Elevated atmospheric CO2 and drought alter carbon allocation above but not belowground in *Eucalyptus saligna*

Courtney E. Campany1, Mark Tjoelker1, Craig Barton1, Remko A. Duursma1.

1 Hawkesbury Institute for the Environment, University of Western Sydney, Locked Bag 1797, Penrith, NSW, Australia

*Corresponding author*: Courtney Campany E: [courtneycampany@gmail.com](mailto:courtneycampany@gmail.com)

# Abstract

Accurately measuring tree carbon (C) allocation 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 photosynthetic C is an essential process in determining future terrestrial C balance. We utilized climate-controlled whole tree chambers (WTC) to measure aboveground net CO2 fluxes of *Eucalyptus tereticornis* trees, which were expected to correlate to harvested tree C mass. This study investigated how treatment manipulations of CO2 and drought affected both tree biomass partitioning and the allocation of photosynthetic C to various above and belowground pools. For each WTC, we calculated total belowground C allocation (TBCA) as the residual between the aboveground net CO2 flux and aboveground C mass. It was hypothesized that that both drought and elevated CO2 would increase both partitioning of biomass to roots and TBCA. The measured cumulative aboveground tree net CO2 flux correlated positively to both whole tree C mass and mean leaf area over the final 11 months of the experiment. Surprisingly, biomass partitioning to roots was not affected by elevated CO2 or drought. Instead, increases in biomass partitioning to leaves and decreases in aboveground wood were detected under elevated CO2. Total C allocation to leaves was increased under elevated CO2, while allocation to aboveground wood and TBCA were not affected by either treatment. Across, the final 11 months of the experiment the daily fraction of C uptake allocated belowground remained relatively constant, regardless of climate change treatment or tree size. Overall, we show that elevated CO2 affects biomass partitioning, beyond ontogeny, aboveground instead of belowground. In addition, the unique design of the WTC also provides evidence that elevated CO2 may not always enhance C allocation belowground, as has been previously shown. These results reveal how climate change factors can impact the investment of photosynthetic C in a Eucalyptus tree species and provide an empirical framework to improve model representations of tree C allocation.

## Key Words

carbon allocation, biomass partitioning, whole tree chambers, elevated CO2, drought

# 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). In resource saturated environments plant should maximize growth by allocating new C to leaves to increase C acquisition (Monsi and Saeki 2005). Environmental stresses such as water, nutrient and light availability, however, may 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 photosynthesis (A) in applied forest models (Friedlingstein et al. 1999, Franklin et al. 2012, Iversen and Norby 2014) and this deficiency is due to the difficulty in defining guiding 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 process-based 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.

This allocation of photosynthetic C above and belowground is an important factor in terrestrial C cycling yet our knowledge of how global change impacts C allocation is incomplete (Litton et al. 2007, Warren et al. 2012). With rising atmospheric CO2 (Ca), forest C allocation has drawn particular interest due to its potential effect on C sequestration and the global C balance (Franklin et al. 2012). A meta-analysis by Poorter et al. (2000) concluded that on average biomass partitioning to stem, root, or leaf mass fractions did not change in plants grown under elevated Ca. Alternatively, the total flux of C belowground (TBCA), which includes all belowground processes, was enhanced under elevated Ca across four forested free-air Ca enrichment experiments (Palmroth et al. 2006). In forest ecosystems this enhancement can be attributed to factors such as increases in C allocation to roots biomass (Iversen 2010) or root exudation (Phillips et al. 2011).

The response of forest to global change also depends on teasing apart 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 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 increasing Ca requires more attention (Duursma et al. 2011).

Despite its importance, data on TBCA remain sparse and 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, TCBA 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). Total belowground C allocation 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). A key assumption of this approach is that C pools are in steady-state conditions (Raich and Nadelhoffer 1989), which is not always the 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 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 whole-tree net CO2 fluxes, allowing A and respiration to be calculated using a mass balance approach (Medhurst et al. 2006, Barton et al. 2010). Generally, measuring canopy A 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). The WTC, however, can resolve net aboveground C gain (canopy A minus respiration of foliage and aboveground woody components), at high temporal resolution, while also controlling temperature and air humidity. Combining the high resolution CO2 flux measurements with an evergreen *Eucalpytus* species, that provides near constant annual production, enables tree C allocation to be tracked over long periods of time. This experimental system can then be used to validate models that scale leaf A to whole canopies with empirical measurements of the response of whole-tree CO2 fluxes and biomass production to global change manipulations (Barton et al. 2010).

Previous findings in this experiment have shown that *Eucalyptus saligna* Sm. trees grown under elevated Ca were smaller than ambient trees and that larger trees had a smaller reduction in canopy transpiration in drought conditions, via deeper rooting access to water resources (Duursma et al. 2011). Therefore, 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 Ca 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 across the final eleven months of the experiment.

(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 then end of the 2 year experiment we expected partitioning of C to roots to increase under elevated Ca, similar to previous studies. We also expected increases in partitioning to roots under drought treatments, as trees should attempt to reduce water limitation.

(3) As shifts in partitioning to root biomass were hypothesized, we expected TBCA to increase through time as cumulative tree C flux became affected by elevated Ca and drought. Additionally, we expected C allocation to leaves and woody tissue components aboveground to remain constant over the final eleven months of the experiment.

# Methods

## Terms

*Mass partitioning*: the relative distribution of biomass between different tree tissue components such as leaves, branches, boles and roots.  
*Carbon allocation*: the fraction of net primary productivity distributed to different ecosystem components such as specific tissue components or total belowground pools.

## Whole tree chamber experimental design

From April 2007 *Eucalyptus saligna* seedlings were grown in 12 WTC 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 below 1 m. Full descriptions of the chamber design and operation are provided in Barton et al. (2010). This multi-factor experimental design included Ca × drought treatments with three replicates in each of four treatments. Six chambers were kept at ambient Ca of 380 ppm (aCa) and six were maintained at eCa of +240 ppm above ambient. Through October 2008 all trees were kept well-watered, with 10 mm of water every 3 days. Half of the chambers 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.

## Aboveground chamber CO2 flux

Floors installed above the soil surface, enclosing the main bole, permitted the chambers to functions as cuvettes and allowed for whole tree fluxes of CO2 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 from April 2008 to March 2009. 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. Cumulative daily net aboveground C fluxes (, g C d-1), representing daily gross aboveground primary productivity of each tree minus respiration of leaves, stems and branches, were summed () to compare to harvested tree C mass, leaf area and C allocation above and belowground.

