The response of coarse root biomass to long-term CO2
enrichment and nitrogen application in a maturing Pinus taeda
stand with a large broadleaved component

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9 **The response of coarse root biomass to long-term CO₂ enrichment and nitrogen application in a**

10 **maturing *Pinus taeda* stand with a large broadleaved component**

11 Running title: Coarse root biomass under elevated CO₂

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1   **Abstract**

2   Elevated atmospheric CO_2 (eCO_2) typically increases aboveground growth in both growth chamber and free-air

3   carbon enrichment (FACE) studies. Here we report on the impacts of eCO_2 and nitrogen amendment on coarse

4   root biomass and net primary productivity (NPP) at the Duke FACE study, where half of the eight plots in a

5   30-year-old loblolly pine (*Pinus taeda*, L.) plantation, including competing naturally regenerated broadleaved

6   species, were subjected to eCO_2 (ambient, aCO_2 plus 200 ppm) for 15-17 years, combined with annual nitrogen

7   amendments (11.2 g N m^{-2}) for 6 years. Allometric equations were developed following harvest to estimate

8   coarse root (> 2 mm diameter) biomass. Pine root biomass under eCO_2 increased 32%, 1.80 kg m^{-2} above the

9   5.66 kg m^{-2} observed in aCO_2, largely accumulating in the top 30 cm of soil. In contrast, eCO_2 increased

10   broadleaved root biomass more than two-fold (aCO_2: 0.81, eCO_2: 2.07 kg m^{-2}), primarily accumulating in the

11   30-60 cm soil depth. Combined, pine and broadleaved root biomass increased 3.08 kg m^{-2} over aCO_2 of 6.46 kg

12   m^{-2}, a 48% increase. Elevated CO_2 did not increase pine root:shoot ratio (average 0.24) but increased the ratio

13   from 0.57 to 1.12 in broadleaved species. Averaged over the study (1997-2010), eCO_2 increased pine,

14   broadleaved, and total coarse root NPP by 49, 373, and 86%, respectively. Nitrogen amendment had smaller

15   effects on any component, singly or interacting with eCO_2. A sustained increase in root NPP under eCO_2 over

16   the study period indicates that soil nutrients were sufficient to maintain root growth response to eCO_2. These

17   responses must be considered in computing coarse root carbon sequestration of the extensive southern pine and

18   similar forests, and in modelling the responses of coarse root biomass of pine-broadleaved forests to CO_2

19   concentration over a range of soil N availability.

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Introduction

The long-term trajectory of forest net primary productivity (NPP) under increasing atmospheric $[CO_2]$ is variable, ranging from strongly positive to no response (Nowak et al. 2004). How forests respond to elevated CO_2 (eCO_2) depends to a large degree on resource limitations of NPP under ambient CO_2 (aCO_2) (e.g., light, water, nutrients) (Kim et al., 2016; Körner 2003ab; Oren et al. 2001). Stimulation of growth from eCO_2 may not occur in ecosystems that are strongly coupled to native nutrient cycling (e.g. *steady-state nutrient cycle-Type III response,* Körner 2006). For example, in an early succession pine stand on nutrient poor sandy soil, a growth response to eCO_2 was only observed with addition of supplemental nutrients (Oren et al. 2001). Similarly, experiments in late succession spruce (Sigurdsson et al. 2013) and eucalyptus (Ellsworth et al. 2017) forests where growth at aCO_2 was limited by nutrient availability, no growth response to eCO_2 were observed. In both studies, eCO_2 induced a growth response after nutrient limitations were alleviated (i.e. by fertilization). Stimulation of tree growth by eCO_2 may be ephemeral if nutrients become progressively scare, being tied up in organic matter (Luo et al. 2004, Norby et al. 2010). In two Free Air Carbon Enrichment (FACE) studies in a pine and broadleaved plantations (Oren et al. 2001, Norby et al. 2010), early increases in tree stem growth from a step increase in $[CO_2]$ were not sustained due to decreasing soil nutrients or nitrogen availability.

Forests allocate 20-65% of NPP belowground to support growth and maintenance of roots and mycorrhizae (Landsberg and Sands 2011), thus a complete accounting of biomass partitioning, particularly belowground, is necessary to understand and predict forest response to eCO_2 (Walker et al. 2019). Long-term eCO_2 experiments performed at the tree and stand scale generally show that CO_2 enrichment increases belowground carbon allocation (Matamala and Schlesinger 2000, Norby et al. 2004, Finzi et al. 2007, Pritchard et al. 2008ab, Iversen et al. 2012) and the magnitude of the response is inversely correlated with aboveground sink strength, which increases with soil fertility (Palmroth et al. 2006). Ecological studies of forest root systems generally distinguish a fine and coarse root fraction based on root diameter as each have different morphological and functional traits, decomposition dynamics, and response to resource availability (Poorter and Nagel 2000, Litton et al. 2007). Fine roots (<2mm diameter) are important for resource acquisition, have high nutrient concentration, and are relatively short-lived, whereas, larger coarse roots (>2 mm diameter) are perennial, support fine root networks, transport water and nutrients, store carbohydrates, and provide physical support for aboveground biomass (Landsberg and Sands 2011). Both root fractions will likely play a key role in the ability of forests to sequester carbon (Johnsen et al. 2001, Norby and Jackson 2000). Fine root production represents a large fraction of annual NPP and provides a large input of soil carbon and nitrogen through rapid root turnover (Ainsworth & Long, 2005; Bonan, 2008). Quantifying the turnover rate of this pool is, however,

1 subject to large uncertainties (Strand et al. 2008). In contrast, coarse roots, which account for up to 80% of

2 belowground biomass (Butnor et al. 2003) and 10-20% of NPP (Giardina and Ryan 2002, Maier et al. 2004),

3 contribute to ecosystem carbon storage through formation of long-lived live wood biomass (Mobley et al.

4 2013) that can persist for decades following senescence (e.g., after forest harvest) (Clark et al. 2001, Johnsen et

5 al. 2001, Ludovici et al. 2002, Anderson et al. 2018). Indeed, ecosystem retention of carbon over the long-term

6 may be determined by the fraction allocated to perennial woody biomass with slow turnover and mean

7 residence time of decades or longer (Walker et al. 2019).

8   Results from large-scale field experiments in conifers and broadleaved species generally show that

9 long-term CO_2 enrichment stimulates fine root production and increases biomass (Matamala and Schlesinger

10 2000, Pritchard et al. 2008a, Phillips et al. 2006, Norby et al. 2004), root length, and rooting depth (Taylor et

11 al. 2014). Interestingly, eCO_2 increased fine root turnover in pine (Pritchard et al. 2008a), but decreased

12 turnover in broadleaved *Liquidambar styraciflua* (Iversen et al. 2008). Compared to the wealth of data for fine

13 roots, less effort has been made to study how eCO_2 affects coarse roots. While it is expected that CO_2

14 enrichment will stimulate coarse root biomass, less is known about how eCO_2 affects above- and belowground

15 biomass partitioning and coarse root structure. This is in part because measuring the effects of eCO_2 on coarse

16 root biomass of trees is difficult owing to the size and scale of the experimental unit and duration of eCO_2

17 exposure necessary to obtain meaningful results. Allometric biomass partitioning theory suggests that there is a

18 stable isometric relationship between coarse root and stem biomass or root:shoot ratio (R/S) (Niklas and Spatze

19 2006) and that coarse root biomass will change in unison with aboveground biomass (McCarthy and Enquist

20 2007). Species-specific R/S are widely used for estimating coarse root biomass in forest ecosystems (Mokany

21 et al. 2006) and developing forest carbon budgets (Cairns et al., 1997; Snowdon et al., 2003, Li et al. 2003),

22 thus a stable relationship between coarse root and shoot biomass would simplify modeling forest response to

23 eCO_2. Nevertheless, evidence for eCO_2 effects on R/S is equivocal. Field experiments using open-top chambers

24 and FACE, found no effect of eCO_2 on R/S ratio in some conifers (Tissue eta al. 1997, Crookshanks et al.

25 1998) and broadleaved species (Norby et al. 1995, Calfapietra et al. 2003, Gielen et al. 2005). In contrast, Day

26 et al. (2013) found for a scrub-oak ecosystem that long-term eCO_2 increased above and belowground biomass,

27 but the absolute increase was greater for root biomass meaning that R/S increased under eCO_2.

28 *Duke Free-Air Carbon Enrichment (FACE) study*

29 The long-term effects of eCO_2 on forest structure and function was studied in a *P. taeda* forest at the Duke

30 FACE experiment from 1994-2010. Exposure to eCO_2 resulted in a sustained increase of photosynthesis in pine

31 (Maier et al. 2008) and broadleaved (Ellsworth et al. 2012) species. Early in the study, 41% greater annual

1   canopy photosynthesis (Schäfer et al. 2003) under eCO_2 resulted in a 13 - 27% increase in tree basal area

2   growth (Moore et al. 2006), a 27% increase in annual wood increment (Hamilton et al. 2002), and a 21%

3   increase in stand NPP (McCarthy et al. 2010). Plot variability in soil nitrogen availability explained much of

4   the variation in NPP, as there was a linear response of NPP to a plot-level index of soil nitrogen availability

5   (Finzi et al. 2002, McCarthy et al. 2010). Kim et al. (2020) further showed that long-term eCO_2 altered the

6   aboveground allometric relationship for pine, specifically an increase in height/diameter ratio. Accounting for

7   shifts in allometry increased the effects of eCO_2 on aboveground pine biomass from 21% to 27%. They found

8   no eCO_2 response in aboveground allometry of broadleaved species.

9   Increased belowground carbon allocation under eCO_2 supported greater nitrogen uptake and sustained

10   aboveground production due to a combination of increased fine root production, soil organic matter

11   decomposition, and carbon allocation to mycorrhizal fungi (Finzi et al. 2007, Drake et al. 2011), increased fine

12   root respiration (Drake et al. 2008) and soil CO_2 efflux (Palmroth et al. 2006, Butnor et al. 2003, Kim et al.

13   2017). Pritchard et al. (2008a,b), using minirhizotrons, found that fine root biomass distribution and

14   mycorrhizal fungi increased at deeper depths, and Taylor et al. (2014), using soil monoliths, found that CO_2

15   enrichment increased fine root length and shifted fine root distribution to smaller size classes (<1mm). There is

16   limited data from the Duke FACE study on the direct effects of eCO_2 on coarse root biomass. Early stand-level

17   estimates of coarse root biomass relied on allometric equations developed prior to CO_2 treatment (Naidu et al.

