

Research Article

Effects of Atmospheric CO₂ Enrichment on Soil CO₂ Efflux in a Young Longleaf Pine System

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The southeastern landscape is composed of agricultural and forest systems that can store carbon (C) in standing biomass and soil. Research is needed to quantify the effects of elevated atmospheric carbon dioxide (CO₂) on terrestrial C dynamics including CO₂ release back to the atmosphere and soil sequestration. Longleaf pine savannahs are an ecologically and economically important, yet understudied, component of the southeastern landscape. We investigated the effects of ambient and elevated CO₂ on soil CO₂ efflux in a young longleaf pine system using a continuous monitoring system. A significant increase (26.5%) in soil CO₂ efflux across 90 days was observed under elevated CO₂; this occurred for all weekly and daily averages except for two days when soil temperature was the lowest. Soil CO₂ efflux was positively correlated with soil temperature with a trend towards increased efflux response to temperature under elevated CO₂. Efflux was negatively correlated with soil moisture and was best represented using a quadratic relationship. Soil CO₂ efflux was not correlated with root biomass. Our data indicate that, while elevated CO₂ will increase feedback of CO₂ to the atmosphere via soil efflux, terrestrial ecosystems will remain potential sinks for atmospheric CO₂ due to greater biomass production and increased soil C sequestration.

1. Introduction

The rural southeastern landscape is dominated by three vegetation types (crops, forests, and pastures), all of which have the ability to store atmospheric carbon (C) as standing biomass (including plant roots) or in soil. One particularly important ecosystem is longleaf pine savannahs. Prior to European settlement, the coastal plains of the southeastern United States were dominated by nearly pure stands of longleaf pine (*Pinus palustris* Mill.) with a diverse understory plant community; some longleaf ecosystems have the highest reported values for species richness, including many threatened and endangered species, in the temperate Western Hemisphere [1]. This system now occupies only 2% of its former range [2], a loss comparable to or exceeding that of most endangered communities throughout the world including the North American tallgrass prairie, the moist tropical coastal forest of Brazil, and the dry forests along

the Pacific coast of Central America [3]. Longleaf pine forests in the southeast currently occupy sites at the more xeric end of the moisture continuum and are often found on soils with low N availability. In fact, it is not unusual to find disjunct longleaf pine communities in the rural farm landscape. Thus, landowner interest in this species has increased dramatically over the last decade, not only due to its ecological significance but also because of superior lumber quality, fire tolerance, and resistance to some of the more devastating southern forest insects (e.g., bark beetles) and diseases (e.g., fusiform rust). Given that longleaf pine systems may become a more important component of the rural farm landscape, it is important to determine how the rising level of carbon dioxide (CO₂) in the atmosphere [4] will impact these systems.

Carbon dioxide is the first molecular link from atmosphere to biosphere. Most plant species increase biomass

production when exposed to above-ambient levels of atmospheric CO₂ (e.g., [5–9]). Positive plant responses to higher CO₂ can be attributed to increased photosynthetic capacity [10], water use efficiency [8, 11], and nutrient uptake and utilization efficiency [6].

The rising level of atmospheric CO₂ has prompted speculation on the ability of terrestrial ecosystems to sequester C as a means of mitigating this rise and its potential impacts on climate. However, as the ability of terrestrial ecosystems to store C (in biomass and/or in soil) is not based solely on net primary productivity [12], elevated atmospheric CO₂ may also impact terrestrial ecosystem C storage through alterations in plant tissue quality, which will impact soil microbes, decomposition processes, and subsequent soil C storage. Plant tissue produced under high CO₂ often has higher C:N ratios [13, 14] and may be structurally different, with alterations in leaf anatomy [15] and epicuticular waxes [16, 17]. Plants grown under elevated CO₂ may also exhibit altered tissue chemistry, including lower N concentrations [18, 19], higher concentrations of carbohydrates [19, 20], and increased levels of defense compounds such as phenolics [21, 22].

