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The fate of carbon in grasslands under carbon dioxide enrichment

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The concentration of carbon dioxide (CO_2) in the Earth's atmosphere is rising rapidly[1], with the potential to alter many ecosystem processes. Elevated CO_2 often stimulates photosynthesis[2], creating the possibility that the terrestrial biosphere will sequester carbon in response to rising atmospheric CO_2 concentration, partly offsetting emissions from fossil-fuel combustion, cement manufacture, and deforestation[3,4]. However, the responses of intact ecosystems to elevated CO_2 concentration, particularly the below-ground responses, are not well understood. Here we present an annual budget focusing on below-ground carbon cycling for two grassland ecosystems exposed to elevated CO_2 concentrations. Three years of experimental CO_2 doubling increased ecosystem carbon uptake, but greatly increased carbon partitioning to rapidly cycling carbon pools below ground. This provides an explanation for the imbalance observed in numerous CO_2 experiments, where the carbon increment from increased photosynthesis is greater than the increments in ecosystem carbon stocks. The shift in ecosystem carbon partitioning suggests that elevated CO_2 concentration causes a greater increase in carbon cycling than in carbon storage in grasslands.

In most ecosystems, leaf and canopy gas-exchange measurements indicate that there is increased carbon uptake in response to experimental doubling of CO_2 concentration[5,6]. However, increased carbon uptake in these one- to ten-year experiments does not necessarily indicate a large potential for carbon storage over 50–100 years, the predicted timescale of atmospheric CO_2 doubling[7]. Ecosystem sequestration of carbon over 50–100 years requires delivery of the extra carbon to large pools with slow turnover (wood and soil organic matter)[8]. However, net gas-exchange measurements do not reveal whether the extra carbon fixed in response to elevated CO_2 is distributed to these pools. The partitioning of this extra carbon among pools with varying turnover times is a critical controller of the potential for terrestrial ecosystems to increase long-term carbon storage.

A consequence of CO_2-stimulated plant growth may be an increased demand for below-ground resources (water and mineral nutrients). If the demands for below-ground resources are not met by increased resource availability or efficiency of resource use[9,10], or if growth potential is constrained[11], plants may increase their loss of carbon through root turnover, respiration or exudation. Carbon allocation to these rapid-turnover pools limits the quantity of long-term carbon storage in response to elevated CO_2 because most of the carbon is quickly returned to the atmosphere as CO_2 (refs 12, 13). Many studies have documented changes in the root biomass of plants grown under elevated CO_2 (refs 14, 15), but root turnover, exudation and respiration are difficult to quantify directly for budgets of total carbon partitioning to roots[16]. We have used two indirect approaches to assess carbon partitioning to roots. First, we used a mass balance model to describe the carbon stocks, partitioning and annual carbon fluxes in two annual grasslands in California after three years of exposure to elevated CO_2. Second, we used isotope labelling of reconstructed ecosystems exposed to elevated CO_2 to measure the partitioning of below-ground carbon fluxes.

Our field experimental system consists of naturally occurring

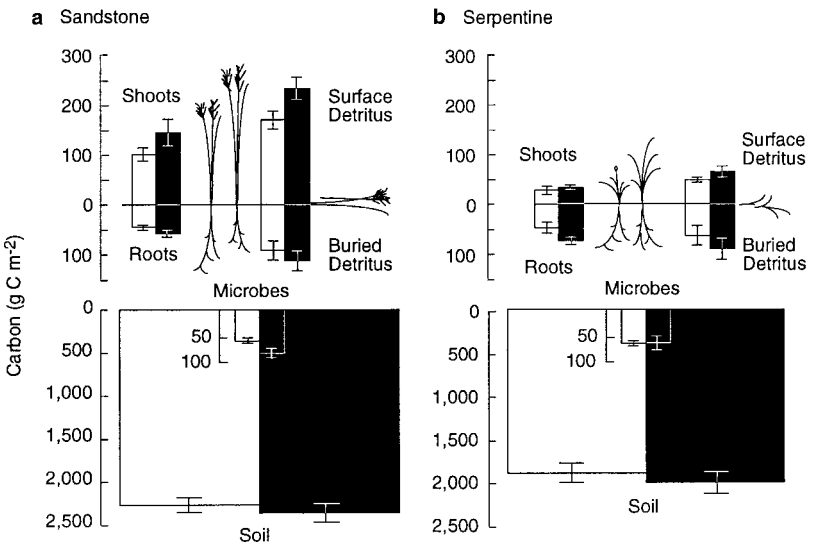

Figure 1 Carbon pools (g C m^{-2}, mean ± s.e.) at peak biomass in 1994 for sandstone (**a**) and serpentine (**b**) grasslands in ambient (open bars) and elevated (filled bars) CO_2.

