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Soil Biology & Biochemistry 33 (2001) 365–373

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Contrasting effects of elevated CO_2 on old and new soil carbon pools

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Received 19 October 1999; received in revised form 18 May 2000; accepted 19 July 2000

Abstract

Soil organic carbon (SOC) is the largest reservoir of organic carbon in the terrestrial biosphere. Though the influence of increasing atmospheric CO_2 on net primary productivity, on the flow of newly fixed carbon belowground, and on the quality of new plant litter in ecosystems has been examined, indirect effects of increased CO_2 on breakdown of large SOC pools already in ecosystems are not well understood. We found that exposure of California grassland communities to elevated CO_2 retarded decomposition of older SOC when mineral nutrients were abundant, thus increasing the turnover time of SOC already in the system. Under elevated CO_2, soil microorganisms appeared to shift from consuming older SOC to utilizing easily degraded rhizodeposits derived from increased root biomass. In contrast to this increased retention of stabilized older SOC under elevated CO_2, movement of newly fixed carbon from roots to stabilized SOC pools was retarded; though root biomass increased under elevated CO_2, new carbon in mineral-bound pools decreased. These contrasting effects of elevated CO_2 on dynamics of old and new soil carbon pools contribute to a new soil carbon equilibrium that could profoundly affect long-term net carbon movement between terrestrial ecosystems and the atmosphere.

Keywords: Elevated CO_2; Soil carbon; Grassland; Soil organic matter; Decomposition; Rhizodeposition; Isotope

1. Introduction

Determining whether terrestrial ecosystems will buffer or exacerbate increasing atmospheric carbon dioxide concentrations over the next century requires knowledge of long-term carbon storage in soil and vegetation. This long-term storage is influenced by the balance among ecosystems' net primary productivity (NPP), the rate of delivery of new organic matter to soil pools, and the decomposition of soil organic matter (SOM). Because of this central role of soil carbon pools in the global carbon cycle, a number of recent studies have explored possible changes in fluxes of newly fixed carbon to soil under elevated CO_2 (e.g. Lekkerkerk et al., 1990; Van Veen et al., 1991; Van de Geijn and Van Veen, 1993; Zak et al., 1993; Leavitt et al., 1994; Rice et al., 1994; Wood et al., 1994; Ineson et al., 1996; Van Ginkel et al., 1997) and changes in decomposition of plant litter produced under elevated CO_2 (reviewed in O'Neill and Norby, 1996). Directly measuring effects of elevated CO_2, however, on breakdown specifically of large, old pools of SOC has received less attention in current ecosystem-level research. Since in grasslands, the only long-term reservoir for carbon storage is soil organic carbon, understanding dynamics of soil organic carbon pools in these ecosystems is essential. We used isotopic labeling to examine how elevated CO_2 influenced the decomposition of older SOM already present in soil, which we planted with California grassland communities. We also followed the fate of newly fixed carbon as it flowed from plants to root litter, particulate organic matter, and mineral-bound carbon pools in soil.

Several approaches have previously been used to investigate effects of elevated CO_2 on SOC pools, ranging from

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0038-0717/01/$ - see front matter
PII: S0038-0717(00)00151-6

simply measuring total SOC pools (e.g. Rice et al., 1994, but see Hungate et al., 1996, for problems with this approach), to quantifying soil respiration, which combines heterotrophic and root respiration (e.g. Luo et al., 1996). Fossil fuel-derived CO_2 (used to maintain elevated CO_2 concentrations in experiments) has provided a useful ^{13}C isotopic signal allowing quantification of the flux of newly fixed carbon belowground (Leavitt et al., 1994; Hungate et al., 1997b), and experiments using isotopic tracers in both elevated CO_2 treatments and ambient controls have been conducted on single plants (Ineson et al., 1996) and stands of agricultural species (Lekkerkerk et al., 1990; Cheng and Johnson, 1998).

One recent study of California grasslands used ^{13}C to pulse-label communities planted in microcosms growing under elevated and ambient CO_2 (Hungate et al., 1997b). Analysis of carbon in microcosms after one year suggested that exposure to elevated CO_2 increased heterotrophic respiration belowground, and, more specifically, increased heterotrophic respiration of newly fixed carbon lost to soil as rhizodeposits. (We define rhizodeposition as all deposition of organic carbon from living root systems to soils, including compounds lost through root exudation, sloughing of dead cells during root growth, and fine root turnover.) Based on this finding, and on literature identifying roots as carbon sources for microbes (e.g. Liljeroth et al., 1994), we hypothesized that increased availability of rhizodeposits to soil microorganisms under elevated CO_2 may reduce microbial dependence on older SOM as a carbon source, as long as mineral nutrients are sufficiently available (Van Veen et al., 1991; Cardon, 1996; Cheng, 1999).

