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Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO_2

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ABSTRACT

Elevated atmospheric CO_2 tends to stimulate plant productivity, which could either stimulate or suppress the processing of soil carbon, thereby feeding back to atmospheric CO_2 concentrations. We employed an acid-hydrolysis-incubation method and a net nitrogen-mineralization assay to assess stability of soil carbon pools and short-term nitrogen dynamics in a Florida scrub-oak ecosystem after six years of exposure to elevated CO_2. We found that soil carbon concentration in the slow pool was 27% lower in elevated than ambient CO_2 plots at 0–10 cm depth. The difference in carbon mass was equivalent to roughly one-third of the increase in plant biomass that occurred in the same experiment. These results concur with previous reports from this ecosystem that elevated CO_2 stimulates microbial degradation of relatively stable soil organic carbon pools. Accordingly, elevated CO_2 increased net N mineralization in the 10–30 cm depth, which may increase N availability, thereby allowing for continued stimulation of plant productivity by elevated CO_2. Our findings suggest that soil texture and climate may explain the differential response of soil carbon among various long-term, field-based CO_2 studies. Increased mineralization of stable soil organic carbon by a CO_2-induced priming effect may diminish the terrestrial carbon sink globally.

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1. Introduction

The global soil organic carbon (SOC) pool contains 200 times the amount of carbon (C) emitted by humans annually. Changes to this vast C reservoir have influenced atmospheric CO_2 concentrations during past climatic cycles (Doney and Schimel, 2007) and could mediate important feedbacks on modern-day climatic change, either moderating or exacerbating the consequences of anthropogenic C emissions (Houghton, 2007). Several global models incorporate these fast-acting biological feedbacks, but great uncertainty remains in their direction and magnitude (Denman et al., 2007; Heimann and Reichstein, 2008). Biologically-mediated changes in the size of the stable SOC pool are one important source of uncertainty. Elevated CO_2 generally increases plant productivity (Dijkstra et al., 2002; Ainsworth and Long, 2005), which also generally increases inputs of plant organic matter into soils (e.g., Hungate et al., 2006). Increased plant inputs should increase the global SOC pool provided they are not offset by an increase in SOC decomposition rates. However, the influence of enhanced plant inputs on microbial mineralization of the existing SOC pool is poorly understood and remains a confounding factor in future global carbon cycle projections.

The presence of plants can increase the decomposition rate of SOC several-fold compared to soils without plants (Kuzyakov, 2002; Cheng et al., 2003). Although the mechanisms are not well-understood, the stimulation of SOC decomposition appears to increase with plant biomass (Dijkstra et al., 2006). Therefore, any perturbation that alters plant productivity, such as elevated CO_2, could change SOC mineralization rates, resulting in what is known as a "priming effect". It has been shown that priming effects brought about by increased plant productivity can outweigh environmental effects on SOC mineralization such as soil warming (Bader and Cheng, 2007). Hereafter, we use the term "priming" for the stimulation of decomposition in slowly cycling SOC pools that results from increased plant growth at elevated CO_2.

Greenhouse-based and short-term field experiments show that elevated CO_2 can either stimulate mineralization of existing SOC, resulting in priming effects (Körner and Arnone, 1992; Zak et al., 1993; Wolf et al., 2007), or have the opposite effect and suppress

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mineralization of existing SOC (Rouhier et al., 1994; Cardon et al., 2001). Although it is difficult to detect priming effects in field experiments, they have been reported in grasslands (Pendall et al., 2003) and forests (Hoosbeek et al., 2004; Carney et al., 2007).

Elevated CO_2-driven priming effects may be more common than reported in the literature because the process is difficult to observe in the field. There are no straightforward methods for separating instantaneous measurements of CO_2 derived from pre- versus post-treatment SOC decomposition. Furthermore, the size of the pre-treatment SOC pool in many ecosystems is so vast and spatially variable that it masks relatively smaller treatment effects (Hungate et al., 1996). These issues help explain why there is no consensus on the magnitude, or even direction, of CO_2-effects on SOC decomposition rates. We used a combination of laboratory incubations, SOC pool partitioning into active, slow and resistant pools, and modeling to detect priming effects.

