Tracing Changes in Ecosystem Function under Elevated Carbon Dioxide Conditions

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Responses of ecosystems to elevated levels of atmospheric carbon dioxide (CO_2) remain a critical uncertainty in global change research. Two key unknown factors are the fate of carbon newly incorporated by photosynthesis into various pools within the ecosystem and the extent to which elevated CO_2 is transferred to and sequestered in pools with long turnover times. The CO_2 used for enrichment in many experiments incorporates a dual isotopic tracer, in the sense that ratios of both the stable carbon-13 (^{13}C) and the radioactive carbon-14 (^{14}C) isotopes with respect to carbon-12 are different from the corresponding ratios in atmospheric CO_2. Here we review techniques for using ^{13}C and ^{14}C abundances to follow the fate of newly fixed carbon and to further our understanding of the turnover times of ecosystem carbon pools. We also discuss the application of nitrogen, oxygen, and hydrogen isotope analyses for tracing changes in the linkages between carbon, nitrogen, and water cycles under conditions of elevated CO_2.

Keywords: elevated carbon dioxide, stable isotopes, radiocarbon, global change, carbon cycle

The responses of terrestrial ecosystems to rising concentrations of atmospheric carbon dioxide (CO_2), and the resulting global changes, are still not fully understood. Humans emitted 6 gigatons of carbon per year into the atmosphere from fossil fuel burning and cement production during the 1990s, yet only about half of this carbon accumulated in the atmosphere. Of the remainder, about half was absorbed by the oceans and half by terrestrial ecosystems (IPCC 2001). The mechanisms that govern the magnitude of net carbon uptake by the terrestrial biosphere are unknown, but enhanced growth under elevated atmospheric CO_2 is commonly proposed (Schimel et al. 2000). Although individual plants often respond to elevated CO_2 by fixing more carbon in photosynthesis and thereby increasing production of biomass, this does not necessarily result in long-term removal of CO_2 from the atmosphere. Long-term removal requires the transfer of fixed carbon into long-lived ecosystem pools such as woody biomass and recalcitrant soil carbon (Körner 2000).

Researchers have exposed a variety of plant communities to elevated CO_2 levels to improve our understanding of whole-ecosystem responses. Such studies have used open-top chambers, closed chambers, natural CO_2 springs, and free-air CO_2 enrichment (FACE). These experiments are critical to our ability to determine the fate of carbon in the terrestrial biosphere. In addition to increasing the rate of fixation of carbon in photosynthesis, elevated atmospheric CO_2 levels may indirectly cause other changes in ecosystem function. Plants may, for example, reduce their uptake of water as a result of closing their stomata in response to elevated levels of atmospheric CO_2 (Morison 1998). The exchange of carbon and water with the atmosphere is also closely related to the cycling of nitrogen, which generally limits growth in temperate systems (Zak et al. 2000). Changes in the ratio of carbon to nitrogen in plant material may influence the quality of food available for herbivores. Thus, elevated atmospheric CO_2 levels can affect populations at different trophic levels (Lindroth 1996a, Owensby et al. 1996).

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The need to develop methods to quantify ecosystem responses to elevated CO_2 in relatively small-scale and short-term experiments presents both challenges and opportunities for ecologists. Measurements of the abundance of various carbon, hydrogen, oxygen, and nitrogen isotopes in samples have long been used in various subdisciplines of ecology as unique tools that integrate physical and physiological processes over space and time (table 1, box 1). New developments in mass spectrometry allow stable isotope analyses to be conducted using very small samples of biomass, soil organic matter, or water. These advances have improved our ability to make inferences about plant physiology, biogeochemistry, and ecosystem function. This is particularly valuable in studies of elevated CO_2, in which destructive measurements must be limited to maintain the integrity of experimental plots for long-term study. Many studies of elevated CO_2 effects are intrinsically isotopic tracer experiments because the ratios of carbon-13 (^{13}C) to carbon-12 (^{12}C) and of carbon-14 (^{14}C) to ^{12}C in the added CO_2 are distinct from the ratios in atmospheric CO_2. Plants grown in conditions of elevated CO_2 that contain such isotopic tracers will incorporate the tracer in carbon-containing compounds. The two labels can then be used to trace the fate of carbon newly incorporated into the ecosystem under high CO_2 conditions and to calculate the residence time of carbon in various pools.

Here we discuss current and potential research that combines isotope ecology and experiments with elevated CO_2 to improve our understanding of interactions between ecosystem function and global change.

Table 1. Isotopes of common elements and their natural abundance.

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
	^{14}C	< 0.001
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.76
	^{17}O	0.04
	^{18}O	0.20

Note: All the isotopes except ^{14}C are stable.

The carbon-13 label

The mean $\delta^{13}C$ (see box 1) of atmospheric CO_2 is currently about –8 parts per mil (‰) (Keeling and Whorf 2002). In contrast, commercial CO_2 originating from geologically ancient carbon (e.g., carbon from petroleum byproducts or wells) is often relatively depleted in ^{13}C, with $\delta^{13}C$ values typically ranging from –27‰ to –45‰. In most experiments with elevated CO_2, the increase in CO_2 concentration is accomplished by mixing commercial CO_2 with ambient air. As a result, short-term fluctuations in canopy CO_2 concentration above and below the targeted concentration may occur, and the concentration may vary from one place to another. Assessing actual exposure of plants to CO_2, therefore, becomes a significant challenge. A further complication is that $\delta^{13}C$ of the commercial CO_2 may vary if the source changes over time. Periodic sampling of the added CO_2 can track its isotopic composition, and subsequent modeling of CO_2 circulation in CO_2 plots can provide estimates of the average exposure of experimental plants to CO_2 and $^{13}CO_2$; however, the best way of measuring plants' CO_2 exposure over time may be to measure of isotopic ratios of carbon in C_4 plants (box 2; Pepin and Körner 2002). Such plants utilize a photosynthetic pathway in which the initial fixation of carbon occurs through the action of the enzyme phosphoenolpyruvate (PEP) carboxylase. This enzyme has a much lower isotopic fractionation factor than rubisco (ribulose bisphosphate carboxylase/oxygenase), the initial carbon-fixing enzyme for plants utilizing the more common C_3 photosynthetic pathway (O'Leary 1981, Farquhar et al. 1989); that is, PEP carboxylase discriminates less against ^{13}C when it fixes carbon. The isotopic composition of C_4 plants therefore changes less in response to factors that influence stomatal opening (such as light, temperature, and humidity) than that of C_3 plants, and its variation tracks the isotopic content of atmospheric CO_2.

This property of C_4 photosynthesis is being used in a Swiss experiment in which a mature temperate forest is fumigated with CO_2 from tubing distributed throughout the canopy. C_4 plants placed in pots throughout the canopy are periodically sampled to calculate the effective atmospheric CO_2 concentration from $\delta^{13}C$ of C_4 plant material (box 2; Pepin and Körner 2002). This method agrees well with estimates of average CO_2 level derived from measuring the CO_2 concentration in sampled air (figure 1) and greatly reduces the need for air turbulence modeling or rapid gas sampling devices while still providing spatial information about the CO_2 gradients that may occur in these experiments. Once the $^{13}CO_2$ exposure of experimental plants has been characterized, the ^{13}C label can then be used to follow the fate of experimentally added CO_2 in the ecosystem.

