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

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38		

40 Abstract

Enhanced CO₂ assimilation by forests as atmospheric CO₂ concentration rises could slow 41 the rate of CO₂ increase if the assimilated carbon is allocated to long-lived biomass. 42 Experiments in young tree plantations support a CO₂ fertilization effect as atmospheric 43 CO₂ continues to increase. Uncertainty exists, however, as to whether older, more mature 44 forests retain the capacity to respond to elevated CO₂. Here we show, aided by tree-ring 45 analysis and canopy laser scanning, that a 180-year-old Quercus robur L. woodland in 46 central England increased the production of woody biomass when exposed to free-air CO_2 47 enrichment (FACE) for seven years. Further, elevated CO2 increased exudation of carbon 48 from fine roots into the soil with likely effects on nutrient cycles. The increase in tree 49 growth and allocation to long-lived woody biomass, demonstrated here, substantiates the 50 major role for mature temperate forests in climate change mitigation. 51

52

53 Main

Carbon uptake and storage by terrestrial vegetation is a major source of uncertainty in 54 projections of future levels of CO_2 in the atmosphere and the resulting effects on climate^{1,2}. 55 Multiple lines of evidence indicate that increasing atmospheric CO₂ concentration in recent 56 57 decades resulting from anthropogenic emissions and land use change have led to higher rates of CO_2 uptake by plants, i.e., the CO_2 fertilization effect, including in forests, which dominate the 58 terrestrial C cycle^{3,4}. Evidence for forest responses to the atmospheric CO₂ concentrations that 59 will be attained in future decades comes from a limited number of decade-long free-air CO₂ 60 enrichment (FACE) experiments in which forest plots were exposed to elevated CO_2 (eCO₂). 61 These "first generation" forest FACE experiments⁵⁻⁷ were established in young tree plantations, 62 63 and questions arise as to the extent to which their responses to eCO₂, including increased growth and primary productivity, are predictive of the responses of older, more established forests⁸⁻¹⁰. 64 There are multiple issues to consider. As forests develop over time, nitrogen, which is often the 65 limiting resource in unmanaged temperate stands, becomes increasingly sequestered in wood or 66 recalcitrant soil organic matter and unavailable to support plant growth, a phenomenon referred 67 to as progressive nitrogen limitation (PNL)¹¹⁻¹³. Furthermore, the development of PNL can be 68 accelerated by eCO₂ (ref. ¹⁴). PNL is of particular interest now, because global atmospheric 69 carbon and nitrogen sources are now moving in opposite directions^{15,16}. Phosphorus limitation 70

might be a critical influence on responses to eCO_2 on sites with old, highly weathered soils^{9,17}.

Apart from nutrient limitations, older trees may be less responsive to eCO₂ than younger trees

because a smaller fraction of the biomass is live tissue contributing to growth.

74

The amount of biomass produced each year—the net primary productivity (NPP)—is a key 75 metric for evaluating forest response to eCO_2 and a benchmark for models¹⁸⁻²⁰. If NPP increases 76 in eCO₂, the key question becomes whether the additional C accumulates in wood or instead is 77 allocated to leaves and fine roots that turn over rapidly and release the C back to the 78 atmosphere²¹. Carbon allocated to wood can persist in the ecosystem for policy-relevant time 79 frames^{22,23}, which provides a negative feedback to atmospheric CO₂. Over longer time frames 80 and landscape scales, C sequestration depends not just on the effects of eCO₂ on tree growth but 81 also on the rate of tree mortality¹⁰, and some evidence suggests that increased growth rate may 82 shorten a tree's lifespan^{24,25}. Nevertheless, the effect of rising CO₂ on NPP and how it is 83 allocated, as revealed in FACE experiments, is critical input to any evaluation of the interaction 84 between terrestrial ecosystems and atmospheric CO₂. 85

86

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

102 **BIFoR FACE facility**

103 The BIFoR FACE facility (https://www.birmingham.ac.uk/research/bifor/face/index.aspx,

Extended Data Fig. 1) is located in central England (52.801°N, 2.301°W, 107 m above sea level).
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
- 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
- by 180-year-old Quercus robur L. trees, which represent 92% of the forest's basal area. The
- 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
- 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
- approximately 30 m diameter. Disturbance to the woodland was avoided during construction of
- 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 $140 \pm$
- 124 38 ppm relative to ambient air due to occasional disruptions in CO₂ supply. Full description of
- 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
- 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