18   1998, Hamilton et al. 2002, Schäfer et al. 2003, Finzi et al. 2006) or estimated coarse root biomass as a

19   function of aboveground biomass (McCarthy et al. 2010) assuming no CO_2 induced shifts in R/S. An exception

20   was Jackson et al. (2009). They measured coarse root biomass (pine + broadleaved) in 5 cm diameter by 15 cm

21   deep soil cores. Periodic measurements from 2003 - 2009 showed that eCO_2 treatments had on average 17%

22   more coarse root biomass, but this difference was not significant. However, a onetime sampling in 2008 from

23   larger 0.13 m^3 pits found a significant doubling of coarse root biomass in eCO_2 treatments, much higher than

24   the 21% increase in aboveground mass over the same period (McCarthy et al. 2010).

25   In this study, we evaluated how up to 17 years of CO_2 enrichment and 6 years of annual nitrogen

26   amendments at the Duke FACE site affected standing coarse root biomass and NPP for dominant pine (*P.*

27   *taeda*) and broadleaved competitors. We developed allometric equations to predict coarse root biomass for the

28   pine (taproot and lateral roots >2mm in diameter) and broadleaved species and explore effects of eCO_2 with

29   and without nitrogen amendment on stand coarse root biomass, vertical distribution with respect to root

30   diameter class, R/S ratio, and how eCO_2 and supplemental nitrogen affected NPP. We hypothesized that eCO_2

31   would increase coarse root biomass for canopy pine and broadleaved trees present both in the pine dominated

1   layer and below (**H1a**), that increased biomass under eCO_2 would shift coarse root biomass distribution with

2   depth (**H1b**), and that smaller diameter roots would be more responsive to eCO_2 than larger diameter roots

3   (**H1c**). We further hypothesized that the amount of increased coarse root biomass would be greater than that

4   observed for aboveground components (i.e. increased R/S) (**H2**). Because the magnitude of aboveground

5   growth response to eCO_2 was constrained by nutrient availability (Oren et al. 2001, McCarthy et al. 2010), we

6   hypothesized nitrogen additions would increase coarse root biomass mostly in the eCO_2 treatment (i.e. a CO_2 x

7   nitrogen interaction) (**H3**). Our results provide a more complete assessment of the long-term effects of eCO_2 on

8   stand development as reflected in carbon production and partitioning in coarse root biomass.

9

10   **Materials and Methods**

11   *Site description*

12   The Duke FACE experiment was located in a 90 ha loblolly pine (*Pinus taeda*, L.) plantation in the Blackwood

13   Division of the Duke Forest (Orange County, NC; 35°97' N, 79°09' W). Three-year-old half-sib seedlings

14   were planted in 1983 at 2.0 x 2.4 m spacing following a chop and burn site preparation treatment. The climate

15   is warm, humid in the summer, and moderate in the winter with a mean annual temperature of 15.5 ° C. Mean

16   annual precipitation is 1,145 mm and is evenly distributed throughout the year. The soils are predominantly

17   Enon silt-loam characterized as a moderately low fertility acidic clay-loam. Soil pH is around 6.0, and pine

18   foliage N (~1.1%) and phosphorus (~0.3%) tend to be at the middle range for mid-rotation *P. taeda*. The site

19   index is 21 m at age 25. Loblolly pine comprised 89% of basal area in 2010. Other common woody plants

20   include sweetgum (*Liquidambar styraciflua*) in the mid- to upper forest canopy, and winged elm (*Ulmas*

21   *alata*), dogwood (*Cornus florida*), and red maple (*Acer rubrum*) in the mid- to lower canopy.

22   The FACE experiment consisted of eight circular plots measuring 30 m in diameter. A prototype

23   (FACEp) plot with elevated CO_2 (eCO_2) and a reference plot with ambient CO_2 (aCO_2) were established in

24   1993 when the trees were 13 years-old. The replicated FACE experiment established six additional plots (three

25   each of eCO_2 and aCO_2) in 1996. The eCO_2 plots received additional CO_2 to maintain atmospheric $[CO_2]$ at

26   ambient + 200 $\mu l \, l^{-1}$, while the aCO_2 plots received only ambient air. Carbon dioxide enrichment commenced

27   in 1994 and 1996, for the FACEp and FACE experiments, respectively, and continued through October 2010.

28   Details on FACE operation and protocols for quality control and assurance are in Hendrey et al. (1999).

29   From 1998 - 2004, the FACEp plots received a nutrient addition treatment. Each plot was split in half

30   by an impermeable barrier down to 70cm, below most of the fine roots (Matamala and Schlesinger 2000), and

one half of each plot was fertilized annually with 5.6 or 11.2 g N m^{-2} as NH_4NO_3 or urea (N) and balanced with other nutrients as in Albaugh et al. (1998). The other half was the non-fertilized reference (R). In 2005, the FACE plots were halved with a similar impermeable barrier and annual fertilization commenced in all plots (including FACEp) with only NH_4NO_3 at 11.2 g N m^{-2} on one-half of each plot. Partitioning was done on a N–S or E–W axis such that each half had similar annual pine biomass increment and litter production rates. The CO_2 and nutrient treatments created 16 plots consisting of four treatment plots each of aCO$_2$/no nutrient (AR), aCO$_2$/ added nutrients (AN), eCO$_2$/no nutrients (ER), and eCO$_2$/added nutrients (EN).

Root sampling

All aboveground mass from approximately 40% of each plot was harvested in early 2011 (Kim et al. 2020). Within each treatment plot, roots from one "large" and "small" diameter pine tree were excavated (32 trees total). Because of the cost in time and labor, lateral roots were not sampled at all depths in each treatment plot. First, all roots (pine and broadleaved) were carefully excavated in a 1.5 x 1.5 m pit (2.25 m^2) centered on each pine tree. All 32 trees were excavated to a depth of 30 cm. Sixteen of the trees (four from each treatment, one from each plot) were excavated to a depth of 60 cm, and four trees (one from each treatment) were excavated down to 90 cm. All coarse roots >2 mm in diameter were removed by sieving soil through 0.64 cm^2 mesh hardware screen. Following manual excavation, a backhoe was used to remove the entire taproot down to 120 cm, if necessary. To sample coarse roots outside the center pit, a 65 x 75 cm (0.49 m^2) side trench was excavated diagonally at the corner of each pit to the sample depth of the pit. Lateral roots collected in the pits and side trenches were considered to belong to the target tree assuming that biomass of roots entering the pit or trench was equal to biomass of roots exiting the pit (Jackson and Chittenden 1981). Root harvests commenced on May 2, 2011 and were completed on June 22, 2011. Approximately 3200 person-hours were used to complete the excavations.

All roots were brought to the lab, washed with de-ionized water, and separated into pine and broadleaved fractions. Pine roots were separated into taproot and lateral coarse roots, and pine and broadleaved roots were further separated into four diameter classes: 2-5, 5-15, 15-30, and >30 mm. All roots were dried to a constant weight at 65° C and weighed. When necessary, large roots were chipped to facilitate drying. Sub-samples from each size class were ground and combusted in muffle furnace at 450° C to determine ash content.

Pine root allometric equations and stand scaling

Separate allometric equations were developed for taproot and lateral root biomass using a simple power function,

1 $$B = a * (D^2)^b \qquad\qquad 1,$$

2 where B is pine root biomass (kg tree^{-1}) of the taproot (B_{tr}) or lateral roots (B_{lr}), D^2 is stem diameter (cm) at

3 breast height squared, and a and b are parameters to be estimated. Roots collected in the pit were used to

4 parameterize equation 1 for B_{tr}. Lateral root biomass for each tree was estimated for three concentric zones

5 around each tree (Figure 1a). Roots collected in the pit were used to scale root biomass to the area of zone 1 as:

6 $$B_{lr_1} = P * \frac{a_1}{2.25} \qquad\qquad 2,$$

7 where B_{lr1} is root biomass in zone 1, P is root biomass in the 2.25 m^2 pit (kg m^{-2}), and a$_1$ is the area of zone 1

8 (3.53 m^2). Roots collected in the trench were used to estimate root biomass in zone 2 as:

9 $$B_{lr_2} = T * (a_2 - a_1) \qquad\qquad 3,$$

10 where, B_{lr2} is root biomass in zone 2, T is root biomass in the 0.49 m^2 trench (kg m^{-2}), and a$_2$ is the area of zone

11 2 (10.29 m^2). Lateral roots can extend up to 7 m from the stem depending on tree diameter (Gilman 1990,

12 Johnsen et al. 2006), well outside of the radius of zone 2 (1.81 m). To estimate lateral root biomass in this area

13 (zone 3), we first estimated the maximum root area (MRA, i.e. root spread) for the tree as,

14 $$MRA = 106.15 * D^{1.18} \qquad\qquad 4,$$

15 where, MRA is the area (m^2) occupied by roots and D is stem diameter (m) (modified from Roering et al.

16 2003). Root biomass in zone 3 was estimated from roots collect from the trench as,

17 $$B_{lr_3} = T * (MRA \text{ - } a_2) * CF \qquad\qquad 5,$$

18 where, B_{lr3} is root biomass in zone 3 and CF (0.2399) is a correction factor (Figure 1b) to account for biomass

19 attenuation with distance from the edge of zone 2 to the edge of MRA. The correction factor was estimated

20 from a normalized decay function derived from a relationship between root cross-sectional area (proportional

21 to biomass) and distance from an inflection point ($\sim$ 1.3 m from the stem) representing a transition from

22 mechanical support and transport function to only transport (Oren, unpublished data). Total lateral root biomass

23 (B_{lr}) was estimated as the sum of B_{lr1} ,$B_{lr2,}$ and B_{lr3}.

24 Equation 1was applied to all trees in the plot to estimate plot level root biomass (PB_r) (kg m^{-2}):

25 $$PB_r = \frac{\sum_{i=1}^{n} B_{tr_i} + \sum_{i=1}^{n} B_{lr_i}}{A_p} \qquad\qquad 6,$$

26 where B_{tri} and B_{lri} is root biomass in the pit for tree i in the plot containing n trees and A_p is treatment plot area

27 (Table S1). Equation 6 was used to estimate pine taproot and lateral root biomass at soil depths 0-30, 30-60,

1 and 60-90 cm and for taproot mass >90 cm. Plot root biomass in each soil layer was partitioned into size

2 classes as the product of PB_{tr} or PB_{lr} and the proportion of each size class at that soil depth (Table S4 and S5)

3 where PB_{lr} was weighted by proportion of root mass in each zone (Figure 1).