## Harvested tree carbon mass

A final destructive harvest was completed in March 2009. Each tree was harvested across 5 canopy layers, set from the floor height and extended through the top of the canopy. 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 roots cores (10 mm diameter), sampled before the harvest, where collected from 0-70 cm in each chamber and biomass from cores was added back to the standing crop total.

Carbon mass was assumed to be 50% of dry biomass for all non-leaf tissue components and this conversion was performed for all harvest and survey data (below). 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).

Additionally, prior to the initiation of the experiment a subset of potted plants of *Eucalyptus saligna* (n=17) were harvested to develop relationships between above and belowground biomass. These seedlings were grown in 25 l pots inside each WTC, while chamber conditions were maintained, until the experiment was started.

## Tree allometry surveys

Tree height was measured bi-weekly and diameters were recorded monthly at regular intervals (30 cm) along the main bole and split stems. Bole diameters at 65 cm height were used as the reference diameter. Diameter and length for every branch, including forked branches, were surveyed seven times between April 2008 and March 2009. Branch diameter measurements were recorded at 5 cm from their individual insertion points. Leaf litter was collected from the chambers bi-weekly, oven-dried and weighed.

## Bole carbon

During the final harvest, diameter measurements were recorded as described above and 1 cm sections were removed from the bole at regular intervals between diameter measurements. 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 diameter intervals from base to tip for each monthly survey. This approach assumed any bole taper was accounted for in the difference in volume between bole sections. The top section was calculated as a cone with a tip radius of .001 cm. The volume below the 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 stem sections with taper assumed as above. All bole volume units were then summed (including forked stems) to calculate total tree volume. Bole mass was calculated as total volume multiplied by WTC specific .

## Branch carbon

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

(1)

where is summed dry mass of all harvested branches, is total branch length (cm), is total branch basal area (cm3), 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. The ratio of measured to was used to generate a WTC-specific .

During each survey period, Mbr was estimated by solving the above equation with and for individual branches with specific to each WTC. As diameters were not recorded at branch insertion points, 5 cm were added back to each branch length in order to represent the entire branch volume. We assumed that did not change through time. Total dry branch mass at each survey point was the total mass of all individual branches.

## Leaf area and carbon

Final harvest total leaf area and dry mass were measured for each of the five canopy layers. Specific leaf area (SLA, cm2 g-1) was calculated by dividing cumulative tree leaf area by leaf mass for each canopy layer. Mean SLA for each chamber 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. 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 (Barton et al. 2010) 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. Specific leaf area was assumed to be constant over the final year of the experiment.

## Tissue C allocation

Tissue specific C allocation represents the fraction of net primary productivity (NPP) 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 + stems) 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 component and is the daily net aboveground C flux (g C d-1). For example, C allocation () to leaves was determined by combining measurements of harvested dry C mass of leaves () with and total cumulative litterfall () such that:

(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. As root turnover was not measured only total belowground C allocation (TBCA) could be calculated (explained below).

## 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 was calculated as:

(8)

where is the gross primary productivity (g C) of each tree aboveground minus respiration of leaves, stems and branches and is the aboveground standing crop C mass (g C) of stems, branches, leaves and cumulative 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 soil respiration, root turnover, root exudation and any unaccounted for root C mass. The use of aboveground allometry to interpolate through time combined with Fc allowed TBCA to be estimated on daily time steps over the final eleven months of the experiment while was calculated at the final harvest.

## Mass balance relationships between and carbon allocation.

The cumulative sum of , at any given time point, represented the net C uptake for each WTC. Daily allocation of C to boles and branches was estimated by linear interpolation between survey measurements and the final harvest, starting at the first branch survey (April 2008). Daily modeled 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 were then tracked from April 2008 to March 2009. The initial estimated C mass of each aboveground component and on the first day were subtracted from all respective daily values so mass balance could be tracked with a 0 starting value. This allowed daily estimates of TBCA to be generated across the final 11 months of the experiment. Additionally, the significant log-linear relationship between aboveground mass of both harvested trees and potted seedlings (R2 = 0.98) was used to predict on the last day from . Mass fractions of leaves, boles+branches and roots were then calculated by dividing their respective total C mass by whole tree C mass at the end of this time period. At the end of the eleven month period, correlations between , whole tree C mass and leaf area with were tested.

## Data analysis

Differences in experimental parameters to the interaction of Ca and drought treatments at the final harvest were analysed 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 to P = 0.05 and findings with 0.05 < P < 0.10 were considered marginally significant.

# Results

## Total aboveground carbon flux, leaf area and whole tree carbon

Over the final year of the experiment, was significantly reduced by 30.5 % under eCa (P = 0.043), while no effects of the drought treatment were detected (Table 1). Similarly, both whole tree C and from the final harvest were reduced under eCa by ca. 32 % (both P < 0.03). was positively correlated with both whole tree C (R2 = 0.74, Figure 1,a) and (R2 = 0.69, Figure 1,b).

Leaf area at the final harvest was significantly reduced by by 31.3 % under eCa (p < 0.001), and this pattern was observed across the final eleven months of the experiment (Figure 2). Overall, was positively correlated with mean daily leaf area (P < 0.001, Figure 3).

## Harverted tree carbon mass and biomass partitioning

At the end of the two year experiment, harvested C mass of tissue components were affected by eCa but not drought treaments (Table 1). Stem C mass was (boles+branches) was reduced 37 % under eCa (P = 0.0151), driven mostly by eCa effects on boles. Neither standing crop leaf C mass or cumultaive litterfall C mass were affected by Ca. Total root C mass was marginally reduced under eCa (P = 0.091).

Leaf mass fraction was increased by 24.2 % under eCa (P = 0.009) but was not affected by the drought treatment. Leaf mass fraction was negatively correlated with whole tree C (P= 0.004, Figure 4a). Stem mass fraction was reduced by 9 % under elevated CO2 (P = 0.018), with no effect of the drought treatment detected. Stem mass fraction was was positively correlated with whole tree C (P = 0.008, Figure 4c). Root mass fraction was not affected by either treatment and was not correlated to whole tree C (Figure 4e).

