

Elevated CO₂ does not increase eucalypt forest productivity on a low-phosphorus soil

David S. Ellsworth^{1*}, Ian C. Anderson¹, Kristine Y. Crous¹, Julia Cooke², John E. Drake¹, Andrew N. Gherlenda¹, Teresa E. Gimeno³, Catriona A. Macdonald¹, Belinda E. Medlyn¹, Jeff R. Powell¹, Mark G. Tjoelker¹, Peter B. Reich^{1,4}

¹Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia, ²School of Environment, Earth and Ecosystem Science, The Open University, Milton Keynes MK7 6AA, UK, ³ISPA, Bordeaux Science Agro, INRA, 33140 Villenave d'Ornon, France, ⁴Department of Forest Resources, University of Minnesota, St. Paul, MN, 55108 USA.

*e-mail: D.Ellsworth@westernsydney.edu.au

1 **Rising atmospheric CO₂ stimulates photosynthesis and productivity of forests, offsetting**
2 **CO₂ emissions^{1,2}. Elevated CO₂ experiments in temperate planted forests yielded ~23%**
3 **increases in productivity³ over the initial years. Whether similar CO₂ stimulation occurs**
4 **in mature evergreen broadleaved forests on low-phosphorus (P) soils is unknown,**
5 **largely due to lack of experimental evidence⁴. This knowledge gap creates major**
6 **uncertainties in future climate projections^{5,6} as a large part of the tropics is P-limited.**
7 **Here, we increased atmospheric CO₂ concentration in a mature broadleaved evergreen**
8 **eucalypt forest for three years, in the first large-scale experiment on a P-limited site. We**
9 **show that tree growth and other aboveground productivity components did not**
10 **significantly increase in response to elevated CO₂ in three years, despite a sustained**
11 **19% increase in leaf photosynthesis. Moreover, tree growth in ambient CO₂ was**
12 **strongly P-limited and increased by ~35% with added phosphorus. The findings suggest**
13 **that P availability may potentially constrain CO₂-enhanced productivity in P-limited**
14 **forests; hence, future atmospheric CO₂ trajectories may be higher than predicted by**
15 **some models. As a result, coupled climate-carbon models should incorporate both**
16 **nitrogen and phosphorus limitations to vegetation productivity⁷ in estimating future**
17 **carbon sinks.**

18

19 Limited understanding of the size of the CO₂-induced fertilisation effect on forest carbon
20 sinks remains among the largest quantitative uncertainties in terms of terrestrial feedbacks to
21 the carbon (C) cycle-climate system^{6,8,9}. Coupled climate-C cycle models project a 24-80%
22 increase of net primary productivity (NPP) for forests in the next 50 years with rising
23 atmospheric CO₂ concentration, with substantial atmospheric CO₂ responses expected for
24 forests in the tropics^{4,10}. These model projections are partly based on elevated CO₂ (eCO₂)
25 experiments in young temperate planted forests, which have yielded on average ~23%

26 increases in production³ over several years with 200 $\mu\text{mol mol}^{-1}$ increases in atmospheric
27 CO_2 concentrations^{4,11}. Due to the lack of experimental evidence, we presently do not know
28 how large the eCO_2 fertilisation response is for mature forests that grow on soils where
29 phosphorus (P) is limiting productivity^{4,10}, as is the case for many evergreen broadleaved
30 forests. This knowledge gap creates major uncertainties in future climate projections⁹ because
31 evergreen broadleaved forests comprise over a third of global forest area, and dominate the
32 atmospheric CO_2 sink at lower latitudes^{5,6}. Many eCO_2 experiments have taken place in
33 young tree plantations³ on relatively P-rich soils, but unlike aggrading forests, mature forests
34 are more likely near nutritional equilibrium with their underlying soils. Hence mature forests
35 may be more appropriate for understanding in situ nutrient limitations to productivity and C
36 storage with rising atmospheric CO_2 . Without clear understanding of this nutrient feedback to
37 the C cycle in evergreen broadleaved forests, we cannot accurately estimate the trajectory of
38 future atmospheric CO_2 , thus limiting our ability to estimate climate change mitigation by
39 such forests and constrain internationally-allowable CO_2 emissions^{9,12}.