To test our hypothesis, we examined how elevated and ambient CO_2 affected both carbon input to and loss of soil carbon from two annual California grassland communities growing in microcosms with low and high soil nutrient availability. If the hypothesized reduction in SOM breakdown were occurring under elevated CO_2, it might be detectable immediately in a decreased flux of CO_2 from SOM to atmosphere. If sustained long enough, an increased retention of carbon in older SOM pools might be detected under elevated relative to ambient CO_2. We examined two experimental grassland communities, one dominated by Mediterranean annual grasses that typically occur on relatively fertile soils derived from sandstone; the other community was dominated by native California forbs and grasses that typically grow on less fertile serpentine soils. The two plant communities grew in open-top chambers at ambient and elevated CO_2, and at high and low soil nutrient availability, for two years in the MECCA (Micro-Ecosystems for Climate Change Analysis) facility at Jasper Ridge Biological Preserve, Stanford, CA (Field et al., 1996).

In order to distinguish the new carbon fixed during the experiment from older soil carbon, we grew these two C3 plant communities on soil obtained from a C4 grassland (Schönwitz et al., 1986) at the Central Plains Experimental Station in Colorado. During photosynthesis, C4 plants discriminate less against ^{13}C than do C3 plants, and the carbon isotope composition of SOM native to the Colorado soil ($\delta^{13}C = -16‰$) reflected the photosynthetic pathway of the dominant C4 grass *Bouteloua gracilis*. The isotopic signature of carbon from the new C3 grassland community, in contrast, was much more negative: $-30‰$ in ambient CO_2 and $-40‰$ in elevated CO_2. We utilized this isotopic tracer to determine the size and origin of soil carbon pools in all experimental treatments. We also measured soil respiration and the isotopic composition of soil atmosphere CO_2 through time, thereby distinguishing the breakdown (respiration) of older C4 carbon in SOM from the breakdown (respiration) of newly fixed C3 carbon through the growing season.

2. Materials and methods

2.1. Microcosms

Avena barbata Link, *Bromus hordeaceus* L., *Hemizonia congesta* DC. ssp. *luzulifolia* (DC.) Babc. and H.M. Hall, *Nassella pulchra* (A. Hitchc.) Barkworth, and *Lotus wrangelianus* Fischer and C. Meyer from the sandstone grassland community, and *Bromus hordeaceus* L., *Hemizonia congesta* DC. ssp. *luzulifolia* (DC.) Babc. and H.M. Hall, *Lasthenia californica* Lindley, *Plantago erecta* Morris, *Vulpia microstachys* (Nutt.) Benth var. *pauciflora* (Beal) Lonard and Gould, *Nassella pulchra* (A. Hitchc.) Barkworth, and *Lotus wrangelianus* Fischer and C. Meyer from the serpentine grassland community were sown at field densities in PVC microcosms 20 cm in diameter, 1 m deep. Soil from the top 15–20 cm of the A horizon was shipped from C4 grassland near Central Plains Experimental Station (Blecker et al., 1997) and pre-germinated by moistening, allowing seeds to germinate, and removing resulting plants. Soil was then mixed 1:1 with sand to improve drainage. For sandstone communities, six replicates of each of the four treatments (high/low CO_2 and high/low nutrients) were planted, for serpentine communities, four replicates. The two plant communities were grown in the MECCA facility at Jasper Ridge Biological Preserve, Stanford, CA (Field et al., 1996), at ambient and elevated CO_2, and at high and low soil nutrient availability, in open–top chambers under natural rainfall and light from October, 1994 through August, 1996. Elevated CO_2 concentration was maintained at 750 ppm. Nutrient availability was manipulated by mixing slow-release 120-day osmocote fertilizer, containing N, P, and K into the top 15 cm of microcosm soil at the beginning of the experiment.

Low and high nutrient microcosms received 3 g/m^2 fertilizer and 20 g/m^2, respectively. Because we mixed soil 1:1 with sand, the low nutrient treatment soil was supplemented with NPK in order to make nutrient availability close to that in undisturbed soil. We based the level of supplementation on information from two studies. Hungate et al. (1997a)

showed 2–3 g N m^{-2} content in aboveground biomass in these California annual grasslands, and 1–2 g N m^{-2} in roots at peak season biomass (Hungate, unpublished data), providing an underestimate of net N mineralization of 3–5 g N m^{-2} yr^{-1}. Schimel et al. (1986) showed net N mineralization for the Colorado C4 grassland where we obtained soil is 3–5.5 g N m^{-2} yr^{-1}. Given these ranges, our addition of 3 g N m^{-2} yr^{-1} to our mix of soil and sand (with 1.5–2.75 g N m^{-2} yr^{-1} from the soil portion in the mix) produced our "low nutrient" substrate with N-availability of 4.5–5.75 g N m^{-2} yr^{-1}. We did not measure available soil nutrient pools or mineralization rates during the experiment; low and high nutrient availability are thus used as relative terms here. After the second growing season, shoot, root, and total plant biomass in the microcosms was significantly increased in the high nutrient relative to the low nutrient treatment (three-way ANOVA, $P < 0.0001$ for shoot and total biomass, $P < 0.05$ for root biomass).

