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Increases in atmospheric CO_2 have little influence on transpiration of a temperate forest canopy

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Summary

- Models of forest energy, water and carbon cycles assume decreased stomatal conductance with elevated atmospheric CO_2 concentration ([CO_2]) based on leaf-scale measurements, a response not directly translatable to canopies. Where canopy–atmosphere are well-coupled, [CO_2]-induced structural changes, such as increasing leaf-area index (L_D), may cause, or compensate for, reduced mean canopy stomatal conductance (G_S), keeping transpiration (E_C) and, hence, runoff unaltered.
- We investigated G_S responses to increasing [CO_2] of conifer and broadleaved trees in a temperate forest subjected to 17-yr free-air CO_2 enrichment (FACE; + 200 μmol mol^{-1}). During the final phase of the experiment, we employed step changes of [CO_2] in four elevated-[CO_2] plots, separating *direct* response to changing [CO_2] in the leaf-internal air-space from *indirect* effects of slow changes via leaf hydraulic adjustments and canopy development.
- Short-term manipulations caused no *direct* response up to 1.8 × ambient [CO_2], suggesting that the observed long-term 21% reduction of G_S was an *indirect* effect of decreased leaf hydraulic conductance and increased leaf shading. Thus, E_C was unaffected by [CO_2] because 19% higher canopy L_D nullified the effect of leaf hydraulic acclimation on G_S.
- We advocate long-term experiments of duration sufficient for slow responses to manifest, and modifying models predicting forest water, energy and carbon cycles accordingly.

Introduction

In most forests, exchanges of energy and mass with the atmosphere are determined by fluxes of CO_2 and water between leaves and the canopy air volume, regulated by stomata. Models of these processes incorporate functions describing stomatal closure with increasing atmospheric CO_2 concentration ([CO_2]) based on leaf-level measurements (Ball *et al.*, 1987; Sellers *et al.*, 1996; Douville *et al.*, 2000). Responses of forest canopies, however, may differ (Medlyn *et al.*, 2001; Wullschleger *et al.*, 2002; Ainsworth & Rogers, 2007), provoking debate regarding the consequences of elevated [CO_2]-induced stomatal responses to ecosystem water use and supply to downstream users (Milly *et al.*, 2005; Gedney *et al.*, 2006; Betts *et al.*, 2007; De Boer *et al.*, 2011; Keenan *et al.*, 2013). Because leaves in most temperate forests are well-coupled to the atmosphere (Jarvis & McNaughton, 1986; Pataki *et al.*, 1998; Ewers & Oren, 2000; Schäfer *et al.*, 2002), reductions in stomatal conductance should scale to canopy fluxes. However, in water limited ecosystems, canopy leaf area (L_D) is predicted to increase with decreasing stomatal conductance, thus conserving canopy conductance and transpiration (Woodward, 1990). Even in moist ecosystems, elevated [CO_2] may increase carbohydrate availability, potentially enlarging L_D (Palmroth *et al.*, 2006), and consequently lowering canopy conductance per unit leaf area (G_S) via light or hydraulic limitation (Schäfer *et al.*, 2002; Ward *et al.*, 2013), also leading to conserved transpiration. Where direct stomatal closure in response to elevated [CO_2] is not countered by increased L_D, and atmospheric demand for and soil supply of water remain unchanged, canopy transpiration should decrease. For example, no increase of L_D is likely if soil nitrogen is limiting (Ågren, 1983) or leaf loss is accelerated by heat damage (Warren *et al.*, 2011).

Where elevated [CO_2] does not elicit a *direct* stomatal response through CO_2 concentration in the leaf-internal air-space, it may do so *indirectly* by changing growth partitioning among leaves, stems, and roots, and by affecting the efficiency of water transport (Domec *et al.*, 2009; Brodribb & Feild, 2010; Ward *et al.*, 2013), or by increasing self-shading of foliage with enhanced L_D (McCarthy *et al.*, 2007). Some global-scale models predict L_D enhancement under high [CO_2] (Douville *et al.*, 2000; Betts *et al.*, 2007), but none to date include an acclimation of the hydraulic system. Tree hydraulic properties often require an extended period for complete acclimation to stepwise [CO_2] changes (Domec *et al.*, 2009, 2010), which may not necessarily be accompanied by increased L_D.

To properly account for and model the effect of increasing [CO_2] on biosphere–atmosphere exchanges, it is necessary to identify all of the primary processes involved and quantify their effect on stomatal conductance of forest canopy that is fully acclimated to increased [CO_2]. Yet, free-air CO_2 enrichment (FACE) studies on intact, hydraulically acclimated canopies are rare, and have not previously been used to separate variables responsible for *direct* vs *indirect* stomatal responses to elevated [CO_2]. The underlying mechanisms for observed high [CO_2] effect on stomatal conductance and, in turn, their effect on tree crown and stand canopy conductance may have been misidentified, leading to incorrect specification in some models.

We used the Duke University FACE experiment in a temperate forest dominated by *Pinus taeda* and *Liquidambar styraciflua*, to evaluate the elevated [CO_2] effects on variables underlying whole-canopy responses of transpiration (i.e. G_S, L_D, and leaf hydraulic conductance). We used a 17-yr long annually integrated dataset to quantify coarse-scale response, and performed a short-term elevated-[CO_2] manipulation during the final phase of the experiment to distinguish the *direct* from *indirect* effects on G_S, providing insight for modeling of future water, energy and carbon cycles in forest ecosystems.

Materials and Methods

Settings

The Duke FACE experiment was in Duke Forest, North Carolina, USA (35°52′N, 79°59′W; 130 m above sea level (a.s.l.)). The forest was 27-yr-old and 25 m tall in 2010. The soil was an acidic, low fertility clay Hapludalf classified in the Enon Series. Mean annual temperature and precipitation were 15.5°C and 1165 mm respectively (Phillips & Oren, 2001). The forest was dominated by *Pinus taeda* L. and *Liquidambar styraciflua* L. with other 50 broadleaved deciduous species in the subcanopy (Schäfer *et al.*, 2002). Under ambient [CO_2], mean annual L_D was 5.4 ± 0.26 (standard error, SE), composed of 55 ± 1% *P. taeda*, and 17 ± 2% *L. styraciflua*, which was assumed representative of the other broadleaved trees. The long-term elevated-[CO_2] treatment was + 200 above ambient (*c.* 385 μmol mol^{-1} at the end of the study) since 1994 ($n = 1$) and 1996 ($n = 4$), using FACE technology on 30-m diameter plots (Hendrey *et al.*, 1999) with untreated plots as reference (Oren *et al.*, 2001; Ward *et al.*, 2013).