Priming of SOC may also alter soil nitrogen (N) dynamics (Hungate et al., 1997a). Though the precise chemical makeup of stabilized soil organic matter is not well described, the elemental ratios of stable SOC appear to follow some universal patterns. Stable soil organic matter pools have a lower C:N ratio than more labile soil pools (Parton et al., 1987), and these differences in C:N ratios are conserved across many ecosystems. As the more resistant, relatively N-rich soil organic matter pools are processed, more N should be mineralized for each unit of C mineralized (Luo et al., 2004). Thus, increased mineralization of the relatively stable and N-rich SOC pools may increase the net release of inorganic N.

An early and still unresolved hypothesis in elevated CO_2 research is that the stimulation of plant growth will sequester N in plant matter and, over time, limited N availability will constrain the plant growth response to elevated CO_2. However, several ecosystems, including our site, do not exhibit as large of a decline in the stimulatory effect of elevated CO_2 on plant growth as predicted by N budgeting (Hungate et al., 2006; Finzi et al., 2007). Recently, Carney et al. (2007) used isotopic tracers to show that elevated CO_2, acting through changes in the soil microbial community, stimulated SOM mineralization in a scrub-oak forest. As such, primed mineralization of organic matter could be one cryptic N source that sustains CO_2-stimulation of productivity in other forest ecosystems (as reported for FACE studies in Wisconsin, North Carolina and Tennessee in Finzi et al., 2007).

We investigated soil C stability and N dynamics in soils from a long-term manipulation of atmospheric CO_2 in a Florida scrub-oak ecosystem. We hypothesized that enhanced SOC mineralization over 6 years of CO_2 manipulation reduced SOC in the relatively N-rich, slow-cycling pools (mean residence time = 15–45 years, Paul et al., 2006) relative to other pools. We measured CO_2 evolution from laboratory incubations of field soils to estimate cumulative effects on the C pool structure of these soils. We combined these data with static measures of total and acid-resistant C to parameterize a two-pool constrained model that partitioned the priming effect into three discrete C pools according to stability. Further, we measured net N mineralization in soils from these same incubations to assess N cycling in the context of priming. We predicted that a CO_2 priming effect would result in: 1) reduced SOC in the slow-cycling pool, and 2) enhanced net N-mineralization rates in the elevated-CO_2 treatment.

2. Methods

2.1. Study site

The study site was located on Merritt Island, a barrier island located at NASA's Kennedy Space Center on the east coast of central Florida, USA (28°38′N, 80°42′W). The climate is subtropical; temperatures reach an average daily maximum of 33.3 °C in July and a minimum of 9.6 °C in January. Annual precipitation averages 131 cm, with most of the precipitation falling from June through October. Three perennial evergreen oaks, *Quercus myrtifolia* Willd., *Quercus geminata* Small, and *Quercus chapmanii* Sarg., constitute up to 90% of the aboveground biomass at the site (Schmalzer and Hinkle, 1992). This ecosystem burns frequently, with an estimated fire-return interval of 8–12 years. The site was burned twice in 1995 prior to the establishment of the chambered sites and commencement of CO_2 fumigation in May 1996. Soils at the site are mapped as the Orsino series; hyperthermic uncoated Spodic Quartzipsamments, which are very deep, moderately well drained, very rapidly permeable soils that form in thick beds of sandy marine or aeolian deposits (NRCS USDA). Other soils that co-occur in the area are the Pomello and Zolfo series; both are sandy, siliceous, hyperthermic Oxyaquic Alorthods.

2.2. Experimental design

Sixteen octagonal open-top chambers (OTCs), 2.5 m tall, each enclosing a surface area of 9.42 m^2, were constructed with PVC framework and covered with rectangular panels of mylar (Melinez 071, Courtaulds Performance Films, Virginia, USA). A frustum atop each chamber narrowed the opening to 5.9 m^2 and reduced wind effects. Since May 1996, eight of the OTCs were maintained at ambient CO_2 concentrations and eight at elevated CO_2 (ambient + 350 μmol CO_2 mol^{-1}). The experimental setup, chamber design, and operation were described in detail by Dijkstra et al. (2002).

