Chapter 4  
Elevated atmospheric CO2 and drought does not alter total belowground carbon allocation in *Eucalyptus saligna*

# 4.1 Abstract

Accurately measuring carbon (C) allocation in large trees above and belowground remains a difficult task and is challenging to represent in models of forest C cycling. Understanding how global change impacts the distribution of tree photosynthate is an essential process in determining future terrestrial C balance. We utilized climate-controlled whole tree chambers (WTCs) to measure cumulative net aboveground CO2 uptake of *Eucalyptus saligna* trees, which was expected to correlate to harvested tree C mass. We then investigated how elevated atmospheric CO2 concentration and a 4-month drought period affected both tree biomass partitioning and the allocation of photosynthetic C to various above and belowground pools. We calculated total belowground C allocation (TBCA) for each WTC, which includes biomass and all belowground fluxes, as the residual between daily aboveground net CO2 uptake and aboveground C mass accrual. It was hypothesized that that both drought and elevated CO2 would increase biomass partitioning to roots, as well as TBCA. Cumulative aboveground net CO2 uptake correlated positively to both whole tree C mass and mean leaf area over the entire 11 month measured chamber flux period. Surprisingly, biomass partitioning to roots and cumulative TBCA were unaffected by either elevated CO2 or drought. As a fraction of total aboveground net C flux, TBCA remained relatively stable (ca. 40%) across the final 11 months of the experiment for all trees. Carbon allocation to leaves increased under elevated CO2, while the effects of a 4 month drought were negligible on biomass production or C allocation of aboveground tissues. The novel approaches used here provide evidence that belowground processes may not be as sensitive to global change as previously thought. These results reveal how quantifying the investment of photosynthetic C beyond biomass production is key to assessing functional tree growth responses, while also providing an empirical framework to test model representations of C allocation in trees.

# 4.2 Introduction

Carbon (C) allocation in trees encompasses investment into biomass production above and belowground as well as fluxes including tissue respiration and exudation (Litton et al. 2007). Trees must allocate C to maximize competitive fitness, reproduction and growth across their life cycle (Dickson 1989). Plants should maximize growth by allocating resources to support leaf growth to increase C acquisition (Monsi and Saeki 2005), however, fluctuations in water, nutrient and light availability cause plants to invest in roots for belowground resources or stem elongation for increased light harvesting (Friedlingstein et al. 1999). These potential changes in C investment are part of a dynamic system: as the tree grows or sink activities are altered, the fate of C assimilate can shift through time. Understanding allocation is vital, as partitioning among plant organs and their feedback processes profoundly impacts plant growth (Friedlingstein et al. 1999, Lacointe 2000, Shipley and Meziane 2002).

Variation in C allocation responses to environmental change combined with a lack of understanding of the mechanisms driving C allocation impede accurate modelling of terrestrial C cycling (Friedlingstein et al. 1999, Landsberg 2003, Litton et al. 2007, Epron et al. 2012, McMurtrie and Dewar 2013). The representation of C allocation lags behind leaf photosynthesis (*An*) in process-based forest models (Friedlingstein et al. 1999, Franklin et al. 2012, Iversen and Norby 2014) and this deficiency is due to the difficulty in defining principles that are valid under a wide range of conditions (Franklin et al. 2012, Mäkelä 2012). Partitioning coefficients or fixed fractions of assimilation to individual components are often used in models of forest C cycling (Litton et al. 2007, Franklin et al. 2012). Unfortunately, using inappropriate or over-simplified allocation schemes can lead to models producing unintended responses or giving the expected answer for the wrong reason (De Kauwe et al. 2014, Fatichi et al. 2014). As a result, there is continued need to empirically measure patterns of tree C allocation under multi-factor global change manipulations to better understand shifts in future forest C balance.

The allocation of photosynthate above and belowground is an important factor in terrestrial C cycling, yet our knowledge of how global change drivers impact C allocation is incomplete (Litton et al. 2007, Warren et al. 2012). With rising atmospheric CO2, forest C allocation has drawn particular interest due to its potential effect on C sequestration and the global C balance (Franklin et al. 2012). Across four forested free-air CO2 enrichment (FACE) experiments the total flux of C belowground (TBCA), which includes biomass and all belowground fluxes, was found to be enhanced under elevated CO2 (eCa) (Palmroth et al. 2006). In FACE experiments this enhancement has been attributed to factors such as increases in C allocation to root biomass production (Iversen 2010) and root exudation (Phillips et al. 2011). Alternatively, a meta-analysis by Poorter et al. (2000) concluded that on average, the distribution of biomass to roots, stems or leaves did not change in herbaceous and woody plants grown under eCa.

Understanding forest responses to global change also depends on disentangling complex relationships between interacting factors (Rustad 2008). For example, drought stress in trees can have deleterious effects on leaf (Bradford and Hsiao 1982, Schulze et al. 1987, Broeckx et al. 2014), stem (Brando et al. 2008) and root production (Meier and Leuschner 2008, Anderegg 2012). It has also been shown that C allocation to root systems can increase in drought environments when the severity and duration of the drought periods are substantial (Poorter et al. 2012). The impacts of leaf water savings during CO2 enrichment may also enhance tree biomass production under drought conditions (Atwell et al. 2007), but sustained enhancement is inhibited by the availability of a limited soil water supply to support larger overall canopies (Health and Kerstiens 1997). The effects of drought may limit C sequestration by the terrestrial biosphere (Iversen and Norby 2014), yet how limitations imposed by drought interact with the growth-stimulating effects of eCa requires more attention (Duursma et al. 2011).

Despite its importance, data on TBCA remain sparse as reliable estimates of root biomass, exudation, turnover and respiration in field conditions are difficult to obtain (Cheng et al. 2005, Litton et al. 2007, Phillips et al. 2008, Strand et al. 2008, Poorter et al. 2012). In forest ecosystems, TBCA has been shown to be equal or greater than aboveground production (Law et al. 1999), yet the controls of this belowground flux are poorly understood (Raich and Nadelhoffer 1989, Giardina et al. 2005). In stand or ecosystem studies, TBCA is often estimated as a residual, by subtracting the changes in C pools of litter, soil and roots from total soil CO2 efflux (Raich and Nadelhoffer 1989, Davidson et al. 2002, Giardina and Ryan 2002, Palmroth et al. 2006, Adair et al. 2009). A key assumption of this approach is that C pools are in steady-state conditions (Raich and Nadelhoffer 1989), which is not always true. Additionally, the reliance on soil respiration in this approach is problematic as studies are often forced to scale up short-term measurements (often monthly) to cumulative yearly fluxes, while also using a variety of measurement techniques. As allocation of C belowground remains one of the most difficult components of tree C budgets to calculate, new approaches are needed to in order accurately track and account for the investment of C belowground.

The whole-tree chambers (WTC), located at the Hawkesbury Forest Experiment, were designed to allow continuous measurement of net tree CO2 fluxes, allowing canopy *An* and respiration to be calculated using a mass balance approach (Medhurst et al. 2006, Barton et al. 2010). Here, we grew a single *Eucalyptus saligna* Sm. tree inside each of these 12 large, outdoor and sunlit WTCs for a period of 2 years. Each WTC can resolve net aboveground C uptake (canopy *An* minus respiration of aboveground woody components), at high temporal resolution, while also controlling temperature and air humidity to track prevailing environmental conditions. Generally, measuring total canopy *An* is difficult as variation in photosynthetic capacity exists within the canopy in response to the environment, requiring leaf measurements and models to upscale to the canopy (Ryan et al. 2010). Combining continuous aboveground net CO2 flux measurements with an evergreen *Eucalpytus* species, which grows throughout the year, enables tree C allocation to be tracked over long periods of time.

Previous findings in this experiment have shown that *Eucalyptus saligna* trees grown under eCa treatments were smaller than ambient trees and that larger trees under ambient CO2 had a smaller reduction in canopy transpiration in drought conditions, via deeper rooting access to water resources (Duursma et al. 2011). The smaller final tree sizes in eCa trees were attributed to an early setback in growth, which may or may not have resulted from effects of the eCa treatment. The specific objectives of this study were to determine the response of biomass partitioning among foliage, aboveground woody components and roots of a native Australian tree species to changes in CO2 and altered water availability. Utilizing the unique WTC design we aimed to test how cumulative net aboveground C gain correlates to whole tree C mass increment, as a function of tree size. We then applied a mass balance approach to track the allocation of C above and belowground throughout the course of an 11 month period under the combined treatments of eCa and drought.

Our hypotheses were:  
(1) As C uptake and growth should be coordinated over long time periods, we expected both total leaf area and harvested tree C mass to correlate with cumulative total aboveground net canopy C uptake.

(2) At the end of the 2-year experiment we expected partitioning of C to harvested roots to increase under eCa. We also expected increases in partitioning to roots under drought treatments to alleviate water limitation.

(3) As increases in partitioning to root biomass were hypothesized, we expected TBCA to increase through time as cumulative tree C flux became affected by eCa and drought.

# 4.3 Methods

## 4.3.1 Terminology

Carbon allocation terminology commonly employed in ecosystem literature is inconsistent (Litton et al. 2007). Here, important distinctions are made between patterns in live tree biomass and the distribution of photosynthate to all tree components. Clarification of these two terms is essential in accurately quantifying and representing C allocation in this work and in future studies.  
*Mass partitioning*: the relative distribution of biomass between different tree tissue components such as leaves, branches, bole and roots.  
*Carbon allocation*: the fraction of canopy photosynthesis distributed to different components such as tissue biomass pools, respiratory C fluxes, non-structural carbohydrate storage pools or root C exudation.

## 4.3.2 Whole tree chamber experiment

From April 2007 *Eucalyptus saligna* seedlings were grown in 12 whole-tree chambers (WTCs) at the Hawkesbury Forest Experiment in Richmond, Australia. One seedling per WTC (9 m high) was grown for 2 years and chamber conditions tracked outside air temperature and humidity. Each WTC was fitted with a root enclosure barrier that extended to the soil hard layer (1 m depth), separating WTC tree roots from neighboring trees. Roots were allowed to grow freely in the chamber soil volume and below 1 m. Neutron probe soil water measurements were utilized to show that trees in this experiment were able extract water deeper than the soil hard layer (Duursma et al. 2011). Soils at the WTC facility are an alluvial formation of low-fertility sandy loam, with clay deposits at a depth of about 1 m. Full descriptions of the chamber design and operation are provided in Barton et al. (2010). This multi-factor experimental design included CO2 × drought treatments with three WTC replicates in each of four treatments. Six chambers were kept at ambient CO2 of 380 ppm (aCa) and six were maintained at elevated CO2 of +240 ppm above ambient (eCa). Through October 2008 all trees were kept well-watered, with 10 mm of water every 3 days. Half of the chambers in each CO2 treatment were then subjected to a drought treatment by completely withholding water (dry) and the remaining six chambers were kept well-watered as an irrigated control (wet). The drought treatment lasted through mid-February 2009 when heavy rainfall ended the drought effect, despite the presence of a root enclosure (Duursma et al. 2011).