In the center of each microcosm, a PVC ring 5.6 cm I.D. and 1.7 cm tall was buried ~0.7 cm in the soil. This small area of the microcosm inside the PVC ring was kept free of aboveground biomass during the course of the experiment to allow soil respiration measurements to be made over undisturbed, bare soil. Sample soil cores taken during summer after the first growing season revealed that roots of surrounding plants grew underneath the ring. Roots developed during the first season remained in the soil. Dead shoots, however, were removed so as not to shade germinating seedlings in other microcosms in the open-top chambers during the 1995–1996 growing season. To track soil moisture content, time domain reflectometry (TDR) probes were buried in eight microcosms at the beginning of the experiment in September 1994 and monitored every two weeks. In order to determine the isotopic signature of soil-respired CO_2 four hollow, stainless steel probes (each 12.5 cm long, 0.5-cm diameter, with four columns of eight 1-mm holes drilled in the terminal 5.5 cm) were vertically buried 12 cm deep in each microcosm.

2.2. Isotopes and soil respiration

Each sampling date, a needleless 60 ml syringe was used to collect six ml of soil atmosphere from each of the four stainless steel hollow probes (described above) buried in the C4 soil of each microcosm. The top ~0.5 cm of each probe was permanently fit aboveground with a Leur valve which linked with the Leur valve of the syringe. The 24 ml of gas from each microcosm were quickly flame-sealed into a glass ampule. Sample $\delta^{13}C$ was analyzed by the University of Utah's Stable Isotope Ratio Facility for Environmental Research. Soil, leaf and root $\delta^{13}C$ were measured at University of California, Berkeley with an isotope ratio mass spectrometer coupled with a combustion system and gas chromatograph (Europa Scientific Limited, Crewe, UK). $\delta^{13}C$ of the total soil carbon was initially −16‰. The isotopic composition of newly fixed carbon in root and shoot tissues of C3 plants was −30‰ in ambient CO_2 and −40‰ in elevated CO_2. Using these C3 plant and C4 soil $\delta^{13}C$ endpoints, and the $\delta^{13}C$ of the soil atmosphere CO_2, we calculated the percentage soil atmosphere CO_2 that came from the C4-SOM vs. C3-plant source for each microcosm on each sampling date.

We used a LiCor 6200 (LiCor Inc., Lincoln, NE) fitted with soil respiration chamber to measure soil respiration from each microcosm after isotopic samples had been taken (Luo et al., 1996). As noted above, a small area of the microcosm inside a 5.6 cm I.D. PVC ring was kept free of aboveground biomass during the course of the experiment. Our soil respiration chamber fit into and sealed onto that ring so that soil respiration could be measured with minimal soil surface disturbance. Using the proportion of respiration derived from C4 vs. C3 sources (determined from isotopic analyses), and multiplying by the soil respiration rate determined using the LiCor 6200, we calculated the rate of mineralization of C4 SOM-derived and C3 root-derived carbon on each date. Time-constraints limited our sampling of soil atmosphere CO_2 to only the microcosms planted with sandstone species.

2.3. Soil fractionation

Soils were harvested in three layers (0–15 cm, 15–45 cm, and 45– ~90 cm) from microcosms after two years exposure to CO_2 and nutrient treatments, and samples from each layer were fractionated. Thirty grams of air-dried 2 mm-sieved soil was dispersed in 100 ml of 5 g l^{-1} sodium hexamethaphosphate and agitated for 18 h. (Fine roots were separated using a 500 μm sieve.) Dispersed soil samples were passed sequentially through a 53 and a 20 mm sieve and rinsed with water. Material remaining on the 53 mm sieve was backwashed onto a nylon filter, and excess water was removed by vacuum. Material was then rinsed into a beaker and the solution brought to a final volume of 50 ml using sodium polytungstate adjusted to a density of 1.85 g cm^{-3}. Samples were separated overnight, after which floating organic material was aspirated from the surface, rinsed with water on a 20 mm nylon filter, dried at 50°C, and ground for 4 min. using a Spex ball mill. This aspirated organic material was the POM fraction (Gale and Cambardella, 2000).

