Altered soil microbial community at elevated CO_2 leads to loss of soil carbon

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Increased carbon storage in ecosystems due to elevated CO_2 may help stabilize atmospheric CO_2 concentrations and slow global warming. Many field studies have found that elevated CO_2 leads to higher carbon assimilation by plants, and others suggest that this can lead to higher carbon storage in soils, the largest and most stable terrestrial carbon pool. Here we show that 6 years of experimental CO_2 doubling reduced soil carbon in a scrub-oak ecosystem despite higher plant growth, offsetting ≈52% of the additional carbon that had accumulated at elevated CO_2 in aboveground and coarse root biomass. The decline in soil carbon was driven by changes in soil microbial composition and activity. Soils exposed to elevated CO_2 had higher relative abundances of fungi and higher activities of a soil carbon-degrading enzyme, which led to more rapid rates of soil organic matter degradation than soils exposed to ambient CO_2. The isotopic composition of microbial fatty acids confirmed that elevated CO_2 increased microbial utilization of soil organic matter. These results show how elevated CO_2, by altering soil microbial communities, can cause a potential carbon sink to become a carbon source.

carbon cycling | global change | microbes | priming effect

Higher carbon storage due to elevated CO_2 may help stabilize atmospheric CO_2 concentrations (1). Many field studies have found that elevated CO_2 leads to higher carbon assimilation by plants (2), and others suggest that this can lead to higher carbon storage in soils, the largest and most stable terrestrial carbon pool (3). However, it has been shown that soils with low nutrient availability have a limited capacity to store soil C at elevated CO_2, and this response is relatively well understood at a mechanistic level (4). Less well understood is the possibility that elevated CO_2 could actually increase carbon losses from ecosystems by stimulating the decomposition of soil organic carbon (5, 6). Only a few field studies have provided evidence that elevated CO_2 may increase soil organic matter decomposition (7–9), and none have directly addressed whether changes in microbial community composition or activity might be responsible for this phenomenon.

A substantial portion of the "extra" carbon fixed by plants grown at elevated CO_2 is labile and rapidly metabolized by microbial communities, whether deposited to soils through increased leaf litterfall, root exudation, or root turnover (10). Because microorganisms mediate critical carbon transformations in soil, their response to extra carbon inputs will influence how much carbon can be stored in soils over the long term. For example, increased plant carbon inputs at elevated CO_2 may suppress soil organic matter decomposition because the simple organic compounds of root exudates are easier for microbes to use than the recalcitrant organic materials found in soil (5). Results from short-term laboratory or greenhouse experiments suggest that suppression of decomposition can occur, although effects vary with time, plant community, and soil nutrient availability (11). Alternatively, the increased influx of labile carbon to soil may stimulate microbial degradation of soil organic matter, an effect known as "priming," due to microbial mining of soil organic matter for nutrients or changes in microbial activity or community composition (8). Despite intensive interest in the fate of carbon with rising CO_2, as well as the importance of microorganisms to ecosystem processes, no studies to date have demonstrated an explicit link between changes in soil microbial activity and composition and long-term carbon storage at elevated CO_2.

To examine the influence of elevated CO_2 on soil carbon storage and how shifts in soil microbial communities might affect long-term soil carbon trends, we used a well replicated, long-term field experiment in a fire-adapted scrub oak ecosystem in Florida (12). The forests at this site have shown a consistent increase in photosynthesis and plant growth at elevated CO_2 (13, 14). At this site, elevated CO_2 has caused very minor effects on leaf chemistry with no discernable effect on litter decomposition (15–17), similar to most other CO_2 enrichment experiments (18). We used standard approaches to detect changes in soil carbon stocks and conducted a laboratory-based decomposition experiment to examine the influence of elevated CO_2 on microbial processes that influence soil carbon pools. In the decomposition experiment, we added the same mass of a constant leaf litter substrate to soils that had been subjected to 6 years of either ambient or elevated CO_2. The leaf litter was collected from the elevated CO_2 chambers of the experiment and thus was far more ^{13}C-depleted ($\delta^{13}C = -39‰$) than either ambient or elevated soils ($\delta^{13}C \geq -30‰$) [Table 1 and supporting information (SI) Table 3]. This large difference between litter and soil allowed us to determine, for both CO_2 treatments, how much of the CO_2 evolved from microbial respiration during the experiment originated from the decomposition of the added litter versus that which evolved from native soil organic matter. We also were able to trace the isotopic signature into microbial fatty acids, which provided an indication of whether specific microbial groups were preferentially using soil carbon.

