes that occur after flowering, consistent with the fruit maturation model. This mode of masting has been linked to the maritime climate of northern UK sites (see Fleuret et al. 2023).

The patterns of variation in the counts of reproductive material across the seven years were broadly consistent between underdeveloped acorns (enlarged cups or immature acorns) and evidence of developed acorns (mature acorns or empty cups) (Fig. 1). In 2018, when mature acorn production failed, however, enlarged cups (the very initial stage of acorn development) was the second most numerous category of reproductive material (Fig. 1). In this year the number of enlarged cups was statistically indistinguishable from those during the mast year of 2015 (2015 = 1.87 per m² vs. 2018 = 0.56 per m²; $P=0.48$). In contrast the counts were much reduced for mature acorns (2015 = 4.53 per m² vs. 2018 = 0.03 per m²; $P<0.001$) and empty cups (2015 = 4.42 per m² vs. 2018 = 0.11 per m²; $P<0.001$). Hence, it is likely that it is the extent of abortion of pollinated flowers early on in acorn development that determines the numbers of mature acorns produced. Fewer pollinated flowers developing acorns wastes less resources and it may be due to selective abortion of an unfavourable pollen source or may indicate limited pollen availability (Boavida et al. 2001). The early abortion of acorns in 2018 (relatively more enlarged cups to immature and mature acorns, Fig. 1) could also be the result of environmental factors, such as summer drought (Espelta et al. 2008) or herbivore-mediated resource limitation (Canelo et al. 2018). In this regard, 2018 and 2019 were

years marked by intense defoliation and powdery mildew infection in Mill Haft (Mayoral et al. 2023), which may have also affected acorn production.

CO₂ treatment effects

Among the treatments, the undisturbed plots produced the most reproductive material overall (Fig. 2). The two CO₂ plots may have been limited in some way. This might have been due to lower soil nitrate or phosphate, important for early-stage flower development (Allen et al. 2017) and acorn development (Sever et al. 2023), or lower soil water (MacKenzie et al. 2021) which reduces mineral and nutrient uptake by trees (Skopp et al. 1990; Alam 1999); or more carbon might have been allocated to the roots and lower stems for increased growth or storage (Dickson and Tomlinson 1996). It is also possible that the two CO₂ treatment areas may have been stressed from the increase in activity, such as trampling, during and after the construction of the enrichment infrastructure (Komatsu et al. 2007).

There was a significant interaction between CO₂ treatment and year on flower numbers (Table 1, Supplementary Table 1). Flower numbers were initially oscillating in synchrony between the treatment groups, however after the CO₂ treatments began the eCO₂ treatment desynchronised with both the aCO₂ and undisturbed plots, producing more flowers than the undisturbed plots during the 2018 failed crop (Fig. 3, eCO₂ / undisturbed: $P < 0.05$); although not significantly more than ambient (Fig. 3, eCO₂ / aCO₂: $P = 0.86$). Flower development uses assimilate stored in the trunk or roots (Hoch et al. 2013). Hence, flower counts may not have been expected to respond to the initial season under elevated CO₂, but greater net photosynthesis under elevated CO₂ (Gardner et al. 2022b) may have refilled stored reserves more quickly later in 2017. The higher-than-expected number of flowers in the eCO₂ plot during 2018 may be evidence of this (Fig. 3). After 2018, flower numbers broadly returned to synchrony among the treatments. It may be that trees under elevated CO₂ acclimatised to the increased CO₂ and/or switched the additional carbon resource from reproduction into maintenance and growth, as evidenced by the higher leaf mass per unit area (Gardner et al. 2022c) found in the eCO₂ cf. aCO₂ arrays at the site.

The undisturbed treatment produced the most mature acorns on average during the recorded period of CO₂ enrichment, with no difference between eCO₂ and aCO₂ (Fig. 2, Supplementary Table 1). To have found no increase in mature acorn number for eCO₂ over aCO₂ is surprising as it disagrees with the results from an open-top chamber study of scrub oaks (Stiling et al. 2004) and for seeds of *Pinus taeda* L. (LaDeau and Clark 2001; Way et al. 2010). There were, however, higher counts of empty cups observed after CO₂ enrichment began and when all evidence of acorns is

combined an increase is detected within the eCO₂ treatment (Figs. 2 and 3). This suggests an increase in acorn production resultant from elevated CO₂ that is being obscured by greater acorn predation rates. Seed predators favour larger, more mature acorns (Gómez 2004). Whilst efforts are made to control the numbers of seed predators at the BIFoR FACE site (Bradwell 2022), it is clear from camera trapping that they remain present. Moreover, the litter traps used were not sealed. Hence, one likely explanation is that post-dispersal acorn predation in the litter traps reduced the counts of mature acorns, leaving behind empty cups and immature acorns; as such, counts of empty cups, immature acorns, and the composite group all acorn evidence are potentially more reliable indicators of the impact of CO₂ on acorn production in this study. The importance of predation in determining the fate of reproductive material under eCO₂ illustrates the importance of process-permitting experimental designs such as FACE in contrast to more closed systems.