The material remaining on the 20-mm sieve was backwashed into a pan and dried at 50°C. This was the coarse silt size fraction. The slurry that passed through the 20 mm sieve contained the three smallest size fractions, which were isolated by sequential centrifugation (Ladd et al., 1977; Cambardella and Elliott, 1994). We grouped coarse silt, fine silt, coarse clay, and fine clay into a "mineral-bound" soil carbon pool for brevity.

2.4. Statistics

For rates of respiration of C4 SOM and fractions of respiration derived from C4-sources, main effects and

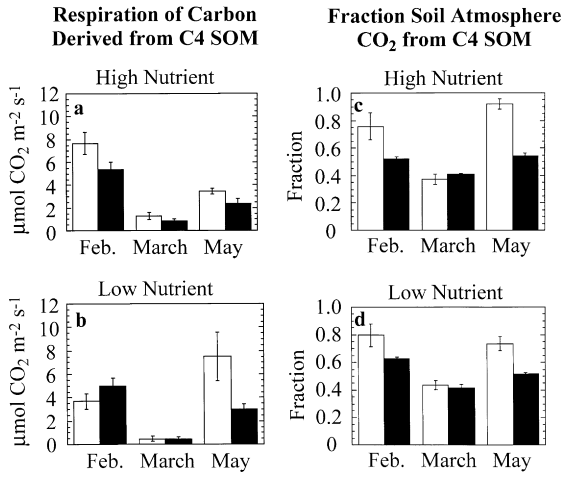

Fig. 1. Absolute amount and fraction C4-derived soil carbon respired from microcosms. Open bars, ambient CO_2; solid bars, elevated CO_2. (a,b) C4-derived carbon in soil surface respiration from microcosms with high (a) and low (b) nutrient availability at three points during the growing season. (c,d) Fraction of respired carbon from C4 sources in microcosms with high (c) and low (d) nutrient availability. All bars represent mean ± 1 s.e.

interactions were tested using two-way analysis of variance at each date. For respiration rates, specific CO_2 effects were identified with Bonferroni-corrected post-hoc means tests (one-tailed) at each nutrient level. For POM and mineral-bound carbon pools in the top 15 cm of soil in the microcosm, main effects and interactions were tested using three-way ANOVA (CO_2, nutrient, and community effects).

3. Results

3.1. Soil respiration

Elevated CO_2 decreased the breakdown of C4-SOC when sandstone plant communities were grown on soil with abundant mineral nutrients (Fig. 1a). This decrease occurred throughout the growing season, from peak vegetative growth rates in February through senescence in May ($P < 0.035$ for February and May, and $P = 0.18$ March). In the low nutrient treatments (Fig. 1b), decomposition of the older SOC was slower under elevated CO_2 on the final sample date in May ($P < 0.034$), but not on the February and March sample dates ($P > 0.25$ for February and March). Elevated CO_2 also increased the fraction of respired CO_2 contributed by C3 root respiration (and/or oxidation of rhizodeposits) relative to respiration of C4-derived substrates (Fig. 1c and d).

3.2. Plant biomass

Elevated CO_2 increased root biomass, shoot biomass, and root:shoot ratio of sandstone and serpentine community plants harvested in 1996 (Table 1, three-way ANOVA, all $P < 0.0001$).

3.3. Soil fractions

Microcosms were harvested after two years' exposure to ambient and elevated CO_2. We did not find consistent differences in averaged total SOC or its component C3-derived and C4-derived SOC pools in elevated vs. ambient CO_2 treatments (Table 1, three-way ANOVA, all $P < 0.32$). Soil bulk density was also unaffected by CO_2 treatments (data not shown).

Differences between ambient and elevated CO_2 treatments were detected after separating C3 and C4 soil carbon pools into particulate organic matter and mineral-bound carbon pools. Less C4-derived carbon in particulate organic matter (POM, Fig. 2a), and slightly more mineral-bound C4 carbon (Fig. 2b), remained in the 0–15 cm soil layer after plant growth under elevated relative to ambient CO_2 (Table 2). The same pattern of increased retention of mineral-bound C4-carbon (Fig. 2b), and decreased POM C4-carbon (Fig. 2a) was apparent in both the high and low nutrient treatments under elevated CO_2. Elevated CO_2 also caused an increase in C3 POM (Fig. 2c) and a decrease in C3 mineral-bound SOC (Fig. 2d) in both high and low nutrient treatments.

When all three microcosm soil layers were included in a four-way ANOVA ($CO_2 \times$ nutrient $\times$ layer $\times$ community), elevated CO_2 had a significant effect on C3-derived POM ($P = 0.003$), C4-derived POM ($P < 0.0001$), C3-derived mineral-bound carbon ($P = 0.006$), but not on the largest pool, C4-derived mineral-bound carbon ($P = 0.7$) (data not shown).

