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Rising atmospheric CO_2 stimulates photosynthesis and productivity of forests, offsetting CO_2 emissions[1,2]. Elevated CO_2 experiments in temperate planted forests yielded ~23% increases in productivity[3] over the initial years. Whether similar CO_2 stimulation occurs in mature evergreen broadleaved forests on low-phosphorus (P) soils is unknown, largely due to lack of experimental evidence[4]. This knowledge gap creates major uncertainties in future climate projections[5,6] as a large part of the tropics is P-limited. Here, we increased atmospheric CO_2 concentration in a mature broadleaved evergreen eucalypt forest for three years, in the first large-scale experiment on a P-limited site. We show that tree growth and other aboveground productivity components did not significantly increase in response to elevated CO_2 in three years, despite a sustained 19% increase in leaf photosynthesis. Moreover, tree growth in ambient CO_2 was strongly P-limited and increased by ~35% with added phosphorus. The findings suggest that P availability may potentially constrain CO_2-enhanced productivity in P-limited forests; hence, future atmospheric CO_2 trajectories may be higher than predicted by some models. As a result, coupled climate-carbon models should incorporate both nitrogen and phosphorus limitations to vegetation productivity[7] in estimating future carbon sinks.

Limited understanding of the size of the CO_2-induced fertilisation effect on forest carbon sinks remains among the largest quantitative uncertainties in terms of terrestrial feedbacks to the carbon (C) cycle-climate system[6,8,9]. Coupled climate-C cycle models project a 24-80% increase of net primary productivity (NPP) for forests in the next 50 years with rising atmospheric CO_2 concentration, with substantial atmospheric CO_2 responses expected for forests in the tropics[4,10]. These model projections are partly based on elevated CO_2 (eCO_2) experiments in young temperate planted forests, which have yielded on average ~23%

increases in production[3] over several years with 200 µmol mol^{-1} increases in atmospheric CO_2 concentrations[4,11]. Due to the lack of experimental evidence, we presently do not know how large the eCO_2 fertilisation response is for mature forests that grow on soils where phosphorus (P) is limiting productivity[4,10], as is the case for many evergreen broadleaved forests. This knowledge gap creates major uncertainties in future climate projections[9] because evergreen broadleaved forests comprise over a third of global forest area, and dominate the atmospheric CO_2 sink at lower latitudes[5,6]. Many eCO_2 experiments have taken place in young tree plantations[3] on relatively P-rich soils, but unlike aggrading forests, mature forests are more likely near nutritional equilibrium with their underlying soils. Hence mature forests may be more appropriate for understanding in situ nutrient limitations to productivity and C storage with rising atmospheric CO_2. Without clear understanding of this nutrient feedback to the C cycle in evergreen broadleaved forests, we cannot accurately estimate the trajectory of future atmospheric CO_2, thus limiting our ability to estimate climate change mitigation by such forests and constrain internationally-allowable CO_2 emissions[9,12].

Soil nutrient limitation may restrict eCO_2-induced biomass enhancement and related C storage processes[11], but it is unclear if the type of nutrient limitation is important. Studies in a temperate grassland and a forest ecosystem under contrasting CO_2 and N supply suggest a large initial stimulation in productivity, often followed by reduced CO_2 stimulation when N is limiting[13,14]. Limited P supply might affect tree growth and ecosystem C sequestration processes differently than the N-supply limitation[15] that has thus far been demonstrated in eCO_2 experiments on N-poor soils. In heavily weathered soils common in tropical and subtropical regions, P is typically bound to Fe and Al oxides, hydroxides and secondary minerals and not available to plants. One possibility is that increased plant carbohydrate availability from eCO_2 leads to increased plant investment in the secretion of organic acids from roots[16] or the investment in P-acquisition by mycorrhizal symbionts. This would thereby reduce P-limitation to broadleaved evergreen forest productivity[17] by increasing plant access to scarce soil P. Consistent with this idea, there is evidence that recent rising CO_2 may have driven a substantial portion of the observed historical increase in tropical forest carbon stocks[18] though future increases remain in question.

Although there is considerable variation in soil fertility across the world, tree growth in highly weathered tropical and sub-tropical soils may be limited by P availability in addition to, or rather than, N availability[19,20]. Hence nutrient availability and the type of nutrient limitation may both be important in regulating forest CO_2 fertilisation responses in those regions[7,17]. There is still little agreement on how to appropriately represent P limitations to productivity in Earth systems models[7,21], and there has been no direct experimental test of the CO_2 fertilisation effect in P-limited forests (Supplementary Fig. 1).