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

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

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

164

165 Net primary productivity

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

179

Most of the observed increase in NPP was attributable to wood production; there was no 180 181 difference in fine-root or leaf mass production, although standing stock of fine roots may have been greater and turnover slower in eCO₂. Leaf mass per unit area (LMA) was ~10% greater and 182 oak leaf area index (LAI) was 5% less in eCO2 (Table 2). LAI in fully occupied sites with 183 relatively high LAI is not expected to increase in eCO₂ (ref.³⁷). Significant increases in fine-root 184 production are common in forest FACE experiments³⁷ but has mainly been observed as a 185 response to eCO₂ in nutrient-limited forest ecosystems³⁸. Although increased fine-root 186 187 production was reported from measurements predominantly under stools of coppiced C. avellana in years one and two of the BIFoR FACE CO₂ treatment³⁹, the absence of a sustained increase in 188 the BIFoR experiment is not unexpected given that the BIFoR FACE forest does not appear to be 189 N-limited (discussed further below). Nevertheless, there was evidence of increased carbon 190 allocation belowground as exudation. Exudation of organic carbon from roots is a component of 191 192 NPP but is rarely included in estimates given the difficulty in measuring and upscaling exudation. We measured rates of net exudation four times between August 2020 and June 2021 193 194 (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
- in 2021 and 2022, with the assumption that exudation rate in the organic horizon applied equally
- to deeper roots and that the average of four measurements in spring, summer, and autumn
- reflected the average exudation rate during the 264-day growing season. Exudation flux was 63%
- greater in eCO₂ in 2021 (P = 0.13) and 43% greater in 2022 (P > 0.20); repeated measures
- analysis indicated a significant overall effect (P = 0.042, $F_{1,4} = 8.64$).
- 201

202 Discussion

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

226

Our results contrast with those of EucFACE, where no increases in dry matter accumulation or 227 NPP were observed¹⁷. BIFoR FACE results argue against a general conclusion that older, mature 228 forest systems have no capacity for response to eCO₂. The difference in response between these 229 two experiments is more likely related to nutrient dynamics rather than simply stand age or 230 maturity. EucFACE responses are thought to be limited by a phosphorus deficiency¹⁷, and 231 mature northern temperate forests are generally thought to be nitrogen limited. However, no 232 clear nitrogen or phosphorus limitation has yet been documented at BIFoR FACE, and leaf 233 nitrogen content has been maintained⁴⁰, although further investigation—and indeed further CO₂ 234 treatment—could change these assessments. As a result of surrounding agricultural activities and 235 regional industry, nitrogen deposition at the BIFoR FACE site is relatively high, 22 kg ha⁻¹ 236 (ref.⁴¹), and not atypical of northern temperate forests. This subsidy of reactive nitrogen may be 237 providing sufficient nitrogen supply to support increased carbon fixation. Furthermore, enhanced 238 soil nitrogen transformations⁴² supported by increased release of bioavailable carbon from root 239 exudation, may be allowing the trees to meet their nitrogen demand under eCO₂. Although 240 241 exudation comprised just 4-7% of total NPP, this flux of highly labile organic C is disproportionately important to ecosystem biogeochemistry. For example, exudation can increase 242 243 the availability of labile C, priming the microbial community and associated nitrogen and phosphorus cycling^{43,44}. Measurements on site have shown that net nitrogen mineralization 244 increased on average by 30% under elevated eCO₂, delivering an extra 24 kg N ha⁻¹ y⁻¹ (ref. ⁴⁵). 245 However, it is possible that the supply of bioavailable nitrogen sourced from decomposition of 246 soil organic matter may be reduced and eventually exhausted over time. Furthermore, nitrogen 247 deposition is declining in the UK¹⁵ and throughout the Global North⁴⁶. Although at present 248 249 nitrogen does not appear to be a limiting factor to tree growth or response to eCO₂, nitrogen limitation may develop at BIFoR FACE as the nitrogen cycle gets tighter and plant demand 250 increases, as was observed in a previous FACE experiment¹⁴. Furthermore, high rates of N 251 deposition have been shown to stimulate P acquisition and alleviate potential P limitation⁴⁷, so it 252 is possible that if N deposition declines, P limitation could develop over time. 253 254

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

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

271

272 Our results directly refute the notion that mature forests cannot respond to eCO_2 , and they emphasize that the important issue is allocation of any increased carbon uptake and the turnover 273 of the tissues that benefit most²¹. Hence, the evidence from BIFoR FACE of a significant 274 increase in woody biomass production is a key result supporting the role of mature forest stands 275 as decadal C stores¹⁰ and, hence, as natural climate solutions in the coming decades while society 276 undertakes deep decarbonization⁴⁸. Quantifying the CO₂ fertilization effect is important for 277 predictions of future atmospheric CO₂ concentrations and the policy decision that derive 278 therefrom, but even if the increase in tree growth translates to a longer-term increase in C storage 279 280 in the ecosystem, CO₂ fertilization cannot be seen as reason to delay reductions in fossil fuel consumption. 281

