Sustained effects of atmospheric [CO_2] and nitrogen availability on forest soil CO_2 efflux

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Abstract

Soil CO_2 efflux (F_{soil}) is the largest source of carbon from forests and reflects primary productivity as well as how carbon is allocated within forest ecosystems. Through early stages of stand development, both elevated [CO_2] and availability of soil nitrogen (N; sum of mineralization, deposition, and fixation) have been shown to increase gross primary productivity, but the long-term effects of these factors on F_{soil} are less clear. Expanding on previous studies at the Duke Free-Air CO_2 Enrichment (FACE) site, we quantified the effects of elevated [CO_2] and N fertilization on F_{soil} using daily measurements from automated chambers over 10 years. Consistent with previous results, compared to ambient unfertilized plots, annual F_{soil} increased under elevated [CO_2] (ca. 17%) and decreased with N (ca. 21%). N fertilization under elevated [CO_2] reduced F_{soil} to values similar to untreated plots. Over the study period, base respiration rates increased with leaf productivity, but declined after productivity saturated. Despite treatment-induced differences in aboveground biomass, soil temperature and water content were similar among treatments. Interannually, low soil water content decreased annual F_{soil} from potential values – estimated based on temperature alone assuming nonlimiting soil water content – by ca. 0.7% per 1.0% reduction in relative extractable water. This effect was only slightly ameliorated by elevated [CO_2]. Variability in soil N availability among plots accounted for the spatial variability in F_{soil}, showing a decrease of ca. 114 g C m^{-2} yr^{-1} per 1 g m^{-2} increase in soil N availability, with consistently higher F_{soil} in elevated [CO_2] plots ca. 127 g C per 100 ppm [CO_2] over the +200 ppm enrichment. Altogether, reflecting increased belowground carbon partitioning in response to greater plant nutritional needs, the effects of elevated [CO_2] and N fertilization on F_{soil} in this stand are sustained beyond the early stages of stand development and through stabilization of annual foliage production.

Keywords: automated soil respiration measurements, FACE, nitrogen fertilization, *Pinus taeda*, primary productivity, soil water content

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Introduction

Aggrading plantation forests have shown enhanced productivity under projected future atmospheric [CO_2] levels, (Oren *et al.*, 2001; Liu *et al.*, 2005; Norby *et al.*, 2005; Liberloo *et al.*, 2006; McCarthy *et al.*, 2006), leading to increased aboveground production of leaf and woody biomass (McCarthy *et al.*, 2007, 2010), as well as belowground production of root biomass (Norby *et al.*, 2004; Pritchard *et al.*, 2008a; Jackson *et al.*, 2009). Increased productivity under elevated [CO_2] typically corresponds to an increase in soil CO_2 efflux (F_{soil}; King

et al., 2004), resulting from higher root respiration (Drake *et al.*, 2008), as well as microbial respiration associated with more soluble carbon (C) released from roots (Phillips *et al.*, 2011) and greater microbial biomass (Bader & Körner, 2010). Thus, increased C uptake by canopies may be offset by losses through ecosystem respiration. Because F_{soil} is the largest component of ecosystem respiration, and a major component of the global atmospheric [CO_2] budget (Ryan & Law, 2005), understanding the response of F_{soil} to elevated [CO_2] is essential for predicting future C cycling.

However, uncertainty remains about the magnitude of the long-term stimulation effect of elevated [CO_2] on F_{soil}, which seems to decrease with stand age, and be affected by species composition (King *et al.*, 2004).

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Initial findings from the Duke Free-Air CO$_2$ Enrichment (FACE) experiment at a *Pinus taeda* L. (loblolly pine) stand showed a 16% increase in F_{soil} under elevated [CO$_2$] in one pair of plots (Butnor *et al.*, 2003); however, longer term studies (using different measurement methods) in replicated plots were inconsistent, showing that enhancement of F_{soil} of the elevated [CO$_2$] relative to ambient treatment may be decreasing (Bernhardt *et al.*, 2006) or increasing (Jackson *et al.*, 2009).

These contrasting results may be attributed to several factors. First, some studies only span a few years, making it difficult to distinguish trends in ecosystem physiological responses to elevated [CO$_2$] from interannual variability in environmental factors. In addition, the measurement frequency of many of these studies may have not provided the temporal resolution to quantify the sensitivity of F_{soil} to environmental drivers (Savage & Davidson, 2003). Finally, most of these studies have spanned an aggrading period of stand development, characterized by increases in height, basal area, and leaf area with potential effects on carbon sources and sinks. Published studies from the Duke FACE experiment covered the forest as it developed from 16 to 27 years of age, characterized by fluctuating, but generally increasing foliage production (McCarthy *et al.*, 2007, 2010). While within and across biomes, F_{soil} tends to increase with aboveground productivity, canopy leaf area, and leaf production (Davidson *et al.*, 2002; Palmroth *et al.*, 2005; Litton *et al.*, 2007; Bond-Lamberty & Thomson, 2010; Chen *et al.*, 2011), there is still uncertainty about the sustained effect of elevated [CO$_2$] after canopy closure. Indeed, advanced age may have been the reason a recent study of a mature broadleaf forest (in a webFACE setting) showed no enhancement of production and F_{soil} under elevated [CO$_2$] (Bader & Körner, 2010).

The effects of elevated [CO$_2$] on F_{soil} cannot be fully understood without consideration of other factors affecting above vs. belowground C allocation. Increased nitrogen (N) availability in N-limited forests – through forest nutrient management, atmospheric deposition, or reflecting variation in native N availability – can increase aboveground production while equally reducing belowground production and respiration, within and among forest stands (McCarthy *et al.*, 2006; Palmroth *et al.*, 2006). In earlier studies at Duke FACE and other loblolly pine plantations, fertilization has been shown to increase aboveground woody biomass, leaf biomass, and also coarse and tap root biomass (Albaugh *et al.*, 1998, 2004, 2008; Oren *et al.*, 2001; Will *et al.*, 2006; McCarthy *et al.*, 2007, 2010; Samuelson *et al.*, 2008; Jackson *et al.*, 2009). However, N fertilization led to decreases in the other belowground components: the fine root and microbial biomass (Lee & Jose, 2003;

Jackson *et al.*, 2009; Janssens *et al.*, 2010; Rifai *et al.*, 2010). Overall, the shift in C partitioning away from belowground components (Butnor *et al.*, 2003; Palmroth *et al.*, 2006) has caused reductions in F_{soil} ranging 10–23% (Butnor *et al.*, 2003; Jackson *et al.*, 2009).

Nutrient availability can limit elevated [CO$_2$]-induced enhancement of biomass production (Oren *et al.*, 2001), resulting in belowground allocation of much of the extra C assimilated (Norby *et al.*, 2004; Palmroth *et al.*, 2006). At Duke FACE, the combination of elevated [CO$_2$] and N fertilization increased net primary productivity (NPP; McCarthy *et al.*, 2006, 2010) and decreased F_{soil} compared to the unfertilized, elevated [CO$_2$] treatment, resulting in F_{soil} similar to the unfertilized, ambient treatment (Butnor *et al.*, 2003; Palmroth *et al.*, 2006; Jackson *et al.*, 2009). Thus, although elevated [CO$_2$] typically increases C supply to production and respiration both aboveground and belowground, high soil nutrient availability may nullify the effect of elevated [CO$_2$] on soil respiration (Palmroth *et al.*, 2006).

The temporal variability in F_{soil} reflects high-frequency dynamics of C assimilation and transport belowground (Johnsen *et al.*, 2007; Stoy *et al.*, 2007; Högberg *et al.*, 2008; Mencuccini & Hölttä, 2010), as well as lower frequency dynamics of soil water content and temperature (T_{soil}). Soil CO$_2$ efflux increases with T_{soil}, reflecting enzyme kinetics, but can be depressed during periods of drought due to reductions in microbial activity, with the sensitivity of F_{soil} to both environmental variables also reflecting their effect on the supply of C belowground (Fang & Moncrieff, 2001; Davidson & Janssens, 2006). Indeed, both spatially and temporally, F_{soil} reflects how assimilated C is allocated among metabolic and structural demands under the biophysical constraints of climate and resource availability (Curiel Yuste *et al.*, 2005; Litton *et al.*, 2007). Thus, because much of the increased belowground C allocation under elevated [CO$_2$] is used in fine root production, particularly at greater soil depths (Norby *et al.*, 2004; Pritchard *et al.*, 2008a), the effects of soil water limitations on F_{soil} may be reduced if these roots provide greater access to water. F_{soil} may also be inversely correlated with leaf area index (LAI), reflecting the combined effects of a shift of C allocation from below- to aboveground with increasing LAI, and reduced soil water content and temperature induced by the higher LAI (Oishi *et al.*, 2013).