The fate of C within plant systems is affected by a chain of biological events starting with transfer of C from air to leaf, transformation within the plant, translocation within the plant/soil system, return of plant residue to the soil, and decomposition and is impacted by the effects of other environmental factors (e.g., temperature, nutrients, and water) on these processes. Therefore, the ability of terrestrial ecosystems to sequester C will depend on C cycling among the various biomass and soil pools and on the residence time of the C within these pools [23].

At many stages in the cycling of C within terrestrial ecosystems, CO₂ is transferred back to the atmosphere by both autotrophic and heterotrophic respiration. Soil respiration is a significant source of CO₂ flux from terrestrial ecosystems to the atmosphere [24], with global estimates ranging from 68 to 100 Pg C yr⁻¹ [25, 26]. Therefore, even small shifts in soil CO₂ efflux could have serious implications for increasing or decreasing atmospheric CO₂ concentration and the resulting impacts on climate change [27]. Through its impact on the quantity and quality of C within the plant/soil system, elevated CO₂ can affect this feedback of C to the atmosphere. For example, increased root growth under elevated CO₂ could increase root respiration [28], while changes in root exudation and/or quality might enhance [23, 29] or suppress [30] microbial respiration. The combined effects on total soil CO₂ respired back to the atmosphere, and the potential for C sequestration, are difficult to predict.

One review of soil and microbial respiration demonstrates that elevated atmospheric CO₂ generally increases belowground respiration, with overall estimates ranging from 40 to 50% for soil respiration and from 20 to 35% for microbial respiration [31]; these estimates agree with another review that reported an overall increase of 37% for forest species [32]. Other elevated CO₂ studies report stimulation of root or total soil respiration in the range of 15–50% [33–35], with even greater stimulation reported in some cases [36, 37]. Enhanced root or soil respiration

under high CO₂ is often related to increased root biomass, that is, autotrophic respiration [31, 34, 36, 37] and/or increases in the size or activity of the microbial community, that is, heterotrophic respiration [31, 34, 37, 38]. However, some cases [30, 33] showed elevated CO₂ to suppress soil respiration or to have no effect [39, 40]. Soil CO₂ efflux can be highly variable on temporal and spatial scales within a single field experiment [34, 41] and among experiments; therefore, even relatively large increases in soil efflux under elevated CO₂ may not be statistically significant [31]. Some of the variation among individual studies may be due to differences in plant species, experimental conditions, or methods used for determination of CO₂ efflux.

A major drawback of most methods for determining soil CO₂ efflux concerns the timescale of measurements (i.e., cumulative totals across hours to days with NaOH traps or discreet points in time with soil collars and gas exchange devices); efflux between measurement periods is then generally assumed to be linearly integrative across the intervening time periods [34, 37]. Given the varying responses of soil CO₂ efflux to elevated atmospheric CO₂ and the limitations of current measurement technology, more research is needed before we can confidently predict the impacts of elevated atmospheric CO₂ on the ability of terrestrial ecosystems to sequester C. The objective of this experiment was to assess the response of soil CO₂ efflux (root plus microbial respiration) to three years of atmospheric CO₂ enrichment in a model regenerating longleaf pine community using a novel, continuous CO₂ efflux monitoring system; correlations of efflux with changes in root biomass, populations of microbes and micro- and mesofauna, and soil C were also investigated.

2. Materials and Methods

2.1. Study Site. A model regenerating longleaf pine-wiregrass ecosystem was constructed in Spring 1998 at the National Soil Dynamics Laboratory in Auburn, AL; descriptions of the study site and model ecosystem have been previously reported [42]. Briefly, an assemblage of five early successional forest species representing major functional guilds within a typical longleaf pine-wiregrass community was chosen for study: longleaf pine (*Pinus palustris*, a C₃ evergreen conifer), wiregrass (*Aristida stricta*, a C₄ bunch grass), sand post oak (*Quercus margaretta*, a C₃ broadleaf tree), rattlebox (*Crotalaria rotundifolia*, a C₃ perennial herbaceous legume), and butterfly weed (*Asclepias tuberosa*, a C₃, nonleguminous, herbaceous perennial). These species are common associates throughout the southeastern USA. The model forest community was assembled in April 1998 on an outdoor soil bin (2 m deep, 6 m wide, and 76 m long) containing a Blanton loamy sand (loamy, siliceous, and thermic Grossarenic Paleudults). The planting regime used [42] reflected densities found in naturally regenerating longleaf pine-wiregrass ecosystems [43, 44].