40

41 Soil nutrient limitation may restrict eCO_2 -induced biomass enhancement and related C
42 storage processes¹¹, but it is unclear if the type of nutrient limitation is important. Studies in a
43 temperate grassland and a forest ecosystem under contrasting CO_2 and N supply suggest a
44 large initial stimulation in productivity, often followed by reduced CO_2 stimulation when N is
45 limiting^{13,14}. Limited P supply might affect tree growth and ecosystem C sequestration
46 processes differently than the N-supply limitation¹⁵ that has thus far been demonstrated in
47 eCO_2 experiments on N-poor soils. In heavily weathered soils common in tropical and
48 subtropical regions, P is typically bound to Fe and Al oxides, hydroxides and secondary
49 minerals and not available to plants. One possibility is that increased plant carbohydrate
50 availability from eCO_2 leads to increased plant investment in the secretion of organic acids

51 from roots¹⁶ or the investment in P-acquisition by mycorrhizal symbionts. This would thereby
52 reduce P-limitation to broadleaved evergreen forest productivity¹⁷ by increasing plant access
53 to scarce soil P. Consistent with this idea, there is evidence that recent rising CO₂ may have
54 driven a substantial portion of the observed historical increase in tropical forest carbon
55 stocks¹⁸ though future increases remain in question.

56

57 Although there is considerable variation in soil fertility across the world, tree growth in
58 highly weathered tropical and sub-tropical soils may be limited by P availability in addition
59 to, or rather than, N availability^{19,20}. Hence nutrient availability and the type of nutrient
60 limitation may both be important in regulating forest CO₂ fertilisation responses in those
61 regions^{7,17}. There is still little agreement on how to appropriately represent P limitations to
62 productivity in Earth systems models^{7,21}, and there has been no direct experimental test of the
63 CO₂ fertilisation effect in P-limited forests (Supplementary Fig. 1).

64

65 To help fill this gap, we established a free-air CO₂ enrichment experiment on six circular 25m
66 diameter plots in mature *Eucalyptus* forest (EucFACE) on a low P soil near Sydney, Australia
67 (23 m elevation; 33° 37' 4" S, 150° 44' 25" E) (Supplementary Fig. 2). The main canopy
68 species, *Eucalyptus tereticornis*, has a distribution through tropical and temperate zones.
69 EucFACE has unique characteristics compared to prior forest elevated CO₂ experiments: the
70 presence of mature broadleaved evergreen trees in natural unmanaged forest, and nutrient-
71 poor soil with a demonstrated P limitation to tree growth²². A gradual CO₂ enrichment began
72 in Sept 2012 at 30 μmol mol⁻¹ above ambient CO₂ concentration, and slowly ramped up to
73 the full-strength eCO₂ treatment of 150 μmol mol⁻¹ above ambient CO₂ concentration²³,
74 which began on 6 Feb 2013. This full CO₂ treatment was maintained throughout the
75 following three years (Feb. 2013-Feb. 2016) that are the focus of this report. We

76 hypothesised 1) a stimulation of photosynthesis and tree growth in early years of the
77 experiment, consistent with many previous experiments^{3,11,17}, but 2) that such enhancement
78 by eCO₂ would be modest (compared to other studies) due to the strong P limitation in this
79 system²⁴.

80

81 Over the first three years of eCO₂, we found a significant enhancement of light-saturated leaf
82 net photosynthesis rate in the tree canopies ($F_{1,4} = 18.20$, $P = 0.013$; Table 1, Fig. 1). Prior to
83 eCO₂ enhancement, there had been no significant pre-treatment difference (Fig. 1). Over ten
84 repeated sampling dates, the average stimulation by eCO₂ of photosynthesis was 19% with a
85 95% confidence interval (CI) between 14.5% and 24.0%. The consistent stimulation of
86 photosynthesis suggests a sustained net positive CO₂ flux into the ecosystem from eCO₂ over
87 three years, in accord with previous experiments¹¹.

88

89 By contrast, this enhanced photosynthesis (Fig. 1) did not translate into increased tree stem
90 growth or aboveground productivity (Fig. 2). Aboveground net primary productivity (ANPP)
91 of the *Eucalyptus* forest averaged 300 g C m⁻² yr⁻¹ and was similar in eCO₂ and the ambient
92 CO₂ treatment (on average -8% across 2013-2015, P -value=0.43; Fig. 2, with a 95% CI for
93 this effect between -25% and +9%). The complete lack of a CO₂ fertilisation effect on
94 productivity was inconsistent with our hypothesis and unexpected based on previous
95 experiments^{3,11,15} and most models^{4,21}. ANPP was not statistically different between CO₂
96 treatments across years (Table 1) or for each year individually (Supplementary Figs. S2 and
97 S3), nor did any ANPP component indicate a positive eCO₂ response. Foliage and fine twig
98 (plus bark) production were the largest components of ANPP (Fig. 2), averaging 48% and
99 28% of the total, respectively. For these components, the estimated eCO₂ effect size
100 encompassed zero (95% CI between -30% and +7% for foliage and between -21% and +24%