The carbon-14 label

If commercial CO_2 is derived from fossil sources, it contains no measurable ^{14}C, because it has effectively all decayed to nitrogen-14 (^{14}N) during its long isolation from the natural source of ^{14}C, the action of cosmic rays in the atmosphere and on Earth's surface. (The half-life of ^{14}C is 5730 years.) The absence of ^{14}C in fossil CO_2 provides a useful isotopic tracer that may show less temporal variation than the ^{13}C isotope ratio. When the ^{14}C-free commercial CO_2 is added to ambient air, ^{14}C concentrations in the air are diluted. This signal is present in the plots with elevated CO_2 in many completed and ongoing FACE experiments, but to date only one has attempted to take advantage of it. At the FACE experiment in Maricopa, Arizona, the ^{14}C-depleted CO_2 added to experimental plots resulted in cotton plants that contained only 70% of the ^{14}C activity of

Box 1. Isotopes in ecology

Isotopes are atoms of an element that differ in the number of neutrons. Heavier isotopes, having more neutrons, form stronger chemical bonds, have lower reaction rates in enzymatic reactions, diffuse more slowly, and are more often found in lower-energy phase states in equilibrium reactions, such as the phase between vapor and liquid water. These properties can be exploited to investigate physical and biochemical processes and are the foundation for stable isotope approaches in ecological studies. In addition, radioactive isotopes such carbon-14 (^{14}C) provide another tracer, particularly useful for dating biological material. Commonly used isotopes in ecology are given in table 1.

Isotopic composition is often expressed as a ratio between the heavier and the lighter isotope. Expression of the absolute ratio is impractical because of the large differences in natural abundance between isotopes of the same element (table 1), so it is more convenient to express an isotope ratio relative to a standard:

(1) $\delta = (R_{sample}/R_{standard} - 1) \times 1000‰$,

where R is the molar ratio of the heavy to the light isotope and ‰ is parts per mil.

Isotope ratios are expressed relative to common international standards, such as the PDB (Pee Dee Belemnite) formation, a standard for the ratio of carbon-13 (^{13}C) to carbon-12 with an R value of 0.0112372.

Isotopic composition can also be expressed as a discrimination (Δ) against heavy isotopes in the reaction between source and product:

(2) $\Delta = R_{source}/R_{product} - 1$.

To calculate Δ from δ:

(3) $\Delta = (\delta_{source} - \delta_{product})/(1 + \delta_{product}/1000)$.

In contrast, ^{14}C is expressed as an activity (concentration). Using conventional radiocarbon (gas proportional counting or liquid scintillation) or accelerator mass spectrometer ^{14}C dating methods, the ^{14}C activity is measured and expressed as percent modern carbon or as fraction modern carbon, in which, by convention, modern carbon activity is defined as the background activity in 1950 (Stuiver and Polach 1977). All activity measurements are corrected for any fractionation taking place in fixation by plants, soil processes, or laboratory procedures by normalizing to a standard $\delta^{13}C$ value of –25‰.

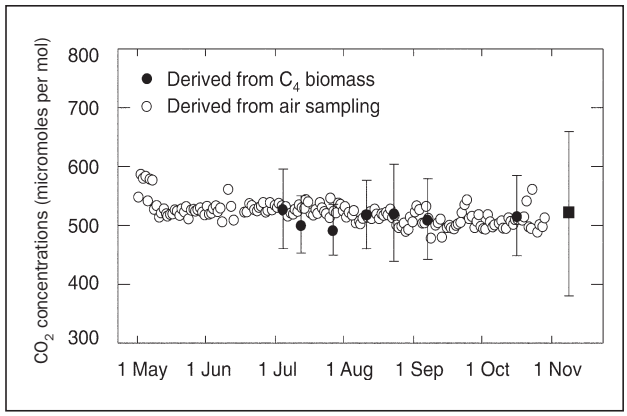

Figure 1. Determination of experimental carbon dioxide (CO_2) concentration at the Swiss Canopy Crane site using two methods: air sampling and the carbon isotope composition of C_4 plants placed in the canopy (± 1 standard deviation). The square on the right refers to the average daytime CO_2 concentration across all enrichment days. Source: Pepin and Körner (2002).

plants in ambient plots—a change that is easily detectable with current methods. Organic carbon pools in soil from plots exposed to elevated CO_2 were also depleted in ^{14}C compared with soil from plots exposed to ambient CO_2 (Leavitt et al. 1994). As with $\delta^{13}C$, any heterogeneity in plants and soils in the enriched plots induced by inhomogeneous broadcast of the commercial CO_2 will lead to unevenness in the distribution of the tracer. Furthermore, because ^{14}C is present in such small concentrations relative to ^{13}C, and because very small samples are used in ^{14}C analysis, effects of heterogeneity and contamination could be magnified in ^{14}C relative to those for ^{13}C.

The depleted ^{14}C signal present in studies of elevated CO_2 that used "dead" CO_2 from petrochemical sources should not be confused with the ^{14}C signal that was artificially generated during thermonuclear bomb testing and contributes to the current "natural" atmospheric background. This "bomb spike" created a background ^{14}C time marker centered in 1964, after which the atmospheric ^{14}C activity declined as the pulse was incorporated into the ocean, groundwater, plants, and soils. This time marker has been very helpful in understanding carbon cycling in soils, among other things (e.g., Dorr and Munnich 1986, Jenkinson et al. 1992, Trumbore 1993), quite apart from the value of ^{14}C in experiments that employ fumigation with tracer CO_2. If ^{14}C depletion in plots with elevated CO_2 is used as a tracer, it is with reference to current levels of atmospheric ^{14}C in ambient plots, which, because of atmospheric tests of thermonuclear bombs, are still above 1950 levels.

Resolving the fate of elevated carbon dioxide

Understanding the future distribution of fixed atmospheric carbon in the terrestrial biosphere is a major goal in many

Box 2. The Farquhar model of photosynthetic discrimination

Discrimination of carbon-13 (^{13}C) during photosynthesis in C_3 plants is caused by fractionation during diffusion through stomatal pores and fractionation by carbon-fixing enzymes, primarily rubisco (ribulose bisphosphate carboxylase/oxygenase). These effects were quantified by Farquhar and colleagues (1982):

(1) $\Delta^{13}C_P = a + (b - a) \times c_i/c_a$,

where c_i/c_a is the ratio of intercellular to ambient carbon dioxide (CO_2) partial pressure, a is the fractionation associated with diffusion (4.4 parts per mil [‰]), and b is the net enzymatic fractionation associated with carboxylation (27‰). Therefore, discrimination is a function of c_i/c_a, which is sensitive to a variety of factors that influence the balance of stomatal conductance and assimilation rate. Equation 1 (above) can also be combined with equation 3 from box 1 to predict the isotopic composition of biomass if the isotopic composition of the air is known.

For C_4 plants, we must separate fractionation factors for rubsico and phosphoenolpyruvate (PEP) carboxlyase. The latter fixes carbon in the mesophyll for transport into bundle sheath cells. In these cells, rubsico is physically isolated from the stomatal cavity. There is no fractionation associated with a product if all of the substrate is utilized. Therefore, we need only consider the fractionation factor for rubsico to the extent that CO_2 or HCO_3^- (hydrocarbonate) leaks out of bundle sheath cells back into the stomatal cavity. These effects were quantified by Farquhar (1983):

(2) $\Delta^{13}C_P = a + (b_4 + b_3\phi - a) \times c_i/c_a$,

where b_4 is the fractionation associated with PEP carboxylase (–5.7‰), b_3 is the fractionation associated with rubisco (30‰), and ϕ is the leakiness of the bundle sheath. For many species, the term $(b_4 + b_3\phi - a)$ is close to zero, such that $\Delta^{13}C$ may show little environmental variation in C_4 plants (Farquhar et al. 1989). In this case, variability in $\delta^{13}C_P$, which is related to $\Delta^{13}C_P$ (box 1, eq. 3), can be interpreted as a function of δ_{source} (see box 1). In using this approach, researchers should take care that ϕ has been characterized in the species of interest.

experiments with elevated CO_2. Carbon fixed in photosynthesis can be transferred into numerous ecosystem pools (figure 2). Will the increase in carbon fixed under elevated atmospheric CO_2 conditions be transferred into long-lived pools, or will the ecosystem carbon cycle merely be accelerated, resulting in no long-term net increase in stored carbon? Tracing the fate of isotopically distinct carbon fixed under elevated atmospheric CO_2 offers a unique means of resolving some of these uncertainties at the ecosystem scale.