Long- and short-term [CO_2] manipulation experiments

In 2010, we separated *direct* vs *indirect* mechanisms of elevated-[CO_2] effect on G_S by observing responses to stepwise [CO_2] changes in the canopy air of the four elevated-[CO_2] plots, using the four ambient-[CO_2] plots as reference. Stomatal response to rising [CO_2] may depend on soil water availability (Schäfer *et al.*, 2002; Domec *et al.*, 2009); we therefore performed the study over a soil dehydration–rehydration cycle (Fig. 1a). Five [CO_2] (ambient, + 100, + 150, + 200 and + 300 above ambient) were administered in eight 5-d periods (Fig. 1b). We avoided potential confounding effects of [CO_2] and soil moisture by allocating one of the four elevated-[CO_2] plots to one of the five [CO_2] treatments during each 5-d period. The four ambient-[CO_2] plots permitted accounting for potential effects of the large variation in soil moisture (expressed as relative extractable water, REW; Pataki & Oren, 2003) on G_S sensitivity to [CO_2]. We partitioned the data

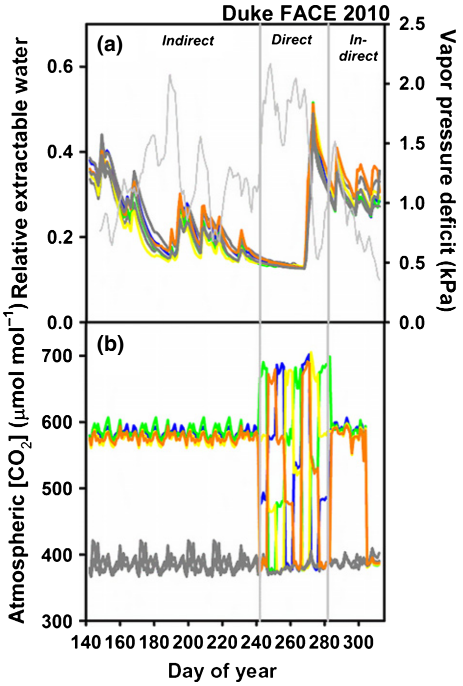

Fig. 1 Site conditions. (a) Soil moisture, expressed as relative extractable water (REW) in each of the eight plots (represented by different colors) and vapor pressure deficit (light gray) during the study period. (b) Atmospheric [CO_2] in each plot during the study period. Ambient [CO_2] in four reference plots are shown in gray, while that of the four elevated-[CO_2] plots are in color. During *Indirect*, elevated-[CO_2] plots were all subjected to +200 μmol mol^{-1}. During *Direct*, [CO_2] in each plot was varied independently for a 5-d period to avoid confounding effects with REW. For example, in an elevated-[CO_2] plot (orange line), [CO_2] was decreased to ambient level during the first period, ramped up to +300 μmol mol^{-1} above ambient in the second period, reduced to +200, +100 μmol mol^{-1}, and to ambient level once again, followed by periods of +300 μmol mol^{-1}, +150 μmol mol^{-1} and finally to the ambient level in the last 5-d period of *Direct* phase, before returning to the long-term enhancement of +200 μmol mol^{-1}.

into two periods in 2010 (Fig. 1): *Direct* (late August to early October, Day of Year (DOY) 242–282) during which the canopy-scale stepwise [CO_2] changes were implemented, and *Indirect* (May to August, DOY 142–241 and late October, DOY 283–304) in which enrichment followed the long-term experimental protocol (+ 200; Oren *et al.*, 2001). The 17-yr long annual values were obtained as described later.

Measurements and calculations of variables

Growing season averaged leaf area index (L_D) and canopy-integrated stomatal conductance per unit leaf area (G_S) through 2008 were synthesized from published data (Phillips & Oren, 2001; McCarthy *et al.*, 2007; Ward *et al.*, 2013), and used to calculate canopy transpiration (E_C) (see Supporting Information Methods S1). We generated similar values from unpublished data for 2009 and 2010.

In 2010, the half-hourly mean sap flux density (J_S; in g $m^{2}_{sapwood}$ s^{-1}), measured at multiple sapwood depths using thermal dissipation probes (Granier, 1985) in 91 *Pinus taeda* and 45 *Liquidambar styraciflua* individuals distributed among the eight plots, was used for the daily analyses. We employed a scaling method utilizing random effects generated from an empirical dynamic model based on a hierarchical Bayesian statistical approach (Ward *et al.*, 2013; Supporting Information Methods S1) to scale the point-measured J_S to sapwood-averaged values ($\bar{J}_S$). Daily values were used to avoid issues related to tree water storage and measurement errors (Phillips & Oren, 1998). The daily E_C was computed from daily sum $\bar{J}_S$ and total sapwood area per unit ground area (Oren *et al.*, 1998). The G_S of a canopy well-coupled to the atmosphere, as in this study, can be computed using the simplified Penman–Monteith equation (Methods S1). In a similar *P. taeda* forest, no vertical gradient of vapor pressure deficit was observed within the canopy, and thus leaf-level stomatal conductance scaled with vertical leaf area distribution was correlated without bias to sap-flux based mean canopy stomatal conductance (Ewers & Oren, 2000). We further verified the close leaf-atmosphere coupling by testing and finding no sensitivity of J_S to wind speed in all study plots (following Kim *et al.*, 2014; $P = 0.66$, $R^2 = 0.003$), consistent with previous findings (Domec *et al.*, 2009).

