Elevated atmospheric CO2 alters carbon allocation above but not belowground in *Eucalyptus saligna*

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# 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. We then 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. We calculated total belowground C allocation (TBCA) for each WTC, which includes all belowground processes, 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 biomass partitioning to roots, as well as TBCA. Cumulative aboveground 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 and cumulative TBCA were unaffected by either elevated CO2 or drought. As a fraction of total aboveground net C flux, TBCA remained relatively constant 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. Overall, these results reveal how climate change factors impact the investment of photosynthetic C in a *Eucalyptus* tree species and provide an empirical framework to improve model representations of C allocation in trees.

## 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 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.

The 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). Across four forested free-air Ca enrichment experiments the total flux of C belowground (TBCA), which includes all belowground processes, was found to be enhanced under elevated Ca (Palmroth et al. 2006). In forest ecosystems this enhancement can be 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 plants grown under elevated Ca.

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 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 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, 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 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). 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.

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 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.

# Methods

## Terminology

*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 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 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 elevated Ca 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 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).

## Aboveground chamber CO2 flux

Floors installed above the soil surface, enclosing the main bole, permitted the chambers to function as cuvettes and allowed for 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). 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 root 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 (see 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). 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 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. 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.

## Bole carbon mass

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 mass

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 (Mäkelä 1997). 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. 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 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 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. This was method was applied by Barton et al. (2012), but we briefly repeat it here for the sake of completeness. 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 + 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 component and is the daily net aboveground C flux (g C d-1). From equation 5, we estimated allocation by rearranging (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 cumulative 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.

## 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 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 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 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 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 daily estimates of TBCA to be generated across the final 11 months of the experiment. Additionally, the significant log-linear relationship between above and belowgground mass of both harvested trees and potted seedlings (R2 = 0.98, Figure S1) was used to predict on the last day from .

## 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, whole tree C mass and leaf area

Both whole tree C and from the final harvest were reduced under eCa by 32 % (both P < 0.03). From April 2008 to March 2009, was significantly reduced by 30.5 % under eCa (P = 0.043), while no effects of the drought treatment were detected (Table 1). was positively correlated with estimates of both whole tree C (R2 = 0.74, Figure 1a) and (R2 = 0.69, Figure 1b) over the same time period. Whole tree C mass estimated over the final eleven months of the experiment represented ca. 75 % of total harvested tree C mass.

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 leaf area (P < 0.001, Figure 3).

## Harvested tissue carbon mass and biomass partitioning

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

Leaf mass fraction (LMF) increased by 15.0 % under eCa (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 4a). Stem mass fraction (SMF) was marginally reduced by 5.8 % under eCa (P = 0.083), with no effect of the drought treatment detected. Stem mass fraction had a weak positive correlation with whole tree C mass (P = 0.09, Figure 4c). Root mass fraction (RMF) was not affected by either treatment and was not correlated to whole tree C mass (Figure 4e).

## 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% under eCa (P = 0.052), with no effect of the drought treatment detected. Leaf C allocation was negatively correlated with (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

Across all treatment combinations, the total C mass of boles, branches, leaves and roots was on average 61.0±0.02 % of (Figure 5). Additionally, was on average 41.2±0.004 % of on any given day. Across a large range in tree size, similar patterns were detected in each individual WTC (Figure S2). As mass balance must be achieved, TBCA and were estimated from Figure 5 as residuals between and whole tree mass excluding and including roots, respectively. Neither cumulative TBCA nor were affected by Ca or drought treatments (Figure 6). 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 constant through time and between treatments (Figure 7).

# Discussion

A whole-tree chamber experiment provided a unique opportunity to study the carbon balance of *Eucalyptus* trees. We found that biomass partitioning and C allocation of component tissues were differentially affected by elevated [CO2]. Minimal effects of a four month drought were detected on total tree C flux, biomass partitioning and tissue 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 and remained constant across daily times steps. 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 assumed.

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

As expected, tree C uptake and growth were coordinated across this two year experiment. Cumulative tree C flux was positively correlated to both canopy leaf area and total biomass produced. The net C uptake of plants should be a function of the canopy leaf area because leaf area index determines canopy light interception (Wilson 1965, Monsi and Saeki 2005) and is correlated to canopy assimilation and tree productivity (Waring 1983, McCarthy et al. 2006, Lindroth et al. 2008). Determining tree canopy C flux, however, is usually inhibited 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). 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. We found that leaf area was consistently reduced under eCa, likely leading to reductions in both tree C flux and whole tree C mass of near identical magnitudes (ca. 30 %).

As empirical measurements of whole tree C flux are rare, relationships with biomass or C allocation patterns are difficult to infer. Estimation of NPP is inextricably linked with estimation of the biomass production and turnover (Valentine 1999), yet 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 photosynthesis 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 whole tree C flux was strongly correlated to tree biomass production across a 2.5 fold size range in *Eucalyptus* trees.