4 *Broadleaved allometry and stand scaling*

5 Broadleaved root biomass was estimated from roots sampled in the pine pits and side trenches. There was no

6 relationship between broadleaved root biomass and pine stem diameter, so plot-level biomass proximal to the

7 2.25 m² pit (BB_{pr}) was determined as the product of the average between the two sample trees and the total pine

8 tree count for the plot (Table S1). Broadleaved root biomass distal to the pit (BB_{dr}) was estimated using trench

9 data multiplied by the plot area not contained in a pit (Table S1). Total plot broadleaved root biomass (kg m⁻²)

10 was estimated as,

11 $$BB_r = \frac{BB_{pr} + BB_{dr}}{A_p} \qquad\qquad 7.$$

12 Broadleaved root biomass in each soil layer was partitioned into size classes as the product of BB_{pr}, BB_{dr}, or

13 BB_r and the proportion of each size class at that soil depth (Table S6).

14 To estimate broadleaved root biomass through time, we used an allometric equation developed by

15 Miller et al. (2006) for broadleaved competitors in a *P. taeda* plantation similar to ours in age, structure, and

16 soil type,

17 $$B_i = c * e^{((lnD * a) - b)} \qquad\qquad 8,$$

18 where, B_i is root biomass (kg) of tree i, lnD is natural log(diameter) (cm), a=1.921950652, b = 2.100356610,

19 and c = exp(0.09344710/2). Plot-level broadleaved root biomass under aCO₂ (kg m²) was estimated as,

20 $$aB_{pred} = \frac{\Sigma_i^n B_i}{A_p} \qquad\qquad 9,$$

21 where n is the number of broadleaved trees in the plot (Table S1). We assumed broadleaved species had the

22 same allometric relationship in all plots prior to CO₂ enrichment (year 0). Root biomass estimates were

23 averaged across N treatments within a ring, because, as we show later, there were no significant effects of N

24 additions (beginning in 2005) on broadleaved root biomass. To account for the CO₂ effect on root biomass, we

25 assumed a linear increase in the ratio of biomass between eCO₂:aCO₂ with time, and corrected the estimate of

26 total broadleaved root biomass in each plot,

27 $$eB_{pred} = aB_{pred} * \left(1 + k * \frac{r-1}{n}\right) \qquad\qquad 10,$$

28 where, eB_{pred} is predicted broadleaved plot root biomass under eCO₂, k is the number years of CO₂ enrichment,

29 r is the eCO₂:aCO₂ root biomass ratio (2.55, see results and Figure 6b), and n is the total number of years,

1   which is two years longer in the prototype plot. Annual broadleaved tree inventories were used to estimate

2   eB_{pred} through time (1996 - 2010).

3   *Biometric variables*

4   Leaf area index (LAI) was estimated based on leaf litterfall samples, which was collected monthly (twice per

5   month from October to January due to high volume) from 12 litter baskets (0.16 m^2 and 0.22 m^2 per basket

6   before and after 2004) in each plot. Because the timing of leaf production and loss of *P. taeda* and broadleaved

7   species were different, their LAI were estimated separately. Details of LAI estimation are given in McCarthy et

8   al. (2007). Annual inventories of plot tree diameters as described in McCarthy et al. (2010) were used to

9   estimate annual PB_r, aB_{pred}, and eB_{pred} (1996 - 2010). Annual pine and broadleaved coarse root net primary

10   productivity (NPP) was estimated with summed coarse root biomass increments of trees surviving two

11   consecutive inventory years and ingrowth in the second year (Clark et al. 2001).

12   *Statistical analyses*

13   The experimental design was a randomized complete block with a split-plot where CO_2 and N amendments

14   were the main and split-plot effects, respectively. All statistical analyses were conducted in SAS (Version 9.3;

15   SAS Institute Inc. Cary, NC, USA). Treatment effects on pine allometric relationships (equation 1) of B_{tr} and

16   B_{lr} were tested for each soil depth and across all depths using Analysis of Covariance (ANCOVA). In most

17   cases, a natural log-log transformation of response and continuous variables was performed to correct for

18   heteroscedasticity. A correction factor based on mean squared error was used to account for bias when $logB$

19   was back transformed to kilograms (Baskerville 1972). Treatment effects on PB_{tr}, PB_{lr}, PB_r, BB_{pr}, BB_{dr}, BB_r,

20   and total coarse root biomass ($TB_r = PB_r + BB_r$) were analyzed using ANOVA (PROC MIXED). Time series

21   estimates of annual standing root mass and NPP were analyzed by repeated measures ANOVA (PROC

22   MIXED). A first-order autoregressive covariance structure (AR(1)) was selected based on AIC fit statistics.

23   Individual plots were used as replicates. Block and Block x CO_2 were treated as random variables with plots

24   blocked to the pairing of plots established at beginning of the experiment (n=4). Significant treatment or

25   interaction effects were further analyzed using multiple comparisons of LSMEANs with Tukey's test. When

26   necessary, plot pine basal area at the beginning of the study was used as covariate to account for variation in

27   plot conditions at the beginning of the study.

28   ANCOVA was used to explore relationships between PB_r and BB_r and stand level variables: basal area

29   (BA), aboveground biomass, and LAI. For this analysis, PB_r or BB_r was the dependent variable, CO_2 and N

30   treatment combination (AR, AN, ER, EN) was the treatment, and pine or broadleaved BA or LAI was used as

31   the quantitative linear covariate. Treatment effects on regression lines were tested using full and reduced

1   models; first by testing the entire regression (i.e. intercepts and slopes simultaneously). If treatment was

2   significant, then a separate analysis for differences in slope or intercepts was performed. Linear contrasts were

3   used to test for differences between regressions and for making pairwise comparisons. To control Type I

4   experiment-wise error, a Bonferroni correction was used to derive the appropriate significance level (α) when

5   multiple comparisons were made (Zarnoch 2009).

6

7   **Results**

8   *Pine diameter and biomass relationships*

9   Pine taproot (B_{tr}) was significantly correlated with D^2 at all soil depths (Figure S1). There was no eCO_2 or N

10   treatment effect on the relationship ($p > 0.05$) at any depth; therefore, allometric equations were developed for

11   data pooled across all treatments (Table S2). Similarly, lateral root biomass (B_{lr}) was correlated with D^2, in the

12   0-30, 30-60, and 60-90 cm depths. In this case, eCO_2 significantly affected the relationship between B_{lr} and D^2

13   for roots in the top 30 cm ($p = 0.052$) (Figure S1b, Table S2). Treatment had no effect on B_{lr} in the 30-60 cm

14   depth. Because only one tree per treatment combination was sampled at the 60-90 cm depth, treatment effects

15   on B_{lr} could not be tested.

16   To simplify estimates of total pine root biomass and calculations of NPP, allometric equations for B_{tr}

17   and B_{lr} were developed for roots summed over all depths (Figure 2, Table S3). In this case, the relationship for

18   B_{lr} for depths 30-60 and 60-90 cm (Table S2) were used to estimate lateral roots of harvested trees not sampled

19   at that depth. When summed across depth, neither eCO_2 nor N affected the allometry of B_{tr}, however the

20   allometric relationship for B_{lr} differed with treatment ($p = 0.061$, Figure 2b). Trees with a similar diameter had

21   greater B_{lr} under eCO_2 indicating a change in allometry. There was no significant bias in the normalized

22   residuals for any of the regression models.

23   *Stand level coarse root biomass*

24   Plot-level pine root biomass was significantly increased under eCO_2 (Table 1). Elevated CO_2 increased total

25   pine root mass (PB_r) 32.5 ± 4.4% or 1.80 kg m^{-2} above the 5.66 kg m^{-2} observed under aCO_2 (se = 0.27, $p =$

26   0.007). Treatment response differed for taproot (PB_{tr}) and lateral root (PB_{lr}) fractions. Elevated CO_2 increased

27   PB_{tr} by 0.53 kg m^{-2} or 22.5 ± 4.1%, over aCO_2 (2.43 kg m^{-2}, se=0.11, $p = 0.013$). In contrast, eCO_2 increased

28   PB_{lr} 40.0 ± 4.7% (A: 3.23, E: 4.50 kg m^{-2}, se=0.13, $p = 0.006$). There was no significant N or CO_2 x N

29   interaction on either root component.

30   Compared to pine, total broadleaved root biomass (BB_r) was 2.55 times higher under eCO_2 (A: 0.81, E:

31   2.06, se = 0.24, $p = 0.033$) (Table 2). This increase was largely due to a significant CO_2 x N effect on

broadleaved roots proximal to a pine stem (BB_{pr}) where the N treatment decreased root mass under aCO_2, but

increased mass in eCO_2. There was no treatment effect on roots distal to the pine stem (BB_{dr}). Total root

biomass (TB_r, pine + broadleaved) ranged between 5.65 and 11.12 kg m^{-2}. Elevated CO_2 increased TB_r by 47.9

± 3.4% (A: 6.47, E: 9.52 kg m^{-2}, se = 0.37; $p < 0.001$) (Figure 3). There was no significant N ($p = 0.206$) or

CO_2 x N ($p = 0.185$) interaction on TB_r. The proportion of BB_r to TB_r was greater under eCO_2 (0.22) than aCO_2

(0.13) (se = 0.04, $p = 0.001$) (Figure 3, inset).

Root size class distribution with depth

Pine root biomass declined asymptotically with depth for all size classes (Figure S2). For all size classes, 45-

75% of root mass in the profile was found in the 0-30 cm depth and less than 25% was found at depths 60-90

and >90 cm (Figure 4). Elevated CO_2 increased PB_{tr} and PB_{lr} in all root size classes (Table 3, Figure 4, inset).

There was a significant CO_2 x N interaction for the 2-5 mm roots where N increased root mass under eCO_2

more than under aCO_2. There was a strong CO_2 x depth interaction for all root categories, where significant

effects of eCO_2 was limited to the 0-30 cm depth and at the 30-60 depth for roots >30 mm (Figure S2). Within

the 0-30 cm depth, eCO_2 had a greater relative effect for PB_{lr} increasing root biomass 41, 45, 52, and 43% for

2-5, 5-15, 15-30, and >30 mm size classes, respectively compared to 22% for PB_{tr}. There was also significant

N x depth interaction for 2-5, 5-15 and >30 mm roots (Table 3); however, a consistent pattern across root

categories was difficult to ascertain.

Broadleaved root biomass distribution was more variable than pine and showed a different distribution

with size and soil depth under eCO_2. Under aCO_2, BB_r declined with depth (Figure S3) and the pattern was

similar for all size classes. For the 2-5 mm size class, 64, 32, and 4% of the root mass was in the 0-30, 30-60,

and 60-90 cm depth, respectively (Figure 5). For root sizes larger than 5mm, >80% of the root mass was in the

0-30 cm soil depth. In contrast, under eCO_2 BB_r increased at the 30-60 cm depth relative to the 0-30 cm depth

(Figure 5 and S3). Summed over size classes, 54% of root mass was in the 30-60 cm depth compared to 41% in

the 0-30cm depth and <10% in the 60-90 cm depth. When summed over depth, eCO_2 increased BB_r in all root

sizes (Table 3), however in contrast to pine, a greater increase in biomass was observed for large roots (>15

mm, >3.5 times) than small roots (<2.1 times). The N treatment had no effect on BB_r or root distribution except

for 15-30 mm size class (Figure S3). This was caused by a large broadleaved taproot in close proximity to a

side trench in one of the ER plots. There were no significant N x depth or CO_2 x N x depth interactions for any

size class.