To examine the long-term effects of elevated [CO$_2$] and soil N on F_{soil}, we used automated soil CO$_2$ efflux systems to take diurnal measurements at the Duke FACE loblolly pine plantation. We expand on previous work at the site by examining the influence of short-term (daily, rather than monthly) variation in

environmental drivers on F_{soil} among all eight FACE plots, including 10 years in two plots and over 5 years in the remaining six plots, a period during which each plot was halved and one half fertilized with N. The approach follows Palmroth *et al.* (2005) and Oishi *et al.* (2013) in quantifying the sensitivity of F_{soil} to the two primary environmental drivers of F_{soil}: T_{soil} and soil water content. These environmental drivers vary seasonally and interannually, but may also be affected by treatment-induced differences in aboveground stand characteristics; as leaf area increases with $[CO_2]$ and N, radiation and water reaching the forest floor may decrease. Therefore, we also examine the linkages between F_{soil} and stand characteristics that are easily observable from aboveground biometric measurements or remote sensing, which may help to refine regional-scale estimates of F_{soil}.

Using automated monitoring at a high temporal resolution, covering wide ranges of environmental conditions, we developed robust functions and assessed treatment effects on the sensitivities of F_{soil} to soil conditions vs. treatment effects on these conditions, and how these effects translate to differences in annual fluxes. We analyzed F_{soil} data with the aim to identify whether long-term trends exist and, if so, whether these reflect changes in C allocation among treatments, while accounting for potential changes in the main driving variables. Our guiding hypotheses were as follows: (H1) Reflecting changes to belowground C supply, for a given T_{soil} and nonlimiting soil water content, F_{soil} will be higher under elevated $[CO_2]$, lower with N fertilization, and, as a result of canceling effects, will be similar under the combined treatment to the reference plots. (H2) Reflecting the contrasting treatment effects on fine root biomass and access to deeper sources of water, the reduction in F_{soil} with decreasing soil water content will be proportionally lower under elevated $[CO_2]$ than under ambient $[CO_2]$, and proportionally higher under N fertilization. (H3) Reflecting increases in LAI and litter fall, the environmental drivers of F_{soil}, T_{soil} and soil water content, will decrease with $[CO_2]$ and N supply, and even more under their combination. We then assess how these responses combine to produce differences in annual F_{soil} among treatments, and explore how well simple metrics of aboveground productivity (LAI and leaf litter fall) explain the variability in annual F_{soil} within and among treatments.

Materials and methods

Study site

The study was conducted at the Duke Forest FACE site in central North Carolina, USA (35°58′N, 79°06′W). The loblolly pine

stand was planted in 1983 at 2×2.4 m spacing and included a substantial component of broadleaved species from natural regeneration. Broadleaf crowns dominated the lower and midcanopy, with some extending into the upper canopy (McCarthy *et al.*, 2007). However, in ambient $[CO_2]$ unfertilized plots, pine comprised 75% of the leaf area (mean of 1999–2003; McCarthy *et al.*, 2007) and >90% of the standing biomass (through 2004; McCarthy *et al.*, 2010). Stem density as of 2004 for pine with DBH >8 cm was 1,214 (SD = 297) trees ha^{-1} and for broadleaf with DBH >2 cm 2,603 (SD = 824) trees ha^{-1} (McCarthy *et al.*, 2010; see Table 1 for other stand characteristics). The experiment consists of eight circular plots with 30 m diameters, four exposed to ambient $[CO_2]$ and four receiving 200 μmol mol^{-1} $[CO_2]$ above ambient. The paired prototype and reference plots (plots 7 and 8, respectively) were established in 1993, with $[CO_2]$ enrichment commencing in 1994. Atmospheric $[CO_2]$ enrichment of the adjacent replicates (plots 1–6) began in 1996. In 1998 plots 7 and 8 were divided in half with an impermeable barrier to a soil depth of 70 cm, below the reach of the majority of fine roots (Matamala & Schlesinger, 2000), and one half received annual N fertilization of ammonium nitrate pellets (11.2 g N m^{-2} yr^{-1}). This fertilization protocol was established in plots 1–6 beginning in 2005. Native N availability was defined as the sum of N mineralization, deposition, and fixation (previously presented in McCarthy *et al.*, 2010) and we assumed that 20% of added N (2.2 g N m^{-2}) was available for uptake (Nason & Myrold, 1992).

Terms relating to the spatial aggregation of samples are described as follows: plot refers to one of the eight circular FACE rings, subplot refers to half of a plot, divided into the fertilized and unfertilized sections, and treatment refers to a group of four subplots receiving the same combination of $[CO_2]$ and N. Plot pairs are also considered as blocks such that each of four blocks contain one of each treatment. Treatment abbreviations are as follows: ambient $[CO_2]$ unfertilized (AU), elevated $[CO_2]$ unfertilized (EU), ambient $[CO_2]$ fertilized (AF), and elevated $[CO_2]$ fertilized (EF).

Table 1 Stand characteristics (AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized)

	AU	EU	AF	EF
Stand biomass (g C m^{-2})*	9330	11 180	9310	10 100
Stand NPP (g C m^{-2} yr^{-1})*	910	1180	1070	1210
Mean annual LAI (m^2 m^{-2})**	3.8	4.6	4.2	4.8
Leaf litter fall (g C m^{-2} yr^{-1})**	301	368	344	377

*Mean from 2001 to 2004; $n = 4$ for AU and EU; $n = 1$ for AF and EF (McCarthy *et al.*, 2010).
**Mean from 2005 to 2010, includes data from McCarthy *et al.* (2007).

Instrumentation

Soil temperature was measured in each FACE plot with a permanently installed thermistor buried at 10 cm (T_{10}). Additional T_{soil} measurements at 5 cm (T_5) from thermocouples installed at the rotating F_{soil} measurement chamber locations were available for noncontinuous portions of the study period (additional details below). Volumetric soil water content (θ) was obtained with four time-domain reflectometry probes (CS-615; Campbell Scientific, Logan, UT, USA) installed vertically at 0–30 cm depth at each plot. Sensors were installed prior to dividing plots for N fertilization, so the four sensors were not always evenly divided among subplots. To increase the generality of our results, soil water content is expressed in this study as relative extractable water [REW $=(\theta-\theta_m)/(\theta_{FC}-\theta_m)$; Granier, 1987], where θ is the volumetric water content, θ_m is the hygroscopic point where soil water is no longer available for plants (0.125 m^3 m^{-3}), and θ_{FC} is θ at field capacity (0.35 m^3 m^{-3}). Incoming precipitation was measured with an above-canopy tipping bucket (TE525M; Texas Electronics, Dallas, TX, USA) installed at the top of a walk-up tower.

F_{soil} was measured using the Automated Carbon Efflux System (ACES, USDA Forest Service, US Patent 6692970). The system has been described in previous studies (Butnor *et al.*, 2003; Palmroth *et al.*, 2005), but briefly, it is an IRGA-based open system that sequentially samples 15 chambers plus one null chamber (491 cm^2 soil chamber measurement area, 10 cm height). Each chamber is sampled for a 10 min cycle and the final record is accepted if air flow rates and CO$_2$ concentrations are stable and within a specified range. Thus, a maximum of nine measurements for each chamber throughout each day is possible. IRGAs (EGM-2, EGM-3, EGM-4; PP Systems, Amesbury, MA, USA) were calibrated monthly with 500 μmol mol^{-1} [CO$_2$] standard gas.

Plots 7 and 8 include data from 2001 to 2010. Additional ACES was added to plots 1–4 and 6 in early 2005, and plot 5 in 2006. Six of the soil chambers were positioned in the unfertilized subplots and five chambers were positioned in the fertilized subplots. The ACES operation and data analysis in this study follow Oishi *et al.* (2013), who analyzed only the AU subplots, but we provide a detailed description below.

Each chamber was switched between two fixed locations in the plot every 3–4 days. Chamber switching was intended to minimize chamber effects on the amount of litter and moisture arriving at the monitored surface, and increased the number of locations sampled. Chamber locations were changed several times during the study period, initially to minimize disturbance to a sampling area and later to examine variability with proximity to trees. The chamber bases have a sharp, metal edge that extended below the soil surface ca. 1 cm, but do not use a permanently installed collar. Forest floor vegetation was sparse and chambers did not cover any vegetation. Any visible aboveground vegetation was removed weekly. Litter fall excluded by the closed-top soil chambers during measurement cycles was replenished with litter collected in baskets within each plot up to 2005 and, from 2005 on, with litter accumulated on the top of each chamber (Oishi *et al.*, 2013).

Automated carbon efflux system is designed to run continuously; however, several factors reduced the amount of usable data. First, individual measurements are filtered to exclude sampling periods where either air flow or CO$_2$ concentrations were out of range. Second, systems were offline periodically for general maintenance and recalibration. Third, over the long duration of the study, systems were offline for major maintenance more frequently. Therefore, the measurements from all systems were not continuous throughout the study period. From 2001 through 2010, we collected a total of 69,996 acceptable individual measures of F_{soil} across the 16 subplots. Of the 55 plot-years, systems were operational for at least 50 days per year for 45 plot-years, at least 100 days for 34 plot-years, and at least 200 days for 19 plot-years. Missing data were gap filled using the T_{soil} and REW response functions described below. Measurements did encompass virtually all the environmental variability that occurred over the past decade, including several droughts and wet growing seasons.

