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LETTERS

No growth stimulation of tropical trees by 150 years of CO_2 fertilization but water-use efficiency increased

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The biomass of undisturbed tropical forests has likely increased in the past few decades[1,2], probably as a result of accelerated tree growth. Higher CO_2 levels are expected to raise plant photosynthetic rates[3] and enhance water-use efficiency[4], that is, the ratio of carbon assimilation through photosynthesis to water loss through transpiration. However, there is no evidence that these physiological responses do indeed stimulate tree growth in tropical forests. Here we present measurements of stable carbon isotopes and growth rings in the wood of 1,100 trees from Bolivia, Cameroon and Thailand. Measurements of carbon isotope fractions in the wood indicate that intrinsic water-use efficiency in both understorey and canopy trees increased by 30–35% over the past 150 years as atmospheric CO_2 concentrations increased. However, we found no evidence for the suggested concurrent acceleration of individual tree growth when analysing the width of growth rings. We conclude that the widespread assumption of a CO_2-induced stimulation of tropical tree growth may not be valid.

Tropical forests store around 25% of the global terrestrial carbon pool and account for a third of net primary production[5] and are therefore a crucial component of the global carbon cycle. Repeated measurements of permanent forest plots across the tropics have shown increased tree growth over the past decades in many cases[1,2], but decelerating or stable growth rates in others[6,7]. One of the main factors suggested to cause an increased tree growth is the rise of the atmospheric CO_2 concentration, as it can increase plant photosynthetic rates[3] and enhance water-use efficiency[4,8]. This latter response is of crucial importance in plant communities subjected to seasonal water shortage or drought periods, because it reduces water stress and extends the growing season. However, direct evidence for a CO_2 fertilization effect on growth rates of individual tropical trees is absent because free-air CO_2 enrichment (FACE) experiments are lacking in this biome[9]. In addition, the reported increase of tropical tree growth, which has been linked to CO_2 fertilization, was obtained on a much shorter timescale than the rise in CO_2 concentration, which started at the onset of the industrial revolution (~1850; ref. 10). Thus, it is unclear whether the rising atmospheric CO_2 concentration has resulted in a long-term stimulation of tropical tree growth.

We address this knowledge gap by analysing growth rings in the wood of long-lived tropical trees[10], thereby obtaining a centennial record of tree growth. We simultaneously quantify the physiological responses to increased atmospheric CO_2 and changes in growth rates over the past 150 years. We did so by studying 1,109 trees of 12 species from three sites distributed across the tropics (Fig. 1). Our study species produce annual growth rings, belong to different functional groups and are locally common to very abundant (Supplementary Table 1). Long-term data on physiological responses to increased atmospheric CO_2 were obtained by measuring stable carbon isotopes ($\delta^{13}C$) in wood cellulose, from which the intercellular CO_2 concentration in leaves (C_i) and the intrinsic water-use efficiency (iWUE) were derived (Supplementary Methods). This iWUE can represent actual water-use efficiency when the gradient in water-vapour pressure between the leaf and the atmosphere remains constant (Supplementary Methods, Fig. 1). Long-term growth changes were assessed by converting tree-ring widths to the growth in the cross-sectional area of tree stems (basal area increment; BAI), a good proxy for tree biomass growth[11]. This is the first pan-tropical study to directly link physiological and growth responses of individual trees to long-term CO_2 rise.

At each of the three sites, we sampled trees in large (145–300 ha) plots in undisturbed old-growth forest. Increment cores and tree discs were collected from trees of all sizes >5 cm diameter at breast height. In our analyses of trends in growth and iWUE over time, we accounted for confounding ontogenetic effects by employing a method that evaluates trends over time at a fixed stem diameter[12] (Figs 2a and 3a). We chose two sizes for our analyses: understorey trees of 8 cm stem diameter (~10 m tall) and canopy trees of 27 cm stem diameter (~30 m tall). These stem diameters were chosen because understorey trees are expected to benefit more strongly from elevated CO_2 than canopy trees[13], whereas canopy trees account for the bulk of forest biomass[2]. A total of ~100,000 rings were measured to determine tree ages, ~9,000 rings were used to calculate BAI and approximately 2,500 $\delta^{13}C$ measurements were carried out.