R/S ratio

1   There was no significant CO_2 or N treatment effect on pine R/S (Table 1). Pine R/S ranged from 0.21 to 0.26

2   and averaged 0.236 ± 0.003. However, PB_r increased linearly with pine BA (Figure 6a), total aboveground

3   biomass (AGM) (Figure 7a), and LAI (Figure 7b). In all cases PB_r, plots with similar BA, AGM, or LAI

4   carried more root biomass under eCO_2 indicating that CO_2 enrichment shifted biomass partitioning

5   belowground.

6   Broadleaved root mass increased with BA (Figure 6b) and the slope of the relationship was greater

7   under eCO_2. In contrast to pines, eCO_2 had no effect on aboveground broadleaved biomass. Thus, the large

8   increase in BB_r under eCO_2 increased R/S from 0.572 under aCO_2 to 1.121 in eCO_2 (se = 0.135) (Table 2).

9   These relationships suggest broadleaved trees increased belowground allocation under eCO_2. Broadleaved BA

10   ranged accounted for $10.5 \pm 1.6\%$ and $11.4 \pm 2.1\%$ of total plot (pine + broadleaved) BA, in aCO_2 and eCO_2

11   treatments, respectively. However, the large increased in broadleaved R/S under eCO_2 increased stand R/S 16.5

12   $\pm 3.1\%$ (A: 0.249, E: 0.292; se = 0.009; $p = 0.018$). There was no N or CO_2 x N effect on broadleaved or stand

13   R/S.

14   *Coarse root net primary productivity (NPP)*

15   Pine root NPP under aCO_2 and eCO_2 was higher during the first five years of the study (1997 - 2001) compared

16   to later years (Figure 8a). Comparing the AR and ER treatments over the study period (1997-2010), eCO_2

17   increased average pine root NPP by $49.4 \pm 1.9\%$ (E: 300.8, A: 202.7 g m^{-2} year^{-1}; se = 17.3; $p = 0.010$). This

18   led to a steady increase in the difference in PB_r between the CO_2 treatments (CO_2 x year: $p < 0.001$) (Figure 8b

19   and S4a). In contrast, mean broadleaved root NPP over the study period was much higher, 3.6 times greater

20   under eCO_2 (E: 144.8, A: 38.8 g m^{-2} year^{-1}; se = 19.0; $p = 0.013$) (Figure 8c). There was a significant CO_2 x

21   year interaction in broadleaved NPP ($p < 0.001$) that led to an increasing difference in BB_r between CO_2

22   treatments (Figure 8d and S4b). The annual pattern in total root NPP and TB_r was similar to pine with respect

23   to eCO_2 (Figure 8e, f and S4c). Elevated CO_2 nearly doubled the average total root NPP (E = 445.1, A = 241.0

24   g m^{-2}; se = 17.4; $p < 0.001$). Nitrogen amendment (2005-2010) had no effect on broadleaved NPP but increased

25   average pine NPP by $19.1 \pm 3.6\%$ (R: 222.8, N: 260.4 g m^{-2} year^{-1}; se = 17.1; $p = 0.011$) and total root NPP by

26   $15.0 \pm 3.0\%$ (R: 319.3, N: 361.6 g m^{-2} year^{-1}; se = 11.7; $p = 0.009$). However, increased NPP from N additions

27   had no effect on standing PB_r ($p = 0.135$) or TB_r ($p = 0.972$) by the end of the study (Figure 8b and f). There

28   was no significant CO_2 x N effect on NPP or standing biomass for any root component.

29   **Discussion**

30   *Coarse root biomass and distribution with depth*

1   Fifteen years of eCO_2 (17 years in FACEp) increased stand coarse root biomass (TB_r) by 3.08 kg m^{-2} over aCO_2

2   (6.46 kg m^{-2}), an increase of 48%. We hypothesized (**H1**) that long-term eCO_2 would increase TB_r, would alter

3   root distribution with depth, and that smaller diameter roots would be more sensitive to eCO_2 than large roots.

4   Our data largely support this compound hypothesis; however, the response differed for pine and broadleaved

5   components. The eCO_2 treatment increased pine coarse root biomass (PB_r) by 1.80 kg m^{-2} above the 5.66 kg m^{-}

6   ^{2} observed under aCO_2, an increase of 32%. This is increase was due to both an increase in tree size (20.5%)

7   and change in root allometry (12.0%). As expected, PB_r dropped precipitously with depth (Albaugh et al.

8   2006b) where 45-75% of root biomass was found in the 0-30 cm depth and less than 25% was found at depths

9   >90 cm. There was a significant CO_2 x depth interaction effect for all root size classes where increased biomass

10   under eCO_2 was only significant at the 0-30 cm depth. At this depth, eCO_2 had a relatively greater effect on

11   lateral roots (>41%) compared to taproots (<25%). Broadleaved trees accounted 13 and 22% of TB_r in aCO_2

12   and eCO_2 treatments, respectively. Compared to the pines, eCO_2 had a much larger effect on broadleaved roots

13   increasing BB_r by 255% (aCO_2: 0.81, eCO_2: 2.07 kg m^{-2}) and altered root distribution with depth. Under eCO_2,

14   54% of BB_r occurred in the 30-60 cm depth compared to less than 15% at the same depth under aCO_2. The

15   reason for the large response at this depth is unknown, but competition with the dominant pine for space in the

16   0-30 cm depth may have precluded a large growth response at this depth or made investment in deeper roots

17   more efficient. Contrary to our hypothesis, the increase in BB_r under eCO_2 was 3.5 times greater for large roots

18   (>15 mm) compared to 2.2 times for small roots (<15 mm).

19   Comparison of root biomass among studies is difficult owing to different sampling methods, sampling

20   depths, and scaling (Addo-Danso et al. 2016). Our values for TB_r at age 30 under aCO_2 and no N (AR

21   treatment) was 6.41 kg m^{-2} similar to the 6.24 kg m^{-2} reported by Miller et al. (2006) for a nearby 23-year-old

22   *P. taeda* plantation receiving a similar site preparation treatment. In their study, broadleaved roots accounted

23   for 12% of total root biomass compared to 11% in our study. In an earlier study at the Duke FACE site,

24   Jackson et al. (2009) sampled coarse roots in 0.41 m^2 pits 32 cm deep. They did not report separated pine and

25   broadleaved components. After 12 years of eCO_2 and two years of N amendment, coarse root biomass was

26   approximately 0.3, 0.45, 0.65, and 1.05 kg m^{-2} in the AR, AN, ER, and EN plots, respectively. Comparing our

27   raw trench data in the 0-30 cm depth, unadjusted for plot dimensions, our estimates were higher, 0.55, 0.78,

28   0.92, and 1.18 kg m^{-2} (se=0.17) for AR, AN, ER, EN plots, respectively. Changes in stand development (2-3

29   years difference between studies) could explain higher biomass for our data. Differences in sampling

30   methodology could also be a factor. For example, we sampled roots after all aboveground vegetation was

31   removed and the randomly positioned trenches probably sampled a more representative portion of broadleaved

1   roots as the trenches contained portions of taproots from small broadleaved and volunteer pines that were not

2   sampled in the Jackson et al. (2009) study.

3   We found that smaller diameter pine lateral roots were more responsive to eCO_2 than taproots

4   (hypothesis **H1c**). This response is consistent with increased fine root production and biomass observed at the

5   Duke FACE site (Pritchard et al. 2008a, Taylor et al. 2014). Only a few studies have examined CO_2 effects on

6   size class distribution of coarse roots. In young Norway spruce, eight years of elevated CO_2 (2x ambient) had

7   no effect on the primary root structure, but increased secondary roots growing on the primary roots by 58%

8   (Pokorný et al. 2013). Jach et al. (2000) reported a 152% increase in total root biomass in young *P. sylvestris*

9   following three years of elevated CO_2 (ambient + 400ppm) and that belowground dry matter partitioning was

10 shifted toward small roots (≈7%). These studies support our observations for a larger relative increase in lateral

11 root vs taproot biomass in pine. However, the opposite occurred for broadleaved roots. Functional equilibrium

12 theory predicts an intrinsic linkage between leaves and fine roots (Cannell and Dewar 1994). Thus, assuming a

13 similar relationship with small broadleaved roots, the lack of a strong CO_2 response may be tied to little or no

14 CO_2 response of broadleaved LAI (Kim et al. 2020). In this case, increased carbon supply from broadleaved

15 photosynthesis (Ellsworth et al. 2012) under eCO_2 was allocated to coarse roots >15 mm.

16 *Root:shoot ratio (R/S)*

17   We hypothesized (**H2**) that increased coarse root biomass under eCO_2 would be greater than that

18 observed aboveground and would result in an increase in R/S. Our data partially supports this premise. Pine

19 R/S ranged from 0.21 - 0.25 under aCO_2, which is within the range reported in a meta-analysis of mature

20 temperate coniferous plantations where aboveground biomass was greater than 150 Mg ha^{-1} (median R/S =

21 0.20; Mokany et al. 2006) and was similar to 0.24 reported for a nearby *P. taeda* plantation with a large

22 broadleaved component (Miller et al. 2006). The 32% increase in PB_r under eCO_2 was larger than the 27%

23 increase in aboveground biomass (AGM) observed at the end of the study (Kim et al. 2020), although there was

24 no significant CO_2 effect on treatment average R/S ($p = 0.146$). However, plot-level PB_r was strongly

25 correlated with AGM (Figure 7a) and in plots with similar AGM, the eCO_2 treatments had greater root

26 biomass. Our observations that eCO_2 had only small effects on pine R/S is consistent with studies that show

27 alleviating soil resource limitations (i.e., nutrients and water), while greatly increasing growth of *P. taeda*, does

28 not strongly influence above/belowground biomass partitioning (Albaugh et al. 1998, King et al. 1999,

29 Samuelson et al. 2004, Coyle and Coleman 2005). In contrast to pine, eCO_2 increased broadleaved R/S from

30 0.57 to 1.12 and combined with pine increased stand R/S from 0.25 under aCO_2 to 0.29 under eCO_2 or 17%.

31 Broadleaved trees were located primarily in the understory, although there were a few large broadleaved trees

1   that shared the canopy with the pine and may have had an overly large influence on the response to eCO_2. For

2   example, we observed a CO_2 x BA interaction (Figure 6b) where the increase in BB_r with BA was greater under

3   eCO_2. This interaction was likely caused by a few large broadleaved trees in two eCO_2 plots (accounting for

4   15.1 and 44.8% of plot broadleaved BA). We could not partition broadleaved overstory versus understory

5   response to eCO_2, confounding species and canopy position.

6   Competition for light and soil resources is a major driver of carbon allocation to root and shoot tissues

7   and may explain the large increase in broadleaved R/S relative to pine (Franklin et al. 2012). McCarthy et al.