In May 2002, five soil cores (7 cm diameter) were taken to a depth of 60 cm in 10 cm increments at each of the 16 plots. The soils were sieved with a 2-mm sieve followed by a 1-mm sieve to remove roots and thoroughly mix the soil. Three of the five cores were randomly chosen for analyses. Subsamples of these soils acquired at depths of 0–10 cm, 10–30 cm, and 30–60 cm were designated for determination of organic matter dynamics in laboratory incubations. Depth increments of 10–30 cm and 30–60 cm required pooling subsamples of soil taken with those depth ranges. Equal soil masses from each 10-cm depth increment (10–30 cm and 30–60 cm) were thoroughly mixed and used to create composite samples for 10–30 cm and 30–60 cm depth increments, respectively. A 10 g subsample was removed from the sieved, homogenous soil for gravimetric soil moisture determination. Soil bulk density was determined by dividing total dry soil mass of each core (described in Brown et al., 2007) by the core volume.

Carbon dioxide evolution from laboratory-incubated soils was used in combination with measurements of the total SOC and acid-resistant SOC to determine discrete SOC pools and dynamics (Townsend et al., 1997; Paul et al., 2001; Paul et al., 2006). Soils (~120 g) were adjusted to and maintained at 60% water holding capacity (10% soil moisture dry wt equivalent), and incubated aerobically in glass jars for 256 d at ~25 °C in the dark. At each sampling date (1, 15, 28, 66, 140, and 256 d), jars were capped and the headspace was sampled at least 5 times at intervals ranging from several minutes to several hours (depending on the respiration rate) through a septum in the jar lid. Before each sample, to avoid drawing down pressure in the jar, a syringe with 2 ml of N_2 was injected into each jar, plunged three times to mix headspace. Two ml of mixed headspace air was taken for CO_2 concentration, against CO_2 calibration curves, with a Li-cor infrared gas analyzer (model Li-7000, Lincoln Nebraska) configured for in-line injection with an 8-port valve (Valco Instruments, Houston, TX) and N_2 as the carrier gas. Rates of C mineralization were estimated using the slope of least squares linear fit of $[CO_2] \times$ time for each jar at each time interval, correcting for dilution by N_2 pre-injection (average $r^2 = 0.996$).

On day 1 and day 15 following CO_2 flux measurements, a 6-g aliquot of soil was removed from each jar for N mineralization.

Aliquots were extracted with 30 ml 2 M KCl, shaken for 1 h, centrifuged for 20 min, and the supernatant was passed through a 20 μm filter. Inorganic nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) concentrations were determined colorimetrically with an Astoria-Pacific Solution Autoanalyzer (Clackamas, Oregon) using the indophenol blue method for NH_4^+ and cadmium reduction followed by diazotization with sulfanilamide for $NO_2^- + NO_3^-$. Net N mineralization was calculated as the final concentration of inorganic nitrogen (μg $NH_4^+ + NO_3$–N g^{-1} dry soil) after 15 d of incubation (T_1), minus the concentration at the start of the incubation (T_0). Total soil [C], total soil [N] and soil C/N, was determined on dried soil by dry combustion/gas chromatography with an elemental analyzer (model 440, Exeter Analytical Inc.).

The total SOC pool (C_{soc}) is the total amount of C in soil at time zero, and is the sum of the active (C_a), slow (C_s), and resistant (C_r) pools.

$$C_{soc} = C_a + C_s + C_r \quad (1)$$

C_{soc} and the acid-resistant C_r fraction were determined on dried soil by dry combustion/gas chromatography with an elemental analyzer (model 440, Exeter Analytical Inc.) (Paul et al., 2001, 2006). The acid-resistant soil fraction (C_r) was determined by refluxing 1 g of the soil with 10 ml of 6 M HCl at 116 °C for 16 h and then rinsing with 100 ml of deionized water. SOC remaining after the acid treatment was considered C_r.