4. Discussion

As is commonly observed (Rogers et al., 1994), elevated CO_2 increased root biomass and root:shoot ratio in these serpentine and sandstone species (Table 1, Jackson and Reynolds, 1996). Elevated CO_2 also decreased the breakdown of C4-SOC in sandstone communities growing on high nutrient soil (Fig. 1a). Greater rhizodeposition, associated with the much larger root mass in elevated CO_2, may have caused this depression of C4-SOM breakdown. We suggest that under elevated CO_2, carbon-limited microbes turned from breaking down older C4-SOM and instead utilized abundant and labile C3 rhizodeposits, especially when ample mineral nutrients were available. A change in soil water status under elevated CO_2 could also influence mineralization rates (Hungate et al., 1997a). However, we measured soil moisture using TDR in a limited number of microcosms every two weeks, and found no significant CO_2-induced difference in soil moisture through time (data not shown).

Though elevated CO_2 decreased C4-derived SOC breakdown by ~30% on all sample dates in the high nutrient treatment (Fig. 1a), in the low nutrient treatments, the pattern was more complex (Fig. 1b). The contrast between

Table 1
Root and shoot biomass, root:shoot ratio, and soil carbon content in microcosms harvested August, 1996 (means (±standard errors) are indicated)

Community	Soil nutrient status	Root biomass (g microcosm^{-1})		Shoot biomass (g microcosm^{-1})		Root:shoot	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Sandstone	Low N	2.76 ± 0.49	12.24 ± 1.24	12.21 ± 0.95	17.53 ± 0.64	0.236 ± 0.057	0.704 ± 0.084
	High N	2.82 ± 0.52	11.23 ± 0.88	29.59 ± 0.78	39.71 ± 2.24	0.096 ± 0.019	0.286 ± 0.028
Serpentine	Low N	2.36 ± 0.50	6.57 ± 0.54	14.00 ± 2.49	15.44 ± 0.53	0.173 ± 0.033	0.425 ± 0.032
	High N	4.00 ± 0.44	11.64 ± 1.72	24.16 ± 1.14	35.05 ± 1.91	0.165 ± 0.015	0.332 ± 0.042
		Total organic soil C in microcosm (mg C g_{soil}^{-1})		C3-derived organic soil C in microcosm (mg C g_{soil}^{-1})		C4-derived organic soil C in microcosm (mg C g_{soil}^{-1})	
		Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Sandstone	Low N	5.27 ± 0.19	4.85 ± 0.18	0.65 ± 0.13	0.55 ± 0.03	4.62 ± 0.14	4.30 ± 0.16
	High N	5.00 ± 0.20	5.41 ± 0.24	0.66 ± 0.06	0.90 ± 0.08	4.34 ± 0.24	4.51 ± 0.16
Serpentine	Low N	4.82 ± 0.25	4.54 ± 0.32	0.71 ± 0.13	0.48 ± 0.06	4.12 ± 0.13	4.06 ± 0.35
	High N	5.06 ± 0.30	4.67 ± 0.18	0.83 ± 0.22	0.59 ± 0.11	4.22 ± 0.17	4.08 ± 0.29

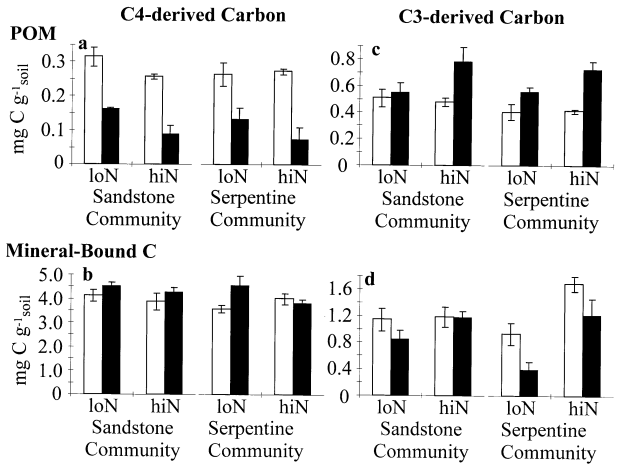

Fig. 2. C4-derived and C3-derived carbon in soil fractions. Open bars, ambient CO_2; solid bars, elevated CO_2. (a) C4-derived carbon in POM. (b) C4-derived carbon in mineral-bound C. (c) C3-derived carbon in POM. (d) C3-derived carbon in mineral-bound C. Only data from the 0–15 cm soil layer of microcosms, where root biomass was concentrated, are shown; each bar represents the mean ± 1 s.e.