## 4.3.3 Aboveground chamber CO2 flux

Floors installed 45 cm above the soil surface, enclosing the main bole, permitted the chambers to function as cuvettes, excluding water and CO2 fluxes from the soil surface and allowed for aboveground whole tree fluxes of CO2 (and H2O) to be monitored once trees were ca. 3.5 m in height. This allowed high resolution CO2 flux data at 14 min intervals to be collected during the final eleven months of the experiment (from April 2008 to March 2009). The raw data were averaged over an hourly time-step and missing CO2 flux data were gap filled with SOLO (self-organizing linear output map) (see Abramowitz 2005). This self-fitting model predicted the flux as a function of photosynthetically active radiation, air temperature, vapor pressure deficit and day of year. In total, 27% of the observations were gap filled. This gap filling included a 5-6 week gap in late winter 2008 when WTC height was extended, as well as shorter periods in which chamber access doors were open during regular use. For each WTC, cumulative 24 hour net aboveground C uptake (, g C d-1) represented daily total canopy *An* of each tree minus respiration of stems and branches. Then was summed over the flux monitoring period () to compare to tree C mass, leaf area and C allocation above and belowground.

## 4.3.4 Harvested tree carbon mass

A final destructive harvest was completed in March 2009. The canopy of each tree was divided into five equal vertical layers, extending from the floor to the top and harvested. Dry biomass of leaves, branches and boles were measured for each layer and summed for each WTC. Root mass was obtained by excavating and sieving all soil inside each root exclusion barrier to the hard layer. Five root cores (10 cm diameter), sampled before the harvest, where collected from 0-70 cm in each chamber. Fine root biomass (<2 mm diameter) was first estimated by scaling-up dry root biomass collected from cores to total chamber area, but highly variable root biomass across cores resulted in unreliable estimates. As a result, root biomass from cores was added back to the standing crop total instead of scaling-up fine root biomass. Consequently, the fine root biomass pool is likely underestimated.

Carbon mass was assumed to be 48% of dry biomass for all non-leaf tissue components and this conversion was performed for all harvest and survey data (see below). This value represents the mean value of wood C of angiosperms from the Dyrad global wood C database, including measurements of stems, twigs, branches, bark, coarse roots and fine roots (Thomas and Martin 2012a, 2012b). Leaf and litter C mass was calculated by multiplying biomass by the WTC specific mean leaf C content (%). Leaf C content was determined from a sub-sample of final harvest dried and milled leaves analyzed using a Leco TruSpec Micro elemental analyzer (LECO corporation, MI, USA). Carbon mass fractions of leaves, boles+branches (stems) and roots were then calculated by dividing their respective total C mass by whole tree C mass at the end of the experiment.

Prior to the initiation of the experiment potted *Eucalyptus saligna* seedlings were harvested to develop relationships between above and belowground biomass (n=17, mean height of 217±17 cm). These seedlings were grown in 25 l pots inside each WTC until the experiment started, using the same soil as each WTC, while chamber [CO2] treatment conditions were maintained.

## 4.3.5 Tree allometry surveys

Tree height was measured every 14 days and diameters were recorded monthly at regular height intervals (30 cm) along the main bole and split stems. Bole diameters at 65 cm height were used as the starting reference diameter for each survey. Diameter and length for every branch, including forked branches, were surveyed seven times between April 2008 and March 2009. The first branch survey coincided with the installation of chamber floors and initiation of whole tree flux measurements. Branch diameter measurements were recorded at 5 cm from their individual insertion points. Leaf litter was collected from the chambers every two weeks, oven-dried and weighed.

## 4.3.6 Bole carbon mass

During the final harvest, diameter measurements were recorded as described above and 1 cm wide cross sections were removed from the bole at equally spaced positions along the bole midpoint between the diameter measurement points. Wood density for each section was calculated by dividing the dry mass by the fresh volume separately for bark and wood. The mean total bole density for each tree (, g cm-3) was then calculated as the total density of bark and wood, weighted by the total diameter of each section. We assumed that did not change through time.

For boles, individual volume units were constructed as concentric cylinders between each diameter measurement from base to tree top for each monthly survey. The tree top section was calculated as a cone with a tip radius of .001 cm. The volume below the starting reference diameter (65 cm) was calculated separately in order to interpolate taper into this section. Using the height of the tree and the standard diameter, the diameters at 30cm and base were estimated by extending the length of the pre-existing cone (from tree top to 65 cm). This resulted in two additional volume units. All volume units were summed,including forked stems, to calculate total bole volume. Bole mass was calculated as total volume multiplied by WTC tree-specific .

## 4.3.7 Branch carbon mass

Measured dry mass, length and basal area of all harvested branches was used to determine the branch wood density () as well as a geometric shape factor (, see Mäkelä 1997) for each WTC tree by rearranging the equation:

(1)

where is summed dry mass of all harvested branches, is summed branch length (cm), is summed branch basal area (cm2), represents the combined density of wood and bark (g cm-3) and corrects branch volume estimates to an intermediate shape between a cone and a cylinder (Mäkelä 1997). The ratio of measured to was used to generate a WTC-specific .

For each survey period, Mbr was estimated by solving the above equation with and for individual branches with specific to each WTC. We assumed that did not change through time. Total dry branch mass at each survey point was the total mass of all individual branches.

## 4.3.8 Leaf area and carbon mass

Total tree leaf area and dry mass were measured for each of the five canopy layers at the final tree harvest in March 2009. Specific leaf area (SLA, cm2 g-1) was calculated by dividing total projected one sided leaf area by dry leaf mass for each canopy layer. Mean SLA for each WTC tree was obtained by weighting SLA of each of the 5 layers by their foliage mass fraction. Estimates of standing leaf area were also obtained in April 2008 from leaf counts for each tree, multiplied by tree-specific mean leaf size (based on a sub-sample).

Canopy leaf area was modeled on daily times steps, between April 2008 and March 2009, using the leaf count census and harvest leaf area estimates, along with height growth and litter fall rates. This was the method applied by Barton et al. (2012). Briefly, leaf growth was assumed to coincide with height growth, so that no leaf growth occurred when height growth had ceased. This method assumes that total cumulative leaf area (i.e. standing leaf area plus that produced by litter fall) followed and allometric relationship with tree height such that:

(2)

where is the total 'potential' leaf area (m2), a and b are tree specific coefficients and H is tree height (m). Then standing leaf area at time *t* are obtained from tree height at time *t* and cumulative litterfall:

(3)

where is the litterfall (m2 t-1) rate at time *t*. Litter was assumed to be produced by all canopy layers. The daily leaf area contribution of litterfall is the difference between and . The mean SLA for each harvested tree was multiplied by daily estimates leaf and litterfall area to calculate biomass. SLA derived from the harvested trees was assumed to be constant over the entire flux measurement period.

## 4.3.9 Tissue C allocation

Tissue specific C allocation represents the fraction of net canopy C uptake distributed to a given tissue, which determines the change in biomass of that tissue through time such that:

(4)

where is the standing C mass of a component (g C), is the allocation to that component (0-1) and is the component specific turnover (d-1).

Here, total C allocation to leaves and aboveground wood (branches + bole) could be estimated from the sums of tissue C mass, net aboveground C flux and tissue turnover for each day of the experiment such that:

(5)

where is the total dry C mass of either leaves or wood and is the daily net aboveground C uptake (g C d-1). From equation 5, we estimated allocation by solving for (as all other components were measured). For example, C allocation to leaves () was determined by combining measurements of harvested dry C mass of leaves () with and total litterfall (), giving:

(6)

and then solving for leaf C allocation:

(7)

Allocation to aboveground wood C was estimated in the same manner with turnover measured as total dry C mass of branch litter collected across the experiment. For roots, only total belowground C allocation (TBCA) could be calculated (explained below) since root turnover was not measured.

## 4.3.10 Total belowground carbon allocation

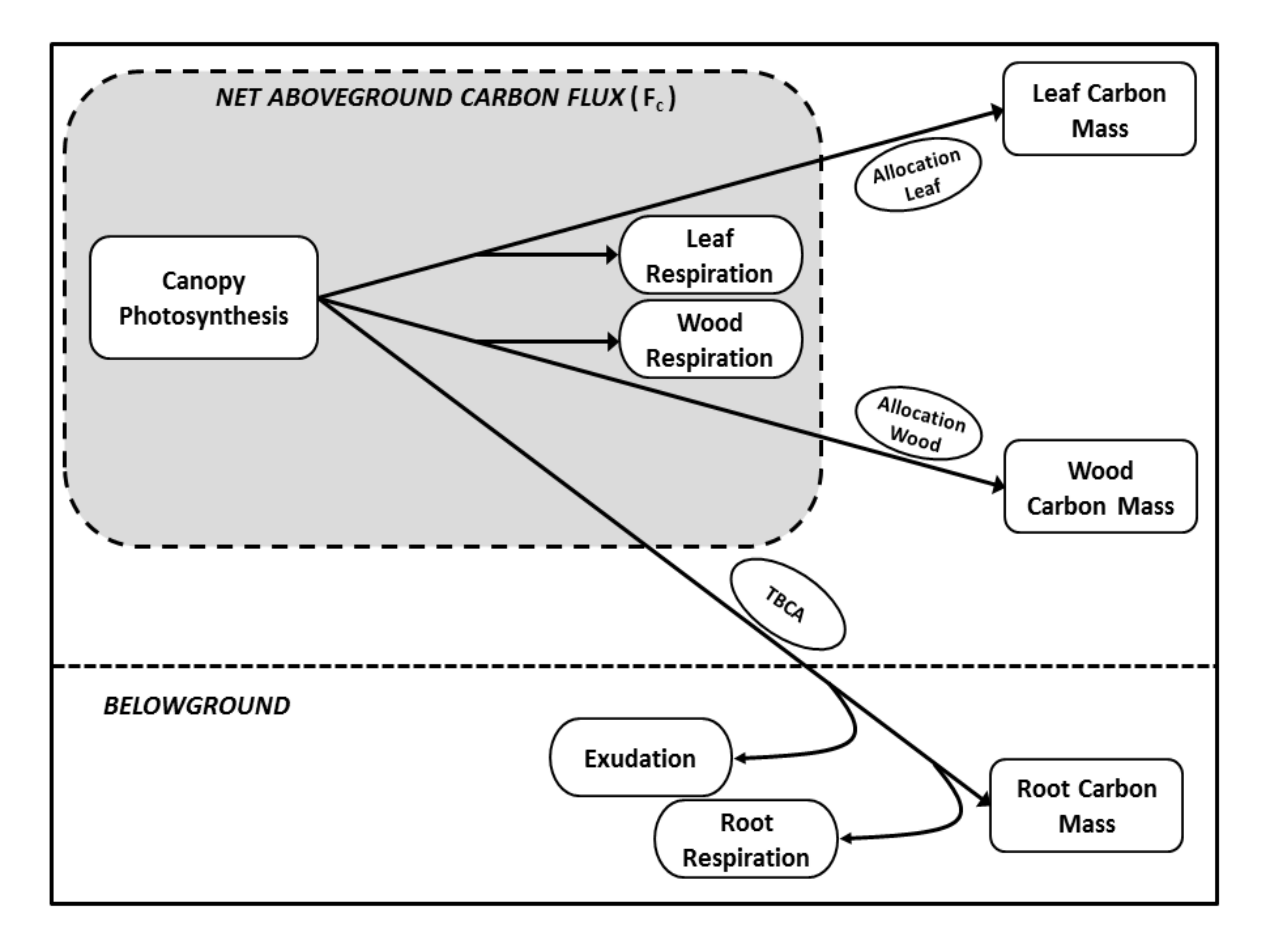
As the installation of chamber floors into each WTC separated the aboveground CO2 uptake from the soil CO2 efflux, TBCA at any time point *t* was calculated as:

(8)

where is the aboveground standing crop C mass (g C) of stems, branches, leaves and total leaf litterfall. As the final standing crop of root biomass was known, TBCA could be further broken down into the total C mass of roots () and the residual belowground C flux (). The residual belowground C flux includes root and microbial respiration, root turnover, root exudation and any unaccounted for root C mass. The use of aboveground allometry to interpolate through time combined with measured daily allowed TBCA to be estimated on daily time steps over the final eleven months of the experiment while cumulative was calculated at the final harvest.