A double-exponential decay model was fit to the CO_2 efflux data from the incubation to determine active pool size, C_a, and decay rate, k_a, and the slow pool decay rate, k_s. Respiration from the resistant pool was assumed to be negligible over the course of the incubation, and so, was excluded from the model (Paul et al., 2001):

$$C_t = C_a k_a e^{-k_a t} + C_s \times k_s e^{-k_s t} \quad (2)$$

where C_t was the SOC released as CO_2 at time, t (Paul et al., 2001). Decay rates per unit time were k_a and k_s for the active and slow pools, respectively. To estimate C_s independently, we substituted ($C_{soc} - C_a - C_r$) for C_s into equation (1) yielding the following equation.

$$C_t = C_a k_a e^{-k_a t} + (C_{soc} - C_a - C_r) \times k_s e^{-k_s t} \quad (3)$$

where C_{soc} and C_r are estimated from the hydrolysis method described. This model was fitted by non-linear regression (Proc NLIN; SAS, 1995). C_s was calculated by subtracting C_a and C_r from the total SOC pool. Turnover of C_a and C_s pools was expressed as mean residence time (MRT; MRT = $1/k$). Carbon concentrations were scaled to an areal basis using bulk density data described above for comparison to other C masses.

2.3. Statistical analyses

Respiration rates for chamber replicates ($n = 3$) were pooled to acquire a single respiration curve for each chamber ($n = 8$) at each depth. Total SOC, resistant SOC, C_s and modeled parameters (C_a, k_a, and k_s) were compared with a mixed model analysis of variance (ANOVA) using CO_2 treatment and soil depth as fixed effects (treatment block) ($n = 8$) (proc mixed, SAS, 1995). A random block ($P = 0.044$) was used for two chambers, one elevated and one ambient, because soil under these chambers had a second, shallower B_h horizon. The shallow B_h horizon (about 100 cm) is far below our deepest point (60 cm) that we examine for SOC in the present study, however, we have found some limited evidence that this horizon may be interacting with plant response to elevated CO_2 and under ambient conditions. We set the significance threshold at $P \leq 0.10$.

3. Results

The slow-cycling carbon pools at 0–10 cm were significantly smaller at elevated CO_2 compared to ambient CO_2 (Table 1). The total SOC pool at 0–10 cm was 21% smaller in elevated than ambient CO_2 ($P = 0.068$) and the slow-cycling SOC pool (C_s) at 0–10 cm was 27% smaller ($P = 0.084$). The 0–10 cm, acid-resistant SOC pool was 16% smaller in elevated CO_2, but this difference was not significant ($P = 0.341$). Elevated CO_2 did not significantly affect total, active, slow, or resistant soil C pools at depths below 10 cm (10–60 cm, $P > 0.10$), though SOC concentration tended to be lower in the elevated-CO_2 treatment (Fig. 1, Table 1). SOC concentration decreased sharply with depth, dropping by ≥80% between the A-horizon and the elluvial horizons (roughly 10–60 cm deep) (Table 1). There were no significant treatment effects on bulk density ($P > 0.1$, Table 2), or area-scaled soil C masses (g m^{-2}) at any depth (0–10, Table 3).

The relative proportion of the active (C_a), slow (C_s), and acid-resistant SOC pools varied with depth (Table 1). The proportion of total SOC in the acid-resistant SOC pool was similar at depths of 0–10 cm (57%) and 10–30 cm (56%), but then increased sharply at 30–60 cm depth (73%). The trend with depth was opposite for the proportion of the total SOC pool that was slow cycling; the 0–10 cm and 10–30 cm depths had similar proportions, ranging from 40 to 44%, followed by a sharp decrease at 30–60 cm to 21–30%. The proportion of the active C pool to the total SOC pool was always greater in the elevated-CO_2 treatment compared to ambient: 1.2% vs. 1.1% at 0–10 cm, 2.0% vs. 1.5% at 10–30 cm, and 1.6% vs. 1.5% at 30–60 cm.

The mean residence time (MRT = $1/k$) ranged from 9 to 14 d at all depth increments for the active SOC pool and 1.3–4.2 years for the slow-cycling SOC pool (Table 1). Because the average laboratory temperature (23 °C) was only slightly higher than the mean annual temperature at the study site (21.7 °C), laboratory and Q_{10}-adjusted field MRTs were similar. The shortest MRT for the slow-cycling SOC pool was at 30–60 cm. At this depth, the elevated-CO_2 treatment had a much shorter MRT (1.3 years) than the ambient treatment (2.1 years) despite also having a larger proportion of slow-cycling SOC (43%).