Leaf hydraulic conductance (K_{leaf}; in mmol m^{-2} MPa^{-1} s^{-1}) was measured on single leaves/fascicles using the timed rehydration method (Brodribb & Holbrook, 2003). We selected an upper and mid-canopy branch from two representative *Pinus taeda* trees in each plot (16 branches per treatment). For *Liquidambar styraciflua*, sampling was based on the distribution of trees in plots with two branches from each of the six trees selected from each [CO_2] treatment (12 branches per treatment). Branches, 30–50 cm long, were collected in early morning, sealed in plastic bags, transported to the laboratory, recut under water and allowed to rehydrate for 4 h. Pressure-volume analyses (Tyree & Hammel, 1972) were conducted on leaves/fascicles taken on the same branches used to determine K_{leaf} (Supporting Information Methods S1).

The influence of canopy leaf area on G_S was inferred from shading effects in the canopy. We employed a one-dimensional, multi-species, multilayer radiative transfer model (Schäfer *et al.*, 2003; Kim *et al.*, 2011) to calculate the average light on leaf surfaces (Q) of each dominant species and for the entire forest. We characterized canopy structure using vertical distributions of shoot clumping, leaf angle distribution, leaf area density and wood surface density of branch and stem based on field measurements (Kim *et al.*, 2011). We partitioned the canopy into 1-m layers, and calculated mean light on leaf surfaces for each canopy layer and for the whole canopy (Supporting Information Methods S1).

Tests for significant elevated-[CO_2] effects

In all analyses, we used data from ambient and elevated [CO_2] plots (each, $n = 4$) during DOY 142–304 in 2010. For the long-term treatment data (*Indirect*; DOY 142–241 & 283–304), we employed regression analysis to analyze E_C response to day-length-normalized vapor pressure deficit ($D_Z = D_D \times (n_d/24)$ where D_D = daytime mean vapor pressure deficit, n_d = number of daylight hours; Phillips & Oren, 2001) and to evaluate the response of G_S, expressed as a ratio of elevated-relative to ambient-[CO_2] values vs [CO_2]-enhancement ratio. We performed *F*-tests for the elevated [CO_2] effect on the E_C vs D_Z response during *Indirect*. We applied Student's *t*-tests on the ratios of G_S, K_{leaf} and Q to test for [CO_2] effects on these variables. Retaining information in the temporal variation, we performed a Randomized Intervention Analysis (Carpenter *et al.*, 1989) on G_S to detect nonrandom change due to treatment effect in the series of observation made before and after treatment application (Supporting Information Methods S1). All computations and analyses were conducted in MATLAB 7.6.0 R2008a (The MathWorks Inc., Natick, MA, USA, 2008).

Results and Discussion

Reviews assessing the response of tree species to elevated-[CO_2], mostly based on seedlings, suggest that G_S would decrease and canopy leaf area would increase (Eamus & Jarvis, 1989; Mousseau & Saugier, 1992; Medlyn *et al.*, 2001; Ainsworth & Long, 2005). These responses were observed at the mixed *Pinus taeda*-*Liquidambar styraciflua* FACE experiment during the early establishment period (Fig. 2a–c). The long dataset produced two clear conclusions: (1) the increased L_D and decreased G_S of *P. taeda* resulted in E_C unaffected by [CO_2] (Fig. 2a). Because *P. taeda* dominated the stand, these responses represent the entire canopy (Fig. 2c). (2) By contrast, the increased L_D of *L. styraciflua* did not compensate for the larger decrease of its G_S, resulting in *c.* 20% lower E_C under high [CO_2] (Fig. 2b). This is consistent with syntheses showing that stomatal conductance of broadleaved species is more sensitive to elevated [CO_2] than that of conifers (Medlyn *et al.*, 2001).

Earlier in the experiment, it was uncertain whether the increased L_D was simply due to accelerated ontogeny or treatment effect (Gunderson & Wullschleger, 1994); now that L_D has stabilized in both treatments, it is clear that L_D enhancement was induced and maintained by increased [CO_2]. However, it has not been possible to determine whether the G_S and L_D trends reflect independent and