the high and low nutrient treatments may be partially explained by differences in plant phenology. In field studies of Jasper Ridge grasslands, growth of late flowering annuals (which continue growing vegetatively beyond May and into September) was consistently favored in unfertilized elevated CO_2 treatments (Field et al., 1996). In companion 4-year microcosm experiments (which overlapped two years with our experiment in the open-top MECCA chambers), Chiariello and Field (1996) found that the late-annual *Hemizonia* constituted 6–12% of aboveground biomass under low nutrient treatments, and aboveground biomass increased as much as eight-fold during summer months from May until September. We did not measure late annual production separate from production in early-flowering species in our experiment, but we speculate that rhizodeposition associated with continuing vegetative growth of late-flowering annuals under elevated CO_2 (Field et al., 1996) may have continued past May (Chiariello and Field, 1996), supplying labile carbon to microbes, and depressing the breakdown of C4 SOM under elevated CO_2 on the May sampling date (Fig. 1b).

Elevated CO_2 also decreased the relative contribution of microbial C4 SOC breakdown to total soil respiration from microcosms (Fig. 1c and d). (The high proportion of older SOM contributing to soil carbon flux in all treatments in Fig. 1c and d is consistent with these soils being disturbed; in undisturbed field soils, the proportion of soil respiration derived from breakdown of older SOM would most likely be smaller and the C3 component larger.) The results in Fig. 1 suggest two mechanisms by which elevated CO_2 alters soil respiration. By stimulating root biomass, elevated CO_2 increases the proportion of soil respiration derived from root respiration and/or oxidation of rhizodeposits (Fig. 1c and d). Also, by increasing the availability of rhizodeposits

Table 2
Results of three-way ANOVA examining effects of CO_2, nutrients, and community type on POM and mineral-bound carbon pools in the top 15 cm of microcosm soils

POM carbon

		C4-derived carbon				C3-derived carbon			
Effect	DF	SS	MS	*F*	*P*	SS	MS	*F*	*P*
Comm.[a]	1	2.23×10^{-9}	2.23×10^{-9}	1.29	0.27	1.84×10^{-8}	1.84×10^{-8}	1.13	0.30
CO_2	1	1.65×10^{-7}	1.65×10^{-7}	95.62	<0.0001	2.52×10^{-7}	2.52×10^{-7}	15.48	0.001
Comm. × CO_2	1	2.70×10^{-11}	2.70×10^{-11}	0.016	0.90	4.73×10^{-9}	4.73×10^{-9}	0.29	0.60
Nutrient	1	1.27×10^{-10}	1.27×10^{-10}	7.32	0.015	5.74×10^{-8}	5.74×10^{-8}	3.52	0.08
Comm. × nutrient	1	2.61×10^{-9}	2.61×10^{-9}	1.51	0.23	2.24×10^{-10}	2.24×10^{-10}	0.01	0.91
CO_2 × nutrient	1	2.83×10^{-9}	2.83×10^{-9}	1.64	0.22	7.20×10^{-8}	7.20×10^{-8}	4.42	0.05
Comm. × CO_2 × nutrient	1	1.09×10^{-9}	1.09×10^{-9}	0.63	0.44	4.04×10^{-9}	4.04×10^{-9}	0.25	0.62
Residual	18	3.11×10^{-8}	1.73×10^{-9}			2.94×10^{-7}	1.63×10^{-8}		

Mineral-bound carbon

		C4-derived carbon				C3-derived carbon			
Effect	DF	SS	MS	*F*	*P*	SS	MS	*F*	*P*
Comm.[a]	1	2.61×10^{-7}	2.61×10^{-7}	1.11	0.31	5.07×10^{-9}	5.07×10^{-9}	0.07	0.79
CO_2	1	9.47×10^{-7}	9.47×10^{-7}	4.02	0.06	6.83×10^{-7}	6.83×10^{-7}	9.64	0.006
Comm. × CO_2	1	7.15×10^{-11}	7.15×10^{-11}	3.04×10^{-4}	0.99	1.91×10^{-7}	1.91×10^{-7}	2.69	0.12
Nutrient	1	2.29×10^{-7}	2.29×10^{-7}	0.97	0.34	1.43×10^{-6}	1.43×10^{-6}	20.21	0.0003
Comm. × nutrient	1	1.28×10^{-8}	1.28×10^{-8}	0.05	0.82	5.62×10^{-7}	5.62×10^{-7}	7.92	0.01
CO_2 × nutrient	1	5.02×10^{-7}	5.02×10^{-7}	2.13	0.16	4.71×10^{-8}	4.71×10^{-8}	0.67	0.43
Comm. × CO_2 × nutrient	1	5.27×10^{-7}	5.27×10^{-7}	2.24	0.15	1.82×10^{-8}	1.82×10^{-8}	0.26	0.62
Residual	18	4.24×10^{-6}	2.36×10^{-7}			1.28×10^{-6}	7.09×10^{-8}		

[a] Grassland community (sandstone or serpentine).