A mixed-effect model revealed a highly significant and exponential increase of C_i (Figs 2 and 3 and Supplementary Table 2a) at each of the three sites, and in both understorey and

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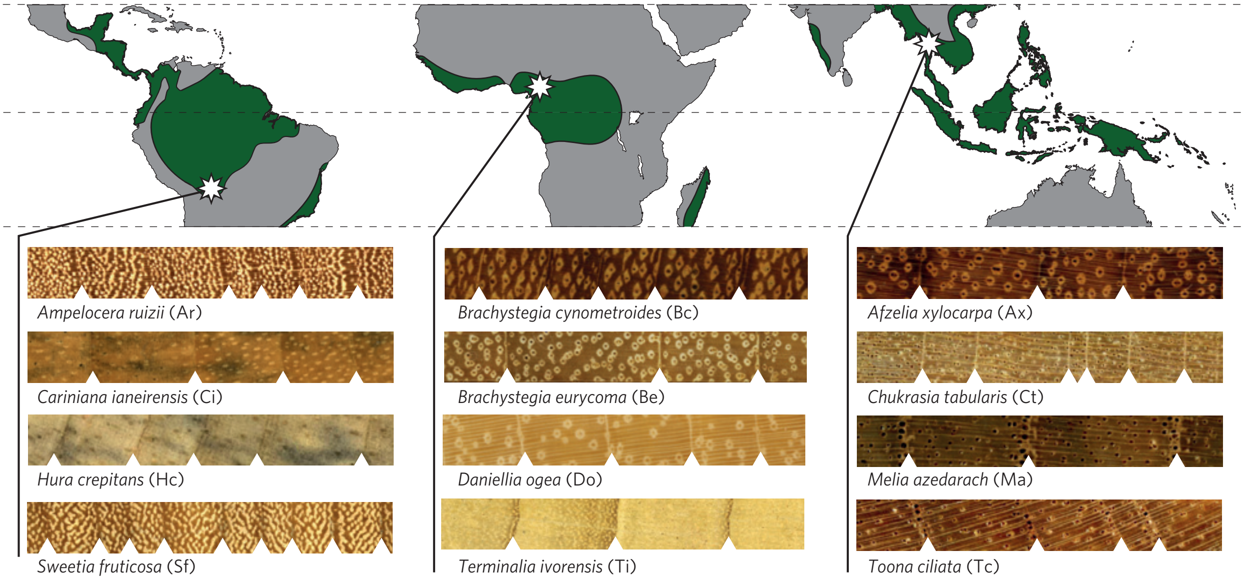

Figure 1 | Study sites and annual growth rings of the study species. Wood samples were collected from three wet tropical forests (≥1,500 mm rainfall per year; green areas). Bolivia: La Chonta forest concession; Cameroon: a forest concession adjacent to Korup National Park and Thailand: Huai Kha Khaeng Wildlife Sanctuary. Pictures show wood samples of the study species, with white triangles indicating growth-ring boundaries.