8   (2010) showed that increased aboveground NPP of pine under CO_2 enrichment was partly due to increased leaf

9   area and was a function of soil nitrogen availability. We found a similar relationship between PB_r and LAI

10   (Figure 7b), where for a given LAI, there was more PB_r in eCO_2. These data suggest that eCO_2 increased coarse

11   root growth efficiency (i.e., root growth per unit LAI). Considering there was little change in R/S, increased

12   PB_r was likely due to enhanced photosynthetic rate and not to whole tree shifts in carbon allocation. These

13   results support a fixed structural biomass partitioning in this species that is not strongly affected by soil

14   resource availability (Retzlaff et al. 2001) or eCO_2. Thus, isometric scaling of pine coarse root biomass from

15   AGM (Figure 7a) is reasonable as long as AGM estimates account for CO_2 effects on root and aboveground

16   allometry (Kim et al. 2020). In contrast, eCO_2 did not stimulate broadleaved AGM (Kim et al. 2020), hence,

17   broadleaved R/S more than doubled under eCO_2. The lack of an eCO_2 effect on AGM is interesting as Schäfer

18   et al. (2003) showed that broadleaved canopy photosynthesis under eCO_2 increased 67%. In a light limiting

19   environment, optimal biomass partitioning theory (Thornley 1972, Franklin et al. 2012) predicts a shift in

20   partitioning away from roots to stems and vice versa under high light, thus a potential increase carbohydrate

21   availability in eCO_2 being allocated to root biomass seems counter intuitive. Alternatively, the observed

22   response is consistent with the photosynthesis growth model proposed by Luo et al. (1994) that when the

23   relative increase in plant photosynthesis under eCO_2 is greater than the relative increase in aboveground

24   growth, the excess carbohydrate is allocated belowground to root growth. Kim et al. (2020) concluded that

25   increased pine LAI (Kim et al. 2016) under eCO_2 reduced understory light availability limiting aboveground

26   broadleaved response to eCO_2. In addition, having to compete with a large increase of pine root biomass in the

27   shallow, nutrient rich soil (0 - 30 cm depth), increased belowground allocation in broadleaved root biomass

28   was forced to deeper soil layers (Figure 5) that likely had lower available nutrients, further limiting

29   aboveground growth response to eCO_2.

30   The large stimulation of broadleaved root biomass in the eCO_2 treatment has implications for estimates

31   of biomass retention under long-term CO_2 enrichment (Walker et al. 2019) and modelling belowground carbon

allocation (De Kauwe et al. 2014) particularly for the understory vegetation. Additionally, increased allocation of biomass to broadleaved roots may confer better survival in shaded environments particularly under dry conditions, thus we may expect increased competition for soil resources under eCO_2. For example, increased root biomass per unit leaf area in pines and greater R/S in broadleaved species indicates an increase in root conductive area relative to leaf area, suggesting that trees growing under eCO_2 may have an advantage during periods of drought, perhaps because larger coarse roots support more fine roots and a higher fine root surface area/leaf area ratio is hydraulically superior, especially under drought (Ewers et al. 2000).

Coarse root NPP

We observed a sustained eCO_2 stimulation of pine and broadleaved root NPP over the 15-year study. While pine root NPP declined with time, the relative difference between eCO_2 and aCO_2 treatments was constant ($\approx$49%). In contrast, broadleaved root NPP in eCO_2 increased with time relative to aCO_2. The combined pine and broadleaved root NPP (Figure 8e) under eCO_2 lead to a continuing expansion of TB_r relative to aCO_2 (Figure 8f). Elevated CO_2 stimulated the production and turnover of fine roots (Pritchard et al. 2008a, Jackson et al. 2009), mycorrhizal associations (Pritchard et al., 2008b, Drake et al., 2011), root exudates (Phillips et al. 2011) and litter fall (Lichter et al., 2008). This increased soil carbon flux may have stimulated microbial nitrogen cycling, and thus enhanced N availability under eCO_2 (Finzi et al. 2007, Averill et al. 2015) supporting greater above- and belowground NPP. Körner (2006) outlined three patterns for forest response to eCO_2 based on the degree of coupling with native soil capacity to supply resources for growth. The sustained increase in root NPP and continuing expansion of root biomass under eCO_2 suggest that this system still has sufficient nutrients to maintain a strong CO_2 effect on root growth (e.g., transitioning between Type II and Type III conditions, Körner 2006) and the stands are not undergoing progressive nitrogen limitation observed in other forest FACE experiments (Norby et al. 2010).

Response to N amendment

In the early FACEp study, Oren et al. (2001) showed that fertilization with balanced nutrients increased stem production under eCO_2 relative to the aCO_2 treatment. McCarthy et al. (2010) further showed that the magnitude of the stand NPP response to eCO_2 was a function of plot-level index of nitrogen availability (Finzi et al. 2007) mediated primarily through increased leaf area. Thus, we would have expected that plots with lower nitrogen availability to respond to N treatments. We found that N additions increased pine root NPP, and the response to N was additive in combination with eCO_2 (i.e. no significant CO_2 x N). However, increased pine root NPP had no effect on final PB_r or TB_r, consistent with no observed N effect on aboveground biomass (Kim et al. 2020). Thus, we reject our hypothesis (**H3**) that root biomass response to eCO_2 will be enhanced by

1 N additions. This contrasts with results of other belowground components where N treatment decreased fine

2 root biomass and production (Pritchard et al. 2008a, Jackson et al. 2009), and increased fine root respiration

3 (Drake et al. 2008) and soil CO_2 efflux (Kim et al. 2017).

4 We caution, however, that this conclusion must be taken in context of the experiment, i.e., mid-rotation

5 *P. taeda* stands may have limited ability to respond to nitrogen fertilization. Response to fertilization varies

6 widely dependent upon stand and site conditions, climate, and type and rate of nutrient application. Increased

7 production from fertilization is primarily mediated through increased LAI as increased foliar nitrogen

8 concentration rarely results in a sustained increased in leaf photosynthesis in *P. taeda* (Fox et al. 2007a).

9 Fertilization of the main study occurred when the trees were 25 years-old when the crowns were closed.

10 Response to fertilization of fully stocked mid-rotation *P. taeda* (where BA > 25 m^2 ha^{-1} and LAI > 3.5) may be

11 limited if light and space are insufficient to support increased foliage biomass (Albaugh et al. 2006a, Fox et al.

12 2007a) or if foliar N concentration is near or above the critical level of 1.2% (Vose and Allen 1988).

13 Furthermore, in contrast to Oren et al. (2001), nitrogen only fertilization was used in the main study. A

14 fertilization response in *P. taeda* is dependent on nutrient application at the correct stoichiometric

15 nutrient/nitrogen ratios (Albaugh et al. 1998, Fox et al. 2007b). For example, nitrogen additions reduced the

16 foliar P/N ratio from 0.075 (se = 0.004) in Reference plots to 0.052 (se = 0.002) (*Aubrey Knier*, *unpublished*

17 *data*) well below the 0.10 considered adequate for growth (Albaugh et al. 2010). It is possible that the nitrogen

18 only fertilization used in this study disrupted the nutrient balance of trees, with adverse effects on growth (Oren

19 et al. 1988).

20 *Uncertainties caused by coarse root biomass sampling and scaling*

21 Measurements of root biomass in forest ecosystems are difficult and time consuming and methods are

22 generally not standardized (Addo-Danso et al. 2016). We used a combination of whole-root harvest in a central

23 2.25 m^2 pit and a sub-sample of lateral roots in a 0.49 m^2 side trench to estimate tree root biomass. This is a

24 common approach for measuring root mass in *P. taeda* plantations where each tree is assumed to occupy a

25 defined area based on planting density (in this study: 2.0 x 2.4 m spacing = 4.8 m^2 per tree) (e.g., Albaugh et al.

26 1998, 2006b, Samuelson et al. 2004, Maier et. al. 2012). Allometric equations for pine taproot mass were

27 robust. For example, a taproot equation developed for 8 to12-year-old *P. taeda* growing in deep sandy soil

28 (Albaugh et al. 1998), predicted stand taproot mass to within 1.5% of our estimates. Comparing estimates of

29 lateral root mass is more problematic as the size of the central pit and pits between trees and sampling depth are

30 usually different between studies. Lateral roots can extend several meters or more from the tree's stem (Gilman

31 1990, Stone and Kalisz 1991, Johnsen et al. 2005) much further than the edge of a typical center pit. We

1 accounted for this by predicting lateral root biomass as function of root spread based on tree diameter and root

2 mass (equations 2-5, Figure 1). The advantage of this approach is it allows for estimating total root biomass for

3 each tree, proximal and distal to the central pit based on stem diameter and can be used to predict change in

4 root biomass over time. An alternative approach for estimating PB_{lr} would be to scale root biomass in the 0.49

5 m^2 trench to all areas in the plot not occupied by pine pit (A_{osp}, Table S1) (e.g., Miller et al. 2006, Albaugh et

6 al. 2006b). This approach predicted 8.5 ± 0.8, 6.1 ± 1.2, 14.3 ± 1.5, and 8.9 ± 3.5 % less root biomass in AR,

7 AN, ER, EN, respectively than our method. The difference was due in part to our estimate of root biomass in

8 zone 3, which accounted for 5.5 ± 0.6 % of PB_r.

9 Relative to the more intensive sampling of pine coarse roots, we measured broadleaved root mass using

10 roots recovered in the pine pits and the proximity to a pine stem may have biased plot-level estimates of

11 broadleaved root mass. As a check on our estimates, we calculated broadleaved root mass using an allometric

12 equation developed by Miller et al. (2006) for broadleaved species growing in nearby ($\approx$100 km) *P. taeda*

13 stands similar to our study in age, soil, and stand structure. The equation was applied to all broadleaved stems

14 that had a measured dbh (Table S1). Using this equation, predicted broadleaved root biomass in the aCO_2

15 treatments was not significantly different from measured BB_r (Figure 3) and predicted values had a similar

16 relationship with broadleaved basal area (Figure 6b). Accordingly, we feel our estimates of BB_r under aCO_2 are

17 realistic, lending confidence that the observed large increase in biomass was a response to CO_2 enrichment.

18 Plot-level TB_r ranged between 5.65 and 11.12 kg m^{-2}, which corresponds to the upper range reported

19 for temperate coniferous forests (Jackson et al. 1996). We likely captured all of the broadleaved roots, as there

20 was little or no root mass in the 60-90 cm depths (Figure S4), however, for pine, 10-20% of lateral roots were

21 in the 60-90 cm depth, and consequently we may have underestimated R/S by not sampling below 90 cm

22 (Robinson 2007). Rooting depth is strongly influenced by soil physical and morphological characteristics and

23 *P. taeda* roots can be found >200 cm (Albaugh et al. 2004). On another Piedmont site with a clay soil texture,

24 *P. taeda* roots were found down to 170 cm, although roots below 90 cm accounted for only 2.2% of the total

25 root mass (Albaugh et al. 2006b). Thus, we feel that our sampling accounted for most of the lateral pine roots.

