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Detecting changes in soil carbon in CO_2 enrichment experiments

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Abstract

After four growing seasons, elevated CO_2 did not significantly alter surface soil C pools in two intact annual grasslands. However, soil C pools in these systems are large compared to the likely changes caused by elevated CO_2. We calculated statistical power to detect changes in soil C, using an approach applicable to all elevated CO_2 experiments. The distinctive isotopic signature of the fossil-fuel-derived CO_2 added to the elevated CO_2 treatment provides a C tracer to determine the rate of incorporation of newly-fixed C into soil. This rate constrains the size of the possible effect of elevated CO_2 on soil C. Even after four years of treatment, statistical power to detect plausible changes in soil C under elevated CO_2 is quite low. Analysis of other elevated CO_2 experiments in the literature indicates that either CO_2 does not affect soil C content, or that reported CO_2 effects on soil C are too large to be a simple consequence of increased plant carbon inputs, suggesting that other mechanisms are involved, or that the differences are due to chance. Determining the effects of elevated CO_2 on total soil C and long-term C storage requires more powerful experimental techniques or experiments of longer duration.

Introduction

Elevated CO_2 often stimulates photosynthesis (Long and Drake, 1992), suggesting that the terrestrial biosphere will sequester carbon in response to rising atmospheric CO_2 (Mooney et al.,1991). However, a long-term increase in terrestrial carbon storage depends on whether the CO_2 stimulation of photosynthesis is sustained (Bowes, 1991), and whether the excess C is allocated to C pools that can sustain rates of input that exceed losses over decades to centuries, the time scale of CO_2-induced climate change. Thus, there is a tenuous relationship between long-term C sequestration in response to the gradual increase in atmospheric CO_2 and short-term C uptake and allocation in response to experimental CO_2 doubling, the most common experimental technique used to explore responses to elevated CO_2. Nevertheless, empirical evidence that short-term CO_2 doubling causes or does not cause increased C allocation to C pools in which long-term storage can occur is an important first step in determining the potential for terrestrial ecosystems to sequester C in the long term.

Soil and woody phytomass are the C pools in terrestrial ecosystems with the greatest potential for long-term C sequestration in response to rising CO_2. The Earth's soils contain 1650 Pg C, about 3 times more than the 550 Pg C in phytomass (Schlesinger, 1990). Grassland ecosystems contain little or no wood, so a long-term stimulation of ecosystem C uptake in grasslands must begin with increased soil organic C, a change which could affect global C storage (Parton et al., 1995).

Even in experiments with a dramatic manipulation like CO_2 doubling, detecting changes in soil C is dif-

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ficult: pools of C in soil are large, vary spatially within ecosystems, and turn over slowly compared to the duration of most field experiments (1–5 years). Here, we report soil C pool sizes in two annual grassland ecosystems exposed to elevated CO_2 for 4 growing seasons. We also present an approach to assess the ability (statistical power) to detect changes in soil C in any elevated CO_2 experiment.

Materials and methods

Site description

Our experimental system consists of naturally occurring annual grasslands in central coastal California, growing on serpentine- and sandstone-derived soils at the Jasper Ridge Biological Preserve of Stanford University, California, USA (37° 24' N, 122° 14' W; elevation 150 m). The climate is mediterranean, with cool, wet winters and hot dry summers. Precipitation for the last 15 years averaged 595 mm, and ranged from 200 to 1200 mm (Chiariello, 1989). The serpentine and sandstone grasslands occur adjacent to one another but differ dramatically in species composition, productivity, and nutrient limitation. Introduced European annual grasses are the dominant plants on the moderately productive sandstone, whereas native forbs are dominant on the less productive, and more strongly nutrient-limited, serpentine (Field et al., 1996; Hickman, 1993). Starting in January, 1992, circular open-top chambers (each covering 0.3 m^2 ground area) have maintained ten ambient and ten elevated (ambient + 350 ppm) CO_2– treated plots on each grassland (Field et al., 1996; Jackson et al., 1994).

Soil C and $\delta^{13}C$ measurements

In April-May of each year from 1993–1995, we removed one soil core from each plot to determine surface soil C concentration and $\delta^{13}C$. In the sandstone, we took 15-cm deep core at all three sampling dates. In 1993 in the serpentine, we took 5-cm deep cores, but switched to 15-cm deep cores in 1994 and 1995. In hopes of obtaining greater sensitivity to the effects of elevated CO_2 on total soil C, we also examined soil C concentrations in the 0–5 cm depth in 1994 in the sandstone, and in the 0–2 and 2–5 cm depths in 1995 in both serpentine and sandstone. In 1994 and 1995, we measured soil bulk density by determining the total mass of solids recovered in the cores of known volume. We also measured soil rock (particles > 2 mm-diameter) content.

For all samples, we sieved (0.2 mm) the dry soil to collect soil free of roots and detritus to use for the soil C and $\delta^{13}C$ analyses. We ground the soil collected through the sieve to a fine powder by milling with steel rods and measured %C and $\delta^{13}C$ by a combustion / GC system interfaced with an isotope-ratio mass spectrometer (Europa Scientific). The serpentine and sandstone soils contain little if any carbonate (Hungate, unpublished data), consistent with the acidic pH values for these soils, 5.5 - 6.6 (Luo et al., 1996). Thus, the $\delta^{13}C$ analyses of total soil C reflect $\delta^{13}C$ of soil organic C.

