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Dynamics of soil CO_2 efflux under varying atmospheric CO_2 concentrations reveal dominance of slow processes

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Abstract

We evaluated the effect on soil CO_2 efflux (F_{CO2}) of sudden changes in photosynthetic rates by altering CO_2 concentration in plots subjected to +200 ppmv for 15 years. Five-day intervals of exposure to elevated CO_2 (eCO_2) ranging 1.0–1.8 times ambient did not affect F_{CO2}. F_{CO2} did not decrease until 4 months after termination of the long-term eCO_2 treatment, longer than the 10 days observed for decrease of F_{CO2} after experimental blocking of C flow to belowground, but shorter than the ~13 months it took for increase of F_{CO2} following the initiation of eCO_2. The reduction of F_{CO2} upon termination of enrichment (~35%) cannot be explained by the reduction of leaf area (~15%) and associated carbohydrate production and allocation, suggesting a disproportionate contraction of the belowground ecosystem components; this was consistent with the reductions in base respiration and F_{CO2}-temperature sensitivity. These asymmetric responses pose a tractable challenge to process-based models attempting to isolate the effect of individual processes on F_{CO2}.

KEYWORDS
Bayesian state space model, carbon dioxide, climate change, nitrogen fertilization, soil respiration

1 | INTRODUCTION

Carbon (C) sequestration in forest ecosystems comprises a small difference between two large fluxes—photosynthesis and respiration (Bond-Lamberty & Thomson, 2010; Schlesinger & Andrews, 2000; Valentini et al., 2000). The largest and most complex component of forest ecosystem respiration takes place in the soil and forest floor, where fine roots, fungi and other microorganisms feed on either recently fixed carbohydrates or on necromass produced in the soil as these constituents turn over, and on litter dropped on the forest floor. Thus, although soil surface CO_2 efflux (F_{CO2}) is coupled with photosynthesis, this relationship may vary in space and time. The flux of carbon belowground is dependent on productivity aboveground, such that when increased leaf area leads to higher photosynthesis, substrate supply belowground can be increased and lead to higher F_{CO2} (Novick, Katul, McCarthy, & Oren, 2012; Palmroth et al., 2006; Schäfer et al., 2003). However, although improved N availability enhances leaf area, and litter delivery to the forest floor, the increased consumption of C in aboveground production comes at the expense of C delivery belowground (Lim et al., 2015; Palmroth et al., 2006); the net effect of increased litterfall and reduced C delivery belowground is lower F_{CO2} (Butnor, Johnsen, Oren, & Katul, 2003; Oishi, Palmroth, Butnor, Johnsen, & Oren, 2013). Disentangling the dependence of F_{CO2} on productivity is complicated by differences in turnover times between exudates, roots, soil organic matter, and leaf litter. Furthermore, environmental drivers affecting respiration and photosynthesis may be out of phase (e.g., because soil temperature is lagging behind canopy temperature), or may affect different processes (e.g., low soil moisture may reduce F_{CO2} both from reductions in photosynthetic production of substrate as well as directly suppressing microbial activity). Indeed, the number of processes interacting to ultimately produce a measured rate of F_{CO2}

frustrates efforts to isolate the effect of each process, and to properly model soil F_{CO2} and ecosystem respiration.

The majority of studies designed to assess the dynamics of F_{CO2} and its response to a step decrease of carbohydrate supply belowground are destructive in nature. In girdling and trenching experiments, reductions in the carbohydrate supply overlap with an increased mortality as roots and mycorrhizal fungi run out of carbohydrates (Bhupinderpal-Singh et al., 2003; Ekberg, Buchmann, & Gleixner, 2007). The onset and rate of root mortality depend on the amount of carbohydrate stored in the tissue prior to treatment initiation, and the temperature of the tissue (Marshall & Waring, 1985). Shading experiments can reduce C allocation to belowground less destructively, allowing evaluation of the effect of reduced carbohydrate source on belowground processes. In combination with enhanced N supply, such experiments can also alter carbohydrate sinks both aboveground and belowground, allowing further definition of processes (Hasselquist et al., 2016). However, the approach has only been employed over short vegetation: crops/grasses (Manderscheid, Pacholski, & Weigel, 2010), seedlings (Mao et al., 2016), or young trees (Hasselquist et al., 2016; Walcroft, Whitehead, Kelliher, Arneth, & Silvester, 2002). Stem chilling (Johnsen et al., 2007) and the recently developed pressure girdling (Henriksson et al., 2015) are both nondestructive methods for reversibly reducing carbohydrate transport to belowground. While the former is impractical for long-term ecosystem-scale manipulation, upon further testing and improvements the latter may become more broadly applicable. Thus, to date, free-air CO_2 enrichment (FACE) experiments at the ecosystem scale allow the only approach to study the effect of changes to belowground carbohydrate supply on soil respiration without causing acute change in ecosystem properties.

Free-air CO_2 enrichment experiments make it possible to manipulate belowground allocation of carbohydrates, while allowing for natural variation in weather, soil hydration and temperature, and the seasonal dynamics of photosynthesis, growth and litter production (McCarthy et al., 2010; Palmroth et al., 2006; Schäfer et al., 2003). These experiments provide empirical estimates of C pool size in, and F_{CO2} from the forest floor–soil system, which can constrain model results (Sulman, Phillips, Oishi, Shevliakova, & Pacala, 2014; Walker et al., 2015; Zaehle et al., 2014; Zhang et al., 2015). Perhaps even more importantly, these experiments can help isolate the effects of short-term variation of recently fixed carbohydrate supply from long-term adjustments of the respiring biomass in the soil and forest floor. They provide insights into processes regulating carbon flow to, and released from belowground, and improved specification of mechanisms in models.

