

Enhanced woody biomass production in a mature temperate forest under elevated CO₂

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1
2 **Enhanced woody biomass production in a mature temperate forest under**
3 **elevated CO₂**
4

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38
39

40 **Abstract**

41 **Enhanced CO₂ assimilation by forests as atmospheric CO₂ concentration rises could slow**
42 **the rate of CO₂ increase if the assimilated carbon is allocated to long-lived biomass.**
43 **Experiments in young tree plantations support a CO₂ fertilization effect as atmospheric**
44 **CO₂ continues to increase. Uncertainty exists, however, as to whether older, more mature**
45 **forests retain the capacity to respond to elevated CO₂. Here we show, aided by tree-ring**
46 **analysis and canopy laser scanning, that a 180-year-old *Quercus robur* L. woodland in**
47 **central England increased the production of woody biomass when exposed to free-air CO₂**
48 **enrichment (FACE) for seven years. Further, elevated CO₂ increased exudation of carbon**
49 **from fine roots into the soil with likely effects on nutrient cycles. The increase in tree**
50 **growth and allocation to long-lived woody biomass, demonstrated here, substantiates the**
51 **major role for mature temperate forests in climate change mitigation.**

52

53 **Main**

54 Carbon uptake and storage by terrestrial vegetation is a major source of uncertainty in
55 projections of future levels of CO₂ in the atmosphere and the resulting effects on climate^{1,2}.
56 Multiple lines of evidence indicate that increasing atmospheric CO₂ concentration in recent
57 decades resulting from anthropogenic emissions and land use change have led to higher rates of
58 CO₂ uptake by plants, i.e., the CO₂ fertilization effect, including in forests, which dominate the
59 terrestrial C cycle^{3,4}. Evidence for forest responses to the atmospheric CO₂ concentrations that
60 will be attained in future decades comes from a limited number of decade-long free-air CO₂
61 enrichment (FACE) experiments in which forest plots were exposed to elevated CO₂ (eCO₂).
62 These “first generation” forest FACE experiments⁵⁻⁷ were established in young tree plantations,
63 and questions arise as to the extent to which their responses to eCO₂, including increased growth
64 and primary productivity, are predictive of the responses of older, more established forests⁸⁻¹⁰.
65 There are multiple issues to consider. As forests develop over time, nitrogen, which is often the
66 limiting resource in unmanaged temperate stands, becomes increasingly sequestered in wood or
67 recalcitrant soil organic matter and unavailable to support plant growth, a phenomenon referred
68 to as progressive nitrogen limitation (PNL)¹¹⁻¹³. Furthermore, the development of PNL can be
69 accelerated by eCO₂ (ref. ¹⁴). PNL is of particular interest now, because global atmospheric
70 carbon and nitrogen sources are now moving in opposite directions^{15,16}. Phosphorus limitation

71 might be a critical influence on responses to eCO₂ on sites with old, highly weathered soils^{9,17}.
72 Apart from nutrient limitations, older trees may be less responsive to eCO₂ than younger trees
73 because a smaller fraction of the biomass is live tissue contributing to growth.

74

75 The amount of biomass produced each year—the net primary productivity (NPP)—is a key
76 metric for evaluating forest response to eCO₂ and a benchmark for models¹⁸⁻²⁰. If NPP increases
77 in eCO₂, the key question becomes whether the additional C accumulates in wood or instead is
78 allocated to leaves and fine roots that turn over rapidly and release the C back to the
79 atmosphere²¹. Carbon allocated to wood can persist in the ecosystem for policy-relevant time
80 frames^{22,23}, which provides a negative feedback to atmospheric CO₂. Over longer time frames
81 and landscape scales, C sequestration depends not just on the effects of eCO₂ on tree growth but
82 also on the rate of tree mortality¹⁰, and some evidence suggests that increased growth rate may
83 shorten a tree’s lifespan^{24,25}. Nevertheless, the effect of rising CO₂ on NPP and how it is
84 allocated, as revealed in FACE experiments, is critical input to any evaluation of the interaction
85 between terrestrial ecosystems and atmospheric CO₂.

86

87 A common question being addressed across the “second generation” forest FACE experiments is
88 whether mature forests will respond to eCO₂ (ref.⁹). Although the definition of “mature” in this
89 context is vague²⁶, the new or developing FACE experiments in the Amazon forest in Brazil,
90 (<https://amazonface.unicamp.br/>), a Eucalyptus stand in Australia¹⁷, and a temperate maritime
91 oak forest at the Birmingham Institute of Forest Research (BIFoR) in central England, UK^{27,28}
92 are in forest stands that are centuries or more older than the young plantations in the ORNL
93 FACE¹⁴ and DukeFACE experiments²⁹ in southeastern United States. For example, the ORNL
94 FACE and BIFoR FACE both are in temperate deciduous forests (albeit with different species)
95 that had attained canopy closure, linear growth, and reproductive maturity, but the *Quercus*
96 *robur* trees that dominate in BIFoR FACE are approximately 180 years old compared to 10-20
97 years old *Liquidambar styraciflua* trees in ORNL FACE, and the stand structure is very
98 different—similar tree basal area per plot (34 cm² m⁻²) is concentrated in 5-7 trees per plot at
99 BIFoR (average tree basal area of 3486 cm²) compared to 90 trees at ORNL FACE (average tree
100 basal area of 118 cm²)(ref.³⁰). Here, we address whether this difference in age and stand structure
101 precludes the response of tree growth to eCO₂ at BIFoR FACE.