Carbon inputs from isotopically labeled plants to the soils can be revealed by shifts in the isotopic composition of soil organic carbon. The larger the difference between the carbon isotopic composition of the plants and soils, the greater the potential for success in quantifying inputs and losses. This tracer signal has now been used in many FACE studies (Allison et al. 1983, Leavitt et al. 1994, 1996, Nitschelm et al. 1997, van Kessel et al. 2000, Leavitt et al. 2001, Schlesinger and Lichter 2001) and chamber studies (Hungate et al. 1997, Torbert et al. 1997) to determine the amount of new carbon that has been incorporated into soils during the course of an experiment. Such isotopic tracers, whether originating from ancient-source CO_2 introduced into experimental plots in FACE experiments or from contrived shifts between C_3 and C_4 vegetation that cause plants and soils to have distinct isotope ratios (the traditional approach), can then be further used to identify the specific organic carbon pools that new carbon has entered (Jastrow et al. 1996). One commonly cited limitation of this method in studies of elevated CO_2 is that there is typically no similarly strong isotope labeling in the control plots, so that comparing the difference in results between the elevated and control CO_2 treatments is not straightforward. This has been overcome by using

- small subplots of soils within the control plots whose carbon isotope composition reflects previous growth of C_4 plants and is therefore distinct from that resulting from currently growing C_3 plants (Allison et al. 1983, Ineson et al. 1996, Cheng and Johnson 1998, Leavitt et al. 2001)
- small subplots exposed to pulsed pure $^{13}CO_2$ within control plots (Hungate et al. 1997, Leavitt et al. 2001)
- carbon dioxide labeled with ^{13}C or ^{14}C and used to fumigate entire control plots in chamber experiments (Lin et al. 1999, 2001)
- the small but quantifiable natural difference between ^{13}C in control plants and in the local soil organic carbon (Nitschelm et al. 1997)

All of these methods distinguish between the newly fixed carbon in control plots and the existing soil carbon in order to follow the transfer of new carbon into various soil pools. In this way, the allocation of new carbon in control plots can be compared to the fate of new carbon in the elevated CO_2 treatment. It should be noted that some of these methods

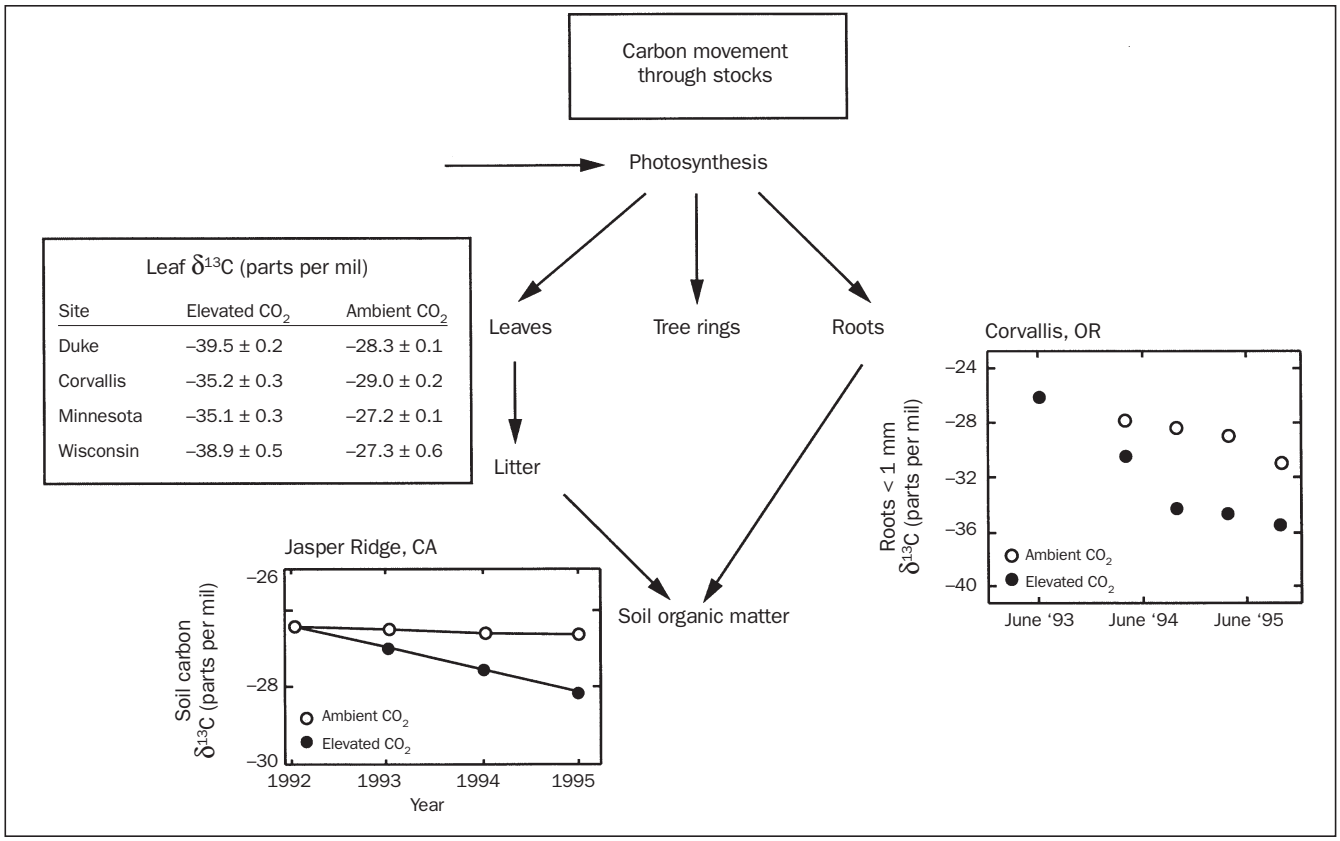

Figure 2. Schematic of ecosystem carbon transfer through various pools, with examples of the carbon isotope ratio ($\delta^{13}C$) of carbon pools from experiments on the effects of elevated carbon dioxide (CO_2). Data from Hungate and colleagues (1997) and Lin and colleagues (2001).

require the application of different experimental methods in the elevated CO_2 treatments and the control, so results should be interpreted with care.

Many experiments with elevated CO_2 have shown increased photosynthetic gain and larger biomass under elevated CO_2 conditions than under ambient conditions (Acklerly and Bazzaz 1995, Drake et al. 1997, DeLucia et al. 1999). However, another common response is an increase in respiratory losses of CO_2 from the soil (Zak et al. 2000). A critical element in understanding the significance of increased soil respiration is the partitioning between the autotrophic (plant-derived) and heterotrophic (soil organism–derived) origin of the respired carbon. When plants are larger, a proportional increase in autotrophic respiration is expected as a result of increased root biomass. Increased heterotrophic respiration may, however, be an indicator of an accelerated carbon cycle, in which plant carbon inputs are quickly decomposed after deposition as litter or senesced roots. Stable isotopes are increasingly being used to distinguish the origins of soil fluxes (Rochette and Flanagan 1997, Cheng and Johnson 1998, Andrews et al. 1999, Lin et al. 1999, 2001, Pendall et al. 2001) and provide an alternative to traditional harvest or disturbance methods (Edwards and Norby 1999, Högberg et al. 2001). It should be noted that exact partitioning of respiration between heterotrophic and autotrophic components is not possible with a single isotopic label, as the microbial community immediately surrounding the roots (the rhizosphere) may rapidly acquire the isotopic signature of labile root exudates. However, the substrate-induced respiration method, in which sugar derived from C_4 plants is applied to soil in an ecosystem containing C_3 plants (Högberg and Ekblad 1996, Ekblad and Högberg 2001), provides good estimates of the relative contributions of heterotrophic and autotrophic respiration, as autotrophic respiration will have a C_3 carbon isotope ratio distinct from microbial decomposition of the C_4 sugar. Furthermore, the use of C_4 plants and elevated CO_2 isotope tracers allows partitioning of the soil CO_2 flux into "old," or pre-label, and "new," or current growing season, components (e.g., Pendall et al. 2001).

