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Carbon budgeting in plant–soil mesocosms under elevated CO_2: locally missing carbon?

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Abstract

Studies have suggested that more carbon is fixed due to a large increase in photosynthesis in plant–soil systems exposed to elevated CO_2 than could subsequently be found in plant biomass and soils — the locally missing carbon phenomenon. To further understand this phenomenon, an experiment was carried out using EcoCELLs which are open-flow, mass-balance systems at the mesocosm scale. Naturally occurring ^{13}C tracers were also used to separately measure plant-derived carbon and soil-derived carbon. The experiment included two EcoCELLs, one under ambient atmospheric CO_2 and the other under elevated CO_2 (ambient plus 350 μL L^{-1}). By matching carbon fluxes with carbon pools, the issue of locally missing carbon was investigated. Flux-based net primary production (NPP_f) was similar to pool-based primary production (NPP_p) under ambient CO_2, and the discrepancy between the two carbon budgets (12 g C m^{-2}, or 4% of NPP_f) was less than measurement errors. Therefore, virtually all carbon entering the system under ambient CO_2 was accounted for at the end of the experiment. Under elevated CO_2, however, the amount of NPP_f was much higher than NPP_p, resulting in missing carbon of approximately 80 g C m^{-2} or 19% of NPP_f which was much higher than measurement errors. This was additional to the 96% increase in rhizosphere respiration and the 50% increase in root growth, two important components of locally missing carbon. The mystery of locally missing carbon under elevated CO_2 remains to be further investigated. Volatile organic carbon, carbon loss due to root washing, and measurement errors are discussed as some of the potential contributing factors.

Keywords: carbon budget, carbon-13, elevated CO_2, *Helianthus annuus*, missing carbon

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Introduction

The continuous increase in atmospheric CO_2 is a well-documented phenomena of global environmental change (Keeling *et al*. 1989). Responses of ecosystems to elevated atmospheric CO_2 constitute critical feedbacks to the global carbon cycle. Many studies have shown that elevated atmospheric CO_2 concentration can significantly increase primary productivity as measured by leaf-level gas exchange (Curtis 1996). Subsequent allocation and the fate of this increased photosynthetically fixed carbon are important determinants of global carbon dynamics (Canadell *et al*. 1996). Partitioning of this extra carbon among pools with different turnover rates is a critical controlling step for carbon cycling and sequestration in terrestrial ecosystems. However, carbon fluxes (i.e. photosynthesis and respiration) mostly measured at the leaf level have rarely been linked to carbon pools (i.e. plant biomass and rhizodeposition). Without an understanding of this linkage, the fate of carbon entering plant–soil systems cannot be determined with certainty.

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Several studies have suggested that more carbon is fixed due to a large increase in leaf-level or canopy-level photosynthesis in systems exposed to elevated CO_2 than could subsequently be found in plant biomass and soils (Norby *et al*. 1992; Diemer 1994; Körner *et al*. 1996). This phenomenon is recognized as 'locally missing carbon' as a separate reference from the missing carbon at the global scale (Watson *et al*. 1992; Sarmiento & Sundquist 1992). For example, in an alpine grassland, a 41% increase in CO_2 uptake was reported during three years of CO_2 enrichment; but no above-ground biomass increase was observed, and only a slight increase in below-ground biomass was detected (Diemer 1994; Körner *et al*. 1996). In a study using yellow poplar (*Liriodendron tulipifera* L.), with continuous exposure to ambient and elevated concentrations of atmospheric CO_2 for three growing seasons, Norby *et al*. (1992) reported no significant effect of CO_2 concentration on dry mass production, despite a sustained increase in photosynthesis and reduced foliar respiration.

Where does all this carbon go? Finding answers to the locally missing carbon question requires simultaneous, accurate measurements of whole system carbon input and the separation of plant-derived carbon from soil-derived carbon. These measurements must be made in addition to conventional measurements of leaf-level photosynthesis, shoot biomass, and root biomass. Accurate measurements of whole system carbon input and output are prerequisites for assessing the quantitative significance of the locally missing carbon. Separating plant-derived carbon from soil-derived carbon is essential for assessing carbon allocation to below-ground components.

At the Great Basin Environmental Research Laboratory of the Desert Research Institute, a unique plant growth facility called EcoCELLs has been constructed and recently tested (Griffin *et al*. 1996). Using EcoCELLs, fluxes of carbon in whole plant–soil systems can be accurately and continuously measured. By employing the ^{13}C natural tracer technique (Cheng 1996), plant-derived carbon can be separated from soil-derived carbon. The special requirements for answering the locally missing carbon question stated above can be met by combining the EcoCELL facility with the ^{13}C natural tracer technique.

The main objectives of this study were: (i) to demonstrate that better carbon budgets can be made by combining the EcoCELL facility with the ^{13}C natural tracer technique; (ii) to address the issue of locally missing carbon; (iii) to investigate the effect of elevated atmospheric CO_2 on plant carbon allocation patterns in the context of carbon balances; and (iv) to assess the accuracy of measuring carbon pools and fluxes at the mesocosm scale.

Materials and methods

Experimental system

Detailed descriptions of EcoCELLs are provided by Griffin *et al*. (1996). Briefly, EcoCELLs are environmentally controlled, naturally lit, open-flow, mass-balance systems at the mesocosm scale. The EcoCELLs have the same theory of operation as leaf-level gas exchange systems but work at a much larger scale, and measure whole-system fluxes continuously. The dimensions of the EcoCELLs are $7.3 \times 5.5 \times 4.5$ m (L × W × D). There is a circulating volume of 162.5 m^3 and a soil volume of 20.1 m^3 in each cell. The soil medium in each cell is contained in three $2.85 \times 1.3 \times 1.8$ m (L × W × D) acrylic-walled boxes that comprise the lysimeter/rhizotron system and closely adjoin each other, forming a continuous plant canopy. Each lysimeter/rhizotron is mounted on a set of four load cells capable of discriminating a change in weight of 250 g out of a total weight of 2.0×10^6 g. The air temperature, relative humidity, and CO_2 concentration in each EcoCELL are controlled automatically.