## 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 total belowground pools, via mass balance. This approach allowed us to evaluate the impacts of climate change treatments on two fundamentally different processes affecting overall tree growth. This is because there are many possible fates for C assimilates beyond 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 mass fractions (SMF) increased with total plant size and was marginally 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 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 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 (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) often increase in under eCa (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 may have resulted in increased leaf TNC 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 actual C allocation when evaluating tree growth responses to climate change.

## 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 under eCa 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 can be used to test and constrain models of allocation.

With high resolution flux data and reliable estimates of aboveground dry mass production we show that TBCA was not affected by eCa or drought over the final eleven months of the experiment. Contrary to expectation, we detected minimal effects of eCa or drought on root biomass partitioning, although it was not possible to differentiate fine and coarse roots pools. Although these findings disagree with results from forested FACE experiments (see Palmroth et al. 2006), comparisons between 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 (Marshall 1986, Meier and Leuschner 2008), reduced root exudation (Iversen and Norby 2014) or reduced C demand via decreases in root respiration rates (Burton et al. 1998). 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 estimations of daily aboveground C mass accrual and measured cumulative whole tree C flux we were 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 measured aboveground processes gives us reliable estimates of the absolute amount of C distributed belowground each day, which appear 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 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.

## Conclusions

Here we use novel aspects of the WTC experimental design 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 disparate responses of above and belowground C allocation to eCa, which has important implications for how C allocation should be represented in applied 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 of questions of the fate of C under global climate change.

# 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. For each variable, P values represent overall differences of Ca or drought main effects and the Ca \* drought interaction.

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**Figure 1**. Whole tree C mass as a function of cumulative aboveground C flux, for each WTC, over the final eleven months of the experiment. The dotted line is the 1:1 relationship and the solid lines represent the significant linear model fit from the equation y = 0.56x + 878.2 (R2 = 0.86).

**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 following Barton et al. (2012). Color 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) from the equation: y = 611.9x + 2791.2.

**Figure 4**. Treatment means of C mass fractions of leaves (a), stems (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 C mass fractions are measured from final harvest biomass totals. Values for C allocation are estimated over the final eleven months of the experiment with cumulative total aboveground net C flux over the same time period. Solid lines represent overall 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).

**Figure 5**. 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 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. Total root C mass, predicted from the logarithimic 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, TBCA, and the residual belowground C flux () at the end of the experiment.

**Figure 7**. 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 combinations.

**Figure 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 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.

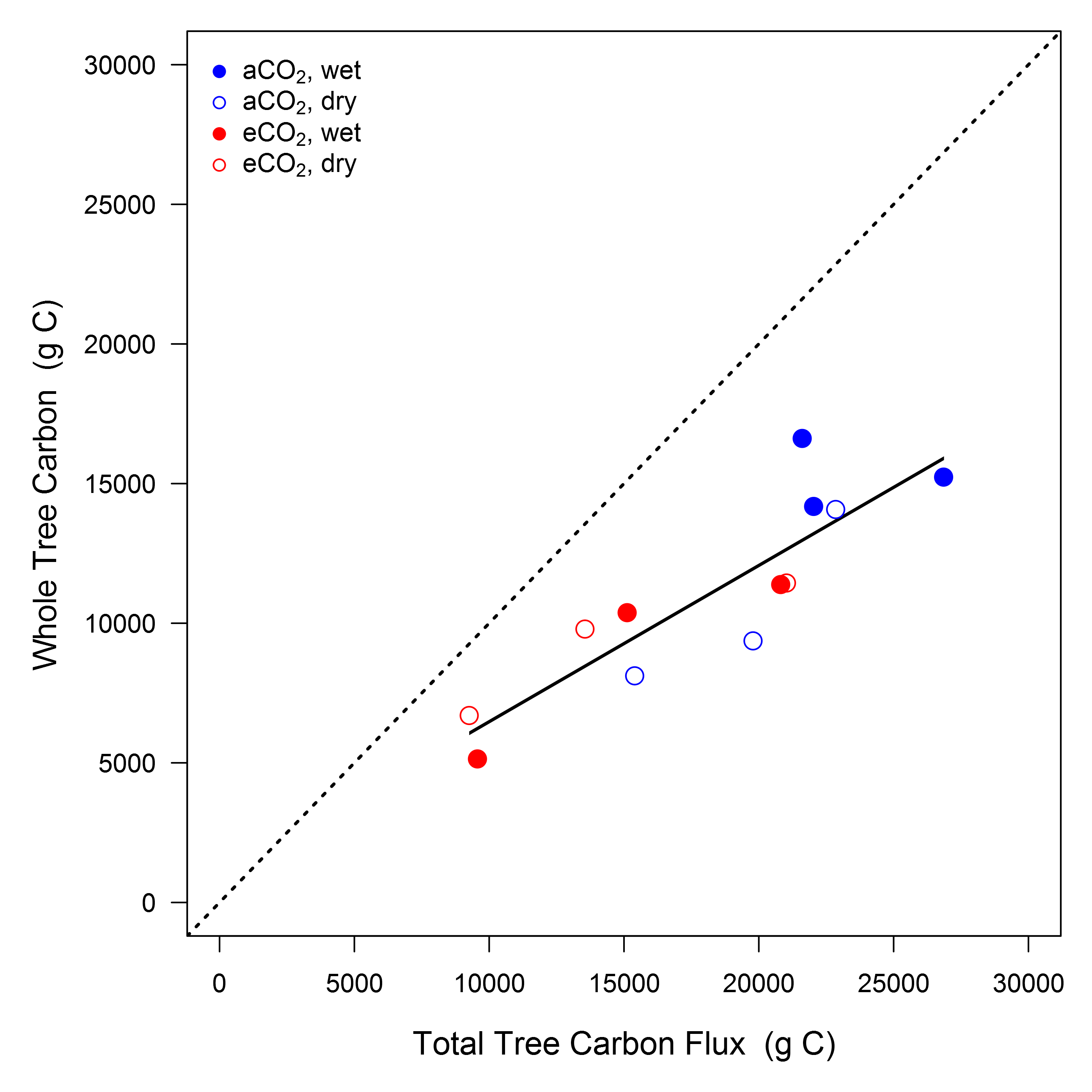
**Figure S2**. Cumulative aboveground net C flux and additive C allocation of individual tree components from 2008-4-15 and 2009-3-16. Panels represent each individual WTC. Both C flux and tissue C allocation where set to 0 on 2008-4-15 in order to track the allocation of C in daily time steps. Total 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