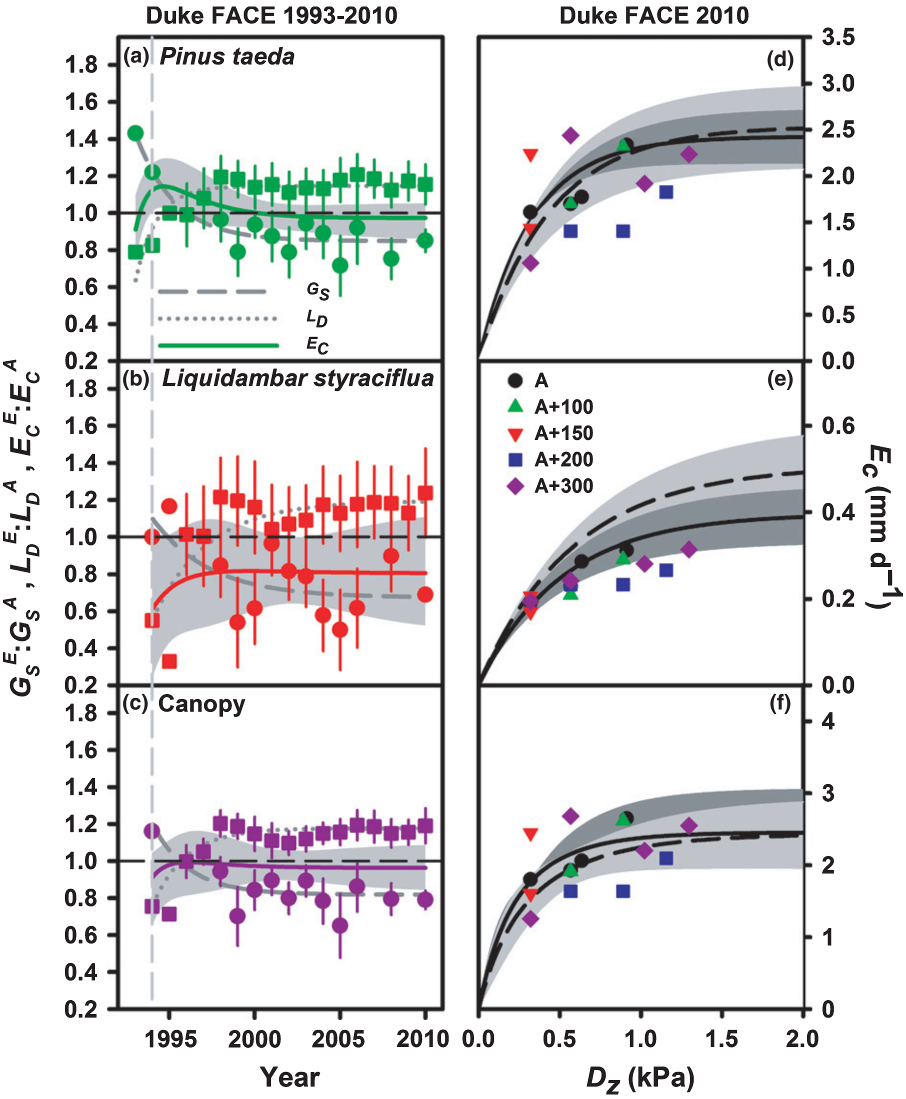

Fig. 2 Elevated-[CO$_2$] effect on canopy transpiration at Duke free-air CO$_2$ enrichment (FACE). (a–c) Mean canopy stomatal conductance (G_S; circles), mean growing season leaf area index (L_D; squares) and canopy transpiration ($E_C = G_S \times L_D$; colored solid lines) expressed as ratios of values in elevated-to-ambient [CO$_2$] from 1993 to 2010 for each dominant species and the entire canopy. Shaded regions represent one standard error (SE) of E_C (Vertical dashed line represents the beginning of elevated-[CO$_2$] treatment in 1994). (d–f) Comparison of E_C response to D_Z (daylength-normalized daytime vapor pressure deficit; see text) during the long-term elevated [CO$_2$] treatment (i.e. +200 μmol mol^{-1} during *Indirect* in 2010) under ambient- (dashed curves) and elevated-[CO$_2$] (solid curves). Shaded regions are 95% confidence intervals associated with each curve (e.g. light gray region corresponds to the fit for ambient-[CO$_2$] data). Symbols represent the 5-d average data from elevated-[CO$_2$] plot(s) during the short-term [CO$_2$] manipulation (*Direct* phase).

direct responses to [CO$_2$], or represent a feedback whereby one variable directly responded to high [CO$_2$], and the other followed indirectly. Because the experiment is in a fairly moist environment, the occasional drought notwithstanding, a feedback of L_D to direct G_S decrease is not likely; a more likely feedback scenario is L_D increasing as a direct response to increasing [CO$_2$] and causing a decrease of G_S (due to more mutual shading in the canopy and reduced hydraulic conductance).

We used the latter part of the last [CO$_2$] enrichment year (termed *Indirect* phase in 2010), focusing on a finer, daily scale, to assess the long-term E_C responses, and to set a baseline for examining the response to stepwise [CO$_2$] changes (termed *Direct* phase; Fig. 1). During *Indirect*, the analysis focused on E_C response to D_Z under nonlimiting light and water conditions, setting a clear baseline for evaluating *Direct* data. The long-term elevated [CO$_2$] did not affect E_C of *Pinus taeda* and the canopy ($P \geq 0.53$) but reduced that of *Liquidambar styraciflua* ($P < 0.001$; see fit lines Fig. 2d–f and Supporting Information Table S1 for statistics). We compared E_C data under varying soil moisture conditions during *Direct* to the baseline by plotting time-averaged values of each treatment in each elevated-[CO$_2$] plot (Fig. 2d–f; symbols). Most (90%) of these values lay within the 99% confidence bounds. Moreover, the E_C values from the *Direct* phase showed no systematic pattern in response to increasing [CO$_2$] relative to the long-term fit, with data below the fit representing the *Direct* drought period, and above the fit representing the period immediately following drought-breaking rains. Therefore, we observed no [CO$_2$] effect on E_C in *Direct* phase. To identify plausible causes for the E_C responses observed over the long term (Fig. 2a–c), we focused further analyses on G_S.

The underlying causes of G_S reduction under rising [CO$_2$] have been elusive (Ward *et al.*, 2013). The plausible causes are not necessarily distinct; for example, hydraulic architecture may acclimate to a long-term decrease in leaf-level stomatal conductance, which may be driven by the increased mutual shading accompanying higher L_D (Whitehead *et al.*, 1984; Tyree & Ewers, 1991; McDowell *et al.*, 2002; Mencuccini, 2002, 2003; Buckley & Robert, 2005). To help assess the potential contribution to the E_C response of increased L_D and changes of hydraulic conductivity, we reanalyzed the data in Fig. 2(d–f) in terms of G_S, assessing the long-term *Indirect* response of G_S to [CO$_2$]-induced changes of L_D and