Two EcoCELLs were used in this experiment, one under ambient atmospheric CO_2 and the other under elevated CO_2 (ambient plus 350 μL L^{-1}). CO_2 level in the ambient treatment was dependent on the outside air, and thus the lower-end set point was constrained by the global average CO_2 concentration and local anthropogenic source emissions. CO_2 concentration was controlled by a three-stage system: (i) a needle valve was used to inject a constant amount of CO_2, approximately 80–90% of the required addition; the CO_2 flow passed through (ii) a mass flow controller for coarse control (0–100 Lpm with 15 Lpm steps) followed by (iii) a fine control mass flow controller (0–15 Lpm). Using this three-stage approach, we were able to obtain CO_2 concentrations well within 2% of the desired set point.

A soil profile was constructed for each soil container in the EcoCELLs. The soil profile consisted of three layers: (i) 0–0.4 m, 1 : 1 mix of top soil from Kansas tallgrass prairie and washed river sand; (ii) 0.4–0.8 m, washed river sand; and (iii) 0.8–1.8 m, washed river bed pebbles. Top soil was obtained from the Konza Prairie Long-Term Ecological Research site. Soil carbon was predominantly C4 plant-derived carbon (^{13}C enriched), which had a $\delta^{13}C$ value of -14.2 ± 0.14‰. Soil was first sieved through a 12.7-mm screen. Plant materials and large stones were removed from the soil by hand-picking before use. The 1 : 1 mix of soil and sand was obtained using a cement mixer. The amount of soil and sand mix added to each soil container was measured using electronic scales.

Sunflowers (*Helianthus annuus* L.) were planted in each soil container for a total of 108 plants per EcoCELL.

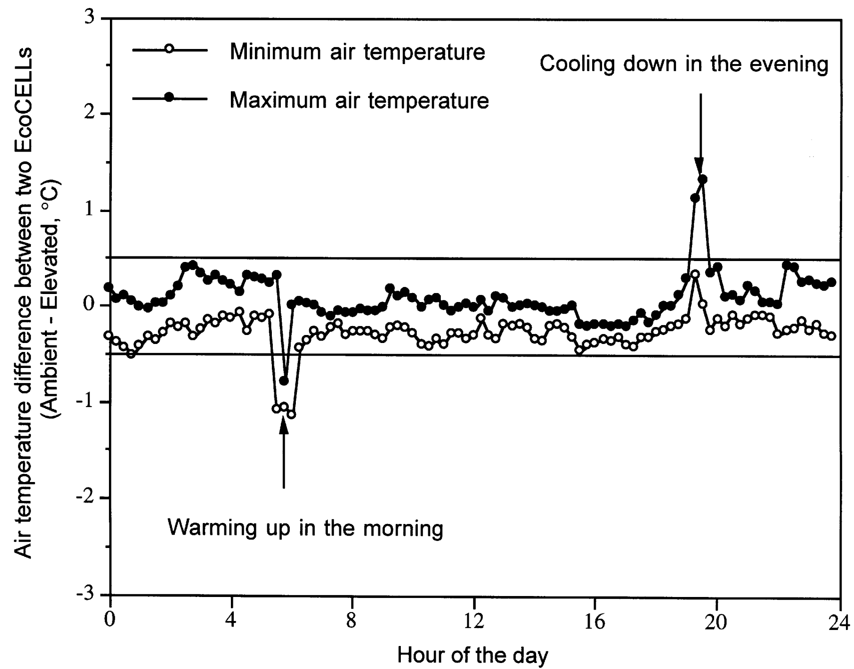

Fig. 1 Maximum and minimum Air temperature difference between two EcoCELLs during a typical day. The difference was within ± 0.5 °C at steady state.

Sunflower was selected because: (i) it represent a typical C3 plant which shows a typical response to elevated CO_2; (ii) its leaf structure allows for simple scaling calculations to a canopy structure; and (iii) measurement of below-ground CO_2 could be carried out with relative ease. The sunflowers were grown for 53 days under nearly identical air temperature, humidity, soil moisture, and irradiance conditions in the two EcoCELLs (Figs 1 and 2). Water was supplied based on whole system weight data and through frequent hand watering; soil water content was maintained between 60% and 100% of field water holding capacity. Day and night air temperatures and relative humidities were set at 28 °C/ 30% and 13 °C/ 60%, respectively. The environmental control system was able to maintain air temperatures in the EcoCELLs within ± 0.5 °C (Fig. 1) and the relative humidity within ± 5% (Fig. 2). Most of the days during this period were cloudless. The appearance of the plants was normal and healthy.

Whole-system carbon budgets

Two carbon budgets were constructed: flux-based and pool-based. The flux-based carbon budget was constructed according to the following relationship:

$$\text{Net ecosystem production } (NEP) = C_{in} - C_{out}, \quad (1)$$

since $C_{in} = GPP$ and $C_{out} = R_{shoot} + R_{rhizo} + R_{soil}$,

$$NEP = GPP - R_{shoot} - R_{rhizo} - R_{soil} \quad (2)$$

or

$$NEP + R_{soil} = GPP - R_{shoot} - R_{rhizo}. \quad (3)$$

Since $NPP = GPP - R_{shoot} - R_{rhizo}$, by definition,

$$NPP_f = NEP + R_{soil}, \quad (4)$$

where NEP = net ecosystem production or whole cell net carbon gain, measured using whole cell gas exchange system; GPP = gross primary production or total carbon fixed by plant photosynthesis (can be calculated using NPP and plant respiration measurements, but GPP is not used in determining the carbon budget); NPP_f = flux-based net primary production; C_{in} = total carbon input from plant photosynthesis; C_{out} = total carbon output; R_{shoot} = shoot dark respiration, i.e. total dark respiration (measured using whole cell gas exchange) minus below-ground respiration (CO_2 loss from roots and soil); R_{rhizo} = rhizosphere respiration, equal to below-ground respiration minus soil respiration measured by open-flow gas exchange system combined with ^{13}C analysis; R_{soil} = soil respiration (carbon loss from original soil organic matter) measured by open-flow gas exchange system combined with ^{13}C analysis.