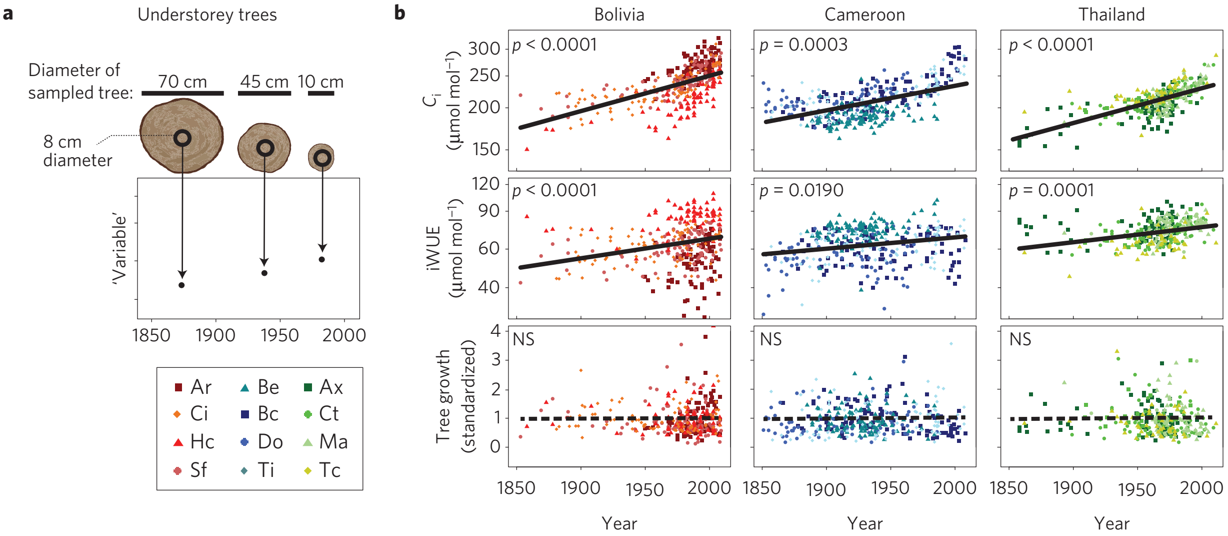

Figure 2 | Temporal trends in C_i, iWUE and tree growth in understorey trees. a, Illustration of our sampling strategy, which explicitly accounts for ontogenetic growth changes. **b**, CO_2 concentration in the leaf intercellular spaces (C_i, on a log scale) and iWUE (on a log scale) significantly increased (p values of mixed-effect models), whereas tree growth (BAI; standardized for clarity) did not ($p > 0.05$; Supplementary Table 2). Black lines represent per-site trends from the linear mixed-effect models (dashed for non-significant models). For species codes in legend see Fig. 1. Individual species figures are in Supplementary Figs 4 and 5. All data can be found in the Supplementary Information.

canopy trees. C_i increased by 43% and 53% over the past 150 years for understorey and canopy trees respectively. Yet, the rate of increase in C_i was consistently lower than that of atmospheric CO_2. This 'active' response[14] to elevated atmospheric CO_2 resulted in a significant and large increase of iWUE. Over the past 150 years, iWUE increased by 30–35% for understorey and canopy trees (Figs 2 and 3). A highly significant increase was also found when relating iWUE to atmospheric CO_2 instead of calendar year (Supplementary Table 2b). Evidence for an increase of tree-level iWUE was previously found for temperate tree species in FACE experiments[9,15] and in field studies for some tropical tree species[16–18], but this study indicates that increased water-use efficiency probably occurred at a pan-tropical scale.

A long-term increase of iWUE indicates that net photosynthesis has increased and/or stomatal conductance (and thus transpiration) was reduced. It is very likely that the century-long increase in atmospheric CO_2 concentration has resulted in both increased photosynthesis and reduced transpiration, in line with results from FACE experiments[9,15]. A sustained increase of photosynthesis and a higher water-use efficiency under elevated CO_2 are expected to stimulate tree growth if carbon and/or water are limiting factors[19]. Such a stimulation of tree growth should become apparent as a long-term increase in tree BAI, because stem growth determines most of the above-ground biomass growth of individual trees[11]. However, we found no evidence for trends in BAI over time in mixed-effect model analyses (Figs 2 and 3 and Supplementary Table 2a).