From FACE experiments, we learned that CO_2 enrichment increases F_{CO2} (King et al., 2004). The greater F_{CO2} under elevated CO_2 (eCO_2) than ambient CO_2 (aCO_2) was attributed to a number of causes, including increased root biomass, specific root respiration rates, root exudates, and root turnover rate (Allen et al., 2000; Drake, Stoy, Jackson, & Delucia, 2008; Jackson, Cook, Pippen, & Palmer, 2009; King et al., 2001; Lukac, Calfapietra, & Godbold, 2003; Norby et al., 2002). In young stands, the extra carbohydrates produced under eCO_2 facilitate a rapid exploitation of the soil volume by roots and mycorrhizae, as trees quickly occupy the canopy volume (Norby et al., 2005). In older stands extra carbohydrates are used to increase photosynthetic efficiency of resource utilization (Drake et al., 2011; Finzi et al., 2015). Higher F_{CO2} under eCO_2 following crown closure is due to the direct contribution of respiration from larger coarse root, fine root and mycorrhizal biomass and more litterfall (Delucia et al., 1999; King et al., 2004; Lukac et al., 2003; Matamala & Schlesinger, 2000; Pritchard et al., 2008, 2014). Combined, more biomass and faster turnover of fine roots and mycorrhizae under eCO_2 (Lukac et al., 2003; Pritchard et al., 2008) increases necromass production, leading to higher microbial respiration. Increased litterfall with eCO_2 also makes more forest floor substrate available for decomposition above the soil surface and belowground, enhancing F_{CO2} (Allen et al., 2000), even without concurrent increase in belowground carbohydrate allocation (Subke et al., 2004). It was shown that increasing the availability of soil nitrogen (N) resulted in a decrease of all of these contributors to soil respiration (Jackson et al., 2009; Pritchard et al., 2014), which was reflected in decreasing F_{CO2} under both aCO_2 and eCO_2 (Oishi et al., 2013; Palmroth et al., 2006). However, these results are based on measurements that were not always coordinated. In FACE studies employed on existing, young stands, each measured pool or process may reflect a different state in the transition between a forest under aCO_2 condition, and a forest under eCO_2.

Interpretation of time series measurements requires that the length of the measurement period and the frequency of the data collected correspond to the time necessary for relevant processes to play out. The decreasing enhancement ratio of F_{CO2} during the early years at the Duke FACE (1999–2003) was interpreted as consistent with decreasing soil respiration as forests age (Bernhardt et al., 2006), while the increasing ratio in the latter years (2003–2009) as consistent with increasing fine root biomass in eCO_2 relative to aCO_2 (Jackson et al., 2009). However, an extended time series (2001–2010) showed no clear pattern (Oishi, Palmroth, Johnsen, McCarthy, & Oren, 2014). Clearly, there must have been a period of time initially during which the system adjusted to eCO_2, and F_{CO2} progressively increased relative to aCO_2 plots. This period could be shorter than a year, requiring frequent monitoring to capture. Unfortunately, these early dynamics were poorly quantified in FACE experiments (Andrews & Schlesinger, 2001; Taneva, Pippen, Schlesinger, & Gonzalez-Meler, 2006). Early in the experiment, the ^{13}C signature was followed at Duke FACE, and showed changes at 20 cm depth 7 days after commencement of enrichment; the enrichment signature moved progressively down the soil profile (Andrews & Schlesinger, 2001). However, measurements of F_{CO2} from the soil surface were made at coarser intervals, thus potentially missing effects on fast process detected within days in C flow reduction experiments (Bloemen et al., 2014; Henriksson et al., 2015; Högberg et al., 2001).

We employed a nondestructive approach to quantify the effect of short-term manipulation of atmospheric CO_2 on F_{CO2} from the forest floor at the Duke FACE experiment during periods of high and low soil moisture, at the end of a growing season. A follow-up, two-year study on the response to termination of CO_2 enrichment, permitted separating the effects of short and longer term reduction in belowground C allocation on soil respiration.

Temporal variability ^{13}C suggests that the isotopic signature of photosynthesis can be observed in F_{CO2} within a week (Andrews, Harrison, Matamala, & Schlesinger, 1999), or, more finely determined, in 3–4 days (Mortazavi et al., 2005). We thus hypothesized that (**H1**) the effect of five-day CO_2 manipulation during the end of the growing season would be reflected in changes of F_{CO2} proportional to the change of CO_2 concentration during the 5 days. The reduction of F_{CO2} has been explained not only by a decrease in sugar flow belowground, but also in terms of rapidly decreasing biomass of mycorrhizae and fine roots (Drake et al., 2012; Hasselquist et al., 2016; Högberg et al., 2001; Jing et al., 2015). Reductions of mycorrhizae may occur within a week (Högberg et al., 2001). However, because increased production of mycorrhizae in eCO_2 was only observed in deep soil (Ekblad et al., 2016; Pritchard et al., 2008), response of mycorrhizae may take longer than a week to be observed. Enrichment termination has less drastic an effect on carbohydrates flow to belowground than girdling, thus the response of fine roots in eCO_2 plots to termination will likely manifest later than the 3 months observed following girdling (Jing et al., 2015). Thus, we hypothesized that, (**H2**) following the termination of eCO_2 at the Duke FACE, reduction of F_{CO2} (reflecting soil respiration) in previously eCO_2 plots will be observed after approximately 3 months, declining to the level of the reference during the following 2 years of monitoring. Alternatively, a larger pool of carbohydrate reserves in roots may buffer against short-term variation in photosynthesis, and a pulse of dying roots may provide for greater microbial activity, maintaining greater F_{CO2} in plots previously subjected to eCO_2.

2 | MATERIALS AND METHODS

2.1 | Site description

This study was conducted at the Duke Forest FACE site, North Carolina, USA (36°58′ N, 79°06′ W) from 2009 to 2012. The mean annual precipitation and air temperature of the site are approximately 1,145 mm and 15.5°C, respectively. *P. taeda* seedlings (3-year-old) were planted after clear-cutting and burning in 1983, and the average height of *P. taeda* reached ~21 m in 2010. Naturally regenerated broadleaved species including *Liquidambar styraciflua*, *Ulmus alata*, *Acer rubrum*, and *Fraxinus americana* accounted for ~41% of total leaf area of the site during the growing season in 2009. Most broadleaved trees were presented in the mid to lower-canopy, but some extended to the upper-canopy (McCarthy et al., 2007).

The study site included eight 30-m-diameter circular plots. The FACE experiment started in a prototype plot under CO_2 enrichment (targeted at 200 μmol/mol above ambient atmospheric CO_2 concentration) and its reference in 1993, and expanded to eight plots (four elevated and four ambient plots) in 1996. The CO_2 enrichment concluded on October 31, 2010. Annual N fertilization with ammonium nitrate pellets (NH_4CO_3; 11.2 g N m^{-2} $year^{-1}$) began on one half of each plot in 1998 for the prototype plot and its reference, and extended to the other six plots in 2005. Each plot was divided with an impermeable barrier inserted 70 cm into the soil, which is the depth of the fine root system at the site (Matamala & Schlesinger, 2000). All above- and belowground biomass in 40% of each plot—half in native soil quadrant and half in N-fertilized quadrant—was harvested from November 2010 to April 2011. The annual N fertilization continued for the remaining half of each plot until 2012.