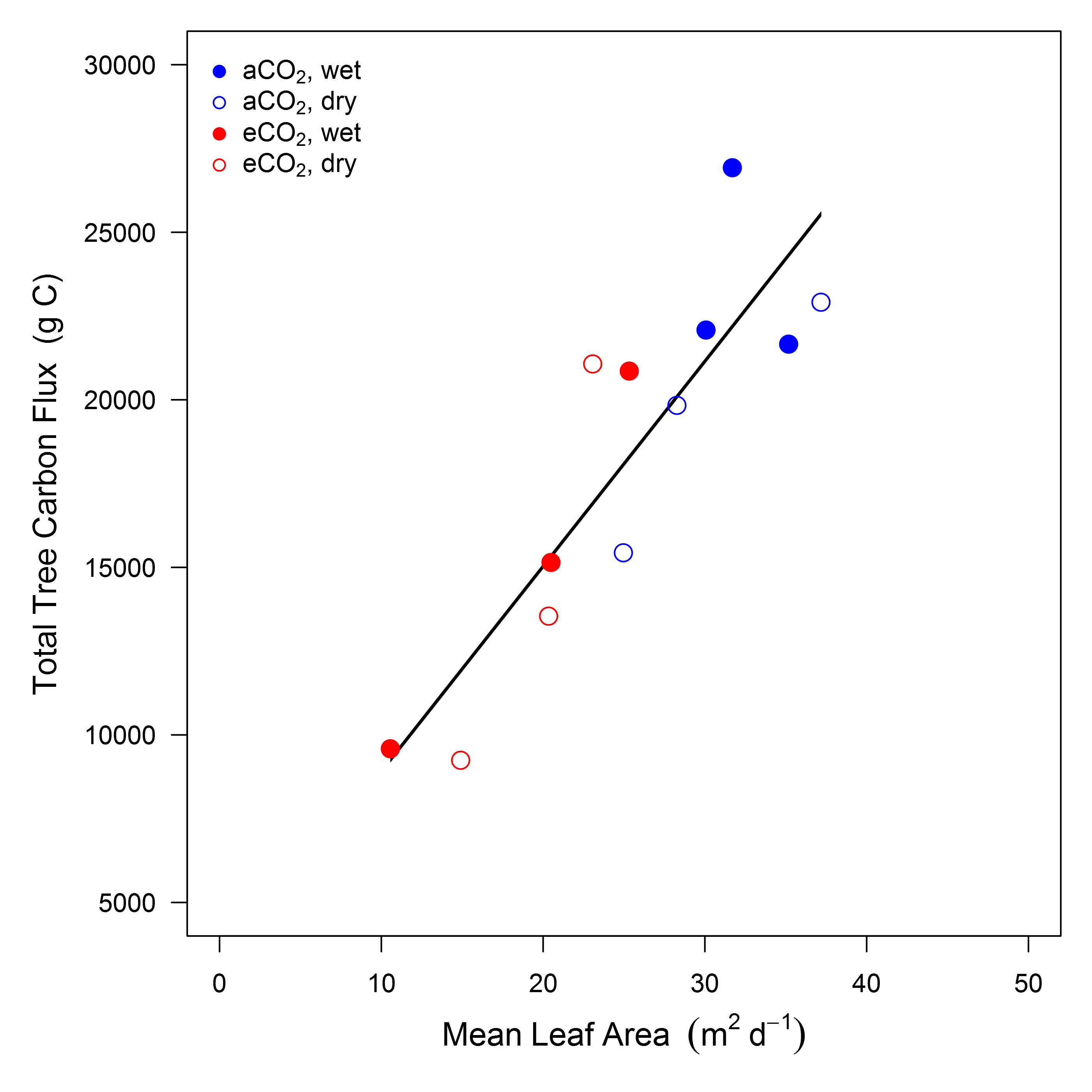
**Table 1**. Final harvest C mass of above and belowground tissues and cumulative aboveground tree C flux. Each value represents the mean (± 1 standard error) for each treatment combination and units for all values are g C. For each component, different letters represent significant differences between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