To help fill this gap, we established a free-air CO_2 enrichment experiment on six circular 25m diameter plots in mature *Eucalyptus* forest (EucFACE) on a low P soil near Sydney, Australia (23 m elevation; 33° 37' 4" S, 150° 44' 25" E) (Supplementary Fig. 2). The main canopy species, *Eucalyptus tereticornis*, has a distribution through tropical and temperate zones. EucFACE has unique characteristics compared to prior forest elevated CO_2 experiments: the presence of mature broadleaved evergreen trees in natural unmanaged forest, and nutrient-poor soil with a demonstrated P limitation to tree growth[22]. A gradual CO_2 enrichment began in Sept 2012 at 30 µmol mol^{-1} above ambient CO_2 concentration, and slowly ramped up to the full-strength eCO_2 treatment of 150 µmol mol^{-1} above ambient CO_2 concentration[23], which began on 6 Feb 2013. This full CO_2 treatment was maintained throughout the following three years (Feb. 2013-Feb. 2016) that are the focus of this report. We hypothesised 1) a stimulation of photosynthesis and tree growth in early years of the experiment, consistent with many previous experiments[3,11,17], but 2) that such enhancement by eCO_2 would be modest (compared to other studies) due to the strong P limitation in this system[24].

Over the first three years of eCO_2, we found a significant enhancement of light-saturated leaf net photosynthesis rate in the tree canopies ($F_{1,4}$ = 18.20, P = 0.013; Table 1, Fig. 1). Prior to eCO_2 enhancement, there had been no significant pre-treatment difference (Fig. 1). Over ten repeated sampling dates, the average stimulation by eCO_2 of photosynthesis was 19% with a 95% confidence interval (CI) between 14.5% and 24.0%. The consistent stimulation of photosynthesis suggests a sustained net positive CO_2 flux into the ecosystem from eCO_2 over three years, in accord with previous experiments[11].

By contrast, this enhanced photosynthesis (Fig. 1) did not translate into increased tree stem growth or aboveground productivity (Fig. 2). Aboveground net primary productivity (ANPP) of the *Eucalyptus* forest averaged 300 g C m^{-2} yr^{-1} and was similar in eCO_2 and the ambient CO_2 treatment (on average -8% across 2013-2015, P-value=0.43; Fig. 2, with a 95% CI for this effect between -25% and +9%). The complete lack of a CO_2 fertilisation effect on productivity was inconsistent with our hypothesis and unexpected based on previous experiments[3,11,15] and most models[4,21]. ANPP was not statistically different between CO_2 treatments across years (Table 1) or for each year individually (Supplementary Figs. S2 and S3), nor did any ANPP component indicate a positive eCO_2 response. Foliage and fine twig (plus bark) production were the largest components of ANPP (Fig. 2), averaging 48% and 28% of the total, respectively. For these components, the estimated eCO_2 effect size encompassed zero (95% CI between -30% and +7% for foliage and between -21% and +24%

for twigs). Similarly, the estimated eCO_2 effect size of wood production was not statistically distinguishable from zero (Figure 2 and table S1). There was no significant eCO_2 effect on stemwood biomass increment across the three years of this study, nor a year × eCO_2 interaction (Table S1; P =0.420). Thus there was no indication of an eCO_2 fertilisation response of any component of ANPP despite a sustained increase in photosynthesis.

We also examined tree-level biomass growth responses across tree size categories between experimental manipulations we did within this forest, either of P availability or of atmospheric CO_2. *Eucalyptus* trees in the forest were capable of higher growth when soil P limitation was alleviated by P-fertilisation[22], as growth of adjacent P-fertilised trees in ambient CO_2 increased by 35% compared to similar sized ambient-grown, unfertilised trees of the same size class over a similar 48-month period (Figure 3). These results suggest that mature trees have the potential to respond to a release from P-limitation. Since growth was greatest for the largest size classes of trees within the overall stand, we also asked whether the eCO_2 effect showed size dependencies. For individual tree biomass increment, the growth of all tree size classes was unaffected by eCO_2 regardless of whether individuals were grouped by dominance (Table S1) or by diameter classes (Fig. 3, Fig. S3). Thus there was no CO_2 fertilisation response observed for any size class of trees on this low-P site, in marked contrast to previous observations in young temperate plantations. Even N-limited plantations showed an initial eCO_2 stimulation in productivity[13,15] whereas no such early eCO_2 response occurred in our P-limited forest. These findings provide key evidence for the debate regarding the capacity for CO_2 fertilisation of the large C stocks maintained in mature forests[1,25] particularly on P-limited soils at mid to low latitudes[4,18] and fill a critical knowledge gap for mature forests responses to eCO_2.