Results and Discussion

We found that elevated CO_2 led to persistent losses of soil carbon content over a 4-year period ($r^2 = 0.98$, $P = 0.009$) (Fig. 1*A* and SI Table 4). This loss of soil carbon amounted to 442 g·m^{-2} C to a depth of 10 cm, which offset ≈52% of the additional carbon that had accumulated at elevated CO_2 in aboveground (212 g·m^{-2} C) and coarse root (646 g·m^{-2} C) biomass by the year 2002 (20) (B.G.D., unpublished data). There was also a loss of soil carbon at 10–30 cm and a small gain from 30 to 60 cm (SI Table 3), suggesting that the loss of soil carbon from the surface was not offset by gains lower in the soil profile, at least to a depth of 60 cm. Future research is needed to examine whether root growth or leaching of dissolved organic carbon affects soil carbon even deeper in the soil profile.

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Abbreviation: PLFA, phospholipid fatty acid.

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Table 1. Characteristics of soil organic carbon fractions sampled in 2002 from 0 to 10 cm depth and separated by density

Soil organic matter density	Mass, ambient CO_2, g·m^{-2}	Mass, elevated CO_2, g·m^{-2}	$\delta^{13}C$, ambient CO_2, ‰	$\delta^{13}C$, elevated CO_2, ‰	Turnover time,* years
<1.5 g·cm^{-3}	1,594 ± 117^{a†}	1,197 ± 157^{a†}	−28.03 ± 0.11	−29.95 ± 0.25	13 ± 2^{a}
1.5–1.8 g·cm^{-3}	359 ± 41^{b}	336 ± 40^{b}	−27.61 ± 0.12	−28.92 ± 0.22	20 ± 3^{a}
1.8–2.2 g·cm^{-3}	146 ± 21^{b}	124 ± 15^{b}	−27.45 ± 0.09	−28.59 ± 0.18	24 ± 5^{a}

Data are mean ± SE. Superior letters a and b denote significant differences ($P < 0.10$, ANOVA and Tukey–Kramer honestly significant difference range test) in comparisons across the three soil organic matter density fractions. Note that Fig. 1 shows a more complete and definitive analysis of the effect of CO_2 on soil carbon concentrations because it incorporates samples taken over multiple time points; the data shown here are from May 2002 only.

*Turnover time could only be estimated for soils exposed to elevated CO_2. See *Materials and Methods* for details.

†The sole significant difference in comparisons across ambient and elevated treatments within a given density fraction ($P = 0.06$, ANOVA).

We examined whether changes in microbial activity or composition could explain the losses in surface soil carbon using a laboratory-based litter decomposition experiment. Specifically, we tested the hypothesis that soil carbon content was reduced through increased microbial decomposition of leaf litter. If input from this important carbon pool decreased (i.e., more of it was decomposed before it could be incorporated into the soil), soil carbon content could potentially decline over the long term. However, we found no difference in litter decomposition rates between soils exposed to elevated versus ambient CO_2 ($P = 0.972$) (SI Table 3), a finding that is consistent with other studies (18).

However, soils from the elevated CO_2 sites demonstrated higher rates of microbial respiration from soil organic matter in the presence of the added litter. In other words, they were more highly primed than soils from ambient sites, degrading soil organic matter more rapidly in the presence of carbon inputs ($P = 0.055$) (SI Table 3). Apparently, elevated CO_2 altered soil microbial communities in the field such that they degraded soil organic matter more rapidly. Importantly, the amount of priming exhibited by soils in the decomposition experiment was correlated to the largest and most rapidly cycling soil carbon pool in the experimental field system, the light fraction ($r^2 = 0.49$, $P = 0.003$) (Fig. 1*B*). This finding suggests that microbial priming can explain the loss of soil organic carbon we observed.