(associated with increased root biomass) to soil microorganisms, elevated CO_2 may reduce microbial utilization of older SOC as a carbon source, as long as mineral nutrients are sufficiently available (Fig. 1a). Through this second mechanism, elevated CO_2 may increase the turnover time of soil carbon already present in terrestrial ecosystems. This decreased decomposition of older SOC may partially explain why increased soil respiration is not universally observed in response to elevated CO_2, even when nutrient availability is high (e.g. Ineson et al., 1998).

If this reduction in breakdown of older SOC is sustained, an increased retention of carbon in older SOC pools might be expected under elevated relative to ambient CO_2. After two years' exposure to ambient and elevated CO_2 treatments, microcosms were harvested and the soil was examined for effects of the decreased respiration from C4-SOM on C4 carbon pools, and increased C3 root biomass, on C3 carbon pools under elevated CO_2. Changes in total soil carbon caused by elevated CO_2 are difficult to detect in short-term experiments because of the long residence times and large sizes of soil carbon pools (Hungate et al., 1996). Not surprisingly, we did not find consistent differences in averaged total SOC or its component C3-derived and C4-derived SOC pools in elevated vs. ambient CO_2 treatments (Table 1).

Upon further partitioning C3 and C4 soil carbon pools into particulate organic matter and mineral-bound carbon pools, however, clear differences between ambient and elevated CO_2 treatments emerged. Less C4-derived carbon in particulate organic matter (POM, Fig. 2a), and slightly more mineral-bound C4 carbon (Fig. 2b), remained after plant growth under elevated relative to ambient CO_2 (Table 2). The C4-derived POM pool, which has no inputs during the course of the experiment, is the only pool in Fig. 2 where effects of decomposition alone influence pool size, and elevated CO_2 significantly enhanced C4-POM breakdown, as did the high nutrient treatment (Table 2). (For contrasting results of high nutrient treatments see Fog, 1988). The greater decomposition of the C4 POM pool observed under elevated CO_2 may have contributed carbon to the increase in the C4 mineral-bound SOC pool under elevated CO_2 (Fig. 2b). However, the increase in the size of the much larger C4 mineral-bound pool under elevated CO_2 (Fig. 2b) appears greater in magnitude than the loss of carbon from the POM pool (Fig. 2a), particularly considering the respiratory loss of carbon during POM processing by microbes.

In this final soil analysis, we observed the same pattern of increased retention of mineral-bound C4-carbon (Fig. 2b), and decreased POM C4-carbon (Fig. 2a), in both the high and low nutrient treatments under elevated CO_2. The decrease in C4-labeled CO_2 flux (Fig. 1), combined with this retention of C4-labeled mineral-bound SOC (Fig. 2b), suggests that the absolute amount of respiration of C4 carbon from mineral-bound fractions may have been reduced under elevated CO_2. Though Fig. 1a and b shows different seasonal patterns of C4-derived soil respiration in the two nutrient treatments, respiration later in the growing season (May, Fig. 1b) may have dominated the total flux of C4-derived CO_2 from the low-nutrient microcosms, leading to the parallel longer-term patterns in low and high nutrient treatments observed in Fig. 2b.

Examining the fate of C3 soil carbon, we found that elevated CO_2 caused an increase in C3 POM (Fig. 2c) and a decrease in C3 mineral-bound SOC (Fig. 2d), suggesting that the movement of C3 carbon from roots to long-lived, mineral-bound pools was retarded. In addition, the six-fold enhancement of root biomass under elevated relative to ambient CO_2 (Table 1) after two growing seasons, compared with only a two-fold enhancement after one growing season (Jackson and Reynolds, 1996), also suggests decreased breakdown of root biomass under elevated CO_2, and thus reduced movement of C3 carbon from roots through POM to mineral-bound pools. (Gorissen et al., 1995, also observed a decrease in grass root decomposition under elevated CO_2). This altered partitioning of belowground carbon under elevated CO_2 in our experiment created a lag in movement of large amounts of new C3 root litter and POM into mineral-bound soil carbon pools (Fig. 2d). However, the loss of older C4 carbon from mineral-bound pools to the atmosphere was also retarded (Fig. 2b), resulting in a similar amount of total mineral-bound soil carbon under ambient and elevated CO_2 treatments.

As noted previously, soil carbon pools are large and turnover times can be very long, restricting the ability of short-term experiments to detect responses of soils to experimental manipulation. Because of this problem, several recent studies have confirmed the usefulness of short-term change in the POM pool as a sensitive early indicator of long-term alterations in dynamics of SOM processing (e.g. Gregorich et al., 1994). Not only did our isotopic approach allow us to detect statistically significant changes in POM pools associated with the increased CO_2 treatment, we also detected significant effects of elevated CO_2 on larger mineral-bound pools (Fig. 2b and d).