The elevated $^{13}CO_2$ label has been used at the Duke FACE experiment in Durham, North Carolina. In this pine forest, soil respiration has increased in response to elevated CO_2 (Andrews and Schlesinger 2001). In 1998, soil respiration increased by about 25%, or 220 grams (g) of carbon per square meter (m^2) per year, in the elevated CO_2 treatments. The heterotrophic component of this flux constituted an increase of 80 g carbon per m^2 per year, which corresponds well with the observation of Högberg and Högberg (2002). According to the isotopic results, about 70% of the total increase in soil respiration in the plots with elevated CO_2 originated from carbon that was fixed since the beginning of the experiment in September 1996. Although the absence of an

isotope tracer prevents the use of a comparable partitioning approach in the control plots, these results are a clear indication that much of the extra carbon fixed under elevated CO_2 conditions may be transferred to short-lived carbon pools that are quickly returned to the atmosphere.

With the use of tracers in control plots, experiments using closed-system mesocosms have found that elevated atmospheric CO_2 stimulates decomposition of recently fixed carbon but suppresses decomposition of older soil organic matter (Lin et al. 2001), particularly in the presence of high nutrient availability (Calder et al. 1992, Cheng and Johnson 1998, Cardon et al. 2001). In the shortgrass steppe open-top chamber (OTC) experiment in Colorado, a change in the proportion of C_3 and C_4 plants, which occurred before the experiment began, caused currently growing plants and soil organic matter from previous vegetation to attain distinct isotope ratios. This has allowed researchers to apply stable isotopes to distinguish between root and rhizosphere respiration and heterotrophic decomposition in three treatments: chambers with elevated CO_2, chambers with ambient levels of CO_2, and nonchambered control plots. In this experiment, elevated CO_2 resulted in doubled decomposition rates but no change in root and rhizosphere respiration relative to ambient conditions (Pendall et al. 2003). In an OTC experiment on a California annual grassland, Hungate and colleagues (1997) found increases in both rhizosphere and heterotrophic respiration under conditions of elevated CO_2, with the enhancement of heterotrophic respiration originating primarily from the decomposition of senesced roots.

These experiments illustrate both the importance of examining changes in the whole-ecosystem carbon cycle in response to elevated CO_2 and the utility of carbon isotopes to serve as valuable tracers. Although plant biomass often increases under elevated CO_2, isotopic methods are providing a unique insight into changes in decomposition rates and the balance of autotrophic and heterotrophic respiration. In general, isotopic evidence points toward greater heterotrophic respiration rates under elevated CO_2. In many studies, this increase has been attributable to greater decomposition of recently fixed labile carbon and to stimulation of microbial activity such that older carbon pools are also utilized. These results have important implications for the long-term sequestration of carbon in the terrestrial biosphere.

Quantifying the turnover rate of carbon in ecosystems

Quantifying the mean residence time of carbon in various ecosystem pools is a principal goal in several areas of carbon cycle research, particularly with regard to belowground fractions of biomass and soil organic matter (Randerson et al. 1999). In this regard, isotope analyses in experiments on elevated CO_2 provide an opportunity to advance our understanding of ecosystem function. These experiments are analogs of labeling studies previously conducted in small greenhouses or closed chambers. It is now possible to understand the time scales of carbon transfer through belowground carbon pools in whole-ecosystem manipulations using labeled CO_2.

In the Duke FACE experiment, within one week of the initiation of fumigation with depleted $^{13}CO_2$, the soil CO_2 efflux in experimental plots became isotopically depleted in ^{13}C relative to the plots with ambient levels of CO_2 (Andrews et al. 1999), providing unequivocal evidence of the rapid carbon fluxes between aboveground and belowground components. This ^{13}C depletion reflects transport of recently fixed carbon below ground, which caused a corresponding change in the isotope ratio of autotrophic respiration. Within a year of the initiation of fumigation, root-free soil showed ^{13}C depletion in the CO_2 efflux, which must have been derived from the heterotrophic component of respiration. Yet after 32 days of incubation with no further supplemental carbon, the ^{13}C-depleted respiration signal returned to values similar to those of soil from ambient plots. This indicated that the newly fixed carbon had been transferred to labile soil organic matter pools that were rapidly exhausted in the absence of continuous inputs (figure 3). These and other results support recent studies of naturally occurring ^{13}C that show correlations between $\delta^{13}C$ of soil-respired and ecosystem-respired CO_2 and environmental factors such as humidity and vapor pressure deficit (Ekblad and Högberg 2001, Bowling et al. 2002), illustrating that a large portion of respired carbon was recently fixed. These isotopic studies allow further delineation of the carbon cycle and provide strong evidence for rapid carbon cycling at the level of short-term photo-

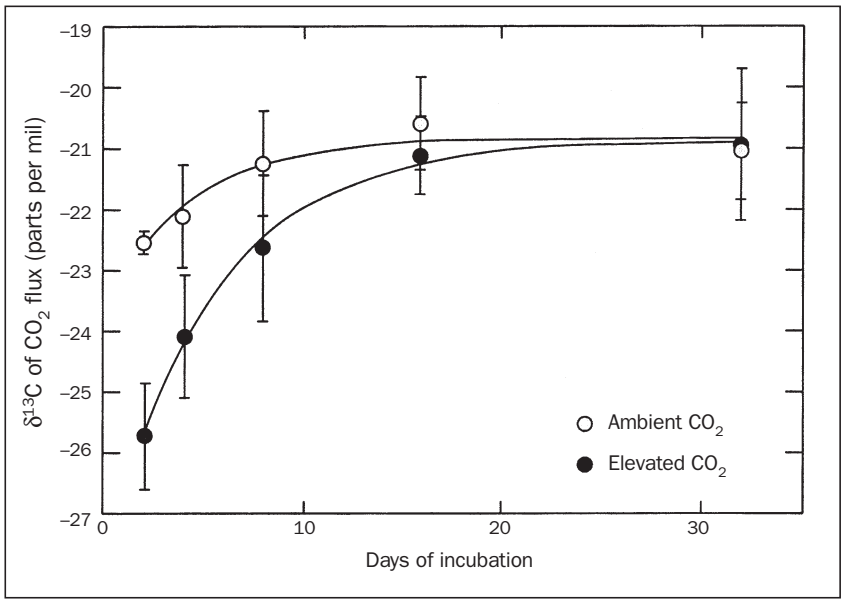

Figure 3. The carbon isotope ratio ($\delta^{13}C$) of soil carbon dioxide (CO_2) flux during root-free incubation of soil from the Duke Forest FACE (free-air CO_2 enrichment) experiment. Source: Andrews and colleagues (1999).

synthesis and respiration. In addition, analyses of naturally abundant radiocarbon in a number of ecosystems have indicated that the carbon evolved in soil respiration has generally been fixed much more recently than the carbon in bulk soil organic matter (Trumbore 2000). Soil CO_2 flux appears to contain a large proportion of carbon from live roots and from decomposition of recently deposited, labile organic matter.