2.2 | Short-term CO_2 response study

In 2010, a short-term CO_2 response study was conducted using stepwise changes of atmospheric CO_2 concentration at the end of the growing season (from late August to early October; day of year 242–282). At the end of the growing season, carbohydrates are preferentially partitioned to belowground (Högberg et al., 2010; Johnsen et al., 2007), thus the study should be taken as a measure of maximum effect. During this short-term experiment, target CO_2 concentrations in the four elevated plots were set to one of five CO_2 concentrations—ambient, 100, 150, 200, and 300 μmol/mol above ambient (A, A + 100, A + 150, A + 200 and A + 300)—at an interval of 5 days (6 days for the last period), and the four ambient plots were used as a reference (Tor-Ngern et al., 2015). The CO_2 concentrations in elevated plots returned to the long-term target (A + 200) on October 10, 2010 (see Table S1 for a complete CO_2 fumigation schedule). A major rainfall event in the middle of the experiment caused a distinct change in soil moisture condition; mean relative extractable water (REW) before and after the rainfall were 0.03 and 0.78, respectively (for detailed representation of conditions during the short-term experiment, see Tor-Ngern et al., 2015). Because the soil moisture condition may affect stomatal responses to CO_2 enrichment (Domec et al., 2009), outgassing of, and soil permeability to, CO_2 (Butnor, Johnsen, & Maier, 2005; Oishi et al., 2013), we divided the study into two periods (low and high soil moisture periods).

We evaluated the short-term responses of F_{CO2} in three ways: (1) to detect whether there was a gradual response with exposure time over the 5 days in each level of CO_2 concentration, we analyzed F_{CO2}, separately for low and high soil moisture periods, searching for increasing or decreasing rates following a corresponding step change in CO_2 concentration; (2) we tested whether the change of F_{CO2} between two intervals was consistent in both amount and direction to the change in CO_2 concentration. We eliminated from this analysis eight of the 64 periods during which soil moisture increased rapidly, thus causing evacuation of soil-CO_2 (Butnor et al., 2005); lastly; and (3) we assessed the daily mean F_{CO2} in relation to CO_2 concentration during low and high soil moisture periods.

2.3 | Data collection

Soil temperature was measured with thermistors (334-NTC102-RC; Xicon Passive Components, Masnfield, TX, USA) buried at 10 cm in each plot. Volumetric soil water content (θ) of the upper 30 cm soil layer was measured with time-domain reflectometry probes (CS615 or CS616L; Campbell Scientific, Logan, UT, USA) at four locations in each plot. θ was used in this study to calculate relative extractable water (REW; Granier, 1987):

$$\mathrm{REW} = \frac{\theta - \theta_m}{\theta_{FC} - \theta_m}, \quad (1)$$

where θ_m is the hygroscopic point where soil water is unavailable for plants (0.125 m^3/m^3), and θ_{FC} is θ at field capacity (0.35 m^3/m^3). Precipitation was measured with a tipping bucket (TE525M; Texas Electronics, Dallas, TX, USA) installed at the top of the tower at the center of plots.

F_{CO2} was measured using the automated carbon efflux system (ACES) developed by the United States Department of Agriculture (USDA) Forest Service (Butnor et al., 2003; Oishi et al., 2014; Palmroth et al., 2005). The ACES is a chamber-based, multiport respiration measurement system that links an infrared gas analyzer (EGM-3 or EGM-4; PP Systems, Amesbury, MA, USA) to 11 soil chambers, randomly distributed in each plot (six under native soil fertility and five under N-fertilized condition). Each soil chamber had a diameter of 25 cm (491 cm^2 measurement area) and a height of 10 cm, and was moved to one of two fixed positions at least once a week to minimize chamber effects on litter input, precipitation and the microenvironment inside the chamber (Oishi et al., 2014). Litterfall which was accumulated on the top of each chamber was added on the forest floor when the chamber was moved to the other position (Oishi et al., 2013). Chambers in harvest quadrants were moved to buffer zones next to unharvested quadrants before the harvest began. Measurements from these chambers were excluded from further analysis from the date the chambers moved to the buffer zones. Each chamber was sampled for 10 min, for a maximum of nine times a day. After allowing the conditions within the chamber to equilibrate with ambient, measurements of the last 3 min were used to calculate mean F_{CO2}. If air flow-rates, or CO_2 concentrations, were unstable or out of a specific range, the data were removed from analysis. Fluxes considered to be abnormal (lower than 0.1 or higher than 30 μmol CO_2 m^{-2} s^{-1}) were also excluded. When more than half of the daily measurements from a soil chamber were available, the daily mean of that soil chamber was used for further analyzes. Filtering out fluxes with unstable or abnormal air flow-rates or CO_2 concentrations created gaps in data. Occasional failure of the gas analyzer, disconnection or leakage of tubing between the ACES and soil chamber, malfunctioning ACES components, power outage, and periodic maintenance for recalibration, also caused missing data. Over the 4 years of study period, ~23% of the time was unavailable for the analyzes.

A Bayesian state space model was used to fill gaps in data and to estimate parameters of interest (e.g., the response of F_{CO2} to atmospheric CO_2 concentration). The Bayesian state space model allows conditional independence between states (x_t); thus, the conditional posterior for the states can be found analytically (Calder, Lavine, Muller, & Clark, 2003). The method also provides full uncertainties of observations, the process model, and parameter estimates (Clark et al., 2011). We modeled $x_t = \log F_{CO2}$ of a chamber at time t as a function of soil temperature (T_t), REW (M_t), daytime mean CO_2 concentration (C_t), N treatment (N_t), and relaxation period (P_t):

$$x_t = \beta_0 + \beta_T T_t + \beta_M M_t + \beta_C C_t + \beta_N N_t + \beta_P P_t + \varepsilon_t, \varepsilon_t \sim N(0, \sigma^2), \quad (2)$$

$$y_t \sim N(x_t, \tau_2), \quad (3)$$

where σ^2 represents process error, y_t represents log of observed soil respiration, and τ^2 represents observation error. In addition to current daytime mean CO_2 concentration, CO_2 concentration with time lags was also tested to identify potential time lags associated with photosynthesis and F_{CO2} (Stoy et al., 2007). We selected conjugate priors for the normal distributions to allow a direct sampling from conditional posteriors and to improve parameter convergence (Clark, 2007). Priors for parameters of interest were represented as normal distributions with mean 0 and variance 100. We used informative priors for process and observation errors (σ^2 and τ^2) based on estimates of variances among chambers and temporal variability in F_{CO2} measurements. σ^2 and τ^2 were represented as inverse gamma distributions with means equal to 0.01 and 0.1, and the prior weights equal to the number of F_{CO2} measurements. Given the hierarchical model structure and conjugate prior distributions, the parameters and latent states in Equations (2) and (3) were drawn directly from the conditional posterior distributions within a Gibbs sampler. When the 95% credible intervals of a posterior mean do not include zero, and the parameter was referred to be significant.