## 4.3.11 Mass balance relationships between and carbon allocation.

The cumulative sum of for each WTC, at any given time point, represented the running total of net C uptake since the chamber floors were installed. Daily allocation of C to boles and branches was estimated by linear interpolation between 14-day survey measurements and the final harvest, starting at the first branch survey (April 2008). These daily estimates of leaf and litter C were added to bole and branch C mass to estimate on any given day. The contribution of each aboveground component to the cumulative sum of was then tracked from April 2008 to March 2009. The initial estimated C mass of each aboveground component and on the day when chamber floors were installed was subtracted from all respective daily values so mass balance could be tracked with a 0 starting value. This allowed cumultaive estimates of TBCA to be generated across the final 11 months of the experiment. Additionally, the significant log-linear relationship between above and belowground mass of both harvested trees and potted seedlings (R2 = 0.98, Figure 4.S1) was used to estimate from on the last day of the 11 month period. The major carbon allocation pathways and subsequent above- and belowground C pools measured in each WTC are further depicted in Figure 4.1.

  
**Figure 4.1**. Conceptual diagram depicting the major components of C flow among plant components including; uptake via photosynthesis, allocation to component tissues, tissue respiration and root exudation. Mass pools of leaves, wood and roots include tissue turnover. Net aboveground C uptake (), shown in the shaded box, represents the flux of C measured within each WTC. With the WTC experimental design, total belowground C allocation (TBCA) is measured as the residual between and total aboveground C mass.

## 4.3.12 Data analysis

Differences in experimental parameters in response to the interactice effects of CO2 and drought treatments at the final harvest were analysed as a completely randomized experimental design with factorial treatment combinations using two-way ANOVA in R (R Development Core Team 2011). Tukey's post-hoc tests were performed in conjunction with ANOVA to determine which specific paired comparisons among climate change treatments were different. Significance level was set at an alpha of 0.05 and findings between 0.05 and 0.10 were considered marginally significant.

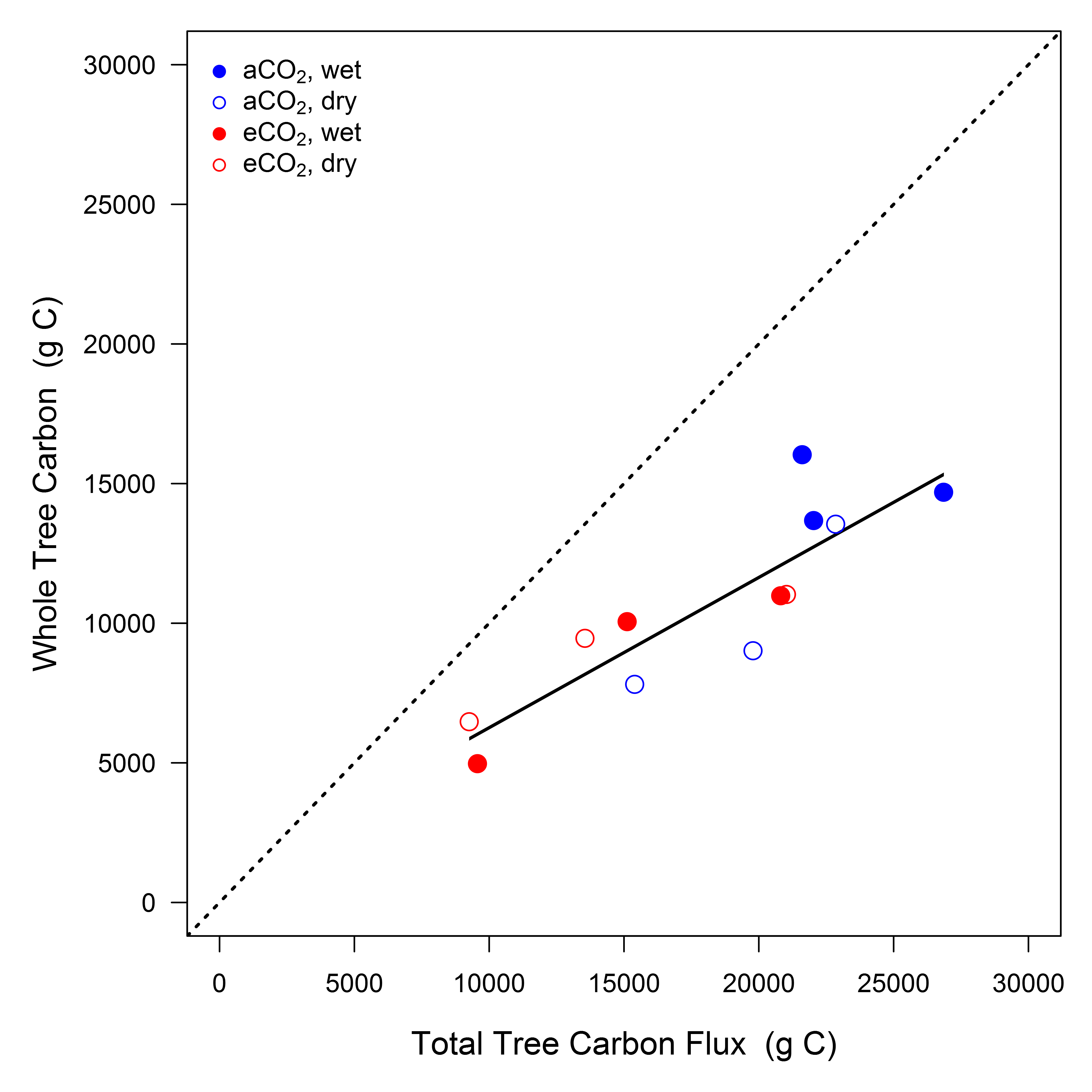
# 4.4 Results

## 4.4.1 Total aboveground carbon flux, whole tree C mass and leaf area

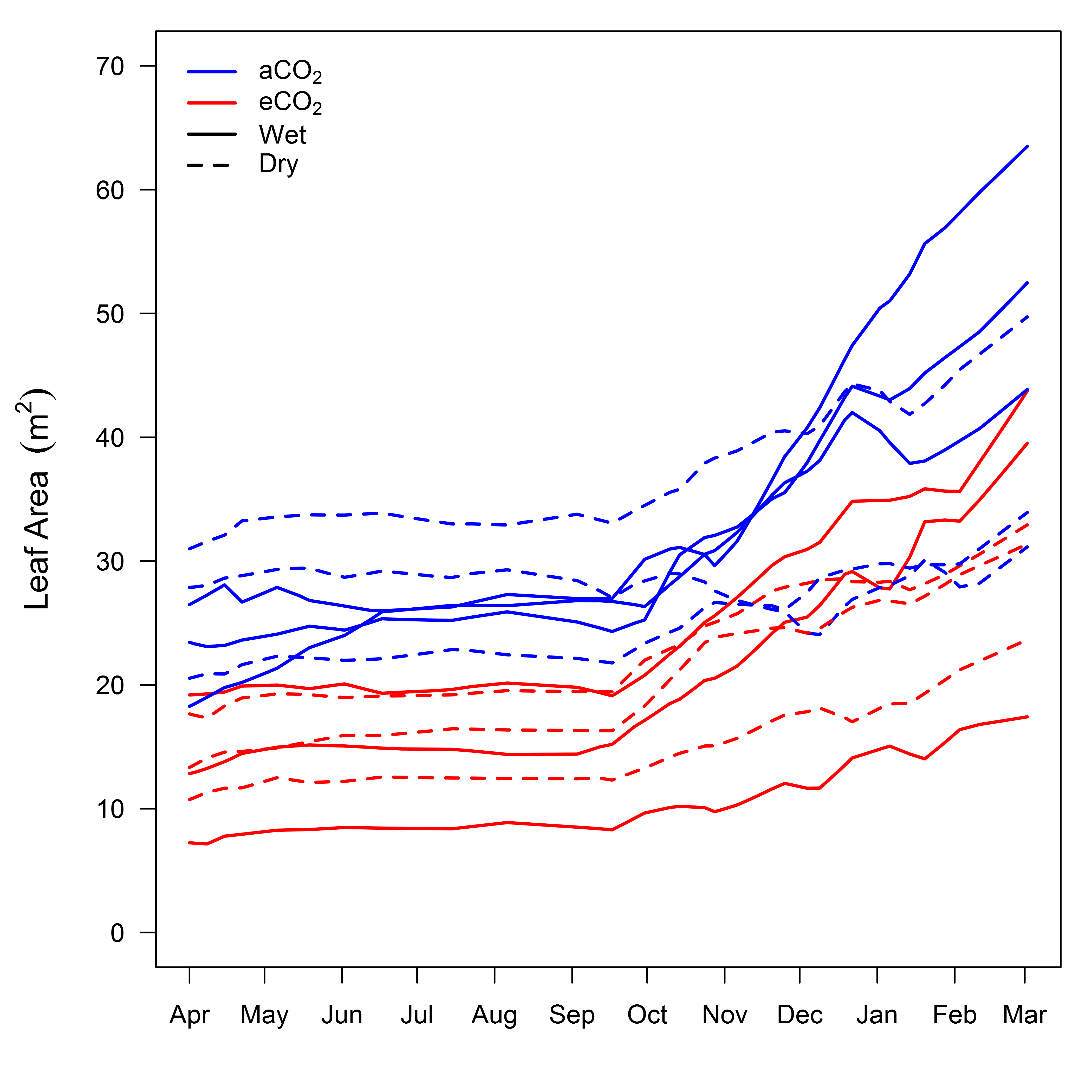
Both whole tree C and from the final harvest were reduced in eCa treatments by 32 % (both P < 0.03). Over the entire 11 month measured chamber flux period the summed aboveground C uptake () was significantly reduced by 30.5 % in eCa treatments (P = 0.043), while no effects of the drought treatment were detected (Table 4.1). was positively correlated with estimates of both whole tree C (R2 = 0.74, Figure 4.2a) and (R2 = 0.69, Figure 4.2b) over the same time period. Whole tree C mass increment estimated during the chamber flux period represented ca. 75 % of total harvested tree C mass. As the majority of biomass production occurred during this period, the allometric estimates of whole tree C were used for comparison to .