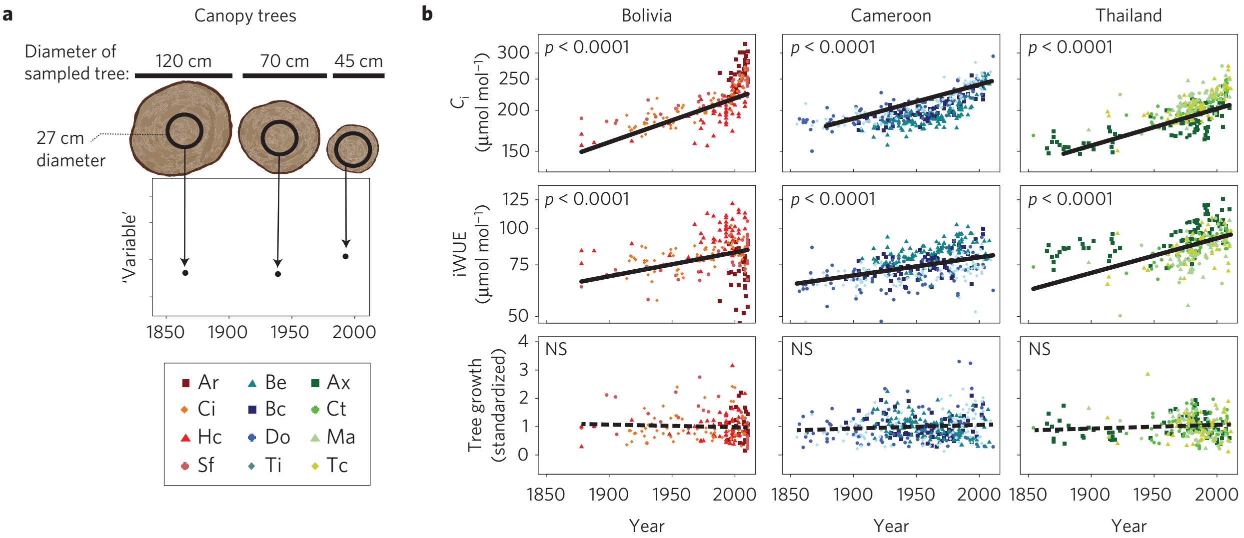

Figure 3 | Temporal trends in C_i, iWUE and tree growth in canopy trees. a, The same as in Fig. 2a, but for canopy trees. **b**, The same as in Fig. 2b, but for canopy trees. C_i and iWUE significantly increased for canopy trees at each site (p values of mixed-effect models, see Supplementary Table 2). Tree growth (standardized BAI) did not change in any of the sites ($p > 0.05$, Supplementary Table 2). Black lines represent per-site trends from the linear mixed-effect models (dashed for non-significant models). Individual species figures are in Supplementary Figs 4 and 5. All data can be found in the Supplementary Information.

This result was consistent across sites and species, and for both understorey and canopy trees (Figs 2 and 3). Thus, for the study species at our three sites, CO_2-induced increases in C_i and iWUE have not resulted in a detectable increase in diameter growth over the past 150 years. To account for the nonlinear increase of atmospheric CO_2 over time, we also examined whether BAI changed with atmospheric CO_2. Again, we found no evidence for a CO_2-induced growth stimulation (no significant relations, except for a negative growth trend for understorey trees in Thailand; Supplementary Table 2b).

We evaluated to what extent sampling biases could have influenced our findings (see 'The potential role of sampling biases' in the Supplementary Methods). There are four potential sampling biases that may generate apparent positive and negative trends in growth rates obtained from tree rings[12,20], or conversely may mask growth trends that are caused by environmental changes (Supplementary Fig. 2). In several additional tests we show that it is very unlikely that these sampling biases have prevented the detection of long-term changes in tree growth in our study (Supplementary Table 3 and 4).