Equation (4), which consists of two measurable terms, was used to construct the flux-based carbon budget.

The pool-based carbon budget was constructed according to the following equation:

$$NPP_p = \text{Shoots} + \text{Roots} + \text{Residue}, \quad (5)$$

where NPP_p = pool-based net primary production; Shoots = shoot biomass; Roots = root biomass; Residue =

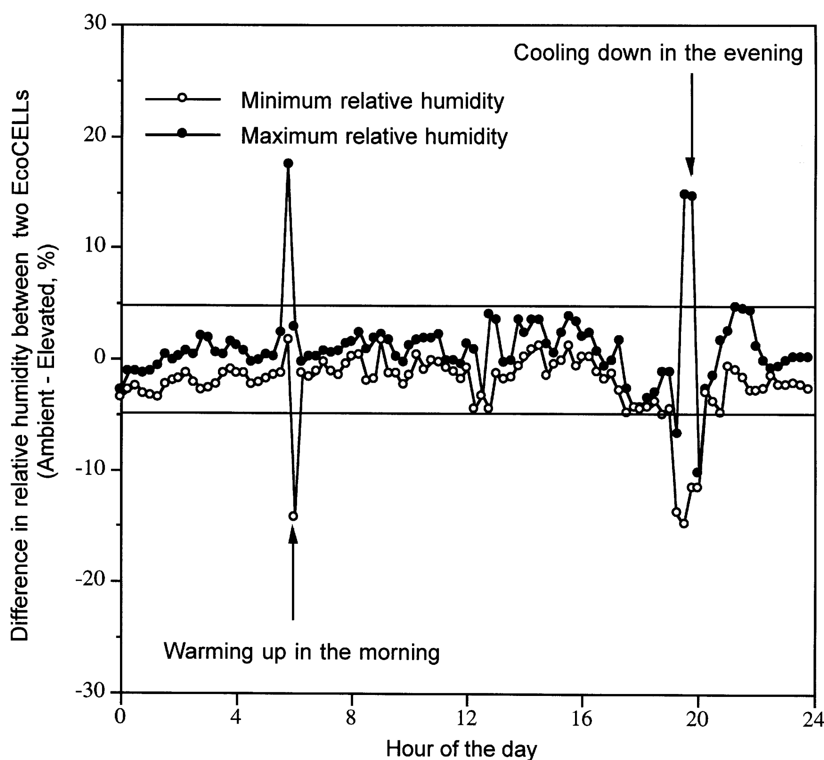

Fig. 2 Difference in maximum and minimum relative humidity between two EcoCELLs during a typical day. The difference was within ±5% at steady state.

plant-derived carbon residue in the soil after the removal of roots, or rhizodeposition.

To construct the two carbon budgets, five measurements were required. The methods and procedures for making these measurements are given below.

NEP: net ecosystem production

Net ecosystem production was measured continuously using the whole-EcoCELL gas exchange system (Griffin *et al.* 1996). Three infrared gas analysers (IRGAs) (LI-COR 6262) were dedicated to CO_2 monitoring. A fourth IRGA was continuously run in differential mode to record the net flux of CO_2 across each EcoCELL. A fifth IRGA was used in absolute mode and sequentially fed with a standard gas, as well as the gas entering and exiting each EcoCELL. All five IRGAs were sampled at 5-s intervals and recorded as 60-s averages. Each IRGA was zeroed and spanned daily with NIST traceable standards (Scott-Marrin 99% accuracy standards) to account for any drift in the calibration coefficients. Cumulative NEP was obtained by integrating the continuous measurements through time.

The mass flow rate of air entering each EcoCELL was measured directly with a multipoint, hot-wire anemometer. Each mass flow meter was individually calibrated *in situ* using a trace gas addition technique (Field *et al.* 1991). System calibration was carried out prior to planting and after the completion of the experiment to account for any drift in the volumetric flow measurements.

Before the start of the experiment, all equipment in the gas exchange system was calibrated either by the manufacturers or by laboratory personnel. During the experiment, the accuracy of the whole system gas exchange was checked five times by injecting a known amount of CO_2 gas through a calibrated mass-flow meter with an accuracy of 98% in comparison to the Scott-Martin standards.

Data points affected by the presence of human activities inside EcoCELLs or by door openings were corrected using daily regression curves between photosynthetic active radiation (PAR) and NEP during daytime and using the average of unaffected data points before and after the affected point(s).