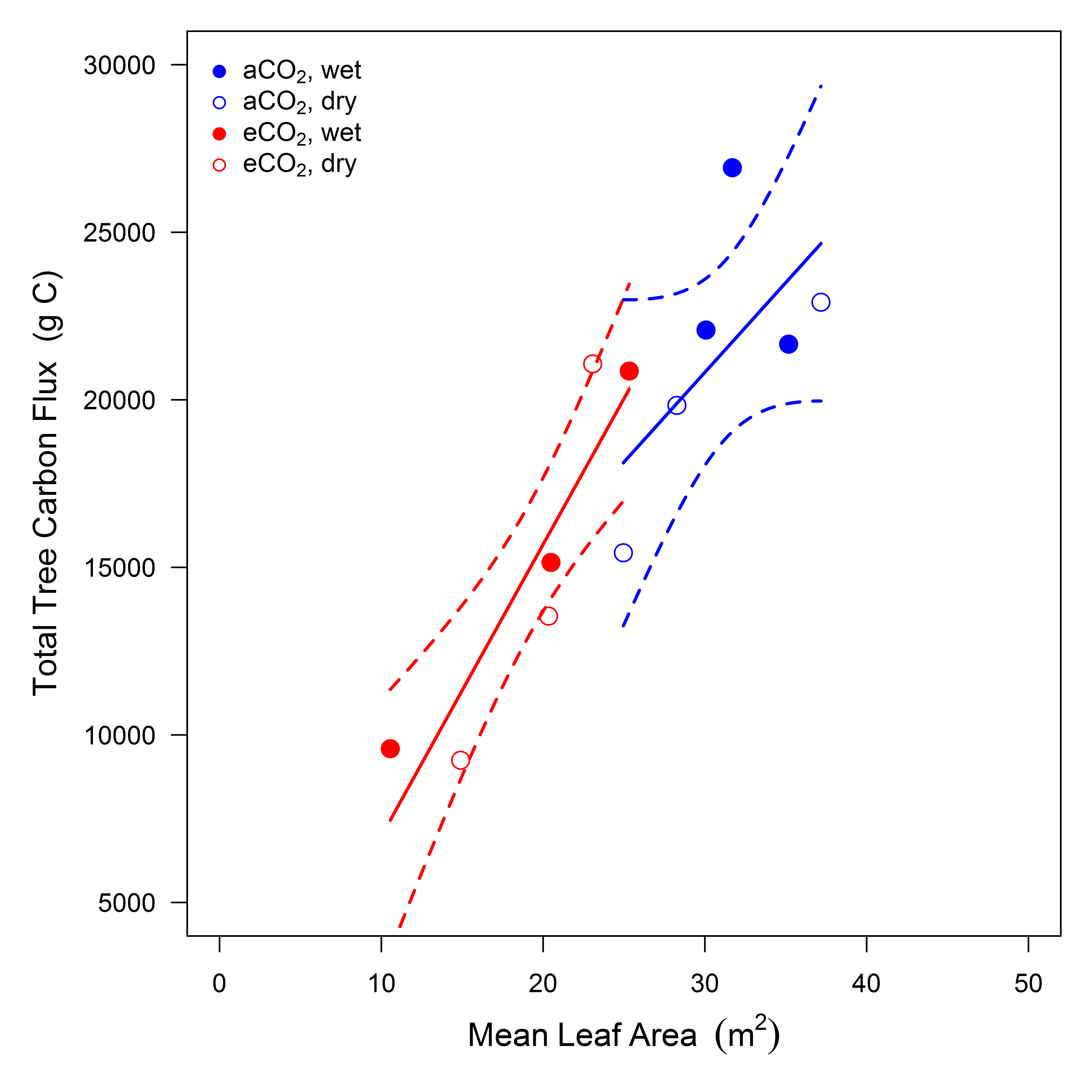
**Table 4.1**. Final harvest C mass of above and belowground tissues, cumulative aboveground tree C uptake () and specific leaf area (SLA). Each value represents the mean (± 1 standard error) for each treatment combination. Units for C mass and are g C, while SLA is cm2 g-1. For each variable, different letters represent significant differences between treatments from the overall model which includes CO2 \* drought interactions. P values represent overall differences of CO2 or drought main effects and the CO2 \* drought interaction.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Bole** | **Branch** | **Leaf** | **Litter** | **Root** | **Fc,T** | **SLA** |
| aCO2-dry | 5231.8 (687.0) b | 2799.2 (628.3) a | 2642.8 (370.7) a | 1129.8 (336.0) a | 3052.9 (500.2) a | 19394.2 (2169.5) a | 83.2 (3.3) a |
| aCO2-wet | 7785.1 (267.1) ab | 3154.6 (687.1) a | 3254.2 (393.5) a | 1043.1 (47.3) a | 3677.4 (316.8) a | 23556.5 (1689.0) a | 87.9 (2.8) a |
| eCO2-dry | 4080.5 (682.5) a | 1926.0 (369.4) a | 2232.1 (235.4) a | 889.4 (82.6) a | 2518.7 (481.6) a | 14620.7 (3456.2) a | 70.6 (3.6) a |
| eCO2-wet | 4026.3 (783.3) a | 1856.8 (474.5) a | 2358.3 (473.6) a | 919.0 (244.3) a | 2213.9 (705.8) a | 15197.9 (3253.5) a | 81.8 (6.1) a |
| CO2 effect (P) | 0.005 | 0.086 | 0.122 | 0.417 | 0.091 | 0.044 | 0.053 |
| Drought effect (P) | 0.085 | 0.803 | 0.358 | 0.897 | 0.766 | 0.413 | 0.089 |
| CO2 \* Drought (P) | 0.075 | 0.712 | 0.539 | 0.792 | 0.397 | 0.532 | 0.454 |

  
**Figure 4.2**. Whole tree C mass as a function of cumulative aboveground C flux for each WTC tree. Values of cumulative aboveground net C flux were measured over the final eleven months of the experiment. Whole tree C mass represents the sum of bole, branch, leaf and root C mass from allometric estimates over the same time period. The dotted line is the 1:1 relationship and the solid line represents the significant overall linear model fit from the equation y = 0.56x + 878.2 (R2 = 0.86).

Leaf area at the final harvest was significantly reduced by 31.3 % under eCa (p < 0.001) and this pattern was observed across the final eleven months of the experiment (Figure 4.3). Specific leaf area was reduced by 10.9 % in eCa treatments (P = 0.053), and by 8.9 % in drought treatments (P = 0.089, Table 4.1). Overall, was positively correlated with mean leaf area (P < 0.001, Figure 4.4). Intercepts and slopes between separate linear regressions of and mean leaf area for aCa and eCa treatments were not different, however, there was negligible overlap of data between treatments. Thus, we were unable to determine if reductions in in eCa treatments were a function of lower mean leaf area or shifts in *An* and tissue respiration rates.

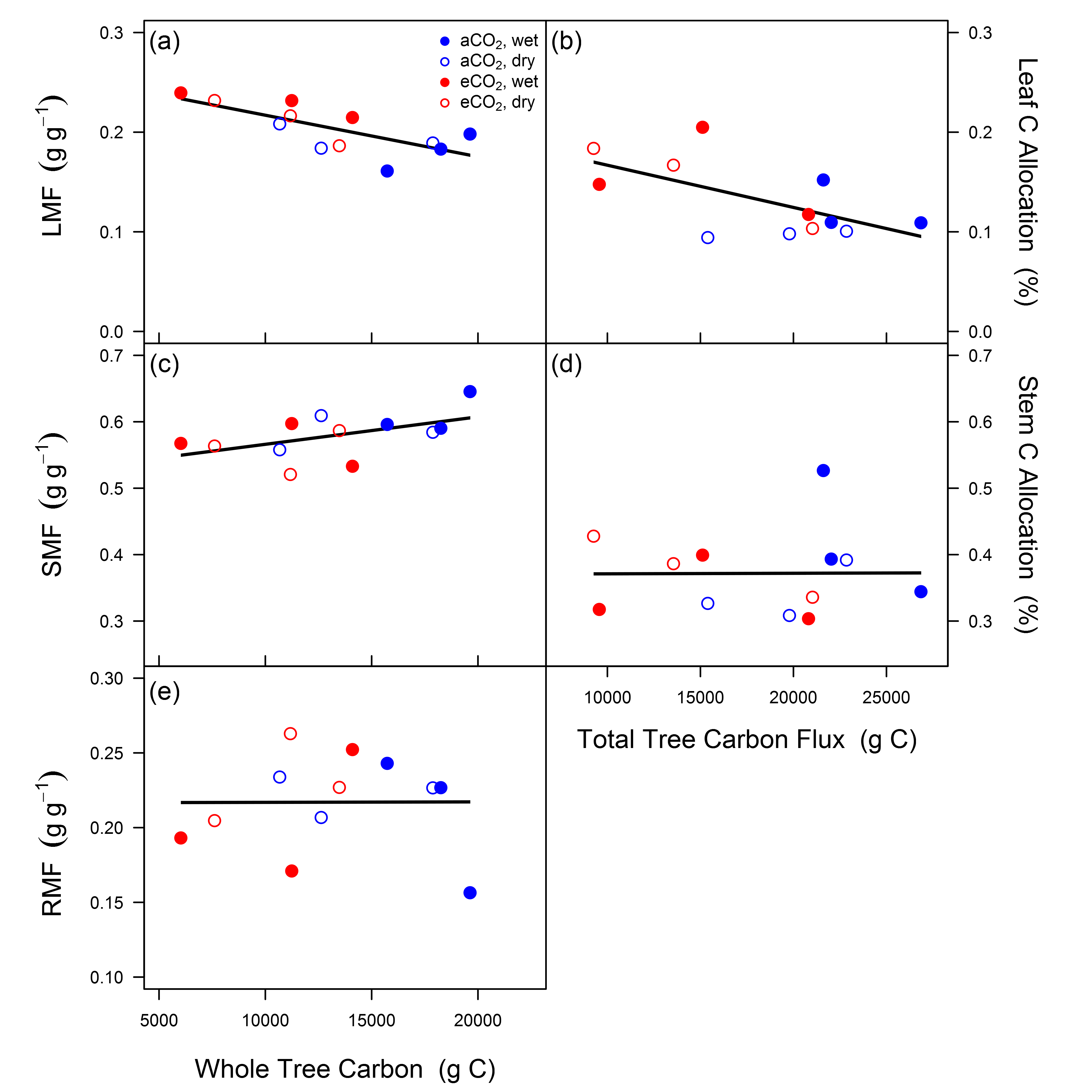
  
**Figure 4.3**. Estimated canopy leaf area for each WTC tree over the final eleven months of the experiment (April 2008 to March 2009). Estimates are based on height growth, litterfall rates and two leaf area estimates following Barton et al. (2012). Color and line type distinguish the treatment combination for each WTC.

  
**Figure 4.4**. Treatment means of cumulative aboveground C flux as a function of mean daily canopy leaf area over the final eleven months of the experiment. The solid line represents the significant overall linear model fit (R2 = 0.77) from the equation: y = 611.9x + 2791.2. Separate 95% confidence intervals are shown for linear regression between and mean leaf area for aCa and eCa treatments.

## 4.4.2 Harvested tissue carbon mass and biomass partitioning

At the end of this two year experiment, harvested C mass of tissue components was affected in eCa but not drought treatments (Table 4.1). Aboveground wood C mass was reduced by 37 % in eCa treatments (P = 0.015), driven mostly by eCa effects on bole wood. Neither standing crop leaf C mass or total litterfall C mass over the study period differed between CO2 treatments. Total root C mass was reduced by 29% in eCa treatments (P = 0.091).