Another potential reason for not finding an acceleration of BAI is low statistical power. We verified to what extent our data set allowed detecting long-term growth trends, by means of a power test (Supplementary Methods). This test revealed that our analysis would detect small growth changes with a high probability. For instance, a 1% growth change per decade is detected with a probability of 68% for understorey trees and >99.9% for canopy trees (Supplementary Fig. 3). If tree growth would have increased by 2% per decade, as reported from permanent monitoring studies in the Amazon[2], our analyses would have detected this change with >99.9% certainty for both size categories (Supplementary Fig. 3). Thus, it is highly unlikely that our study species experienced a growth stimulation over the past 150 years. These results contrast with those obtained from permanent sample plots showing increased tree growth at a stand level over time[1,2]. Differences in time period analysed (centennial versus decadal), unit of analysis (individual trees versus stands), plot size (large versus small) and spatial replication (few versus many plots) do not allow a straightforward comparison of plot-based and ring-based studies and such a comparison was not our goal here. Nonetheless, our findings do clearly indicate that, contrary to the common assumption, CO_2 rise did not stimulate tree growth of our study species on a centennial timescale.

Why has increased C_i and/or iWUE not resulted in increased growth rates? We discuss three possible explanations, all of which deserve considerable research attention. First, a CO_2-induced stimulation of photosynthesis may not have resulted in increased growth owing to an external climate-related stressor, such as increased temperature or decreased precipitation. There is little evidence for decreased precipitation at our study sites, but mean daily temperatures did increase by 0.02–0.2 °C per decade since 1950 (Supplementary Methods). At the leaf level, however, temperatures may have increased more if evaporative cooling of leaves is reduced at higher CO_2 levels[21]. On the short-term, higher temperature increases respiration rates, but plants are able to acclimate relatively quickly to such changes[22]. It is not clear though, how the potential negative effects of higher (leaf) temperatures interact with the positive effects of atmospheric CO_2 rise in the longer run. A second explanation is that the additional assimilates generated owing to a CO_2-stimulated photosynthesis have not been invested in stem growth and could thus not be detected in tree rings (or in tree diameter measurements). Such allocation shifts might include increased fruit production[23] and investment in root biomass[24]. The third possible explanation for the absence of a CO_2-driven growth increase is the limitation of resources other than CO_2 or water, for example, a persistent limitation of nutrients (for canopy trees) or a gradual reduction in light levels by increased leaf area index (for understorey trees). FACE experiments using temperate trees have shown that elevated CO_2 does not lead to a sustained increase of tree growth under most field conditions, a result that has been related to limited nutrient availability[9,19,25]. Our findings are consistent with those experimental results. We anticipate that the planned FACE experiment in the Amazon will shed light on the factors limiting a CO_2 fertilization response in mature tropical trees[26].

The increased iWUE of the study trees at each of our sites (Figs 2 and 3) has probably been partially caused by a lowered stomatal conductance over time and hence a reduced transpirational water loss. If tropical trees are in general responding to atmospheric CO_2 rise by reducing stomatal conductance, this may importantly change hydrologic cycles. Decreased transpiration by plants can lead to lower humidity, higher air temperatures and a reduction of the recycling of precipitation[27]. It is not clear to what extent hydrologic cycles have already changed owing to reduced transpiration because these alterations have occurred concomitantly with other changes such as deforestation.

As tropical forests are a crucial component of the global carbon cycle, it is important to predict their responses to atmospheric change. Such predictions are made using dynamic global vegetation models (DGVMs). At present, most—if not all—DGVMs predict increases in tropical forest biomass as a result of CO_2 fertilization[28–30]. Most model simulations therefore suggest that tropical forests will have the capacity to act as CO_2 sinks for the coming century, thus reducing global warming. However, if a CO_2 fertilization effect on tree growth is absent—as our findings suggest—current DGVMs overestimate the sink capacity of tropical forests.

Methods

Study sites, tree sampling and measuring growth. The study was carried out in three forest sites (Fig. 1): La Chonta logging concession in Bolivia (15.84° S, 62.85° W; average precipitation of 1,580 mm yr^{-1}), Huai Kha Khaeng Wildlife Sanctuary in Thailand (15.60° N, 99.20° E; average precipitation of 1,473 mm yr^{-1}) and Management Unit 11.001 of Transformation REEF in Cameroon (5.23° N, 9.10° E; average precipitation of ~4,000 mm yr^{-1}). Climate diagrams for each site are shown in Supplementary Fig. 6.