## Aboveground carbon allocation

Treatment effects on tissue C allocation were determined by using total mass values obtained from allometric equations over the final eleven months of the experiment with cumultaive C flux over the same time period. Total C allocation to leaves marginally increased under eCa (+28 %, P = 0.052), with no effect of the drought treatment detected. Leaf C allocation was was negatively correlated with adjusted (P = 0.031, Figure 4b). Alternatively, C allocation to aboveground wood was not affected by either treatment and was not correlated to whole tree C (Figure 4d).

## Belowground carbon allocation

Within each treatment combination the cumulative C mass of each tree component (boles, branches, leaves and roots) did not achieve mass balance with (Figure 5). Across a large range in tree size, similar patterns were detected in each individual WTC (Figure S1). It was therefore necessary to account for allocation to TBCA and . Neither TBCA nor were affected by Ca or drought treatments (Figure 6). TBCA and adjusted 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 constant through time and between treatments (Figure 7). TBCA was positively correlated with mean daily leaf area (R2 = 0.44, P = 0.019).

# Discussion

Utilizing the WTC experimental design we show that biomass partitioning and C allocation were differentially affected by eCa in a Eucalyptus species. We detected minimal effects of the drought treatment on total tree C flux, biomass partitioning or C allocation, despite previous findings of negative effects of drought on leaf and canopy physiology (see Duursma et al. 2011, Crous et al. 2012). Using a novel methodological framework, we show that TBCA was unchanged by either eCa or drought over the final eleven months of the experiment and remained constant across daily times steps. By combining mass balance approaches with novel measurements of whole tree C flux we highlight how impacts to C allocation of a component tissue may not always result in similar changes to tissue biomass production over longer time periods. The consistency of TBCA, at both daily and annual time scales, suggests that TBCA may not be as sensitive to the effects of climate changes as previously thought.

## Do biomass partitioning and C allocation respond the same to climate change

Here, we 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 total belowground pools, via mass balance. This approach allowed us to evaluate the impacts of climate change treatments on these two fundamentally different processes affecting overall tree growth. This is because there are many possible fates for C assimilates and only one of these is the production of plant biomass (**???**). Changes in C allocation encompass effects of tissue turnover, the storage and use of carbohydrates, root exudation to stimulate microbial activity, with each representing significant tree and ecosystem responses to environmental change. Thus, patterns in biomass partitioning and C allocation may not be consistent with respect to the tissue or ecosystem pool in question, which contributes to the current uncertainty in modelling tree growth responses to interacting climate change factors.

We found that SMF increased with total plant size and surprisingly 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). As a result, it is possible that observed patterns in SMF 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. We found that C allocation to stems was unaffected by eCa which infers 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 negatively affected other tree or ecosystem processes, unrelated to stem production, which first decreased overall tree size.

Contrary to expectation we found that both LMF and C allocation to leaves increased under eCa. 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. Leaf respiration during the day was increased under eCa in this study (Crous et al. 2012), similar to other studies (see Davey et al. 2004, Gonzalez-Meler et al. 2004, Leakey et al. 2009), and was attributed to higher energy demand from increased photosynthesis rates. This potential increase in C demand could account for observed increases in C allocation, however, respiration rates would need to increase relative to photosynthesis rates (as in Wang et al. 2001) for the entire canopy. In addition, concentrations of leaf non-structural carbohydrates (TNC) are known to increase in under eC~a (Roden and Ball 1996, Picon et al. 1997, Poorter et al. 1997, Loewe et al. 2000, Walter et al. 2005). Increased C allocation to leaves could result an increase in TNC, which could fulfil increased canopy respiratory demands or meet sink demands of other tissues. Contrary to stems, these results highlight how changes in tissue C allocation can respond to climate change factors without measureable effects on harvested biomass.

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

Despite increased attention of 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 under eC~a 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 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 over an eleven month period, both of which should improve representation of C allocation in models.

With high resolution flux data and reliable estimates of aboveground dry mass production we show that cumulative TBCA was not affected by eCa or drought across the final eleven months of the experiment. Contrary to expectation, we detected minimal effects of eCa or drought on root biomass partitioning, although we did not differentiate fine and coarse roots pools. Although our findings disagree with results from forested FACE experiments (see Palmroth et al. 2006), comparing a single tree ecosystem 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 necessarily infer that belowground processes were not affected by either treatment. In trees under drought stress, TBCA might increase with higher allocation to root systems to alleviate water stress (Poorter et al. 2012), which could by offset increased root mortality and turnover [marshall1986drought; meier2008belowground], reduced root exudation (Iversen and Norby 2014) or reduced C demand via decreases in root respiration rates [burton1998]. Alternatively, the lack of belowground competition for soil mineral resources in this single tree system might have delayed enhancement of TBCA to eCa treatments, such as enhanced root production and exudation.

With estimations of daily aboveground C accrual and measured cumulative whole tree C flux we were then able to uniquely track dynamic short term effects of eCa or drought on TBCA. Across daily time steps, we observed a relatively constant 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 observed aboveground processes gives us reliable estimates of the absolute amount of C distributed belowground each day, which appear to be insensitive to sustained eCa fumigation 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 residency time in any tissue or soil component. As a result, the consistency of TBCA across daily intervals along with lack of a cumulative response of TBCA raises questions about the regularity of belowground response to climate change 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.

## Summary

# List of Tables

**Table 1**. Final harvest C mass of above and belowground tissues and cumulative aboveground tree C flux. Each value represents the mean (± 1 standard error) for each treatment combination and units for all values are g C. For each component, different letters represent significant differences between treatments with the overall model which includes Ca \* Drought interactions. Each P value represent overall differences within individual components of the main treatment effects of the Ca or Drought and treatment interactions of eCa and Drought.

# List of Figures

**Figure 1**. Treatment means of harvested whole tree carbon mass (a) and aboveground carbon mass (b) as a function of cumulative aboveground C flux over the final year of the experiment. The dotted line is the 1:1 relationship and the solid lines represent the significant linear model fit for whole tree C (R2 = 0.86) and aboveground C mass (R^2 = 0.78).

**Figure 2**. 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 leaf area estimates at two dates. Color and and line type distinguish the treatment combination for each individual chamber.

**Figure 3**. Treatment means of cumulative aboveground C flux as a function on mean daily leaf area over the final eleven months of the experiment. The solid line represents the significant linear model fit (R2 = 0.77).