As no root production and turnover data are available for the first year and a half of the experiment, we do not know whether belowground productivity was influenced by eCO_2, though there is evidence of an initial stimulation in root and/or rhizosphere respiration returning CO_2 back to the atmosphere[23]. Assessing belowground productivity is challenging given difficulties in accessing deep roots and methodological problems with all approaches for quantifying belowground NPP[26]. Given that ANPP is typically 75-80% of total forest NPP globally[26], we demonstrated no eCO_2 response on productivity for an important set of components of aboveground C balance in a P-limited forest ecosystem. A meta-analysis of open-top chamber and free-air studies mostly in N-limited grassland ecosystems suggested that root biomass might be stimulated slightly more than shoot biomass under eCO_2 (+28% versus +22%, respectively), but cautioned that a lack of data on root and shoot biomass measured simultaneously within long-term experiments precluded a definitive answer to that question[27]. Due to a paucity of studies, such data are not widely available for low P ecosystems. Experiments involving eCO_2 on low-P sites are rare but in the glasshouse, ref. 24 found that neither root C nor total belowground C was significantly affected by eCO_2 until P was added to a native soil. Lack of an aboveground growth response to eCO_2 in EucFACE, lack of preferential belowground C stimulation of root growth in prior long-term eCO_2 studies[14] and lack of a belowground response to eCO_2 by P-limited plants in a glasshouse[24] are all no guarantee that there will also be no belowground eCO_2 response in EucFACE. However, these studies collectively suggest a large belowground C storage response of the EucFACE to eCO_2 may be unlikely, though we cannot rule out the possibility. Given these uncertainties, further work is needed to quantify the full stand C cycle response to eCO_2.

Our results are consistent with models accounting for nutrient limitations, suggesting that P-limited forest ecosystems should show a constrained eCO_2–induced productivity enhancement[21,28]. These models are generally not well-constrained by empirical evidence[4,21] such as large-scale free-air CO_2 experiments, and the biogeochemistry of P availability in the context of environmental change is not well understood[7,17]. As a single tree species dominates the forest overstory in our study, it may still be possible that species-rich tropical forests show a larger composite response to eCO_2 than observed here[29]. In this P-limited woodland, we observed a complete lack of wood, twig, or foliage growth enhancement with CO_2 fertilisation. As forests vary in their degree of nutrient limitation[20], there is no reason to posit that a complete absence of a productivity response to eCO_2 should be the norm in mature forests on P-limited soils. However, given the prevalence of P limitations in subtropical and tropical regions[20,30], our results strongly suggest that these forests might show a muted productivity increase with CO_2 fertilisation, especially when compared with the strong positive responses seen in young temperate forests on more fertile, P-rich soils[11]. If this were generally the case, it would indicate a constrained capacity of P-limited, mid- to low-latitude mature forests to sequester additional C from the atmosphere in a CO_2-enriched world, resulting in smaller reductions in atmospheric CO_2 concentrations and thus smaller allowable emissions reductions than anticipated by models that do not consider P limitations.

Methods

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

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Author Contributions

D.S.E, I.C.A. and B.E.M. designed the eCO_2 experiment. D.S.E., K.Y.C., T.E.G., designed the photosynthesis measurements and carried out and analysed them with J.C. and J.E.D. K.Y.C., J.C., J.R.P., D.S.E. and A.G. did the litterfall collections and measurements. D.S.E., 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 P.B.R. wrote the draft of the paper. All authors contributed to subsequent versions.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints.

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Competing financial interests

The authors declare no competing financial interests.