Leaf mass fraction (LMF) increased by 15.0 % in eCa treatments (P = 0.011) but was not affected by the drought treatment. Leaf mass fraction was negatively correlated with whole tree C mass (P= 0.007, Figure 4.5a). Stem mass fraction (SMF) was marginally reduced by 6.0 % under eCa (P = 0.077), with no effect of the drought treatment detected. Stem mass fraction had a weak positive correlation with whole tree C mass (P = 0.08, Figure 4.5c). Root mass fraction (RMF) was not affected by either treatment and was not correlated to whole tree C mass (Figure 4.5e).

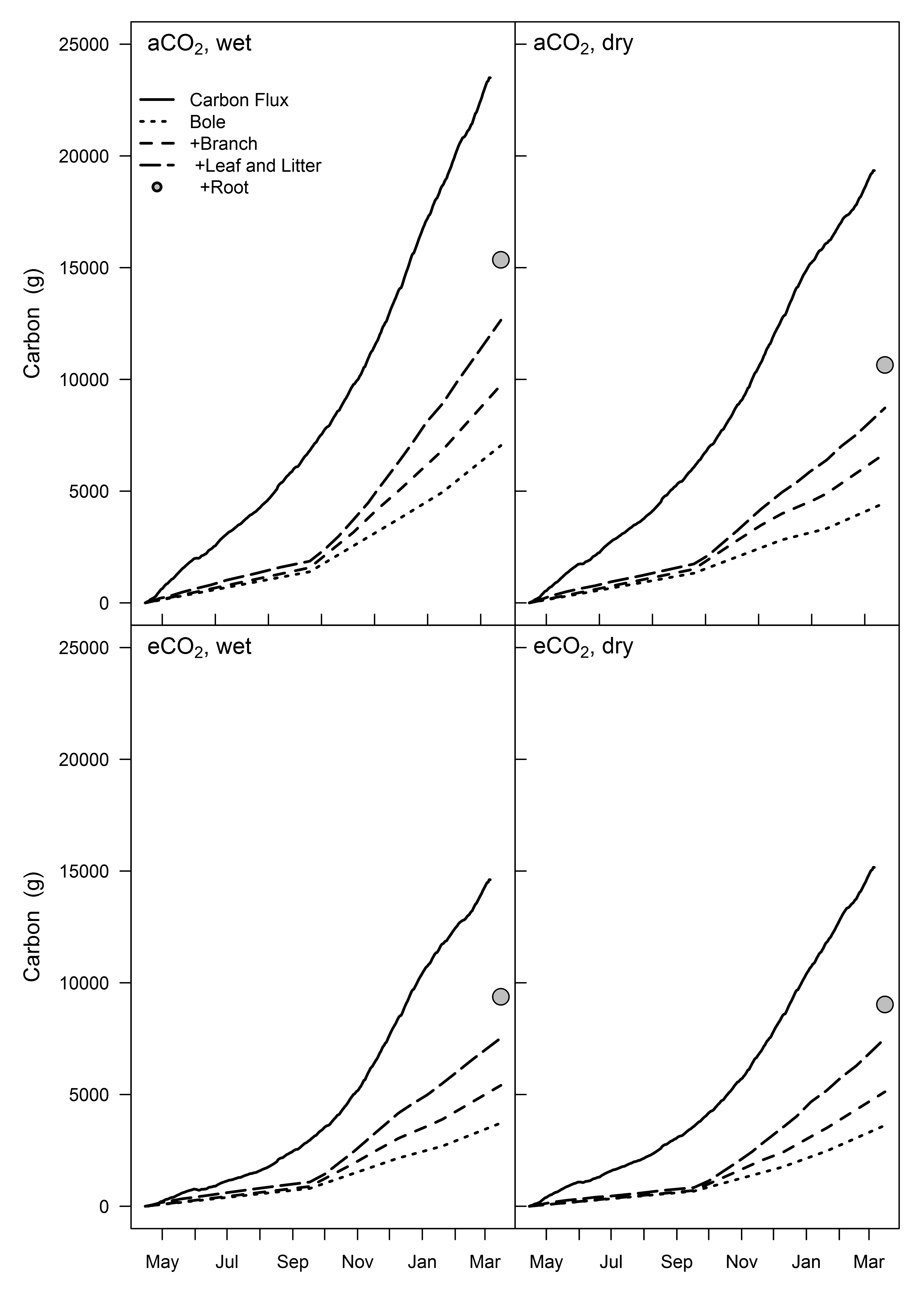
  
**Figure 4.5**. Treatment means of C mass fractions of leaves (a), stems (branches+boles) (c) and roots (e) as a function of tree size, via whole tree C mass. Treatment means of C allocation to leaves (b) and stems (d) as a function of cumulative aboveground net C flux. Root C allocation could not be estimated as root turnover was not known. Values for C mass fractions are calculated from final harvest biomass totals. Values for C allocation are estimated from cumulative total aboveground net C flux over the final eleven months of the experiment. Solid lines represent overall linear model fit for leaf, stem and root mass fractions (R2 = 0.53, 0.26 and 0.01, respectively), as well as leaf and stem C allocation (R2 = 0.39, 0.01, respectively).

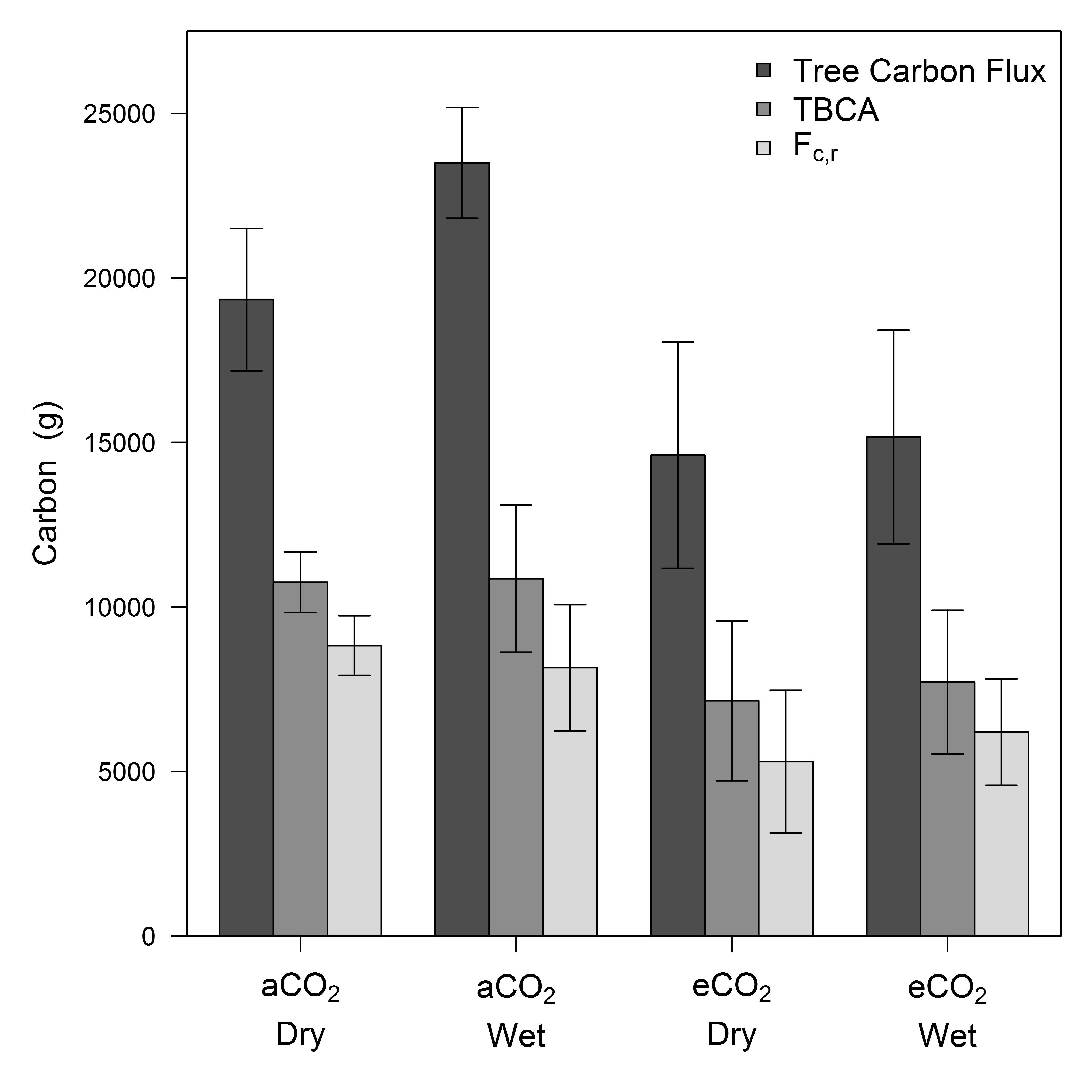
## 4.4.3 Aboveground carbon allocation

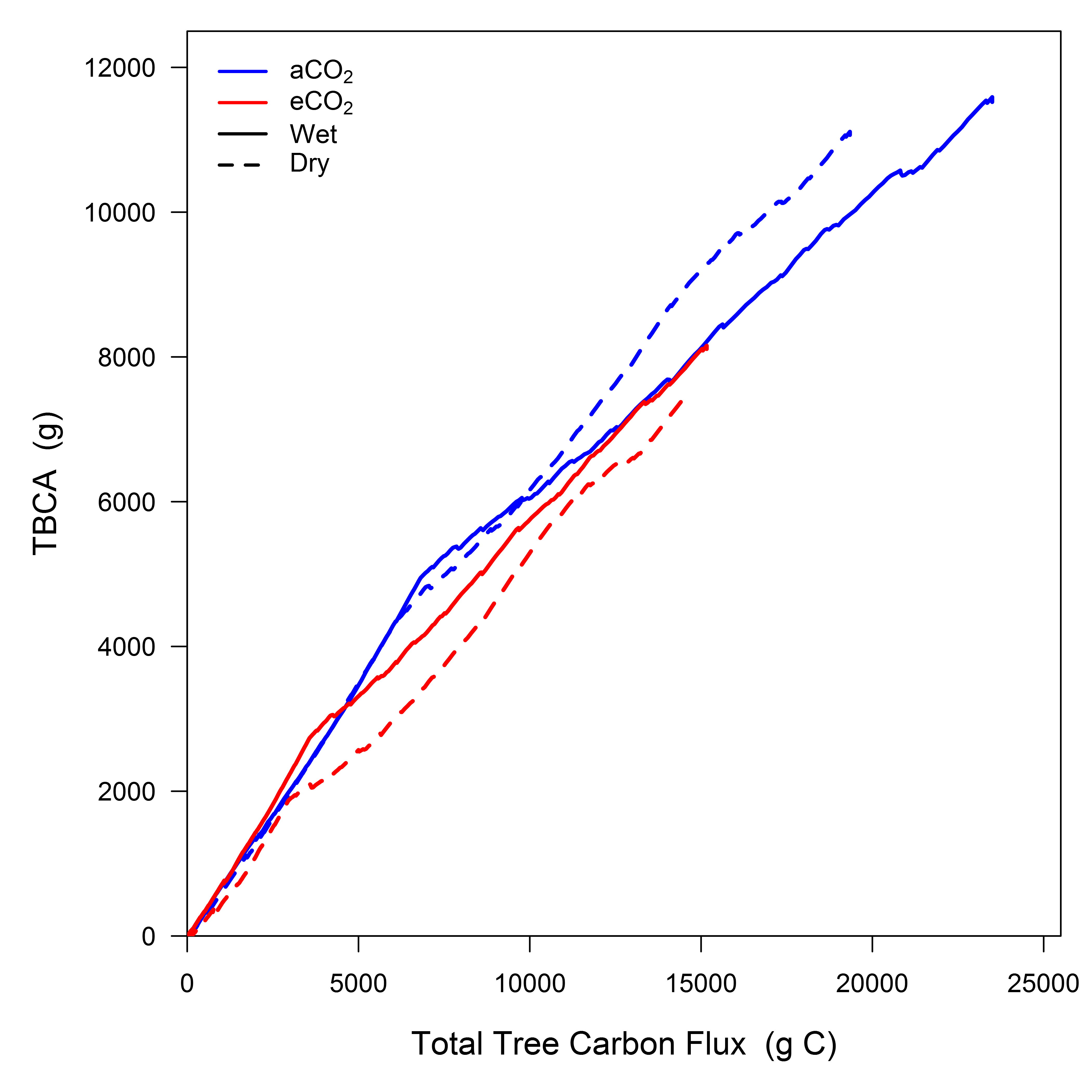
Treatment effects on tissue C allocation were determined from C mass estimates obtained from allometry over the final eleven months of the experiment and over the same time period. Total C allocation to leaves increased by 28% in eCa treatments (P = 0.052), with no effect of the drought treatment detected. Leaf C allocation was negatively correlated with (P = 0.031, Figure 4.5b). Alternatively, C allocation to aboveground wood was not affected by either treatment and was not correlated to whole tree C (Figure 4.5d).