At each site, we sampled trees of four species (Supplementary Table 1). Species were selected on the basis of their abundance and the possession of clear annual growth rings. At each site, 100 trees per species were randomly selected (ranging in size from 5 to >100 cm diameter at breast height; dbh) in 145–300 ha of undisturbed forest (see Supplementary Methods). From each tree, a wood sample was collected as a stem disc or as increment cores (of 0.5 cm diameter) taken in at least three different directions. After drying, the surface of discs and cores were either cut or polished depending on what gave the best visibility of ring boundaries. Growth rings were identified using a LINTAB 6 measuring table and TSAPWin software (Rinntech) or using high-resolution scans (1,600 dpi) and WinDendro software (Regent Instruments). The quality of the dating of tree rings was checked in several ways, including chronology building and radiocarbon dating (see Supplementary Methods and Table 5). Ring widths were measured for each tree in at least three different directions and averaged. Measured tree-ring widths were converted to growth in cross-sectional area of the tree (BAI, in square centimetres).

Correcting for tree ontogeny. As diameter growth of trees changes with tree size (and age), it is important to separate ontogenetic growth changes from potential growth changes over time. We therefore compared growth rates in the years around two fixed diameters: understorey trees (8 cm dbh) and canopy trees (27 cm dbh). For understorey trees, we assessed changes in growth over time by selecting the ring formed when a tree reached the target size of 8 cm dbh. We measured this 'central' ring as well as the two rings formed before and after the central ring, and averaged the widths of these five rings. By using five rings, we obtained an estimate of tree growth that was largely unaffected by year-to-year variation in growth due to climatic fluctuations. As we collected trees ranging in size from 5 to >100 cm dbh, the rings formed around the 8 cm diameter differ in age and allow comparisons of growth rates over long time spans for trees of the same size (Fig. 2a). The same procedure was used for canopy trees, but then for a target dbh of 27 cm (Fig. 3a).

Stable carbon isotopes and statistical analyses. The analysis of trends in the CO_2 concentration in the intercellular spaces (C_i) and iWUE was done in a similar way as those for BAI, again using canopy and understorey trees. By using the same selected tree rings from which we obtained BAI values, we could directly compare physiological responses to elevated CO_2 levels with growth responses during the same period. We cut wood samples from the selected tree rings (around 8 and 27 cm dbh) and obtained 5-year bulk samples from which cellulose was extracted. Carbon isotope ratio ($\delta^{13}C$) of the cellulose samples was measured in a continuous flow mode with an element analyser coupled to a mass spectrometer (Sercon Hydra 20-20) at the Leicester Environmental Stable Isotope Laboratory, University of Leicester, UK. From $\delta^{13}C$, estimates of C_i and iWUE were derived (Supplementary Methods). We analysed long-term changes in C_i, iWUE and BAI with a linear mixed-effect model and included 'calendar year' as a fixed factor and 'tree species' as a random factor in the analysis of trees per site and both 'tree species' and 'site' as random factors in the analysis of an overall pan-tropical trend (that is, combining all trees measured).

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Author contributions

P.A.Z. conceived the study in discussion with N.P.R.A. and T.L.P. The study was supervised by P.A.Z. and F.B. P.G., M.V. and P.v.d.S. sampled the trees in the field and measured growth rings. G.T., P.v.d.S. and A.B. performed the stable isotope analyses. P.v.d.S. and P.A.Z. performed the data analyses and wrote the manuscript. All authors contributed to the interpretation of the results and helped to improve the manuscript.

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. Correspondence and requests for materials should be addressed to P.v.d.S. or P.A.Z.

Competing financial interests

The authors declare no competing financial interests.