FIGURES AND TABLES

Figure 1 | Pattern of leaf net photosynthesis in the canopy over the first three years of elevated CO_2. (**a**) Photosynthesis for canopy leaves at prevailing seasonal temperatures and growth CO_2 concentration across time, including pre-treatment values (left) and the mean over the experimental period (right panel). For pretreatment (left panel), photosynthesis in both plot types was measured at the same ambient CO_2 concentration of 395 µmol mol^{-1} prior to CO_2 enrichment. **(b)** The CO_2 fertilisation response ratio for photosynthesis over time, with grey areas representing two-sided 95% confidence intervals for the CO_2 fertilisation response ratio for each of the measurement timepoints. The mean response ratio with lower and upper 95% confidence limits is shown by the grey area around the square, taken across all timepoints (right panel). The leaf photosynthesis in **(a)** was significantly different overall between CO_2 treatments (*P* = 0.013) and there was no time × CO_2 treatment interaction (repeated-measures ANOVA from mixed-model analysis; Table 1). Means ± 1 s.e. for *N*=3 plots per treatment are shown across ten different measurement periods, with open symbols for ambient and closed symbols for eCO_2. The s.e. bars may be obscured by points.

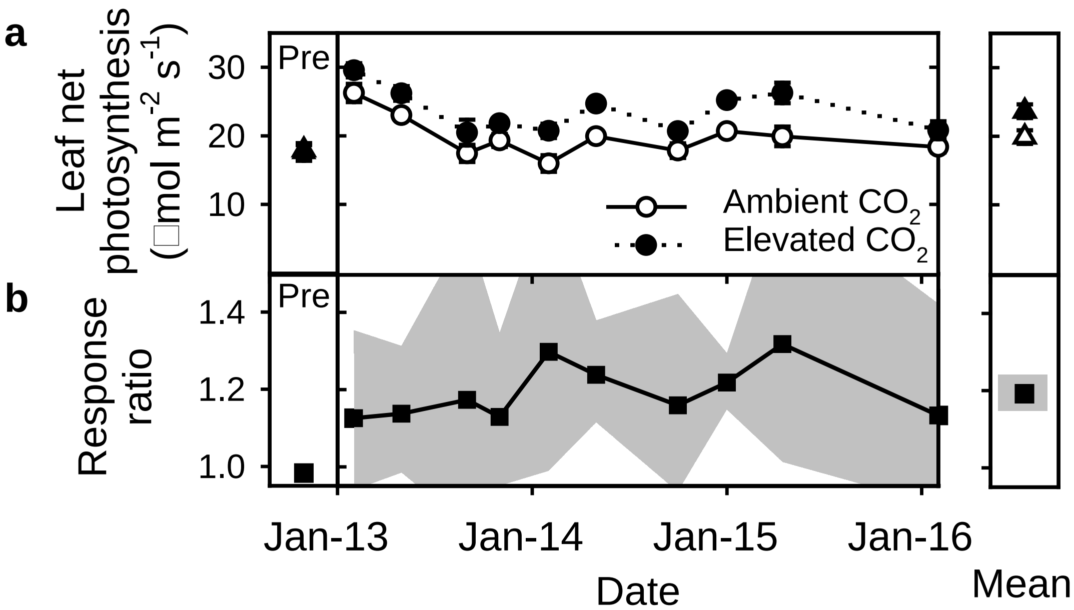

Figure 2 | Aboveground net primary production (ANPP) in a mature *Eucalyptus* stand and its components across three years of elevated CO_2. Total ANPP is represented by the combination of stemwood biomass production (stippled), fine twig and bark production (striped), seed and capsule production (hatched), and leaf production (solid). Stemwood production is determined as the annual biomass increment, and foliage+fine twig production are measured as annual biomass turnover collected monthly in permanent litter baskets. Reproductive structures (“capsules”) were measured in all three years but are small and obscured in 2014 and 2015. Ambient plots are shown with white backgrounds, and elevated CO_2 plots have grey/black backgrounds. Stem biomass increment, total foliage+fine twig turnover, and total ANPP were not significantly different across CO_2 treatments (P = 0.85, 0.41, and 0.38 respectively). Means ± 1 s.e. for N=3 plot replicates are shown for total ANPP, with yearly means shown for each component.