annual grasslands in central coastal California, growing on serpentine- and sandstone-derived soils at the Jasper Ridge biological preserve of Stanford University, California (37° 24′ N, 122° 14′ W; elevation 150 m). The climate is Mediterranean-type, with a winter rainy season and a summer drought. The serpentine and sandstone grasslands occur adjacent to one another but differ dramatically in species composition and productivity. Introduced European annual grasses are the dominant plants on the moderately productive sandstone, whereas native forbs are dominant on the less productive serpentine grassland[17,18].

On each grassland, open-top chambers maintain ten ambient and ten elevated (ambient + 360 p.p.m.) CO_2-treated plots[18,19]. We focus on results from the third growing season (1994) after establishing the CO_2 manipulation. Live shoot, live root, surface detritus, buried detritus, soil microbial and soil carbon pools were measured by destructive harvest when plants were approaching their maximum biomass during the 1994 growing season. We determined the effect of elevated CO_2 on total ecosystem carbon uptake by comparing carbon stocks in the ambient and elevated CO_2 treatments. We combined these measurements of carbon stocks with the annual carbon flux in below-ground respiration to calculate carbon partitioning to labile fractions below ground by mass balance. We also determined rates of carbon input to soil by using the ^{13}C-depleted isotopic signature of the CO_2 added to the enriched plots. The added CO_2 is depleted in ^{13}C ($\delta^{13}C$ −35‰ versus $\delta^{13}C$ −8‰ for atmospheric CO_2) because it is derived from fossil fuel. We quantified short-term carbon partitioning in below-ground respiration in a separate experiment using a $^{13}CO_2$ pulse label in these grassland communities grown in outdoor microcosms[18]. By monitoring the rate and $\delta^{13}C$ of CO_2 released from the soil, we could distinguish CO_2 produced from root and heterotrophic sources.

In the field experiment, elevated CO_2 significantly increased carbon pools in roots, surface detritus, and soil microorganisms in the sandstone grassland, and there was a 37% increase in the total amount of carbon in these carbon pools (Fig. 1 and Table 1). Similarly, although roots were the only carbon pool (when analysed separately) to increase significantly under elevated CO_2 in the serpentine grassland (Fig. 1; one-way analysis of variance (ANOVA) $P = 0.048$), the sum of plant, detrital and microbial carbon pools increased by 25% in the serpentine grassland (Table 1). Increases in these rapidly cycling carbon pools on both grasslands (two-way ANOVA, $P = 0.002$) resulted in an increase in total ecosystem carbon (Table 1; two-way ANOVA, $P = 0.056$), even though soil carbon did not change (two-way ANOVA, $P = 0.30$)[20].

Significant changes in the large pool of total soil carbon (2,111 and 2,311 g C m^{-2} for the serpentine and sandstone grasslands, respectively) are difficult to detect over the short time course of this experiment (discussed in detail in ref. 20). As expected, the $\delta^{13}C$ of soil carbon was lower in elevated compared to ambient CO_2 (Fig. 2), owing to the incorporation in elevated CO_2 of 266 and 303 g C m^{-2} of the fossil-fuel-derived (^{13}C-depleted) carbon into the serpentine and sandstone soils (0–15 cm depth), respectively, after four years[20]. These are substantial amounts of carbon, but they represent only 13% of the soil organic carbon pool. Small increases in soil carbon can, however, lead to large increases in soil respiration if the carbon is delivered to one or more highly labile fractions in the soil[21].

Table 1 Ecosystem carbon stocks

Ecosystem	Active C Pools* (g C m^{-2})			Total C Pools (g C m^{-2})		
	Ambient	Elevated	Change (%)	Ambient	Elevated	Change (%)
Sandstone	468 ± 24†	640 ± 47	37%‡	2,658 ± 85	2,868 ± 107	7.9%
Serpentine	241 ± 32	301 ± 32	25%	2,066 ± 103	2,255 ± 107	9.1%

Data are for serpentine and sandstone grasslands under ambient and elevated atmospheric CO_2 treatments.
* The sum of plant, detrital, and microbial carbon pools.
† Values are means ± standard error (n = 9–10).
‡ The percentage increase caused by the elevated CO_2 treatment.