C input to soil

The bottled CO_2 added to the elevated CO_2 plots is derived from fossil fuel, and is thus depleted in ^{13}C ($\delta^{13}C$ -35 ‰) compared to atmospheric CO_2 ($\delta^{13}C$ -8‰). The isotopic composition of the CO_2 in the elevated CO_2 plots reflects a mixture of atmospheric and bottled CO_2 (Jackson et al., 1994), providing a continuous C isotope tracer in the elevated CO_2 plots (Figure 1; Leavitt et al., 1994). This ^{13}C tracer allows one to quantify the net flux into soil of C fixed since the beginning of the experiment. Plants grown in the elevated CO_2 atmosphere will be depleted in $\delta^{13}C$, reflecting the tank $\delta^{13}C$ signature, compared to plants grown in ambient CO_2. As litter from these elevated CO_2-grown plants decomposes and becomes incorporated into soil organic matter, the $\delta^{13}C$ of soil C will decrease.

We calculated the flux into soil of carbon fixed since the beginning of the experiment using the ^{13}C tracer in the elevated CO_2 plot. First, we determined the change through time in $\delta^{13}C_{plantC}$ in the elevated CO_2 plots. Within each grassland, the chambers are arranged in a randomized complete-block design, with paired elevated and ambient CO_2 plots within each block. For each block at each harvest, we subtracted $\delta^{13}C_{soilC}$ in the ambient CO_2 plot from $\delta^{13}C_{soilC}$ in the elevated CO_2 plot, thereby obtaining ten independent estimates in each ecosystem of the difference in $\delta^{13}C_{soilC}$ between ambient and elevated CO_2 plots; we refer to this difference as $\Delta\delta^{13}C_{soilC}$.

We used $\delta^{13}C_{plantC}$ from Jackson et al. (1994) from the 1993 growing season to determine the difference in isotopic signature between plants grown under elevated and ambient CO_2 $\Delta\delta^{13}C_{plantC}$ in order to establish the input signal for the ^{13}C isotope tracer. $\delta^{13}C_{plantC}$ in 1994 and 1995 were similar to 1993 (Jackson, unpublished data), and we assumed that the live tissue $\delta^{13}C$

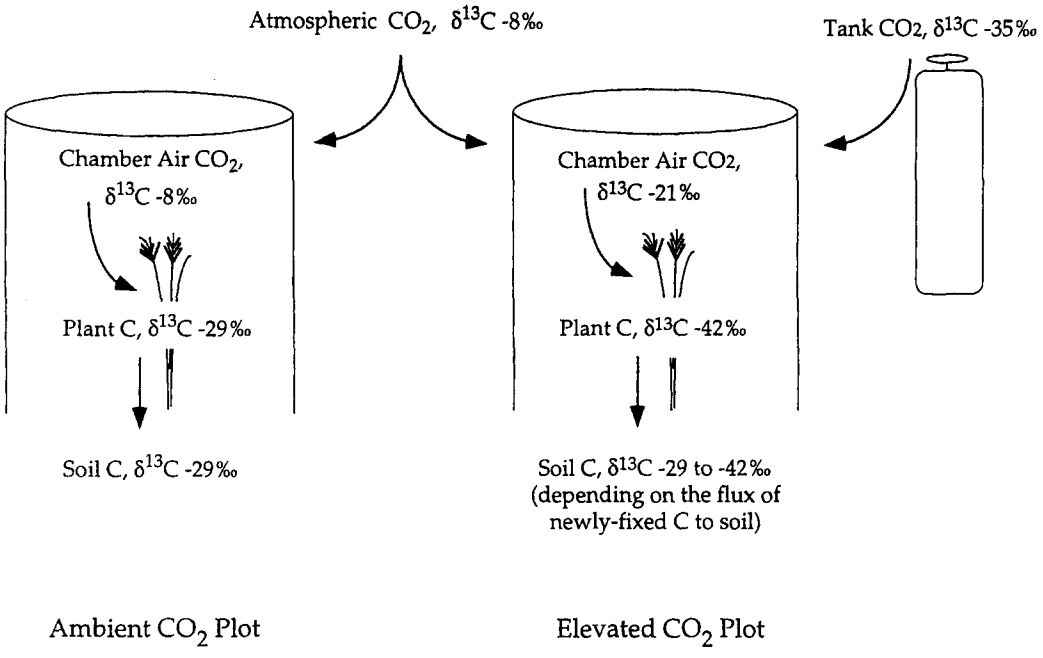

Figure 1. The $\delta^{13}C$ tracer in the elevated CO_2 plots. In the elevated CO_2 treatment, the $\delta^{13}C$ value of CO_2 in the chamber atmosphere is depleted compared to atmospheric CO_2 (-21‰ compared to -8‰, respectively), reflecting the 1:1 mixture of atmospheric CO_2 and the tank CO_2 (-35‰) added to the elevated CO_2 chambers. Thus, plants in the elevated CO_2 chambers have a lower $\delta^{13}C$ value (-42‰) than plants in the ambient CO_2 chambers (-29‰). Through time, soil C in the elevated CO_2 plots will be depleted in ^{13}C compared to ambient CO_2, according to the amount of C incorporated into soil organic matter that reflects the depleted tank $\delta^{13}C$ source.

is representative of the $\delta^{13}C$ value of senesced plant material.