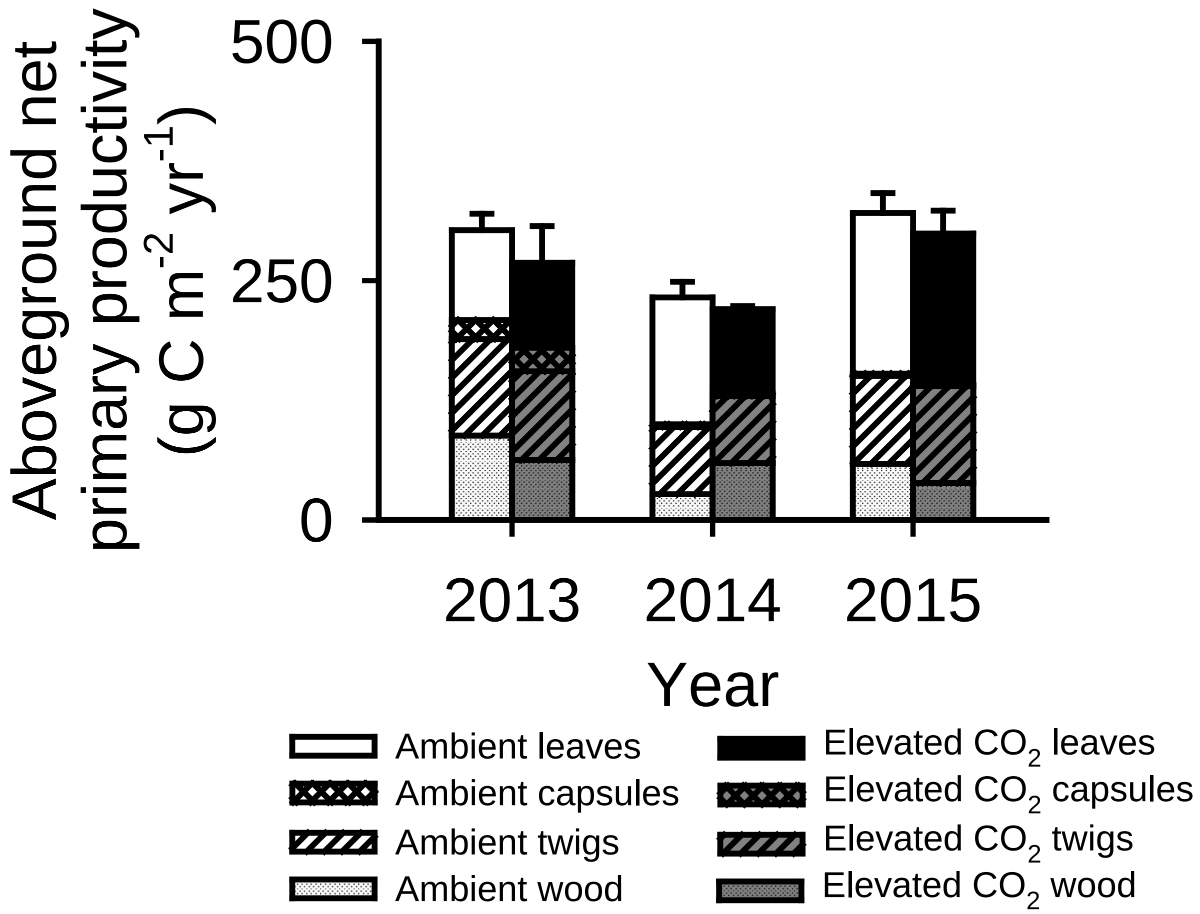

Figure 3 | Biomass increment of five different size classes of *Eucalyptus* trees. Shown is the biomass increment over 4 years from Dec. 2011 to Dec. 2015 within each size-class for ambient (open bars, mean ± s.e.) and elevated CO_2-grown trees (dark bars, mean ± s.e.), and ambient-grown trees with four years of P fertilisation (striped bar, mean ± s.e.). Diameter-classes are defined as the diameter in Dec. 2011 prior to the start of treatments. The biomass increment for elevated CO_2 trees in the first size class (15-20 cm) were not different from zero. Each tree diameter-class by treatment combination contained 9 unsuppressed trees on average (*N*=5 trees for P-fertilised). Bars are means + 1 s.e. within each size class. The P-fertilised tree increment is significantly different from the ambient tree increment for the appropriate size class ($P = 0.031$; one-tailed *t*-test).