Table 1
Estimates of mean carbon concentration for each pool (±se) and turnover for active (C_a, k_a) and slow pools (C_s, k_s) to a depth of 60 cm in a scrub-oak ecosystem exposed to elevated and ambient CO_2 concentrations ($n = 8$), in central Florida using a two-pool constrained model. Asterisks (*) represent $P \leq 0.10$ for treatments at the same depth. The mean annual temperature for the study site is 21.7 °C, incubation temperature was 25 °C and the Q_{10} correction is $2^{(25-21.7)/10}$ to derive field MRT.

Depth (cm)	Treat.	C_{soc} (mg kg^{-1})	C_r (mg kg^{-1})	C_a (mg kg^{-1})	k_a (day^{-1})	MRT (day)		C_s (mg kg^{-1})	k_s (y^{-1})	MRT (year)	
						Lab	Field			Lab	Field
0–10	A	25,018(1694)*	13,791(1452)	270(32)	0.113(0.02)	8.8	11.1	10,958(1372)*	0.31(0.04)	3.19	4.02
	E	19,784(2378)*	11,583(1677)	256(38)	0.105(0.04)	9.5	12.0	7946(1163)*	0.21(0.07)	4.01	5.05
10–30	A	4787(611)	2702(261)	74(7)	0.084(0.01)	11.9	15.0	2011(436)	0.27(0.11)	3.79	4.78
	E	4112(501)	2267(198)	82(11)	0.072(0.01)	13.9	17.5	1763(319)	0.24(0.11)	4.24	5.35
30–60	A	1625(187)	1166(124)	25(4)	0.116(0.02)	8.6	10.8	433(189)	0.47(0.26)	2.10	2.65
	E	1857(344)	1216(172)	25(3)	0.112(0.01)	8.9	11.2	615(268)	0.75(0.4)	1.33	1.68

C_{soc} = total soil organic carbon; C_r, C_a and C_s = resistant, active and slow carbon pools, respectively; k_a and k_s = active and slow decay constants; MRT = mean residence time.

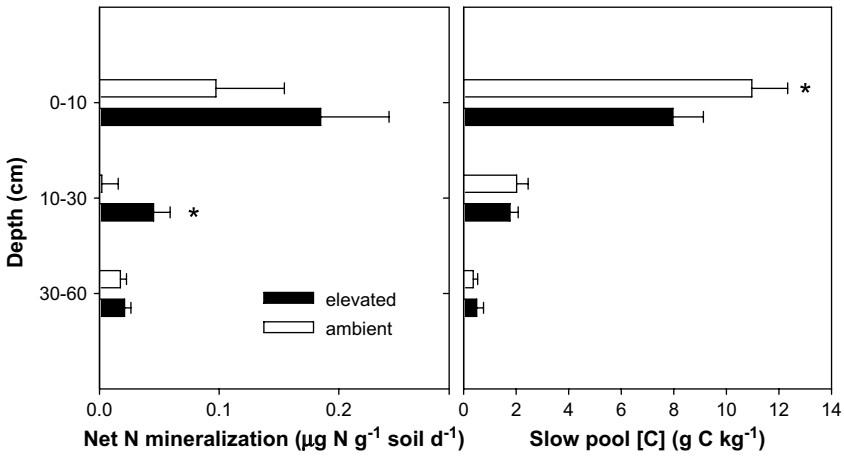

Fig. 1. Slow C pool size and net N mineralization throughout the profile. White bars represent means of soils from ambient CO_2 plots (±se), dark bars represent elevated CO_2. Asterisks (*) represent treatment effects ($P \leq 0.10$) at an individual depth.

Mean net N mineralization was positive in both treatments at all depths, and tended to increase at elevated CO_2 (Fig. 1). At the 10–30 cm depth, net N mineralization was significantly higher in the elevated-CO_2 treatment than in the ambient CO_2 treatment (ANOVA, $P = 0.030$). Total soil N followed patterns of soil C, decreasing with depth (Table 2). There was no treatment effect on total soil N or total soil C:N at any depth.