26 *Implications*

27 We found that long-term eCO_2 stimulated coarse root NPP and carbon accumulation in root biomass (48%) and

28 that the magnitude of the response differed between dominant pine and broadleaved species. An accurate

29 estimate of coarse root biomass response to eCO_2 is important as this pool sequesters carbon on-site for longer

30 periods than aboveground biomass. Decomposing root systems provide many benefits to forests including

31 biogeochemical cycling of nutrients, improved soil physical characteristic important for live root development,

and is a source for a large carbon flux into the soil matrix. Our stands were cut at age 30. Using a coarse root decay rate of 0.0534 yr^{-1} established for *P. taeda* in the Duke Forest (Ludovici et al. 2002), 21% or 1.19 and 1.57 kg m^{-2} of coarse root biomass in aCO_2 and eCO_2 treatments, respectively would still be present after a subsequent 30-year rotation. This suggests that increased pine coarse biomass under elevated CO_2 would promote soil carbon sequestration over successive rotations assuming eCO_2 does not accelerate soil carbon cycling (De Graff et al. 2006) or alter other factors that regulate long-term decomposition (Pendall et al. 2003).

Average overstory pine R/S was not affected by eCO_2. These results suggest that isometric scaling from aboveground metrics (AGM, BA, LAI) may be acceptable for predicting coarse root biomass response to eCO_2 in closed canopy pine forests; however, because of large increases in R/S in broadleaved species, isometric scaling would greatly underestimate root biomass of the understory component and stand R/S. Our data on coarse root biomass under eCO_2 should be helpful for estimating stand carbon pools and allocation and for testing and constraining models predicting forest response to eCO_2.

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Author Contributions

Sampling and scaling design: Oren, Maier, Johnsen; Field sampling: Anderson, Oren, Palmroth, Maier, Kim, Johnsen; Root NPP data: Kim, Maier, Oren; LAI data: McCarthy, Oren; Data analysis: Maier, Oren; Writing: Maier. All authors provided comments on the interpretation and edited the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

1 Data available on request from the authors

2 **Supplementary Information**

3 Additional supporting information may be found online in the Supporting Information section.

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Table 1. Averaged pine tree diameter at breast height (dbh), stand-level basal area (BA, m^2 ha^{-1}), root, stem, and total aboveground (AGM = stem + branch + foliage) biomass, the ratio of root to stem (root:stem), the ratio of root to AGM (R/S), and results of ANCOVA. Total pine root biomass (PB_r) was partitioned into taproot (PB_{tr}) and lateral roots (PB_{lr}) summed over 0-90 cm soil depth. Biomass values are least square means (kg m^{-2}) and standard error (se). Treatments are: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition.

Treatment	dbh	BA	PB_r[2]	PB_{tr}	PB_{lr}	Stem mass[3]	AGM[3]	Root: stem	R/S	LAI
AR	21.12	47.02	5.42	2.32	3.10	21.1	23.5	0.258	0.231	3.55
AN	22.57	50.40	5.90	2.54	3.36	23.1	25.6	0.257	0.232	3.84
ER	24.44	55.96	7.40	2.94	4.46	27.9	30.9	0.267	0.241	4.19
EN	24.46	57.23	7.52	2.98	4.54	28.6	31.4	0.265	0.241	4.16
se	1.18	1.72	0.27	0.12	0.15	1.8	1.9	0.008	0.007	0.19
Effect										
CO_2	0.029	0.012	0.007	0.013	0.006	0.030	0.030	0.234	0.146	0.001
Nitrogen (N)	0.482	0.125	0.176	0.195	0.169	0.181	0.241	0.745	0.915	0.251
C x N	0.493	0.451	0.398	0.355	0.444	0.488	0.477	0.882	0.869	0.147
Ipba[1]	ns	0.001	0.027	0.036	0.021	0.098	0.099	ns	ns	0.011

[1] Initial (1996) pine basal area. If "ns", then $p > 0.10$ and ipba is not included in the model.

[2] $PB_r = PB_{tr} + PB_{lr}$

[3] Stem mass and AGM are from Kim et al. (2020)

Table 2. Average broadleaved basal area (BA, m^2 ha^{-1}), coarse root biomass proximal (BB_{pr}) and distal (BB_{dr}) to a 2.25 m^2 area around a pine stem, total root biomass (BB_r), aboveground wood mass (AGM), the ratio of root to AGM (R/S), broadleaved peak leaf area index (LAI), and results of ANCOVA. Biomass values are least square means (kg m^{-2}) and standard error (se). Treatments are: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition.

Treatment	BA	BB_{pr}	BB_{dr}	BB_r	AGM[2]	R/S	LAI
AR	6.77	0.35	0.62	0.99	1.67	0.67	2.53
AN	4.46	0.18	0.50	0.63	1.36	0.47	2.63
ER	7.40	0.81	1.17	2.02	2.07	1.01	2.70
EN	6.63	1.14	0.97	2.11	1.84	1.23	3.47
se	1.01	0.13	0.50	0.29	0.43	0.19	0.21
Effect							
CO_2 (C)	0.270	<0.001	0.243	0.033	0.390	0.014	0.082
Nitrogen (N)	0.130	0.480	0.546	0.588	0.351	0.947	0.059
C x N	0.417	0.041	0.886	0.370	0.873	0.279	0.123
Ipba[1]	0037	ns	ns	0.094	ns	ns	0.004

[1] Initial (1996) pine basal area. If "ns", then $p > 0.10$ and ipba was not included in the model.

[2] AGM is from Kim et al. (2020)

Table 3. Probability of significance (p values) for treatment effects on pine (PB_r) and broadleaved (BB_r) root biomass with soil depth. Data for PB_r is partitioned into taproot (PB_{tr}) and lateral root (PB_{lr}) by size class. Data for BB_r is partitioned into size class.

	PB_r	\multicolumn{4}{c}{PB_{lr}}	PB_{tr}
Effect		**2-5 mm**	**5-15 mm**	**15-30 mm**	**>30 mm**	
CO$_2$ (C)	0.006	0.001	0.006	0.003	0.017	0.011
Nitrogen (N)	0.452	0.041	0.264	0.816	0.160	0.157
C x N	0.436	0.001	0.173	0.365	0.296	0.304
Depth (D)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C x D	<0.001	<0.001	<0.001	<0.001	<0.001	0.004
N x D	0.806	0.037	0.060	0.444	<0.012	0.909
C x N x D	0.987	0.195	0.392	0.001	0.218	0.959
Ipba[1]	0.034	0.005	0.026	0.027	ns	0.039
**Pine summed over depth**						
CO$_2$ (C)		0.001	0.006	0.003	0.017	0.011
Nitrogen (N)		0.073	0.185	0.739	0.301	0.213
C x N		0.012	0.117	0.223	0.434	0.355
Ipba		0.005	0.023	0.026	ns	0.040

Table header: **Pine Root size class**

Broadleaved Root size class					
Effect	BB_r	**2-5 mm**	**5-15 mm**	**15-30 mm**	**>30 mm**
CO$_2$ (C)	0.003	0.032	0.103	<0.001	0.003
Nitrogen (N)	0.632	0.915	0.258	0.014	0.472
C x N	0.896	0.446	0.337	0.128	0.905
Depth (D)	<0.001	<0.001	0.003	0.015	0.001
C x D	0.088	0.340	0.402	0.656	0.036

N x D	0.836	0.682	0.661	0.489	0.489
C x N x D	0.533	0.486	0.787	0.302	0.185
Ipba[1]	0.019	ns	0.032	ns	0.026
Broadleaved summed over depth					
CO_2 (C)	0.019	0.002	0.038	0.002	0.008
Nitrogen (N)	0.710	0.843	0.089	0.023	0.245
C x N	0.566	0.178	0.184	0.144	0.838
Ipba	ns	ns	ns	ns	0.026

[1] Initial (1996) pine basal area. If "ns", then $p > 0.10$ and ipba was not included in the model.

Figures

Figure 1. Approach for estimating total lateral root biomass from roots excavated in a 2.25 m^2 pit centered on the tree stem and a 0.49 m^2 side trench. The bold 'X' in the center denotes the target tree and the grey "X" are neighbor trees on 2.0 x 2.4 m spacing. Root biomass was determined for three zones. From the target tree, roots in zone 1 extend 1.06 m (area = 3.53 m^2) and in zone 2 extends to 1.81 m (area = 6.76 m^2). Roots in zone 3 extend to maximum root area (MRA, m^2), estimated as MRA = 106.15 * $D^{1.18}$, where D is tree diameter at breast height (m) (modified from Roering et al. 2003). For example, a 25 cm tree would have a MRA = 20.7 m^2 and roots would extend to 2.57 m. Roots excavated from the pit and trench were used to estimate biomass in zones 1 and 2, respectively. Biomass in zone 3 was estimated from the trench multiplied by a correction factor (b) to account for root biomass attenuation from the edge of zone 2 to the edge of MRA. The correction factor was estimated from a normalized decay function derived from a relationship between root cross-sectional area (proportional to biomass) and distance from an inflection point ($\sim$ 1.3 m from the stem) representing a transition from mechanical support and transport function to only transport (Oren, unpublished data).

Figure 2. Pine coarse root biomass (kg tree^{-1}) versus tree diameter at breast height squared (D^2) for roots summed over 0-120 cm for taproot (B_{tr}) (a) and 0 - 90 cm for lateral roots (B_{lr}) (b). Symbols represent treatments (legend: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition), and each data point is an individual tree. In panel a, the regression line is fitted to all treatments combined. In panel b, the line is fitted to each CO_2 treatment.

Figure 3. Stand-level pine (PB_r), broadleaved (BB_r), and total (TB_r) root biomass and the ratio of broadleaved to total root biomass (inset). The bar labeled "Miller" are allometric estimates of broadleaved root biomass based on the equation from Miller et al. (2006). Data are least square means and standard errors (n=4). Treatments are: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition.