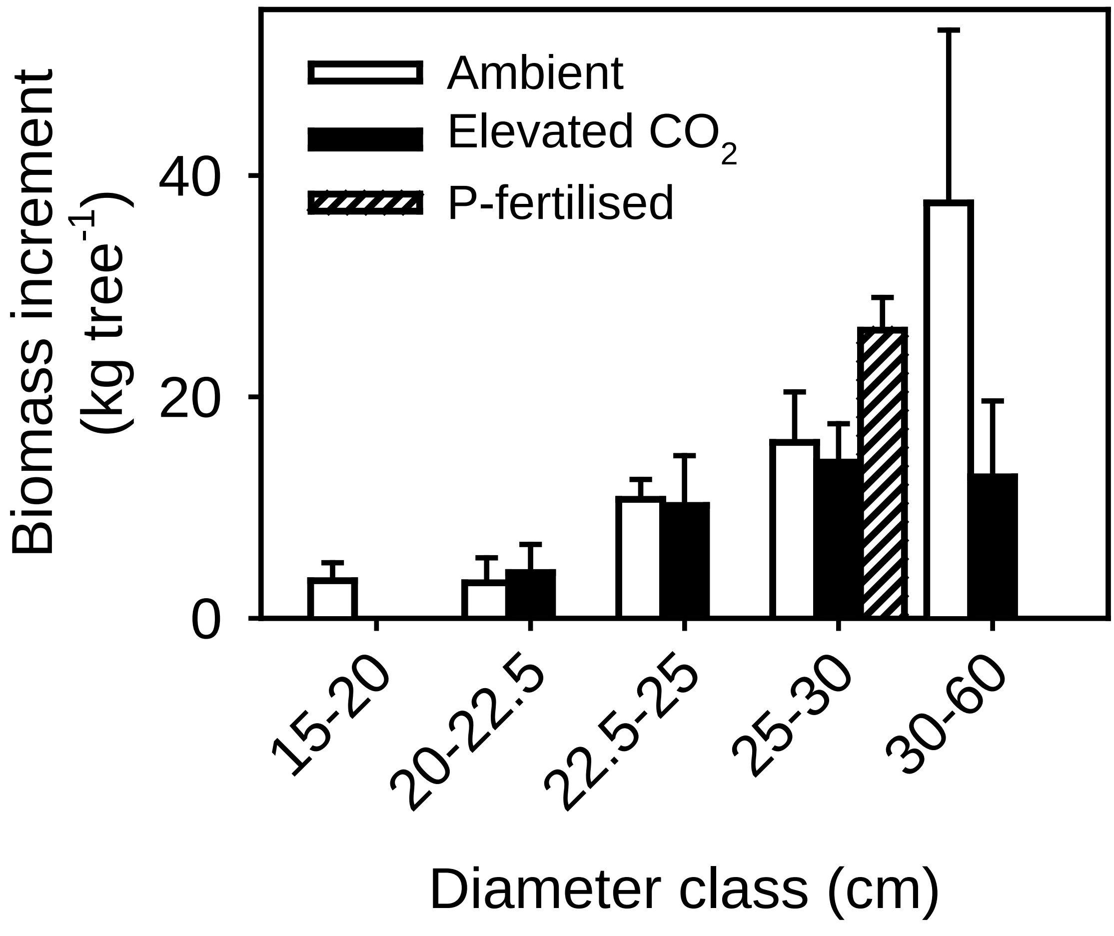

Table 1 | Repeated-measures analysis of variance of CO_2 treatment and time effects. These effects are shown for leaf net photosynthesis (a, left side) and aboveground net primary production, ANPP from 2013 to 2015 (b, right side). The mixed-model repeated-measures analysis for photosynthesis was done using data shown in Fig. 1**a)**, with the time term indicating sampling date across three years. For ANPP, the time term is 'year', the first to third year of the full eCO_2 treatment. In both analyses, a mixed-model repeated-measures analysis was done using a fixed treatment (CO_2) and a random plot effect, and Type III sums of squares computed using restricted maximum likelihood estimates for *F*-tests. The numerator and denominator degrees of freedom (df) for each *F*-test are shown.

	a) Photosynthesis			**b)** ANPP		
Source	**df**	***F*-ratio**	***P*-value**	**df**	***F*-ratio**	***P*-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

Methods (online)