4. Discussion

4.1. Evidence for priming of soil carbon

Carney et al. (2007) reported that elevated CO_2 tended to reduce total soil C at the Florida scrub-oak CO_2 site and asserted that a priming effect had stimulated SOC mineralization. After six years of CO_2 fumigation, the soils in the elevated treatment had 21% lower C concentrations than ambient treatment soils in the top 10 cm (Table 1). Based on density fractionation they found that the losses occurred in the low-density fraction of SOC, which was assumed to be rapidly cycling, and therefore not entirely consistent with priming. But, our data indicate that most of the difference between treatments in soil C concentration exists in the slow-cycling SOC fraction, which was 27% lower in elevated than ambient CO_2. Because bulk density did not differ among treatments (Table 2), scaling soil C concentrations to masses showed similar patterns (0–10 cm, Table 3). That soil C masses did not differ between treatments partly owed to propagated error in deriving areal estimates from several independent parameters which lead to greater variability in soil C masses than that in soil C concentrations. For this reason, C concentrations of each pool, which are directly estimated from the technique, are the values typically analyzed and interpreted for the acid-hydrolysis-incubation method (Paul et al., 2006).

Table 2
Estimates of mean soil organic nitrogen (N_{son}) concentration, carbon to nitrogen (C:N) ratios, and soil bulk density mean (±se) to a depth of 60 cm ($n = 8$). Superscript letters represent significantly different treatment means, treatment by depth.

Depth (cm)	Treat.	N_{son} (mg kg^{-1})	C:N (g g^{-1})	Bulk Density (g cm^{-3})
0–10	A	750(67)^{a}	32(1)^{a}	0.80(0.08)^{a}
	E	639(83)^{a}	31(1)^{a}	0.84(0.04)^{a}
11–30	A	134(17)^{b}	35(2)^{a}	1.12(0.06)^{b}
	E	108(13)^{b}	36(2)^{a}	1.19(0.03)^{b}
31–60	A	42(5)^{b}	38(3)^{a}	1.49(0.04)^{c}
	E	43(6)^{b}	35(2)^{a}	1.49(0.03)^{c}

Because the soil C stability analysis has only been performed at one point in time, treatment differences do not necessarily represent trends. Moreover, previous work indicates that elevated CO_2 plots had a non-significant tendency of higher total soil C in pre-treatment measurements (Johnson et al., 2003). But, given the trajectory of treatment effects on total soil C (a widening gap between elevated and ambient; Carney et al., 2007), it is likely that the difference in the size of the slow C pool results, at least partly, from a priming effect.

The majority of studies that investigated the response of SOC pools to elevated CO_2 reported no significant treatment effects on stabilized SOC fractions (for meta-analyses and reviews, see Jastrow et al., 2005; van Groenigen et al., 2006; de Graaff et al., 2006). Given the large and sustained plant productivity increases resulting from CO_2 exposure in most ecosystems (Ainsworth and Long, 2005), increases in the soil C pool that range from null to modest have perplexed scientists. A few studies have reported evidence for priming (Hoosbeek et al., 2004), or increased rates of SOC mineralization (Hungate et al., 1997b). Our study suggests CO_2-induced priming of slow-cycling soil C may offset increased C inputs resulting in the small or negligible changes in SOC pool sizes observed at other CO_2 sites (Jastrow et al., 2005). The reason that priming yielded measurable differences in the SOC pool after just six years in our site may be explained by a combination of soil properties and climate.

First, the sandy soils at our study site generally lack clay minerals that can physically protect SOC against microbial mineralization (Christensen, 1996). The slow-cycling and resistant SOC pools at our site presumably arose through other mechanisms such as selective preservation or microbial transformation into more resistant chemical compounds. We propose that SOC stability arising primarily from chemical composition yields C pools that are more susceptible to CO_2-induced priming than mineral-protected SOM. At our study site, a priming response was accompanied by an increase in the relative abundance of fungi and increased phenol oxidase activity (Carney et al., 2007). This shift would be expected to favor more efficient degradation of the chemically-protected SOC that dominates our site but may have a more muted effect in soils dominated by physically-protected SOC.