We converted $\Delta\delta^{13}C_{soilC}$ into pools of soil C derived from C fixed since the beginning of the experiment. We refer to this soil C pool as soil C_{new}. By isotopic mass balance, soil C_{new} (in g C m^{-2}) is:

$$\text{soil } C_{new} = \text{soil } C_{total} \times (\Delta\delta^{13}C_{soilC}/\Delta\delta^{13}C_{plantC}), \quad (1)$$

where soil C_{total} is the total C pool in the soil and $\Delta\delta^{13}C_{soilC}$ and $\Delta\delta^{13}C_{plantC}$ are the differences, respectively, in $\delta^{13}C_{soilC}$ and $\delta^{13}C_{plantC}$ between paired elevated and ambient CO_2 plots.

To determine the experimental duration to obtain statistical power of 0.8, we used a simple model with a constant rate of C input to soil and a first order decomposition term (e.g. Townsend et al., 1995) to extrapolate the rate of accumulation through time of soil C_{new}. In this model,

$$C(t) = I/\tau \times (1 - e^{-\tau t}), \quad (2)$$

where C(t) is soil C_{new} at time, t, I is the rate of input of C to the soil pool, and τ is the turnover time of soil C_{new}.

Effect size and power analysis

The soil $\delta^{13}C$ data constrain the magnitude of the possible increase ("effect size") in soil C due to elevated CO_2. As there must be some soil C_{new} in ambient CO_2, an increase in total soil C due to elevated CO_2 must be smaller than the measured soil C_{new} in elevated CO_2. Thus, given the hypothesis that elevated CO_2 increases soil C_{new}, the $\delta^{13}C$-determined value of soil C_{new} in the elevated CO_2 treatment is an upper, absolute reference for postulated relative effect sizes of elevated CO_2 on soil C_{new}: as the effect size increases, the postulated value of soil C_{new} for ambient CO_2 decreases.

Within this framework, we examine a range of relative effect sizes of elevated CO_2 on soil C_{new}. CO_2 from the atmosphere enters soil through plants, so the 70% stimulation of plant photosynthesis (per unit leaf area) observed for the dominant species in the sandstone grassland (Jackson et al., 1994) sets a reasonable upper bound of 70% on the putative increase in soil C_{new} caused by elevated CO_2, since we have not observed pronounced increases in leaf area index and have observed somewhat smaller increases in leaf-level photosynthesis for the serpentine domi-

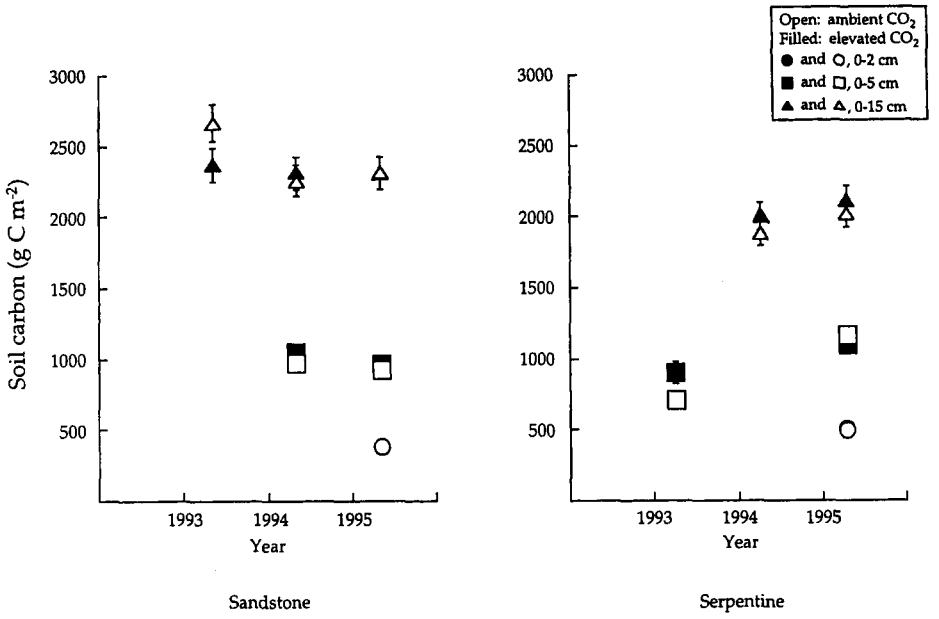

Figure 2. Surface soil C pools for 1993–1995 in serpentine and sandstone grasslands under ambient and elevated CO_2. Triangles, squares, and circles show values for the 0-15, 0–5, and 0–2 cm depths, respectively. Values are means±sem (n=10). See Table 1 for ANOVA results.

nants (Field et al., unpublished). The 35 to 140% stimulation of soil respiration by elevated CO_2 (Luo et al., 1996) at least partly counteracts the CO_2 stimulation of photosynthesis, so a more reasonable effect size of elevated CO_2 on soil C_{new} may be only 10–50% of the stimulation of instantaneous photosynthesis. Thus, we examine three effect sizes: a 7%, 35%, and 70% stimulation of soil C_{new} caused by elevated CO_2. This is a broad range of effect sizes, underlining the inherent uncertainty in the estimate; however, these relative effect sizes have less influence in the power analysis than the absolute reference provided by the ^{13}C tracer.