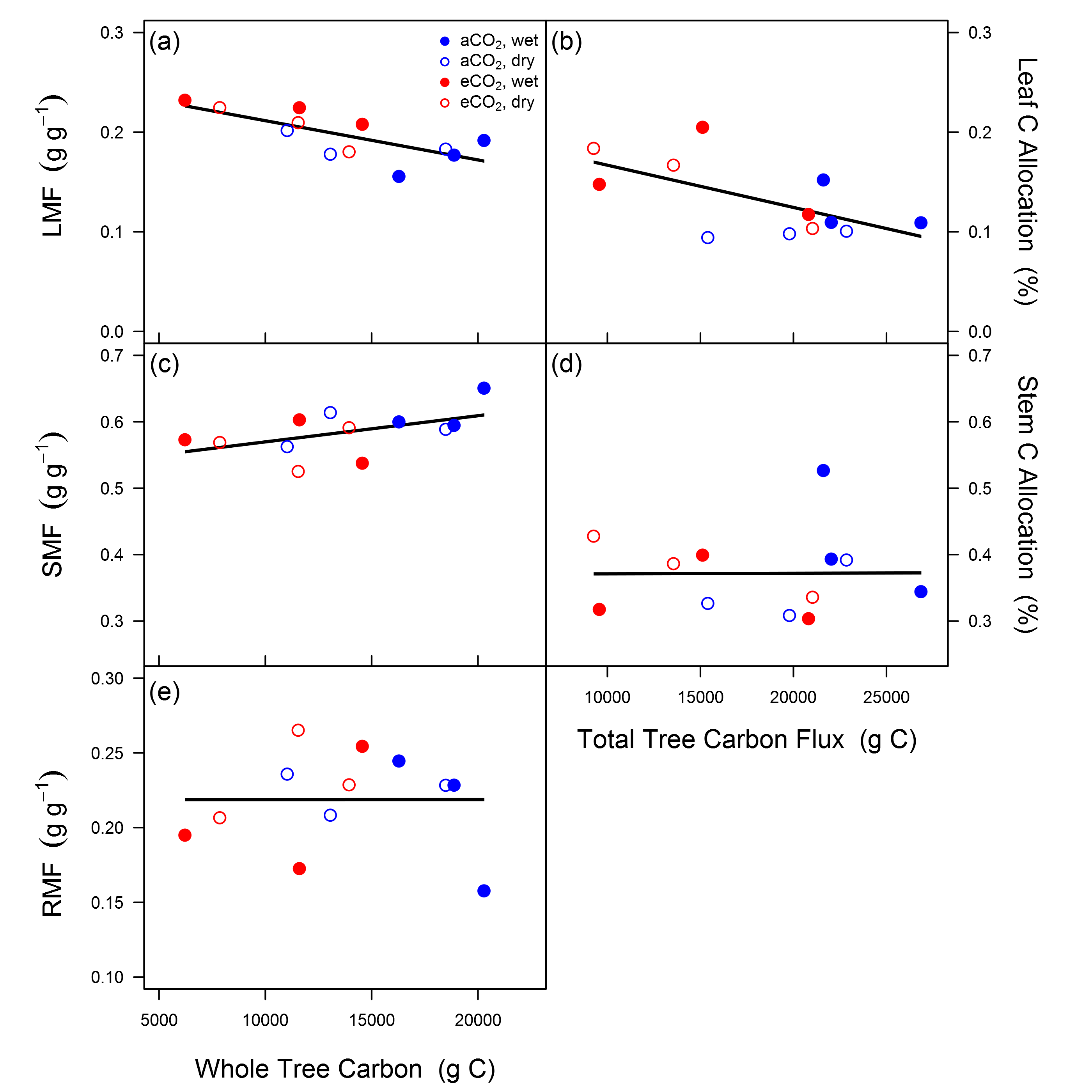
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Bole** | **Branch** | **Leaf** | **Litter** | **Root** | **Fc,T** |
| aCO2-dry | 5449.8 (715.6) b | 2915.9 (654.4) a | 2642.8 (370.7) a | 1129.8 (336.0) a | 3180.1 (521.0) a | 19394.2 (2169.5) a |
| aCO2-wet | 8109.4 (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 |