Precise determination of root turnover rates is difficult in the field, although it has been recognized that root turnover is likely to be a major component of soil carbon inputs (Jackson et al. 1996). The ^{13}C-depletion signature used to trace the added carbon in FACE experiments can also be used to determine turnover rates of carbon in different pools, including roots of different class sizes. The mean residence time of carbon can be calculated from an exponential decay function by monitoring the remaining $\delta^{13}C$ values and biomass of a given root size class over the course of a long-term CO_2 fumigation experiment (table 2). Results from this isotopic approach have indicated that the mean residence time of carbon in roots in the Duke FACE experiment varied from 4 to 9 years depending on root size class and treatment. These root ages are much greater than previously estimated with other techniques (Matamala and Schlesinger 2000). Yet the estimated turnover rates based on ^{13}C measurements are close to root age estimates based on another isotopic approach, the natural abundance of ^{14}C (Gaudinski et al. 2001). Isotopic methods appear to suggest much longer fine-root turnover times than traditional methods. Luo (2003) suggests that the influence of older, pretreatment carbon reserves on the isotope ratio of fine roots could account for much of this discrepancy, although conventional methods of estimating root production and turnover (such as periodic soil sampling or the use of belowground cameras) are also subject to significant uncertainty (Lauenroth 2000). This issue merits further investigation because of the important implications for ecosystem carbon balance. For example, at the Duke FACE site, if carbon has long mean residence times in live roots, root turnover cannot explain the observed increase in soil respiration under elevated CO_2. Isotopic techniques thus suggest that other rhizosphere processes, such as microbial decomposition of root exudates, increased mycorrhizal activity, and decomposition of very fine root tips, must be a significant component of the observed increase in respiration of new carbon under elevated CO_2.

Carbon and nitrogen interactions

Ecosystem carbon and nitrogen cycles are tightly integrated, and isotope-based approaches can help elucidate changes under elevated CO_2 conditions. Organic material in the form of roots, litter, and woody debris is decomposed by the soil microbial community and releases mineral nitrogen available for plant uptake in the form of ammonium and nitrate (nitrogen mineralization). In temperate

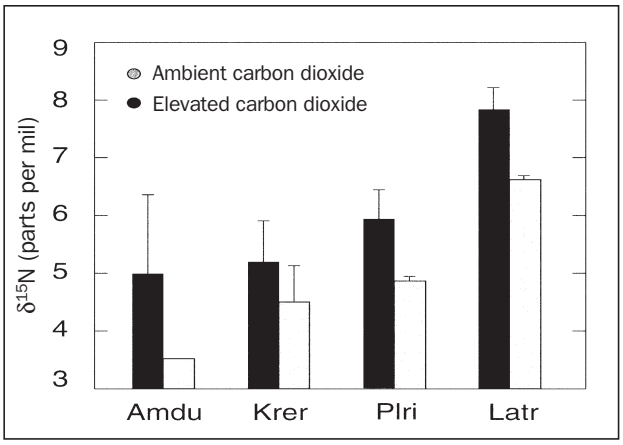

Figure 4. The nitrogen isotope ratio ($\delta^{15}N$) of leaves of* Ambrosia dumosa *(Amdu),* Krameria erecta *(Krer),* Pleuraphis rigida *(Plri), and* Larrea tridentata *(Latr) at the Nevada Desert FACE (free-air carbon dioxide enrichment) facility, September 1999. Modified from Billings and colleagues (2002).

ecosystems, the availability of nitrogen is often a limiting factor for plant growth. To sustain increased growth under elevated CO_2 in these ecosystems, plants must either increase their nitrogen use efficiency (carbon fixed per unit of plant nitrogen) or increase their uptake of nitrogen. Increased nitrogen uptake may result from greater uptake of existing mineral nitrogen, from increased rates of mineralization, or from increased fixation by symbiotic or free-living organisms that can utilize nitrogen from the atmosphere and make it available to plants.

The number of possible alterations to the nitrogen cycle that could occur under elevated CO_2 introduces uncertainty into interpretations of experimental results. It is essential to determine whether short-term observations of increased plant growth under elevated CO_2 can be sustained in the future. This question hinges in part on an understanding of carbon and nitrogen interactions. To examine these interactions, ecologists have studied variations in naturally occurring levels of nitrogen-15 (^{15}N) and conducted experiments using artificially applied ^{15}N. These studies present considerable opportunities for understanding carbon–nitrogen interactions in experiments with elevated CO_2.

Table 2. Mean residence times of fine roots in the Duke Forest FACE (free-air carbon dioxide enrichment) site, estimated using carbon-13 and carbon-14 isotope tracers.

	Mean residence times of fine roots	
Fine root class	**Ambient carbon dioxide**	**Elevated carbon dioxide**
< 1 mm	4 to 5 years	4.2 years
1 to 2 mm	6 to 7 years	5.9 years
> 2 mm	8 to 9 years	6.0 years

Note: Incorporation of the isotope tracer into a given root size pool is measured as the quantity of roots that have the initial isotope ratios as a function of time fitted to an exponential function ($F[t] = e^{-kt}$, mean residence time = $1/k$ years).

Lessons from natural abundance of nitrogen-15. The nitrogen isotope ratio ($\delta^{15}N$) is a useful indicator of change in ecosystem nitrogen cycles. The $\delta^{15}N$ of plant-available nitrogen is a function of several variables, including nitrogen inputs into the ecosystem, $\delta^{15}N$ of substrates used by soil microbial populations, rates of microbial transformations, gaseous losses from the soil, and changes in active plant rooting depths. Changes in any of these factors as a result of growth under elevated CO_2 should alter the $\delta^{15}N$ of nitrogen available to plants and subsequently the $\delta^{15}N$ of the plants themselves. Therefore, a significant shift in plant $\delta^{15}N$ can be a conclusive indicator of changes in the ecosystem nitrogen cycle long before small shifts in the overall nitrogen content of different ecosystem components can be detected.

Recently, a large shift in plant $\delta^{15}N$ under simulated conditions of elevated CO_2 was observed at the Nevada Desert FACE facility. Billings and colleagues (2002) documented a 3‰ increase in $\delta^{15}N$ of the dominant shrub *(Larrea tridentata)* following exposure to elevated CO_2 and seasonal shifts of 2‰ for shrubs grown under both ambient and elevated CO_2 conditions. In September 1999, four shrub species showed enrichment in $\delta^{15}N$ under conditions of elevated CO_2 relative to ambient conditions (figure 4). No significant differences were observed in root growth and water sources between the two treatments. Moreover, plant $\delta^{15}N$ was actually greater than soil $\delta^{15}N$, suggesting enrichment of ^{15}N in the pool of nitrogen available to plants as a result of mineralization. This could be caused by an increase in gaseous nitrogen loss or by immobilization in microbial biomass, because both processes are accompanied by significant fractionation, that is, preferential loss of ^{14}N and enrichment of the remaining pool in ^{15}N (Högberg 1997). The shift in plant $\delta^{15}N$ and other supporting evidence in the Nevada study strongly suggest an increase in microbial activity under conditions of elevated CO_2 associated with the greater inputs of organic substrate available for decomposition.

The numerous transformations that occur in the nitrogen cycle prohibit ^{15}N at natural abundance levels from being used as a tracer of nitrogen movement in ecosystems (Evans 2001, Robinson 2001). One exception is that ^{15}N discrimination is not observed during plant uptake of inorganic nitrogen at typical soil concentrations; therefore, whole-plant $\delta^{15}N$ will accurately trace that of the source if plants absorb ammonium or nitrate (Mariotti et al. 1982, Yoneyama and Kaneko 1989, Evans et al. 1996). A complication is that ^{15}N fractionation may occur during mycorrhizal uptake and transfer of nitrogen to the host, but ^{15}N discrimination will not be observed if most of the nitrogen absorbed by the fungus is transferred to the host (Hobbie et al. 1999).