102 **BIFoR FACE facility**

103 The BIFoR FACE facility (<https://www.birmingham.ac.uk/research/bifor/face/index.aspx>,
104 Extended Data Fig. 1) is located in central England (52.801°N, 2.301°W, 107 m above sea level).
105 The 19-ha Mill Haft deciduous forest woodland was established as a plantation of oak
106 ‘standards’ (i.e. timber trees) with hazel coppice in the mid-19th century and has been very
107 lightly managed since that time, the understory hazel trees being last coppiced ~1985. About two
108 thirds of the 400-ha estate surrounding Mill Haft were arable and pasture until 2010, since when
109 they have been converted into mixed broadleaf plantation and no-till organic herbal ley (a
110 mixture of grasses, legumes, and herbs) in roughly equal proportion³¹. The forest is dominated
111 by 180-year-old *Quercus robur* L. trees, which represent 92% of the forest’s basal area. The
112 forest canopy is 24-26 m high with a leaf area index of the overstory of about 6. The forest
113 understory comprises *Corylus avellana* L. coppice, *Acer pseudoplatanus* L., *Crataegus*
114 *monogyna* Jacq., and a few individuals of other broadleaf species³². The dominant soil is Dystric
115 Cambisol with a sandy-clay texture. Underlying geology is a Helsby sandstone formation. Mean
116 annual maximum and minimum temperatures (1991 – 2020) were 13.5 and 6.0 °C with 676 mm
117 precipitation³³. The FACE facility comprises six experimental arrays surrounding plots of
118 approximately 30 m diameter. Disturbance to the woodland was avoided during construction of
119 the FACE infrastructure, fitting the infrastructure between the trees so that no oak standards or
120 coppice stools were removed, and using no concrete foundations or guy wires²⁷. CO₂-enriched
121 air (1-2% by volume) is released from vertical vent pipes in three of the arrays to attain a target
122 CO₂ concentration at plot center that is 150 ppm above ambient CO₂. Actual daytime CO₂
123 concentration enrichment during the 2017-2022 growing seasons (April-November) was 140 ±
124 38 ppm relative to ambient air due to occasional disruptions in CO₂ supply. Full description of
125 the FACE facility and its operating characteristics are available²⁷.

126

127 **Aboveground biomass production**

128 Evaluation of treatment effects required accounting for pretreatment differences among plots.
129 Tree ring analysis (see Methods) revealed high variability in the size and growth of the 180-year-
130 old *Q. robur* trees on plots within the experimental arrays (Fig. 1a), as is often typical of mature
131 forest stands; this variability complicates detection of possible effects of eCO₂, while underlining
132 the realism of the BIFoR FACE context and increasing its applicability to other real-world

133 forests. Furthermore, basal area increment (BAI) in plots randomly assigned to receive eCO₂
134 starting in 2017 was significantly greater than in plots for ambient CO₂ (aCO₂) prior to the
135 initiation of the CO₂ treatment (Fig. 1b). Hence, these pretreatment differences were accounted
136 for in evaluating treatment differences after eCO₂ exposure was initiated. We used the average
137 plot-specific BAI from 2011-2015 (based on tree ring analysis) to normalize subsequent annual
138 growth data, thereby accounting for site and stand differences that would otherwise obscure
139 treatment effects. A departure in the normalized tree ring chronologies between aCO₂ and eCO₂
140 plots was apparent beginning in 2017, the first year of eCO₂ exposure (Fig. 1c).