3 | RESULTS

The Bayesian state space model explained most of the variations in daily mean F_{CO2} in 2009 and 2010. In 2009, the last complete year of data collection before the termination of eCO_2, the model shows only a slight underestimation of the rarely measured high fluxes (Fig. S1). The model also estimated parameters separately for each of the 2 years following termination of eCO_2, and for the 2 years combined. The outcome from the two approaches was the same for both aCO_2 and eCO_2 (Fig. S2). Thus, parameter estimates based on the combined data collected during the two last years of enrichment (2009–2010) and during the combined two-year postenrichment (2011–2012) were used to gap fill daily mean F_{CO2}.

The short-term CO_2 response study was divided into two periods based on soil moisture, before and after a major rainfall event. We evaluated whether there was a gradual response with exposure time over the 5 days in each level of CO_2 concentration. Among the 20 available combinations (two soil moisture periods × two soil nutrient conditions × five CO_2 concentrations), we found that none were significant (see Table S2). Given these results, we tested whether the change of F_{CO2} between two intervals was consistent in both amount and direction to the change in CO_2 concentration. We found that there were no consistent responses corresponding to the changes of CO_2 concentration during low, high, and combined soil moisture periods (Fig. S3). Assessing the daily mean F_{CO2} in relation to CO_2 concentration, we found that the regression coefficients for the main effect of CO_2 concentration and the interaction effect between CO_2 concentration and soil temperature were significant, but close to zero (0.03 and 0.04, respectively; Fig. S4); other interaction effects involving CO_2 concentrations (with soil moisture and N fertilization) were not significant. Regardless of soil moisture, daily mean F_{CO2} did not respond to changes in CO_2 concentration at five-day intervals (Figure 1). Yet, consistent with the long-term behavior observed at the site (+17% during last 10 years of CO_2 enrichment; Oishi et al., 2014), plots receiving eCO_2 for many years, maintained higher F_{CO2} when measured under ambient concentration than plots receiving long-term aCO_2—the difference was significant in all but the most restrictive condition of low moisture and N addition (Figure 1c; compare open and closed symbols under aCO_2 concentrations). As commonly observed, F_{CO2} increased with soil water availability.

The gap-filled data showed typical seasonal dynamics corresponding roughly with those of soil temperature, slightly lagging air temperature, with excursions above and below the general pattern corresponding to variation of soil moisture (Figure 2a,b). The difference in F_{CO2} under eCO_2 versus aCO_2 increased in midgrowing season and decreased to a minimum in winter (Figure 2c,d). After terminating eCO_2 in late fall 2010, F_{CO2} in plots of the two treatments converged as in previous winters, remaining similar in the following spring. However, by midsummer, F_{CO2} of previously eCO_2 plots was less than that of aCO_2 plots, a pattern that became even more obvious in the 2012 growing season. The dynamics of F_{CO2} in the N-fertilized portions of the plots were similar, but the amplitude was smaller, without affecting the ratio of F_{CO2} in eCO_2 relative to aCO_2 plots.

Response ratio of F_{CO2} data showed that F_{CO2} in previously eCO_2 plots became similar to that of aCO_2 plots approximately 10 weeks after termination of enrichment and, despite some seasonal variations, remained lower than those of aCO_2 plots for the remainder of the study (Figure 2e). During the last 2 years of enrichment, annual F_{CO2} under eCO_2 was ~210 g C m^{-2} $year^{-1}$ more than that under aCO_2 regardless of soil fertility, while during the 2 years following termination, F_{CO2} decreased in previously enriched plots to ~250 g C m^{-2} $year^{-1}$ less than aCO_2 in native soil fertility plot portions, but to only ~100 g C m^{-2} $year^{-1}$ less than aCO_2 in N-fertilized portions. The change from before to after termination was from an eCO_2/aCO_2 F_{CO2} of 1.20 to 0.77 under native soil, and 1.27 to 0.89 under N-fertilized conditions, or a reduction of ~35% following termination.

We also summarized the long-term responses into annual values, and merged with previous data obtained at the site based on the same methodology (Figure 3). F_{CO2} was higher under eCO_2 in most years before termination of enrichment and was depressed similarly by N addition under aCO_2 (999 and 1,003 g C m^{-2} $year^{-1}$ under

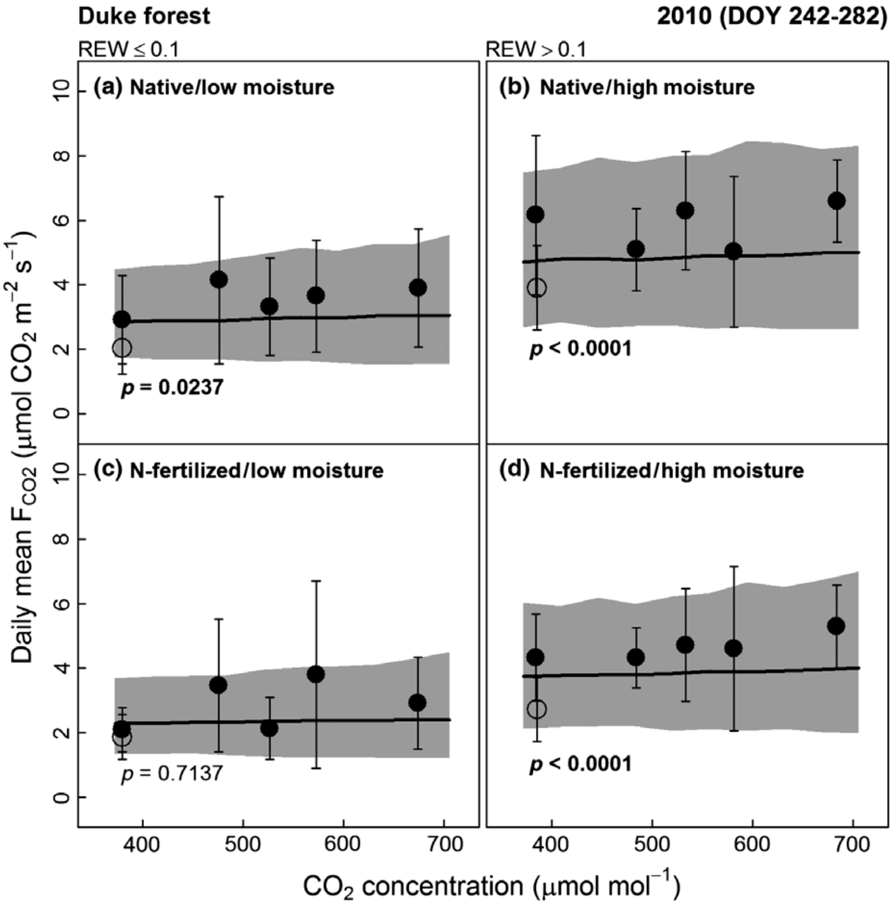

FIGURE 1 Response of daily mean soil CO_2 efflux (F_{CO2}) to five-day manipulation of atmospheric CO_2 concentration under native soil (a, b) and N-fertilized (c, d) conditions. Closed symbols represent long-term elevated CO_2 plots, and open symbols represent ambient CO_2 plots (no CO_2 manipulation). Error bars are 95% confidence intervals of observed F_{CO2}. Solid lines and gray area are mean predicted values and 95% prediction intervals. p-Values test differences of F_{CO2} between ambient and elevated CO_2 plots under ambient CO_2 concentration