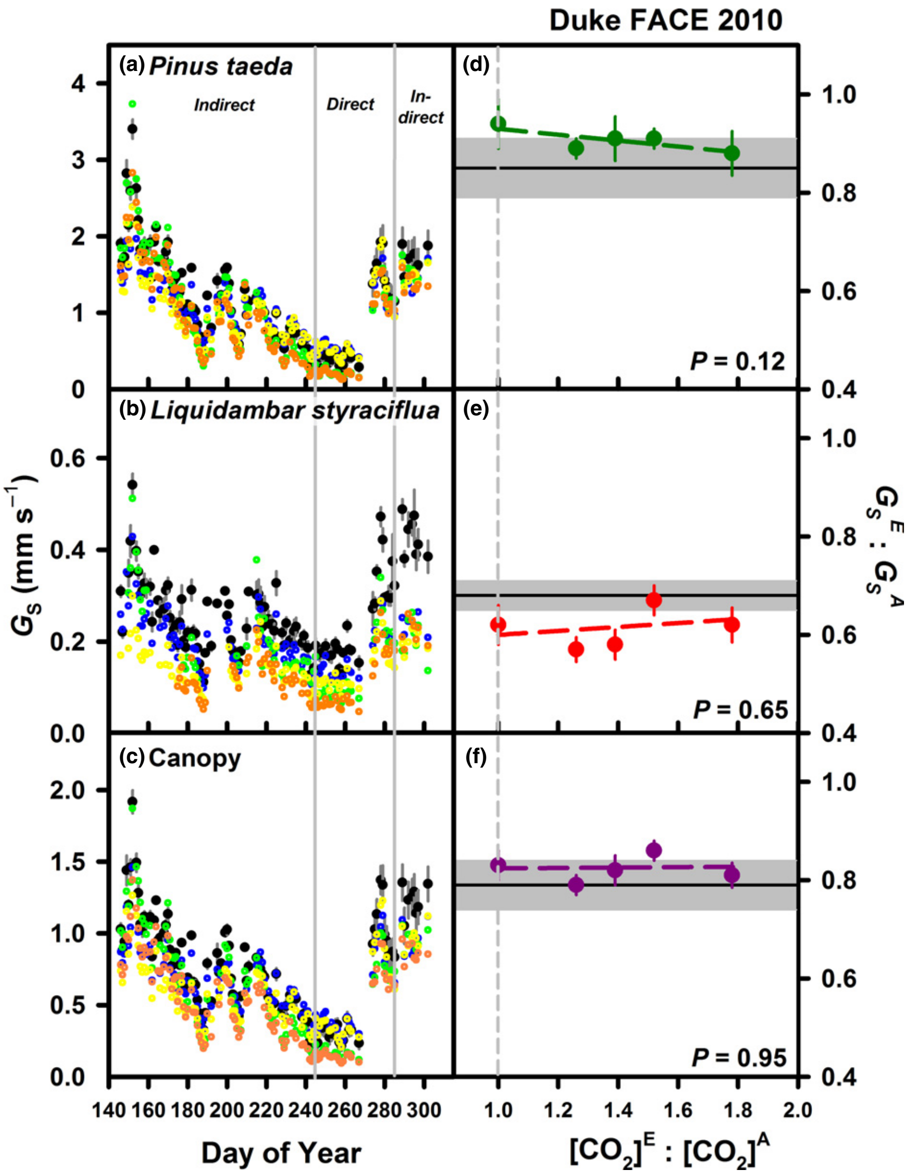

Fig. 3 Mean canopy stomatal conductance response to elevated [CO_2]. (a–c) Time series of daily means of mean canopy stomatal conductance (G_S) over the study period, including *Direct* and *Indirect* phases. Data for reference plots were averaged ($n = 4$, black circles with one SE); data for each elevated-[CO_2] plot are shown in different colors. (d–f) During *Direct*, the apparent decrease of G_S under elevated-[CO_2] relative to ambient-[CO_2] ($G_S{}^E$:$G_S{}^A$) with increasing [CO_2] was small, < 1% per 10% increase of [CO_2]^E:[CO_2]^A, and insignificant in either major species or the entire canopy, indicating no direct response. The *P* values are statistical results for *t*-tests on the regression slopes. The reduction of G_S observed during *Indirect* is depicted as a constant over the entire range of enhancement employed during the [CO_2]-response of *Direct* (horizontal solid lines, with one SE shown as a gray range). Because no direct response was detected in *Direct*, the reductions observed in *Indirect* are due to other mechanisms.

K_{leaf}. During both *Indirect* and *Direct* phases, G_S was highly variable, reflecting mostly soil drying–rewetting cycles and associated atmospheric conditions (Fig. 3a–c). *Pinus taeda* was more sensitive to drought than *Liquidambar styraciflua* (Schäfer *et al.*, 2002). The background conditions of [CO_2] enrichment (+ 200 μmol mol^{-1} above ambient) reduced the elevated-to-ambient [CO_2] G_S ratio ($G_S{}^E$:$G_S{}^A$) (*Indirect* phase) to 0.85, 0.67 and 0.79 of ambient [CO_2] for *P. taeda*, *L. styraciflua*, and the entire canopy (Fig. 3d–f; horizontal solid lines), similar to results from an analysis of an 11-yr long dataset (0.87 and 0.69 for the two species respectively; Ward *et al.*, 2013). We then related $G_S{}^E$:$G_S{}^A$ of *Direct* to the enhancement ratio of [CO_2] ([CO_2]^E:[CO_2]^A). As [CO_2]^E:[CO_2]^A increased, $G_S{}^E$:$G_S{}^A$ of neither species, nor that of the canopy changed significantly (Fig. 3d–f; least-square regression on plot-scale data). The apparent slight trend seen for *P. taeda* represented only a 1% decrease for a 10% increase of [CO_2], with *L. styraciflua* showing a slight, opposite trend. These weak responses made the G_S of the canopy insensitive to short-term changes in [CO_2]. Testing (one-sample *t*-test) whether $G_S{}^E$:$G_S{}^A$ at each CO_2 level differed from the mean of *Indirect* data showed that there was a tendency for difference only under two [CO_2] levels for *L. styraciflua* and one level for the canopy (Supporting Information Tables S2, S3). Testing individual plots in each enrichment period based on Randomized Intervention Analysis showed that none displayed a $G_S{}^E$:$G_S{}^A$ different from the *Indirect* values (Supporting Information Fig. S1; tested against $\alpha = 0.05$). Thus, neither E_C nor G_S show evidence of direct response to increasing [CO_2]. We note that the finding of no G_S response can be accommodated within the Ball–Berry framework (Ball *et al.*, 1987) considering, for example, that net photosynthesis can increase proportionally with [CO_2].

Because G_S reduction under high [CO_2] cannot be attributed to *direct* stomatal response, we assessed changes in hydraulic conductance (Domec *et al.*, 2010) and increased mutual shading (McCarthy *et al.*, 2007) as potential causes of the observed decline of G_S.