Six large circular plots (0.05 ha each) were established in 2010 in a mature eucalypt woodland on an alluvial spodosol in western Sydney, Australia. The location receives 800 mm of precipitation per annum on average and has a mean annual temperature of 17.5°C (www.bom.gov.au). Mean maximum temperature in the warmest month is 30°C and mean minimum temperature in the coldest month is 3.6°C, with monthly mean temperatures always > 10°C. The CO_2 treatment was implemented in three of the plots using free-air CO_2 enrichment under computer control using the pre-dilution approach starting in Sept. 2012. After a period where the [CO_2] increased gradually over approximately 6 months[23], the plots received ambient +150 μmol mol^{-1} CO_2 during daylight hours over all days of the year, for Feb. 2013 onward. The mean 5-minute [CO_2] in the tree crowns was kept within ± 50% of the desired target of ambient +150 μmol mol^{-1} for 98% of the daylight hours over 2013-14 (Fig. S2). A separate set of trees within the stand ($N = 5$), located at least 60 m from the eCO_2 plots, were fertilised with 50 kg P ha^{-1} yr^{-1} starting in 2011, in two lots of superphosphate fertiliser applied within the drip-line of the trees during the growing season[22]. Root barriers were established prior to any fertilisation by trenching and inserting a plastic barrier to 50 cm depth in the soil around a set of fertilised and control trees. The P-addition treatments were maintained through the duration of the study, resulting in 4 years of P-fertilisation concurrent with the 3-year eCO_2 study.

Net photosynthesis. Light-saturated net photosynthesis of leaves was measured at high light, the growth CO_2 concentration and prevailing seasonal temperature at the top of three dominant or co-dominant trees in each plot using a pair of temperature- and CO_2-controlled portable photosynthesis systems (Li-6400, Li-Cor Inc.). Access to the ca. 22 m treetops was by construction cranes permanently located adjacent to each plot[31]. A smaller set of measurements on shaded foliage within the tree crowns was used to confirm results from the upper-crown measurements in terms of the CO_2-enhancement effect on photosynthesis, thus the entire crown can be expected to behave similarly.

Aboveground productivity measurements. Wood production was estimated from measured stem diameter changes for *N*=146 trees across the ambient and elevated plots. The diameter of each tree was measured at 1.3 m height at approximately monthly intervals starting February 2011, 2 years prior to commencement of the full CO_2 treatment. Manual band dendrometers were used to monitor stem diameter changes. The permanently-placed bands consisted of plastic straps graduated with a vernier scale placed around a tree (D1 Permanent Girth Tape, UMS GmbH, München, Germany) to detect changes in diameter to the nearest $0.01 \cdot \pi$ cm. As 99% of the tree stems measured represented by *E. tereticornis*, a species-specific allometric regression for *E. tereticornis*[32] was used to convert these increments to aboveground biomass increment. Of a total of 146 trees measured across the ambient and elevated plots, 49 suppressed trees, 6 co-dominant trees with trunk defects, and 4 trees showing shrinkage possibly preceding mortality were omitted from the mixed-model analysis. We thus used a total of *N*=87 trees measured across all years and without stem defects, suppression or shrinkage in the mixed-model analyses.

Foliage and twig production were measured as litterfall, collected monthly in ~0.2 m^2 circular fine-mesh traps at eight random locations per plot[33]. Litter was sorted into leaf, twigs and bark, and other material, dried at 40°C and weighed. A subsample was reweighed when dried at 70°C and a small moisture correction was applied to the leaf component of the whole dataset. We use litterfall to estimate annual foliage and twig production, but acknowledge that this approach assumes steady-state for these pools as would be expected in mature forest without any recent major disturbance. A steady-state status for foliage pools in 2013 and

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

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

Statistical analyses. We analysed the photosynthesis data[35] using a mixed-model repeated-measures analysis of variance in R v3.3.1 using the 'lme4' function within the 'nlme' package, with CO_2 treatment as a fixed factor and plot as a random factor nested within CO_2 treatment. There were no pre-treatment differences in photosynthesis at light-saturation and prevailing temperatures amongst the plots measured at the same [CO_2] ($P > 0.10$). Outcomes from type III *F*-tests are reported. A similar model was used to analyse annual above-ground net productivity, including leaf production, twig and bark production, and total stem growth. Confidence intervals for the CO_2 effect size estimate were computed in R (http://cran.r-project.org) using the function ‘confint’, which applies quantile functions for the *t*-distribution after model-fitting. We further analysed stemwood increment[35] on an individual tree basis for the largest 15 trees in each plot, using pre-treatment growth (biomass increment from Feb. 2011 – June 2012) as a covariate. For this analysis both plot and tree were treated as random factors. Pre-treatment was comprised of 2011 and the first six months of 2012 where no additional CO_2 was added to the plots[23,31]. All data were checked for normality using the Q-Q plots and Levene’s test, and residuals from model fitting were checked for evidence of heteroscedasticity. Constant error variances were confirmed by this approach, and if not, then an appropriate transformation was employed to ensure constant variances.

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

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