101 for twigs). Similarly, the estimated eCO₂ effect size of wood production was not statistically
102 distinguishable from zero (Figure 2 and table S1). There was no significant eCO₂ effect on
103 stemwood biomass increment across the three years of this study, nor a year × eCO₂
104 interaction (Table S1; $P = 0.420$). Thus there was no indication of an eCO₂ fertilisation
105 response of any component of ANPP despite a sustained increase in photosynthesis.

106

107 We also examined tree-level biomass growth responses across tree size categories between
108 experimental manipulations we did within this forest, either of P availability or of
109 atmospheric CO₂. *Eucalyptus* trees in the forest were capable of higher growth when soil P
110 limitation was alleviated by P-fertilisation²², as growth of adjacent P-fertilised trees in
111 ambient CO₂ increased by 35% compared to similar sized ambient-grown, unfertilised trees
112 of the same size class over a similar 48-month period (Figure 3). These results suggest that
113 mature trees have the potential to respond to a release from P-limitation. Since growth was
114 greatest for the largest size classes of trees within the overall stand, we also asked whether
115 the eCO₂ effect showed size dependencies. For individual tree biomass increment, the growth
116 of all tree size classes was unaffected by eCO₂ regardless of whether individuals were
117 grouped by dominance (Table S1) or by diameter classes (Fig. 3, Fig. S3). Thus there was no
118 CO₂ fertilisation response observed for any size class of trees on this low-P site, in marked
119 contrast to previous observations in young temperate plantations. Even N-limited plantations
120 showed an initial eCO₂ stimulation in productivity^{13,15} whereas no such early eCO₂ response
121 occurred in our P-limited forest. These findings provide key evidence for the debate
122 regarding the capacity for CO₂ fertilisation of the large C stocks maintained in mature
123 forests^{1,25} particularly on P-limited soils at mid to low latitudes^{4,18} and fill a critical
124 knowledge gap for mature forests responses to eCO₂.

125

126 As no root production and turnover data are available for the first year and a half of the
127 experiment, we do not know whether belowground productivity was influenced by eCO₂,
128 though there is evidence of an initial stimulation in root and/or rhizosphere respiration
129 returning CO₂ back to the atmosphere²³. Assessing belowground productivity is challenging
130 given difficulties in accessing deep roots and methodological problems with all approaches
131 for quantifying belowground NPP²⁶. Given that ANPP is typically 75-80% of total forest NPP
132 globally²⁶, we demonstrated no eCO₂ response on productivity for an important set of
133 components of aboveground C balance in a P-limited forest ecosystem. A meta-analysis of
134 open-top chamber and free-air studies mostly in N-limited grassland ecosystems suggested
135 that root biomass might be stimulated slightly more than shoot biomass under eCO₂ (+28%
136 versus +22%, respectively), but cautioned that a lack of data on root and shoot biomass
137 measured simultaneously within long-term experiments precluded a definitive answer to that
138 question²⁷. Due to a paucity of studies, such data are not widely available for low P
139 ecosystems. Experiments involving eCO₂ on low-P sites are rare but in the glasshouse, ref. 24
140 found that neither root C nor total belowground C was significantly affected by eCO₂ until P
141 was added to a native soil. Lack of an aboveground growth response to eCO₂ in EucFACE,
142 lack of preferential belowground C stimulation of root growth in prior long-term eCO₂
143 studies¹⁴ and lack of a belowground response to eCO₂ by P-limited plants in a glasshouse²⁴
144 are all no guarantee that there will also be no belowground eCO₂ response in EucFACE.
145 However, these studies collectively suggest a large belowground C storage response of the
146 EucFACE to eCO₂ may be unlikely, though we cannot rule out the possibility. Given these
147 uncertainties, further work is needed to quantify the full stand C cycle response to eCO₂.
148
149 Our results are consistent with models accounting for nutrient limitations, suggesting that P-
150 limited forest ecosystems should show a constrained eCO₂-induced productivity