Second, the climate at the Florida site may contribute to the differences between our findings and those of other studies. Other multi-year, forest CO_2 manipulations have mean winter (December–February) temperatures ranging from −11 to 5 °C (NOAA); winter temperatures at our site average 18 °C. The subtropical climate allows for high rates of microbial activity sustained throughout the year, which may yield relatively rapid

Table 3
Summary of results from two C pool partitioning methods performed on the top 10 cm of soil taken from the FL site in 2002.

Method			Soil [C] ($g\,m^{-2}$)		CO_2 effect 100*(E-A)/A	Partition (% of total)	
			Amb	Elev		Amb	Elev
Density fractionation[a]	From Carney et al., 2007	Light (<1.5 $g\,cm^{-3}$)	1594	1197	−25	76	72
		Intermediate (1.5–1.8 $g\,cm^{-3}$)	359	336	−6	17	20
		Heavy (1.8–2.2 $g\,cm^{-3}$)	146	124	−15	7	7
Acid-hydrolysis-incubation	Present study	Active	20	20	−3	1	1
		Slow	903	662	−26	45	41
		Resistant	1076	946	−12	54	58

[a] Data are taken from Table 1 of Carney et al. (2007).

changes in soil C balance. Moreover, two of the other long-term, field-based CO_2 studies that have found support for priming (Jasper Ridge in California, Hungate et al., 1997a,b; POPFACE in Italy, Hoosbeek et al., 2006) are sites that also occur on low-clay loamy soils and in climates with relatively mild winter temperatures. The response observed here may provide insight into soil C dynamics due to changing atmospheric conditions in subtropical and tropical ecosystems, which remain understudied in the context of rising CO_2.

4.2. Soil C pool partitioning in CO_2 studies

Acid-hydrolysis-incubation analyses performed on soils from other field-CO_2 studies have revealed increases in the active SOC pool (Haile-Mariam et al., 2000; Dijkstra et al., 2005; Hoosbeek et al., 2006, non-significant increase; Pendall and King, 2007). These studies reported C pool distributions similar to our soils, with the active pool contributing <1% of total C, while the stable and resistant pools each contributed nearly half of total SOC (except Hoosbeek et al., 2006). Ours is the only study to show a difference in any individual pool C concentration with elevated-CO_2 exposure. None of the studies, including ours, has shown a significant CO_2 effect on the MRT of any soil C pool (Haile-Mariam et al., 2000; Dijkstra et al., 2005; Pendall and King, 2007). We observed a non-significant reduction in the MRT and a non-significant increase in the size of the slow C pool at 30–60 cm depth due to elevated CO_2. The trend in these data indicates enhanced inputs of root-derived C (previously reported at this site by Dilustro et al., 2002; Langley et al., 2003), which would increase the size, while reducing the residence time, of this pool.

We observed the largest SOC difference in the slow C pool, which is consistent with our understanding of priming effects. The active pool is composed of newer, more easily degraded C that is thought to initiate a priming response but does not itself respond to priming. The resistant pool is considered too stable to change appreciably over relatively short intervals like the 6 years of this study. Yet, we did observe a non-significant tendency of lower stable SOC pool of 130 $g\,m^{-2}$ in the 0–10 cm interval (Table 3). Considering the size of the stable SOC pool, this pattern suggests that elevated-CO_2 treatment could lead to even more dramatic losses over longer time periods (Fontaine et al., 2007).

4.3. Priming and progressive N limitation

It has been suggested that stimulation of productivity with elevated CO_2 ties up N in plant litters, which, if not offset by increases in N-use efficiency or N supply, will limit the ecosystem CO_2 response (Reich et al., 2006). We found that elevated CO_2 tended to increase net N mineralization in the top 10 cm, but the strongest effects were observed in the 10–30 cm depth increment. Other long-term field studies have found that CO_2 either has a negative effect or no effect on N mineralization (for review, see de Graaff et al., 2006), although the N status of a particular ecosystem appears to play a role in determining the N-mineralization response (van Groenigen et al., 2006; Reich et al., 2006). At our scrub-oak site, plants exposed to elevated CO_2 acted to redistribute soil N to plant biomass and litter organic matter, a pattern that should have rendered N less available (Hungate et al., 2006). Yet, because plant biomass and canopy N measurements revealed little indication of increased plant N limitation over several years of elevated-CO_2 exposure (Hungate et al., 2006), N may have been liberated from less-available soil pools. Indeed, the soil N stock appears to follow patterns of SOC depletion (Table 2). Our results support the hypothesis that elevated CO_2 sequestered N in biomass and litter, while simultaneously stimulating the mineralization of additional N from more decay-resistant SOM pools (Hungate et al., 2006).