We calculated statistical power to detect changes in soil C and in soil C_{new} to soil using these hypothetical effect sizes, constrained by the ^{13}C tracer. We calculated the hypothetical difference between ambient and elevated CO_2 for soil C and soil C_{new} for each effect size scenario and calculated power to detect differences in these hypothetical means, using the 1995 soil samples to estimate variance in total soil C pools and in soil C_{new}. Coefficients of variation for total soil C in 1995 (6% sandstone, 13% serpentine) were the lowest from four years of sampling and are similar to, or lower than, coefficients of variation observed from other studies in these grasslands (Huenneke et al., 1990; Jackson et al., 1990). We used the normal approximation of the noncentral t distribution to determine power (Winer et al., 1991) for α=0.05 and for 10 replicates of each treatment. We also determined the experimental duration necessary to obtain power of 0.8-chosen arbitrarily, but with some precedence, e.g. Osenberg et al. (1994), by extrapolating our first-order model of soil C_{new} through time with a 7%, 35%, or 70% stimulation of C input to soil (I, Equation 2).

Results and discussion

The sandstone grassland contains more C in the top 15 cm of soil than the serpentine (Figure 2, $p < 0.001$), even though C concentrations in the serpentine are higher than in the sandstone (2.53±0.10 and 1.67±0.03%C, respectively, for 1995, 0–15 cm). Bulk density is lower in the serpentine (0.97 g cm^{-3}) than the sandstone (1.19 g cm^{-3}) and the serpentine is also much rockier, containing 29% rocks (particles > 2 mm) by mass, compared to < 1% in the sandstone. The lower bulk density and high rock content in the serpentine soil offsets the difference in C concentrations, yielding the smaller soil C pool in the serpentine.

Elevated CO_2 did not significantly alter soil C at any depth in 1994 or 1995 ($p > 0.05$, Figure 2, Table 1). In 1993, C in the top 15 cm of soil was 200 g C m^{-2} higher in the elevated CO_2 plots in the serpentine, and

Table 1. Results from 2-way analyses of variance for surface soil C pools, sampled at various depths, from 1993 to 1995. F_{df} is the F-ratio for the effect of elevated CO_2, difference between ecosystems, and the CO_2 by ecosystem interaction, with residual degrees of freedom noted (subscript), and p is the p-value of the test

Factor	1993[a]		1994		1995					
			0–15 cm		0–2 cm		0–5 cm		0–15 cm	
	F_{36}	p	F_{df}	p	F_{df}	p	F_{df}	p	F_{df}	p
CO_2	0.37	0.55	1.14	0.29	0.05	0.83	0.04	0.84	0.37	0.55
Ecosystem	382	<0.001	14.35	0.001	21.9	<0.001	16.5	<0.001	10.6	0.003
CO_2 × Ecosystem	7.9	0.008	0.03	0.87	0.004	0.95	0.63	0.43	0.38	0.54

[a] For 1993, sandstone samples were 0–15 cm, serpentine samples were 0–5 cm.

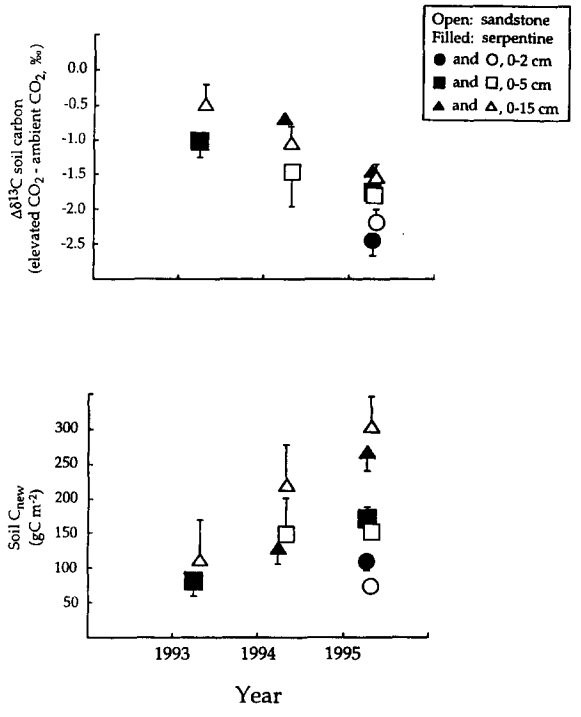

Figure 3. Soil $\Delta\delta^{13}C$ (**A**) and soil C_{new} (**B**) for serpentine (filled symbols) and sandstone (open symbols) grasslands exposed to elevated atmospheric CO_2. Triangles, squares, and circles show values for the 0–15, 0–5, and 0–2 cm depths, respectively.

300 g C m^{-2} lower in the elevated CO_2 plots in the sandstone. These differences were significant at the $p < 0.05$ level (t-tests for each ecosystem), but are surprisingly large when considered in the context of the annual C cycle in these grassland ecosystems (see power analysis, below). Furthermore, these differences did not persist in 1994 and 1995. We suggest they are due to sampling error. Elevated CO_2 did not significantly alter soil C pools in the 0–2 or 2–5 cm depths in 1995 nor in the 0–5 cm depth in the sandstone in 1994 (Figure 2, Table 1).