Open top chambers [45], encompassing 7.3 m² of ground surface area, were used to deliver target CO₂ concentrations of 365 μmol mol⁻¹ (ambient) or 720 μmol mol⁻¹ (elevated)

beginning June 1998 using a delivery system described by Mitchell et al. [46]. The study area was divided into six blocks, and each CO₂ treatment was randomly assigned to one open top chamber within each block; therefore, the experimental design was a randomized complete block design, with blocks occurring along the length of the soil bin.

2.2. Soil Respiration Measurements. Soil CO₂ efflux was measured using the Automated Carbon Efflux System (ACES) (US Patent 6,692,970), developed at USDA Forest Service, Southern Research Station Laboratory in Research Triangle Park, NC; a description of the ACES has been previously reported [47]. Briefly, ACES is a chamber-based, multi-port respiration measurement system, which uses open system, dynamic soil respiration chambers measuring 25 cm diameter (491 cm²) equipped with air and soil thermocouples (soil thermocouples were inserted to depth of 5 cm). The soil chambers are designed with pressure equilibration ports to ensure that differences in chamber pressure do not compromise the quality of the respiration measurement [48]. Each ACES has 15 sample chambers and one null calibration chamber, which are measured sequentially for 10 minutes each, allowing a complete run every 2 hours and 40 minutes or nine complete runs per day. When not being actively sampled, all chambers are refreshed with reference air to prevent buildup of CO₂. The ACES units constructed for our study were modified to allow use of reference air from two sources, owing to the differential atmospheric CO₂ concentrations employed; soil chambers in ambient CO₂ open top chambers were refreshed with ambient CO₂ air, while those in elevated open top chambers were refreshed with elevated CO₂ air. Ambient CO₂ reference air was obtained by placing an air compressor in an additional, empty, ambient open top chamber located on an adjacent soil bin and using the same CO₂ delivery system as the main study; elevated CO₂ reference air was similarly obtained by placing a second air compressor in an additional, empty, elevated open top chamber. The air compressors replace the ballast tanks commonly used with the ACES, which provide reference air for the ACES that is buffered against fluctuations in atmospheric CO₂ concentration [47].

Constraints on distance between soil respiration chambers and the main ACES unit (housing the infrared gas analyzer and datalogger) necessitated use of two ACES units in this study; one was used for blocks 1–3 and a second for blocks 4–6. Two soil chambers were placed into each of the 12 open top chambers; the three additional soil chambers for each system were placed outside of open top chambers. Calibration chambers were placed into the ambient open top chamber nearest to each main ACES unit. A soil moisture probe was placed adjacent to each calibration chamber and inserted to a depth of 20 cm.

To minimize the effect of precipitation exclusion on the soil substrate within the soil chambers, soil chambers were moved every 3–4 days between two sample points (A and B) within each open top chamber. Litter on the soil surface was not removed from each sample point, but all points were kept free of live vegetation. The ACES units were

installed on March 6, 2001, at which time the study had been continuously exposed to CO₂ treatments for 33 months. The ACES units were run continuously until June 4, 2001 (day of year (DOY) 65 through 155), with the exception of brief periods for maintenance or due to system/power failures; at this time they were removed to allow for a complete destructive harvest of the study. Details of the harvest, along with associated biomass and plant and soil C data, have been previously reported [49].