Soil respiration

The loss of original soil carbon due to soil respiration was determined by subtracting rhizosphere-respired carbon (i.e. C3 plant-derived carbon) from the total respired carbon below-ground. The flux of below-ground CO_2 during a 24-h period was measured weekly using a continuous open-flow gas exchange system equipped

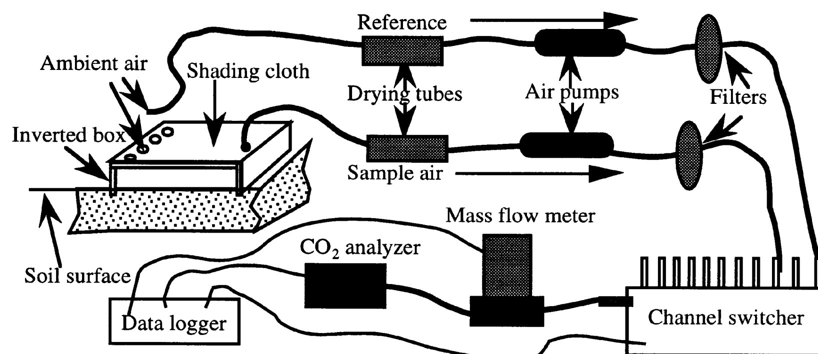

Fig. 3 A continuous open-flow gas exchange system for measuring below-ground CO_2 evolution.

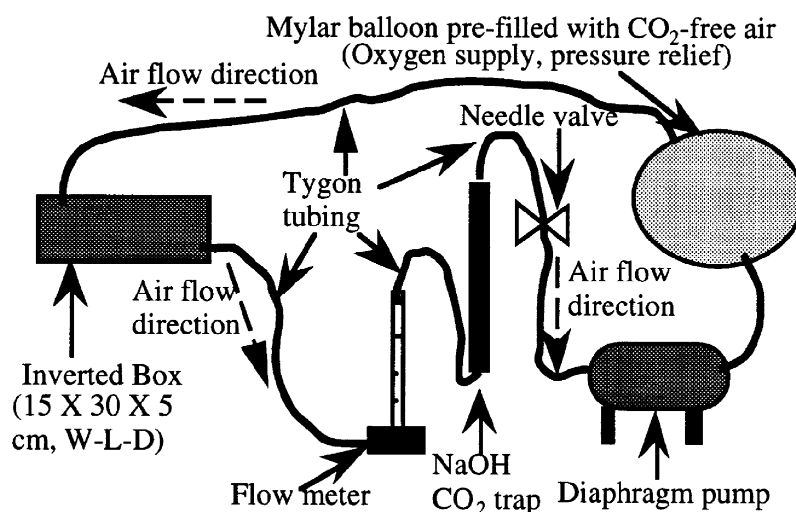

Fig. 4 A closed-circulation CO_2 trapping system. The problems of air leaks, diffusion fractionation, and diurnal fluctuation are avoided by using this system. The CO_2 concentration inside the inverted box is controlled by adjusting the air flow rate.

with six sampling units (inverted boxes) within each EcoCELL (Fig. 3). This open-flow system was calibrated using the whole EcoCELL gas exchange system before planting. The $\delta^{13}C$ value of below-ground CO_2 was determined weekly using a closed-circulation CO_2 trap system (Fig. 4) with subsequent analysis of ^{13}C abundance of the trapped CO_2 during each 24-h period (Harris *et al.* 1997). Plant-derived, rhizosphere-respired carbon in total below-ground CO_2 was partitioned from soil-derived carbon using the ^{13}C natural tracer method (Cheng 1996).

The ^{13}C natural tracer method is based on the difference in ^{13}C:^{12}C ratio (often reported in $\delta^{13}C$ value) between plants with the C3 and C4 photosynthetic pathway (Smith & Epstein 1971) and on the subsequent difference between soil organic matter derived from the two types of plants. Soil organic matter derived from C4 plant (C4-derived soil), such as tallgrass prairie and tropical grasslands, has $\delta^{13}C$ values ranging from −12 to −20‰, whereas $\delta^{13}C$ values of soil organic matter derived from cold and temperate forests (C3-derived soil) range from −24 to −29‰. If one grows C3 plants such as sunflowers in C4-derived soil, or vice versa, the carbon entering the soil via roots will have a different $\delta^{13}C$ value than the $\delta^{13}C$ value of the soil. The following equation can be used to partition soil-derived C4 carbon from plant-derived C3 carbon:

$$C3 = C_t(\delta_t - \delta_4)/(\delta_3 - \delta_4), \quad (6)$$

where $C_t = C3 + C4$, the total carbon from below-ground CO_2; $C3$ = the amount of carbon derived from C3 plants; $C4$ = the amount of carbon derived from C4 soil; δ_t = the $\delta^{13}C$ value of the C_t carbon; δ_3 = the $\delta^{13}C$ value of the C3 plant carbon; δ_4 = the $\delta^{13}C$ value of the C4 soil carbon.

Shoot and root biomass

Shoot biomass was measured by complete harvesting. Root biomass was measured by washing roots out of nine replicate soil columns of single-plant equivalent (30 × 30 × 40 cm, L × W × D) from each EcoCELL. The sampling depth of 40 cm was adequate because virtually

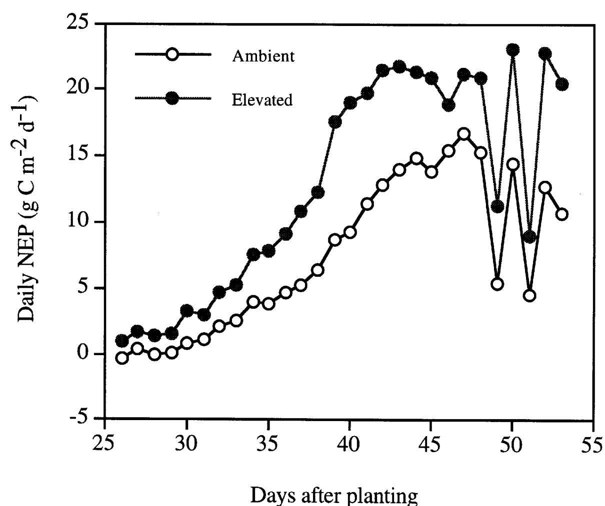

Fig. 5 Daily net whole ecosystem carbon production (gain).

