Altered soil microbial community at elevated CO₂ leads to loss of soil carbon

Karen M. Carney†, Bruce A. Hungate‡, Bert G. Drake*, and J. Patrick Megonigal**

*Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037; and ‡Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011

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Increased carbon storage in ecosystems due to elevated CO₂ may help stabilize atmospheric CO₂ concentrations and slow global warming. Many field studies have found that elevated CO₂ 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 CO₂ doubling reduced soil carbon in a scrub-oak ecosystem despite higher plant growth, offsetting ≈52% of the additional carbon that had accumulated at elevated CO₂ 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 CO₂ 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 CO₂. The isotopic composition of microbial fatty acids confirmed that elevated CO₂ increased microbial utilization of soil organic matter. These results show how elevated CO₂, by altering soil microbial communities, can cause a potential carbon sink to become a carbon source.

H igher carbon storage due to elevated CO₂ may help stabilize atmospheric CO₂ concentrations (1). Many field studies have found that elevated CO₂ 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 CO₂, and this response is relatively well understood at a mechanistic level (4). Less well understood is the possibility that elevated CO₂ 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 CO₂ may increase soil organic matter decomposition (7–9), and none have directly addressed position and activity. Soils exposed to elevated CO₂ had higher decline in soil carbon was driven by changes in soil microbial composition and activity or community composition (8). Despite intensive interest in the fate of carbon with rising CO₂, 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 CO₂.

To examine the influence of elevated CO₂ 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 CO₂ (13, 14). At this site, elevated CO₂ has caused very minor effects on leaf chemistry with no discernable effect on litter decomposition (15–17), similar to most other CO₂ 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 CO₂ 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 CO₂. The leaf litter was collected from the elevated CO₂ chambers of the experiment and thus was far more 13C-depleted (δ13C = −39‰) than either ambient or elevated soils (δ13C ≥ −30‰) [Table 1 and supporting information (SI) Table 3]. This large difference between litter and soil allowed us to determine, for both CO₂ treatments, how much of the CO₂ 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 CO₂ led to persistent losses of soil carbon content over a 4-year period ($r^2 = 0.98, P = 0.009$) (Fig. 1A 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 CO₂ 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.

†To whom correspondence should be addressed. E-mail: megonigalp@si.edu.

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However, soils from elevated CO2 sites had higher phenol oxidase activity were correlated (in part through their production of lignolytic enzymes, such as lignin, which accumulate in soil organic matter (23). Therefore, increased fungal abundance not only promoted lignolytic enzyme activities but also improved the degradation of highly recalcitrant organic materials, such as lignin. The ratio of fungi to bacteria was higher in soils from elevated CO2 sites (P < 0.01) compared to those from ambient CO2 sites (P > 0.05) (SI Table 3). This finding suggests that microbial priming can explain the loss of soil organic carbon 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 CO2 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 CO2 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 CO2 preferentially promotes fungi (25–27).

Overall, our findings indicate that microbial community responses to elevated CO2 will constrain the potential for net gains
in soil carbon storage by enhancing the decomposition of soil carbon. This response suggests a limited capacity of Earth’s ecosystems to stabilize atmospheric CO2 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. All 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 predates 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 fractions. Soil samples were suspended in a solution of sodium polytungstate adjusted to a density of 1.5 g cm−3. Samples were agitated and then allowed to settle for 24–48 h. The supernatant containing material <1.5 g 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, δ15N, and δ13C 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 cm−3, and then a third time with a solution at 2.2 g 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 CO2 treatment, we used bootstrapping to estimate the slope of the relationship between the absolute effect of CO2 versus time. We used the program Resampling Stats V.5.0 (Resampling Stats, Arlington, VA) to generate eight random estimates of the absolute CO2 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 CO2 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 m−2 yr−1 with a 95% confidence interval of −200 to −44 g m−2 yr−1.

Turnover time of soil carbon was calculated from the incorporation of 13C-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 δ13C of pretreatment soil organic matter was the mean δ13C for each density fraction in the ambient treatment. The change in the δ13C of plant biomass inputs was calculated as the difference in the mean ambient treatment δ13C and the δ13C of individual elevated CO2 replicates (n = 8). For the δ13C of plant biomass we used the isotopic ratio of wood harvested in 2003, which provided an integrated estimate of 13C inputs to soil since the study began in 1998. Turnover time was calculated as the inverse of the average annual incorporation of 13C.