**Figure 4**. Treatment means of C mass fractions of leaves (a), stems (boles + branches) (c) and roots (e) as a function of tree size, via total tree C mass. Treatment means of total C allocation to leaves (b) and stems (d) as a function of total aboveground net C flux. Root C allocation could not be estimated as root turnover was not known. Values for mass fractions and C allocation are estimated over the final eleven months of the experiment and total aboveground net C flux is the cumulative total over the same time period. Solid lines represent overall model fit for leaf, stem and root mass fractions (R2 = -0.57, 0.52 and 0.02, respectively), as well as leaf and stem C allocation (R2 = -0.39, 0.01, respectively).

**Figure 5**. Cumulative aboveground C flux and additive C allocation to individual tree components from 2008-4-15 to 2009-3-16. Each panel represents mean values for each treatment combination (n=3). Both 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.

**Figure 6**. Treatment means ± 1 standard error of total aboveground net C flux, total belowground c allocation, and the residual belowground C flux at the final harvest.

**Figure 7**. Total belowground c allocation as a function of cumulative aboveground C flux across the final eleven months of the experiment. Carbon allocation aboveground was estimated from allometric surveys, interpolated on a daily time scale and then subtracted from the cumulative aboveground C flux to quantify TBCA. Individual colored lines represent treatment means and the dotted black line is the 1:1 relationship.

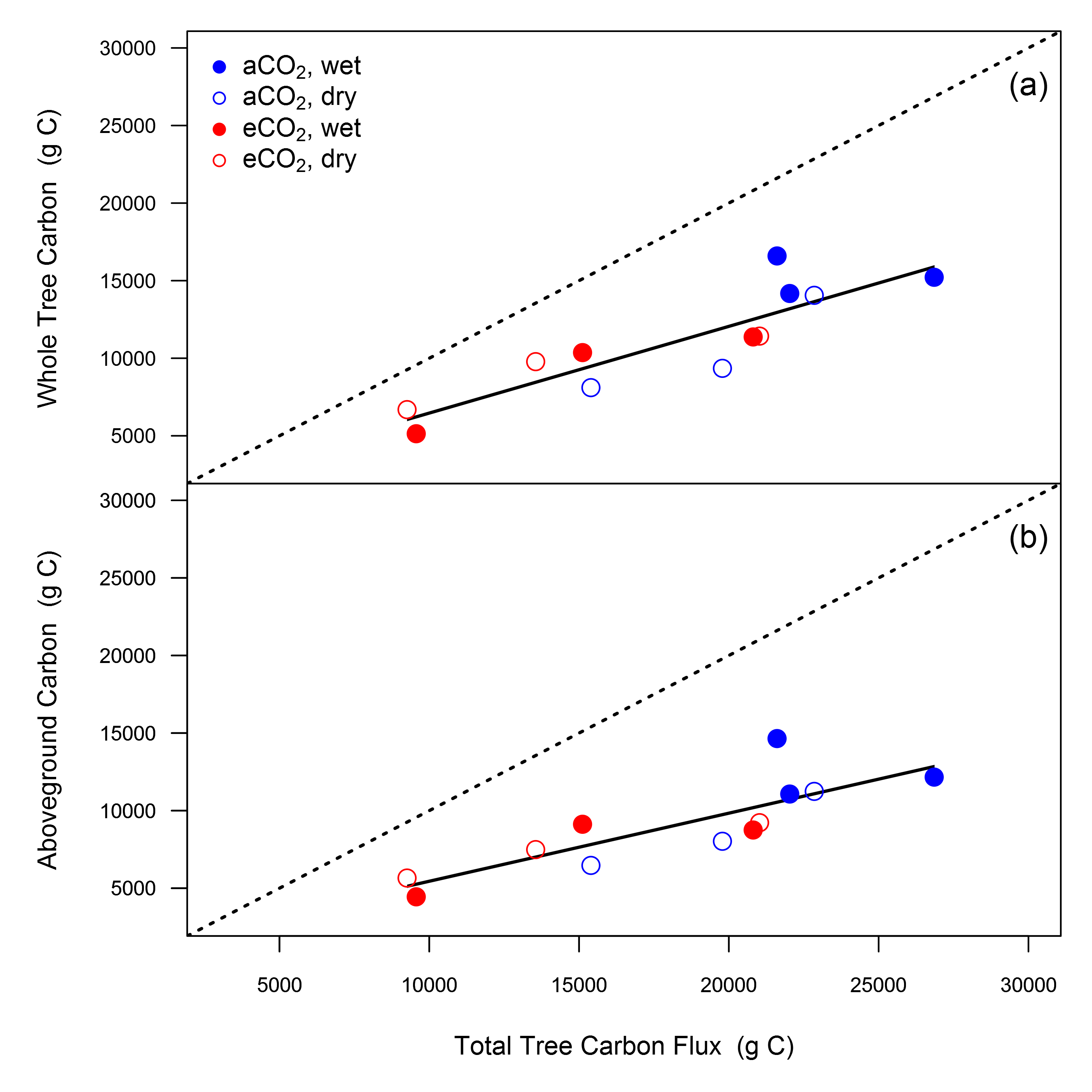
**Figure S1**. Cumulative aboveground C flux and additive C allocation of individual tree components from 2008-4-15 and 2009-3-16. Panels represent each individual WTC. Both C flux and tissue C allocation where set to 0 on 2008-4-15 in order to track the allocation of C in daily time steps. Total root 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.