The generation of a large pool of undecomposed root biomass (Table 1) may be transient; such a pool could create a feedback operating through the nitrogen cycle, ultimately decreasing NPP. Nitrogen is supplied to plants through the decomposition of SOM; in this study, SOM had an average C:N ratio of 10. Microbes also utilize the nitrogen supplied by breakdown of SOM as they decompose root biomass, which in this study had an average C:N ratio of 50–70. The buildup of root biomass (with very high C:N ratio) under elevated CO_2 is likely to generate an increase in the microbial demand for nitrogen, causing competition for nitrogen between microbes and plants to intensify. This may reduce the stimulation of NPP by elevated CO_2, and thus the rate of addition of new root biomass to soil, unless nitrogen is supplied from another source (e.g. N_2 fixation or N deposition).

By linking our microcosm results with other field

measurements in grasslands at Jasper Ridge, a clearer picture of the movement of older and newly fixed carbon through vegetative and soil pools under elevated CO_2 is emerging. Although the California serpentine and sandstone communities in our experiment were growing on non-native Colorado soil, the calculated fluxes of newly fixed carbon from plants to the top 15 cm of soil in the microcosms exposed to elevated CO_2 (113 ± 13 and 111 ± 6 g C $m^{-2}\,yr^{-1}$ from serpentine and sandstone plant communities, respectively) were of the same magnitude as those detected under elevated CO_2 in a companion study in field plots at Jasper Ridge Biological Preserve (81 ± 8 and 92 ± 13 g C $m^{-2}\,yr^{-1}$ for serpentine and sandstone communities, Hungate et al., 1997b). A mass-balance approach (Raich and Nadelhoffer, 1989) used in the field at Jasper Ridge suggested that elevated CO_2 causes a stimulation of photosynthesis and production, and proportionally more of the new photosynthate may be partitioned to short-lived belowground pools (exudation, respiration, and turnover) in elevated relative to ambient CO_2 (Hungate et al., 1997b). We found decreased mineralization of the largest pool of older soil carbon (the mineral-bound pool) under elevated CO_2 (Fig. 2b), in support of our hypothesis that elevated CO_2 may reduce microbial dependence on older SOM as a carbon source. At the same time, though the absolute amount of newly fixed carbon invested belowground in new root biomass increases under elevated CO_2 (Table 1), the movement of newly fixed carbon into long-lived, mineral-bound pools is retarded (Fig. 2d). The resulting buildup of root litter with high C:N ratio, combined with decreased decay of older SOC, could limit nitrogen availability to plants and eventually decrease NPP, unless nitrogen is supplied from a source external to the ecosystem.

As noted initially, it is the balance among NPP, the rate of delivery of new organic matter to soil pools, and the decomposition of SOM that determines the capacity of terrestrial ecosystems to store carbon. In our study, contrasting dynamics of C3- and C4-labeled soil carbon pools clearly demonstrate altered soil carbon processing and turnover under elevated CO_2, which may also ultimately result in a feedback on NPP through the nitrogen cycle. These contrasting effects of elevated CO_2 on dynamics of old and new soil carbon pools contribute to a new soil carbon equilibrium that could profoundly change long-term net carbon movement between terrestrial ecosystems and the atmosphere.

Acknowledgements

Thanks to Chris Lund and Jim Randerson for TDR measurements, Nona Chiariello for care of the microcosms, William H. Whitted for technical assistance, Ron Amundson and Hope Jahren for advice on isotopic sampling, Cynthia Lodding and Yvonne Troya for harvesting and sample processing, Melanie Hanes, Paul Brooks, and Don Herman for help with mass spectrometry, and Kevin Harrison, David Hooper, and Manuel Lerdau for comments on the manuscript. Thanks also for harvest assistance to Anne-Blanche Adams, Geeske Joel, Barbara Mortimer, Erik Nelson, Sue Thayer, Hailin Zhong and numerous others. Z.G.C. was supported by a DOE Global Change Distinguished Postdoctoral Fellowship administered by the Oak Ridge Institute for Science and Engineering Education. Undergraduate assistance and sample processing costs were supported by a grant from the Andrew W. Mellon Foundation to Z.G.C. B.A.H. was supported by a National Defense Science and Engineering Graduate Fellowship and an Alexander Hollaender Distinguished Postdoctoral Fellowship administered by the Oak Ridge Institute for Science and Engineering Education. The Jasper Ridge CO_2 Experiment was supported by grants from the US National Science Foundation to C.B.F., H.A.M., and F.S.C. III. The National Center for Atmospheric Research (NCAR) is sponsored by the National Science Foundation. This is Carnegie Institution of Washington, Department of Plant Biology, publication number 1398.

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