151 enhancement^{21,28}. These models are generally not well-constrained by empirical evidence^{4,21}
152 such as large-scale free-air CO₂ experiments, and the biogeochemistry of P availability in the
153 context of environmental change is not well understood^{7,17}. As a single tree species dominates
154 the forest overstory in our study, it may still be possible that species-rich tropical forests
155 show a larger composite response to eCO₂ than observed here²⁹. In this P-limited woodland,
156 we observed a complete lack of wood, twig, or foliage growth enhancement with CO₂
157 fertilisation. As forests vary in their degree of nutrient limitation²⁰, there is no reason to posit
158 that a complete absence of a productivity response to eCO₂ should be the norm in mature
159 forests on P-limited soils. However, given the prevalence of P limitations in subtropical and
160 tropical regions^{20,30}, our results strongly suggest that these forests might show a muted
161 productivity increase with CO₂ fertilisation, especially when compared with the strong
162 positive responses seen in young temperate forests on more fertile, P-rich soils¹¹. If this were
163 generally the case, it would indicate a constrained capacity of P-limited, mid- to low-latitude
164 mature forests to sequester additional C from the atmosphere in a CO₂-enriched world,
165 resulting in smaller reductions in atmospheric CO₂ concentrations and thus smaller allowable
166 emissions reductions than anticipated by models that do not consider P limitations.

167

168 **Methods**

169 Methods, including statements of data availability and any associated references, are
170 available in the online version of this paper.

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234

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246

247 **Author Contributions**

248 D.S.E, I.C.A. and B.E.M. designed the eCO₂ experiment. D.S.E., K.Y.C., T.E.G., designed
249 the photosynthesis measurements and carried out and analysed them with J.C. and J.E.D.
250 K.Y.C., J.C., J.R.P., D.S.E. and A.G. did the litterfall collections and measurements. D.S.E.,
251 P.B.R., J.R.P., K.Y.C., M.G.T. and B.E.M did the analyses and statistical tests. D.S.E. and
252 P.B.R. wrote the draft of the paper. All authors contributed to subsequent versions.

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255 **Additional information**

256 Supplementary information is available in the online version of the paper. Reprints and

257 permissions information is available online at www.nature.com/reprints.

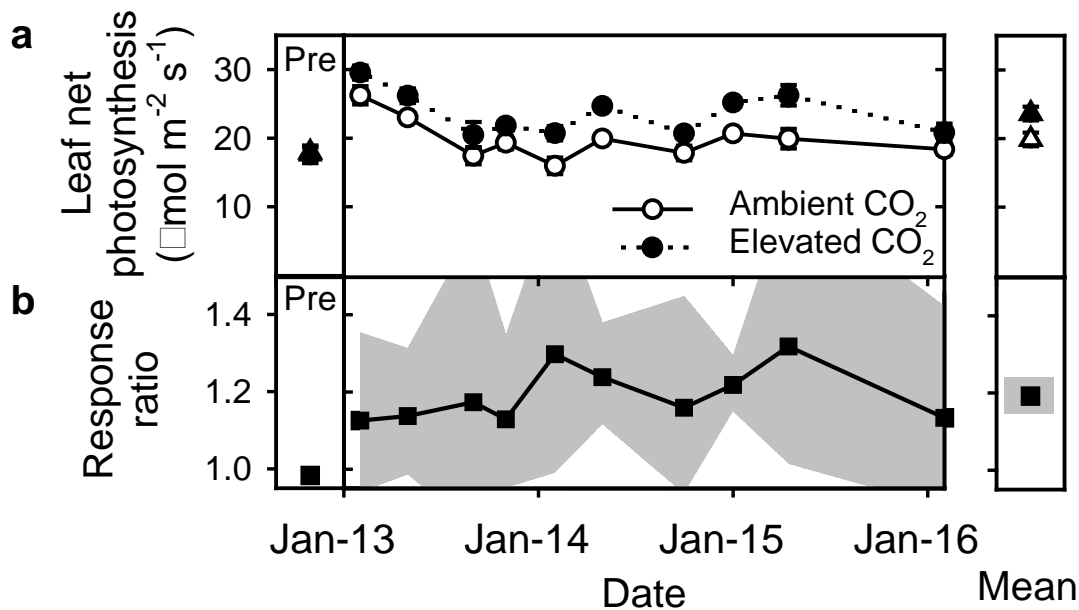
258 Correspondence and requests for materials should be addressed to D.S.E.