Lessons from tracer nitrogen-15. An alternative approach to the use of natural abundance nitrogen isotopes is to study nitrogen cycling with the application of an artificial ^{15}N-enriched tracer. An important consideration is that the application of an artificial tracer excludes the possibility of subsequent studies of natural abundance, even if the tracer is applied to a small subsection of the experimental plot. Artificial ^{15}N tracers are far more enriched in ^{15}N than naturally occurring organic material, with ^{15}N concentrations that are 1000 to 10,000 times higher. It is difficult to avoid contamination under these circumstances.

One of the strengths of the ^{15}N tracer technique is the possibility of constructing a complete ^{15}N budget by following the tracer into the various plant and soil pools. This provides an opportunity to address the question of whether elevated CO_2 leads to greater nitrogen use efficiency of plants, increased available nitrogen, or decreased nitrogen losses from the plant–soil system. Increases or decreases in nitrogen availability could occur depending on whether elevated CO_2 stimulates or suppresses soil organic matter decomposition. A number of studies have assessed how plant nitrogen use and total ^{15}N losses have been altered under elevated CO_2 conditions. Losses of applied ^{15}N were found to be slightly lower under elevated CO_2 in a microcosm study when the grass *Danthonia richardsonii* was grown under a high level of applied mineral nitrogen (Lutze and Gifford 1998). A FACE experiment that made use of ^{15}N-labeled fertilizer indicated that elevated CO_2 concentration had no significant effect on total ^{15}N losses (soil and plant) in swards of *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) after 4 years of CO_2 enrichment (Hartwig et al. 2002). These studies suggest that the total nitrogen in the

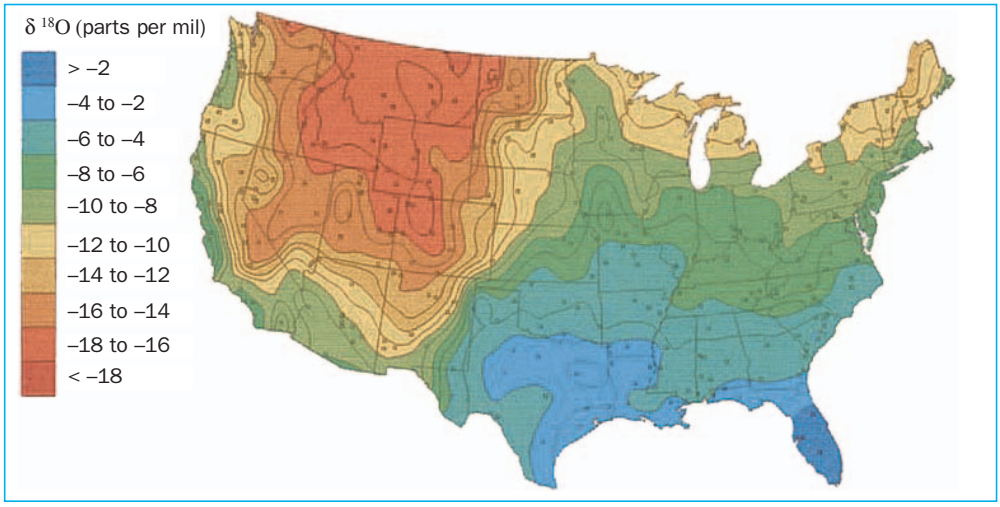

Figure 5. Isolines of the oxygen isotope ratio ($\delta^{18}O$, parts per mil) in precipitation (relative to Vienna Standard Mean Ocean Water) in the United States. Source: Kendall and Coplen (2001).

plant–soil system is not altered under elevated CO_2 in these ecosystems. Moreover, in the *Lo. perenne* system after 8 years of elevated and ambient CO_2 concentrations, ^{15}N enrichments were similar in the various soil organic matter fractions, showing that the soil organic matter–nitrogen dynamics were unaffected by prolonged exposure to elevated CO_2 (van Groenigen et al. 2002).

Nitrogen fixation plays a key role in the overall nitrogen cycle, and the total input in terrestrial ecosystems is estimated at around 100 teragrams per year (Mosier 2001). Estimates for molecular nitrogen (N_2)–fixing activity in the field are based on the dilution of ^{15}N in N_2-fixing legumes compared with non-N_2-fixing reference plants. Using the ^{15}N dilution approach, the percentage of nitrogen derived from N_2 fixation by *T. repens* grown under elevated CO_2 (FACE) conditions increased by 8% over a 3-year period (Zanetti et al. 1996). Similar findings were reported for free-living, N_2-fixing bacteria in stands of a C_3 sedge and a C_4 grass grown under elevated CO_2 (Dakora and Drake 2000). It will be of interest to determine whether the increase in N_2 fixation under elevated CO_2 conditions will be sustained. Once the feedback mechanisms between the demand of nitrogen by the plant, available soil nitrogen, and N_2-fixing activity have reached a new equilibrium under elevated CO_2, the increase in N_2 fixation may subside.

Integrating altered water balance

In water-limited ecosystems, water availability has been shown to be the critical variable controlling plant growth responses under elevated CO_2 (Mooney et al. 1999). Elevated CO_2 can improve plant water relations, increase soil water availability, and indirectly alter organic matter decomposition, nutrient cycling, and carbon storage. In addition, alterations to evapotranspiration and biosphere–atmosphere exchange of water vapor may have important implications for climate and the hydrologic cycle. Stable isotopes of carbon, oxygen, and hydrogen record the influence of environmental conditions and plant water sources on plant function. Isotopic water balance studies take advantage of the large environmental gradients in the isotopic composition of precipitation and soil water. Such gradients provide natural tracers that avoid the need for artificial labeling (figure 5). Unlike short-term measurements of leaf gas exchange and temperature, isotopic methods may be used to study long-term changes in plant energy and water balance.

Lessons from carbon isotopes. The carbon isotope ratio of plant organic matter ($\delta^{13}C_p$) is an integrated measure of photosynthetic discrimination ($\Delta^{13}C$) during CO_2 fixation. The parameter $\Delta^{13}C$ takes into account variations in $\delta^{13}C$ of source air ($\delta^{13}C_{source}$; box 1) in the formation of plant material. It is particularly important to take into account $\delta^{13}C_{source}$ in studies of elevated CO_2, in which $\delta^{13}C_{source}$ is often significantly different from ambient levels. When effects of $\delta^{13}C_{source}$ are removed, $\Delta^{13}C_p$ in C_3 plants is a function of the ratio of the partial pressures of CO_2 inside and outside the leaf (c_i/c_a; box 2). This ratio reflects the balance between CO_2 diffusion into the leaf as regulated by stomatal conductance and CO_2 removal as regulated by the photosynthetic rate. Therefore, the c_i/c_a ratio can be considered an integrated set point of plant gas exchange (Ehleringer and Cerling 1995). By applying estimates of $\delta^{13}C_{source}$ and measurements of $\delta^{13}C_p$ to estimating c_i/c_a integrated over time in studies of elevated CO_2, we can assess the effects of elevated CO_2 on this set point, that is, how stomatal regulation of photosynthetic gas exchange has responded to a higher-CO_2 environment.

Short-term, instantaneous leaf-level estimates of c_i/c_a can be obtained with traditional gas exchange measurements. While several studies have reported no short-term changes in instantaneous c_i/c_a under elevated CO_2 (see Morison 1985, Drake et al. 1997), longer-term measurements using $\delta^{13}C_p$ to estimate assimilation-weighted c_i/c_a values have suggested otherwise. One of the authors (G. L.) estimated c_i/c_a values from plants grown under elevated CO_2 in a variety of ecosystem facilities, including chambers, micro- and mesocosms, FACE experiments, and CO_2 springs, and concluded that about two-thirds of studies examined showed significant increases in the c_i/c_a ratio under elevated CO_2, while the rest showed no change in c_i/c_a. This is a key observation, because long-term increases in c_i/c_a are associated with increased water-use efficiency, defined as the ratio of carbon fixed to water lost (Farquhar et al. 1989). These ^{13}C measurements will not only help resolve uncertainty in physiological changes in response to rising CO_2 concentrations over the long term but also improve parameterization of ecological models, for which c_i/c_a is often a critical variable (see Katul et al. 2000).