The larger priming response of the elevated CO_2 microbial community was consistent with changes in microbial enzyme activities. We examined the activities of two enzymes critical to carbon cycling in soils: β-glucosidase and phenol oxidase (21). β-Glucosidase is important to the degradation of cellulose (22), a common material in plant cell walls. Phenol oxidase is critical to the degradation of highly recalcitrant organic materials, such as lignin, which accumulate in soil organic matter (23). β-Glucosidase activities did not differ across treatments ($P = 0.34$) (SI Table 3). However, soils from elevated CO_2 sites had higher phenol oxidase activities than those from ambient CO_2 sites ($P = 0.090$) (Fig. 2*A*). These data are consistent with higher rates of soil organic matter decomposition in elevated CO_2 soils.

The differences in enzyme activity may have been driven by differences in soil microbial community composition. We found that the ratio of fungi to bacteria was higher in soils from elevated than ambient CO_2 sites ($P = 0.041$) (Fig. 2*B*). Fungi are widely known to be key to the degradation of recalcitrant organic materials, in part through their production of lignolytic enzymes, such as phenol oxidase (24). In fact, fungal abundance and phenol oxidase activity were correlated ($r^2 = 0.33$, $P = 0.031$) (SI Fig. 3). This work shows that increased fungal abundance not only promoted lignolytic enzyme activity, but also reduced soil carbon storage.

Many studies across different ecosystems have found that elevated CO_2 increased fungal abundance in soils (25–27). Lower nitrogen availability at elevated CO_2 may in part explain these increases in fungi; fungi tend to have higher carbon:nitrogen ratios than bacteria, which lessens fungal demand for nitrogen (28). It is also possible that higher root turnover at elevated CO_2 promotes fungal growth (29).

The carbon isotopic composition of microbial fatty acids provided direct evidence that elevated CO_2 increased microbial utilization of the carbon compounds in soil organic matter. We added litter that was depleted in ^{13}C relative to soils in the experimental system, and we expected the isotopic composition of microbial fatty acids to shift to more depleted values as the added carbon was incorporated into microbial biomass. The ^{13}C-depleted signature was incorporated into microbial fatty acids, although the shifts were generally larger in the ambient compared with the elevated CO_2 treated plots (Fig. 2*C*). Correcting for the fact that soil microbes at elevated CO_2 already received organic matter that is depleted in ^{13}C in the field, an isotope mixing model showed that microbes in elevated CO_2 soils used more carbon from soil organic matter than those in ambient CO_2 soils (Table 2). Thus, elevated CO_2 both accelerated the oxidation of soil organic carbon to CO_2 by soil microorganisms (Fig. 1*B* and SI Table 3) and increased their use of this carbon source as a substrate for biomass production (Table 2).

The elevated CO_2-induced priming effect and decline in long-term soil carbon storage can theoretically occur in any terrestrial ecosystem. However, such effects will be difficult to detect in many ecosystems on short time scales because of the large size and long mean residence times of soil carbon pools (30). In our study system, we were able to detect changes in soil carbon over a relatively short time period because the carbon pool is small and turns over relatively quickly. The most active soil carbon fraction (i.e., the lowest density fraction) had a mean residence time of 13 years and constituted 75% of the total soil carbon pool (Table 1). Moreover, the soil carbon in the highest density fractions, which are usually considered to be "slow" or "passive" (i.e., turn over on decadal or millennial timescales, respectively), have mean residence times of <25 years (Table 1). This compares to mean residence times for bulk soils (i.e., light and heavy fractions combined) that range from 20 to 1,000 years for forests (31). The quick turnover times at our site are consistent with the poorly developed soil structure and the low silt and clay mineral content of the sandy soils at our site (32). The similar turnover times among all density fractions suggest that these heavier pools may also be sensitive to priming-induced losses of soil carbon (Table 1). In fact, there was a trend for losses in the heavier fractions, although the changes after 4 years were not statistically significant. These results are not consistent with the expectation that elevated CO_2 will increase soil carbon in this system, even after prolonged exposure.

The phenomenon we observed may be difficult to detect in well developed soils with high silt and clay concentrations, yet the underlying mechanisms we document may be general. For example, soil microbial shifts and associated soil priming could help explain why increases in soil carbon content in response to elevated CO_2 are small or absent even when plant biomass has increased substantially; this has been observed in a variety of temperate grasslands and forests, as well as agricultural and small scale experimental systems (3, 4, 7). This mechanism may be particularly pertinent to ecosystems in which elevated CO_2 preferentially promotes fungi (25–27). Overall, our findings indicate that microbial community responses to elevated CO_2 will constrain the potential for net gains