259

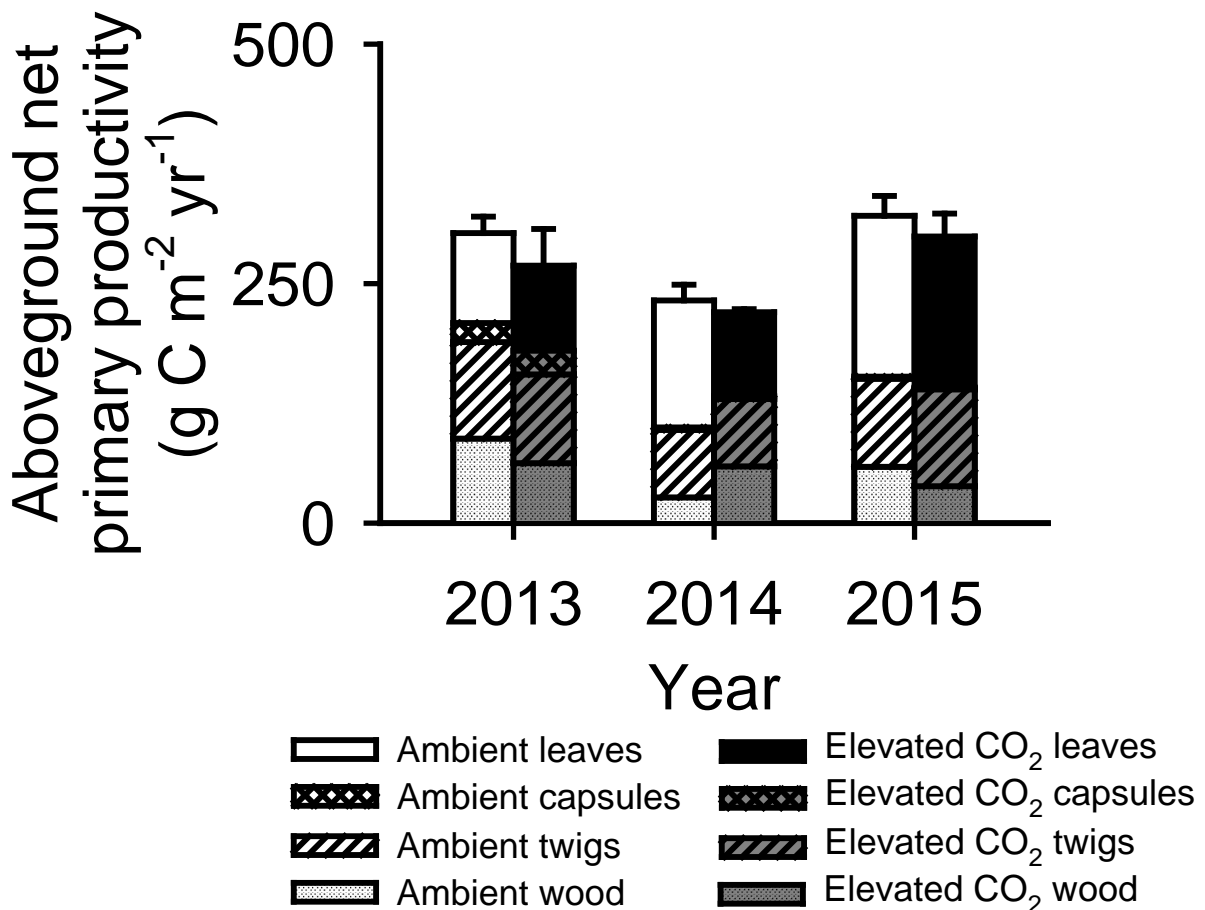
260 **Competing financial interests**

261 The authors declare no competing financial interests.

263 **Figure 1 | Pattern of leaf net photosynthesis in the canopy over the first three years of**
 264 **elevated CO₂.** (a) Photosynthesis for canopy leaves at prevailing seasonal temperatures and
 265 growth CO₂ concentration across time, including pre-treatment values (left) and the mean
 266 over the experimental period (right panel). For pretreatment (left panel), photosynthesis in
 267 both plot types was measured at the same ambient CO₂ concentration of 395 μmol mol⁻¹ prior
 268 to CO₂ enrichment. (b) The CO₂ fertilisation response ratio for photosynthesis over time,
 269 with grey areas representing two-sided 95% confidence intervals for the CO₂ fertilisation
 270 response ratio for each of the measurement timepoints. The mean response ratio with lower
 271 and upper 95% confidence limits is shown by the grey area around the square, taken across
 272 all timepoints (right panel). The leaf photosynthesis in (a) was significantly different overall
 273 between CO₂ treatments ($P = 0.013$) and there was no time × CO₂ treatment interaction
 274 (repeated-measures ANOVA from mixed-model analysis; Table 1). Means ± 1 s.e. for $N=3$
 275 plots per treatment are shown across ten different measurement periods, with open symbols
 276 for ambient and closed symbols for eCO₂. The s.e. bars may be obscured by points.