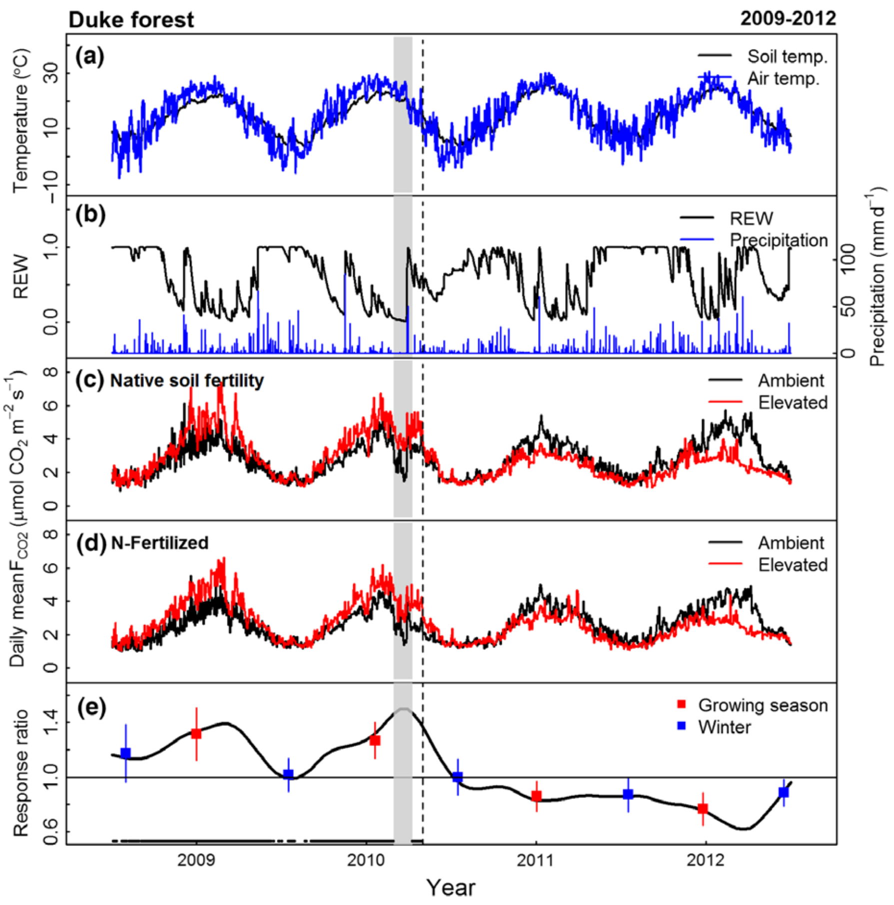

FIGURE 2 Daily mean air and soil temperature (a), relative extractable water (REW) and precipitation (b), soil CO_2 efflux (F_{CO2}) in ambient and elevated CO_2 under native soil fertility (c), F_{CO2} under N-fertilized condition (d), and ratio of F_{CO2} in elevated over ambient CO_2 (e; combined cubic smoothing spline fits to the daily F_{CO2} response ratio from two fertility conditions). Vertical dashed line shows CO_2 enrichment termination. Gray area represents short-term CO_2 response study period. Horizontal solid line is 1:1 line. Squares and error bars of (e) indicate mean response ratios and 95% confidence intervals during growing season (red) and winter (blue). Dots on the bottom of (e) indicate dates with CO_2 enrichment (ambient + 200 ppmv) [Colour figure can be viewed at wileyonlinelibrary.com]

native soil and 856 and 817 g C m^{-2} $year^{-1}$ under N-fertilized condition in 2009 and 2010, respectively) and eCO_2 (1,205 and 1,193 g C m^{-2} $year^{-1}$ under native soil and 1,096 and 1,023 g C m^{-2} $year^{-1}$ under N-fertilized condition), with slightly (but not significantly) higher CO_2 enhancement ratio under N-fertilized conditions than under native soil conditions. The enhancement ratios were unrelated to annual mean soil temperature or growing season REW. Following termination of enrichment the annual F_{CO2} in previously eCO_2 plots (848 and 820 g C m^{-2} $year^{-1}$ under native soil and 823 and 807 g C m^{-2} $year^{-1}$ under N-fertilized condition in 2011 and 2012, respectively) decreased to less than that of aCO_2 plots (1,029 and 1,137 g C m^{-2} $year^{-1}$ under native soil and 873 and 968 g C m^{-2} $year^{-1}$ under N-fertilized condition), with the decrease apparently greater under native soil (Figure 3).

The model parameter estimates showed 10% higher base respiration (at 15°C) in eCO_2, falling to 22% lower following termination under native soil conditions (+14% and −11%, respectively, in N-fertilized plot portions; Table 1). In contrast, the temperature sensitivity of F_{CO2} was the same in both CO_2 treatments during the enrichment period, but decreased 9% after termination, regardless of soil fertility. The sensitivity to soil moisture was ~5% lower under eCO_2 and did not change after termination.

To further investigate potential causes of the large decrease in F_{CO2} after the termination of enrichment, we related the flux to estimates of leaf area index (L) obtained from the same data and methodology as in McCarthy et al. (2007), available throughout the study (Figure 4). We found that under aCO_2, maximum monthly mean L was somewhat lower (~0.5 m^2/m^2) in the final 2 years of observation (2011–2012) relative to the previous 2 years (2009–2010), yet monthly F_{CO2} at a given L increased significantly (~25% change in slopes), slightly more in N-fertilized than native soil conditions (Figure 4a,c). The increase in F_{CO2} was consistent with warmer and wetter conditions in the latter 2 years; mean (and standard deviation) of air temperature and growing season REW in the former 2 years were 14.7 (9.1)°C and 0.38 (0.31), whereas those in the latter 2 years were 15.7 (8.0)°C and 0.51 (0.34). In contrast, termination of enrichment in eCO_2 plots resulted in a much greater reduction of L (~1.5 m^2/m^2), as well as a large reduction of F_{CO2} at a given L, with the effect slightly less pronounced under N-fertilized conditions. These observations were consistent in all plots. When normalized by L in aCO_2 plots (thus accounting for decrease in L unrelated to termination of eCO_2), the reduction of L in eCO_2 plots was ~15%, while the reduction of the slope (similarly normalized) was ~50%. There was a time lag of 2 months between maximum canopy-scale photosynthesis and peak L at the site (McCarthy et al., 2007; Schäfer et al., 2003). Attempting to lag correlate the two time series did not change the outcomes, but reduced the proportion of variation in F_{CO2} explained by L by >30%.