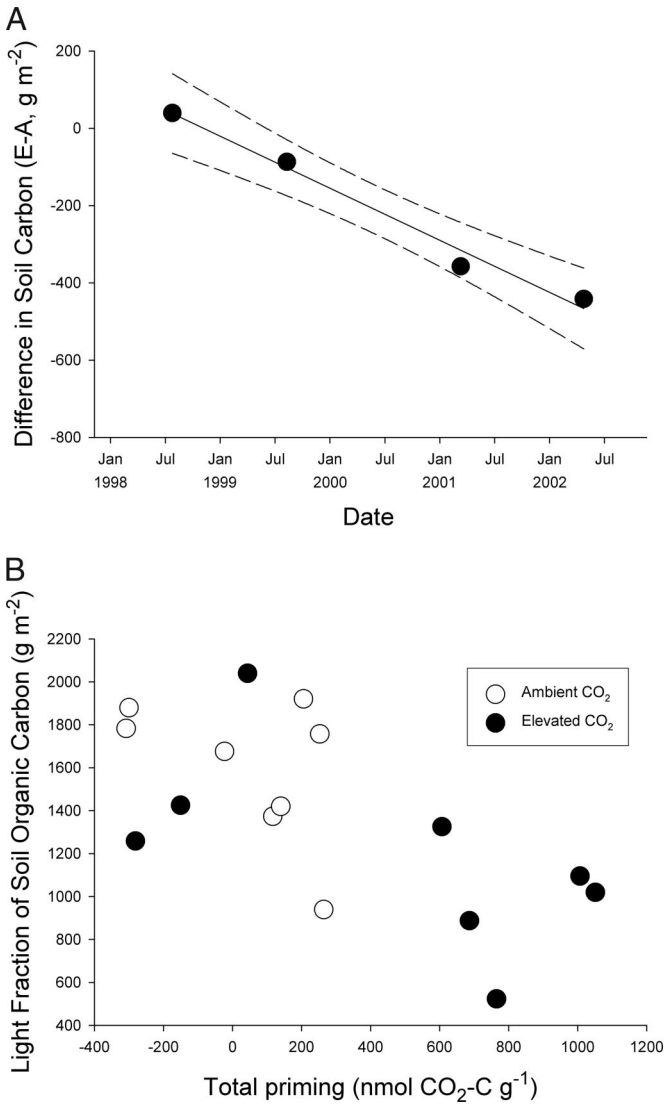

Fig. 1. Declines in soil carbon content over time are driven by the acceleration of soil organic matter decomposition. (*A*) The difference between mean soil carbon content at elevated and ambient CO_2 over time. The dashed lines show upper and lower 95% confidence intervals of the regression line. (*B*) The relationship between the priming observed during the laboratory experiment and light fraction soil C content in April 2002. Here priming is defined as $(C_l - C_c)$, where C_l is the amount of CO_2 generated from soil organic matter decomposition in soils to which litter was added and C_c is the amount of CO_2 generated from soil organic matter decomposition in control soils. The light fraction is the largest and most rapidly cycling soil carbon pool. We also found a negative relationship between priming in the laboratory and total soil C content ($r^2 = 0.28$, $P = 0.034$).

in soil carbon storage by enhancing the decomposition of soil carbon. This response suggests a limited capacity of Earth's ecosystems to stabilize atmospheric CO_2 and slow global warming.