## 4.4.4 Belowground carbon allocation

Across all treatment combinations, the total C mass of boles, branches, leaves and roots produced through the course of the measured flux measurement period was on average 61.0±0.02 % of (Figure 4.6). As mass balance must be achieved, TBCA and the residual belowground C flux () were estimated from Figure 4.6 as residuals between and whole tree mass excluding and including estimates of roots over the flux measurement period, respectively. Total belowground C allocation was on average 49.9±0.02 of and ranged from 46.1 to 54.9 % across treatment combinations. Across a large range in tree size among the treatment combinations and replicate WTCs, similar patterns were detected for each tree (Figure 4.S2). Neither cumulative TBCA nor were affected by CO2 or drought treatments (Figure 4.7). The time course of cumulative daily TBCA and were positively correlated over the final 11 months of the experiment (R2 = 0.78, P < 0.001) and the proportion of C allocated belowground was relatively stable through time and between treatments (Figure 4.8).

  
**Figure 4.6**. Cumulative aboveground net C flux and additive C allocation to individual tree components from 15 April 2008 to 16 March 2009. Each panel represents mean values for each treatment combination (n=3). Both aboveground net C flux and tissue C allocation where set to 0 on 15 April 2008 in order to track the allocation of C in daily time steps. Root C mass, predicted from the logarithmic relationship between above and belowground mass partitioning of pre-planting seedlings and harvested trees, is shown on the last date.

  
**Figure 4.7**. Treatment means ± 1 standard error of cumulative aboveground net C flux, TBCA, and the residual belowground C flux (). Values of cumulative aboveground net C flux were measured over the final eleven months of the experiment. Values for TBCA are the residual between the cumulative C flux and total C mass aboveground estimated from allometric surveys over the same time period. Values for were calculated as the residual between TBCA and root C mass predicted on the last date of the eleven month period.

  
**Figure 4.8**. Total belowground C allocation as a function of cumulative aboveground net C flux across the final eleven months of the experiment. Carbon mass aboveground was estimated from allometric surveys, interpolated on a daily time scale and then subtracted from the aboveground net C flux to quantify TBCA. Individual lines represent treatment means, with color and line type distinguishing treatment

# 4.5 Discussion

A whole-tree chamber experiment provided a unique opportunity to study the C balance of *Eucalyptus* trees. We found that biomass partitioning and C allocation of component tissues were differentially affected by eCa. Despite previous findings of negative impacts of drought on leaf and canopy physiology in this study (see Crous et al. 2011, 2012, Duursma et al. 2011), minimal effects of a four month drought were detected on total tree C flux, biomass partitioning and tissue C allocation. Using a novel methodological framework, we show that TBCA may be less sensitive to climate change factors than previously assumed. As reliable estimates of TBCA are notoriously hard to obtain, we provide essential empirical data that can be compared to model predictions where C allocation is represented.

## 4.5.1 Relationships between tree C flux, leaf area and tree C mass

A novel aspect of this study was the ability to measure whole tree C fluxes directly and compare these fluxes to observed patterns in leaf area and growth. Tree C uptake and growth were strongly coordinated across this two year experiment. The net C uptake of plants should be a function of the canopy leaf area and light interception (Wilson 1965, Monsi and Saeki 2005) and correlate to canopy assimilation and tree productivity (Waring 1983, McCarthy et al. 2006, Lindroth et al. 2008). Estimates of tree canopy C flux, however, are limited by simple upscaling of single leaf measurements (Amthor 1994), oversimplification of big leaf models (De Pury and Farquhar 1997) or parameterization of more complex models with assumptions of canopy behavior (Leuning et al. 1995). We found that leaf area was consistently reduced in eCa treatments, likely leading to reductions in both tree C uptake and whole tree C mass of near identical magnitudes (ca. 30 %).

A shortage of empirically measured whole tree C fluxes also makes inferring relationships with biomass or C allocation difficult. Biomass and C fluxes have been found to be poorly related in forest ecosystems due to difficulty in accounting for C retention of different tissues (Litton et al. 2007). This partial accounting of C likely inhibits the ability of many studies to precisely test the coordination between canopy *An* and growth. The advantage of the WTC approach is the ability to compare cumulative whole tree C fluxes to absolute biomass production over long time periods. Here, we show empirically measured aboveground tree C uptake (Fc) was strongly correlated to tree biomass production across a 2.5 fold size range in *Eucalyptus* trees.

## 4.5.2 Responses of biomass partitioning and C allocation to climate change

We first used final harvest biomass to determine patterns of biomass partitioning to leaves, stems and roots. We then combined cumulative tree C fluxes with tissue biomass production and turnover to measure C allocation to stems, leaves and TBCA, via mass balance. This approach allowed us to evaluate the impacts of climate change treatments on tree growth through potential shifts in tissue biomass production or C allocation. This is because there are many possible fates for C assimilates beyond just the production of plant biomass (Körner et al. 2005). Changes in C allocation encompass effects of tissue turnover, the storage and use of carbohydrates and root exudation to stimulate microbial activity, with each representing significant tree or ecosystem responses to environmental change. Thus, patterns in biomass partitioning and C allocation may not be consistent with respect to the tissue in question, which contributes to the current uncertainty in modelling tree growth responses to interacting climate change factors.

We found that stem C mass was reduced in eCa treatments. Opposite responses of stem growth under eCa have been found across different forested FACE experiments, including no effect in a mixed deciduous forest at WEB-FACE (Körner et al. 2005) and a positive enhancement in a loblolly pine forest at duke FACE (DeLucia et al. 2005). It is possible that observed patterns in stem C mass were related to allometric trajectories as a function of plant size (Tjoelker et al. 1998, Müller et al. 2000) more than direct effects of eCa on stem biomass production. Stem mass fractions (SMF) were found to increase with total plant size and were marginally reduced in eCa treatments. Carbon allocation to stems was unaffected in eCa treatments, however, inferring that patterns in SMF were a consequence of size-dependent relationships between larger aCa trees compared to smaller eCa trees. Trees in this experiment followed commonly observed developmental patterns in biomass partitioning, with increases in SMF and decreases in LMF as tree became larger (Poorter et al. 2015). Thus, it is likely that eCa treatments negatively affected other tree processes which first decreased overall tree size.

Contrary to expectation, we found that both LMF and C allocation to leaves increased in eCa treatments independent of tree size effects. As leaf production and turnover were not subsequently affected in the smaller eCa trees, it is likely that changes in other physiological processes were necessary to explain observed increases leaf C allocation. Previously reported increases in leaf respiration under eCa treatments (Crous et al. 2011, 2012) are intrinsically included in the measurement of , thus observed increases in leaf C allocation in terms of leaf biomass production are independent of shifts in respiration. Decreases in SLA were detected in WTC trees under eCa treatments, which is often found across eCa enrichment studies (Yin 2002, Ainsworth and Long 2005, Wang et al. 2012). Concentrations of leaf non-structural carbohydrates (TNC) often increase under eCa (Roden and Ball 1996, Picon et al. 1997, Poorter et al. 1997, Loewe et al. 2000, Walter et al. 2005) and are often associated with subsequent decreases in SLA in trees (Barron-Gafford et al. 2005, Körner et al. 2005). Here, increased C allocation to leaves may have resulted in the observed increase in leaf TNC under eCa (see Crous et al. 2011) to fulfill increased canopy respiratory demands or meet sink demands of other tissues. Taken together, results for aboveground tissues highlight the importance of separating impacts on measured biomass from those of total C allocation associated with growth when evaluating tree responses to climate change.

## 4.5.3 TBCA response to climate change in a single-tree ecosystem

Despite increased attention to the effects of climate change on belowground processes, the difficulty in measuring TBCA currently hinders our ability to make well-founded empirical conclusions. One of our specific objectives was to use a novel method to calculate TBCA to test the hypothesis that TBCA was enhanced in eCa treatments and then to evaluate potential shifts in TBCA across shorter times scales. For example, changes in TBCA to eCa or drought could occur as sustained or pulsed responses through time. Enhancement of TBCA has been reported across forested FACE experiments (Palmroth et al. 2006) but the single-tree ecosystem design of the WTC allowed us to evaluate the effects of climate change factors without the inherent environmental complexity of a forest community. The unique design of the WTC allowed us to track TBCA as a cumulative total and across daily time steps, both of which can be used to validate and constrain models where C allocation is represented.