We also analyzed F_{CO2} data during growing season from four FACE experiments in temperate forests (Aspen FACE, Duke FACE, ORNL FACE and POPFACE; Allen et al., 2000; Andrews &

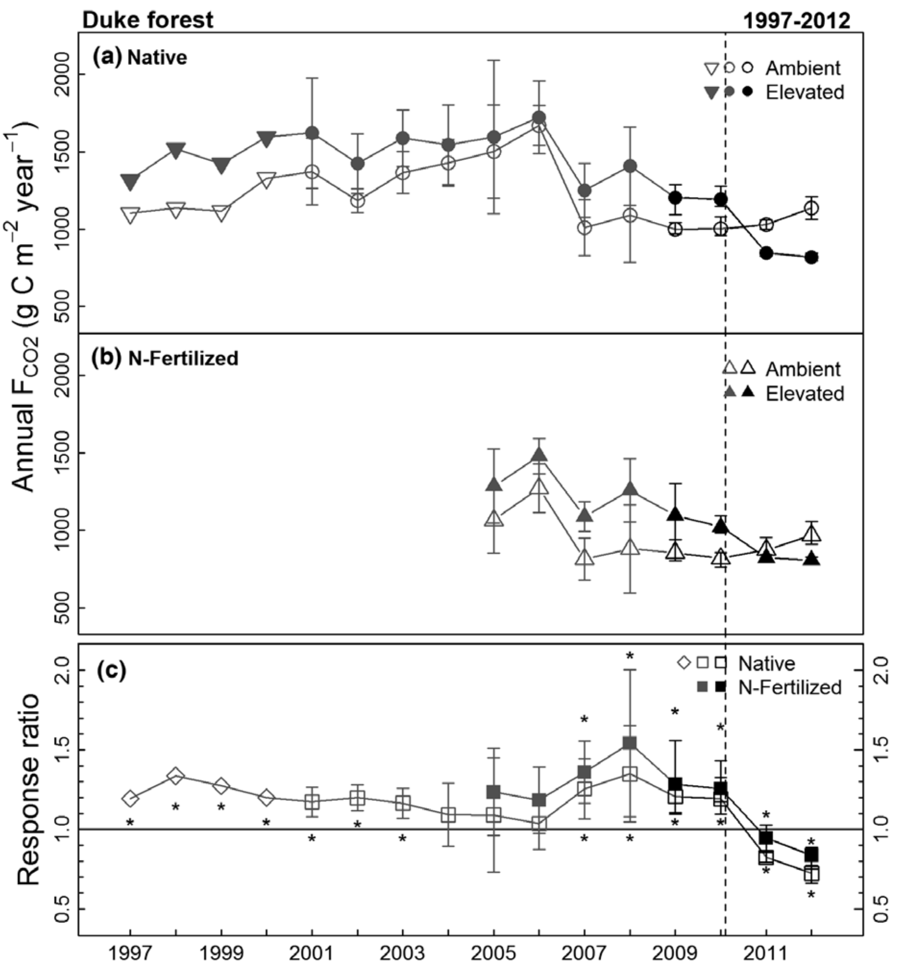

FIGURE 3 Annual soil CO_2 efflux (F_{CO2}) under native soil fertility (a; inverted triangles and circles), annual F_{CO2} under N-fertilized condition (b; triangles), and F_{CO2} response ratio (c; diamonds and squares) from 1997 to 2012. Error bars are 95% confidence intervals. Values in 1997–2000 (gray inverted triangles and diamonds) are adjusted means from King et al. (2004), Bernhardt et al. (2006) and Jackson et al. (2009); values in 2001–2008 (gray circles, triangles and squares) are from Oishi et al. (2014). Asterisks indicate significant difference of the response ratio from 1.0. Vertical dashed line shows CO_2 enrichment termination. Horizontal solid line in (c) is 1:1 line

TABLE 1 Means (and 95% credible intervals) of base respiration (β_0), temperature sensitivity (β_T) under nonlimiting soil moisture condition, and moisture sensitivity (β_M) under mean soil temperature condition before and after the termination of CO_2 enrichment

		Native			N-fertilized		
Parameter	Period	Ambient	Elevated	E/A	Ambient	Elevated	E/A
β_0 (μmol CO_2 m^{-2} s^{-1})	Before	2.70 (2.68, 2.72)	2.98 (2.96, 3.01)	1.10[a]	2.29 (2.27, 2.31)	2.60 (2.58, 2.63)	1.14[a]
	After	2.63 (2.62, 2.65)	2.06 (2.04, 2.09)	0.78[a]	2.23 (2.21, 2.25)	1.99 (1.96, 2.02)	0.89[a]
β_T	Before	1.48 (1.47, 1.49)		1.00	1.48 (1.47, 1.49)		1.00
	After	1.42 (1.41, 1.43)	1.29 (1.29, 1.30)	0.91[a]	1.42 (1.41, 1.43)	1.29 (1.29, 1.30)	0.91[a]
β_M	Before	1.01 (1.01, 1.01)	0.97 (0.97, 0.97)	0.96[a]	1.01 (1.01, 1.01)	0.97 (0.97, 0.97)	0.96[a]
	After	1.01 (1.01, 1.01)	0.96 (0.96, 0.97)	0.95[a]	1.01 (1.01, 1.01)	0.96 (0.96, 0.97)	0.95[a]

[a] Ratio is significantly different from 1.

Schlesinger, 2001; Jackson et al., 2009; King et al., 2004) and those from studies using other approaches to reduce or eliminate carbohydrate movement belowground (Bhupinderpal-Singh et al., 2003; Drake et al., 2012; Hasselquist et al., 2016; Högberg et al., 2001; Jing et al., 2015; Johnsen et al., 2007). Under FACE treatment, which increases the C supply belowground, the response ratio of F_{CO2} became higher than one approximately 400 days after the initiation of the treatment at Duke FACE; when combined, growing season F_{CO2} in broadleaved deciduous forests from the other three FACE experiments became greater than those under ambient condition approximately 180 days after the initiation of the treatment (Figure 5). When C transport belowground was hindered by girdling, trenching, stem compression, shading, or chilling, the response ratio of F_{CO2} became lower than one approximately 10 days after the commencement of the treatment. When treatment was terminated at Duke FACE, the ratio of F_{CO2} became lower than its long-term average enhancement ratio (set as one in Figure 5) approximately 120 days after the termination.