# Tables

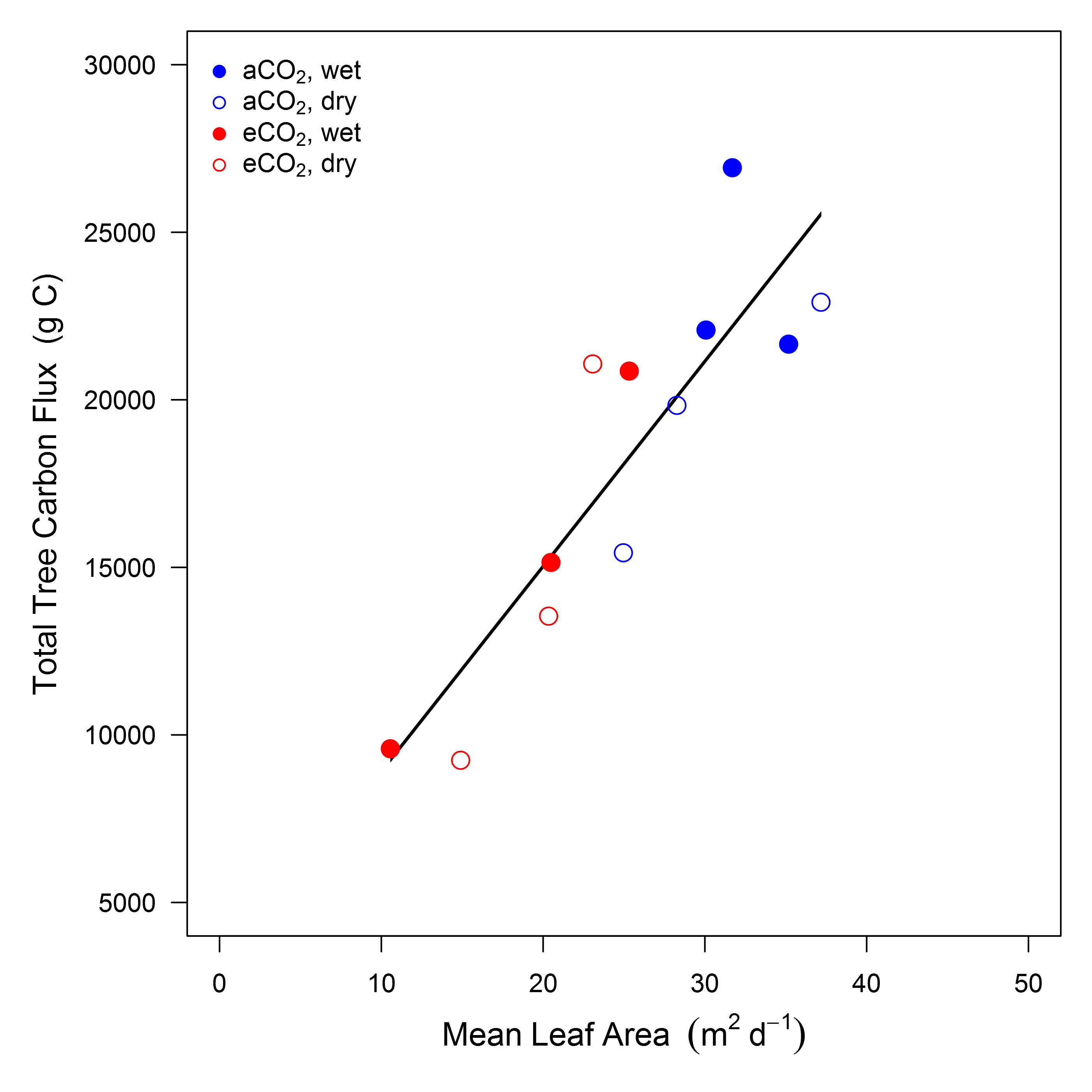
**Table 1**.

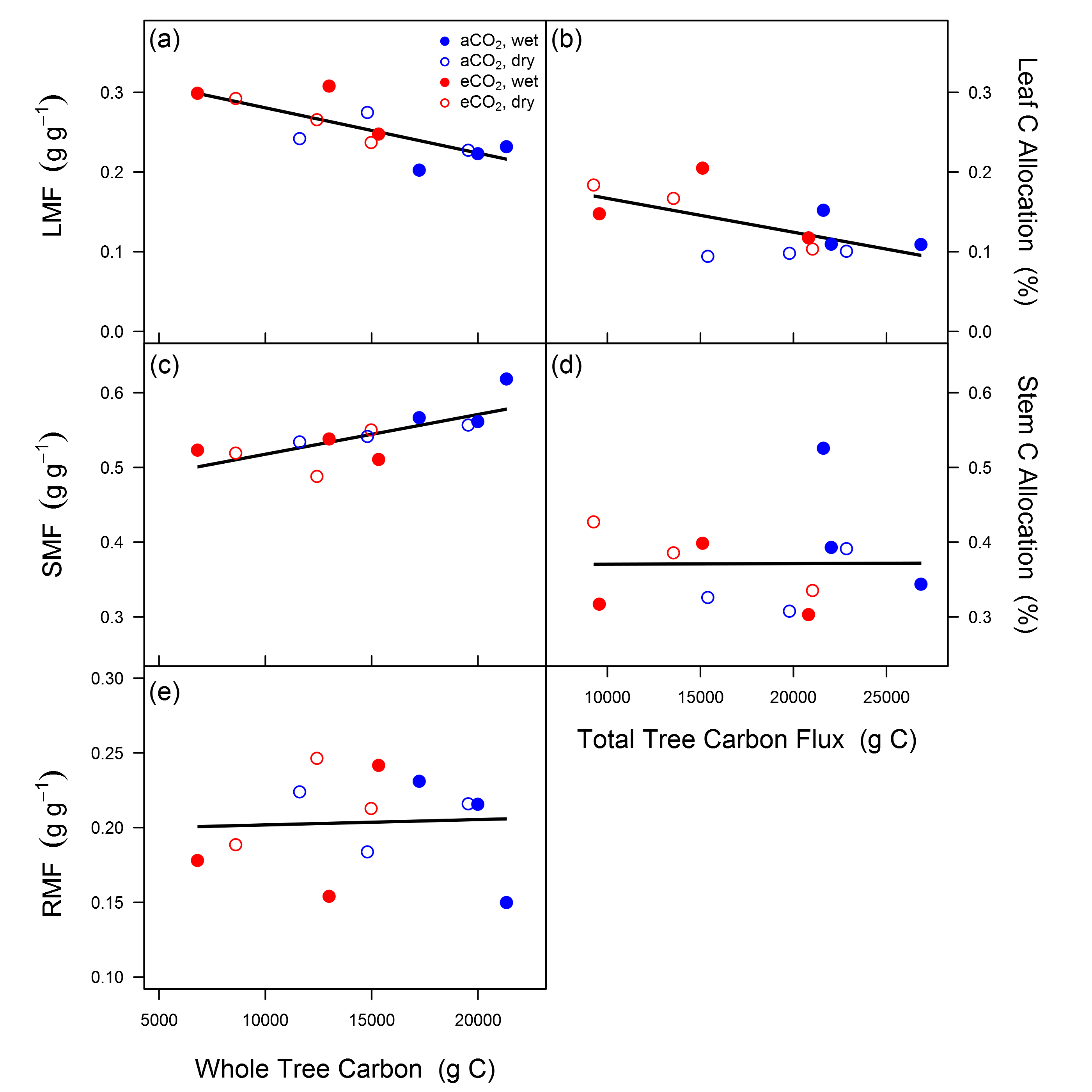
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Bole** | **Branch** | **Leaf** | **Litter** | **Root** | **Fc,T** |
| aCO2-dry | 5449.8 (715.6) b | 2915.9 (654.4) a | 2642.8 (370.7) a | 1129.8 (336.0) a | 3180.1 (521.0) a | 19394.2 (2169.5) a |
| aCO2-wet | 8109.4 (278.2) ab | 3286.0 (715.7) a | 3254.2 (393.5) a | 1043.1 (47.3) a | 3830.6 (330.1) a | 23556.5 (1689.0) a |
| eCO2-dry | 4250.6 (710.9) a | 2006.3 (384.8) a | 2232.1 (235.4) a | 889.4 (82.6) a | 2623.6 (501.7) a | 14620.7 (3456.2) a |
| eCO2-wet | 4194.1 (816.0) a | 1934.2 (494.3) a | 2358.3 (473.6) a | 919.0 (244.3) a | 2306.1 (735.2) a | 15197.9 (3253.5) a |
| CO2 effect (P) | 0.005 | 0.086 | 0.122 | 0.417 | 0.091 | 0.044 |
| Drought effect (P) | 0.085 | 0.803 | 0.358 | 0.897 | 0.766 | 0.413 |
| CO2 \* Drought (P) | 0.075 | 0.712 | 0.539 | 0.792 | 0.397 | 0.532 |