$\Delta\delta^{13}C$ decreased through time in both serpentine and sandstone grasslands, reflecting the rate of incorporation of newly-fixed C into soil (Figure 3). Accordingly, soil C_{new} in the elevated CO_2 plots increased through time. After 4 years of treatment, soil C_{new} in the top 15 cm of soil was 266 ± 26 g C m^{-2} y^{-1} for the serpentine and 303 ± 43 g C m^{-2} y^{-1} for the sandstone. These rates were not significantly different from each other, even though there is more total soil C in the sandstone. In contrast, soil C_{new} in the top 0–2 cm of soil was significantly higher in the serpentine than the sandstone (Figure 3, $p < 0.05$), possibly a consequence of higher concentration in the serpentine of roots in the top 0–2 cm of soil. For both serpentine and sandstone, soil C_{new} per gram soil C was significantly higher in the 0–2 cm depth than the 2–5 or 5–15 cm depths (data not shown) indicating faster turnover and suggesting that changes in soil C may be easier to detect in the top 0–2 cm of soil.

The ^{13}C-determined rates of incorporation of newly-fixed C into soil provide a basis for determining the ability to detect possible effects of elevated CO_2 on total soil carbon. The soil C_{new} values for ambient CO_2 presented in Figure 4 reflect the hypothetical scenarios of elevated CO_2 increasing soil C_{new} by 70%, 35%, or 7%. Projected changes in total soil C pools after 4 growing seasons (January 1992 to May 1995) in elevated CO_2 are relatively small under all three scenarios, ranging from 0.7-9.0% (Figure 4). Although these hypothetical changes in soil C are small, even a 7% increase in soil C_{new} would be a substantial change in the C cycle.

In this experiment, the statistical power to detect these changes in soil C is low (Figure 4), even in

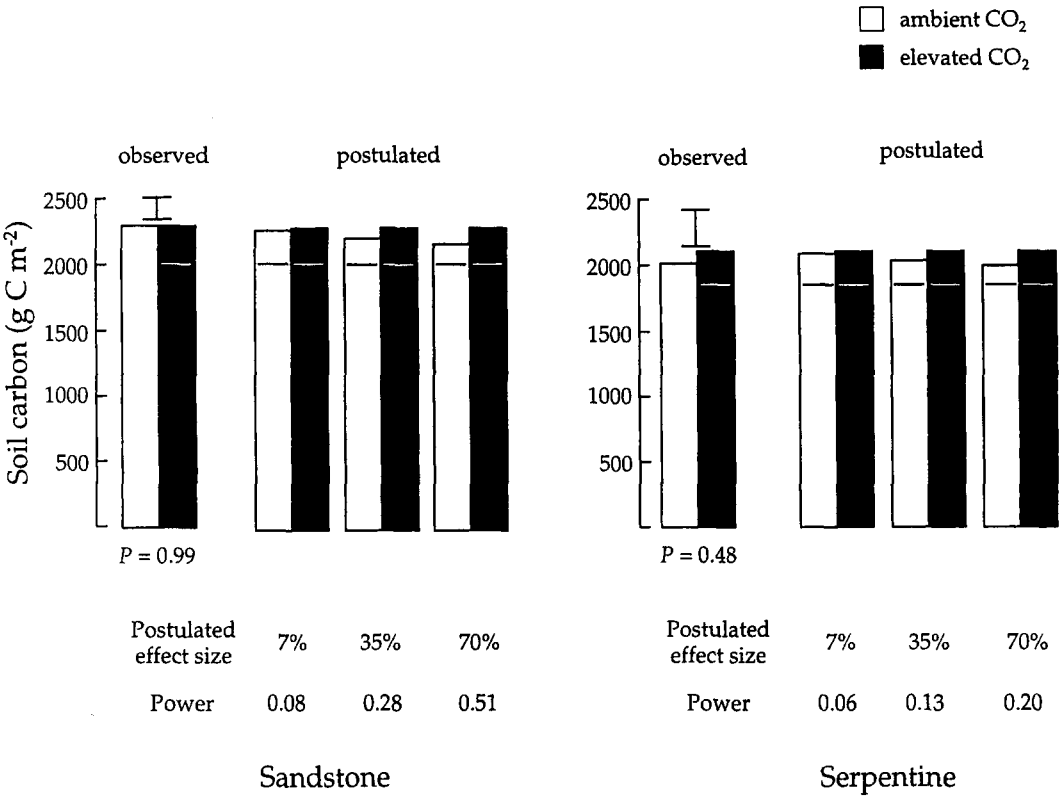

Figure 4. Observed and postulated values of total soil C and soil C_{new} for 0–15 cm in 1995. Observed values are means (n=10); the 95% confidence interval for the difference between elevated and ambient CO_2 treatments is shown above each pair of bars; the probability (p, t-test) that elevated and ambient CO_2 differ in total soil C is shown below each pair of bars. Postulated values are means. The top portion of each bar shows soil C_{new} (observed for the elevated CO_2 plots only, postulated for both elevated and ambient CO_2). The statistical power to detect a significant difference, if it exists, is indicated for each postulated effect size (values are probabilities of obtaining a significant result, given a true effect of the magnitude shown).

the largest effect size scenario (70% stimulation); our chances of detecting an effect of this magnitude in the top 15 cm of soil are 51% in the sandstone and only 20% in the serpentine. (Though C input and turnover were relatively more rapid in the top 2 and top 5 cm of soil, the coefficients of variation for soil C were higher in these depths, and thus power to detect changes in soil C was no greater than for the top 15 cm of soil. For simplicity, we present power only for the top 15 cm of soil.) If elevated CO_2 increases soil C_{new} by only 35% or 7%, the power to detect changes in soil C is substantially lower, ranging from 0.28 to 0.06. In other words, our chances of detecting these differences, if they exist, are as low as 6% (Figure 4). The lower statistical power in the serpentine is primarily due to a higher coefficient of variation for soil C: 6% for the sandstone, 13% for the serpentine.