Figure 4. Relative distribution by depth of pine lateral root (PB_{lr}) by size class, taproot (PB_{tr}), and total root (PB_r) biomass. Symbols are the means and standard error (n=4) for each treatment (legend: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition). Inset graph is PB_r summed over all depths in kg m^{-2} under ambient (A) or elevated (E) CO_2. Data are least square means and standard error. Statistics for the main panel and inset are in Table 3.
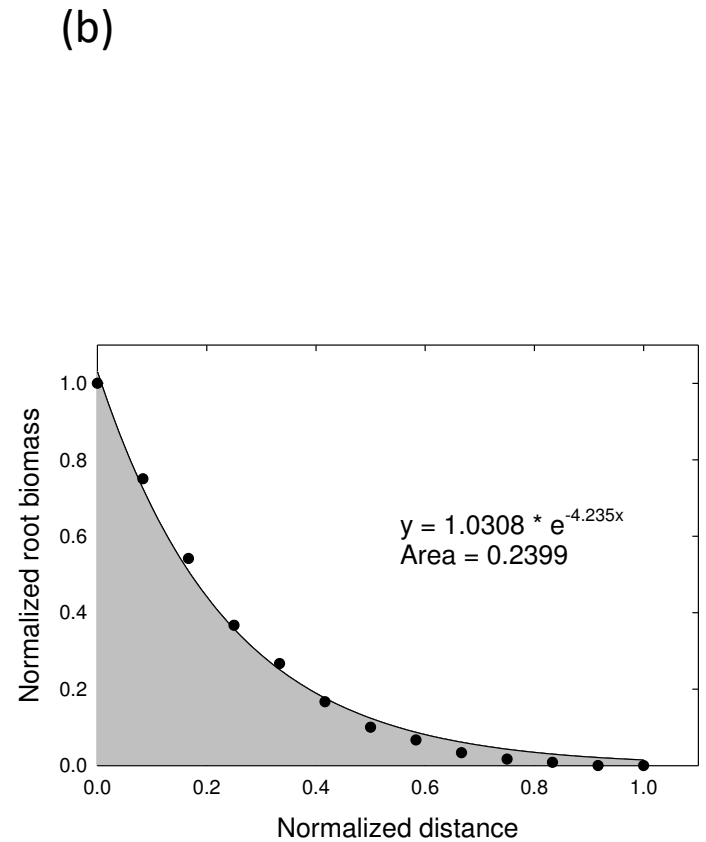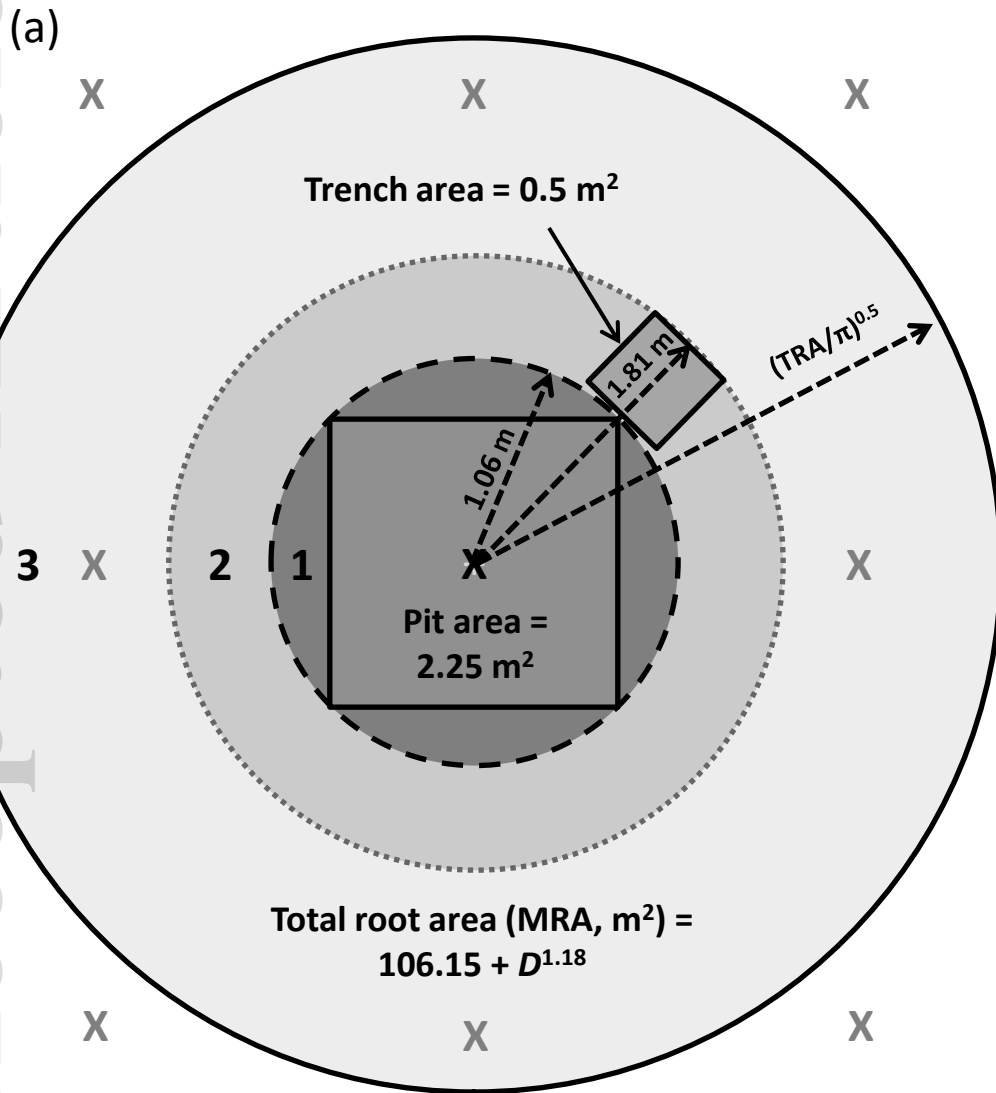
Figure 5. Relative distribution by depth of broadleaved root biomass by size class and total (BB_r). Symbols are the means and standard error (n=4) for each treatment (legend: A – ambient CO_2; E – elevated CO_2; R – native
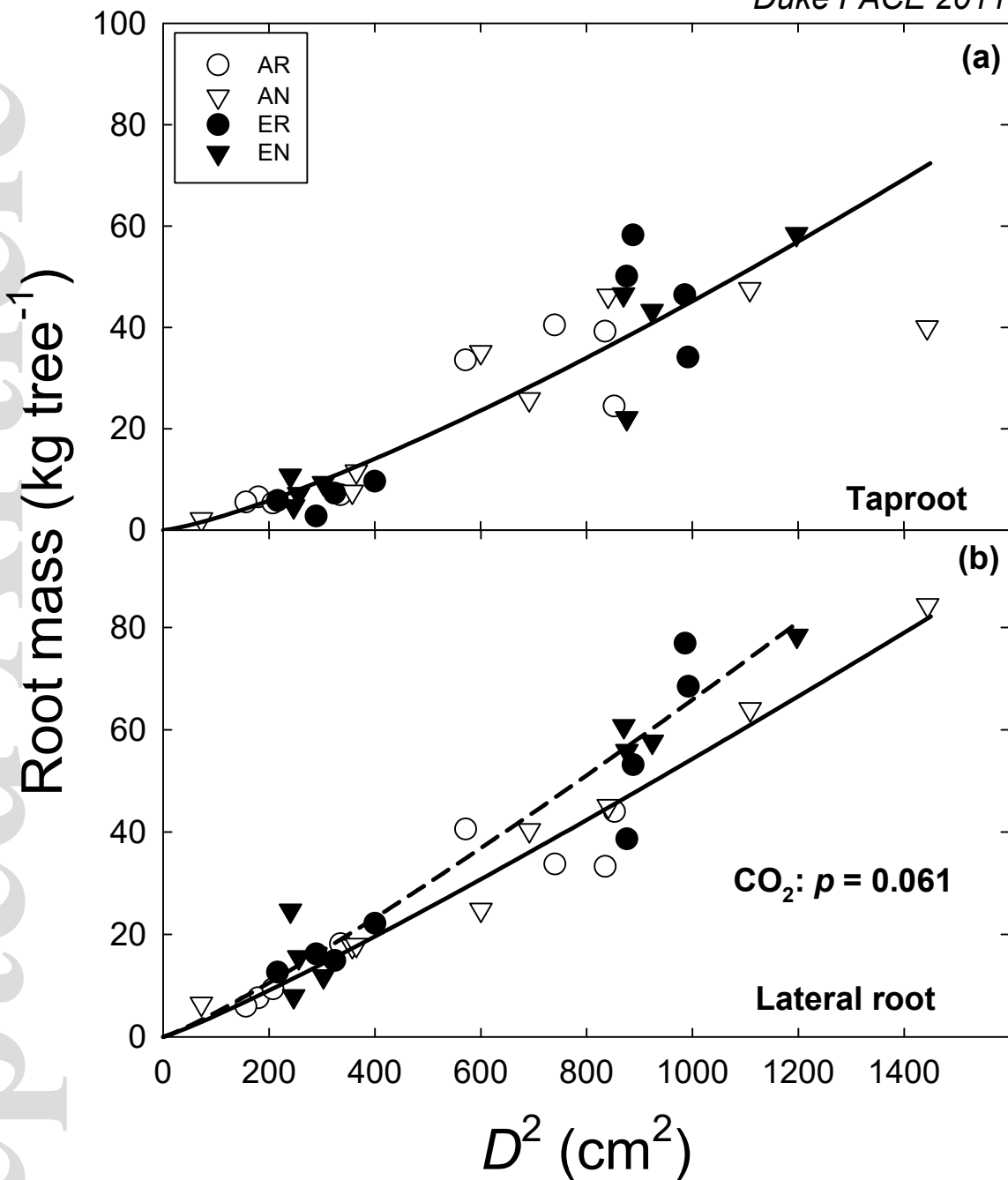
nitrogen; N – nitrogen addition). Inset graph is total root biomass (BB_r) over all depths in kg m^{-2} under ambient (A) or elevated (E) CO_2. Data are least square means and standard error. Statistics for the main panel and inset are in Table 3.
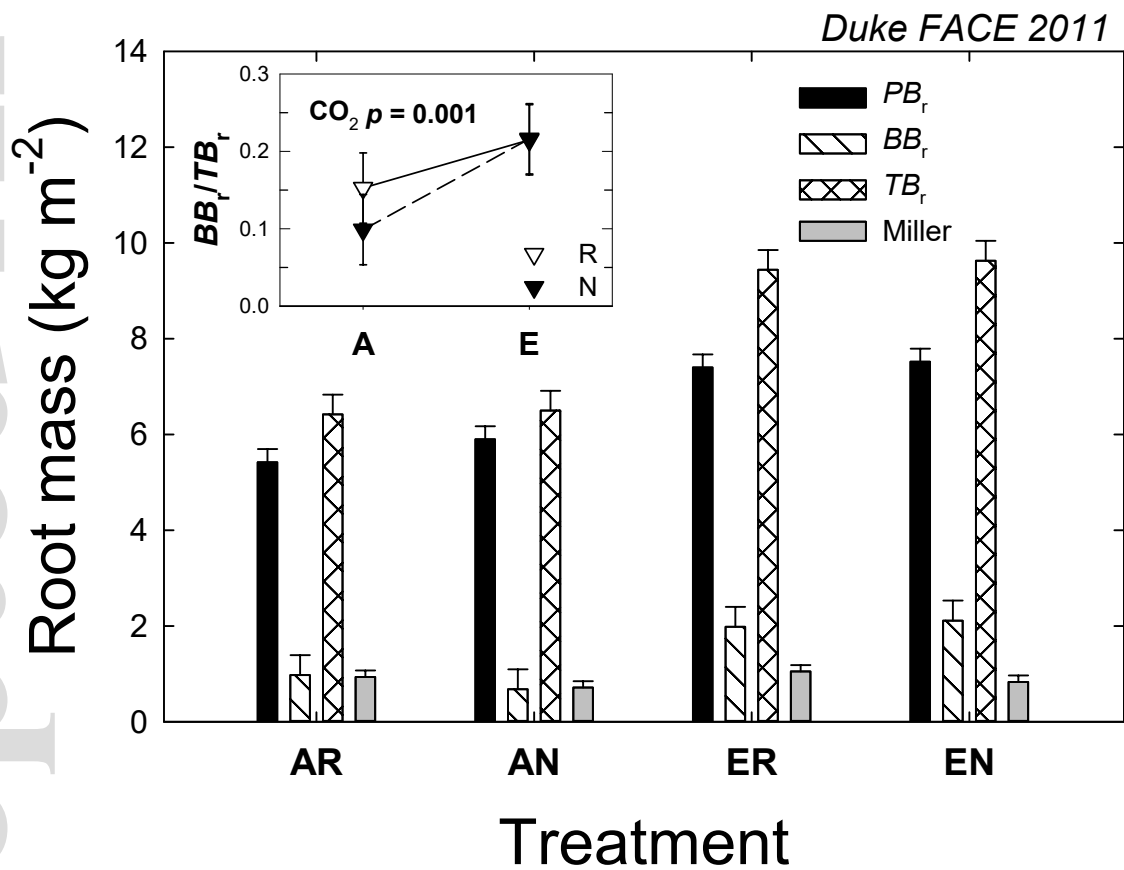
Figure 6. The relationship of pine root biomass (PB_r) and pine basal area (BA) (a) and broadleaved root biomass (BB_r) and broadleaved BA (b). Symbols are plot level data where treatments are: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition. The solid black and dashed lines are regressions of the aCO_2 and eCO_2 treatments, respectively. The grey regression line in panel b is the estimate of broadleaved root biomass in the aCO_2 treatment based on an allometric equation from Miller et al. (2006).
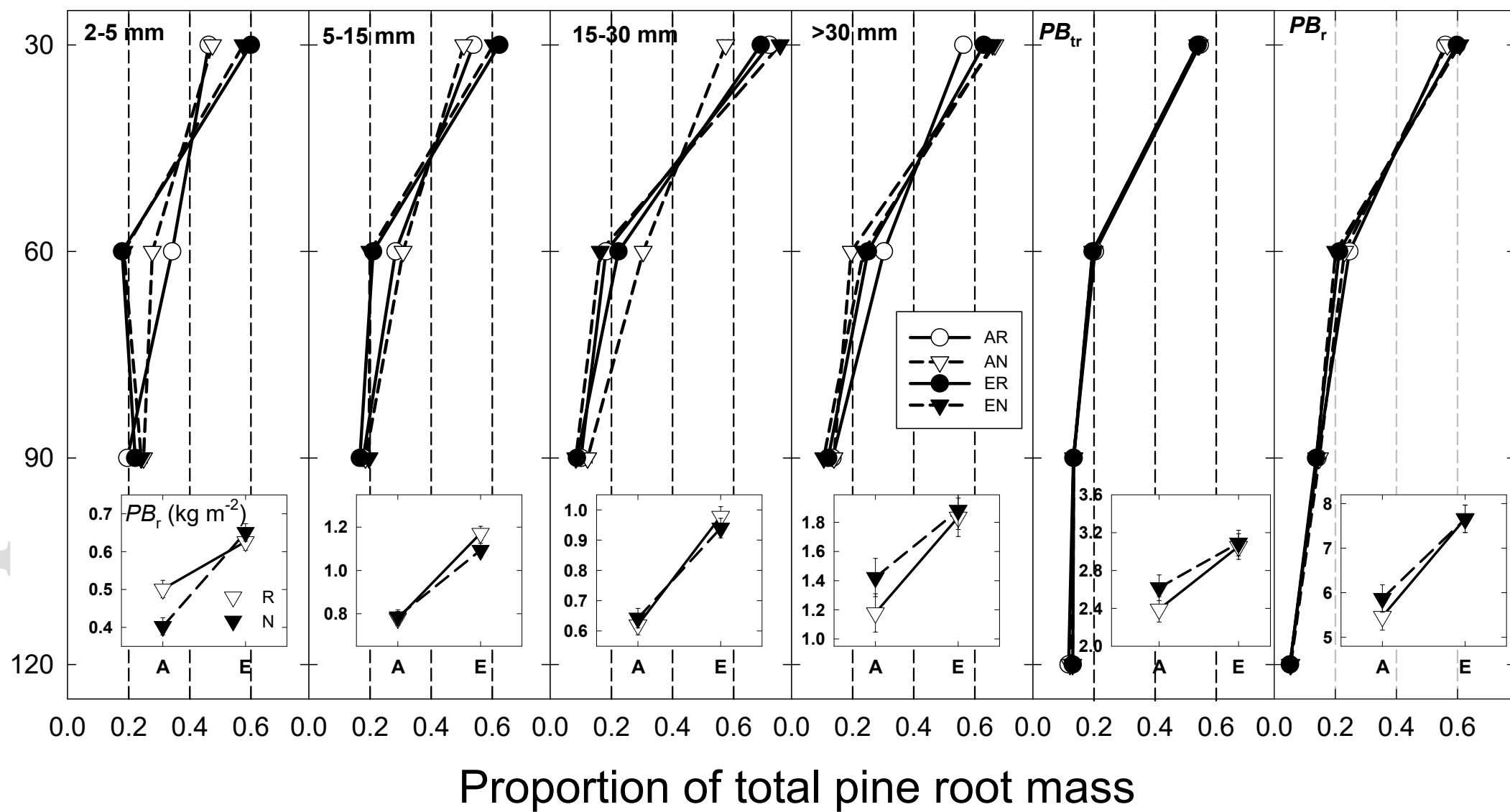
Figure 7. Relationship between pine root biomass (PB_r) and total aboveground pine biomass (stem + branch + foliage) (a) and PB_r and leaf area index (LAI) (b). Symbols are plot level data where treatments are A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition. The solid black and dashed lines are regressions of the aCO_2 and eCO_2 treatments, respectively.