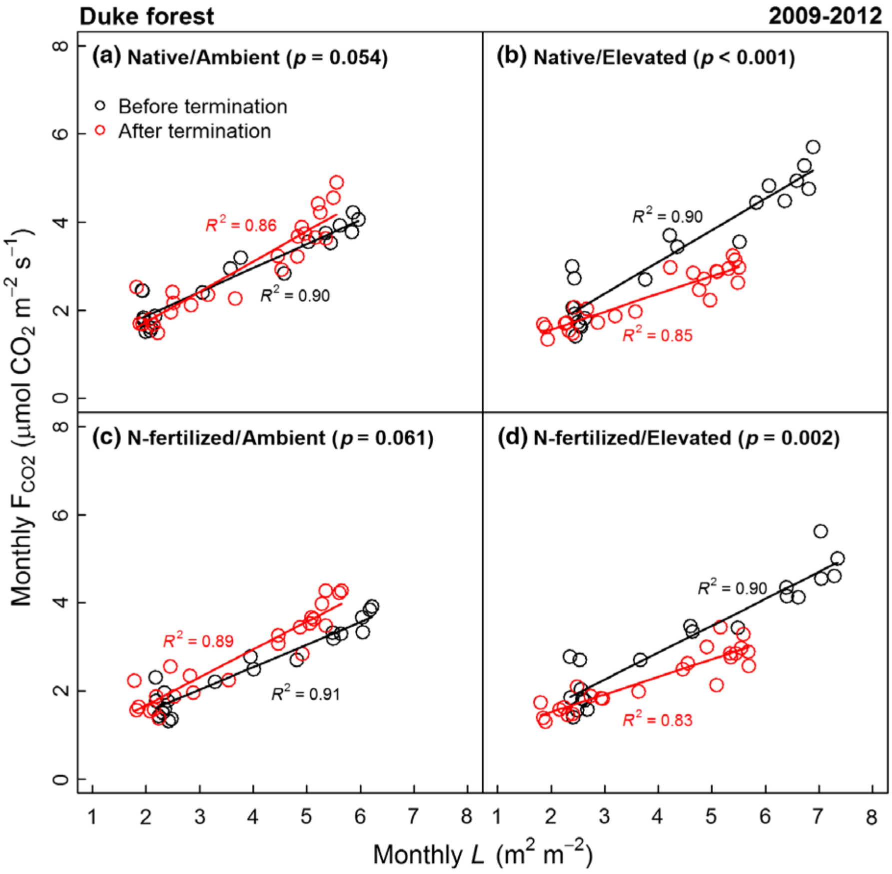

FIGURE 4 Relationship between monthly mean soil CO_2 efflux (F_{CO2}) and monthly mean leaf area index (*L*) under native soil (a, b) and N-fertilized (c, d) conditions. Black and red symbols indicate values before and after the termination of CO_2 enrichment. Displayed *p*-values test for differences between linear fits before and after. All linear fits are significant ($p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

F_{CO2} in eCO_2 plots was not affected by short-term (five-day) manipulation of atmospheric CO_2 concentration ranging from ambient to 300 ppmv above ambient. However, prolonged exposure to aCO_2 after the termination of the long-term CO_2 enrichment reduced F_{CO2} in previously eCO_2 plots to lower than that in aCO_2 plots for at least 2 years.

Allocation of extra carbohydrates belowground under eCO_2 facilitate growth of roots and mycorrhizae, improve the efficiency of resource utilization, increase substrate (necromass and litterfall) available for decomposition, and enhance F_{CO2} (Allen et al., 2000; Drake et al., 2011; Finzi et al., 2015; Pritchard et al., 2008). These changes in the forest floor–soil system occur slowly, taking months to years to affect F_{CO2} and be observed (Allen et al., 2000; Andrews & Schlesinger, 2001; King et al., 2004).

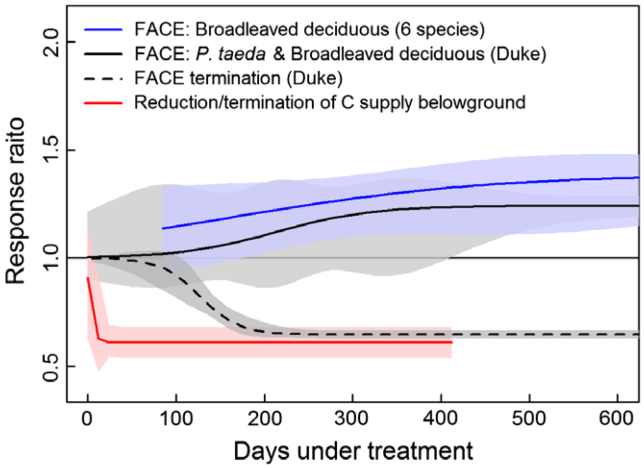

FIGURE 5 Temporal patterns of F_{CO2} response ratios (elevated/ambient) following the initial free-air CO_2 enrichment (FACE) in three experiments on six broadleaved deciduous species (blue solid line) and one experiment (Duke) in a *Pinus taeda* dominated stand (black solid line), the termination of the long-term Duke FACE (dashed line) and the reduction of carbohydrate supply belowground from 13 experiments on six species (red solid line). Gray, blue, and red areas represent 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]

In contrast to the forest floor–soil system, changes in photosynthetic rates affect the delivery of carbohydrates belowground within days (Andrews et al., 1999; Mortazavi et al., 2005). On sandy soils, temporary blockage of sugar transport to roots reduced F_{CO2} within ~6 days, but only in fall (Henriksson et al., 2015; Johnsen et al., 2007), when recently produced carbohydrates are used in the production of fine roots and mycorrhizae (Högberg et al., 2001). At that time, nonstructural carbohydrate reserves in fine roots are typically low (Oren et al., 1988), potentially causing a strong coupling between the activity of the belowground constituents in these ecosystems and the supply of recently assimilated carbohydrates. However, when CO_2 concentration was manipulated in eCO_2 plots to between ambient and nearly 80% over ambient, causing a similar effect on carbohydrate production (Tor-Ngern et al., 2015), F_{CO2} did not change under either soil N availability regime (Figure 1), thus refuting **H1**. Although lower stomatal conductance when the soil was dry (Tor-Ngern et al., 2015) may reduce photosynthesis and carbohydrate supply belowground, the carbohydrate reserve under eCO_2 appeared to have been sufficient to meet the demand of carbohydrate-dependent constituents. Thus, the large fluctuations of photosynthetic rates imposed by short-term step changes of CO_2 concentration had no effect on F_{CO2} in eCO_2 plots, which has been exposed to long-term eCO_2, regardless of soil moisture (Figure 1). A somewhat lower belowground activity during drought (Figure 1) may reflect restricted microbial activity under reduced soil water availability (Borken & Matzner, 2009). Carbohydrate concentrations in fine roots were unrelated to any treatment at the Duke FACE, although no measurements were made in fall (Drake et al., 2008). If the similarity of carbohydrate reserves among treatments held through the fall, the results suggest that carbohydrate demand was less than the combined availability from reserves and the up to ~30% lower photosynthesis expected when eCO_2 plots were subjected to aCO_2 (Schäfer et al., 2003).