2.3. Soil Biology Assessments. Root-zone soil, from the 0–15 cm depth increment, was collected using large soil cores (24.5 cm diameter × 60 cm deep) and an extraction method of our own design [50]. The soil was then passed through a 2 mm mesh stainless steel sieve until 10–20 g of sieved soil was collected. Dehydrogenase activity, a reliable index of microbial activity in soil [51], was determined from modified procedures described by Tabatabai [52]. Sieved soil (≈1 g) for triplicate subsamples from each plot was placed in test tubes (15 × 100 mm), covered with 1 mL of 3% aqueous (w/v) 2,3,5-triphenyltetrazolium chloride and stirred with a glass rod. After 96 hr incubation (27°C), 10 mL of methanol was added to each test tube, and the suspension was vortexed for 30 sec. Tubes were then incubated for 1 hr to allow suspended soil to settle. The resulting supernatant (≈5 mL) was carefully transferred to clean test tubes using Pasteur pipets. Absorbance was read spectrophotometrically at 485 nm, and formazan concentration was calculated using a standard curve produced from known concentrations of triphenyl formazan. One subsample of sieved soil (≈1 g) from each soil sample was used for determination of soil moisture so that formazan concentrations could be expressed per gram soil dry weight.

Soil taken from the previously described cores was extracted for relative populations of Collembola and Acari by a modified version of the Tullgren system as described by Wiggins et al. [53]. Soil samples in large funnels, with stems positioned over water in a collecting tube, were arranged in series under 40 W light bulbs. The animals, migrating in advance of the slowly drying soil (5–7 days), were collected live. Populations, counted under a dissecting microscope, were expressed as numbers per kg of air-dried soil. A subsample of the soil collected for soil animals was sent to the Department of Entomology and Plant Pathology, Auburn University for assessment of nematode populations.

2.4. Data Analysis. All soil respiration data were analyzed for system and power failures; obvious “systematic” errors were parsed from the data set. A total of 18,813 soil CO₂ efflux observations were taken over the 90-day measurement period; of these, 94.4% were deemed acceptable for analysis. Data analysis was conducted using the mixed model procedures (Proc Mixed) of the Statistical Analysis System [54]. Data were initially analyzed to determine if differences existed between the two ACES units employed in the study or between soil chamber positions (A versus B); as no significant unit or positional effects were noted, data were not segregated prior to analysis. Effects of CO₂

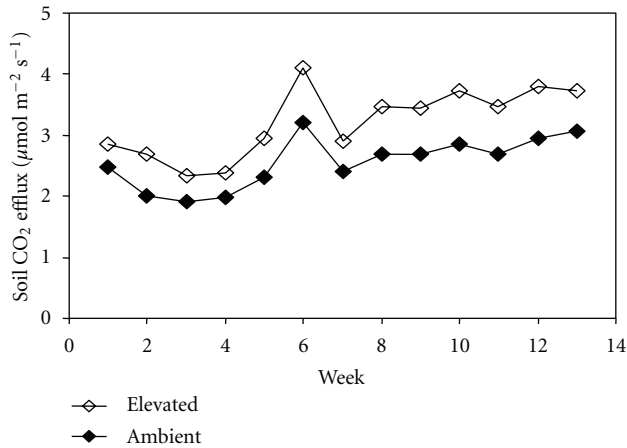


FIGURE 1: Weekly average soil CO₂ efflux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for ambient ($365 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and elevated ($720 \mu\text{mol CO}_2 \text{ mol}^{-1}$) CO₂ plots in a model regenerating longleaf pine-wiregrass ecosystem.

concentration were determined using the analysis of variance statistics derived from the mixed procedure of SAS; effects were determined by day, by week, and across the entire measurement period. All data from each ACES chamber (total = 24) were then averaged for 1.0°C intervals of soil temperature measured at a depth of 5 cm at each ACES chamber, regardless of DOY; averaging served to reduce the influence of outliers on the response of soil CO₂ efflux to temperature throughout the experiment. Linear regression [55] was then used on the averaged data to determine the relationship between soil CO₂ efflux and soil temperature; a similar procedure was used to investigate the relationship between soil CO₂ efflux and soil moisture. The relationship of soil CO₂ efflux with components of root biomass and with soil C and N (previously reported by Runion et al. [49]) was also investigated using linear regression. Specific respiration rates were calculated by dividing cumulative soil CO₂ efflux (g C m^{-2}) over the entire measurement period by total root biomass, total root C and N, and total soil C and N (g m^{-2}) to give g C respired per g root dry weight, root C or N, or per g soil C or N.