Materials and Methods

Soil Carbon Analyses. We measured the organic matter content of soils by collecting cores from each of the experimental plots over time. In June, July, September, and December 1998, September 1999, and April 2001, we collected three cores at random locations within each plot using a 1.9-cm-diameter punch auger. In May 2002 we collected five cores from each plot using a larger, 7-cm-diameter corer. Samples from 1998 and 1999 were collected from the top 0–15 cm of soil, in 2001 from the top 0–9 cm of soil, and in May 2002 from the top 0–10 cm of soil. In all cases, cores were taken from the top of the A horizon after clearing away the organic horizon from the coring location. We focused on the A horizon because we were interested in tracking the fate of soil carbon that predated the beginning of the experiment; because the sites were burned, no O horizon existed at the start of the experiment. In May 2002 samples were also taken from 10 to 60 cm, which contained ≈42% of the total soil carbon in the top 60 cm of the soil profile. All soil samples were passed through a 1-mm sieve to remove roots and coarse fragments and composited into a single sample for each plot. Subsamples (30 g) of dried soil were subjected to sequential density fractionations. Soil samples were suspended in a solution of sodium polytungstate adjusted to a density of 1.5 $g{\cdot}cm^{-3}$. Samples were agitated and then allowed to settle for 24–48 h. The supernatant containing material <1.5 $g{\cdot}cm^{-3}$ was then aspirated onto a glass filter, rinsed, oven-dried (105°C), weighed, ground to a fine powder, and analyzed for % N, % C, $\delta^{15}N$, and $\delta^{13}C$ using isotope-ratio mass spectrometry at the Colorado Plateau Stable Isotope Laboratory of Northern Arizona University. The procedure was then repeated with the remaining material with a solution of sodium polytungstate adjusted to 1.8 $g{\cdot}cm^{-3}$, and then a third time with a solution at 2.2 $g{\cdot}cm^{-3}$. As the density of soil increases along this continuum, it is generally assumed that carbon turnover increases from minutes to years, to decades, to millennia (33). Total carbon content was determined by summing over density fractions.

Changes in soil carbon content were calculated as the difference in mean soil carbon mass to a depth of 9–15 cm depending on sample year. A best-fit linear regression and confidence intervals for mean differences versus time were calculated with Sigmaplot V9 (Systat, San Jose, CA). To further explore the role of between-chamber variability on the calculated loss of soil organic matter in the elevated CO_2 treatment, we used bootstrapping to estimate the slope of the relationship between the absolute effect of CO_2 versus time. We used the program Resampling Stats V.5.0 (Resampling Stats, Arlington, VA) to generate eight random estimates of the absolute CO_2 effect (random samples of E-A, with replacement) for each year (1998, 1999, 2001, and 2002), thereby simulating the actual experiment where $n = 8$ per treatment. With each trial, the absolute effect of elevated CO_2 was regressed against year and the slope recorded; confidence intervals around each slope were then estimated by using the 1,000 trials. The mean slope was -118 $g{\cdot}m^{-2}{\cdot}yr^{-1}$ with a 95% confidence interval of -200 to -44 $g{\cdot}m^{-2}{\cdot}yr^{-1}$.

Turnover time of soil carbon was calculated from the incorporation of ^{13}C-depleted plant biomass into soil organic matter density fractions from 1998 to 2002 (depths ≤15 cm) by using a two end-member mixing model. The $\delta^{13}C$ of pretreatment soil organic matter was the mean $\delta^{13}C$ for each density fraction in the ambient treatment. The change in the $\delta^{13}C$ of plant biomass inputs was calculated as the difference in the mean ambient treatment $\delta^{13}C$ and the $\delta^{13}C$ of individual elevated CO_2 replicates ($n = 8$). For the $\delta^{13}C$ of plant biomass we used the isotopic ratio of wood harvested in 2003, which provided an integrated estimate of ^{13}C inputs to soil since the study began in 1998. Turnover time was calculated as the inverse of the average annual incorporation of ^{13}C.

Overall Carbon Budget. Differences between treatments in total aboveground wood biomass were based on a census of stem diameters that were converted to biomass by using species-specific allometric equations and data from the 2002 growing season (B.G.D., unpublished data). For belowground biomass we used data from Stover *et al.* (20), who reported course root biomass (>5 mm in diameter) for the year 2005. For an estimate of 2002 coarse root biomass, we first calculated the ratio of aboveground:belowground biomass for 2005 and assumed that this ratio was constant across years. We then used this ratio and aboveground biomass for 2002 from Stover *et al.* (20) to calculate coarse root biomass for 2002. Biomass was converted to approximate units of C by using unpublished % C data for course roots and wood biomass.