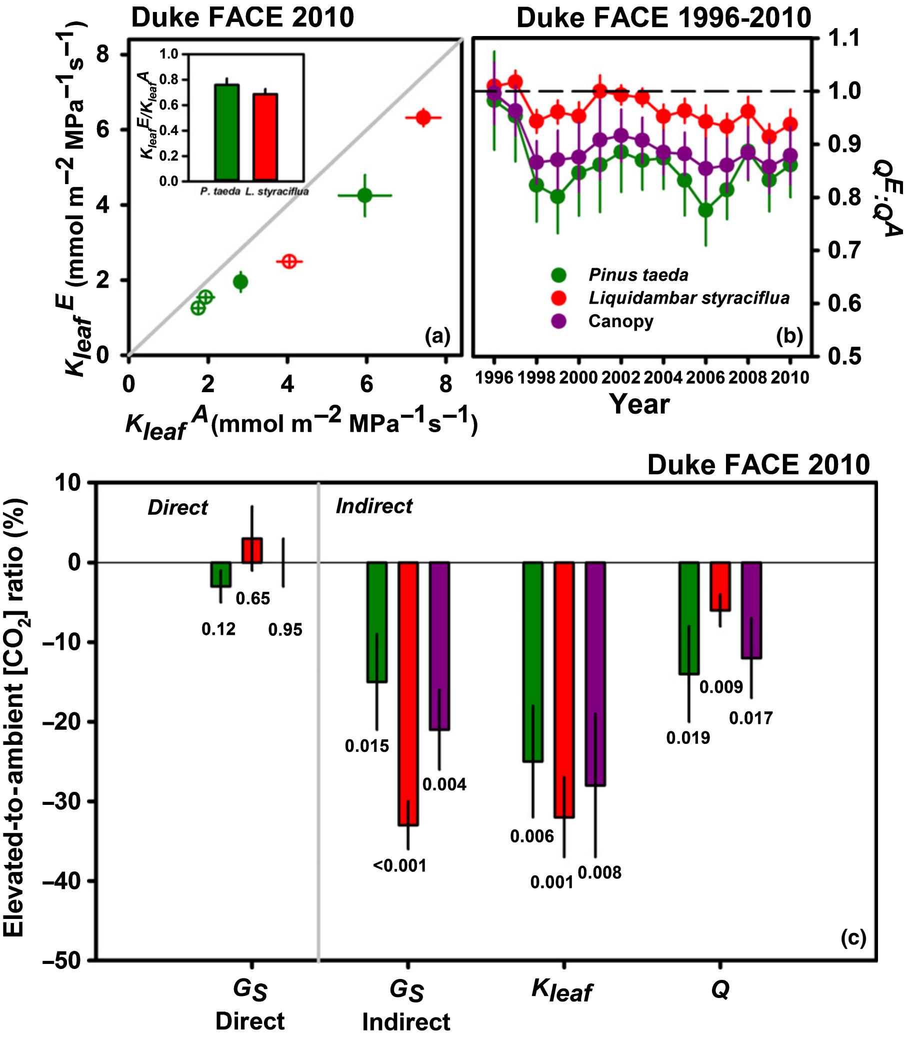

Fig. 4 The elevated-[CO$_2$] effects for +200 μmol mol^{-1} enrichment. (a) Mean leaf hydraulic conductance (K_{leaf}, in mmol s^{-1} MPa^{-1} m^{-2}, with one SE) of the two dominant species measured at low (open circles) and high (filled circles) relative extractable water (REW). Elevated-to-ambient [CO$_2$] ratios of the mean annual K_{leaf} scaled from the two measurements in 2010 are presented in the inset. Repeated-measures analysis of variance (ANOVA) yielded significant reductions with $P = 0.029$ and $P < 0.001$ in *Pinus taeda* and *Liquidambar styraciflua* respectively. (b) Responses of light intensity on leaf surfaces to high [CO$_2$] through the increased-[CO$_2$]-induced effect on L_D during the active free-air CO$_2$ enrichment (FACE) years ($n = 4$ plots). Error bars represent one SE. The general decreasing trend of elevated-to-ambient [CO$_2$] ratio of average light on leaf surfaces (Q^E:Q^A) results from increasing mutual shading following the L_D enhancement (Fig. 2a–c; squares). (c) The mean elevated-[CO$_2$] impacts on stomatal conductance (with one SE) under stepwise [CO$_2$] exposure (*Direct*, were calculated from the slopes in Fig. 3d–f; $G_{S,direct}$) and long-term elevated-[CO$_2$] exposure (*Indirect*, $G_{S,indirect}$). The results show nonsignificant direct and significant indirect G_S responses to high [CO$_2$]. Decreased leaf hydraulic conductance (K_{leaf}) through hydraulic acclimation, and reduced average light intensity on leaf surfaces (Q) through mutual shading caused by increased L_D contributed to the reduction of $G_{S,indirect}$. Numbers under bars are P values from Student's t-tests ($\alpha = 0.05$). Color notations are the same as (b).

Elevated-[CO$_2$] induced reduction of K_{leaf} of both dominant species regardless of soil moisture (Fig. 4a) or leaf water status (Supporting Information Fig. S2), averaging 25 ± 7% and 32 ± 5% for *Pinus taeda* and *Liquidambar styraciflua* respectively. These reductions are large enough to explain the observed long-term $G_S{}^E$:$G_S{}^A$ (Fig. 3d–f; horizontal solid lines) and reflect an *indirect* effect, because K_{leaf} represents the structural capacity of the leaf transport system to deliver water (Sack & Holbrook, 2006; Brodribb, 2009).

During *Indirect*, L_D averaged 19 ± 9% enhancement in the canopy, somewhat lower during severe drought years and years of acute weather events (Fig. 2a–c; squares; McCarthy *et al.*, 2007). Elevated-[CO$_2$]-induced L_D enhancement of *Liquidambar styraciflua* (24 ± 15%) was more pronounced than that of *Pinus taeda* (17 ± 8%). The enhanced L_D resulted in 14 ± 6% decrease of Q of the upper canopy *P. taeda*, and 6 ± 2% decrease for mid-canopy *L. styraciflua* (Fig. 4b), less than the effect of the hydraulic acclimation (Fig. 4c). However, hydraulic conductivity is not independent of light intensity, that is, lower light levels can reduce hydraulic conductivity (Sellin & Kupper, 2005) making the effect of higher L_D under elevated [CO$_2$] nonadditive with the reduction in hydraulic efficiency.