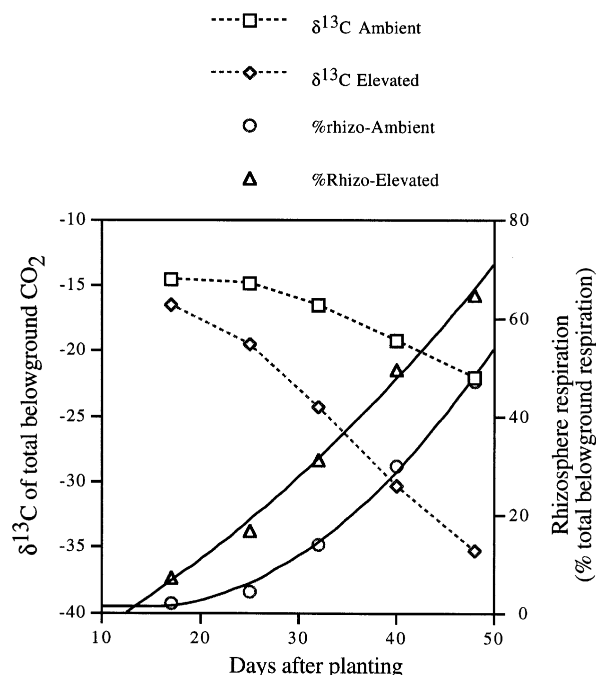

Fig. 6 $\delta^{13}C$ values and corresponding rhizosphere respiration in both ambient CO_2 and elevated CO_2 treatments.

no roots were found below the top soil layer in this experiment.

Plant-derived carbon in the soil

Nine soil cores (2 cm in diameter) were taken randomly from each soil container and bulked together. Visible roots in the sample were removed by hand picking. The bulked soil samples were first ground and homogenized in large quantities (300 g) and then pulverized in smaller quantities with a ball mill (Spex Mill) before ^{13}C analysis. A mass spectrometer (Finnigan Delta-C plus) facility at the Institute of Ecology, University of Georgia, was used for all ^{13}C analysis. This configuration of sample preparation and analytical equipment produced $\delta^{13}C$ data with random errors less than 0.04‰. The amount of plant-derived carbon in the soil after root removal was calculated using (6). To account for all possible carbon pools, the change in inorganic carbon pool was determined by measuring the concentration of inorganic carbon in the soil before and after the experiment. The amount of both organic and inorganic carbon in the soil leachate was measured using a total organic carbon analyser (Shimadzu 5050 A).

Results

As plant growth progressed, daily NEP increased sigmoidally regardless of CO_2 levels (Fig. 5), except for a few cloudy days toward the end of this experiment. The daily NEP under elevated CO_2 was consistently higher than under the ambient treatment.

The $\delta^{13}C$ values of total below-ground CO_2 decreased over time under both CO_2 treatments (Fig. 6), indicating increased rhizosphere respiration (i.e. root respiration plus rhizosphere microbial respiration using sunflower-derived carbon substrates). The $\delta^{13}C$ values of total below-ground CO_2 were consistently lower under elevated CO_2 than ambient CO_2. Two factors caused this $\delta^{13}C$ difference: (i) the relative contribution of rhizosphere respiration (C3 plant carbon) to total below-ground CO_2 and (ii) the contribution to photosynthesis of a fossil-fuel atmospheric CO_2 (^{13}C depleted) source. Because of the use of a ^{13}C-depleted fossil-fuel CO_2 source under the elevated CO_2 treatment, the $\delta^{13}C$ value of plant carbon under this treatment was lower than plant carbon under ambient CO_2 (Table 1). The average $\delta^{13}C$ value of root tissue was −31.0‰ under ambient CO_2 and −46.8‰ under elevated CO_2, a difference of > 15‰. The average $\delta^{13}C$ value of atmospheric CO_2 during the entire experiment was −12.63‰ under the ambient treatment and −22.89‰ under the elevated CO_2 treatment, respectively.

The contribution of rhizosphere respiration to total below-ground respiration was calculated using (6) and the data shown in Fig. 6 and Table 2. Results indicated that rhizosphere respiration under the elevated CO_2 treatment was consistently higher than under the ambient treatment (Fig. 6). Rhizosphere respiration as a

percentage contribution to total below-ground respiration over time (days after planting) under both treatments could best be fitted with quadratic equations. At the end of this experiment, almost 70% of the below-ground respiration was rhizosphere respiration under elevated CO_2, and 46% was rhizosphere respiration under ambient CO_2.

Total CO_2 evolved from below-ground components (i.e. rhizosphere respiration and soil microbial respiration utilizing original soil carbon) was measured five times during this experiment. There was little change in total below-ground respiration under ambient CO_2 during the course of this experiment. However, the rate of total below-ground respiration under elevated CO_2 was lower than the ambient treatment initially, increased as plant growth progressed (Fig. 7a), and eventually surpassed the ambient treatment during the later part of this experiment.

Using (6) and $\delta^{13}C$ data, total below-ground respiration was partitioned into soil respiration and rhizosphere respiration (Fig. 7b,c). Original soil respiration rates declined over time, but this decline was faster under ambient CO_2 than elevated CO_2. Rhizosphere respiration increased exponentially during the experimental period for both treatments. Rhizosphere respiration rates under elevated CO_2 were consistently higher than under the ambient CO_2 treatment.