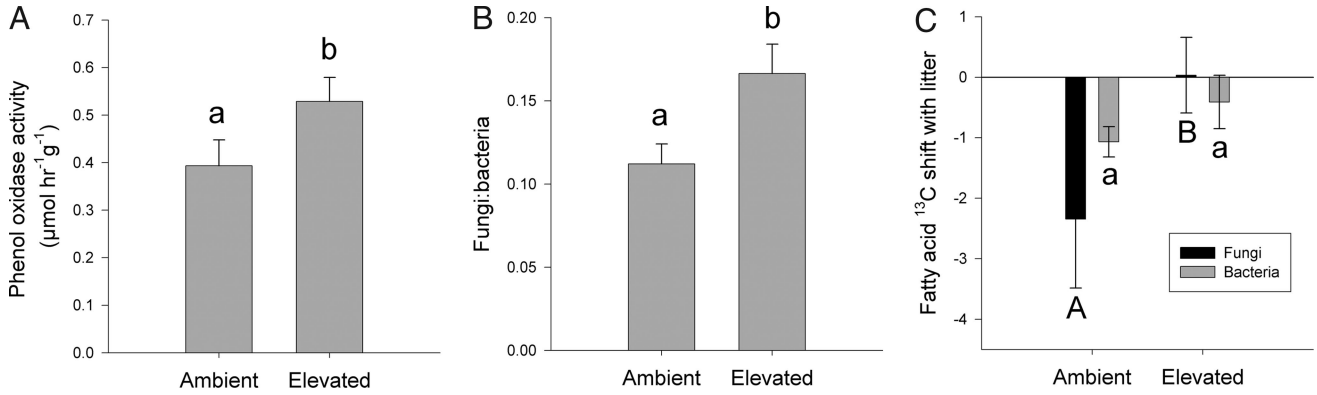

Fig. 2. Soil microbial activity and composition are altered by elevated CO_2. (*A*) Phenol oxidase activity (μmol·h^{-1}·g^{-1}) before the beginning of the decomposition experiment ($\pm$1 SE). (*B*) Fungi:bacteria values derived from analyses of phospholipids fatty acid contents of soil microbial communities ($\pm$1 SE). During analysis, samples from two elevated CO_2 chambers were lost; therefore, for the elevated CO_2 treatment $n = 6$, and for ambient CO_2 $n = 8$. (*C*) The shift in the isotopic signatures of fungal and bacterial fatty acids in soils to which depleted ^{13}C litter was added ($\pm$1 SE); this is expressed relative to the fatty acid signatures of control soils. Uppercase letters denote differences across treatments in fungal fatty acid shifts, and lowercase letters are used for comparisons of bacterial fatty acid shifts at $P < 0.10$.

Soil Sampling and Processing for Decomposition Experiment. In July 2004 we took three 5-cm-diameter cores to 10-cm depth, including both organic and A layers of soil, from each of the ambient and elevated chambers ($n = 8$). We included the organic layer to ensure that we were capturing the most microbially active parts of the soil horizon. We composited cores from each chamber and sieved the resulting soil through 1-mm mesh. We transported soils on ice to the Smithsonian Environmental Research Center, where we conducted soil nitrogen (N) analyses within 3 days of soil sampling and froze subsamples of soil for microbial community analysis at a later date. Subsamples of the remaining soil were air-dried for soil pH, dried at 105°C for soil moisture content, or used fresh in the decomposition or enzyme assays described below.

Litter Decomposition Experiment. We measured litter decomposition in soils from elevated and ambient CO_2 sites using laboratory mesocosms (120-ml airtight jars with septa for gas sampling) that were kept at constant soil moisture (11%) and temperature (25°C) to control for as many factors as possible other than microbial community differences. After conditioning each soil for 10 days at 11% moisture and 25°C, we added leaf litter that was collected from the O horizon in the elevated CO_2-treated plots. The litter carried a depleted ^{13}C signature ($\delta^{13}C = -39‰$), reflecting the signature of the supplemental CO_2 added to this treatment.

For each elevated and ambient soil there were two treatments: a litter treatment and a control, each of which was run in duplicate (i.e., there were four jars total per each field chamber). In the litter treatment, 133 mg of ground leaf litter was added to the soil and mixed; the soil in the control treatment was simply mixed without the addition of litter. We sampled the headspace of the jars at days 5, 15, 19, and 60 to determine both its CO_2 concentration its CO_2-C isotopic composition. For the former we analyzed gas samples immediately using a LI-7000 (LI-COR Biosciences, Lincoln, NE) with an N_2 carrier gas and a sample injection loop. For the latter we stored and shipped samples in 15-ml Hungate tubes that were preflushed with nitrogen gas and evacuated; CO_2 samples were analyzed for ^{13}C composition at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University with a Delta Plus Advantage gas isotope-ratio mass spectrometer (Thermo Electron, Bremen, Germany). After sampling at each time point, soil moisture for each soil was adjusted and each jar was aired out and reset to outdoor ambient CO_2 concentrations. It was then resealed and returned to the constant temperature chamber until the next sampling.