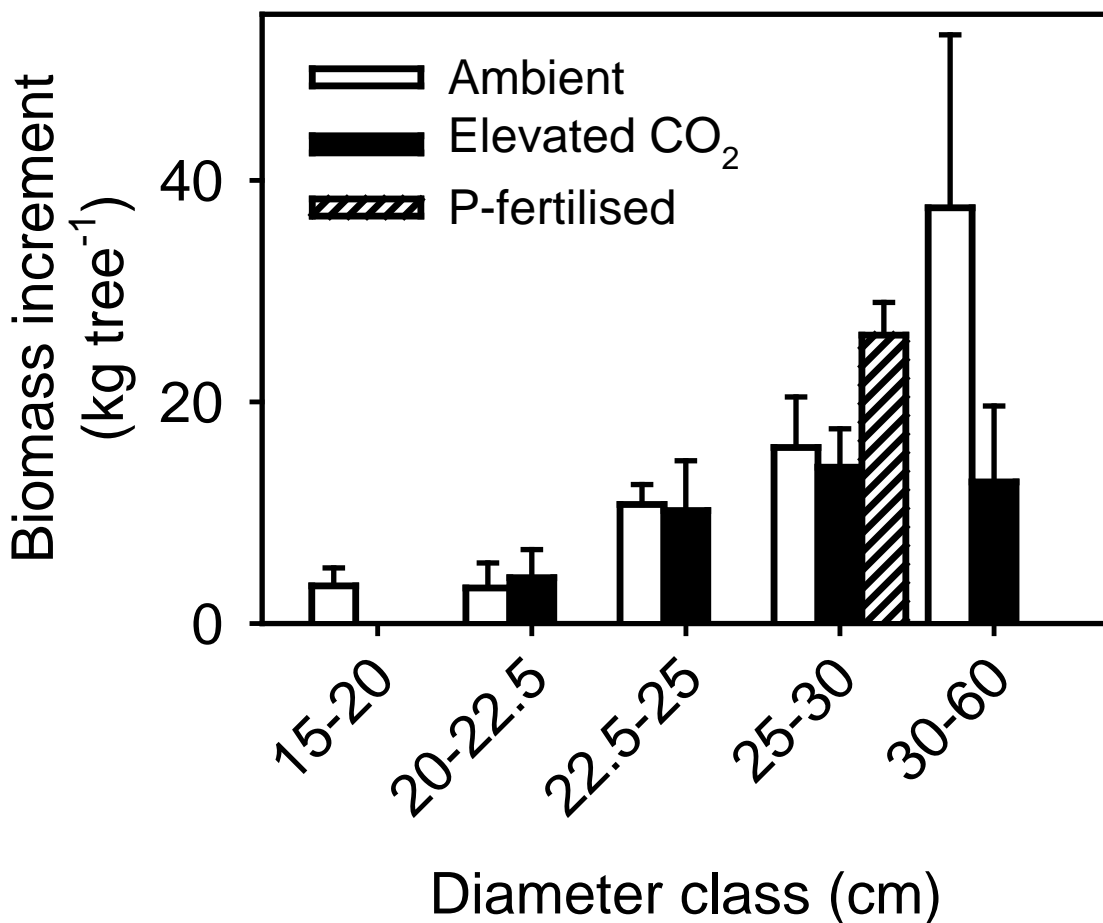
278 **Figure 2 | Aboveground net primary production (ANPP) in a mature *Eucalyptus* stand**
 279 **and its components across three years of elevated CO₂.** Total ANPP is represented by the
 280 combination of stemwood biomass production (stippled), fine twig and bark production
 281 (striped), seed and capsule production (hatched), and leaf production (solid). Stemwood
 282 production is determined as the annual biomass increment, and foliage+fine twig production
 283 are measured as annual biomass turnover collected monthly in permanent litter baskets.
 284 Reproductive structures (“capsules”) were measured in all three years but are small and
 285 obscured in 2014 and 2015. Ambient plots are shown with white backgrounds, and elevated
 286 CO₂ plots have grey/black backgrounds. Stem biomass increment, total foliage+fine twig
 287 turnover, and total ANPP were not significantly different across CO₂ treatments ($P = 0.85$,
 288 0.41, and 0.38 respectively). Means \pm 1 s.e. for $N=3$ plot replicates are shown for total ANPP,
 289 with yearly means shown for each component.