To analyze the treatment effects on the sensitivity of F_{soil} to environmental variables, we utilized the model for F_{soil} as a function of T_{soil} and REW previously described in Palmroth *et al.* (2005):

$$F_{soil} = F^*_{soil} \times f_{REW} \tag{1}$$

where F_{soil} is mean daily soil CO$_2$ efflux (μmol CO$_2$ m^{-2} s^{-1}), F^*_{soil} is potential F_{soil} for a given T_{soil} under nonlimiting REW, and f_{REW} is the reduction function for soil water content–limited conditions, see Eqn (4). We aggregated F_{soil} by subplot, first taking the daily mean from individual chamber locations where >4 daily measurements were available, then calculating the daily mean F_{soil} for each subplot as the mean of the daily mean from each chamber. We then estimated F^*_{soil} by fitting mean daily F_{soil} as a function of mean daily T_{10} under nonlimiting soil water conditions. These conditions were defined as REW > 0.33 (Oishi *et al.*, 2013; equivalent to the volumetric soil water content, $\theta > 0.20$ m^3 m^{-3} in Palmroth *et al.*, 2005). Soil water content exceeding field capacity did not limit F_{soil} in AU (Oishi *et al.*, 2013). The equation used is

$$F^*_{soil} = R_{b10}e^{b(T_{10}-10)} \tag{2}$$

where R_{b10} is estimated 'basal' respiration at 10 °C (μmol CO$_2$ m^{-2} s^{-1}), and b is the temperature-sensitivity parameter ($e^{b \times 10} = Q_{10}$). The values of F_{soil} were natural log transformed to reduce heteroscedasticity and to fit the data as a linear function. Thus, Eqn (2) can be rearranged to the equation

$$\log(F^*_{soil}) = \beta_0 + \beta_1 \times T_{10} \tag{3}$$

where β_0 and β_1 are parameter estimates.

To test for differences in temperature and moisture sensitivities among treatments, we first compared the significance of parameters among models that included [CO$_2$], N, and [CO$_2$] × N interaction effects with a full vs. reduced model F-test, removing parameters, one at a time, associated with a given treatment. This approach allowed testing for differences among all the relationships, as well as among the parameters describing the relationships, utilizing as a population the daily data from all years and subplots within a treatment. As a more conservative approach acknowledging limitations on randomness and independence of data points in the experimental

design, we also estimated parameters for individual subplots and tested for treatment effects on the parameters based on an ANOVA with a split-plot, randomized block design with years as repeated measures. These parameters, estimated for individual subplots, were used to fill gaps in data coverage.

Linear regressions were not possible for all subplots in all years due to gaps for reasons described previously, as well as uneven representation of T_{10} ranges. For example, during the drought year of 2005 there were very few days when temperature was above the annual mean while soil water content was nonlimiting. Least-squares fitting of these data led to some unreasonable Q_{10} values (e.g., <0). Therefore, we constrained each subplot's regressions by assuming a constant Q_{10} parameter across years, but allowing for varying R_{b10}.

Limitations to F_{soil} imposed by REW, f_{REW}, were accounted for by fitting the relative reduction from daily F^*_{soil} under nonlimiting REW using the following function for each subplot:

$$f_{REW} = 1 - e^{-c \times W + d} \qquad (4)$$

where W represents REW and c and d are coefficients describing the sensitivity of F_{soil} to low REW. The combination of few low-REW days during some years and gaps in observations in some plots in other years did not allow us to test for interannual differences in the effects of treatments on the soil water content limitation coefficients. However, for each of the two driest years, 2005 and 2007, we were able to fit f_{REW} for sufficient number of subplots in each treatment to allow testing for interannual differences in the coefficients. Neither c nor d was different between the 2 years for any treatment ($P > 0.31$; F-test comparison of parameter models). Thus, data were pooled across years to allow estimation of f_{REW} parameters over sufficiently wide range of soil water content in a manner representing the entire study period. As with the temperature-response function, we first tested for differences among treatment populations comparing the full model to reduced models, removing treatment parameters. We subsequently analyzed parameter fits for each subplot, accounting for the randomized block, split-plot design, but without repeated measures.

To summarize, Matlab (Version 6.0.1.450, Release 12.1, Mathworks Inc., Natick, MA, USA) was used to process raw data and for linear regressions and statistical tests. Analysis of variance using a randomized block, split-plot design (plot pairs as blocks, [CO_2] as whole plots, and N fertilization as split plot), with years as repeated measures was performed to test for treatment effects using the R software package (R Foundation for Statistical Computing, Vienna, Austria). Additional linear and nonlinear curve fitting was performed with R (ANOVA, lm, lme, and nls functions) and SigmaPlot (v8.0.2, Systat Software Inc., San Jose, CA, USA). Given the small number of replicates ($n = 4$), we report statistical significance as strong ($P < 0.05$) and weak ($0.05 \leq P < 0.10$).

Long-term data integration

To examine the combined effects of long-term treatments and interannual variability in environmental drivers on F_{soil}, we expanded the scope of our analysis to include data extending back to the initiation of the Duke FACE experiment in 1997. King *et al.* (2004) and Bernhardt *et al.* (2006) published estimates of annual F_{soil} based on monthly portable IRGA measurements. Jackson *et al.* (2009) published monthly data from portable IRGA and soda-lime chamber measurements, from which we estimated annual F_{soil}.

Leaf litter fall, leaf area index, and foliage production

Leaf area and litter fall dynamics were published by McCarthy *et al.* (2007) through 2003, which we have extended in this study through 2010 using the same methodology (briefly summarized here; Table 1). We define leaf litter as total foliar biomass, including pine needles and deciduous leaves. Leaf litter was collected biweekly during peak litter fall (September through December) and monthly for the remainder of each year with an array of baskets in each subplot. Samples were oven dried, separated into pine or deciduous, and weighed to generate annual totals. Foliage biomass was converted into C using a C content of 0.48 for pine foliage, and a C content of 0.46 for hardwood foliage (Schäfer *et al.*, 2003). To establish a continuous estimate of LAI, we combined leaf litter data with temporal dynamics of leaf production and loss, which differed between deciduous and evergreen species, as well as within vertical sections of the canopy.

Net primary productivity of foliage follows McCarthy *et al.* (2010) and extends their published data beyond 2004. For *P. taeda* foliage production was determined by lagging collected leaf litter fall masses by 2 years to account for foliage longevity, correcting when necessary for the effects of droughts and storms (see McCarthy *et al.*, 2007); for hardwood species, foliage production was based on that year's litter fall mass (McCarthy *et al.*, 2007). Leaf production of pines in 2009 and 2010 was taken to be the average of production derived from the above procedure, and from the proportions of 2009 and 2010 leaf cohorts present when the (subsets of) plots were harvested in spring 2011.

Results

Sensitivity of F_{soil} to temperature and soil water content

We first tested for differences among treatments by comparing responses of F_{soil} to T_{10} and soil water content. Compared to a common relationship among all treatments, incorporating parameters for [CO_2] and N treatments into the model for the temperature sensitivity of F_{soil} under nonlimiting soil water content improved the fit (F-test comparison of reduced models; $P < 0.001$). The Q_{10} parameter decreased with elevated [CO_2] and N fertilization ($P < 0.0001$; Fig. 1a); however, no interaction was observed ($P = 0.21$). Compared to the ambient [CO_2] unfertilized treatment (AU), R_{b10} was higher under elevated [CO_2] unfertilized (EU) and lower under ambient [CO_2] fertilized (AF; $P < 0.001$; Fig. 1b). In addition, there was a significant interaction

term ($P < 0.001$), such that the reduction in R_{b10} with N fertilization was lower under elevated [CO_2] compared to ambient (i.e. elevated [CO_2] fertilized treatment; EF).