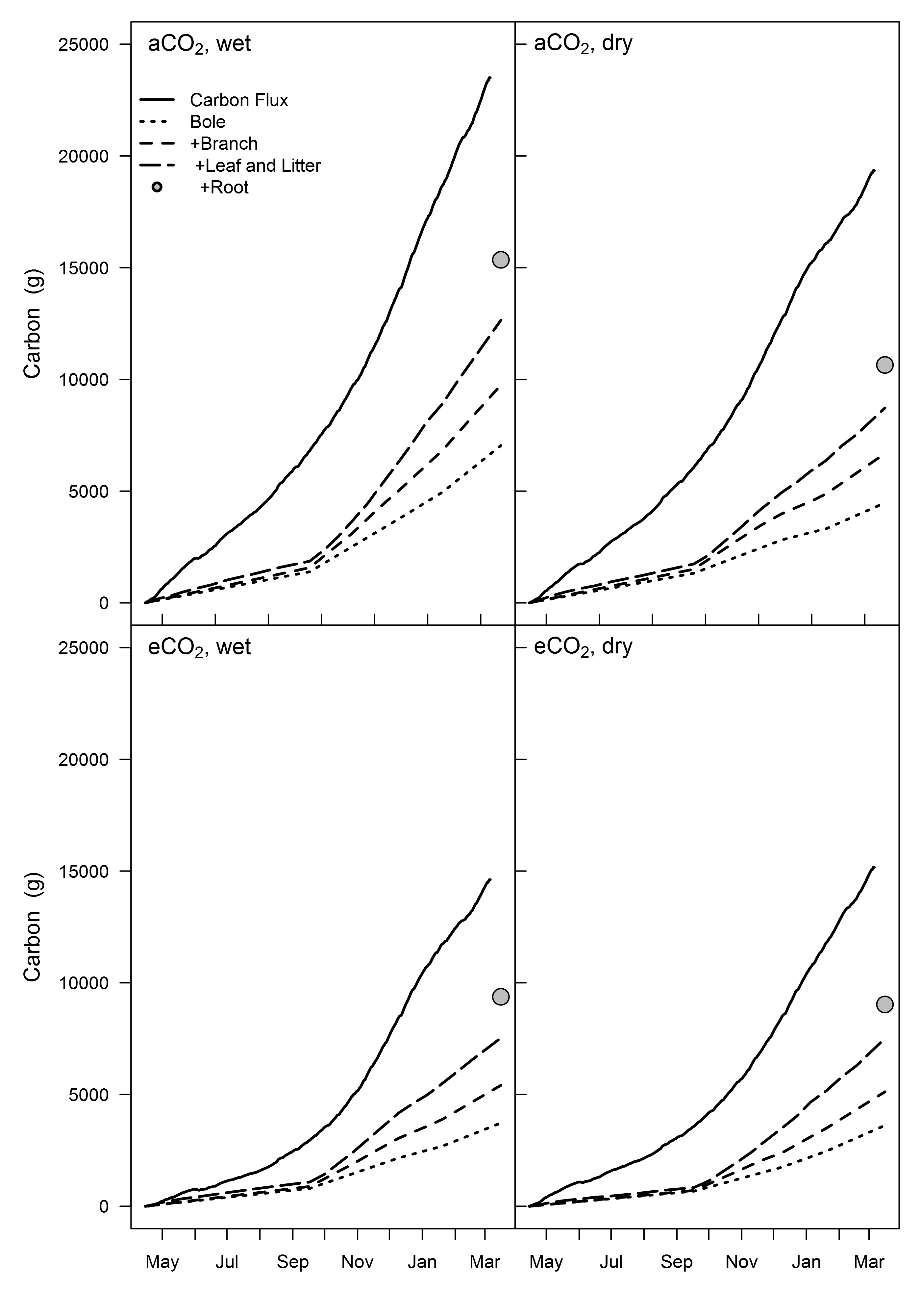
# Figures

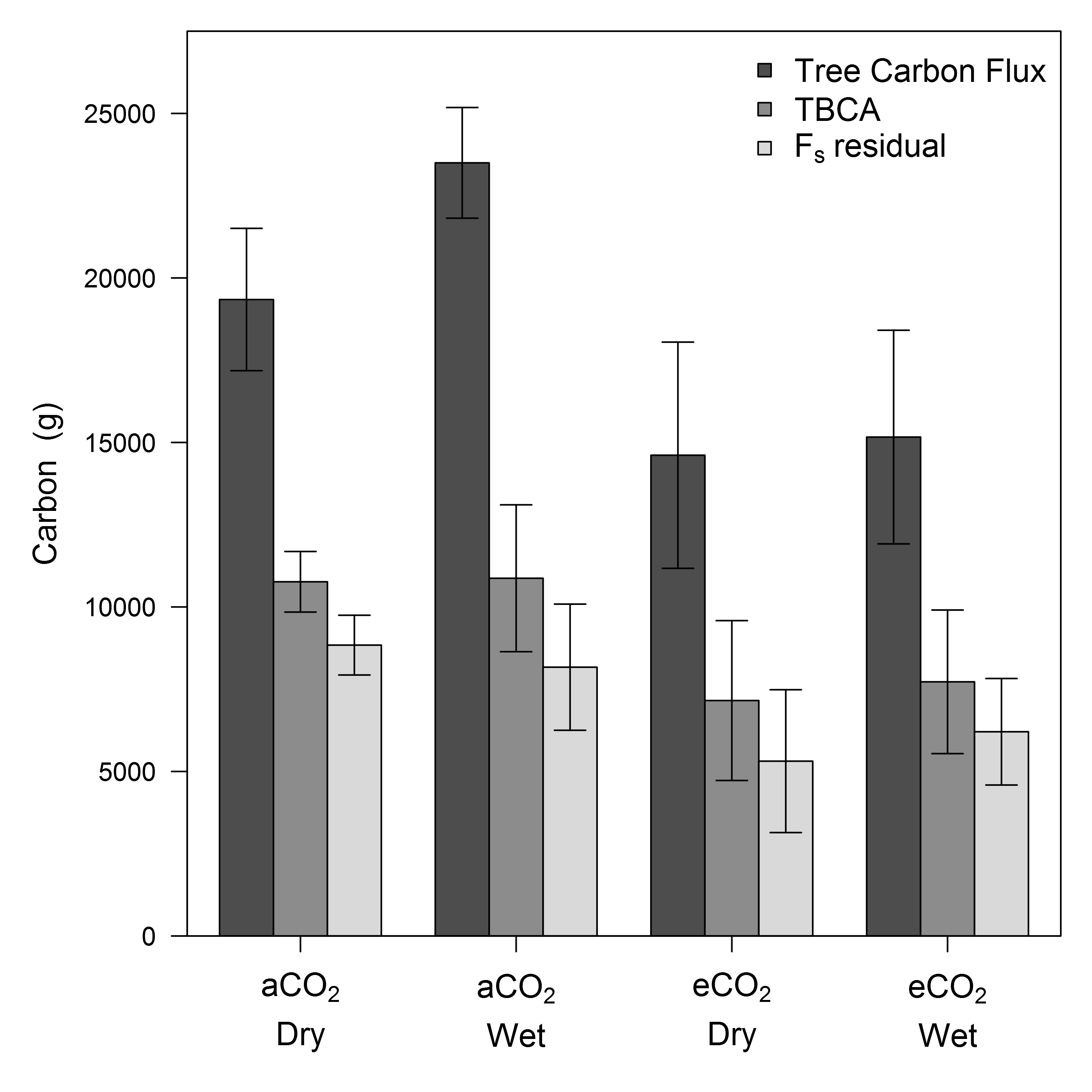
  
**Figure 1**.

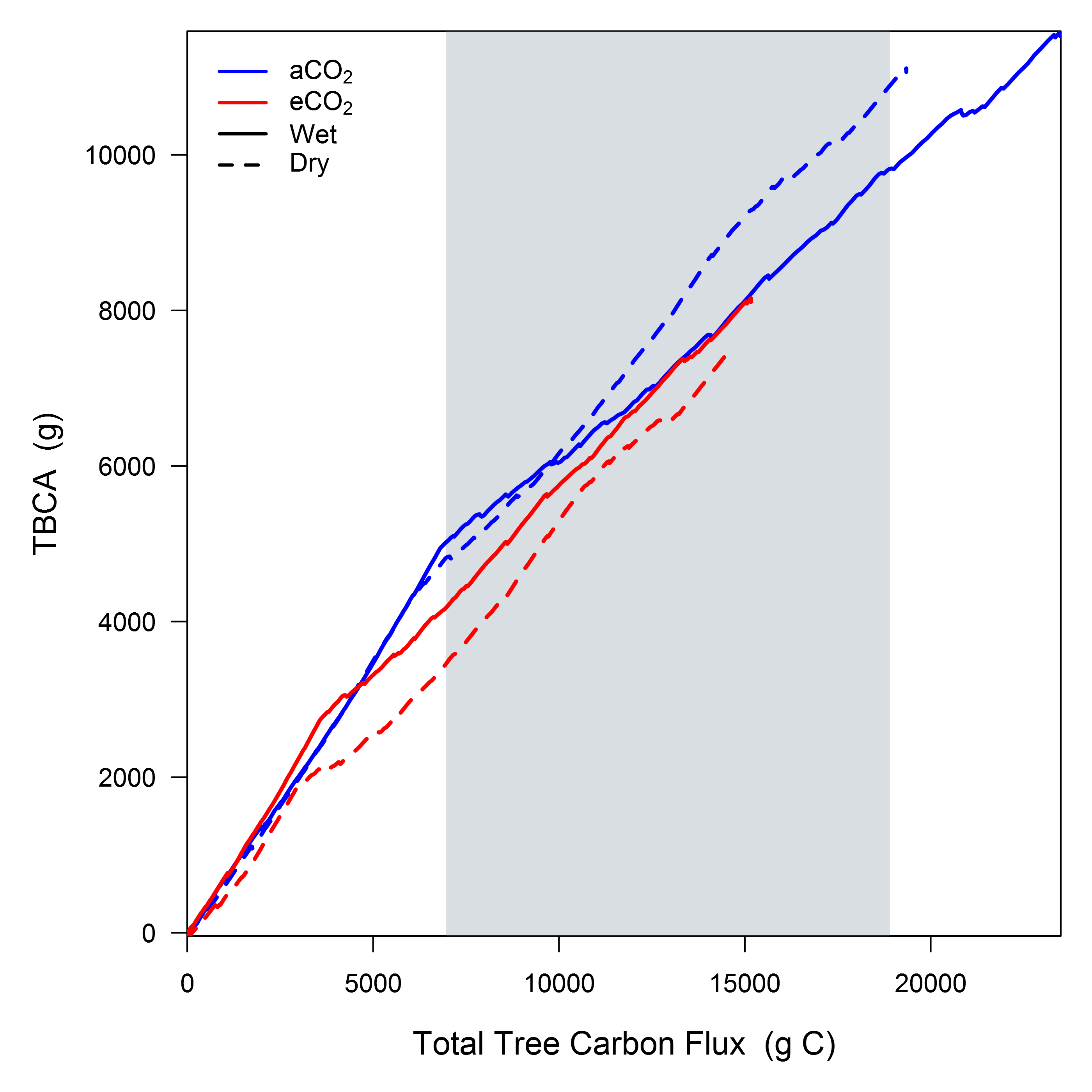
  
**Figure 2**.

  
**Figure 3**.

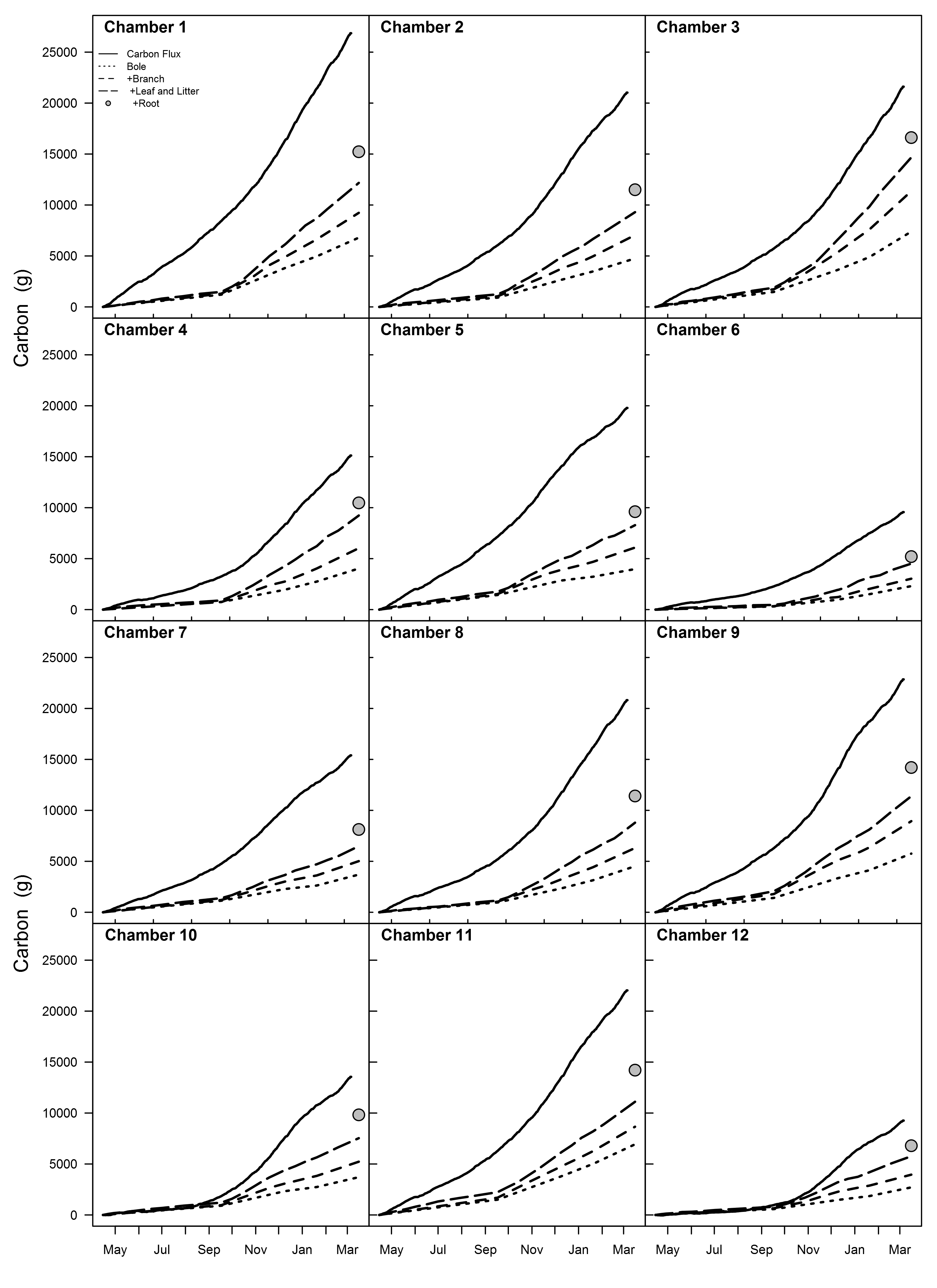
  
**Figure 4**.

  
**Figure 5**.

  
**Figure 6**.

  
**Figure 7**.

# Supporting Information

  
**Figure S1**.

# References

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

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

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 CO 2 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.

Cheng W, Fu S, Susfalk RB, Mitchell RJ (2005) Measuring tree root respiration using 13C natural abundance: rooting medium matters. 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.

Davey PA, Hunt S, Hymus GJ, DeLucia EH, Drake BG, Karnosky DF, Long SP (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO2], but is increased with long-term growth in the field at elevated [CO2]. Plant Physiology 134:520–527.

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.