141
142 It has become clear that application of published allometric equations for temperate forests can
143 create considerable errors in biomass estimation when applied to a different site with a different
144 stand structure than that from which the relationship was established³⁴. Therefore, we developed
145 a site-specific allometry (Extended Data Fig. 2) based on a terrestrial laser scan (TLS) of the site
146 to calculate annual wood production from diameter measurements (see Methods). TLS provides
147 highly accurate estimates of tree volume that are translated into estimates of aboveground
148 biomass as long as internal stem damage is not large³⁵, and tree cores did not indicate any
149 internal stem damage. Annual diameter increments as measured in the field with manual
150 dendrometers and calipers were consistent with independent measures using automated
151 dendrometers that measure circumference changes continuously (Extended Data Fig. 3) and with
152 the tree ring chronologies. Dry matter increment (DMI) per tree, as calculated from diameter and
153 adjusted by pretreatment differences among plots, was greater in eCO₂ than in aCO₂ every year
154 of CO₂ enrichment except for 2019 (Fig. 2); over the 7 years of treatment, tree growth was 10%
155 greater in eCO₂. The loss of response in 2019 may have been related to differential defoliation by
156 the winter moth (*Operophtera brumata* L.) and other foliar feeding insects, which was greater in
157 eCO₂ based on litter trap collections³⁶ (Extended Data Fig. 4). There also was an insect outbreak
158 in 2018, which was not seen in the litter collection data and had no apparent effect on the growth
159 response. Although the apparent difference in DMI in any year was not statistically significant,
160 repeated measures analysis indicated a significant CO₂ effect over the 2017-2023 period ($P =$
161 0.028 , $F_{1,4} = 5.48$). Total DMI over the experimental period was linearly related to tree size
162 (basal area) with no clear discontinuities in the relationship or in the distribution of ambient vs.
163 elevated trees around the distribution (Extended Data Fig. 5).

164

165 **Net primary productivity**

166 With additional data on fine-root and leaf mass production, understory production, reproductive
167 output, and exudation (see Methods), NPP and its response to eCO₂ were estimated for 2021 and
168 2022, the 5th and 6th years of eCO₂ treatment (Table 1). Aboveground *Q. robur* wood production
169 comprised the largest fraction (40-48%) of NPP, followed by leaf production and fine-root
170 production. After accounting for pretreatment differences in wood and leaf production, total NPP
171 was 9.7% greater in eCO₂ in 2021 and 11.5% greater in 2022, but neither of these differences
172 were statistically significant ($P > 0.20$). Given the variance structure of the forest stand in the six
173 plots, a CO₂ enhancement of 45% would have been required in a given year to achieve a
174 statistically significant ($P < 0.05$) difference, greatly exceeding what has been observed in
175 previous forest FACE experiments⁵. Alternatively, given the measured means and variance, 28
176 arrays per treatment would have been required to achieve type I and II errors of 0.05 and 0.20.
177 Taking the two years together in a repeated measures framework, NPP increased 10.6% ($P =$
178 0.099 , $F_{1,4} = 4.56$), equivalent to ~1.7 tonnes dry matter per hectare per year.

179

180 Most of the observed increase in NPP was attributable to wood production; there was no
181 difference in fine-root or leaf mass production, although standing stock of fine roots may have
182 been greater and turnover slower in eCO₂. Leaf mass per unit area (LMA) was ~10% greater and
183 oak leaf area index (LAI) was 5% less in eCO₂ (Table 2). LAI in fully occupied sites with
184 relatively high LAI is not expected to increase in eCO₂ (ref.³⁷). Significant increases in fine-root
185 production are common in forest FACE experiments³⁷ but has mainly been observed as a
186 response to eCO₂ in nutrient-limited forest ecosystems³⁸. Although increased fine-root
187 production was reported from measurements predominantly under stools of coppiced *C. avellana*
188 in years one and two of the BIFoR FACE CO₂ treatment³⁹, the absence of a sustained increase in
189 the BIFoR experiment is not unexpected given that the BIFoR FACE forest does not appear to be
190 N-limited (discussed further below). Nevertheless, there was evidence of increased carbon
191 allocation belowground as exudation. Exudation of organic carbon from roots is a component of
192 NPP but is rarely included in estimates given the difficulty in measuring and upscaling
193 exudation. We measured rates of net exudation four times between August 2020 and June 2021
194 (see Methods) and scaled data expressed as grams C per unit root mass to grams dry mass

195 equivalent per square meter land based on fine-root mass per unit area from soil cores collected
196 in 2021 and 2022, with the assumption that exudation rate in the organic horizon applied equally
197 to deeper roots and that the average of four measurements in spring, summer, and autumn
198 reflected the average exudation rate during the 264-day growing season. Exudation flux was 63%
199 greater in eCO₂ in 2021 ($P = 0.13$) and 43% greater in 2022 ($P > 0.20$); repeated measures
200 analysis indicated a significant overall effect ($P = 0.042$, $F_{1,4} = 8.64$).