Responses of F_{soil} to soil water content limitations (i.e., f_{REW}) also differed among treatments, expressed by an increase in the c parameter with [CO_2], N, and [CO_2] × N ($P < 0.046$; Fig. 1c) and a decrease in the d parameter with [CO_2] ($P = 0.038$; $P > 0.28$ for the other factors; Fig. 1d).
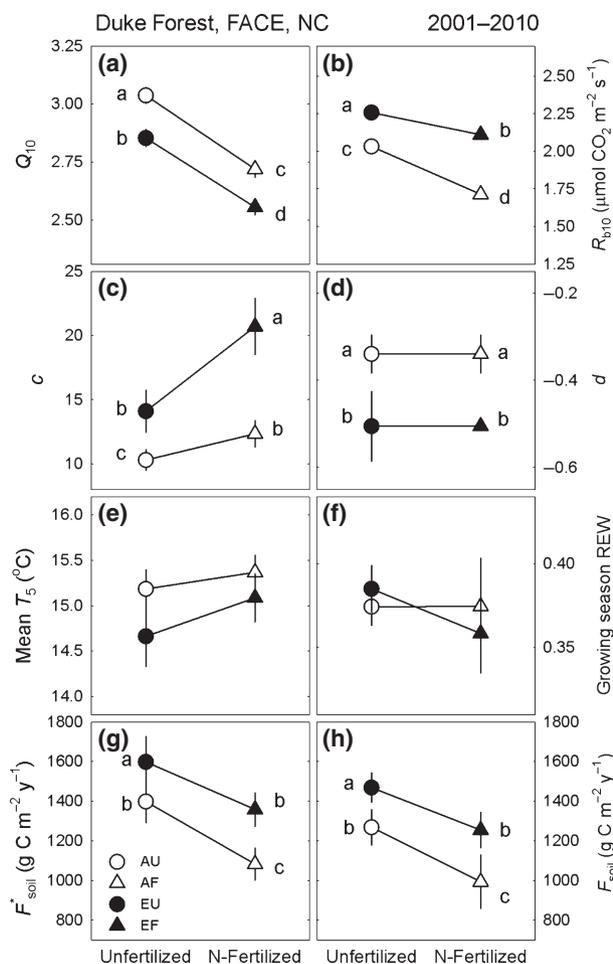

Fig. 1 Treatment mean of temperature-response parameters of potential soil CO_2 efflux under nonlimiting soil water content [F^*_{soil}, see Eqn (2)], (a) Q_{10}, and (b) R_{b10}; of parameters of the sensitivity of soil CO_2 efflux (F_{soil}) to relative extractable water [REW; f_{REW}, see Eqn (4)], (c) c, and (d) d; mean growing season environmental variables, (e) soil temperature at 5 cm (T_5), and (f) REW; annual sums of (g) F^*_{soil} and (h) F_{soil}. Variables are expressed in relation to fertilization treatment on the x-axis. Open and closed symbols represent ambient and elevated [CO_2] plots, and circles and triangle represent unfertilized and fertilized plots, respectively (AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized). Means accompanied by similar letters are not different (Tukey's least significant difference, $P < 0.05$). Error bars represent ±1 SE.

As individual temperature- and soil water content-response functions were also generated at the subplot level, we also performed ANOVAS on the parameter estimates, utilizing the split-plot, randomized block design with repeated measures. Similar to the general patterns emerging from the less constrained analysis above, Q_{10} showed a decrease under both [CO_2] and N ($P < 0.071$; Table 2). Also, R_{b10} increased with [CO_2] ($P = 0.028$) and decreased under N ($P = 0.069$); yet no interaction was observed ($P = 0.96$). Analyzing the sensitivity of soil CO_2 efflux to soil water content with the more constrained design produced a contrasting outcome, whereby none of the treatments affected either of the f_{REW} parameters (Table 2).

The treatment effect on the temperature function parameters is reflected in the exponential temperature response of potential soil CO_2 efflux under nonlimiting soil water content conditions (F^*_{soil}) in all treatments (Fig. 2a; Table 3). Expressing treatments' F^*_{soil} relative

Table 2 P-values for significance of treatment effects on temperature-sensitivity parameters [Q_{10} and R_{b10}; Eqn (2)], soil water content-sensitivity parameters [c and d; Eqn (4)], growing season environmental variables [whole-plot soil temperature at 10 cm (T_{10}), below-chamber soil temperature at 5 cm (T_5), and relative extractable soil water (REW)], and annual soil CO_2 fluxes [potential soil CO_2 efflux under nonlimiting soil water conditions (F^*_{soil}) and after accounting for soil water limitations (F_{soil})]. ANOVAS were performed to test for treatment effects as a blocked, split-plot experiment with years as repeated measures. Single values were used for all years for Q_{10}, c, and d. Subplot T_{10} was not available, so N effects could not be tested. We discuss responses that are strongly significant ($P < 0.05$; in bold) and weakly significant ($0.05 \leq P < 0.10$; in italics). We do not discuss single factors effect if the interaction is significant

	CO_2	N	[CO_2] × N	Year
T_{10} parameters				
Q_{10}	*0.055*	*0.071*	0.405	n/a
R_{b10}	**0.028**	*0.069*	0.961	**<0.0001**
f_{REW} parameters				
c	0.286	0.440	0.898	n/a
d	0.730	0.269	0.539	n/a
Growing season variables				
T_{10}	0.650	n/a	n/a	**<0.0001**
T_5	0.369	0.515	0.818	**<0.0001**
REW	0.581	0.761	0.133	**<0.0001**
Annual Fluxes				
F^*_{soil}	**0.026**	**0.005**	0.595	**<0.0001**
F_{soil}	**0.027**	**0.002**	0.490	**<0.0001**

Duke Forest, FACE, NC   2001–2010
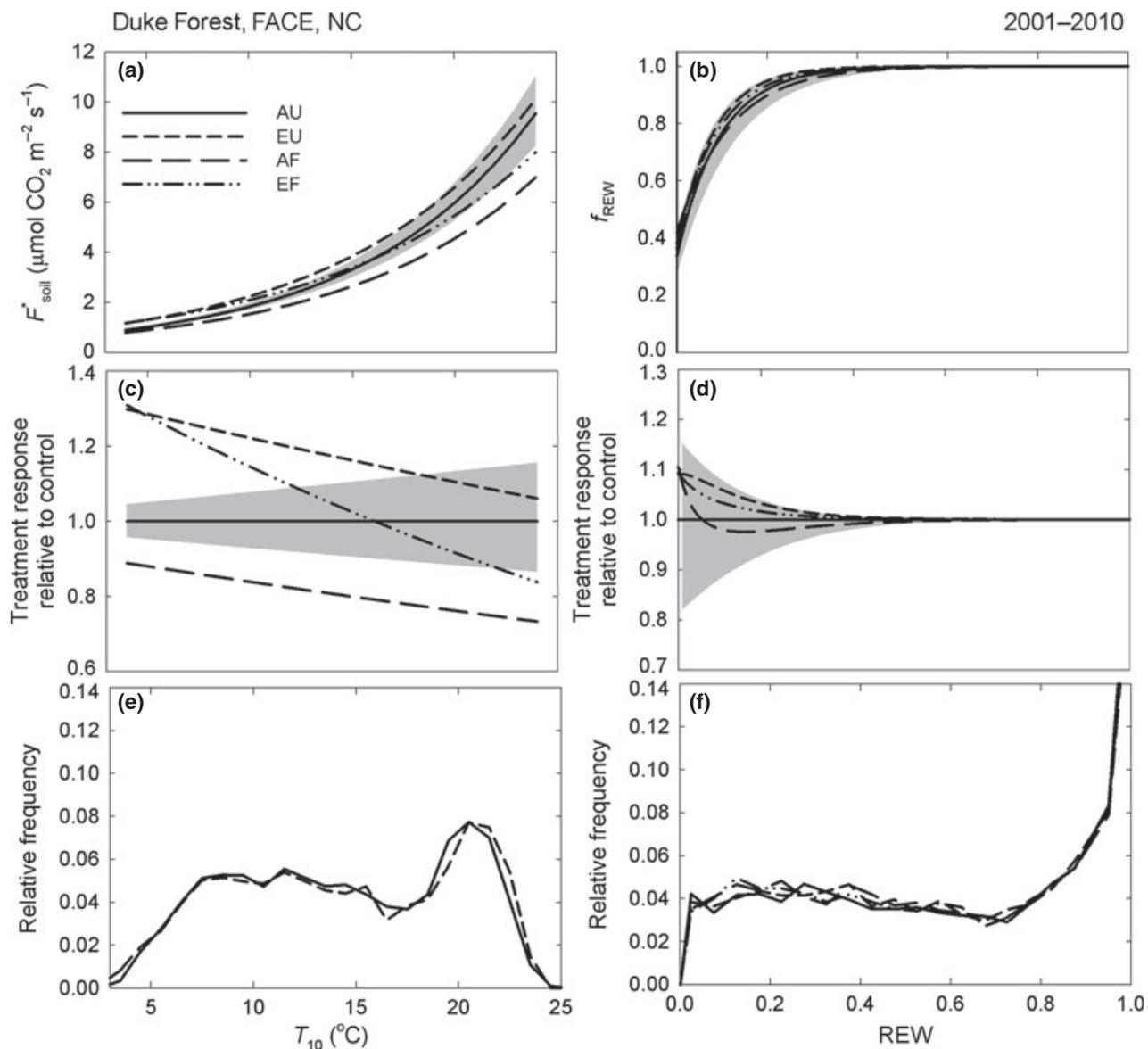

Fig. 2 (a) Potential soil CO_2 efflux under nonlimiting soil water content (F^*_{soil}) as a function of soil temperature at 10 cm [T_{10}; see Eqn (2)], and (b) limitation to soil CO_2 efflux (F_{soil}) by relative extractable soil water [REW; f_{REW}, see Eqns (1) and (4)]. Treatment responses of (c) F^*_{soil} and (d) f_{REW} relative to control plots. Relative frequency distribution of (e) T_{10} and (f) REW. AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized. Gray areas in (a–d) represent ±1 SE around the control plots.

to AU shows that the increase in F^*_{soil} with T_{10} was lower in all other treatments, as indicated by the negative slopes in Fig. 2c. Unfertilized, elevated [CO_2] plots had higher F^*_{soil} than AU plots at soil temperatures below 17 °C. At higher temperatures, including the temporal mode of T_{10} (Fig. 2e), the lower Q_{10} values in elevated treatments compensated for higher R_{b10} values, and F^*_{soil} at EU and EF was similar to that at AU (Fig. 2c). The combination of low R_{b10} and Q_{10} at AF led to lower F^*_{soil} than at AU across the entire T_{10} range.