Lessons from oxygen and hydrogen isotopes. The isotopic composition of plant stem water reflects the isotopic composition of the plant's water source, because, with the exception of several halophytes (Lin and Sternberg 1993), there is no fractionation against the heavy hydrogen isotope deuterium and oxygen ($\delta^{18}O$) isotopes during plant water uptake (White et al. 1985, Dawson and Ehleringer 1991, Lin and Sternberg 1993). However, because of evaporative effects, leaf water becomes isotopically enriched in ^{18}O by 15‰ to 30‰ and in deuterium by 40‰ to 60‰ relative to stem water (box 3). In the leaf, oxygen in CO_2 readily exchanges with oxygen in liquid water, a rapid reaction in the presence of the plant enzyme carbonic anhydrase. Because plant leaves contain much more water than CO_2, the $\delta^{18}O$ of CO_2 takes on the $\delta^{18}O$ value of leaf water. Farquhar and colleagues (1993) and Farquhar and Lloyd (1993) have shown how measurements of the $\delta^{18}O$ values of leaf water and CO_2 can be used to estimate gross photosynthetic rates. In addition, organic matter fixed within leaves (i.e., sugars and cellulose) records the enriched $\delta^{18}O$ values of leaf water. Because ^{18}O and deuterium have similar applications and are subject to similar mechanisms of evaporative enrichment, we will restrict our discussion below to ^{18}O for convenience.

Models predicting the isotopic composition of leaf water and plant cellulose are shown in box 3. These

Box 3. Models of fractionation in plant water isotopes

Although there is no fractionation in plant water uptake or transport (White et al. 1985), leaf water is isotopically enriched relative to the plant water source because of evaporation. To quantify this effect, plant leaves can generally be treated as evaporative pools of water. However, unlike large water reservoirs, leaves have a high ratio of evaporative flux to water volume and have nearly equal amounts of water entering from the xylem and leaving from evaporation. Under these steady-state conditions, we can predict the isotopic composition of leaf water at the site of evaporation with a simplified version of the model given by Craig and Gordon (1965):

$$(1) \qquad \delta_{Ls} = \delta_s + \varepsilon_{eq} + \varepsilon_k + (\delta_a - \delta_s - \varepsilon_k) \times e_a/e_i,$$

where δ stands for $\delta^{18}O$; the subscript a is ambient water vapor; ε_{eq} is the equilibrium fractionation, which is temperature dependent (Majoube 1971); ε_k is the kinetic fractionation of water, which depends on the molecular diffusion of ^{18}O in air and the aerodynamic nature of the boundary conditions (Merlivat 1978); and e_a/e_i is the ratio of the partial pressure of water vapor outside and inside the leaf (Farquhar and Lloyd 1993, Yakir 1998). The ^{18}O signal from leaf water at the site of evaporation is transferred to organic matter (mostly cellulose) via biosynthesis from sucrose and starch with an averaged oxygen fractionation value of 27‰ (DeNiro and Epstein 1979, Sternberg et al. 1989, Yakir 1992).

Although equation 1 (above) is useful for predicting the isotopic composition of water at the site of evaporation, it is often inadequate to predict the isotope ratio of bulk leaf water. Not all parts of the leaf are equally exposed to evaporation, and significant spatial heterogeneity within leaves has been found (Yakir et al. 1989, Luo and Sternberg 1992, Yakir et al. 1994, Helliker and Ehleringer 2000). Some of this heterogeneity may be caused by patchiness (Terashima et al. 1988), leaf compartmentalization (Yakir et al. 1993), and incomplete mixing away from the site of evaporation at the cell walls (Farquhar and Lloyd 1993). To incorporate the effect of the decay of the ^{18}O enrichment signal between the surface of evaporation and the isotopic signature of the source water entering the leaf, Farquhar and Lloyd (1993) proposed

$$(2) \qquad \delta_{LW} = \delta_s + (\delta_{Ls} - \delta_s) \times (1 - e^{-P}) / P,$$

as modified by Yakir (1998), where δ_{LW} is the $\delta^{18}O$ bulk leaf water, δ_{Ls} in equation 1, and P is the Péclet number. P is related to the transpiration rate (moles per square meter per second) and the effective maximal mixing path length as well as physical constants. An alternative approach to estimating transpiration rate to determine δ_{LW} has been proposed by Roden and Ehleringer (1999), who apply best-fit empirical coefficients to leaf water mixing.

equations can be used to interpret the effects of elevated CO_2 on $\delta^{18}O$ of bulk leaf water, which may occur as a result of several possible changes in plant function and canopy structure. Leaf water of plants grown under elevated CO_2 can be expected to become enriched in $\delta^{18}O$ if transpiration is reduced (box 3, eq. 2), and even more so if this reduction causes an increase in leaf temperature and therefore an increase in the leaf saturation vapor pressure (box 3, eq. 1). Conversely, reduced enrichment is expected if transpiration increases under elevated CO_2 because of increased plant size and rooting volume (box 3, eq. 2).

Preliminary results from a Swiss forest experiment showed that leaves of deciduous trees exposed to elevated CO_2 were enriched in ^{18}O relative to those grown under ambient CO_2. However, the humidity, leaf temperature, and air temperature in the canopy under elevated CO_2 did not differ significantly from ambient conditions. Thus, it is likely that reduced transpiration under elevated CO_2 caused the enrichment in $H_2{}^{18}O$ (see box 3, eq. 2). Cooper and Norby (1994) found species-specific effects on $H_2{}^{18}O$ enrichment: Both *Quercus alba* and *Liriodendron tulipifera* showed reductions in transpiration under elevated CO_2, but only *Li. tulipifera* showed ^{18}O-enriched leaf water and leaf cellulose (figure 6). These results demonstrate that subtle changes in water and energy balance, which may be difficult to detect with traditional meteorological and physiological measurements, may occur in plants grown under elevated CO_2. Isotopic approaches allow detection of the integrated effects over time of changes in the isotopic composition of leaf water and organic components. In addition, these studies have consequences for the interpretation of changes in isotope ratios in long-term data sets, such as tree ring data, that encompass historical changes in atmospheric CO_2 concentrations.

A mechanistic assessment of the effects of elevated CO_2 on water fluxes is facilitated by the use of stable isotopes in soil water. Soil water near the surface undergoes evaporation and becomes ^{18}O enriched, whereas water from deeper layers is unchanged isotopically from precipitation inputs. Since plants do not fractionate soil water during uptake, and water vapor leaving the leaf is in isotopic equilibrium with stem water, water evaporating from soil and water transpired by plants often have isotopically distinct compositions. This difference affords a means of comparing the magnitudes of the soil and plant components of evapotranspiration.

This approach was applied at the Colorado OTC experiment, which had as its subject a perennial grassland. In this study, the oxygen isotope ratio of soil CO_2 was used as a proxy for $\delta^{18}O$ of soil water. Measurements of $\delta^{18}O$ in soil

CO_2, soil water, and precipitation, as well as the amount of precipitation, were used in a mass balance model to assess evaporation and transpiration (Ferretti et al. 2003). Soil water $\delta^{18}O$ in chambers with ambient CO_2 was more enriched than in chambers with elevated CO_2. The mass balance showed that this enrichment was in part a consequence of year-to-year variability in the effects of elevated CO_2 on ecosystem water balance. During an unusually dry growing season, transpiration in chambers with elevated CO_2 was higher than in those with ambient CO_2, reflecting the larger plant biomass under elevated CO_2 conditions. In years of average precipitation, however, transpiration was similar under both treatments. Despite these differences, transpiration use efficiency, defined as the amount of biomass fixed per unit of transpiration, was greater in chambers with elevated CO_2 in dry and normal years. These results illustrate a unique application of stable isotope tracers to understanding changes in ecosystem water use under elevated CO_2. Such applications could be particularly valuable in light of the difficulties in distinguishing the components of evapotranspiration with nonisotopic methods.