201

202 **Discussion**

203 The responses observed in FACE experiments require careful consideration of temporal and
204 spatial scales and levels of biological complexity before their implications for the global carbon
205 cycle and feedbacks to climate change can be properly interpreted. Forest responses to eCO₂ start
206 with an enhancement of leaf-level photosynthesis, as has been documented at BIFoR FACE³². In
207 many forest systems, the enhancement of photosynthesis scales up to increased NPP⁴, although
208 nutrient limitations can inhibit the translation of increased photosynthesis to increased NPP¹⁷. At
209 BIFoR FACE there is evidence of increased NPP in eCO₂ over 2 years, a response that may
210 become statistically more compelling if it is sustained for more years. It has long been
211 recognized that although NPP might be enhanced by eCO₂ in most terrestrial ecosystems, a more
212 important question is whether an eCO₂ response results in greater wood mass or instead is
213 allocated to fast-turnover pools (e.g., leaves and fine roots)²¹. Aboveground woody biomass is
214 the component of NPP that is most relevant for decades-long (and policy relevant) carbon
215 balance evaluations and the basis of evaluations of past responses to eCO₂. Much of the carbon
216 allocated to wood (other than that in small cast-off branches) will persist in the ecosystem for
217 many decades, whereas much of the carbon allocated to the fast-turnover tissues will quickly
218 return to the atmosphere, although leaf and fine-root necromass also contribute to recalcitrant
219 soil organic matter. At BIFoR FACE additional carbon, taken up in response to eCO₂ through the
220 stimulation of leaf-level photosynthesis³², accumulated in wood, with no increases observed in
221 production of leaves and fine roots. Long-term C sequestration is also determined by tree
222 turnover²⁵, which cannot be assessed at the scale of FACE experiments, but model simulations
223 coupled with inventory analysis in unmanaged temperate forests demonstrated that enhanced tree
224 growth increases biomass stocks despite simultaneous decreases in carbon residence time and
225 tree longevity¹⁰.

226

227 Our results contrast with those of EucFACE, where no increases in dry matter accumulation or
228 NPP were observed¹⁷. BIFoR FACE results argue against a general conclusion that older, mature
229 forest systems have no capacity for response to eCO₂. The difference in response between these
230 two experiments is more likely related to nutrient dynamics rather than simply stand age or
231 maturity. EucFACE responses are thought to be limited by a phosphorus deficiency¹⁷, and
232 mature northern temperate forests are generally thought to be nitrogen limited. However, no
233 clear nitrogen or phosphorus limitation has yet been documented at BIFoR FACE, and leaf
234 nitrogen content has been maintained⁴⁰, although further investigation—and indeed further CO₂
235 treatment—could change these assessments. As a result of surrounding agricultural activities and
236 regional industry, nitrogen deposition at the BIFoR FACE site is relatively high, 22 kg ha⁻¹
237 (ref.⁴¹), and not atypical of northern temperate forests. This subsidy of reactive nitrogen may be
238 providing sufficient nitrogen supply to support increased carbon fixation. Furthermore, enhanced
239 soil nitrogen transformations⁴² supported by increased release of bioavailable carbon from root
240 exudation, may be allowing the trees to meet their nitrogen demand under eCO₂. Although
241 exudation comprised just 4-7% of total NPP, this flux of highly labile organic C is
242 disproportionately important to ecosystem biogeochemistry. For example, exudation can increase
243 the availability of labile C, priming the microbial community and associated nitrogen and
244 phosphorus cycling^{43,44}. Measurements on site have shown that net nitrogen mineralization
245 increased on average by 30% under elevated eCO₂, delivering an extra 24 kg N ha⁻¹ y⁻¹ (ref. ⁴⁵).
246 However, it is possible that the supply of bioavailable nitrogen sourced from decomposition of
247 soil organic matter may be reduced and eventually exhausted over time. Furthermore, nitrogen
248 deposition is declining in the UK¹⁵ and throughout the Global North⁴⁶. Although at present
249 nitrogen does not appear to be a limiting factor to tree growth or response to eCO₂, nitrogen
250 limitation may develop at BIFoR FACE as the nitrogen cycle gets tighter and plant demand
251 increases, as was observed in a previous FACE experiment¹⁴. Furthermore, high rates of N
252 deposition have been shown to stimulate P acquisition and alleviate potential P limitation⁴⁷, so it
253 is possible that if N deposition declines, P limitation could develop over time.

254

255 These BIFoR FACE results have illustrated the importance of and challenges in documenting
256 meaningful ecosystem-scale responses to eCO₂ in mature forests that are inevitably heterogenous

257 in tree size, productivity, and spatial distribution and in experiments in which engineering
258 constraints limit plot size and financial constraints limit replication and duration. Employing
259 multi-decadal tree ring analysis to account for plot differences prior to the onset of the CO₂
260 treatment markedly improved our confidence in attribution of eCO₂ effects. A single year of
261 observation is unlikely to provide convincing evidence of a tree growth or NPP response of the
262 expected possible magnitude; sustained and consistent response over multiple years is necessary.
263 Assessment of dry matter increment is highly dependent on the allometric relationship used to
264 scale nondestructive measurements of tree diameter to biomass. A harvest of trees at the
265 experimental site is precluded, as it is in most forest FACE experiments. Reliance on published
266 allometric equations developed in forests with different stand structures can introduce significant
267 uncertainty in assessment of forest carbon stocks³⁴, and is especially problematic for larger, more
268 mature stands. The development of site-specific allometry developed from nondestructive
269 estimates of *Q. robur* tree volume using terrestrial laser scanning significantly increased our
270 confidence in the assessment of tree biomass in BIFoR FACE.