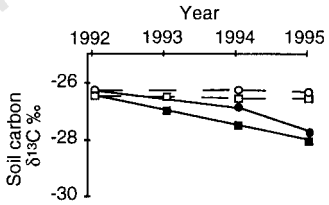

Figure 2 Soil $\delta^{13}C$ (0–15 cm) as a function of time. Soil sampling and $\delta^{13}C$ analyses are described in detail in ref. 20. Values shown are mean ± s.e. for ambient (open squares) and elevated (filled squares) CO_2 treatments on the sandstone, and ambient (open circles) and elevated (filled circles) CO_2 treatments on the serpentine grasslands. $\delta^{13}C$ declines in elevated CO_2 owing to the incorporation into soil of the ^{13}C-depleted CO_2 added to these plots. Rates of carbon flux to soil were quire similar in the two grasslands, despite the two-fold greater above-ground productivity in the sandstone, underscoring the higher relative below-ground allocation in the serpentine grassland[22].

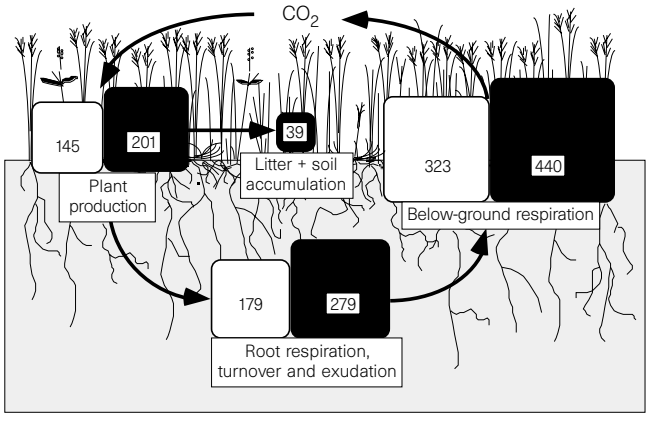

Figure 3 Annual carbon fluxes in g C m^{-2} yr^{-1} for 1994 in the sandstone grassland in ambient (white boxes) and elevated (black boxes) CO_2. Spot measurements in the field experiment and measurements over a 7-month period in the microcosm experiments show that the CO_2 stimulation of below-ground respiration in the serpentine is similar to that observed in the sandstone grassland[22] (measurements in the field were too infrequent to calculate the annual flux in the serpentine).

The small stature and annual turnover of plants in these grasslands make it possible to calculate carbon allocation to labile fractions below ground by mass balance. Annual litter input to soil, excluding root exudation and turnover, can be determined as above-ground plant production plus standing root mass. Below-ground respiration includes total carbon lost through decomposition of soil organic matter and root respiration. We measured above-ground plant production, peak-season root mass, and the annual loss of carbon through below-ground respiration (measured repeatedly throughout the year in the sandstone grassland and integrated to an annual flux, as described in ref. 22). From these measurements we calculated the annual carbon input by roots, which is the sum of root respiration, intra-annual root turnover, and root exudation: $RR + T + E = BR + C_{acc} - (ANPP + RB)$, where RR, T and E are root respiration, turnover and exudation, BR is carbon loss through below-ground respiration, C_{acc} is net carbon accumulation in detritus and soil, ANPP is above-ground plant production, and RB is peak-season root mass[23]. By measuring RB, we can distinguish carbon allocation to standing root mass from allocation to root respiration, turnover and exudation. We assessed changes in C_{acc} caused by elevated CO_2 as the difference between the two treatments (paired by block) in litter and soil pools under ambient and elevated CO_2, thereby avoiding the equilibrium assumption in the model of ref. 23.

Elevated CO_2 increased below-ground respiration in the sandstone and serpentine grasslands (Figs 3, 4)[22]. By the mass-balance calculation, elevated CO_2 increased root respiration, turnover and exudation by 56% in the sandstone grassland (Fig. 3), substantially more than the 25% increase in root biomass. Independent measurements using minirhizotron imaging and in-growth cores in the sandstone grassland show an increase of 0–15% in root turnover in response to elevated CO_2 (ref. 24), considerably less than the 56% increase in the sum of root respiration, turnover and exudation shown here, suggesting that root respiration and exudation increase disproportionately. The isotope experiment supported this idea, showing increased root respiration (Fig. 4a; one-way ANOVA, $P < 0.001$) and heterotrophic respiration (Fig. 4b; two-way ANOVA, $P = 0.004$), the latter resulting primarily from increased oxidation of carbon derived from roots (Fig. 4c; two-way ANOVA, $P = 0.038$). This was especially pronounced under nutrient enrichment, which increased above-ground productivity in these microcosms to levels typical of the sandstone grassland[25].