Table 1 $\delta^{13}C$ values for CO_2 from the soil without roots and sunflower (*Helianthus annus*) roots grown for 53 days in the EcoCELLs under both ambient and elevated CO_2 treatments. Numbers are means of three replicates with standard error

	Ambient CO_2	Elevated CO_2
$\delta^{13}C$-roots (‰)	-31.01 ± 0.03	-46.87 ± 0.17
$\delta^{13}C$-soil CO_2 (‰)	-14.21 ± 0.11	-14.21 ± 0.11

Flux-based NPP (NPP_f) was calculated by integrating NEP and soil respiration data though time. Based on (4), flux-based carbon budgets under both CO_2 treatments were obtained by summing total NEP and soil respired carbon (Table 2). Soil respiration data for the entire experimental period were obtained through extrapolation of the five 24-h measurements by linear curve fitting (Fig. 7b). Data of pool-based NPP (NPP_p) were calculated by summing shoot biomass, root biomass, and rhizodeposits (eqn 6). Under ambient CO_2, the amount of NPP_f was close to NPP_p, indicating only a small amount of missing carbon ($12\,g\,C\,m^{-2}$, or 4% of NPP_f). However, under elevated CO_2, the amount of NPP_f was much greater than NPP_p, resulting in missing carbon of approximately $80\,g\,C\,m^{-2}$ or 19% of NPP_f.

There was a 49% increase in NPP_f and a 27% increase in NPP_p under elevated CO_2 compared to ambient CO_2. This difference was primarily due to the above-mentioned missing carbon under elevated CO_2. Of the two components of NPP_f, there was a 70% NEP increase and a 16% decrease in soil respired carbon under elevated CO_2 compared to ambient CO_2. Net ecosystem production was the major component of NPP_f, contributing 75% of NPP_f under ambient CO_2 and 86% under elevated CO_2. There was a 27% increase in shoot biomass carbon and a 50% increase in root biomass carbon under elevated CO_2 compared to ambient CO_2. The percentage increase in shoot biomass (27%) was similar to the percentage increase in NPP_p (26%), whereas the percentage increase in root biomass was similar to the percentage increase in NPP_f (49%). The difference in rhizodeposition between the two CO_2 treatments was difficult to evaluate due to the large error associated with the measurement.

The error columns in Table 2 were given for the assessment of measurement accuracies. NPP_f was calculated from two components: (i) net ecosystem production (NEP), and (ii) soil-derived CO_2. The accuracy of NEP measurements was equal or better than 95% as calibrated using national CO_2 standards. The accuracy of the soil-

Table 2 Carbon budgets of an experiment with sunflowers (*Helianthus annus*) grown for 53 days in the EcoCELLs under both ambient and elevated CO_2 treatments. NPP_f: flux-based net primary production; NEP: net ecosystem production; NPP_p: pool-based net primary production. Errors are given in the same unit as each measurement

	Ambient CO_2		Elevated CO_2		Increase
Components	$g\ C\ m^{-2}$	Error	$g\ C\ m^{-2}$	Error	%
NPP_f	280	< 12	417	< 18	49
NEP	210	< 10	358	< 18	70
soil respiration	70	11	59	6	−16
NPP_p	268	< 13	337	< 5	26
shoot mass	175	< 4	223	< 5	27
root mass	40	3	60	3	50
rhizodeposition	53	13	54	4	2
NPP-missing	12		80		567

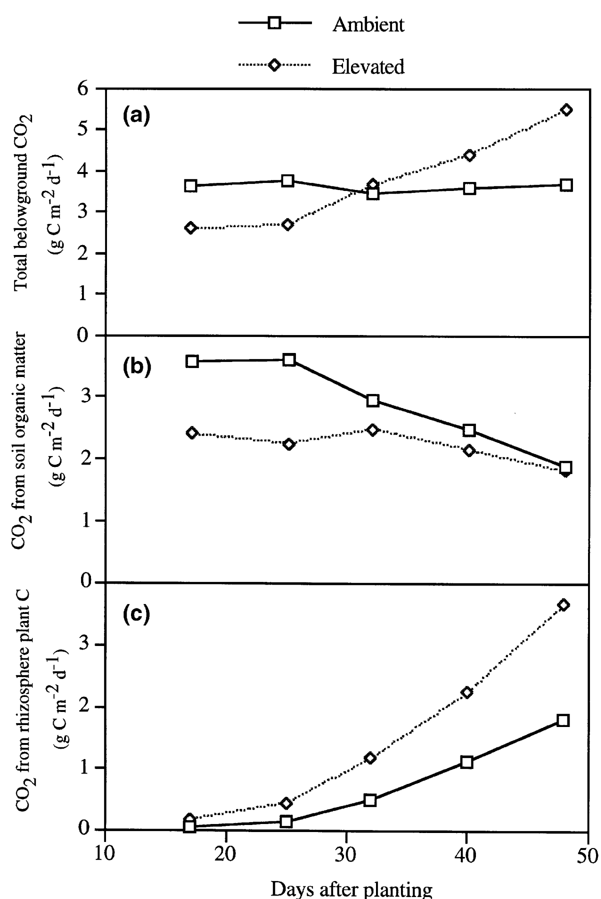

Fig. 7 Total below-ground CO_2 evolution (a), soil respiration (utilizing original soil C) (b), and rhizosphere respiration (C) in the ambient CO_2 and elevated CO_2 treatments.