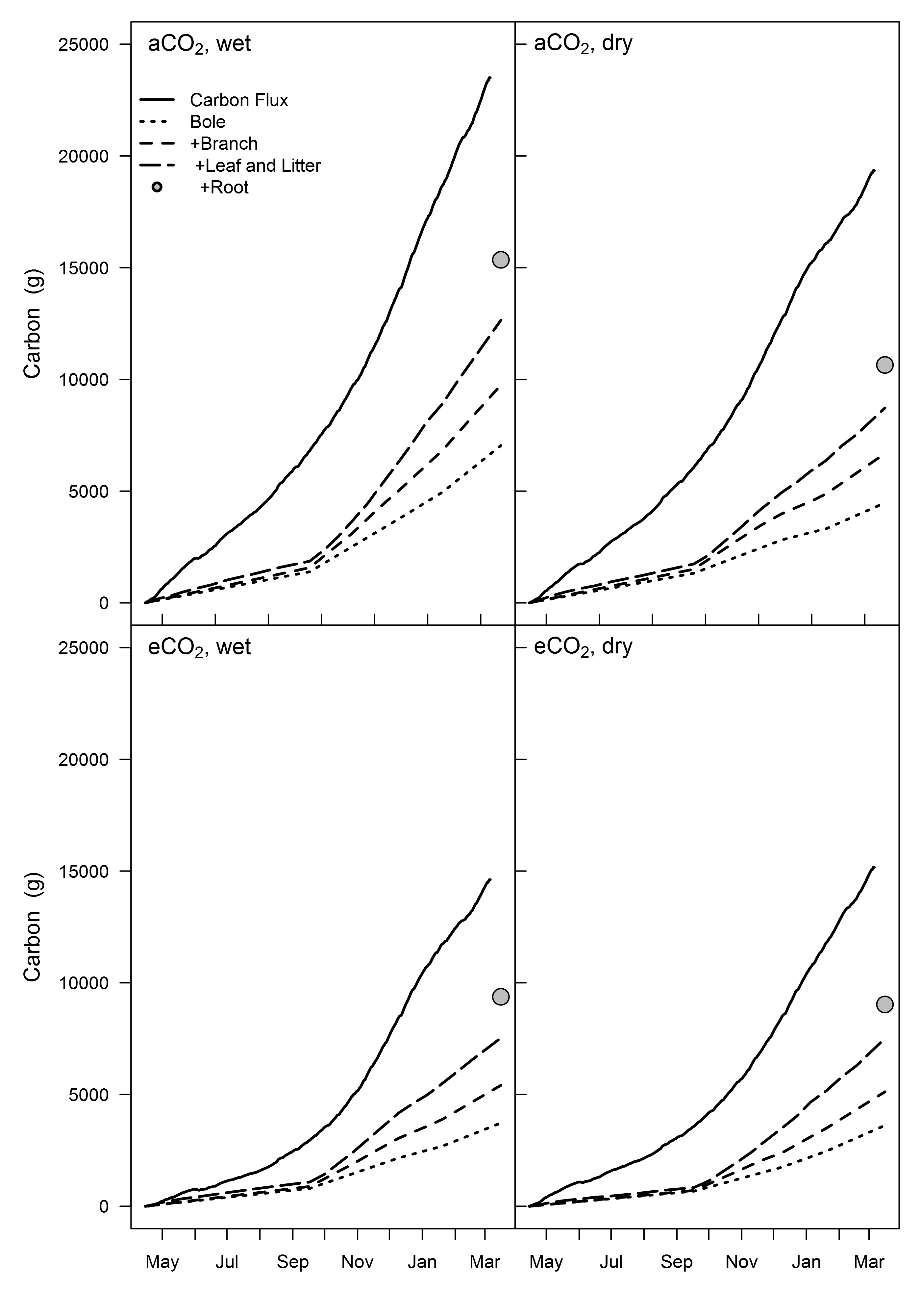
# Figures

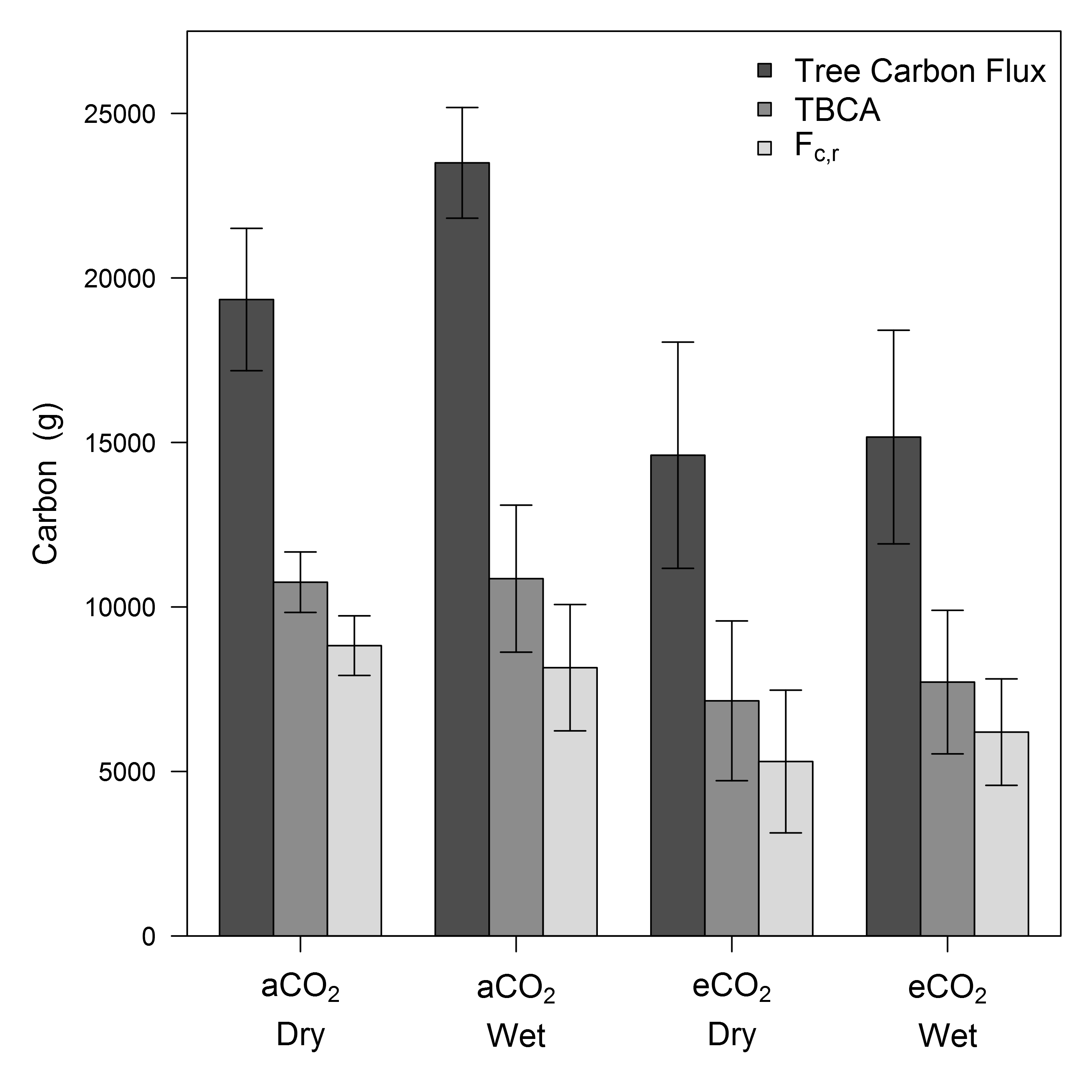
  
**Figure 1**.