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.D. Stover, 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.
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 CO2 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 CO2-treated plots. The litter carried a depleted 13C signature (δ13C = −39‰), reflecting the signature of the supplemental CO2 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 CO2 concentration its CO2-C isotopic composition. For the former we analyzed gas samples immediately using a LI-7000 (LI-COR Biosciences, Lincoln, NE) with an N2 carrier gas and a sample injection loop. For the latter we stored and shipped samples in 15-ml Hungate tubes that were prefilled with nitrogen gas and evacuated; CO2 samples were analyzed for 13C 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 CO2 concentrations. It was then rescaled and returned to the constant temperature chamber until the next sampling.

For each jar we calculated how much CO2 originated from litter versus soil organic matter using a two-member isotope mixing model. One end member corresponded to the δ13C signature of the CO2 respired from soils without litter added. Because the high CO2 plots had a legacy of exposure to 13C-depleted CO2, this end member had a lower δ13C value for the high CO2 treatment. The other end member was the δ13C of the litter itself (−39.08‰). The equation for the calculation was

\[ C_{\text{lit}} = C_{13C}(\delta_{13C} - \delta_{\text{soil}})/(\delta_{13C} - \delta_{\text{soil}}) \]

where \( C_{\text{lit}} \) is the amount of CO2 derived from litter decomposition, \( C_{13C} \) is the total amount of CO2 respired over the incubation period, \( \delta_{13C} \) is the δ13C of the respired CO2-C, \( \delta_{\text{soil}} \) is the isotopic signature of the CO2-C respired from soil alone, and \( \delta_{\text{soil}} \) is the δ13C of the litter. In this article priming is defined as \( (C_1 - C_2) \), where \( C_1 \) is the amount of CO2 generated from soil organic matter decomposition in soils to which litter was added and \( C_2 \) is the amount of CO2 generated from soil organic matter decomposition in control soils.

Our interpretation of the δ13C data may be influenced by the fact that the soils in elevated CO2 chambers had already incorporated depleted 13C organic matter over the course of the field experiment (Table 1 and SI Table 3). If litter addition caused microbial communities from elevated CO2 chambers to preferentially use the depleted 13C portion of soil organic matter, such a shift would be interpreted as litter decomposition. In such a case our calculation of microbial use of soil organic matter after litter addition would be underestimated, and our conclusion that elevated CO2 stimulated the priming response would be conservative.

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 pNP-β-glucopyranoside and pyrogallol (5
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., ([i5:0 + a15:0] + i16:0 + i15:0 2OH + 16:1 o7c + 16:1 o9c + 16:1 o10c + 10Me 16:0 + i17:0 + a17:0 + cy17:0 + 17:0 + 18:1 o7c + 18:1 o7t + 18:1 o5c + 10Me 18:0; all bacterial markers)/ (18:2 o6; fungal biomarker) (19, 35).

PLFA Isotopes and Mixing Model. Leaf litter for the incubations was collected from the O horizon in the elevated CO₂-treated plots. This litter carried a depleted 13C signature, reflecting the strong reliance on litter carbon was reflected in relatively large decreases in 13C values during the incubation with the 13C-depleted litter, whereas reliance on soil organic carbon was reflected by 13C values that stayed relatively constant. For each field plot we calculated the shift in 13C composition of fungal and bacterial PLFAs as the difference between 13C in the presence and absence of litter after incubation.

For the mixing model, the soil organic matter end members were determined as the 13C 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 CO₂ sites have already been exposed to depleted 13C 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 13C isotopic signature of litter from elevated and ambient CO₂ plots (10.3‰). For the elevated CO₂ soils it was the difference between this 10.3‰ and the shift in the microbial isotope signature that had already occurred during the experimental because of depleted carbon inputs; the end member for these soils was 5.7‰. Proportional reliance on litter carbon was calculated as the 13C 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 CO₂ treatment from comparable 13C shifts in PLFAs of ambient and elevated plots. Therefore, this approach is conservative for detecting the priming phenomenon at elevated CO₂. 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 NH₄-N and NO₃-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 NH₄-N and NO₃-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/Congruency 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. 1A) is described in Soil Carbon Analyses. To test for differences between the CO₂ treatments, we used one-way ANOVA. Because of a significant interaction between elevated CO₂ 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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