3. Results

Soil CO₂ efflux, averaged across the entire 90 day measurement period, from ambient plots was $2.54 (\pm 0.008; n = 8884) \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, while elevated plots averaged $3.22 (\pm 0.011; n = 8878) \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; this represented a significant increase ($P < 0.0001$) of 26.8% or a total increase of $\approx 60 \text{ g C m}^{-2}$. When averaged on a weekly basis (Figure 1), elevated CO₂ plots consistently had significantly higher ($P < 0.0001$) soil respiration rates than did ambient plots, with the increase ranging from 15 to 33%. Further, when analyzed on a daily average basis (data not shown), elevated CO₂ significantly increased ($P < 0.05$) soil respiration on all but two days (DOY 79, $P = 0.08$, and DOY 80, $P = 0.99$);

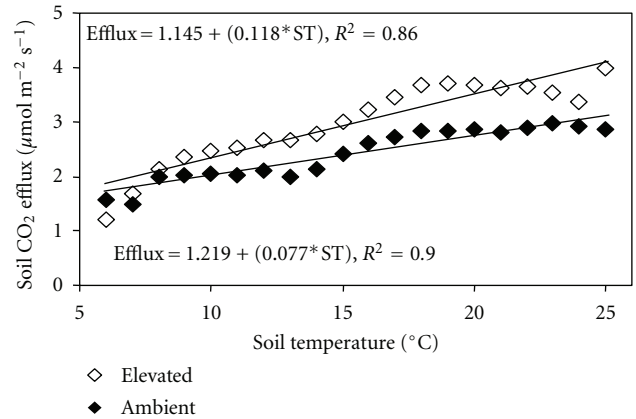


FIGURE 2: Response of soil CO₂ efflux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) to soil temperature for ambient ($365 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and elevated ($720 \mu\text{mol CO}_2 \text{ mol}^{-1}$) CO₂ plots in a model regenerating longleaf pine-wiregrass ecosystem. Equations describing each line, with fit statistics, are provided above (elevated) and below (ambient) the lines.

these two days had the lowest daily average soil temperatures recorded during the entire duration of the study (7.3 and 8.0, resp.).

Regression of averaged soil CO₂ efflux on soil temperature (Figure 2) showed strong positive linear relationships for both ambient and elevated CO₂ plots ($r^2 = 0.90$ and 0.86 for ambient and elevated, resp.). Assessment of non-linear models did not improve the fit of these data over the linear models. The slope of the line for elevated CO₂ plots was significantly steeper ($P < 0.01$) than for ambient plots; Y-intercepts for these two regression lines did not differ ($P = 0.15$). Using these regressions, we calculated the change in soil CO₂ efflux for a 10°C change in soil temperature for each set of plots; these values were 0.77 and $1.18 \mu\text{g m}^{-2} \text{ s}^{-1}$ for ambient and elevated CO₂ plots, respectively, indicating a 53% increase in the response of efflux to increasing temperature under elevated CO₂. Soil moisture, collected only in two ambient CO₂ plots (Figure 3), also showed a strong linear correlation with soil CO₂ efflux, albeit a negative relationship ($r^2 = 0.76$). However, these data showed a better fit when a quadratic function was employed ($r^2 = 0.96$).

Soil CO₂ efflux was not correlated with fine, coarse, or total root biomass (r^2 range = 0.01 to 0.35), whether analyzed for each plant species or for total across all species. Similar trends were observed when correlating soil CO₂ efflux with either root N or C (data not shown).