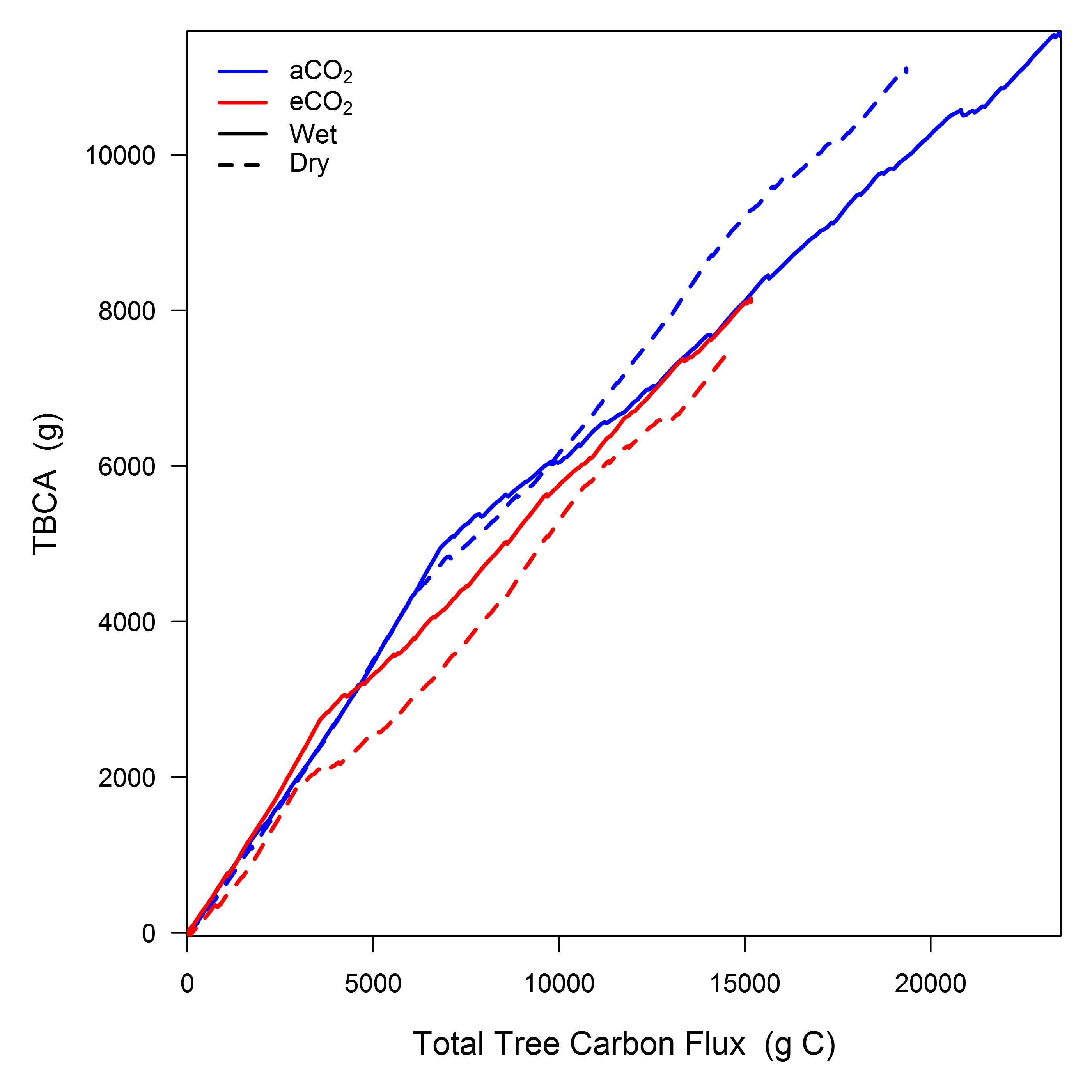
  
**Figure 2**.

  
**Figure 3**.

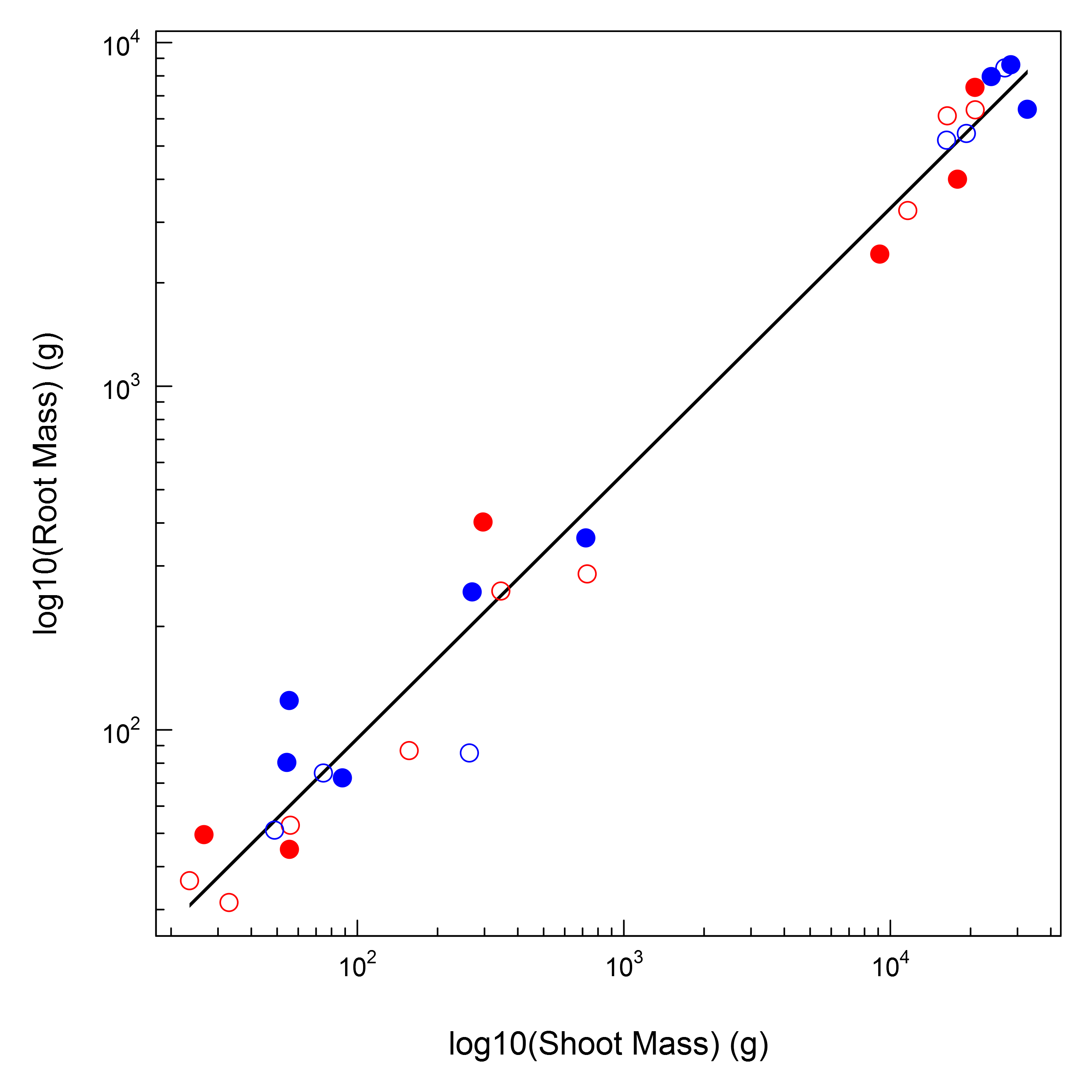
  