Power increases with the duration of the experiment, as the soil C_{new} pool constitutes an ever larger proportion of total soil C. In the sandstone, extrapolating the first-order model of soil C_{new} through time, power to detect increased soil C due to a 70% CO_2 -stimulation of C input to soil will be 0.8 for the 1996 sampling (Figure 5). To detect increased soil C due to a 35% stimulation of C input to soil, the experiment must reach a total duration of 9 years, continuing until 2001 (Figure 5). With the 7% stimulation scenario in the sandstone (and for all scenarios in the serpentine), soil C_{new} in the first-order model reaches steady state before the difference in soil C between ambient and elevated CO_2 treatments is large enough for power of 0.8. Thus, obtaining power of 0.8 in these cases will require maintaining the experiment until the slow and passive soil C pools (sensu Parton et al., 1987) respond to increased C input in elevated CO_2.

Examining confidence intervals for total soil C complements the power analysis presented above. Because elevated CO_2 did not significantly increase

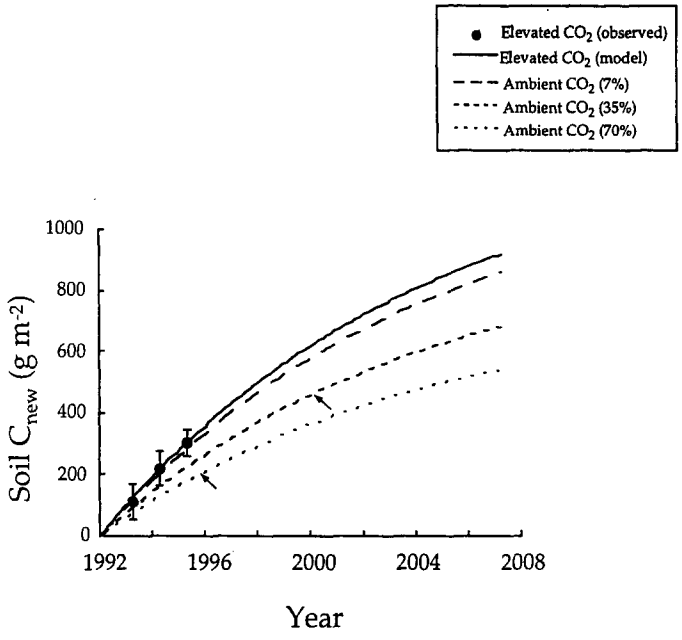

Figure 5. Changes in soil C_{new} through time for the elevated CO_2 treatment, based on extrapolation through time of the first-order model for soil C_{new}, and for the ambient CO_2 treatment, based on three postulated effect sizes (7%, 35%, and 70%) of increased soil C_{new} in elevated CO_2. For simplicity, only data and model output for the 0–15 cm depth in the sandstone are presented. For the three effect size scenarios, arrows indicate the time at which power = 0.8 to detect the difference in total soil C between elevated and ambient CO_2 treatments. (Power asymptotes at 0.77 for the 7% effect size scenario when the first-order model for soil C_{new} reaches steady state).

soil C (Figure 2), we can reject an increase in soil C larger than the upper limit of the 95% confidence interval for the difference in soil C between high and low CO_2. In the sandstone, this upper limit is 162 g C m^{-2} (Figure 4). Thus, we can reject the hypothesis that elevated CO_2 increased soil C by more than 162 g C m^{-2} over the four years of this experiment. An increase of this magnitude corresponds to a 115% stimulation of soil C_{new}, which, though outside the range of effect sizes we consider plausible in this experiment, is modest compared to observed increases in soil C_{new} caused by elevated CO_2 (Ineson et al., 1996) or to increases in C input to soil required to explain observed changes in total soil C caused by elevated CO_2 in other experiments (Leavitt et al., 1994; Rice et al., 1994; Wood et al., 1993; see discussion below). In the serpentine, we can rule out any stimulation of C input to soil that causes an increase in total soil C by more than 274 g C m^{-2} (Figure 4). In this case, the difference we can rule out is larger than soil C_{new} for elevated CO_2; i.e. increased C input in elevated CO_2 could not cause a difference of 274 g C m^{-2}, so we can reject an effect of this magnitude based on the $\delta^{13}C$ constraint.

The incorporation of the ^{13}C-depleted CO_2 into soil organic matter in the elevated CO_2 plots provided a valuable constraint on putative CO_2 effects total soil C and a basis for determining our ability to detect those effects. Given the slow turnover rate and large sizes of soil C pools, the low power to detect these changes in soil C pools is not surprising. C pools in the top 15 cm of soil are around 2000 g C m^{-2} in both ecosystems, but even after 4 growing seasons of exposure to elevated CO_2 with the largest plausible stimulation of C input to soil, differences in total soil C between CO_2 treatments will be only about 100 g C m^{-2}; detection of this difference with standard statistical criteria is difficult. The smaller 7% stimulation of net C flux to soil would cause differences in soil C of only about 20 g C m^{-2}, detection of which is extremely unlikely.