No effects of CO₂ treatment on dehydrogenase were observed ($P = 0.40$). Soil CO₂ efflux was not correlated with dehydrogenase ($r^2 = 0.40$ and 0.05 for ambient and elevated CO₂ treatments, resp.). While numbers of both nematodes and soil animals were higher under elevated CO₂, these effects were not significant ($P = 0.40$ and 0.15 for nematodes and soil animals, resp.). Soil CO₂ efflux was, again, not correlated with numbers of nematodes or soil animals (r^2 range = 0.01 to 0.35).

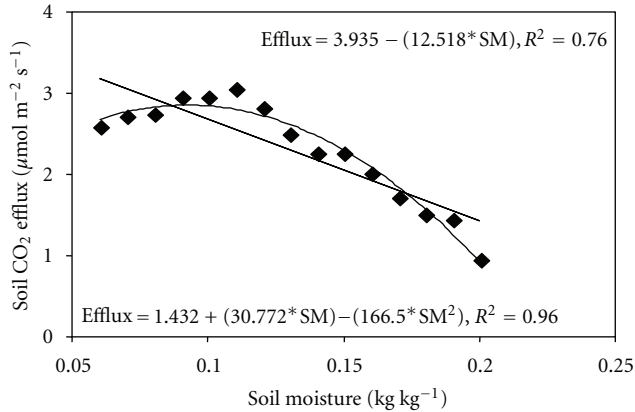


FIGURE 3: Response of soil CO₂ efflux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) to soil moisture for ambient ($365 \mu\text{mol CO}_2 \text{ mol}^{-1}$) CO₂ plots only in a model regenerating longleaf pine-wiregrass ecosystem. Equations describing this line, with fit statistics, are provided above (linear) and below (quadratic) the lines.

Soil C and N content did not differ among plots at initiation of the study (1852 and 197 g m^{-2} for soil C and N, resp.). These variables also varied little at study termination (3042 and 2975 g C m^{-2} and 165 and 163 g N m^{-2} for ambient and elevated plots, resp.). Soil CO₂ efflux was positively correlated with both soil C and N content measured at the end of the study; the significance of these correlations varied by soil profile depth (Table 1). Correlations of soil CO₂ efflux with soil C tended to be stronger than correlations with soil N.

Specific soil CO₂ efflux rates per g root dry weight or per g root C were significantly lower in elevated than in ambient CO₂ plots; specific respiration rate per g root N was not different between CO₂ treatments (Table 2). However, specific respiration rates per g soil N and C were significantly higher in elevated than in ambient CO₂ plots.

4. Discussion

The observed increase in soil CO₂ efflux under elevated CO₂ in this study is consistent with other reports in the literature (e.g., [31]). The $\approx 60 \text{ g m}^{-2}$ increase observed across the 90-day measurement period is also comparable with the $\approx 178 \text{ g C m}^{-2}$ increase reported by Butnor et al. [47] who used ACES over a 220-day period in a 17-year old loblolly pine (*P. taeda*) stand. The consistency of the increase we observed, on a weekly or daily basis, further demonstrates that growth under high CO₂ had a sustained impact on soil CO₂ efflux in the model longleaf pine community following 33–36 months of constant exposure to a twice ambient concentration of atmospheric CO₂.

Temperature is known to strongly influence soil respiration [56, 57], with efflux increasing as temperature increases, as observed in this study. Soil CO₂ efflux has generally been shown to increase as an exponential function of temperature [57]; however, in the present study this relationship was more than adequately described using a linear function for both

ambient and elevated CO₂ treatments. Under elevated CO₂, the increased responsiveness (i.e., steeper slope) of soil CO₂ efflux to temperature might suggest increased feedback of C to the atmosphere under global warming. However, when we attempted to fit quadratic relationships of soil respiration to soil temperature (data not shown), we observed differences in the inflection points of the curves (30 and 24°C for ambient and elevated plots, resp.), as well as a slight increase in fit statistics ($r^2 = 0.92$ and 0.94 for ambient and elevated plots, resp.). This analysis suggested that, at soil temperatures above 24°C , the increase in soil CO₂ efflux under elevated CO₂ was reduced; extrapolation of these curves indicated that efflux for both CO₂ treatments would be nearly equal at $\approx 33^\circ\text{C}$. Additional research is needed to verify these extrapolations.