Figure 8. Time series of pine (a), broadleaved (c), and total (e) coarse root net primary productivity (NPP) and the ratio of pine (PB_r) (b), broadleaved (BB_r) (d), and total (TB_r) (e) biomass under AN, ER, and EN to that under AR. Symbols are the means and standard error (n=4) for each treatment (legend: A – ambient CO_2; E – elevated CO_2; R – native nitrogen; N – nitrogen addition).
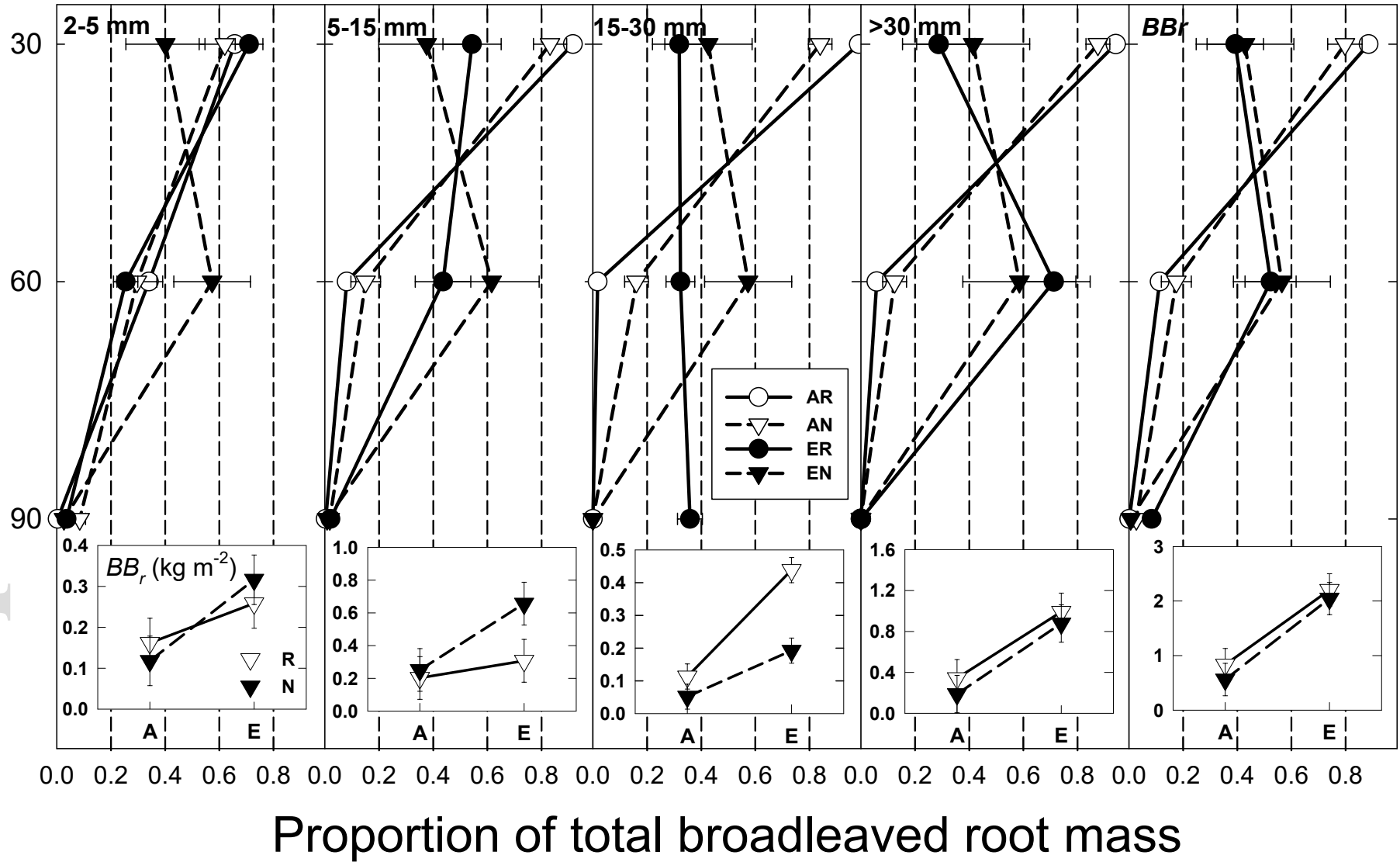
(a)

Trench area = 0.5 m²

1.81 m

1.06 m

$(TRA/\pi)^{0.5}$

3   X   2   1   X

Pit area =
2.25 m²

Total root area (MRA, m²) =
$106.15 + D^{1.18}$

(b)

$y = 1.0308 * e^{-4.235x}$
Area = 0.2399

Normalized root biomass

Normalized distance

Duke FACE 2011

Duke FACE 2011

Duke FACE 2011

Duke FACE 2011

Duke FACE 2011

Duke FACE 2011