For each jar we calculated how much CO_2 originated from litter versus soil organic matter using a two-member isotope mixing model. One end member corresponded to the $\delta^{13}C$ signature of the CO_2 respired from soils without litter added. Because the high CO_2 plots had a legacy of exposure to ^{13}C-depleted CO_2, this end member had a lower $\delta^{13}C$ value for the high CO_2 treatment. The other end member was the $\delta^{13}C$ of the litter itself ($-39.08‰$). The equation for the calculation was $C_{lit} = C_t(\delta_t - \delta_{som})/(\delta_{lit} - \delta_{som})$, where C_{lit} is the amount of CO_2 derived from litter decomposition, C_t is the total amount of CO_2 respired over the incubation period, δ_t is the $\delta^{13}C$ of the respired CO_2-C, δ_{som} is the isotopic signature of the CO_2-C respired from soil alone, and δ_{lit} is the $\delta^{13}C$ of the litter. In this article priming is defined as $(C_l - C_c)$, where C_l is the amount of CO_2 generated from soil organic matter decomposition in soils to which litter was added and C_c is the amount of CO_2 generated from soil organic matter decomposition in control soils.

Our interpretation of the ^{13}C data may be influenced by the fact that the soils in elevated CO_2 chambers had already incorporated depleted ^{13}C organic matter over the course of the field experiment (Table 1 and SI Table 3). If litter addition caused microbial communities from elevated CO_2 chambers to preferentially use the depleted ^{13}C portion of soil organic matter, such a shift would be interpreted as litter decomposition. In such a case our calculations of microbial use of soil organic matter after litter addition would be underestimates, and our conclusion that elevated CO_2 stimulated the priming response would be conservative.

Table 2. Estimates of the reliance (%) of soil microbes on added litter as opposed to soil organic matter

Microbial group	Ambient CO_2*	Elevated CO_2†	*P* value‡
Overall community	11.8 ± 3.8	−4.9 ± 5.9§	0.019
Fungi	22.8 ± 11.8	0.6 ± 11.7	0.175
Bacteria	10.4 ± 2.6	−5.1 ± 6.0§	0.023

*Values presented are treatment means ± SE.
†These data are corrected for the fact that soil microbes in elevated CO_2 sites had already been exposed to depleted ^{13}C inputs in the field before the incubation (see *Materials and Methods*).
‡From one-way ANOVA for comparisons across CO_2 treatments.
§These values are not significantly different from zero ($P \geq 0.10$).

Soil Enzyme Activities. Immediately before running the litter decomposition experiment we analyzed the activities of two soil enzymes known to be important to carbon cycling in soil (21) in each of the soil mesocosms: β-glucosidase and phenol oxidase. The substrates for each were *p*NP-β-glucopyranoside and pyrogallol (5

mM and 50 mM, respectively). Two grams of soil was added to 60 mM acetate buffer solution (pH 5.0), and the mixture was blended for 1 min. A total of 0.750 ml of the soil homogenate was then mixed with 0.750 ml of substrate in a 2-ml Eppendorf tube. Tubes for β-glucosidase were placed on a shaker in the dark for 1 h, and those for phenol oxidase were incubated for 2 h. After incubation, the tubes were spun at 8,000 $\times$ *g*. For phenol oxidase the absorbance of the supernatant at 460 nm was measured immediately on a spectrophotometer (Hitachi U-2000). For β-glucosidase 0.075 ml of 1 M NaOH was added to the supernatant to develop its color. The solution was diluted with 1 ml of water, and its absorbance was measured at 410 nm. There were three analytical replicates and two controls for soil in each enzyme assay.

Phospholipid Fatty Acid (PLFA) Analyses. We used PLFA composition to determine microbial community composition. We extracted 4 g of lyophilized soil using a modified Bligh and Dyer extraction (34) and identified and quantified individual fatty acids using gas chromatography and mass spectrometry. We compared microbial community composition with the mole percent of the fatty acids identified in each soil, all of which were present in all soils. Extracted fatty acid methyl esters were quantified at the University of Michigan by using a Delta Plus mass spectrometer (Thermo Electron) with a GC/C III interface coupled to an HP 5973 GC (Agilent Technologies, Palo Alto, CA). Peaks were quantified by using an internal 19:0 standard, and the identities of peaks were determined with a standard qualitative mix of known bacterial fatty acid methyl esters. Fatty acids were expressed in nmol·g^{-1} dry soil.