Soil moisture is known to affect soil CO₂ efflux through both physical (displacing soil gases) and biological (impacts on root and microbial activity) means [56]. Although the relationship is generally positive, negative relationships (as observed in the present study) have been reported [56, 58]. The improved fit of our data to a quadratic relationship suggests the existence of a soil moisture content at which soil CO₂ efflux is maximized; this would, obviously, be dependent on soil type. Therefore, as most prior soil CO₂ efflux data have been collected using a series of spot measurements or measurements integrated across relatively short time scales, it is possible that the varying responses (i.e., positive versus negative) of soil CO₂ efflux to soil moisture in previous studies [56, 58] might be explained by knowing where data fell on the quadratic response curve (Figure 3).

Increased rates of soil CO₂ efflux under high CO₂ have often been shown to be related to increases in root biomass [31, 34, 36, 37]. Course, fine, and total root biomass were all increased by elevated CO₂ in this experiment [49]. Therefore, the lack of strong correlations between soil CO₂ efflux and root biomass, root C, or root N was unexpected. Most likely, this lack of correlation was due to high variability within the data, particularly variability among species [49]. The lower specific respiration rates for root dry weight and root C under elevated CO₂ are primarily due to the fact that root biomass increased more than soil CO₂ efflux. In contrast, the higher specific respiration rates per g soil C or N are primarily due to the fact that elevated CO₂ increased soil CO₂ efflux to a greater degree than soil C or N.

Assessments of soil microbial activity and populations of soil micro- and mesofauna at study termination showed no differences between CO₂ exposure treatments, again suggesting that root respiration was primarily responsible for the observed differences in soil CO₂ efflux. However, since microbial parameters are highly variable even on short temporal scales, it is likely that assessment of these parameters solely at the end of the study does not accurately reflect their overall contribution to soil CO₂ efflux across the 90-day measurement period. Further, the strong correlations of CO₂ efflux with soil N and, especially, C might also indicate a greater contribution of heterotrophic respiration to total soil efflux than the microbial assessments suggested. Separation of heterotrophic and autotrophic respiration would have aided explanation of these trends.

TABLE 1: Regression parameters and statistics for relationships of total soil CO₂ efflux (g C m⁻²) over the 90-day measurement period with soil N and C content.

CO ₂ ^a	Soil profile depth	Soil elemental content (g m ⁻²) ^b	Intercept	Variable	Significance of variable (<i>pr</i> > <i>t</i>)	<i>r</i> ²
Ambient	0–15 cm	N	163.970	1.550	0.17	0.42
	15–30 cm	N	172.578	1.516	0.17	0.41
	30–45 cm	N	136.918	2.381	0.12	0.49
	45–60 cm	N	185.687	1.101	0.30	0.26
	0–60 cm	N	165.534	0.403	0.18	0.39
Elevated	0–15 cm	N	169.059	2.805	0.07	0.60
	15–30 cm	N	179.336	2.908	0.26	0.30
	30–45 cm	N	207.544	2.197	0.22	0.35
	45–60 cm	N	220.245	1.795	0.30	0.26
	0–60 cm	N	190.030	0.633	0.18	0.39
Ambient	0–15 cm	C	57.834	0.215	0.21	0.36
	15–30 cm	C	-34.372	0.379	0.05	0.67
	30–45 cm	C	-130.076	0.499	0.04	0.69
	45–60 cm	C	-20.394	0.315	0.12	0.50
	0–60 cm	C	-142.144	0.123	0.02	0.79
Elevated	0–15 cm	C	-61.083	0.443	0.01	0.88
	15–30 cm	C	-79.153	0.528	0.08	0.57
	30–45 cm	C	75.940	0.309	0.11	0.51
	45–60 cm	C	19.100	0.356	0.14	0.46
	0–60 cm	C	-120.255	0.139	0.01	0.83

^a Ambient CO₂ ≈ 365 μmol mol⁻¹; elevated CO₂ ≈ 720 μmol mol⁻¹. ^bN: soil nitrogen; C: soil carbon.