290

291

292 **Figure 3 | Biomass increment of five different size classes of *Eucalyptus* trees.** Shown is
293 the biomass increment over 4 years from Dec. 2011 to Dec. 2015 within each size-class for
294 ambient (open bars, mean \pm s.e.) and elevated CO₂-grown trees (dark bars, mean \pm s.e.), and
295 ambient-grown trees with four years of P fertilisation (striped bar, mean \pm s.e.). Diameter-
296 classes are defined as the diameter in Dec. 2011 prior to the start of treatments. The biomass
297 increment for elevated CO₂ trees in the first size class (15-20 cm) were not different from
298 zero. Each tree diameter-class by treatment combination contained 9 unsuppressed trees on
299 average ($N=5$ trees for P-fertilised). Bars are means + 1 s.e. within each size class. The P-
300 fertilised tree increment is significantly different from the ambient tree increment for the
301 appropriate size class ($P = 0.031$; one-tailed t -test).



302

303

304 **Table 1 | Repeated-measures analysis of variance of CO₂ treatment and time effects.**
 305 These effects are shown for leaf net photosynthesis (a, left side) and aboveground net primary
 306 production, ANPP from 2013 to 2015 (b, right side). The mixed-model repeated-measures
 307 analysis for photosynthesis was done using data shown in Fig. 1a), with the time term
 308 indicating sampling date across three years. For ANPP, the time term is ‘year’, the first to
 309 third year of the full eCO₂ treatment. In both analyses, a mixed-model repeated-measures
 310 analysis was done using a fixed treatment (CO₂) and a random plot effect, and Type III sums
 311 of squares computed using restricted maximum likelihood estimates for *F*-tests. The
 312 numerator and denominator degrees of freedom (df) for each *F*-test are shown.

313

a) Photosynthesis				b) ANPP		
Source	df	<i>F</i> -ratio	<i>P</i> -value	df	<i>F</i> -ratio	<i>P</i> -value
CO ₂	1,4	18.20	0.013	1,4	0.76	0.432
Time	9,36	9.10	<0.0001	2,8	5.85	0.084
CO ₂ x Time	9,36	0.73	0.682	2,8	0.094	0.911

314

315 **Methods (online)**

316 Six large circular plots (0.05 ha each) were established in 2010 in a mature eucalypt
317 woodland on an alluvial spodosol in western Sydney, Australia. The location receives 800
318 mm of precipitation per annum on average and has a mean annual temperature of 17.5°C
319 (www.bom.gov.au). Mean maximum temperature in the warmest month is 30°C and mean
320 minimum temperature in the coldest month is 3.6°C, with monthly mean temperatures always
321 > 10°C. The CO₂ treatment was implemented in three of the plots using free-air CO₂
322 enrichment under computer control using the pre-dilution approach starting in Sept. 2012.
323 After a period where the [CO₂] increased gradually over approximately 6 months²³, the plots
324 received ambient +150 μmol mol⁻¹ CO₂ during daylight hours over all days of the year, for
325 Feb. 2013 onward. The mean 5-minute [CO₂] in the tree crowns was kept within ± 50% of
326 the desired target of ambient +150 μmol mol⁻¹ for 98% of the daylight hours over 2013-14
327 (Fig. S2). A separate set of trees within the stand (*N* = 5), located at least 60 m from the eCO₂
328 plots, were fertilised with 50 kg P ha⁻¹ yr⁻¹ starting in 2011, in two lots of superphosphate
329 fertiliser applied within the drip-line of the trees during the growing season²². Root barriers
330 were established prior to any fertilisation by trenching and inserting a plastic barrier to 50 cm
331 depth in the soil around a set of fertilised and control trees. The P-addition treatments were
332 maintained through the duration of the study, resulting in 4 years of P-fertilisation concurrent
333 with the 3-year eCO₂ study.