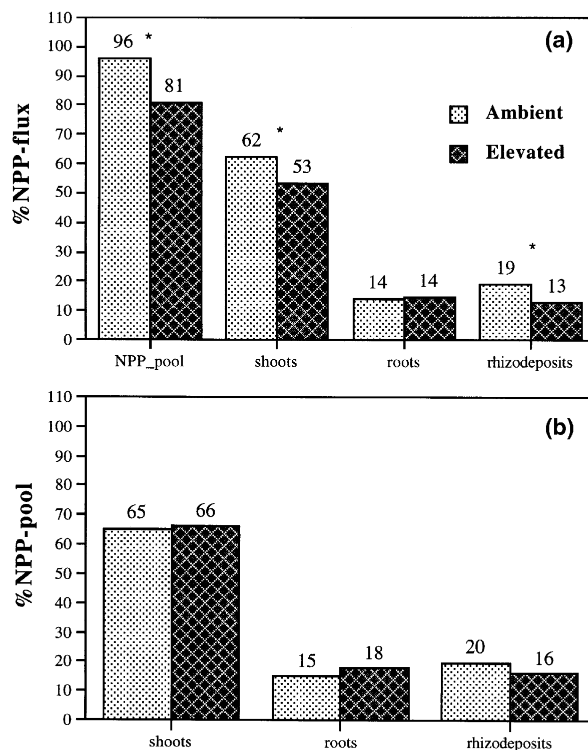

Fig. 8 Plant carbon allocation based on two carbon budgets, indicating the changed patterns of carbon allocation depending on the use of different NPP bases. Bars with * in the middle are significantly different from each other ($P < 0.05$). The maximum error is less than 5% of the total NPP.

derived CO_2 measurements was limited by random errors associated with ^{13}C isotope measurements of trapped CO_2 and spatial heterogeneity in total below-ground respiration. The standard errors of the $\delta^{13}C$ measurements were equal or smaller than 0.2‰, which translates into an accuracy of 98%. Errors due to soil spatial heterogeneity (16% of the mean for the ambient CO_2 and 10% for the elevated CO_2) were larger than $\delta^{13}C$ measurement errors therefore were chosen for the overall errors of soil-derived CO_2 measurements. The largest error among the two components was less than 5% of NPP_f. The accuracy of NPP_f was at least 95%. Error values associated with shoot biomass measurements was assumed to be 2% of the mean or less since shoot biomass was measured by 100% harvesting. Error values associated with root biomass were calculated using the standard errors of the nine replicate root samples from each EcoCELL. Plant biomass carbon contents were determined using a CHN analyser with an accuracy of 99.5%. The error associated with rhizodeposition measurements under ambient CO_2 was very high (approximately 25% of the mean) due to the disparity between the very small amount of rhizodeposition compared to the large total soil carbon pool. Thus, the input of plant-derived rhizodeposition only caused a small shift (0.21‰) in the $\delta^{13}C$ value of total soil carbon from the $\delta^{13}C$ value of the original soil carbon. A small random variation (SE = 0.052‰) in $\delta^{13}C$ values among replicate samples produced a large error. The rhizodeposition measurement error under elevated CO_2 was much smaller than under ambient CO_2 because the $\delta^{13}C$ value shift (0.44‰) was larger and the standard error (0.011‰) among replicates was lower than under ambient CO_2. Based on these error values, the accuracy of NPP_p was at least 95%.

The patterns of plant carbon allocation were different depending on which type of NPP measurement (flux-based or pool-based) was used (Fig. 8a,b). Distribution patterns among carbon pools were similar between the two CO_2 treatments if NPP_p was used as the 100% base (Fig. 8b). But the patterns differed if NPP_f was used since more carbon was missing under elevated CO_2 (19% of

NPP_f) than under ambient CO_2 (only 4% of NPP_f) (Fig. 8a). Above-ground plant biomass accounted for 53% of NPP_f under elevated CO_2 and was 62% of NPP_f under ambient CO_2, a 9% difference which was much greater than the maximum error of 5%. Rhizodeposition accounted for 13% of NPP_f under elevated CO_2 and 19% of NPP_f under ambient CO_2, a 6% difference which was slightly greater than the maximum error of 5%.

Discussion

By combining the EcoCELL facility with the ^{13}C natural tracer technique, two separate carbon budgets were obtained for each EcoCELL, one from the sum of fluxes and another from the sum of the pools. By matching carbon fluxes with carbon pools, the issue of locally missing carbon was addressed with this experiment in EcoCELLs. The flux-based net primary production (NPP_f) was similar to the pool-based net primary production (NPP_p) under the ambient-CO_2 conditions, and the discrepancy between the two carbon budgets ($12\,g\,C\,m^{-2}$, or 4% of NPP_f) was less than measurement errors. In other words, there was no significant 'missing carbon' under ambient CO_2 since virtually all carbon entering the system was found in the plant and soil pools at the end of the experiment. Under elevated CO_2, however, the amount of NPP_f was much higher than NPP_p, resulting in missing carbon of approximately $80\,g\,C\,m^{-2}$ or 19% of NPP_f.

The only difference between the two EcoCELL treatments was the concentration of atmospheric CO_2. Other experimental conditions including air temperature, humidity, lighting, and soils were almost identical (Figs 1 and 2). This result suggests that elevated CO_2 was the cause of the missing carbon, although this inference could not be derived from statistical analysis because CO_2 treatments were not replicated. Replications were not included because there were only two EcoCELLs available at the time of this experiment.

The missing carbon under the elevated CO_2 treatment was derived primarily using a mass balancing approach. The accuracy of each measurement was more critical than the precision of mass balancing. Our careful assessment of the accuracies of all measurements provides reasonable assurance of the validity of the conclusion. The 49% increase in NPP_f under elevated CO_2 compared to the ambient CO_2 treatment is nearly equal to the overall mean increase of approximately 50% in photosynthesis reported in 83 independent studies (Curtis 1996). The 27% increase in shoot biomass carbon and the 50% increase in root biomass carbon under elevated CO_2 compared to ambient CO_2 are also within the normal range reported in elevated CO_2 studies (Rogers *et al*. 1994; Körner *et al*. 1996). The increase in rhizosphere respiration under elevated CO_2 is consistent with the result from the pot study of Cheng & Johnson (1998) using the same soil and is also consistent with reported results from the literature (Gorissen 1996; Hungate *et al*. 1997). The decrease in original soil organic matter decomposition under elevated CO_2 compared to ambient CO_2 is also consistent with the result from the pot study of Cheng & Johnson (1998) and with other reports (Kuikman *et al*. 1990; Lekkerkerk *et al*. 1990; Rouhier *et al*. 1996). In general, all the effects of elevated CO_2 in our experiment are consistent with results reported in the literature.