Total microbial biomass was estimated as the sum of the nanomoles of each of the fatty acid groups present in a given soil. We determined the ratio of bacterial:fungal biomass using the ratio of the relative abundances of the marker fatty acids; i.e., (i15:0 + a15:0 + i16:0 + i15:0 2OH + 16:1 ω7c + 16:1 ω9c + 16:1 ω5c + 10Me 16:0 + i17:0 + a17:0 + cy17:0 + 17:0 + 18:1 ω7c + 18:1 ω7t + 18:1 ω5c + 10Me 18:0; all bacterial markers)/(18:2 ω6; fungal biomarker) (19, 35).

PLFA Isotopes and Mixing Model. Leaf litter for the incubations was collected from the O horizon in the elevated CO_2-treated plots. This litter carried a depleted ^{13}C signature, reflecting the signature of the supplemental CO_2 added to this treatment. We used the ^{13}C composition of PLFAs to quantify microbial utilization of soil organic matter and litter as carbon sources. Strong reliance on litter carbon was reflected in relatively large decreases in $\delta^{13}C$ values during the incubation with the ^{13}C-depleted litter, whereas reliance on soil organic carbon was reflected by $\delta^{13}C$ values that stayed relatively constant. For each field plot we calculated the shift in $\delta^{13}C$ composition of fungal and bacterial PLFAs as the difference between $\delta^{13}C$ in the presence and absence of litter after incubation.

For the mixing model, the soil organic matter end members were determined as the ^{13}C composition of the PLFAs in the soils to which no litter had been added. For the labeled litter end member we needed to account for the fact that soil microbes in elevated CO_2 sites have already been exposed to depleted ^{13}C inputs in the field; we therefore had different end members for ambient and elevated soils. For the ambient soils the end member for the labeled litter was simply the difference between the ^{13}C isotopic signature of litter from elevated and ambient CO_2 plots (10.3‰). For the elevated CO_2 soils it was the difference between this 10.3‰ and the shift in the microbial isotope signature that had already occurred during the experiment because of depleted carbon inputs; the end member for these soils was 5.7‰. Proportional reliance on litter carbon was calculated as the $\delta^{13}C$ shift after litter addition divided by the difference between soil organic matter and litter end members. Our mixing overestimates the proportional contribution of litter in the elevated CO_2 treatment from comparable $\delta^{13}C$ shifts in PLFAs of ambient and elevated plots. Therefore, this approach is conservative for detecting the priming phenomenon at elevated CO_2. This approach was applied to PLFAs representing bacteria and fungi separately and to the microbial community as a whole by using weighted averages and observed relative abundances of fungal and bacterial PLFAs (Table 2).

Soil Characteristics. We measured extractable NH_4-N and NO_3-N, moisture, pH, C:N, and % C in each soil composite. For extractable nitrogen, 10-g subsamples of field-moist soil were extracted with 100 ml of 2 N KCl. After shaking the slurry and letting it set for 24 h, the extract was passed through Whatman no. 1 filters, and the filtrate was analyzed for NH_4-N and NO_3-N colorimetrically by the Soil, Water, and Forage Analytical Laboratory of Oklahoma State University (Stillwater, OK). Soil gravimetric moisture content was determined by oven-drying 15-g subsamples of field-moist soil at 105°C for 48 h. Soil pH was measured on air-dried soil in deionized water by using a 1:2 (wt/vol) soil:liquid ratio (Accumet Dual Channel pH/Ion/Conductivity Meter; Thermo Fisher Scientific, Waltham, MA). Soil nutrient concentrations were expressed on an oven-dry basis and analyzed by using JMP 4.0 (SAS Institute, Cary, NC).

Statistical Analyses. The statistical approach for analyzing soil carbon content trends (Fig. 1*A*) is described in *Soil Carbon Analyses*. To test for differences between the CO_2 treatments, we used one-way ANOVA. Because of a significant interaction between elevated CO_2 and SOM density fraction, we tested for differences in these factors with separate one-way ANOVAs. We used the Tukey–Kramer honestly significant difference post hoc range test when necessary. In all cases, we considered differences significant at $P < 0.10$.

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