Consistent with our findings, most other studies that have examined the effect of elevated CO_2 on total soil C concentrations have found no significant changes (Arnone and Körner, 1995; Johnson et al., 1994; Rogers and Prior, 1992; Ross et al., 1995; Zak et al., 1993). Several of these studies have also acknowledged the difficulty of detecting changes in soil C in their experiments (Johnson et al., 1994; Ross et al., 1995). However, a study of tallgrass prairie (Rice et al., 1994) and another of cotton (Leavitt et al., 1994; Wood et al., 1994) reported that elevated CO_2 sig-

nificantly increased or tended to increase total soil C concentrations. Here, we express soil C concentrations from these studies as soil C pools, an exercise that shows that the magnitude of these reported increases in soil C are surprisingly large compared to measured or estimated rates of C input to soil.

For example, Rice et al. (1994) found that, after three growing seasons in the tallgrass prairie ecosystem, elevated CO_2 significantly ($p < 0.1$) increased soil organic C when N fertilizer was also added. The reported difference in soil C concentrations is equivalent to approximately 1760 g C m^{-2} more soil organic C in elevated CO_2 (Table 2), a dramatic increase in soil C. In the same experiment, Owensby et al. (1993) found that elevated CO_2 stimulated plant biomass, but even the largest measured increase in aboveground and belowground biomass, extrapolated over three years, is about four times too low to account for 1760 g C m^{-2} increase in soil C (Table 2). Since Owensby et al. (1993) measured standing shoot biomass by destructive harvest and root growth with ingrowth cores, it is possible that the unmeasured components of plant production, such as root turnover and exudation, could account for this large increase in soil C (see below).

In a study of cotton responses to elevated CO_2, Leavitt et al. (1994) suggest that "there is more [soil] carbon in the FACE (i.e., elevated CO_2) plots". Leavitt et al. (1994) also determined the rate at which newly fixed C is incorporated into soil by following the fossil-fuel $\delta^{13}C$ tracer in their FACE plots, in a manner analogous to that presented here. Converting their total soil C concentrations and $\delta^{13}C$ data to pools and fluxes shows that the difference in total soil C between elevated and ambient CO_2 plots is larger than soil C_{new} in the elevated CO_2 plots (Table 2); as soil C_{new} is certainly not less than zero in ambient CO_2, increased C input to soil alone can not account for the large change in total soil C. In the same experiment, Wood et al. (1994) conclude that increased cotton production in elevated CO_2 caused "trends toward increased [soil] organic C". If soil C_{new} for the top 20 cm is 230 g C m^{-2} (assuming that 90% of soil C_{new} in the top 30 cm of soil is in the top 20 cm of soil), then elevated CO_2 must have stimulated soil C_{new} by 230% (Table 2) to explain the difference in soil C between elevated and ambient CO_2 of 160 g C m^{-2} (i.e. soil C_{new} in ambient CO_2 must have been 70 g C m^{-2}).

Thus, the reported increases in soil organic C are quite large compared to increased plant biomass (in the tallgrass prairie study) and to C input to soil (in the cotton study). There are several possible explanations for this: (1) In the tallgrass prairie, elevated CO_2 augments soil C through root turnover and exudation. Although the estimate (Table 2) of increased C input to soil from the plant biomass response does not include these processes (whereas the $\delta^{13}C$ approach does), root exudation and turnover would have to be about 400 g C m^{-2} yr^{-1} higher in the elevated CO_2 treatment (with no corresponding increase in belowground respiration) in order to explain the difference in total soil C. An increase of this magnitude would be considerable, as root growth into ingrowth cores is 80–140 g C m^{-2} yr^{-1} (Owensby et al., 1994). (2) In addition to stimulating C input, elevated CO_2 caused decreased loss of older soil organic C as well. If soil microorganisms preferentially consume labile organic compounds released from roots for C and energy, and if these compounds are abundant in elevated CO_2, decomposition of native soil organic matter could be retarded (Cardon, 1996; Lekkerkerk et al., 1990). Decreased loss of old soil C would contribute to increased total soil C concentrations and may explain the large effects of elevated CO_2 on total soil C found in these studies. (3) It is also possible that the apparent differences in soil C concentrations, though significant or marginally so, were due to chance and not to an effect of elevated CO_2.

This analysis is simplistic because it ignores the considerable variance in the estimates and measurements of C input to soil and in soil C content. Nevertheless, this exercise shows how estimating plausible "effect sizes" on changes in total soil C in elevated CO_2 experiments can help evaluate whether detectable changes in total soil C can be expected, whether observed effects are likely due to increased C input or must also include decreased C loss, and, if on the margins of statistical significance, whether the observed trends are plausible. In CO_2 experiments, the $\delta^{13}C$ tracer from using fossil-fuel derived CO_2 in the elevated CO_2 treatment offers a powerful approach to estimating effect sizes on C input to soil.