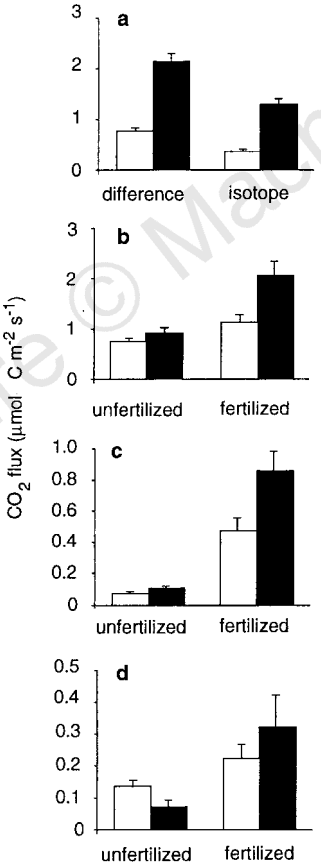

Figure 4 Partitioning below-ground respiration using the ^{13}C isotope tracer in the microcosm experiment. Values are mean + s.e. for ambient (open bars) and elevated (filled bars) CO_2 ($n = 6$–7). Note the changes in scale for the different graphs. **a**, Root respiration rates for the unfertilized treatment only, determined either by difference or by isotope. **b**, Heterotrophic (soil microbial) respiration rates determined by laboratory incubations of root-free soil. **c**, The heterotrophic respiratory flux associated with the decomposition of rhizodeposited substrates. **d**, The heterotrophic respiratory flux associated with decomposition of above- and below-ground litter produced during the previous growing season.

These findings indicate that elevated CO_2 enhances carbon partitioning to roots, a prediction that has not received wide support by studies measuring standing root biomass[14–16]. Our results indicate that this shift in partitioning manifests primarily as increased root exudation and respiration, and is thus extremely difficult to detect, providing a possible explanation for the difficulty in accounting for all the additional carbon fixed under elevated CO_2 (refs 6, 26). All of the carbon allocated to root respiration is immediately returned to the atmosphere as CO_2. The carbon allocated to exudation is distributed between microbial respiration and labile soil carbon[27]. Carbon additions to labile pools in the soil can drive substantial but difficult to measure sequestration of carbon in the short term[21]. The small size and high turnover of the labile pools, however, prevents them from providing quantitatively important long-term carbon storage. Only a small portion of the labile carbon added to soil through exudation can become stabilized in soil organic matter through interactions with clays; exudates will not remain in soil owing to their chemical nature, in contrast to other more recalcitrant plant carbon constituents, such as lignin and cellulose, which may persist in soil for many years[27,28]. Thus, compared with similar carbon additions through increased plant litter production, increased root exudation may cause relatively small increases in the carbon content of soil.

Our measurements are consistent with modelling studies that predict increased carbon uptake by grassland vegetation in response to elevated CO_2 (ref. 29), and with experimental results from tallgrass prairie where elevated CO_2 increased carbon uptake over a 34-day period[30]. Our data provide further experimental support for increased grassland carbon uptake in response to elevated CO_2. Yet the distribution of the extra carbon, particularly the increase in carbon allocation to labile pools below ground, suggests that the net carbon balance obtained in short-term CO_2-enrichment experiments tend to overestimate the potential for grasslands to sequester carbon in soils in the long term. □

Methods

Field experiment. Circular open-top chambers (0.65 m in diameter and 1 m tall) were established in each grassland in January 1992. A blower forces ambient air (either unsupplemented or with additional CO_2) into the lower portion of each chamber and out of the open top at a rate of 0.08 m^3 s^{-1}, maintaining CO_2 concentrations of approximately 720 p.p.m. in the elevated treatment (for more details see refs 18, 19). Live shoot and surface litter mass were determined by harvesting a circular area (10-cm diameter) from each plot as described[18] on 5 April 1994 in the serpentine and 4 May 1994 in the sandstone grassland. At the same time, 5-cm diameter by 15-cm deep cores were removed from each plot. Roots and detritus were removed from a subsample of the core by sieving (0.5 mm) followed by hand separation under a dissecting microscope; this cleaned soil was used to assess total soil and soil microbial carbon content. Roots and buried detritus (dead plant fragments, identified by colour) were recovered by washing the remainder of

the soil core. Root, detrital and soil carbon content were determined by combustion/gas chromatography (Europea Scientific); combustion/gas chromatographic determinations of shoot carbon content values from the peak biomass harvest in 1992 were used to calculate shoot carbon pools. Microbial carbon content was determined by chloroform fumigation.