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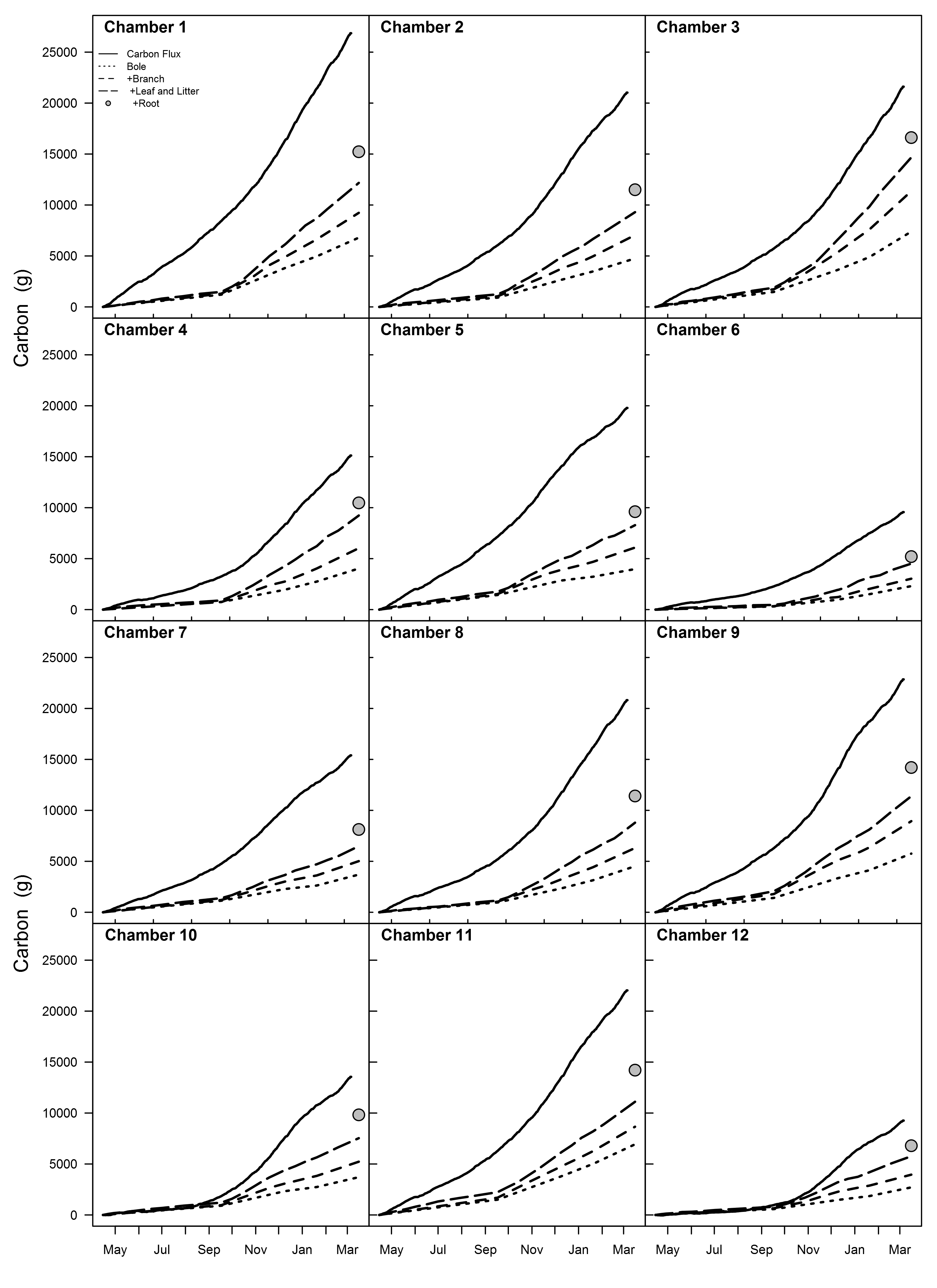
  
**Figure 5**.

  
**Figure 6**.

  
**Figure 7**.

# Supporting Information

  
**Figure S1**.

  
**Figure S2**.

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