The treatment effect on the moisture reduction function parameters is reflected in the moisture response of F_{soil}. Soil CO_2 efflux decreased with relative extractable soil water (REW) in all treatments (Fig. 2b). Both EU and EF showing slightly higher f_{REW} than the AU and AF plots (i.e., less reduction from potential F_{soil} under dry conditions); however, these responses were within 1 SE of that of AU (Fig. 2d, Table 3). Nevertheless, the similarity in soil water content distributions of the treatments (Fig. 2f) meant that differences in sensitivity to moisture translated to effects on CO_2 fluxes.

Table 3 Parameters for temperature sensitivity of soil CO$_2$ efflux under nonlimiting soil water content conditions [F^*_{soil}; Eqn (2)] and for the sensitivity F^*_{soil} to relative extractable water (REW) [f_{REW}; Eqn (4)] presented as mean (with SE) by plot and treatment. R_{b10} is in μmol CO$_2$ m^{-2} s^{-1}, all other parameters are unitless

| | Block | Plot | F^*_{soil} | | | | f_{REW} | | | |
			Q_{10}		R_{b10}		c		d	
AU	1	1	3.17	(0.08)	2.15	(0.03)	8.81	(1.50)	−0.349	(0.103)
	2	5	3.42	(0.08)	1.96	(0.02)	16.50	(5.18)	−0.634	(0.215)
	3	6	2.98	(0.08)	1.65	(0.02)	6.59	(1.27)	−0.390	(0.112)
	4	8	2.83	(0.05)	2.13	(0.02)	11.06	(1.28)	−0.067	(0.076)
EU	1	2	2.81	(0.06)	2.80	(0.03)	35.56	(15.44)	−0.374	(0.482)
	2	3	3.33	(0.08)	1.94	(0.02)	16.99	(6.91)	−0.962	(0.280)
	3	4	2.96	(0.06)	2.28	(0.02)	18.28	(3.23)	−0.107	(0.160)
	4	7	2.61	(0.05)	2.15	(0.02)	6.35	(0.83)	−0.416	(0.080)
AF	1	1	2.95	(0.06)	1.61	(0.02)	6.08	(1.06)	−0.552	(0.102)
	2	5	3.47	(0.08)	1.55	(0.02)	21.02	(8.26)	−0.695	(0.308)
	3	6	2.67	(0.07)	1.53	(0.02)	6.10	(1.23)	−0.458	(0.117)
	4	8	2.46	(0.05)	1.84	(0.02)	13.26	(2.14)	−0.246	(0.109)
EF	1	2	2.57	(0.05)	2.33	(0.03)	10.10	(2.14)	−0.411	(0.146)
	2	3	3.03	(0.07)	1.89	(0.02)	11.65	(2.97)	−0.649	(0.168)
	3	4	2.61	(0.05)	2.39	(0.03)	43.32	(16.45)	−0.272	(0.484)
	4	7	2.38	(0.05)	1.94	(0.02)	5.18	(1.03)	−0.914	(0.117)

Treatment effects on soil temperature and moisture

Treatments may affect not only the T_{soil} and REW sensitivities but also these soil variables directly. Annual T_{10} was similar ($P > 0.13$) in both [CO$_2$] treatments, as were the interannual mean (Table 2) and the frequency distributions of T_{10} (Fig. 2e). Mean annual T_{10} varied less than 1 °C among years and did not exhibit a linear trend ($P > 0.35$). Because T_{10} was only measured in the whole plots, so to test for treatment effects on T_{soil}, we compared T_5 measurements beneath each soil chamber. Annual, growing season, and winter T_5 were similar among all treatments (ANOVA $P > 0.36$; Table 2; Fig. 1e).

Neither [CO$_2$] nor N affected the interannual growing season means of REW (Table 2; Fig. 1f), nor the frequency distributions (Fig. 2f). At monthly timescales, REW at EF was lower than at EU for 3 of the 120 months in this study ($P < 0.05$; t-tests to compare monthly REW). In only 1 of the 3 months, soil water availability was low (REW < 0.33) resulting in large reductions in F_{soil} (Fig. 2b). Fertilization in ambient plots (AF) reduced REW relative to AU during 12 months ($P < 0.05$), two of which were dry (REW < 0.33). Elevated [CO$_2$] (EU and EF) was associated with lower REW than ambient [CO$_2$] treatments (AU and AF) during 19 months in the study period ($P < 0.05$), five of which occurred during the growing season and had mean REW < 0.33. In general, the four treatment combinations experienced similar soil temperature and drought conditions over the study period.

Interannual variability and long-term trends in F_{soil}

Mean annual F^*_{soil} and F_{soil} decreased with N and increased with [CO$_2$] (Table 2; Fig. 1g, h). The lack of an interactive effect of [CO$_2$] × N ($P > 0.49$) meant that absolute reductions in both quantities caused by N fertilization were similar under elevated and ambient [CO$_2$]. Annual F_{soil} of AU averaged 1268 (SD = 232) g C m^{-2} yr^{-1}, varying between a minimum of 1009 g C m^{-2} yr^{-1} and maximum of 1670 g C m^{-2} yr^{-1} (Fig. 3a). Annual F_{soil} of EU averaged 1468 (SD = 174) g C m^{-2} yr^{-1}, generally higher than at AU (average 17%, SD = 10%; Fig. 3a). Differences between AU and EU were strongly significant ($P < 0.05$; paired t-test among blocks) in 4 of the 10 years, and weakly significant ($0.05 \leq P < 0.10$) in 2 (Fig. 3b). Mean annual F_{soil} of AF was 993 (SD = 167) g C m^{-2} yr^{-1}, a 21% (SD = 4%) reduction from AU, and lower in all years ($P < 0.05$). Fertilization under elevated [CO$_2$] led to annual F_{soil} that was similar to AU all years ($P > 0.1$), averaging 1254 (SD = 134) g C m^{-2} yr^{-1}.

After accounting for the effect of REW, absolute values of annual F_{soil} did not show a linear trend over time in any treatment ($P > 0.11$; Fig. 3a). Relative to AU, F_{soil} at EF showed a weak increasing trend between 2005 and 2010 ($P = 0.093$; Fig 3b). F_{soil} at EU and AF did not change relative to AU over time ($P > 0.31$).

Expanding the scope of our analysis to the initiation of the Duke FACE experiment, neither T_{10} nor REW showed a linear temporal trend over the 1997–2010 or
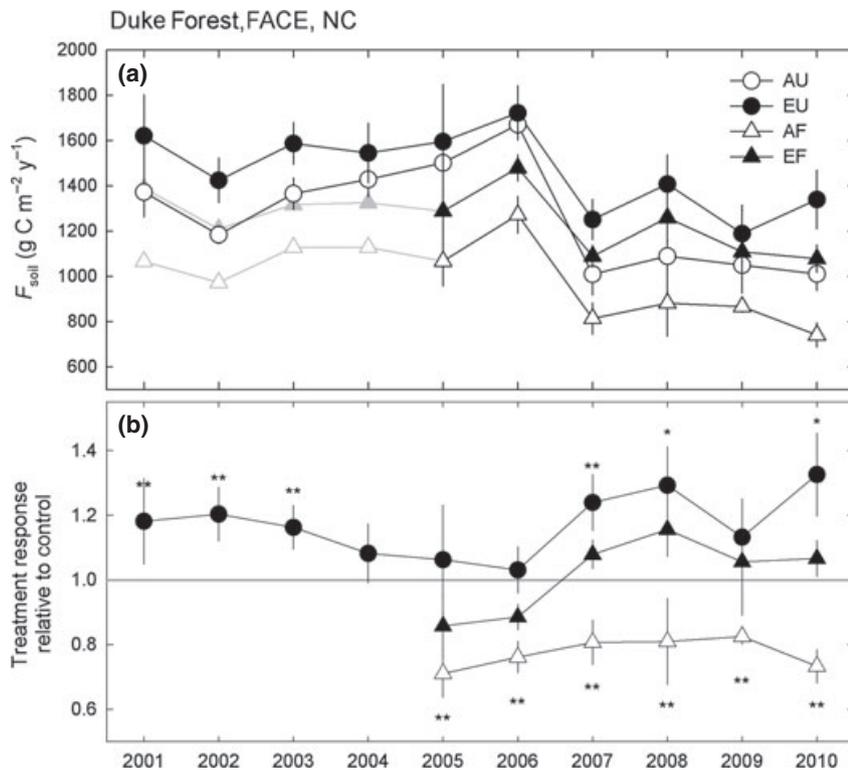
Fig. 3 (a) Annual soil CO_2 efflux (F_{soil}) by treatment and (b) treatment response of F_{soil} as a proportion of control plot. Only one plot under ambient and elevated $[CO_2]$ received nitrogen fertilization prior to 2005 (FACE reference and prototype plots), so stand-level estimates were scaled based on the relationship between these two plots and the other six plots using data from 2005 to 2010 (gray symbols). AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized. Significant differences between the control and treatment plots are denoted by ** for $P < 0.05$ and * for $0.05 \leq P < 0.10$ (paired *t*-test). Error bars represent ± 1 SE.