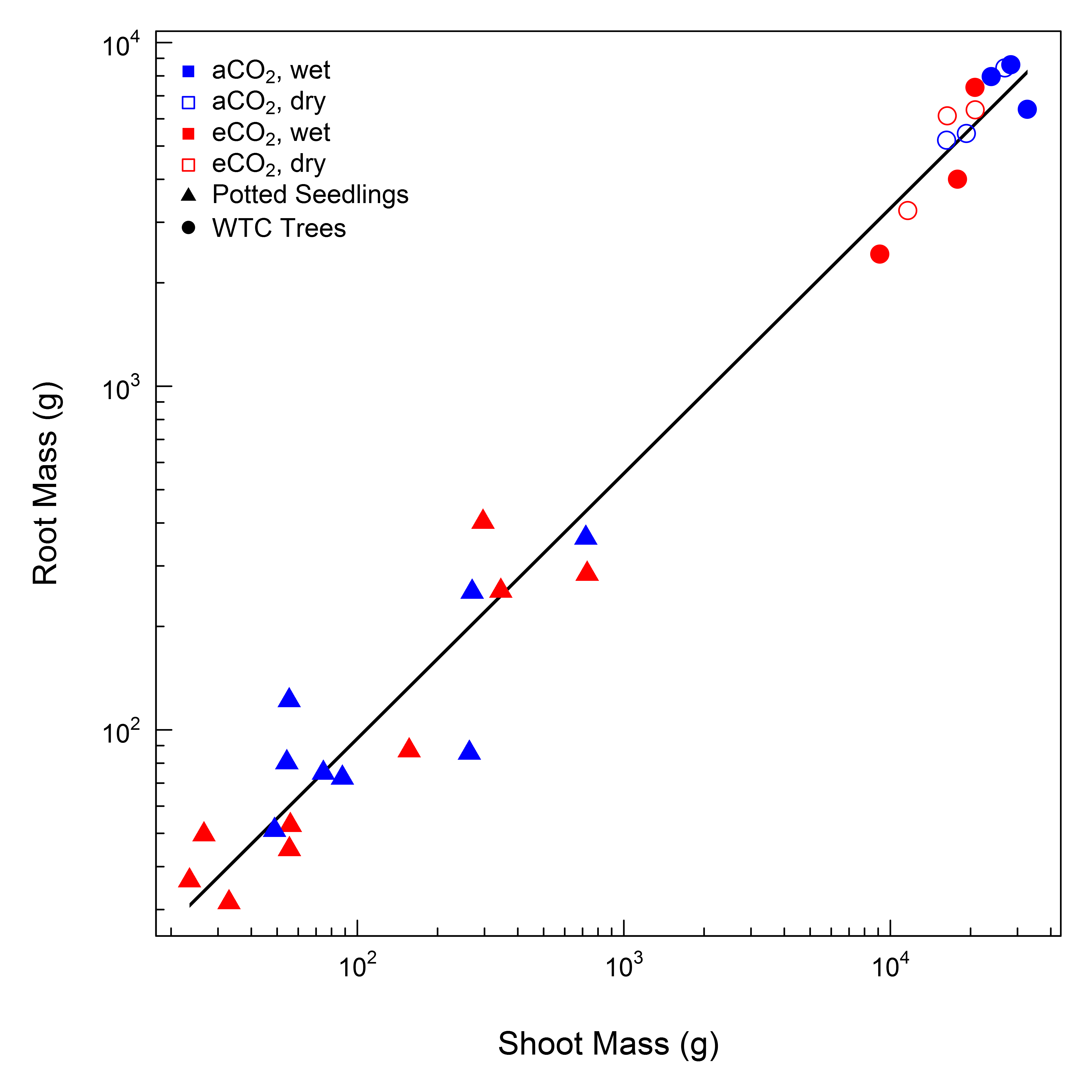
With high resolution flux data and reliable estimates of aboveground dry mass production we show that TBCA was not affected by eCa or drought treatments over the final eleven months of the experiment. Contrary to expectation, we detected minimal effects of eCa or drought treatments on root biomass partitioning, although it was not possible to differentiate fine and coarse root production and turnover. Although these findings disagree with TBCA results from forested FACE experiments (see Palmroth et al. 2006), comparisons between single-tree studies with evidence from forest ecosystem experiments should be made with caution. Nevertheless, we show that TBCA in *Eucalyptus* trees may be less sensitive to climate change factors than expected over a ~1 year period. However, a lack of cumulative change in TBCA does not imply that belowground processes were unaffected by either treatment. For example, trees under drought stress may increase C allocation to root systems to alleviate water stress (Poorter and Nagel 2000), but this increase could be offset by reduced root exudation (Iversen and Norby 2014), reduced C demand via decreases in root respiration rates (Burton et al. 1998) or increased root mortality and turnover (Marshall 1986, Meier and Leuschner 2008). Alternatively, the lack of belowground competition for soil mineral resources in this single tree ecosystem might have delayed enhancement of TBCA to eCa treatments, such as increased root production and exudation.

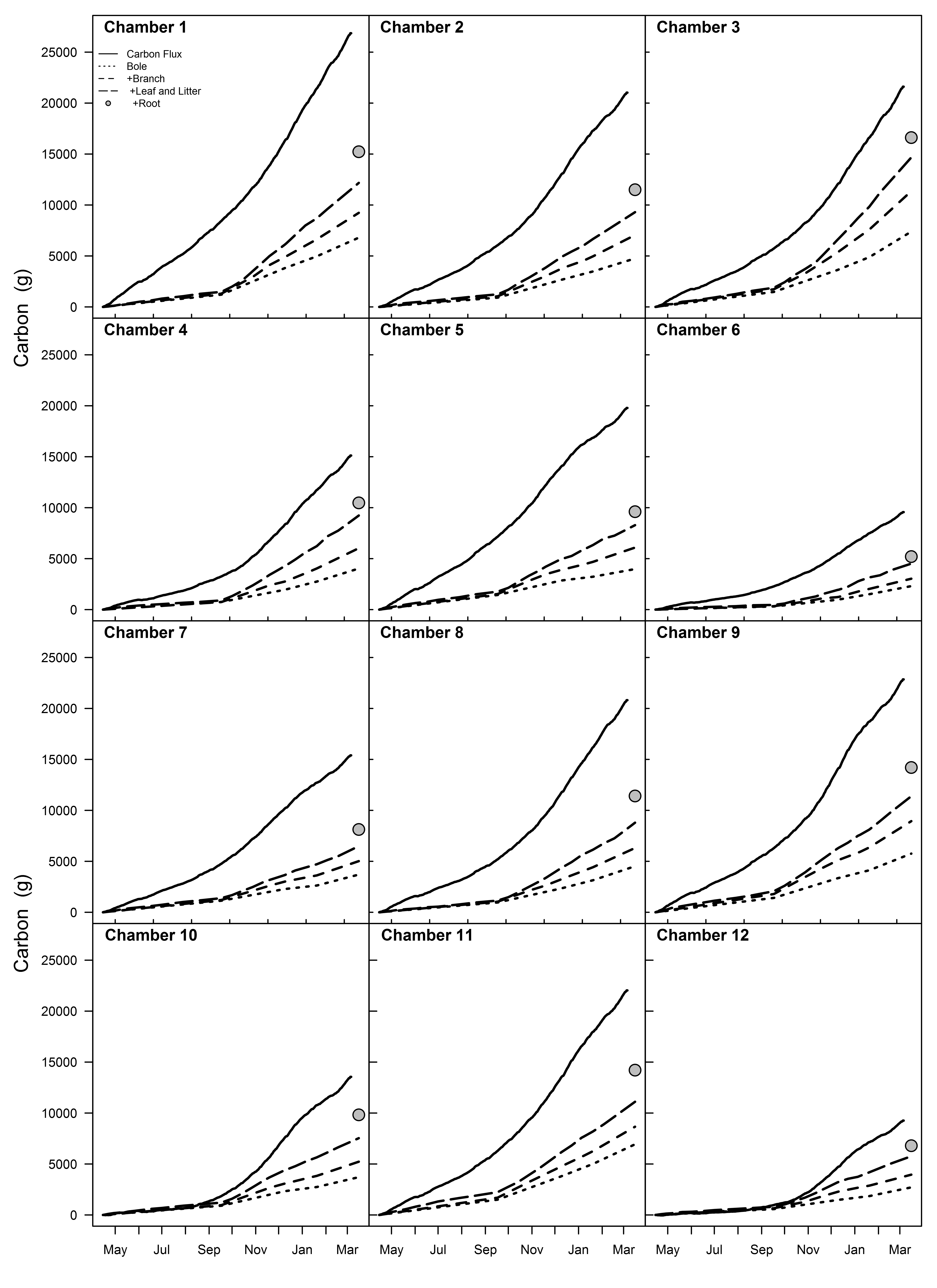
With an estimation of daily aboveground C mass gain and measured cumulative whole tree C uptake we were able to uniquely track dynamic short term effects of eCa or drought on TBCA. Across daily time steps, we observed a relatively stable fraction of total tree C flux distributed to TBCA over a period of eleven months. The ability to calculate TBCA as a simple residual between measured aboveground processes gives us reliable estimates of the absolute amount of C distributed belowground each day, which appears to be insensitive to sustained exposure to eCa and a four month drought. Similar to Palmroth et al. (2006) we cannot quantify allocation to specific belowground pools, but our approach with the WTC design does not have to make assumptions about C residence time in any tissue or soil component. As a result, the lack of a cumulative response of TBCA raises questions about the consistency of belowground responses to climate change factors often reported. Our results confirm the need for more reliable estimates of TBCA in future studies, which are crucial for predicting forest responses to climate change.

## 4.5.4 Conclusions

Here we use novel aspects of the WTC experimental facility to show that whole tree C flux and tree growth were highly correlated, while patterns in biomass partitioning alone were insufficient to explain eCa effects on tree growth. With individual *Eucalyptus saligna* trees we show different responses of above and belowground C allocation to eCa treatments, which may have important implications for how C allocation should be represented in process-based forest models. As empirical measurements of belowground processes are still difficult to obtain, models may have to assume that responses of aboveground tissues to global change represent those of belowground tissues (Giardina et al. 2005). As a result, continued empirical measurements to define C allocation patterns constrained by functional relationships with biomass production are needed to reduce uncertainty and improve model predictions (De Kauwe et al. 2014). Continuing to apply novel approaches to better evaluate TBCA and empirically measure whole tree C fluxes, such as the WTC experiment, are the way forward in addressing questions regarding the fate of assimilated C under global climate change.

# 4.6 Supporting Information

  
**Figure 4.S1**. Root mass as a function of shoot mass in *Eucalyptus saligna* for potted seedlings harvested before planting of WTC trees (n=17) and WTC trees harvested after 2 years (n=12). Potted seedlings were grown in 25 l pots inside each WTC, while chamber [CO2] treatments conditions were maintained. The solid line represents the significant log-log model fit (R2 = 0.98) from the equation: log(x) = 0.77(log(y)) + 0.43.

  
**Figure 4.S2**. Cumulative aboveground net C flux and additive C allocation to individual tree components from 15 April 2008 to 16 March 2009. Panels represent each individual WTC. Both aboveground net C flux and tissue C allocation where set to 0 on 2008-4-15 in order to track the allocation of C in daily time steps. Total root C mass, predicted from the log relationship between above and belowground mass partitioning of pre-planting seedlings and harvested trees, is shown on the last date.

# References

Abramowitz G (2005) Towards a benchmark for land surface models. Geophysical Research Letters 32

Adair EC, Reich PB, Hobbie SE, Knops JMH (2009) Interactive effects of time, CO2, N, and diversity on total belowground carbon allocation and ecosystem carbon storage in a grassland community. Ecosystems 12:1037–1052.

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytologist 165:351–372.

Amthor JS (1994) Scaling CO2-photosynthesis relationships from the leaf to the canopy. Photosynthesis Research 39:321–350.

Anderegg WRL (2012) Complex aspen forest carbon and root dynamics during drought. Climatic Change 111:983–991.

Atwell BJ, Henery ML, Rogers GS, Seneweera SP, Treadwell M, Conroy JP (2007) Canopy development and hydraulic function in *Eucalyptus tereticornis* grown in drought in CO2-enriched atmospheres. Functional Plant Biology 34:1137–1149.

Barron-Gafford G, Martens D, Grieve K, Biel K, Kudeyarov V, McLain JET, Lipson D, Murthy R (2005) Growth of Eastern Cottonwoods (*Populus deltoides*) in elevated [CO2] stimulates stand-level respiration and rhizodeposition of carbohydrates, accelerates soil nutrient depletion, yet stimulates above-and belowground biomass production. Global Change Biology 11:1220–1233.