Trophic-level interactions

Changes in plant tissue composition and chemistry induced by elevated CO_2 have important implications not only for carbon and nutrient cycles but also for interactions between plants and communities of organisms at higher trophic levels. Although there have been few studies of the effects of elevated CO_2 on fauna, effects on leaf carbon-to-nitrogen (C:N) ratios, and on the levels and amounts of the secondary metabolic compounds that deter herbivory, have been shown to change leaf consumption by herbivorous insects and consequently the insects' growth rate (Lindroth 1996a, 1996b). There is considerably less information available on the effects of elevated CO_2 on the chemistry of fine roots and root exudates, but such changes have been hypothesized to have a strong influence on soil microbial community dynamics and subsequently on the processes of nitrogen mineralization and immobilization that control the availability of nitrogen to plants (Zak et al. 2000).

The isotopic composition of animals reflects that of their food source when tissue-dependent metabolic fractionation effects have been accounted for (DeNiro and Epstein 1978, Tiezen et al. 1983, Focken and Becker 1998). Therefore, stable carbon isotopes have been used extensively in studies of terrestrial, aquatic, and marine food webs (Rounick and Winterbourn 1986). Carbon dioxide fumigation with a highly ^{13}C-depleted source creates a unique isotopic signature in plant material, which can be traced to higher trophic levels. This provides a unique opportunity to evaluate changes in diet in a high-CO_2 environment.

In a novel application of this idea, Cotrufo and Drake (2000) collected litter from several FACE experiments with contrasting $\delta^{13}C$ values of approximately –27‰ in plots with ambient levels of CO_2 and –40‰ in plots with elevated levels of CO_2. Litter from plots with elevated CO_2, litter from plots with ambient CO_2, and a 50–50 mixture of the two were then inoculated with isopods and incubated. The CO_2 respired from each incubation was collected and analyzed for ^{13}C. The results showed that CO_2 respired from the 50–50 mixture had a carbon isotope ratio of 34‰, an amount intermediate between the ratios in CO_2 evolved from litter from plots with ambient and with elevated CO_2. The results indicate that the detritivores showed no preference for litter from plots with ambient CO_2 over litter from plots with elevated CO_2. This finding contrasts with hypotheses based on the commonly observed increase in leaf C:N ratios under elevated CO_2. These hypotheses propose that decomposition should occur more slowly in litter with a higher C:N ratio, reducing the rate of litter decomposition under elevated CO_2 (Strain and Bazzaz 1983). However, the results were consistent with a recent meta-analysis that found no overall CO_2 effect on litter decomposition rates from the range of results reported to date (Norby et al. 2001), despite the differences in live leaf C:N ratios and the increased levels of secondary compounds, both of which affect herbivory, in plants grown under elevated CO_2. Similar approaches may be used to understand herbivore preferences for plant material grown under elevated CO_2 and ambient conditions.

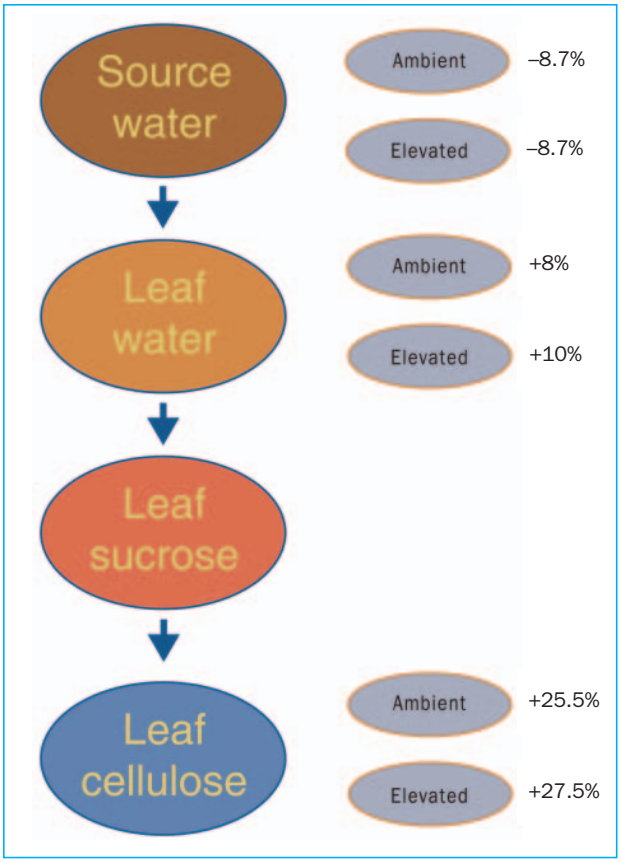

Figure 6. Oxygen isotope ratios of* Liriodendron tulipifera *and its source water (relative to Vienna Standard Mean Ocean Water) grown under elevated (+300 parts per mil) and ambient concentrations of carbon dioxide. Data from Cooper and Norby (1994).

In addition to the CO_2 fumigation tracer, other labeling techniques have been used to examine the flow of carbon into the soil microbial community under elevated CO_2. One such technique is pulse labeling with ^{14}C. In this technique, plants are exposed to a brief pulse of CO_2 containing elevated ^{14}C. In an experiment on the effects of elevated CO_2 on crops, pulse labeling showed that high CO_2 caused reduced allocation of carbon into the microbial carbon pool, probably the result of a change in the types and amounts of carbon compounds available to microbes under elevated CO_2 (Patterson et al. 1996). It is also likely that alterations in the amount and composition of carbon deposited to the soil can affect microbial community composition as well as biomass, but this has been difficult to assess. Radajewski and colleagues (2000) reported that taxonomic groups with distinct metabolic processes could be distinguished by supplying a ^{13}C-enriched substrate to the soil. Microbes that utilize the substrate incorporate the isotope into their DNA, and density-gradient centrifugation can then be employed to separate DNA containing the added ^{13}C isotope from DNA without the added isotope. Sequence analysis of the DNA thus separated can identify the phylogenetic group that metabolizes a particular substrate. This technique holds promise for evaluating shifts in microbial community composition in response to environmental perturbations such as elevated CO_2. Thus, a variety of isotopic tracers can be used to evaluate hypothesized shifts in plant–animal interactions and microbial food webs resulting from changes in plant chemistry.

Conclusions

Carbon, nitrogen, oxygen, and hydrogen isotope ratios at natural abundance levels provide valuable tracers for integrated analyses of terrestrial ecosystems in experiments with elevated levels of CO_2. The additional CO_2 supplied in these experiments provides a useful label that can be used to trace carbon flows and turnover rates in different pools, both under elevated CO_2 treatment conditions and after supplemental CO_2 is withdrawn. In experiments in which isotope ratio mass spectrometry is not available, dry biomass and soil samples can be archived for future isotopic analysis. Frozen leaf and soil samples or, if possible, water extracted and stored in sealed vials can provide valuable information about the effect of elevated atmospheric CO_2 on leaf water budgets and possibly on leaf energy balance. Linking these stable isotope analyses with traditional methods in elevated-CO_2 ecosystem studies provides a unique opportunity for tracing short-term and long-term changes in the carbon cycle and their impacts on key ecosystem biogeochemical cycles as well as trophic-level interactions.

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