282

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- 299 R.M.T. collected data and contributed to analysis. G.C. and D.B. curated data. R.J.N. wrote the
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- 303
- 304 **Competing interests:** Authors declare that they have no competing interests.
- 305
- 306

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

308 All data are the means of three plots in ambient CO_2 and three plots in elevated $CO_2 \pm$ standard

309 error, expressed as grams dry matter per square meter. None of the differences within year are

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

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

senescing leaves and the assumption that nitrogen was exported as glutamine³⁰. Plot-level leaf

mass production was normalized to the pretreatment (2016) values. Production of fine roots (< 2

mm diameter) was determined from in-growth cores installed to 30 cm depth and scaled to 1 m

depth based on the relative depth distribution of fine-root standing crop in core samples. E/A is

the ratio of the measure in eCO_2 to the measure in aCO_2 .

319

320

	<i>Q. robur</i> bole + branch	<i>Q. robur</i> coarse root	Understory wood	Total wood	Fine root	Exudation	Reprod. tissue	Leaves	Total NPP
2021				1	NPP (g m ⁻²)				
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				Fraction	al distributior	n (%)			
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									
2022				10.9 1 2 9	105 00	56102	40 ± 0.2	201-11	
Ambient				40.8 ± 2.8	10.5 ± 0.9	3.0 ± 0.3	4.9 ± 0.3	30.2± 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

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

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,

 $F_{1,4} = 4.2$ 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 aCO_2 .
- 330

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

Figure Legends

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

- (A) annual basal area increment (BAI) of each tree. Orange lines are trees that began receiving
- elevated CO₂ in 2017; blue lines are trees that remained in ambient CO₂. (**B**) Cumulative BAI
- per plot area since 2010; data are the means of trees in three ambient CO₂ plots and three
- elevated CO_2 plots \pm standard error. (C) Cumulative BAI adjusted by dividing data in (B) by the
- mean BAI from 2020 to 2015 relative to the overall mean for the period.

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- Fig. 2. Dry matter increment per tree. Data are the mean \pm standard error of trees in three plots
- 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
- 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, $F_{6,24} = 0.46$).

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469 Methods

470 <u>Wood production</u>

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

478

Previous analysis of tree biomass at this site employed a published allometric equation for Q.

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

collected within the six experimental arrays in January and February 2022, when canopy was in

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

extraction of single trees from the co-registered point cloud was done in CloudCompare

(https://www.cloudcompare.org/). Determining appropriate parameters for, and the construction

of, the Quantitative Structure Models (QSMs) with subsequent calculation of component

490 volumes was done using the open-source software of optQSM

491 (<u>https://github.com/apburt/optqsm</u>) and TreeQSM, version 2.4.1

492 (<u>https://github.com/InverseTampere/TreeQSM</u>), respectively. The optimal cylinder model per

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

- using a wood density of 0.58, an average of trunk and branch density reported for Q. robur⁵¹.
- 496 The regression of ln(biomass, kg) vs ln(diameter, cm), with diameter being that calculated from
- 497 dendroband measurements in December 2021 is: $\ln(\text{biomass}, \text{kg}) = 2.312 \times \ln(\text{diameter}, \text{cm}) -$
- 498 1.0863; $r^2 = 0.77$ (Extended Data Fig.2).

Aboveground wood mass (kg) of other species was calculated using allometric equations from 499 Forrester *et al.*⁵⁰, where M_{stem} is dry matter (kg) and D is diameter (cm): 500 For *Acer pseudoplatanus*, $\ln(M_{stem}) = -2.3116 + 2.4186 \times \ln(D)$ 501 For other species: $\ln(M_{\text{stem}}) = -2.1653 + 2.4143 \times \ln(D)$ 502 Annual wood production per tree was calculated as the dry matter increment (DMI) during the 503 504 vear. 505 Coarse root production for understory species was calculated similarly using the equation⁵⁰: 506 $\ln(M_{root}) = -2.6183 + 2.1353 \times \ln(D)$ 507 The equation⁵⁰ for *Q*. robur coarse root biomass $[\ln(M_{root}) = -2.863 + 2.208 \times \ln(D)]$ 508 is assumed to be inaccurate to the same extent as the difference between the equivalent 509 above ground equation⁵⁰ $[\ln(M_{stem}) = -2.9128 + 2.7442 \times \ln(D)]$ and the site-specific TLS-based 510 allometry (Extended Data Fig.2), which varied from 56% greater to 40% less. Hence, O. robur 511 coarse root biomass was calculated by multiplying aboveground biomass (from the TLS 512 allometry) by the ratio of coarse root to above ground biomass from the published⁵⁰ equations. 513 514 DMI of the multi-stemmed stools of coppiced Corylus avellana was determined by measuring 515 516 the five thickest sprouts on each hazel stool within the plots irrespective of whether their DBH was below 10 cm, as described⁵². Coarse root production of *C. avellana* was not estimated as a 517 518 function of D but was assumed to be 26% of aboveground wood production, as determined from

520

519

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

the other understory species.