Barton CVM, Duursma RA, Medlyn BE, Ellsworth DS, Eamus D, Tissue DT, Adams MA, Conroy J, Crous KY, Liberloo M, Others (2012) Effects of elevated atmospheric [CO2] on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. Global Change Biology 18:585–595.

Barton CVM, Ellsworth DS, Medlyn BE, Duursma RA, Tissue DT, Adams MA, Eamus D, Conroy JP, McMurtrie RE, Parsby J, Others (2010) Whole-tree chambers for elevated atmospheric CO2 experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. Agricultural and Forest Meteorology 150:941–951.

Bradford KJ, Hsiao TC (1982) Physiological responses to moderate water stress. In: Physiological plant ecology iI. Springer, pp 263–324.

Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazon forest: results of a throughfall reduction experiment. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1839–1848.

Broeckx LS, Verlinden MS, Berhongaray G, Zona D, Fichot R, Ceulemans R (2014) The effect of a dry spring on seasonal carbon allocation and vegetation dynamics in a poplar bioenergy plantation. GCB Bioenergy 6:473–487.

Burton AJ, Pregitzer KS, Zogg GP, Zak DR (1998) Drought reduces root respiration in sugar maple forests. Ecological Applications 8:771–778.

Cheng W, Fu S, Susfalk RB, Mitchell RJ (2005) New Phytologist 167:297–307.

Crous KY, Zaragoza-Castells J, Ellsworth DS, Duursma RA, Loew M, Tissue DT, Atkin OK (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO2 and summer drought. Plant, cell & environment 35:966–981.

Crous KY, Zaragoza-Castells J, Loew M, Ellsworth DS, Tissue DT, Tjoelker MG, Barton CVM, Gimeno TE, Atkin OK (2011) Seasonal acclimation of leaf respiration in *Eucalyptus saligna* trees: impacts of elevated atmospheric CO2 and summer drought. Global Change Biology 17:1560–1576.

Davidson EA, Savage K, Bolstad P, Clark DA, Curtis PS, Ellsworth DS, Hanson PJ, Law BE, Luo Y, Pregitzer KS, Others (2002) Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. Agricultural and Forest Meteorology 113:39–51.

De Kauwe MG, Medlyn BE, Zaehle S, Walker AP, Dietze MC, Wang Y-P, Luo Y, Jain AK, El-Masri B, Hickler T, Others (2014) Where does the carbon go? A model–data intercomparison of vegetation carbon allocation and turnover processes at two temperate forest free-air CO2 enrichment sites. New Phytologist 203:883–899.

De Pury DGG, Farquhar GD (1997) Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. Plant Cell and Environment 20:537–557.

DeLucia EH, Moore DJ, Norby RJ (2005) Contrasting responses of forest ecosystems to rising atmospheric CO2: implications for the global C cycle. Global Biogeochemical Cycles 19

Dickson RE (1989) Carbon and nitrogen allocation in trees. In: Annales des sciences forestières. EDP Sciences, pp 631s—–647s.

Duursma RA, Barton CVM, Eamus D, Medlyn BE, Ellsworth DS, Forster MA, Tissue DT, Linder S, McMurtrie RE (2011) Rooting depth explains [CO2] x drought interaction in *Eucalyptus saligna*. Tree physiology 31:922–931.

Epron D, Nouvellon Y, Ryan MG (2012) Introduction to the invited issue on carbon allocation of trees and forests. Tree physiology 32:639–643.

Fatichi S, Leuzinger S, Körner C (2014) Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. New Phytologist 201:1086–1095.

Franklin O, Johansson J, Dewar RC, Dieckmann U, McMurtrie RE, Brännström Å, Dybzinski R (2012) Modeling carbon allocation in trees: a search for principles. Tree Physiology 32:648–666.

Friedlingstein P, Joel G, Field CB, Fung IY (1999) Toward an allocation scheme for global terrestrial carbon models. Global Change Biology 5:755–770.

Giardina CP, Ryan MG (2002) Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach. Ecosystems 5:487–499.

Giardina CP, Coleman MD, Hancock JE, King JS, Lilleskov EA, Loya WM, Pregitzer KS, Ryan MG, Trettin CC (2005) The response of belowground carbon allocation in forests to global change. In: Tree species effects on soils: Implications for global change. Springer, pp 119–154.

Health J, Kerstiens G (1997) Effects of elevated CO2 on leaf gas exchange in beech and oak at two levels of nutrient supply: consequences for sensitivity to drought in beech. Plant Cell and Environment 20:57–67.

Iversen CM (2010) Digging deeper: fine-root responses to rising atmospheric CO2 concentration in forested ecosystems. New Phytologist 186:346–357.

Iversen C, Norby R (2014) Terrestrial Plant Productivity and Carbon Allocation in a Changing Climate. In: Global environmental change. Springer, pp 297–316.

Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Peláez-Riedl S, Pepin S, Siegwolf RTW, Zotz G (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO2. Science 309:1360–1362.

Lacointe A (2000) Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science 57:521–533.

Landsberg J (2003) Modelling forest ecosystems: state of the art, challenges, and future directions. Canadian Journal of Forest Research 33:385–397.

Law BE, Ryan MG, Anthoni PM (1999) Seasonal and annual respiration of a ponderosa pine ecosystem. Global Change Biology 5:169–182.

Leuning R, Kelliher FM, Pury DGG de, Schulze E-D (1995) Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. Plant, Cell & Environment 18:1183–1200.

Lindroth A, Lagergren F, Aurela M, Bjarnadottir B, Christensen T, Dellwik E, Grelle A, Ibrom A, Johansson T, Lankreijer H, Others (2008) Leaf area index is the principal scaling parameter for both gross photosynthesis and ecosystem respiration of Northern deciduous and coniferous forests. Tellus B 60:129–142.

Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. Global Change Biology 13:2089–2109.

Loewe A, Einig W, Shi L, Dizengremel P, Hampp R (2000) Mycorrhiza formation and elevated CO2 both increase the capacity for sucrose synthesis in source leaves of spruce and aspen. New Phytologist:565–574.

Marshall JD (1986) Drought and shade interact to cause fine-root mortality in Douglas-fir seedlings. Plant and Soil 91:51–60.

Mäkelä A (1997) A carbon balance model of growth and self-pruning in trees based on structural relationships. Forest Science 43:7–24.

Mäkelä A (2012) On guiding principles for carbon allocation in eco-physiological growth models. Tree physiology 32:644–647.

McCarthy HR, Oren R, Finzi AC, Johnsen KH (2006) Canopy leaf area constrains [CO2]-induced enhancement of productivity and partitioning among aboveground carbon pools. Proceedings of the National Academy of Sciences 103:19356–19361.

McMurtrie RE, Dewar RC (2013) New insights into carbon allocation by trees from the hypothesis that annual wood production is maximized. New Phytologist 199:981–990.

Medhurst J, Parsby J, Linder S, Wallin G, Ceschia E, Slaney M (2006) A whole-tree chamber system for examining tree-level physiological responses of field-grown trees to environmental variation and climate change. Plant, cell & environment 29:1853–1869.

Meier IC, Leuschner C (2008) Belowground drought response of European beech: fine root biomass and carbon partitioning in 14 mature stands across a precipitation gradient. Global Change Biology 14:2081–2095.

Monsi M, Saeki T (2005) On the factor light in plant communities and its importance for matter production. Annals of Botany 95:549–567.

Müller I, Schmid B, Weiner J (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics 3:115–127.

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 below ground and its [CO2]-induced enhancement. Proceedings of the National Academy of Sciences 103:19362–19367.

Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble root exudates in forest soils. Functional Ecology 22:990–999.

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. Ecology letters 14:187–194.

Picon C, Ferhi A, Guehl J-M (1997) Journal of Experimental Botany 48:1547–1556.

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO2, nutrients and water: a quantitative review. Functional Plant Biology 27:1191.

Poorter H, Jagodzinski AM, Ruiz-Peinado R, Kuyah S, Luo Y, Oleksyn J, Usoltsev VA, Buckley TN, Reich PB, Sack L (2015) How does biomass distribution change with size and differ among species? An analysis for 1200 plant species from five continents. New Phytologist 208:736–749.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist 193:30–50.

Poorter H, Van Berkel Y, Baxter R, Den Hertog J, Dijkstra P, Gifford RM, Griffin KL, Roumet C, Roy J, Wong SC (1997) The effect of elevated CO2 on the chemical composition and construction costs of leaves of 27 C3 species. Plant, Cell & Environment 20:472–482.

R Development Core Team R (2011) R: A Language and Environment for Statistical Computing Team RDC (ed). 1:409. <http://www.r-project.org>

Raich JW, Nadelhoffer KJ (1989) Belowground carbon allocation in forest ecosystems: global trends. Ecology 70:1346–1354.

Roden JS, Ball MC (1996) The Effect of Elevated [CO2] on Growth and Photosynthesis of Two Eucalyptus Species Exposed to High Temperatures and Water Deficits. Plant Physiology 111:909–919.

Rustad LE (2008) The response of terrestrial ecosystems to global climate change: towards an integrated approach. Science of the Total Environment 404:222–235.

Ryan MG, Stape JL, Binkley D, Fonseca S, Loos RA, Takahashi EN, Silva CR, Silva SR, Hakamada RE, Ferreira JM, Others (2010) Factors controlling *Eucalyptus* productivity: How water availability and stand structure alter production and carbon allocation. Forest ecology and management 259:1695–1703.

Schulze E-D, Robichaux RH, Grace J, Rundel PW, Ehleringer JR (1987) Plant water balance. BioScience:30–37.

Shipley B, Meziane D (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. Functional Ecology 16:326–331.

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R (2008) Irreconcilable differences: fine-root life spans and soil carbon persistence. Science 319:456–458.

Thomas SC, Martin AR (2012a) Data from: Carbon content of tree tissues: a synthesis. <http://dx.doi.org/10.5061/dryad.69sg2>

Thomas SC, Martin AR (2012b) Carbon content of tree tissues: a synthesis. Forests 3:332–352.

Tjoelker MG, Oleksyn J, Reich PB (1998) Temperature and ontogeny mediate growth response to elevated CO2 in seedlings of five boreal tree species. New Phytologist 140:197–210.

Walter A, Christ MM, Barron-gafford GA, Grieve KA, Murthy R, Rascher U (2005) The effect of elevated CO2 on diel leaf growth cycle, leaf carbohydrate content and canopy growth performance of *Populus deltoides*. Global Change Biology 11:1207–1219.

Wang D, Heckathorn SA, Wang X, Philpott SM (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO2. Oecologia 169:1–13.

Waring RH (1983) Estimating forest growth and efficiency in relation to canopy leaf area. Adv Ecol Res 13:327–354.

Warren JM, Iversen CM, Garten CT, Norby RJ, Childs J, Brice D, Evans RM, Gu L, Thornton P, Weston DJ (2012) Tree physiology 32:799–813.

Wilson JW (1965) Stand structure and light penetration. I. Analysis by point quadrats. Journal of applied Ecology:383–390.

Yin X (2002) Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO2 enrichment: a retrospective synthesis across 62 species. Global Change Biology 8:631–642.