Taken together, these analyses suggest little, if any, *direct* [CO$_2$] effect on G_S for either species, and appreciable *indirect* [CO$_2$]-induced G_S reduction in *Pinus taeda* and *Liquidambar styraciflua*, with the canopy response falling close to that of *P. taeda* (Fig. 4c) due to its dominance of stand E_C (Fig. 2). Assuming all hardwoods responded similarly to *L. styraciflua*, the combined effects of [CO$_2$] on G_S and L_D produced no reduction of total canopy E_C during *Indirect* of +200 μmol mol^{-1} [CO$_2$] (−4 ± 12%, $P = 0.58$) as 19 ± 9% increase of L_D compensated for reduced G_S. Considering that higher L_D would also increase canopy rainfall interception, we conclude that water yield and export to rivers draining such ecosystems will not increase. There are conflicting predictions on the effects of reduced transpiration resulting from elevated-[CO$_2$]-induced stomatal closure, ranging from increased stream flow observed during the past two decades (Gedney *et al.*, 2006) and projected from results of general circulation models (Milly *et al.*, 2005; Betts *et al.*, 2007; Cao *et al.*, 2010), to intensified droughts resulting from decreases in cloud formation and precipitation (Katul *et al.*, 2012). Unless our results are atypical for forested ecosystems, such projections must consider increases of L_D as well as decreases in G_S with increasing [CO$_2$].

Whether stomatal responses to high [CO_2] are *direct* or *indirect* is important not only for proper modeling of ecosystem response to elevated [CO_2], but also when considering investments in expensive forest FACE experiments, several of which have recently come online or are in planning. Such experiments require an initial phase during which an existing stand must acclimate to elevated [CO_2] or a newly established stand matures to a quasi-steady state. Information collected during this initial phase is of limited use for modeling the responses of forests to increasing [CO_2]. Effects of high [CO_2] on G_S may take longer to manifest in established coniferous forest than in broadleaved forest (Li *et al.*, 2003; Wang *et al.*, 2005), possibly because many conifers retain foliage for many years. For species retaining hydroactive xylem in stems and needles for several years, especially when growing slowly in cold or dry climates, or poor soil, many years will be needed to transition to tissues whose function represent the new [CO_2] regime. We hope that the results of this study will serve as a model for future experiments, so that elevated-[CO_2] acclimation will enlarge our understanding and ability to predict ecosystem responses.

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References

Ågren GI. 1983. Nitrogen productivity of some conifers. *Canadian Journal of Forest Research* **14**: 494–500.

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165**: 351–372.

Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**: 258–270.

Ball JT, Woodrow IE, Berry JA. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In: Biggins J, ed. *Progress in photosynthesis research.* Dordrecht, the Netherlands: Springer, 221–224.

Betts RA, Boucher O, Collins M, Cox PM, Falloon PD, Gedney N, Hemming DL, Huntingford C, Jones CD, Sexton DMH *et al.* 2007. Projected increase in continental runoff due to plant responses to increasing carbon dioxide. *Nature* **448**: 1037–U5.

Brodribb TJ. 2009. Xylem hydraulic physiology: the functional backbone of terrestrial plant productivity. *Plant Science* **177**: 245–251.

Brodribb TJ, Feild TS. 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters* **13**: 175–183.

Brodribb TJ, Holbrook NM. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**: 2166–2173.

Buckley TN, Robert DW. 2005. How should leaf area, sapwood area and stomatal conductance vary with tree height to maximize growth? *Tree Physiology* **26**: 145.

Cao L, Bala G, Caldeira K, Nemani R, Ban-Weiss G. 2010. Importance of carbon dioxide physiological forcing to future climate change. *Proceedings of the National Academy of Sciences, USA* **107**: 9513–9518.

Carpenter SR, Frost TM, Heisey D, Kratz TK. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. *Ecology* **70**: 1142–1152.

De Boer HJ, Lammertsma EI, Wagner-Cremer F, Dilcher DL, Wassen MJ, Dekker SC. 2011. Climate forcing due to optimization of maximal leaf conductance in subtropical vegetation under rising CO_2. *Proceedings of the National Academy of Sciences, USA* **108**: 4041–4046.

Domec J-C, Palmroth S, Ward EJ, Maier CA, Thérézien M, Oren R. 2009. Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO_2 (free-air CO_2 enrichment) and N-fertilization. *Plant, Cell & Environment* **32**: 1500–1512.

Domec J-C, Schäfer KVR, Oren R, Kim H-S, McCarthy HR. 2010. Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO_2 concentration. *Tree Physiology* **30**: 1001–1015.

Douville H, Planton S, Royer J-F, Stephenson DB, Tyteca S, Kergoat L, Lafont S, Betts RA. 2000. Importance of vegetation feedbacks in doubled-CO_2 climate experiments. *Journal of Geophysical Research* **105**: 14841–14861.

Eamus D, Jarvis PG. 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. In: Begon A, MacFadyen A, eds. *Advances in ecological research.* London, UK: Academic Press, 1–55.

Ewers BE, Oren R. 2000. Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. *Tree Physiology* **20**: 579–589.

Gedney N, Cox PM, Betts RA, Boucher O, Huntingford C, Stott PA. 2006. Detection of a direct carbon dioxide effect in continental river runoff records. *Nature* **439**: 835–838.

Granier A. 1985. Une nouvelle méthode pour la mesure du flux de séve brute dans le tronc des arbres. *Annals of Forest Science* **42**: 193–200.

Gunderson CA, Wullschleger SD. 1994. Photosynthetic acclimation in trees to rising atmospheric CO_2: a broader perspective. *Photosynthesis Research* **39**: 369–388.

Hendrey GR, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* **5**: 293–309.

Jarvis PG, McNaughton KG. 1986. Stomatal control of transpiration: scaling up from leaf to region. *Advanced Ecological Research* **15**: 1–49.

Katul GG, Oren R, Manzoni S, Higgins C, Parlange MB. 2012. Evapotranspiration: a process driving mass transport and energy exchange in the soil–plant–atmosphere–climate system. *Reviews of Geophysics* **50**: RG3002.

Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson AD. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* **499**: 324–327.

Kim D, Oren R, Oishi AC, Hsieh C-I, Phillips N, Novick KA, Stoy PC. 2014. Sensitivity of stand transpiration to wind velocity in a mixed broadleaved deciduous forest. *Agricultural and Forest Meteorology* **187**: 62–71.