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 foresti{è}res. 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]times drought interaction in Eucalyptus saligna. Tree physiology:tpr030.

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, Koerner 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:tpr138.

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.

Gonzalez-Meler MA, Taneva L, Trueman RJ (2004) Plant Respiration and Elevated Atmospheric CO2 Concentration: Cellular Responses and Global Significance. Annals of Botany 94:647–656. <http://aob.oxfordjournals.org/content/94/5/647.abstract>

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.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60:2859–2876. <http://jxb.oxfordjournals.org/content/60/10/2859.abstract>

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.

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.

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) Concentration and 13C of leaf carbohydrates in relation to gas exchange in Quercus robur under elevated CO2 and drought. Journal of Experimental Botany 48:1547–1556. <http://jxb.oxfordjournals.org/content/48/8/1547.abstract>

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

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. <http://dx.doi.org/10.1046/j.1365-3040.1997.d01-84.x>

R Development Core Team R (2011) R: A Language and Environment for Statistical Computing Team RDC (ed). R foundation for statistical computing 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. <http://www.plantphysiol.org/content/111/3/909.abstract>

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.

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. <http://dx.doi.org/10.1111/j.1365-2486.2005.00990.x>

Wang X, Lewis JD, Tissue DT, Seemann JR, Griffin KL (2001) Effects of elevated atmospheric CO2 concentration on leaf dark respiration of Xanthium strumarium in light and in darkness. Proceedings of the National Academy of Sciences 98:2479–2484. <http://www.pnas.org/content/98/5/2479.abstract>

Warren JM, Iversen CM, Garten CT, Norby RJ, Childs J, Brice D, Evans RM, Gu L, Thornton P, Weston DJ (2012) Timing and magnitude of C partitioning through a young loblolly pine (Pinus taeda L.) stand using 13C labeling and shade treatments. Tree physiology 32:799–813.