When eCO_2 plots were subjected to aCO_2 for 5 days, F_{CO2} remained higher than that of aCO_2 plots under all but dry soils under N-fertilized conditions (Figure 1), reflecting the difference in belowground biomass and activity (~50% more fine root biomass and 90% more mycorrhizal standing crop in native soil, and 20% more fine roots in N-fertilized soil; Drake et al., 2008; Pritchard et al., 2014). Thus, while carbohydrate reserves under eCO_2 may have been sufficient to buffer short-term fluctuations in CO_2 concentration, a prolonged reduction of photosynthesis after the termination of enrichment must impact the larger biomass of belowground constituents in eCO_2 plots. The soils of the site are relatively warm, which should cause a rapid utilization of root carbohydrate reserves (Marshall & Waring, 1985), followed by a large contraction of the root system. This proposed response is consistent with the large drop in base respiration (Table 1), which was somewhat less in N-fertilized subplots in which the root-mycorrhizal system had decreased following N addition. The concurrent reduction in the temperature sensitivity of respiration (Table 1) may reflect downregulation matching the curtailed carbohydrate supply. The changes in base respiration and temperature sensitivity were reflected in a rapid decline of postenrichment F_{CO2} in eCO_2 plots (Figures 2 and 3). The daily mean F_{CO2} data showed that the eCO_2/aCO_2 response ratio of F_{CO2} dropped to one ~10 weeks after termination of enrichment, and remained approximately 0.8 for the final 2 years of the study, forcing a rejection of **H2**.

A large contraction of the root system may enhance microbial activity by providing extra substrates (necromass of fine root and mycorrhizal fungi mass). However, the stimulation of microbial activity was not manifested as an increase in F_{CO2} in this study. Reduced labile carbohydrate supply belowground may have diminished microbial activity (Drake et al., 2012). It is also possible that increased C input through litterfall and fine root turnover under long-term FACE treatment (Lichter et al., 2008; Pritchard et al., 2008) may have already provided an abundant C source for microorganisms, and thus diminishing the effect of extra substrate on heterotrophic respiration. It is also possible that early responses of microorganisms to termination of FACE were suppressed by low soil temperature (Bhupinderpal-Singh et al., 2003).

The contraction of the root–mycorrhizal system exceeded what can be expected by simple scaling to lowered canopy photosynthesis (Kim, 2016). Canopy photosynthetic C uptake and stand level net primary productivity before termination were ~40% and ~28% higher in eCO_2 than aCO_2 plots (McCarthy et al., 2010; Schäfer et al., 2003), reflecting the higher atmospheric CO_2 concentration (Schäfer et al., 2003), and ~17% greater *L* (McCarthy et al., 2007), and the largely unaffected ratio of the leaf internal to external concentration of CO_2 (Ellsworth et al., 2012) and stomatal conductance (Kim, 2016; Tor-Ngern et al., 2015). Following eCO_2 termination, deeply shaded leaves may have no longer been able to support themselves, and certainly could not export carbohydrates to support the root–mycorrhizal system (Sprugel, 2002). Thus, reductions in both *L* and F_{CO2} were expected and observed (Figure 4). However, while the reduction of *L* in previously eCO_2 plots resulted in *L*, and likely photosynthesis, becoming similar to that observed in aCO_2 plots, the reduction brought F_{CO2} of previously eCO_2 plots to ~20% less than that under aCO_2 (Figure 3). Although we could not directly quantify the response of belowground constituents to reductions in carbohydrate availability, the seasonal analysis may shed some light on the dynamics of the response.

In *P. taeda* stands, maximum canopy-scale photosynthesis occurs in mid-June, preceding the peak *L* time by 2 months (McCarthy et al., 2007; Schäfer et al., 2003). Even though the dynamics of *L* and photosynthesis are not synchronized, those of *L* and F_{CO2} are matched both before and after termination of enrichment (Figure 4). Assuming, based on the above, that canopy photosynthesis in previously eCO_2 plots following the termination was similar to that in aCO_2 plots, the reduction of F_{CO2} in previously eCO_2 plots at the same level of *L* suggests a large contraction of the live fine root–mycorrhizal system that is both disproportionate to the contraction of canopy *L*, and not balanced by an increased mass and activity of organism decomposing the swelling necromass pool.

We wish to note that, unlike the short-term experiment, early responses of F_{CO2} after termination of enrichment were observed through late fall and winter when the responses of F_{CO2} to eCO_2 were small or none during the long-term enrichment period (Jackson et al., 2009). Thus, the initial response of F_{CO2} to termination of enrichment likely reflects a yearly diminishing effect in winter combined with reduced C supply belowground. Furthermore, our postenrichment monitoring did not last long enough to determine when the belowground system recovered a similar activity to aCO_2 plots, and the end of time series may have confounded dormant season F_{CO2} with recovery (Figure 2). Nevertheless, we note that during the last dormant season, F_{CO2} of previously eCO_2 plots has not yet fully matched that of aCO_2 plots, as it had done commonly during the enrichment period (Figure 2e).

The necromass of roots and associated mycorrhizae enlarges when carbohydrate allocation to belowground increases, because the live biomass pool size increases and turns over faster (Drake et al., 2008; Lukac et al., 2003). The size of the necromass pool also enlarges when belowground allocation decreases, because of extensive fine root and mycorrhizal mortality of the contracting root system. While the size of the necromass pool increases with both increasing and decreasing belowground C allocation, the amount of living, respiring fine root mass (and associated mycorrhizae) increases or decreases in concert with carbohydrate allocation (Hasselquist et al., 2016; Högberg et al., 2001; Jing et al., 2015). We found that the aforementioned changes, and associated changes in rhizosphere microorganisms that live on root exudates and necromass (Luo et al., 2001; Phillips, Finzi, & Bernhardt, 2011), cause treatments that enhanced C supply belowground to increase F_{CO2} at appreciably longer temporal lags (~180 and ~400 days in broadleaved and *P. taeda* stands, respectively) than those reducing it (~10 days), even though the same constituents are involved (Figure 5). Reducing carbohydrate production upon termination of eCO_2 in the latter stand had an intermediate lag (~120 days). These asymmetric responses pose a tractable challenge to process-based models attempting to isolate the effects of individual processes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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