An unexpected result was that the two carbon budgets did not match under elevated CO_2. In addition to the increase in rhizosphere respiration and root growth as two important components of the locally missing carbon, we found that there was still approximately 19% of total net primary production missing under elevated CO_2. The net change in soil inorganic carbon during the experiment was less than $2\,g\,m^{-2}$, which was negligible in the total carbon budget. Total carbon in the leachate collected during the entire experimental period was also negligible (less than $2\,g\,m^{-2}$). All exposed surfaces in both EcoCELLs are painted with a special inert paint, so that carbon sources can neither be absorbed nor released from these surfaces. No herbivore insects or senesced leaves were noticed during the entire experiment. Based on current knowledge, the following factors might have contributed to the missing carbon under elevated CO_2: (i) not-measured non-CO_2 carbon volatilized from the plant-soil system; (ii) loss of plant-derived carbon during root washing; and (iii) measurement errors.

Volatile organic carbon has rarely been measured in experiments involving CO_2 enrichments. However, whole-system biogenic organic carbon emissions have been mostly assumed to be insignificant for carbon budgets, even though some recent studies have indicated that biogenic non-CO_2 carbon emissions could significantly increase in response to elevated CO_2 (Turner *et al*. 1991; Penuelas & Llusia 1997). Increased output of volatile organic carbons under elevated CO_2 may have potentially far reaching effects on the atmosphere. The issue of whether volatile carbon emissions will change in response to increasing atmospheric CO_2 concentrations needs to be rigorously addressed.

Loss of plant-derived carbon during root washing was not assessed in this study, which might have contributed to the missing carbon under elevated CO_2. One study (Swinnen *et al*. 1994) using ^{14}C pulse-labelling reported that loss of plant-derived carbon during root washing could be as high as 21% of root mass if root samples were stored by oven-drying, freeze-drying, or frozen before washing. The carbon loss was suggested to be primarily in the forms of soluble compounds and rhizosphere micro-

bial materials. As shown above, elevated CO_2 significantly increased root growth and rhizosphere respiration in this experiment. Differential losses of plant-derived carbon due to root washing between the two CO_2 treatments could have partially resulted in the missing carbon under elevated CO_2. If this carbon loss had reached the maximum of 21% root biomass under elevated CO_2, the amount of missing carbon would have reduced by 12.6 g C m^{-2}, which would still be less than the maximum measurement error of 18 g C m^{-2}. However, the amount of carbon loss due to root washing in this experiment should be much less than the maximum percentage (21%) reported by Swinnen *et al*. (1994) because samples in this study were stored in a refrigerator (2–6 °C) for less than three weeks before being washed. Root integrity is better preserved by refrigeration than by oven-drying, freeze-drying, or under frozen conditions.

Among measurement errors which might have contributed to the missing carbon (Table 2), larger absolute errors were associated with flux measurements under both CO_2 treatments and with rhizodeposition under ambient CO_2. However, the amount of missing carbon under the elevated CO_2 treatment was more than four times the largest measurement error (Table 2). Therefore, it was unlikely that measurement errors alone could have resulted in the amount of missing carbon under elevated CO_2.

Finding the locally missing carbon using the carbon budgeting approach may have some technical similarities with finding the globally missing carbon based on global carbon budgets. In virtually all budgeting processes, the accuracy of each measurement is more critical than the precision. In this study, we have paid special attention to the accuracies of all measurements. By combining the EcoCELL facility and the ^{13}C natural tracer technique, we have demonstrated that two separate carbon budgets can be constructed with approximately 95% accuracies. Any discrepancies less than 5% of the total NPP do not warrant much attention because of measurement errors. This issue is also relevant to the recent debate about missing carbon on the global scale (Taylor 1993). A best efforts global atmospheric carbon budget for 1980–89 (Sarmiento & Sundquist 1992; Watson *et al*. 1992) suggests that there may be an imbalance of approximately 1.8 gigatonnes of carbon per year between total source and total sink. Many efforts have been made to find the sink(s) for this missing carbon since this atmospheric carbon budget has been published (Dai & Fung 1993; Houghton 1993; Dixon *et al*. 1994). However, attention has rarely been paid to the accuracy of the global atmospheric carbon budget and some subsequent estimates of the identified carbon sink in the northern hemisphere. Estimated values of the identified missing carbon sink have ranged from 0.8 to 1.6 gigatonnes per year, which may not be much different from the error value of 1.4 gigatonnes for the missing carbon in the 1980–89 carbon budget. Although this missing carbon issue has stimulated valuable research for finding or identifying the missing carbon sink(s), increasing the accuracy or reducing the uncertainty of the global carbon budget seems to be the first requirement.

Virtually all existing results relevant to the locally missing carbon issue have been obtained from small pot studies using partial carbon budgets (i.e. Ineson *et al*. 1996; Gorissen 1996; Hungate *et al*. 1997). To the best of our knowledge, carbon budget at the mesocosm scale has rarely been reported in the literature. Without better carbon budgets, altered carbon allocation patterns under elevated CO_2 conditions cannot be fully investigated. As shown in Fig. 8, the percentage distribution patterns of different carbon pools were similar between the two CO_2 treatments if partial carbon budgets of NPP_p were used as the 100% base. The patterns were different, however, if more complete NPP_f carbon budgets were used. Recent studies have indicated that changes in carbon allocation under elevated CO_2 can be as important as the CO_2 fertilization effect, since allocation is the first process determining the ultimate fate of the increased carbon input under elevated CO_2 (Canadell *et al*. 1996; Luo *et al*. 1997).

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