the 2001–2010 periods (Fig. 4a, b; $P > 0.24$). Annual NPP of foliage in all treatments generally increased from 1997, and stabilized in 2004, following a large decline in 2002–2003 caused by a drought and ice storm (Fig. 4c). From 2005 to 2010, when fertilization in all FACE plots began and ACES was installed in all subplots, there has been no linear trend in foliage NPP ($P > 0.26$).

Comparing our results with data presented in previous F_{soil} studies at Duke FACE, annual F_{soil} in AU was often of a similar magnitude (Fig. 4d), but our estimated annual F_{soil} values showed no correlation with other studies (linear regression; Bernhardt *et al.*, 2006; $P = 0.96$, $n = 3$; Jackson *et al.*, 2009; $P > 0.51$, $n = 8$). Despite the fact that the other studies used a common source of data from monthly portable IRGA measurements, different approaches to annual estimates led to no correlation among annual F_{soil} values ($P > 0.26$). We note that annual estimates based on the portable IRGA and soda-lime methods (Jackson *et al.*, 2009) were also not correlated among years ($P = 0.78$, $n = 12$). All studies showed a consistent positive relative response to

elevated $[CO_2]$; however, no consistent temporal trend emerged (Fig. 4e.). We used a stepwise additional multiple linear regression to test whether a combination of year, T_{10}, REW, and foliage NPP could explain interannual variability in the relative response to elevated $[CO_2]$, but no trend emerged ($P > 0.11$)

Among treatments, R_{b10} increased until approximately 2005, declined for about 2 years and apparently stabilized (Fig. 5). This trend was captured by a second-order polynomial ($P < 0.081$). Variation in R_{b10} was not related to T_{10} ($P > 0.57$) or REW ($P > 0.25$).

Sources of variation in F^*_{soil}

The combined effects of R_{b10}, Q_{10}, and T_{10} are reflected in annual F^*_{soil}, which decreased with increasing LAI and leaf litter fall among ambient $[CO_2]$ plots, forming a single relationship over both fertility levels (Fig. 6a, b). Although elevated $[CO_2]$ plots had higher F^*_{soil} than ambient plots at a given level of leaf production, these plots did not form a significant relationship ($P > 0.29$). Replacing LAI and annual leaf litter fall with N
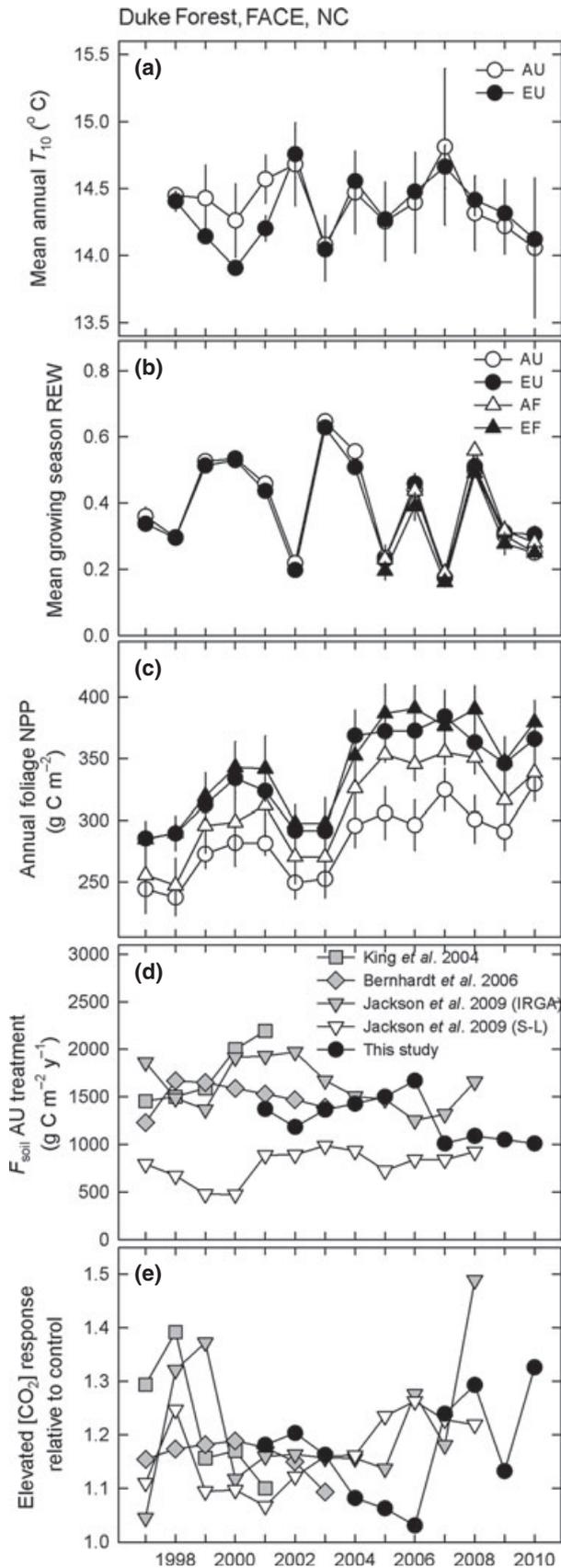
Fig. 4 (a) Mean annual soil temperature at 10 cm depth (T_{10}), (b) mean growing season soil relative extractable water (REW), (c) annual net primary productivity (NPP) of foliage, (d) annual soil CO$_2$ efflux (F_{soil}) under ambient unfertilized conditions for this study and previous studies, and (e) response of F_{soil} to elevated [CO$_2$], relative to the control. Error bars represent ±1 SE.
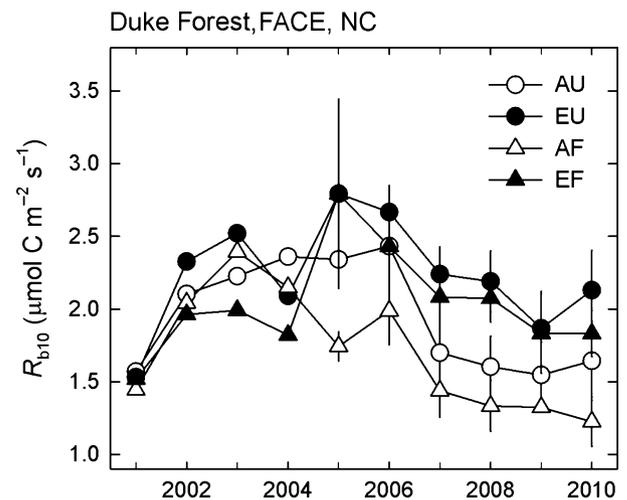

Fig. 5 Annual estimate of base respiration at 10 °C. AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized. Error bars represent ±1 SE.

availability improved the fit for ambient plots ($P = 0.004$) and produced a significant relationship among elevated plots ($P = 0.036$; Fig. 6c). For a given N level, F^*_{soil} was consistently 255 (SE = 31) g C m^{-2} yr^{-1} higher in elevated compared to ambient [CO$_2$] plots ($P = 0.003$, comparing intercepts in a linear model). The two populations had a common slope ($P = 0.9$); F^*_{soil} declined by 114 (SE = 23) g C m^{-2} yr^{-1} for an increased availability of 1 g N m^{-2}.