TABLE 2: Specific respiration rates (g C respired per g) for root biomass, root C and N and soil C and N.

CO ₂ ^a	Root dry weight	Root N	Root C	Soil N	Soil C
Ambient	0.172	34.493	0.375	1.437	0.076
Elevated	0.146	32.262	0.315	1.848	0.098
ANOVA	<i>P</i> = 0.03	<i>P</i> = 0.32	<i>P</i> = 0.02	<i>P</i> < 0.01	<i>P</i> < 0.01

^a Ambient CO₂ ≈ 365 μmol mol⁻¹; elevated CO₂ ≈ 720 μmol mol⁻¹.

Previous research with container-grown longleaf pine seedling showed that N was the controlling factor; under low N conditions, longleaf growth response to elevated CO₂ was negligible [14]. In the current study, Torbert et al. [59] found increased soil N mineralization under elevated CO₂, indicating that N resources should not be limiting for either microbial decomposition of residues or plant growth in future regenerating longleaf pine systems. Therefore, despite this study receiving no N additions throughout the three years, a positive growth response to elevated CO₂ was observed for longleaf pine [49].

We assessed the overall impact of CO₂ enrichment on this model longleaf pine community through its first three years of growth by extrapolating biomass (above- and belowground, as well as litter), soil C and N [49], and soil respiration data from this study. Elevated CO₂ resulted in a significant increase of 4.07 Mg C ha⁻¹ yr⁻¹ sequestered in

standing biomass (ambient CO₂ = 6.29 Mg C ha⁻¹ yr⁻¹; elevated CO₂ = 10.36 Mg C ha⁻¹ yr⁻¹) with an additional significant increase of 0.54 Mg C ha⁻¹ yr⁻¹ in litter (ambient CO₂ = 0.72 Mg C ha⁻¹ yr⁻¹; elevated CO₂ = 1.26 Mg C ha⁻¹ yr⁻¹). The change in soil C was not significantly different between CO₂ treatments at termination of the study (3.97 and 3.74 Mg C ha⁻¹ yr⁻¹ for ambient and elevated CO₂, resp.). Therefore, the entire system showed a gain of 4.38 Mg C ha⁻¹ yr⁻¹ due to exposure to elevated atmospheric CO₂ (ambient CO₂ = 10.98 Mg C ha⁻¹ yr⁻¹; elevated CO₂ = 15.36 Mg C ha⁻¹ yr⁻¹). It should be noted that soil respiration rates from the final three months of exposure were used to estimate soil CO₂ efflux over the three-year study period. Also, we did not assess nighttime plant respiration; it is unlikely this would have significantly impacted the analysis since plant respiration has been shown to be relatively unresponsive to elevated CO₂ [60]. Despite

increased soil respiration of 2.54 Mg CO₂-C ha⁻¹ yr⁻¹ under elevated CO₂, our estimates suggest a net increased storage of 1.84 Mg C ha⁻¹ yr⁻¹ with the majority of the added C residing in plant biomass. Torbert et al. [59] found decreased soil C turnover under elevated CO₂, suggesting that increased C sequestration in soil is possible in these longleaf systems.

In general, elevated CO₂ increases soil CO₂ efflux due to increases in autotrophic respiration from increased root growth and/or increased heterotrophic respiration associated with microbial use of increased C inputs [31, 34]. However, despite increased soil CO₂ efflux under elevated atmospheric CO₂, terrestrial ecosystems can still be potential sinks for atmospheric CO₂ due to greater biomass production and increased soil C sequestration. This may be particularly true for forest systems. For example, our research indicates that regenerating longleaf pine systems have the potential to be sinks for atmospheric CO₂ in a future elevated CO₂ environment. These findings are especially important given that longleaf pines currently occupy less productive, low N sites and given the increasing landowner interest in this species due to its superior economic and ecological attributes.

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