271
272 Our results directly refute the notion that mature forests cannot respond to eCO₂, and they
273 emphasize that the important issue is allocation of any increased carbon uptake and the turnover
274 of the tissues that benefit most²¹. Hence, the evidence from BIFoR FACE of a significant
275 increase in woody biomass production is a key result supporting the role of mature forest stands
276 as decadal C stores¹⁰ and, hence, as natural climate solutions in the coming decades while society
277 undertakes deep decarbonization⁴⁸. Quantifying the CO₂ fertilization effect is important for
278 predictions of future atmospheric CO₂ concentrations and the policy decision that derive
279 therefrom, but even if the increase in tree growth translates to a longer-term increase in C storage
280 in the ecosystem, CO₂ fertilization cannot be seen as reason to delay reductions in fossil fuel
281 consumption.

282 283 **Acknowledgments**

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297 administered the experiment. R.J.N. designed the data synthesis and analysis. R.J.N., N.J.L.,
298 C.M., A.R.S, M.K.R., K.V.W., M.S.A., D.B., M.E.C., G.D., R.T.G., R.L.H., A.G.J., A.K. and
299 R.M.T. collected data and contributed to analysis. G.C. and D.B. curated data. R.J.N. wrote the
300 manuscript with substantial input from A.R.MK., N.J.L., C.M., S.U., A.R.S., M.K.R., I.P.H. and
301 K.V.W. All authors edited and approved the manuscript.

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303

304 **Competing interests:** Authors declare that they have no competing interests.

305

306

307 **Table 1. Net primary productivity (NPP) and its fractional distribution.**

308 All data are the means of three plots in ambient CO₂ and three plots in elevated CO₂ ± standard
 309 error, expressed as grams dry matter per square meter. None of the differences within year are
 310 statistically significant ($P > 0.20$); repeated measures analysis across years indicated an effect of
 311 CO₂ on NPP at $P = 0.099$, $F_{1,4} = 4.56$. Wood production by plot was adjusted to account for
 312 average pretreatment BAI 2010-2015 as determined by tree ring analysis. Leaf mass production
 313 was assumed to be 14% greater than litter mass based upon the amount of nitrogen resorbed from
 314 senescing leaves and the assumption that nitrogen was exported as glutamine³⁰. Plot-level leaf
 315 mass production was normalized to the pretreatment (2016) values. Production of fine roots (< 2
 316 mm diameter) was determined from in-growth cores installed to 30 cm depth and scaled to 1 m
 317 depth based on the relative depth distribution of fine-root standing crop in core samples. E/A is
 318 the ratio of the measure in eCO₂ to the measure in aCO₂.

319
320

	<i>Q. robur</i> bole + branch	<i>Q. robur</i> coarse root	Understory wood	Total wood	Fine root NPP (g m ⁻²)	Exudation	Reprod. tissue	Leaves	Total NPP
2021									
Ambient	755 ± 95	83 ± 12	12 ± 7	849 ± 103	203 ± 28	76 ± 20	124 ± 13	501 ± 9	1753 ± 114
Elevated	835 ± 166	88 ± 21	15 ± 7	938 ± 183	201 ± 11	124 ± 15	133 ± 31	529 ± 49	1924 ± 263
E/A	1.106	1.060	1.269	1.104	0.989	1.633	1.075	1.054	1.097
2022									
Ambient	534 ± 67	58 ± 8	16 ± 1	608 ± 74	154 ± 8	83 ± 8	72 ± 1	562 ± 22	1479 ± 78
Elevated	621 ± 87	67 ± 10	17 ± 10	706 ± 87	156 ± 15	118 ± 25	95 ± 28	575 ± 88	1650 ± 140
E/A	1.164	1.141	1.087	1.161	1.014	1.426	1.318	1.023	1.115
2021									
Fractional distribution (%)									
Ambient				48.1 ± 2.7	11.6 ± 1.7	4.2 ± 0.8	7.2 ± 1.1	28.8 ± 1.9	
Elevated				47.8 ± 4.7	10.8 ± 1.5	6.5 ± 0.4	6.9 ± 1.2	27.9 ± 1.6	
E/A				0.995	0.929	1.542	0.967	0.967	
2022									
Ambient				40.8 ± 2.8	10.5 ± 0.9	5.6 ± 0.3	4.9 ± 0.3	38.2 ± 2.2	
Elevated				42.7 ± 3.8	9.5 ± 1.0	7.2 ± 1.6	6.1 ± 2.0	34.5 ± 2.5	
E/A				1.045	0.908	1.174	1.243	0.904	

321
322
323

324 **Table 2.** Leaf area index (LAI), calculated from litter mass and green leaf mass per unit area
 325 (LMA) and adjusted to pretreatment (2016) values. Green leaf mass is assumed to equal litter
 326 mass \times 1.14 (ref. ³⁰). All data are means \pm standard error of three plots per treatment. No
 327 differences are significantly different ($P > 0.20$) except litter mass and LMA in 2021 ($P = 0.110$,
 328 $F_{1,4} = 4.2$ and $P < 0.082$, $F_{1,4} = 5.33$, respectively). E/A is the ratio of the value in eCO₂ to the
 329 value in aCO₂.