Soil water availability controls over interannual variation in F_{soil}

Because interannual variability in soil temperature was generally small, interannual variability in F_{soil} was driven by soil water availability. As the significance of treatment effects on the c and d parameters were not consistent among statistical approaches (Fig. 1; Table 2), we examined the integrated effects of f_{REW} (Fig. 2b, d) on annual soil CO$_2$ efflux by taking the ratio of annual F_{soil} to annual estimated F^*_{soil}, thus generating annual f_{REW}. Annual f_{REW} was compared with mean growing season REW, as growing season soil water content has the greatest effect on respiration rates and annual CO$_2$ fluxes, and showed that annual F_{soil} departed from F^*_{soil} by as much as 20% (Fig. 7). The
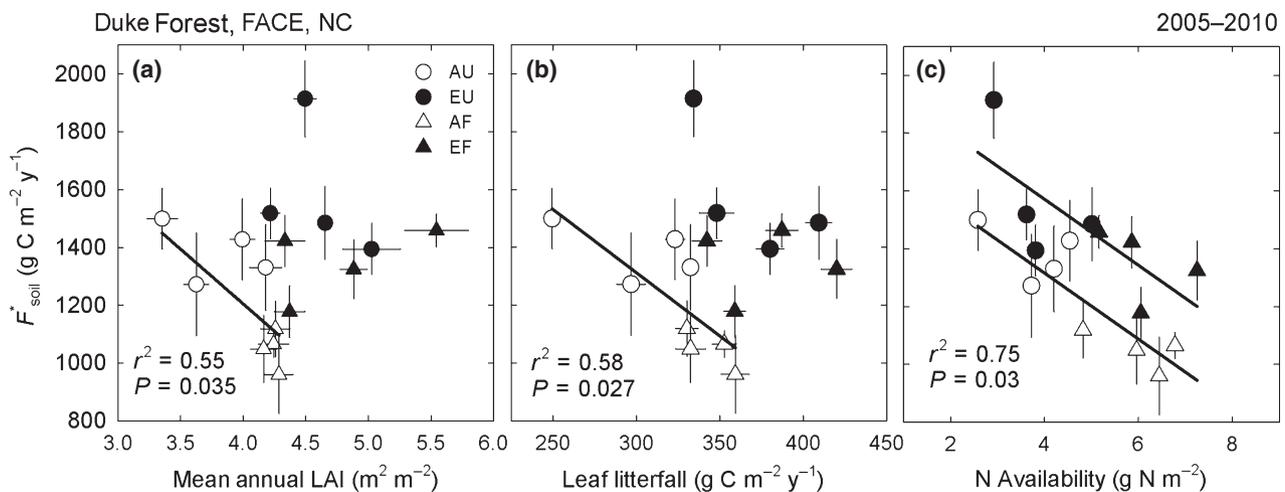
Fig. 6 Annual sums of potential soil CO_2 efflux under nonlimiting soil water content conditions (F^*_{soil}) as a function of mean annual leaf area index (LAI) (a), leaf litter fall (b), and soil N availability (c). AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized. Error bars represent ±1 SE.
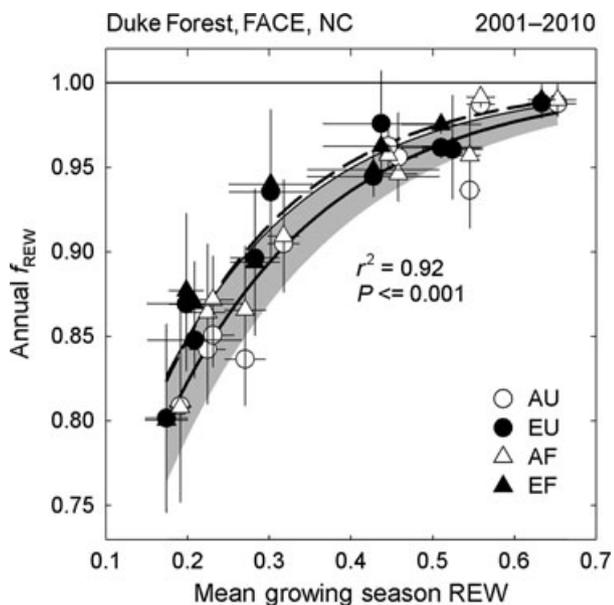

Fig. 7 Mean growing season relative extractable water content (REW) compared to annual reduction in soil CO_2 efflux due to soil water content limitation (f_{REW}). AU, ambient/unfertilized; EU, elevated/unfertilized; AF, ambient/fertilized; EF, elevated/fertilized. Error bars represent 1 SE. Gray areas represent ±1 SE around the ambient [CO_2] plots.

decline followed the same function form used for daily data (Eq. 4), but the sensitivity of annual F_{soil} began at a higher REW. Nevertheless, annual F_{soil} was above 90% of its potential down to a REW of 0.33. As soil dried further, a reduction in REW of 5% (e.g., from 0.30 to 0.25) led to a reduction in f_{REW} of ca. 3.5%. Based on regression analysis, the c parameter was different among the two [CO_2] treatments ($P < 0.0001$; F-test

comparison of models). This meant that compared to ambient [CO_2], F_{soil} under elevated [CO_2] was less sensitive to REW, resulting in between 0.007 and 0.025 higher f_{REW} across the range of observed REW. Thus, in elevated [CO_2] treatments, despite lower relative sensitivity to REW (Fig. 7), higher F^*_{soil} (Fig. 1g) resulted in only 20 g C m^{-2} yr^{-1} smaller decrease in F_{soil} due to soil water content limitations than in ambient [CO_2] plots.

Discussion

Elevated atmospheric [CO_2] has been shown to have no effect on the temperature sensitivity of F_{soil} (King *et al.*, 2004), whereas N fertilization has been shown to increase the sensitivity (Butnor *et al.*, 2003). Here, on the basis of more replicates and greater temporal density of measurements than previous studies (Bernhardt *et al.*, 2006; Jackson *et al.*, 2009), we find that, consistent with (H1), the temperature sensitivity of F_{soil} (i.e., Q_{10}) decreased with both elevated [CO_2] and greater N supply, and additively by their combination (Fig. 1a); however, these decreases did not necessarily lead to lower F_{soil} than at the control (AU). Higher base respiration (R_{b10}; Fig. 1b) in EU than in AU led to higher potential F_{soil} (F^*_{soil}) up to 17 °C (T_{10}; Fig. 2a, c). In contrast, compared to AU, fertilization (AF) decreased both R_{b10} and Q_{10} and, thus, F^*_{soil} at all temperatures. Fertilization under elevated [CO_2] (EF) led to a combination of higher R_{b10} and lower Q_{10} than AU and, thus, similar soil CO_2 fluxes along much of the temperature range.

Over the course of our study, R_{b10} in all treatments followed a general trend of increasing, then decreasing

(Fig. 5). Estimates of R_{b10} prior to 2005 are based on only one pair of plots, so caution must be used when interpreting the trends over that period. Indeed, no consistent pattern emerged in previous studies over this same period of increasing foliage NPP (King *et al.*, 2004; Bernhardt *et al.*, 2006; Jackson *et al.*, 2010; Fig. 4c, d). However, our measurements in the fully replicated experiment, beginning in 2005 and coinciding with a period of stable foliage NPP (Fig. 4c), show a decrease in R_{b10}, followed by an apparent stabilization (Fig. 5).

While changes in F_{soil} with temperature showed clear differences among treatments under nonlimiting soil water content conditions, differences among treatments in the responses to decreasing soil water content were less apparent (H2) and different statistical approaches led to contrasting results. Thus, although F_{soil} sensitivity to decreasing soil water appeared similar among all treatments (Fig. 2b, d) and did not show differences in the individual parameters based on our ANOVA (Table 2), the integrated effect of the differences observed in the parameters (Fig. 1c, d) led to a small, but significantly higher annual f_{REW} in elevated [CO$_2$] plots compared to ambient plots irrespective of N treatment (Fig. 7). This outcome is consistent with a portion of (H2). The reduction in fine root biomass from 0 to 15 cm under N amendment (Jackson *et al.*, 2009) seems to have no effect on annual f_{REW}, perhaps indicating that their vertical distribution and thus access to water was unchanged. However, the enhanced fine root biomass under elevated [CO$_2$] (Jackson *et al.*, 2009) accompanied by a shift from shallow to deeper soil layers (Norby *et al.*, 2004; Pritchard *et al.*, 2008a), possibly buffered trees from water limitations (Domec *et al.*, 2012).

The sharp reduction in F_{soil} below REW = 0.33 observed at fine temporal scales (Fig. 2b) is tempered and extended to higher soil water content at annual-scale F_{soil} (Fig. 5), the latter quantity affected by both the sensitivity to soil water content and soil water content distributions (Fig. 2f). Variation in canopy photosynthesis quickly affects carbohydrate supply belowground, with effects on the mass-specific rate fine root respiration noticeable after 1 day (Drake *et al.*, 2008). It is therefore not surprising that short-term droughts at sites of limited water storage can curtail canopy gas exchange when soil water content drops below a similar value (Oren *et al.*, 1998) and quickly impact F_{soil}. Such an impact may not be captured by monthly F_{soil} measurements (Bernhardt *et al.*, 2006). Furthermore, prolonged droughts reduce fine root production (Pritchard *et al.*, 2008a) and microbial respiration (Drake *et al.*, 2012). The greater availability of C under elevated [CO$_2$] (Schäfer *et al.*, 2003; McCarthy *et al.*, 2010) likely helped buffer f_{REW} against drying soil (Fig. 7), but this averaged only ca. 20 g C m^{-2} yr^{-1} greater F_{soil}.