Kim H-S, Palmroth S, Thérézien M, Stenberg P, Oren R. 2011. Analysis of the sensitivity of absorbed light and incident light profile to various canopy architecture and stand conditions. *Tree Physiology* **31**: 30–47.

Li J-H, Dugas WA, Hymus GJ, Johnson DP, Hinkle CR, Drake BG, Hungate BA. 2003. Direct and indirect effects of elevated CO_2 on transpiration from *Quercus myrtifolia* in a scrub-oak ecosystem. *Global Change Biology* **9**: 96–105.

McCarthy HR, Oren R, Finzi AC, Ellsworth DS, Kim H-S, Johnsen KH, Millar B. 2007. Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO_2. *Global Change Biology* **13**: 2479–2497.

McDowell N, Barnard H, Bond B, Hinckley T, Hubbard RM, Ishii H, Köstner B, Magnani F, Marshall JD, Meinzer FC *et al.* 2002. The relationship between tree height and leaf area: sapwood area ratio. *Oecologia* **132**: 12–20.

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E *et al.* 2001. Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. *New Phytologist* **49**: 247–264.

Mencuccini M. 2002. Hydraulic constraints in the functional scaling of trees. *Tree Physiology* **22**: 553.

Mencuccini M. 2003. The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**: 163–182.

Milly PCD, Dunne KA, Vecchia AV. 2005. Global pattern of trends in streamflow and water availability in a changing climate. *Nature* **438**: 347–350.

Mousseau M, Saugier B. 1992. The direct effect of increased CO_2 on gas exchange and growth of forest tree species. *Journal of Experimental Botany* **43**: 1121–1130.

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, McCarthy HR, Hendrey G, McNulty SG *et al.* 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature* **411**: 469–472.

Oren R, Phillips N, Katul G, Ewers BE, Pataki DE. 1998. Scaling xylem sap flux and soil water balance and calculating variance: a method for partitioning water flux in forests. *Annals of Forest Science* **55**: 191–216.

Palmroth S, Oren R, McCarthy HR, Johnsen KH, Finzi AC, Butnor JR, Ryan MG, Schlesinger WH. 2006. Aboveground sink strength in forests controls the allocation of carbon belowground and its CO_2-induced enhancement. *Proceedings of the National Academy of Sciences, USA* **103**: 19362–19367.

Pataki DE, Oren R. 2003. Species differences in stomatal control of water loss at the canopy scale in a mature bottomland deciduous forest. *Advances in Water Resources* **26**: 1267–1278.

Pataki DE, Oren R, Tissue DT. 1998. Elevated carbon dioxide does not affect average canopy stomatal conductance of *Pinus taeda* L. *Oecologia* **117**: 47–52.

Phillips N, Oren R. 1998. A comparison of daily representations of canopy conductance based on two conditional time-averaging methods and the dependence of daily conductance on environmental factors. *Annals of Forest Science* **55**: 217–235.

Phillips N, Oren R. 2001. Intra- and inter-annual variation in transpiration of a pine forest. *Ecological Applications* **11**: 385–396.

Sack L, Holbrook NM. 2006. Leaf hydraulics. *Annual Review of Plant Biology* **57**: 361–381.

Schäfer KVR, Oren R, Ellsworth DS, Lai C-T, Herrick JD, Finzi AC, Richter DD, Katul GG. 2003. Exposure to an enriched CO_2 atmosphere alters carbon assimilation and allocation in a pine forest ecosystem. *Global Change Biology* **9**: 1378–1400.

Schäfer KVR, Oren R, Lai C-T, Katul GG. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. *Global Change Biology* **8**: 895–911.

Sellers PJ, Bounoua L, Collatz GJ, Randall DA, Dazlich DA, Los SO, Berry JA, Fung I, Tucker CJ, Field CB *et al.* 1996. Comparison of radiative and physiological effects of doubled atmospheric CO_2 on climate. *Science* **271**: 1402–1406.

Sellin A, Kupper P. 2005. Effects of light availability versus hydraulic constraints on stomatal responses within a crown of silver birch. *Oecologia* **142**: 388–397.

Tyree MT, Ewers FW. 1991. Tansley Review No. 34. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**: 345–360.

Tyree MT, Hammel HT. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* **23**: 267–282.

Wang K, Kellomäki S, Zha T, Peltola H. 2005. Annual and seasonal variation of sap flow and conductance of pine trees grown in elevated carbon dioxide and temperature. *Journal of Experimental Botany* **56**: 155–165.

Ward EJ, Oren R, Bell DM, Clark JS, McCarthy HR, Kim H-S, Domec J-C. 2013. The effects of elevated CO_2 and nitrogen fertilization on stomatal conductance estimated from 11 years of scaled sap flux measurements at Duke FACE. *Tree Physiology* **33**: 135–151.

Warren JM, Norby RJ, Wullschleger SD. 2011. Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology* **31**: 117–130.

Whitehead D, Edwards WRN, Jarvis PG. 1984. Conducting sapwood area, foliage area, and permeability in mature trees of *Picea sitchensis* and *Pinus contorta*. *Canadian Journal of Forest Research* **14**: 940–947.

Woodward FI. 1990. Global change – translating plant ecophysiological responses to ecosystems. *Trends in Ecology and Evolution* **5**: 308–311.

Wullschleger S, Gunderson C, Hanson PJ, Wilson K, Norby R. 2002. Sensitivity of stomatal and canopy conductance to elevated CO_2 concentration – interacting variables and perspectives of scale. *New Phytologist* **153**: 485–496.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Results from Randomized Intervention Analysis (RIA) to test for *direct* effects of elevated-[CO_2].

Fig. S2 Elevated-to-ambient [CO_2] ratios of K_{leaf}.

Table S1 Regression statistics of *Indirect* E_C response to D_Z in Fig. 2(d–f)

Table S2 The *t*-test result for comparison of *direct* vs *indirect* effects of elevated-[CO_2]

Table S3 Plot-level results from the Randomized Intervention Analysis (RIA)

Methods S1 Additional Materials and Methods.