330

CO ₂ treatment	2020	2021	2022
	Litter mass (g m ⁻² land)		
ambient	330 \pm 28	291 \pm 19	358 \pm 21
elevated	348 \pm 20	332 \pm 5	392 \pm 23
E/A	1.053	1.139	1.093
	LMA (g m ⁻² leaf)		
ambient	66.5 \pm 5.5	60.0 \pm 2.6	60.1 \pm 0.7
elevated	73.3 \pm 9.8	67.3 \pm 1.8	64.2 \pm 5.5
E/A	1.102	1.121	1.068
	LAI (m ² m ⁻²)		
ambient	6.02 \pm 0.47	5.85 \pm 0.38	7.16 \pm 0.22
elevated	5.65 \pm 0.94	5.51 \pm 0.94	6.85 \pm 0.96
E/A	0.939	0.941	0.956

331

332

333 **Figure Legends**

334 **Fig. 1. Tree ring chronologies of *Quercus robur* L. trees in the FACE experimental plots.**

335 (A) annual basal area increment (BAI) of each tree. Orange lines are trees that began receiving
336 elevated CO₂ in 2017; blue lines are trees that remained in ambient CO₂. (B) Cumulative BAI
337 per plot area since 2010; data are the means of trees in three ambient CO₂ plots and three
338 elevated CO₂ plots ± standard error. (C) Cumulative BAI adjusted by dividing data in (B) by the
339 mean BAI from 2020 to 2015 relative to the overall mean for the period.

340
341 **Fig. 2. Dry matter increment per tree.** Data are the mean ± standard error of trees in three plots
342 in ambient CO₂ (blue) and elevated CO₂ (orange). Numbers above the points represent the
343 percentage increase (or decrease) in eCO₂ relative to aCO₂. CO₂ exposure began in 2017;
344 pretreatment (2016) data calculated from tree ring analysis. Repeated measures analysis of
345 variance indicated that the effect of CO₂ was significant at $P = 0.0279$, $F_{1,24} = 5.48$; CO₂ × year
346 was not significant ($P = 0.830$, $F_{6,24} = 0.46$).

347

348

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466

467

468

469 **Methods**

470 Wood production

471 Every tree greater than 10 cm DBH within the array of vent pipes was fitted with a dendroband⁴⁹
472 at 1.3 m height, or as close as possible to 1.3 m as necessary to avoid a large branch or stem
473 abnormality. Forty-three trees were outfitted in 2016, and five trees were fitted in later years. The
474 initial tree diameter (D) and initial offset of the dendroband were recorded, and the dendroband
475 offset was measured approximately bimonthly between early spring (prior to leaf out) and late
476 autumn with digital calipers. Diameter and basal area increments (BAI; $BA = \pi \times (D/2)^2$) were
477 calculated from the change in circumference and initial diameter.

478
479 Previous analysis of tree biomass at this site employed a published allometric equation for *Q.*
480 *robur* from Forrester *et al.*⁵⁰, which was based primarily on trees harvested in northwestern
481 Spain. This approach was not considered reliable because the largest tree in that data set was
482 smaller than 49% of the BIFoR FACE *Q. robur* trees. Instead, we established a site-specific
483 allometry based on terrestrial laser scans (TLS). 3D point clouds of the forest stands were
484 collected within the six experimental arrays in January and February 2022, when canopy was in
485 leaf-off condition, using a RIEGL VZ-400i laser scanner (RIEGL Laser Measurement Systems
486 GmbH). Registration of separate scans was done in the RiScanPro software (RIEGL) and manual
487 extraction of single trees from the co-registered point cloud was done in CloudCompare
488 (<https://www.cloudcompare.org/>). Determining appropriate parameters for, and the construction
489 of, the Quantitative Structure Models (QSMs) with subsequent calculation of component
490 volumes was done using the open-source software of optQSM
491 (<https://github.com/apburt/optqsm>) and TreeQSM, version 2.4.1
492 (<https://github.com/InverseTampere/TreeQSM>), respectively. The optimal cylinder model per
493 tree was selected based on lowest point model distance out of the five iterations. Tree volume
494 was calculated as the sum of bole and branch volume, and volume was converted to dry mass
495 using a wood density of 0.58, an average of trunk and branch density reported for *Q. robur*⁵¹.
496 The regression of $\ln(\text{biomass, kg})$ vs $\ln(\text{diameter, cm})$, with diameter being that calculated from
497 dendroband measurements in December 2021 is: $\ln(\text{biomass, kg}) = 2.312 \times \ln(\text{diameter, cm}) -$
498 1.0863 ; $r^2 = 0.77$ (Extended Data Fig.2).