Microcosm experiment. Seeds of plants representative of the serpentine and sandstone grassland communities were planted in 0.4-m diameter tubes with a 0.95-m deep column of serpentine soil in September–October 1992 and grown in open-top enclosures supplied with ambient (360 p.p.m.) or elevated (720 p.p.m.) CO_2. By the second growing season, all were similar in plant species composition and ecosystem properties, and so are combined for presentation and analysis. Nitrogen, phosphorus and potassium ($20\,g\,m^{-2}$) were supplied once each season as a slow-release fertilizer to half of the tubes in a factorial design. In mid-March 1993, a subset of the tubes was pulse labelled by closing the chambers and supplying 1 litre of 99% $^{13}CO_2$ to the chamber atmosphere during a 5-h period (8:00 to 13:00). At the height of flowering the following year (18–20 April 1994), we determined the $\delta^{13}C$ of soil CO_2 ($^{13}CO_2$ BR) collected from perforated stainless steel tubes inserted into the soil (0–15 cm depth) and the amount and $\delta^{13}C$ of CO_2 produced by soil heterotrophs during 96-h laboratory incubations of root-free soil ($^{13}CO_2$ HR). We assumed that root $\delta^{13}C$ was the same as the $\delta^{13}C$ of CO_2 produced by root respiration ($^{13}CO_2$ RR) and calculated the proportional contributions from roots (y) and soil heterotrophs ($1-y$) to below-ground respiration: $^{13}CO_2BR = y(^{13}CO_2RR) + (1-y)(^{13}CO_2HR)$. We also calculated root respiration by difference, as below-ground respiration rates[22] minus CO_2 production rates in the soil incubations. The ^{13}C signal associated with the CO_2 enrichment of the atmosphere and the pulse label allowed us further to partition the heterotrophic respiration flux into three components. The soil organic carbon (SOC) contribution to heterotrophic respiration (HR) was calculated using the elevated CO_2 treatment with no pulse label, where x is the proportional contribution of SOC to HR: $^{13}CO_2HR = x(^{13}C\ SOC) + (1-x)(^{13}C\ plant)$. Rhizodeposition and previous year's litter contributions to HR were calculated using ambient and elevated CO_2 treatments that had been pulse labelled, where y is the proportional contribution of rhizodeposition to HR and x is from above: $^{13}CO_2HR = x(^{13}C\ SOC) + y(^{13}C\ rhizodeposition) + (1-x-y)\ (^{13}C\ litter)$.

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Net transfer of carbon between ectomycorrhizal tree species in the field

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Different plant species can be compatible with the same species of mycorrhizal fungi[1,2] and be connected to one another by a common mycelium[3,4]. Transfer of carbon[3–5], nitrogen[6,7] and phosphorus[8,9] through interconnecting mycelia has been measured frequently in laboratory experiments, but it is not known whether transfer is bidirectional, whether there is a net gain by one plant over its connected partner, or whether transfer affects plant performance in the field[10,11]. Laboratory studies using isotope tracers show that the magnitude of one-way transfer can be influenced by shading of 'receiver' plants[3,5], fertilization of 'donor' plants with phosphorus[12], or use of nitrogen-fixing donor plants and non-nitrogen-fixing receiver plants[13,14], indicating that movement may be governed by source–sink relationships. Here we use reciprocal isotope labelling in the field to demonstrate bidirectional carbon transfer between the ectomycorrhizal tree species *Betula papyrifera* and *Pseudotsuga menziesii*, resulting in net carbon gain by *P. menziesii*. *Thuja plicata* seedlings lacking ectomycorrhizae absorb small amounts of isotope, suggesting that carbon transfer between *B. papyrifera* and *P. menziesii* is primarily through the direct hyphal pathway. Net gain by *P. menziesii* seedlings represents on average 6% of carbon isotope uptake through photosynthesis. The magnitude of net transfer is influenced