In a previous analysis of only the AU plots, Oishi *et al.* (2013) found lower growing season T_{soil} in plots with higher LAI and a positive relationship between T_{soil} and F_{soil}. In their analysis, both T_{soil} and LAI explained a similar amount of variability in F_{soil}, and they could not distinguish which factors, environmental or physiological, were affecting T_{soil}. The treatments in this study extended the range of LAI in Oishi *et al.* (2013) by >2 m^2 m^{-2}, and, similar to the previous study, no relationship emerged between LAI and T_{soil}. This is reflected in no treatment effects on T_{soil} (Fig. 1e, Table 2). Higher LAI in treatment plots also corresponds to lower stomatal conductance (Domec *et al.*, 2009), which likely contributed to no treatment effects on REW (Fig. 1f; Table 2). Thus, addressing (H3), we found that although treatments led to substantial increases in productivity, canopy leaf area, and forest floor organic matter (McCarthy *et al.*, 2006, 2007; Lichter *et al.*, 2008), soil conditions (T_{soil} and REW) were unaffected (Figs 1e, f and 2e, f; Table 2). Because soil temperature and moisture were similar among treatments, differences in annual soil CO$_2$ efflux were governed by treatment effects on the response of F_{soil} to these variables.

The combined effects of environmental conditions and the physiological response to these conditions are integrated in annual F_{soil} (Fig. 3a). Relative to AU, F_{soil} was generally higher in EU and always lower in AF (Fig. 3b). The magnitude of the reduction in F_{soil} with fertilization of 11.2 g N m^{-2} yr^{-1} was similar to the increase with 200 ppm [CO$_2$], and as no interaction was observed (Fig. 1h; Table 2), the canceling effects of the two treatments resulted in EF having similar F_{soil} to AU. We also note that 2007 marked the beginning of lower F_{soil} among all treatments (Fig. 3). We attribute low F_{soil} in 2007 to the combination of low R_{b10}, which persisted through 2010 (Fig. 5) and a severe drought (Fig. 4b). Although 2005 was also marked by a severe drought, we note that annual F_{soil} remained high due to high R_{b10}, possibly the result of the phase of stand development (characterized by increasing foliage NPP; Fig 4c) and a legacy effect of decomposition of leaf biomass deposited as a result of the 2002 ice storm and drought.

An early synthesis of soil CO$_2$ efflux across four forest FACE experiments (King *et al.*, 2004) suggested elevated [CO$_2$]-induced enhancement of annual F_{soil} decreased with stand age, consistent with a later observation of no enhancement in a mature forest (Bader & Körner, 2010). We show that after an additional decade of growth and accumulation of ca. 1.5 times additional aboveground biomass (ca. 25% more under elevated [CO$_2$]), continuous exposure to elevated [CO$_2$] resulted in a relatively persistent 17% increase in annual soil

CO_2 efflux [ca. 200 (SD = 95) g C m^{-2} yr^{-1}, comparing AU to EU; Table 2; Figs 1g, h, and 3]. A midterm initiation of nitrogen fertilization led to a 21% reduction in annual F_{soil} in AF compared to AU [ca. 274 (SD = 86) g C m^{-2} yr^{-1}; Fig. 3], similar to earlier reports (Butnor *et al.*, 2003; Jackson *et al.*, 2009), whereas the addition of N under elevated [CO_2] (EF) led to a 15% reduction, effectively restoring annual F_{soil} to the values of AU. Although the relative decrease was greater under ambient than elevated [CO_2], the absolute reduction was similar [ca. 214 (SD = 67) g C m^{-2} yr^{-1}] and, like the effects of elevated [CO_2], essentially persistent over time.

The responses of F_{soil} to the treatments at Duke FACE is consistent with greater belowground C pools and fluxes under high [CO_2] and smaller pools and fluxes following fertilization (Butnor *et al.*, 2003; Bernhardt *et al.*, 2006; Palmroth *et al.*, 2006; Jackson *et al.*, 2009). Briefly, elevated [CO_2] has increased, while fertilization has reduced *in situ* measurements of fine root respiration scaled to stand level (Drake *et al.*, 2008), fine root biomass (Pritchard *et al.*, 2008a; Jackson *et al.*, 2009), forest floor C and heterotrophic respiration (Lichter *et al.*, 2008), root exudation rates (Phillips *et al.*, 2011), rhizomorphs (Pritchard *et al.*, 2008b), as well as fungal and microbial activity (Billings & Ziegler, 2008). We note, however, that although qualitatively our results match these patterns, the absolute differences among treatments in F_{soil}, in this study and others, are much larger than the observed treatment-induced changes in the sum of individual soil C pools and fluxes.

The longevity and magnitude of the effect of elevated [CO_2] on F_{soil} remain an important question. As the duration of [CO_2] enrichment increases, the additional flux of C to some belowground plant components (e.g., fine roots) can be greatly reduced (Norby *et al.*, 2010), allowing the extra carbohydrates to be exuded by roots, stimulating or priming microbial biomass and rapidly cycling C back to the atmosphere. As microbes search for N, the turnover of older soil organic matter may increase (Drake *et al.*, 2011; Ziegler & Billings, 2011), further adding to the heterotrophic component of F_{soil} and depleting soil C. However, no evidence was found at this site that elevated [CO_2] decreased soil C (Lichter *et al.*, 2008). In contrast, a survey of fertilized loblolly pine plantations in the southeastern United States detected a 10% increase in mineral soil C (Rifai *et al.*, 2010), possibly the result of suppression of lignin decomposition under high N (Fog, 1988). Thus, deceleration in the soil turnover rate may also play a role in reduced F_{soil} where N is added.

We suggest that the variation in the response of F_{soil} to soil temperature and water content among treatments, and thus in the annual fluxes, was controlled by processes related to belowground C partitioning in response to plant nutritional needs. Across biomes, ecosystems of higher productivity support higher canopy leaf area, gross primary production (GPP), and respiration, as well as higher C flux belowground and F_{soil} than lower productivity biomes (DeLucia *et al.*, 2007; Litton *et al.*, 2007). Although within a biome, increasing nutrient availability also leads to higher GPP, R_A/GPP tends to decrease because a larger portion of C is allocated to wood production and smaller amount to support fast turnover soil pools (Vicca *et al.*, 2012), mostly associated with mycorrhizal fungi, the respiration of which is difficult to separate from R_A (Högberg & Read, 2006). Indeed, there is extensive evidence from pine plantations that multiple years of fertilization increase aboveground productivity and decrease belowground C supply (Linder *et al.*, 1987; Albaugh *et al.*, 1998, 2004; Maier & Kress, 2000; Maier *et al.*, 2004; McCarthy *et al.*, 2010). Within a single climate zone, a synthesis by Palmroth *et al.* (2006) showed that stands on sites in the medium-to-high fertility range have canopy leaf area that absorbs most available solar energy, so having higher leaf area in more fertile sites does not increase GPP proportionally. There, and within stands beyond the establishment phase where GPP does not increase with soil fertility, enhanced fertility causes an increased proportion of C to be allocated to aboveground production, lowering F_{soil} (Palmroth *et al.*, 2006). Consistent with this observation, we found that F^*_{soil} decreased as productivity (indicated by LAI and leaf litter fall) increased, but only under ambient atmospheric [CO_2] (Fig. 6a, b). This trend was not apparent under elevated [CO_2] where C is more plentiful. McCarthy *et al.* (2006) showed that aboveground production increased with both N availability and LAI in both [CO_2] treatments, with the elevated plots increasing at a greater rate than those under ambient conditions. Here, we show that the more direct measure of soil fertility, N availability, best explained the variation in F^*_{soil} across [CO_2] treatments. With increased soil N availability, more C was allocated to aboveground production (McCarthy *et al.*, 2010), reducing allocation belowground (Palmroth *et al.*, 2006) and F^*_{soil} (Fig. 6c) by similar amounts in both treatments.

In conclusion, in this temperate forest, higher base respiration rates under elevated [CO_2] increased potential annual soil CO_2 efflux (F^*_{soil}), averaging 127 g C m^{-2} per 100 ppm [CO_2], and actual F_{soil} (reflecting soil water limitations) averaging 100 g C m^{-2} per 100 ppm [CO_2]. Nitrogen fertilization reduced both temperature sensitivity of soil CO_2 efflux and the base respiration, resulting in a reduction in annual F^*_{soil} by ca. 114 g C m^{-2} per 1 g m^{-2} increase in soil N, regardless of [CO_2] level (Fig. 6c).

Base respiration appeared to decrease after the stabilization of canopy development (Fig. 5), but the treatment effects persisted (Fig. 3). Soil temperature and soil water content did not vary appreciably among treatments, nor did they exhibit a linear pattern over time (Fig. 4a, b); however, interannual variability in soil water content produced reductions in F_{soil} from potential values. Among the drier years, for each 1% reduction in REW, F_{soil} was depressed by an additional 0.7%, an effect that was only slightly buffered by elevated [CO$_2$].

Acknowledgements

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