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

547

548 Leaf Production

Leaf production was determined from leaf litter mass. Leaf litter was collected in two or three 549 $1-m^2$ traps per plot (2016-2020) and six 0.25-m² traps (2020-2022). Litter was collected monthly 550 from March to October and weekly from mid-October to mid-December, separated by species, 551 oven dried, and weighed. Some leaves were retained on the trees and fell during the winter and 552 early spring. Hence, annual litter mass production was calculated as the litter collected from 553 April through March. Leaf production exceeds litter mass because of resorption of N-rich 554 organic compounds during senescence. Based on the difference in N concentration between 555 green leaves and litter and assuming N is resorbed as glutamate, green leaf production was set to 556 be 14% greater than litter production. Leaf production in 2017-2021 was relativized to 557 pretreatment (2016) values to account for spatial differences. 558

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),

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 <u>Fine-root production</u>

- 567 Fine-root production was measured from in-growth cores. Five 5-cm diameter \times 30-cm long
- mesh columns filled with root-free soil from the O horizon (0-7 cm depth, 0.64 g cm⁻³), A

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 mm diameter)

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

fine-root standing crop in the top 30 cm (approximately 65%).

575

576 <u>Exudation</u>

Root exudation was measured four times between August 2020 and June 2021. *Q. robur* roots in the O horizon were identified based on surveys of root morphology outside of experimental arrays. Root boxes within 1 m of a *Q. robur* trees were installed to permit access to new root growth during this period, and exudates were collected from six root systems per plot. The collection procedure was adapted from Phillips *et al.* ⁵³. Roots (less than 2 mm diameter) were

washed with a nutrient solution (carbon free, NH4NO3 40 mg l^{-1} ; KH2PO4 13.6 mg l^{-1} ; K2SO4

583 349 mg l^{-1} ; CaCl₂ 441 mg l^{-1} and MgSO₄.7H₂O 0.3705 g l^{-1}) to remove adhered soil and placed

584 in a glass syringe filled with glass beads (750 µm) and nutrient solution (10 ml) to mimic the soil

environment. Roots were allowed to recover for 48 h, then the nutrient solution was replaced

- (carbon and nitrogen free, KH₂PO₄ 13.6 mg l^{-1} ; K₂SO₄ 349 mg l^{-1} ; CaCl₂ 441 mg l^{-1} and
- 587 MgSO₄.7H₂O 0.3705 g l^{-1}). Exudates were collected for 24 h, and the nutrient solution was
- analyzed for dissolved organic carbon content (Shimadzu TOC-L Organic Carbon Analyzer,
- 589 LOD 0.01 mg C l^{-1}). Roots were dried (40 °C for 48 h) to determine dry mass. Net exudation per
- $figure{1}$ gram of dry root per m² was calculated using fine-root standing stock from soil cores (n=5)

calculation of NPP, mass of carbon in exudates was converted to equivalent dry matter units 592 assuming a carbon content of 48%, and the rate per day was scaled to a 246-day growing season. 593 Fine-root mass measured in soil cores sampled in 2022 were used to scale the exudation rates for 594 2022. 595 596 **Statistics** 597 Statistical analyses were performed with Stata software. The effect of CO₂ on dry matter 598 599 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 treatment years were analyzed by two-sided *t*-test. NPP was analyzed similarly, except only two 601 602 years were included in the repeated measures analysis. The Stata command for repeated measures analysis of DMI (and similarly for NPP) was: anova dmi co2 plot|co2 year year#co2. 603 604 **Data availability** 605 606 All data are publicly available without restriction at Dryad (https://datadryad.org/stash) DOI: 10.5061/dryad.z612jm6jw (ref. 54). Biological samples (leaf litter, tree cores) were collected at 607 608 the BIFoR research site (52.801°N, 2.301°W) and are archived at the University of Birmingham. 609 **Code availability** 610 Quantitative Structure Models and calculation of tree volume from TLS data (QSMs) used the 611 612 open-source software of optQSM (https://github.com/apburt/optqsm) and TreeQSM, version 2.4.1 (https://github.com/InverseTampere/TreeQSM). 613 614 **Methods-only references** 615

collected within 1-2 weeks of the exudate collections. To support inclusion of exudation in the

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