The additional N liberated by priming of relatively N-rich SOM may have partially alleviated progressive N limitation. Further evidence for this hypothesis is that the ratio of net N-min to C-min increased from 0.0001 to 0.021 at 10–30 cm (data not shown, $P = 0.023$) with elevated CO_2. This ratio shows that more N was released per unit of C respired at elevated than ambient CO_2. Because elevated CO_2 has not altered the C:N ratio of litter entering the soil at this site (Hall et al., 2005), this pattern presumably reflects a shift in the metabolism of elevated-CO_2 microbes toward the use of a relatively recalcitrant SOC pool with a lower C:N ratio. Such a shift is consistent with the observation that elevated CO_2 increases soil fungi and phenol oxidase activity (Carney et al., 2007).

4.4. Methodological evaluation

As a technical assessment, we compared our incubation-model results to those from density fractionations performed on the same soils (reported in Carney et al., 2007; Table 3). Crow et al. (2007) concluded that separating SOM pools by density may have utility for general soil characterization, but these operationally-defined fractions are often not relevant to the three pools that are commonly recognized as functionally discrete according to stability. Moreover, they suggested that simple substitution of density-defined pools for stability-defined pools has caused confusion among soil scientists and poor predictive power in ecosystem models.

Our findings support the conclusions of Crow et al. (2007) in that the organic matter pools previously delineated by density fractionation apparently did not agree with our functionally defined pools (Table 3). For instance, light organic matter is often interpreted to be synonymous with active C pools. Here, we found that the active pool constituted only 1% of total soil C, yet the light SOC fraction constituted 75% of total organic C (Carney et al., 2007). By necessity, a large portion of the light fraction must have a long residence time. Indeed, using the isotopic tracer present in the elevated-CO_2 treatment, Carney et al. (2007) found that the light fraction had a C mean residence time of 13 years, which is (1) longer than actively cycling C by most definitions (Trumbore, 2000), and (2) only slightly shorter than the intermediate (MRT = 20 years) or heavy pools (24 years). Although density fractionation may be

equivalent to functional pools of organic matter in some soils, the sandy soils at this site likely do not generate the organo-mineral complexes that allow SOC to group similarly with both methods. Or more simply, the stage of organic degradation for a given pool does not accurately predict the future residence time. This difference between the two methods of fractionating the SOC pool further substantiates our assertion that C-stabilizing mechanisms in these soils are different from those in soils where these methods have been in better agreement.

5. Conclusions

Elevated CO_2 had less soil C in the slow-cycling soil pool, which has a mean residence time of 3–4 years. The diminished slow-cycling soil C, along with pre-established trend of declining total soil C (Carney et al., 2007), suggests that elevated CO_2 stimulated microbial respiration of the slow pool enough to outpace increased C inputs to soils. Previous work showed that elevated CO_2 increased litterfall by 19–59% in the four years leading up to the present study (Hungate et al., 2006). Elevated CO_2 also increased N mineralization at depths of 10–30 cm. Release of N from this depth may have allowed the sustained CO_2 effect on productivity in this scrub-oak forest. The extent to which similar CO_2-induced priming effects occur, and are detectable, in other ecosystems may depend on the presence of clay minerals. Clay minerals were largely absent from the upper meter of soil at our site, but are known to protect soil C from decomposition in other soils. Enhanced mineralization of formerly stable soil C could exert a positive feedback on atmospheric CO_2 in other ecosystems (Heimann and Reichstein, 2008), or at least partially negate C gains from increased plant productivity. Such an effect may contribute to the apparent decline in the global terrestrial C sink (Canadell et al., 2007).

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