334

335 **Net photosynthesis.** Light-saturated net photosynthesis of leaves was measured at high light,
336 the growth CO₂ concentration and prevailing seasonal temperature at the top of three
337 dominant or co-dominant trees in each plot using a pair of temperature- and CO₂-controlled
338 portable photosynthesis systems (Li-6400, Li-Cor Inc.). Access to the ca. 22 m treetops was
339 by construction cranes permanently located adjacent to each plot³¹. A smaller set of

340 measurements on shaded foliage within the tree crowns was used to confirm results from the
341 upper-crown measurements in terms of the CO₂-enhancement effect on photosynthesis, thus
342 the entire crown can be expected to behave similarly.

343

344 **Aboveground productivity measurements.** Wood production was estimated from measured
345 stem diameter changes for $N=146$ trees across the ambient and elevated plots. The diameter
346 of each tree was measured at 1.3 m height at approximately monthly intervals starting
347 February 2011, 2 years prior to commencement of the full CO₂ treatment. Manual band
348 dendrometers were used to monitor stem diameter changes. The permanently-placed bands
349 consisted of plastic straps graduated with a vernier scale placed around a tree (D1 Permanent
350 Girth Tape, UMS GmbH, München, Germany) to detect changes in diameter to the nearest
351 $0.01 \cdot \pi$ cm. As 99% of the tree stems measured represented by *E. tereticornis*, a species-
352 specific allometric regression for *E. tereticornis*³² was used to convert these increments to
353 aboveground biomass increment. Of a total of 146 trees measured across the ambient and
354 elevated plots, 49 suppressed trees, 6 co-dominant trees with trunk defects, and 4 trees
355 showing shrinkage possibly preceding mortality were omitted from the mixed-model
356 analysis. We thus used a total of $N=87$ trees measured across all years and without stem
357 defects, suppression or shrinkage in the mixed-model analyses.

358 Foliage and twig production were measured as litterfall, collected monthly in ~ 0.2 m²
359 circular fine-mesh traps at eight random locations per plot³³. Litter was sorted into leaf, twigs
360 and bark, and other material, dried at 40°C and weighed. A subsample was reweighed when
361 dried at 70°C and a small moisture correction was applied to the leaf component of the whole
362 dataset. We use litterfall to estimate annual foliage and twig production, but acknowledge
363 that this approach assumes steady-state for these pools as would be expected in mature forest
364 without any recent major disturbance. A steady-state status for foliage pools in 2013 and

365 2014 has been demonstrated in Ref. 32 but foliage litterfall was a month earlier in all rings in
366 2015 than prior years due to an outbreak of psyllids (*Cardiaspina* sp.)³⁴.

367 Annual C turnover by trunk bark production was not accounted for. For the leaf
368 component, the productivity was computed as the sum of annual litterfall whilst for twigs we
369 assume strictly annual turnover across the three years. We assume that all biomass
370 components are comprised of 47% C for the purpose of calculating annual C storage and
371 turnover comprising aboveground net productivity.

372

373 **Statistical analyses.** We analysed the photosynthesis data³⁵ using a mixed-model repeated-
374 measures analysis of variance in R v3.3.1 using the 'lme4' function within the 'nlme' package,
375 with CO₂ treatment as a fixed factor and plot as a random factor nested within CO₂ treatment.
376 There were no pre-treatment differences in photosynthesis at light-saturation and prevailing
377 temperatures amongst the plots measured at the same [CO₂] ($P > 0.10$). Outcomes from type
378 III *F*-tests are reported. A similar model was used to analyse annual above-ground net
379 productivity, including leaf production, twig and bark production, and total stem growth.
380 Confidence intervals for the CO₂ effect size estimate were computed in R ([http://cran.r-](http://cran.r-project.org)
381 [project.org](http://cran.r-project.org)) using the function 'confint', which applies quantile functions for the *t*-
382 distribution after model-fitting. We further analysed stemwood increment³⁵ on an individual
383 tree basis for the largest 15 trees in each plot, using pre-treatment growth (biomass increment
384 from Feb. 2011 – June 2012) as a covariate. For this analysis both plot and tree were treated
385 as random factors. Pre-treatment was comprised of 2011 and the first six months of 2012
386 where no additional CO₂ was added to the plots^{23,31}. All data were checked for normality
387 using the Q-Q plots and Levene's test, and residuals from model fitting were checked for
388 evidence of heteroscedasticity. Constant error variances were confirmed by this approach,
389 and if not, then an appropriate transformation was employed to ensure constant variances.

390 **Data availability.** The datasets generated during and/or analysed during the current study are
391 available in a Research Data Australia repository (<http://doi.org/10.4225/35/57ec5d4a2b78e>).

392

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