499 Aboveground wood mass (kg) of other species was calculated using allometric equations from
500 Forrester *et al.*⁵⁰, where M_{stem} is dry matter (kg) and D is diameter (cm):

501
$$\text{For } \textit{Acer pseudoplatanus}, \ln(M_{\text{stem}}) = -2.3116 + 2.4186 \times \ln(D)$$

502
$$\text{For other species: } \ln(M_{\text{stem}}) = -2.1653 + 2.4143 \times \ln(D)$$

503 Annual wood production per tree was calculated as the dry matter increment (DMI) during the
504 year.

505

506 Coarse root production for understory species was calculated similarly using the equation⁵⁰:

507
$$\ln(M_{\text{root}}) = -2.6183 + 2.1353 \times \ln(D)$$

508 The equation⁵⁰ for *Q. robur* coarse root biomass [$\ln(M_{\text{root}}) = -2.863 + 2.208 \times \ln(D)$]

509 is assumed to be inaccurate to the same extent as the difference between the equivalent

510 aboveground equation⁵⁰ [$\ln(M_{\text{stem}}) = -2.9128 + 2.7442 \times \ln(D)$] and the site-specific TLS-based

511 allometry (Extended Data Fig.2), which varied from 56% greater to 40% less. Hence, *Q. robur*

512 coarse root biomass was calculated by multiplying aboveground biomass (from the TLS

513 allometry) by the ratio of coarse root to aboveground biomass from the published⁵⁰ equations.

514

515 DMI of the multi-stemmed stools of coppiced *Corylus avellana* was determined by measuring

516 the five thickest sprouts on each hazel stool within the plots irrespective of whether their DBH

517 was below 10 cm, as described⁵². Coarse root production of *C. avellana* was not estimated as a

518 function of D but was assumed to be 26% of aboveground wood production, as determined from

519 the other understory species.

520

521 DMI of the *Q. robur* trees and understory species was expressed per square meter by dividing

522 total DMI by plot area. The area of the plots, which were not circular to avoid removing large

523 trees during construction, was determined as the area of an irregular polygon with the vertices set

524 to maintain at least a 2.5 m buffer from any vent pipe in the experimental array. The resulting

525 areas as determined with ArcGIS ranged from 574 m² to 678 m². DMI was adjusted for

526 pretreatment differences using tree core analysis as described below.

527

528 Tree ring analysis

529 Increment cores (approximately 4 mm diameter) were collected from the *Q. robur* trees to
530 provide a pretreatment time series of BAI. Cores were collected in June 2021 and August 2022
531 from the south side of the tree at approximately 1.3 m height. The cores were stored temporarily
532 in straws and air dried prior to preparation and ring width measurement. The cores were surfaced
533 using progressively finer grades of abrasive paper to reveal the ring boundaries. Ring widths
534 were measured under magnification using TSAP-Win software (Rinntech, Inc.) to 1/100 mm.
535 The series were measured in duplicate and the series internally cross-matched. Tree diameter
536 (not including bark) from 2019 to 2010 was back calculated by sequentially subtracting ($2 \times$ ring
537 width) from each year's calculated diameter, starting with the measured diameter in 2020 minus
538 ($2 \times$ average bark thickness). BAI from 2011 to 2020 was then calculated as the annual increase
539 in BA, with $BA = \pi D^2/4$. To account for pretreatment differences in tree growth across the six
540 plots, the total BAI from 2011 to 2015 of each tree was averaged for each plot, and the relative
541 difference from the overall average BAI for 2011-2015 was used to normalize the data between
542 plots to the site average pre-treatment level. The BAI calculated from ring width analysis was on
543 average 15% less than BAI from dendrobands. This is likely because the dendrobands that
544 integrate information on the entire tree circumference are subject to moisture dependent
545 fluctuations in tree volume and include measures of inner and outer bark thickness, which were
546 not included in the ring width analysis of the air-dried cores.

547

548 Leaf Production

549 Leaf production was determined from leaf litter mass. Leaf litter was collected in two or three
550 1-m² traps per plot (2016-2020) and six 0.25-m² traps (2020-2022). Litter was collected monthly
551 from March to October and weekly from mid-October to mid-December, separated by species,
552 oven dried, and weighed. Some leaves were retained on the trees and fell during the winter and
553 early spring. Hence, annual litter mass production was calculated as the litter collected from
554 April through March. Leaf production exceeds litter mass because of resorption of N-rich
555 organic compounds during senescence. Based on the difference in N concentration between
556 green leaves and litter and assuming N is resorbed as glutamate, green leaf production was set to
557 be 14% greater than litter production. Leaf production in 2017-2021 was relativized to
558 pretreatment (2016) values to account for spatial differences.