Though there are undoubtedly some exceptions, low power to detect changes in total soil C concentrations is probably a general characteristic of elevated CO_2 field experiments. Power increases with sample size, as larger samples afford more confident estimates of variance. However, in the context of ongoing CO_2 enrichment experiments and budget constraints, we ought to consider alternative approaches to address this question. Using soil fractionation techniques that separate labile from recalcitrant soil C pools (e.g. Cambardella and Elliot, 1992) may increase statistical pow-

Table 2. Comparing increases in soil C concentrations with estimates of C input to soil in tallgrass prairie and cotton elevated CO_2 studies. Soil C (%) is the average soil C concentrations reported in these studies, weighted by core depth. Δ soil C is the change in the total soil C pool (g m^{-2}), calculated assuming that bulk density is 1 in the tallgrass prairie and 1.33 in the cotton FACE study (average from Rogers and Prior, 1992). For the tallgrass prairie study, C input (Δ plant biomass) is the stimulation of C input to soil by elevated CO_2, estimated from the largest measured increase in aboveground (120 g C m^{-2} in 1991) and belowground (40 g C m^{-2} in 1990) plant C pools from Owensby et al. (1994, Figures 2 and 3, assuming that plant biomass is 40% C), multiplied by three growing seasons. Soil C_{new} (reported here for the cotton FACE study) is defined in Equation 1 (see Methods) as the pool of soil C derived from C fixed since the CO_2 manipulation began, as determined by the ^{13}C tracer (from Leavitt et al., 1994), and assuming that 90% of soil C_{new} in the 0–30 cm depth is in the 0–20 cm depth

Ecosystem	Soil C (%)		Depth	Δ soil C	C input (g C m^{-2})		Comments	Reference
	Ambient CO_2	Elevated CO_2	(cm)	(g C m^{-2})	Δ plant biomass	Soil C_{new}		
Tallgrass prairie	3.30	4.47	0–15	1760	480	n.d.[a]	Stimulation of C input 4 times too low to explain increased soil C	Rice et al. (1994)
Cotton	0.56	0.64	0–30	320		255	C input smaller than change in soil C	Leavitt et al. (1994)
	0.61	0.67	0–20	160		230	Requires 230% stimulation of C input by elevated CO_2	Wood et al. (1994)

[a] n.d. not determined

er by focusing the analysis on the soil C pools that are likely to show the largest responses on the time scale of elevated CO_2 experiments. Of course, the labile pools of soil C are the least likely to be important in long-term carbon storage. Models of soil organic matter dynamics provide one method for estimating the consequences for long-term storage of changes in the size of one or more labile pools.

Another approach would be to add a ^{13}C tracer to the ambient CO_2 plots comparable to that already in the elevated CO_2 plots, so that soil C_{new} could be measured in both treatments. In our experiment at Jasper Ridge, this would increase power considerably; for example, for the 70% stimulation scenario, power to detect changes in soil C_{new} would be 0.89 for the top 15 cm in the serpentine (as compared to 0.20 for total soil C). Adding such a tracer could be accomplished by continuously enriching the chamber atmosphere with ^{13}C-CO_2, but because of the high volume of air flow through the open top chamber, adding enough ^{13}C to increase $\delta^{13}C$ of plants by 10‰ would be extremely expensive. It may be possible to remove CO_2 from the air entering the ambient CO_2 plots, replacing it with tank CO_2 bearing the distinct fossil-fuel-derived $\delta^{13}C$ signature, but this approach would also be costly.

Alternatively, Cardon (1996) and Ineson et al. (1996) maintained continuous ^{13}C tracers in elevated CO_2 experiments, not by altering the ^{13}C-CO_2 composition of the atmosphere, but by using soils that developed under C_4 plants with a $\delta^{13}C$ value distinct from their C_3 plants. The use of an imported soil in these experiments is certainly a departure from natural conditions, and may cause artifacts, but provides a powerful isotope tracer in both CO_2 treatments. Because fractionation against ^{13}C-CO_2 during photosynthesis is greater in C_3 plants than in C_4 plants (O'Leary, 1981), plant and soil C are isotopically distinct in these experiments. For example, in a grassland experiment (Cardon, 1996), $\delta^{13}C$ of the C_3 plants in ambient CO_2 is about 12% less, in elevated CO_2 about 22‰ less, than $\delta^{13}C$ of the C_4 soil. This is of comparable magnitude to the ^{13}C tracer discussed in this paper (Figure 2). Thus, power to detect changes in C flux to soil in these experiments is considerably higher than the power afforded by examining total soil C pools. Indeed, Ineson et al. (1996) detected a significant increase in C input to soil in elevated CO_2 (i.e. more soil C reflecting the plant (C_3) $\delta^{13}C$ value) in their birch seedling microcosms grown in a C_4 soil.

It is difficult to detect changes in soil C in elevated CO_2 experiments because the soil C pool is large and heterogeneous relative to annual plant inputs. However, detection of even small CO_2 effects on soil C is crucial to our understanding of global C cycling. Pow-

er analysis helps identify when a "negative result" (i.e. no significant difference) can be interpreted with confidence to mean no biologically meaningful difference or simply the lack of statistical power to detect a small but important change. Finally, by focusing on those soil C pools that are most likely to be affected and by using unique isotopic signatures of soil or atmospheric CO_2 in an experiment, we greatly increase our power to detect meaningful changes in C cycling of ecosystems in response to elevated CO_2.

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