There were significant interactions between treatment and year for immature acorns, empty cups, and all acorn evidence, suggesting these groups are increasing after CO₂ enrichment began (Table 1; Figs. 2 and 3). At the start of the experiment the number of empty cups, immature acorns and all evidence of acorns were lowest in the eCO₂ plots, but post-treatment application the eCO₂ plots were producing the highest counts of these reproductive material categories (Table 1; Figs. 2 and 3). Treatments were assigned to FACE arrays to pair aCO₂ and eCO₂ treatments as geographically close as practicable, but the variation observed in the pre-enrichment period suggests that other underlying differences remain between the aCO₂ and eCO₂ treatment arrays. The increase in these groups of reproductive material over this period might indicate an increase in post-fertilisation production of acorns under elevated CO₂. That the number of mature acorns does not increase in line with this might indicate inadequate nutrient supply to support acorn development to maturity. The numbers of immature acorns (i.e., early-aborting acorns) were greatest in the eCO₂ treatment for 2020 and 2021 (Fig. 3). There is, however, no evidence for nutrient limitation in leaf composition at BIFoR FACE (Gardner et al. 2022c). Moreover, post-hoc pairwise comparison showed the higher immature acorn counts in the eCO₂ plots in these years was not statistically significant, however; as the experiment continues it is worth monitoring this putative trend. If elevated CO₂ were to increase the numbers of immature acorns produced each year this might damage the management of *Q. robur* by negating the seed predator defence of masting (Bogdziewicz et al. 2020). The higher numbers of immature acorns could provide a bridging effect between mast years, maintaining consistently higher seed predator populations that would eat higher proportions of mature acorns during the mast year.

Attacks from pre-dispersal predators, in this case exclusively oak knopper galls (*Andricus quercuscalicis* Burgsdorf), showed no significant effects of the plots on galls in the two years before the CO₂ treatments were applied (Fig. 2), but thereafter galls were more common in the undisturbed and eCO₂ treatments (Table 1 and Fig. 2). The greater numbers of mature acorns in the undisturbed plot might explain why more galls were found (i.e., with more acorns one may expect more galls). However, the eCO₂ treatment had the lowest mature acorn count on average and yet the number of galls was three times higher than the aCO₂ treatment (Fig. 2). If one assumes that each gall could have developed into a mature acorn if it had not been attacked, then eCO₂ would have produced more acorns than aCO₂ on average: galls plus mature acorns were 3.43 per m² of litter trap under eCO₂ and 2.15 under aCO₂; and this difference would be yet greater if the numbers of immature acorn were also added (see Fig. 2). The higher attack rate by galls observed may thus have contributed to more acorns failing to reach maturity in the eCO₂ plots. These results suggest that the presence of more developing acorns in elevated CO₂ may be of greater benefit to seed predators than to the regeneration of the trees themselves, as noted by Bogdziewicz et al. (2020). Moreover, higher growth under CO₂ may result in higher mortality rates in mature trees (Büntgen et al. 2019; Brienen et al. 2020). And so, the potential benefits from future increase in CO₂ may, conversely, result in fewer juvenile trees with shorter lifespans.

Elevated CO₂ has been found to increase herbivore numbers in some years (Kampichler et al. 2008), but this effect may be specific to species and functional groups because other species have shown the opposite result (Hall et al. 2005; Roberts et al. 2022). In this study the difference in the numbers of galls in the periods before and after enrichment was not statistically significant under any treatment (Fig. 2), agreeing with previous research at the site into herbivory per unit area of leaf on *Q. robur* (Roberts et al. 2022). Pre-enrichment counts suggest that the eCO₂ plots were already experiencing higher pre-dispersal seed predation, with two years of data giving insufficient power to pick up on this difference statistically.

Studies of this type are exceedingly rare due to the resources required to set up such large, long-duration experiments. Moreover, the requirement to build such investigations around long-established trees in mature woodlands means that the experimental sites cannot be as homogeneous as with, for example, experimental grounds with annual plants. Nonetheless, this study provides strong evidence of bottlenecks to fruit production and the ultimate fate of fruit in old temperate forest subject to predation by granivorous vertebrates. Our results suggest that the effects of elevated CO₂ on acorn production increase

over time (Fig. 3, 'all evidence' panel) and so may be cumulative, especially as masting works on multi-year cycles. Hence it will be important to carry on the study for some years to determine if more pronounced effects emerge over time.

Conclusions

Using FACE to increase the atmospheric CO₂ concentration by 150 μL L⁻¹ in old, temperate deciduous woodland, had no significant direct effect on the phenomenon of masting in *Quercus robur* L., nor on the numbers of either enlarged cups or mature acorns, but it did increase significantly the numbers of empty cups, immature acorns, and all evidence of acorns. All categories of reproductive material were affected by year, with *Q. robur* at this site conforming to fruit maturation masting behaviour. We suggest that elevated CO₂ increased the initial numbers of acorns developing, but this was not reflected by the mature acorn counts due to (1) higher load of pre-dispersal seed predation in the elevated CO₂ plots, and (2) post-dispersal seed predators taking mature acorns. We have no evidence currently for a third possible explanation of nutrient limitations under eCO₂ preventing full acorn development. The number of immature acorns also increased under eCO₂ from being the lowest of the three treatments in the early years to the highest by the final two studied, suggesting that the effects of elevated CO₂ may be cumulative—which requires further study. Flower number was also affected by the interaction of CO₂ treatment with year, suggesting elevated CO₂ may affect the cycle of resource expenditure and storage, but in this regard the trees appeared to acclimatise to this increase in CO₂ quickly.

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