559

560 Flower and fruit production

561 Flowers and fruit were collected from the litter baskets as described above, dried, and weighed.
562 This material comprised flowers, enlarged cups (i.e., cups with visible premature acorns),
563 immature acorns, fully mature acorns, empty cups (i.e., empty acorn cups without acorns), and
564 galls (acorn development prevented by insect attack).

565

566 Fine-root production

567 Fine-root production was measured from in-growth cores. Five 5-cm diameter \times 30-cm long
568 mesh columns filled with root-free soil from the O horizon (0-7 cm depth, 0.64 g cm^{-3}), A
569 horizon (7-16 cm depth, 1.03 g cm^{-3}), and B horizon (16-30 cm depth, 1.30 g cm^{-3}) were installed
570 in each plot. Cores were retrieved and replaced every 4-5 months. Fine roots ($<2 \text{ mm}$ diameter)
571 were removed from the soil, oven dried, and weighed. To extend the fine-root production from
572 the 30 cm deep cores to 1 m deep, adjacent soil cores were collected to 1 m depth and fine-root
573 mass quantified. Production in in-growth cores was extended to 1 m based on the fraction of total
574 fine-root standing crop in the top 30 cm (approximately 65%).

575

576 Exudation

577 Root exudation was measured four times between August 2020 and June 2021. *Q. robur* roots in
578 the O horizon were identified based on surveys of root morphology outside of experimental
579 arrays. Root boxes within 1 m of a *Q. robur* trees were installed to permit access to new root
580 growth during this period, and exudates were collected from six root systems per plot. The
581 collection procedure was adapted from Phillips *et al.*⁵³. Roots (less than 2 mm diameter) were
582 washed with a nutrient solution (carbon free, NH_4NO_3 40 mg l^{-1} ; KH_2PO_4 13.6 mg l^{-1} ; K_2SO_4
583 349 mg l^{-1} ; CaCl_2 441 mg l^{-1} and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3705 g l^{-1}) to remove adhered soil and placed
584 in a glass syringe filled with glass beads ($750 \text{ }\mu\text{m}$) and nutrient solution (10 ml) to mimic the soil
585 environment. Roots were allowed to recover for 48 h, then the nutrient solution was replaced
586 (carbon and nitrogen free, KH_2PO_4 13.6 mg l^{-1} ; K_2SO_4 349 mg l^{-1} ; CaCl_2 441 mg l^{-1} and
587 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3705 g l^{-1}). Exudates were collected for 24 h, and the nutrient solution was
588 analyzed for dissolved organic carbon content (Shimadzu TOC-L Organic Carbon Analyzer,
589 LOD 0.01 mg C l^{-1}). Roots were dried ($40 \text{ }^\circ\text{C}$ for 48 h) to determine dry mass. Net exudation per
590 gram of dry root per m^2 was calculated using fine-root standing stock from soil cores ($n=5$)

591 collected within 1-2 weeks of the exudate collections. To support inclusion of exudation in the
592 calculation of NPP, mass of carbon in exudates was converted to equivalent dry matter units
593 assuming a carbon content of 48%, and the rate per day was scaled to a 246-day growing season.
594 Fine-root mass measured in soil cores sampled in 2022 were used to scale the exudation rates for
595 2022.

596

597 Statistics

598 Statistical analyses were performed with Stata software. The effect of CO₂ on dry matter
599 increment from 2017 to 2023 was analyzed by repeated measures analysis of variance, with plot
600 as the experimental unit and year as the repeated measure. Individual years and the total over the
601 treatment years were analyzed by two-sided *t*-test. NPP was analyzed similarly, except only two
602 years were included in the repeated measures analysis. The Stata command for repeated
603 measures analysis of DMI (and similarly for NPP) was: `anova dmi co2 plot|co2 year year#co2.`

604

605 **Data availability**

606 All data are publicly available without restriction at Dryad (<https://datadryad.org/stash>) DOI:
607 10.5061/dryad.z612jm6jw (ref. ⁵⁴). Biological samples (leaf litter, tree cores) were collected at
608 the BIFoR research site (52.801°N, 2.301°W) and are archived at the University of Birmingham.

609

610 **Code availability**

611 Quantitative Structure Models and calculation of tree volume from TLS data (QSMs) used the
612 open-source software of optQSM (<https://github.com/apburt/optqsm>) and TreeQSM, version
613 2.4.1 (<https://github.com/InverseTampere/TreeQSM>).

614

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