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PI – David Ellsworth

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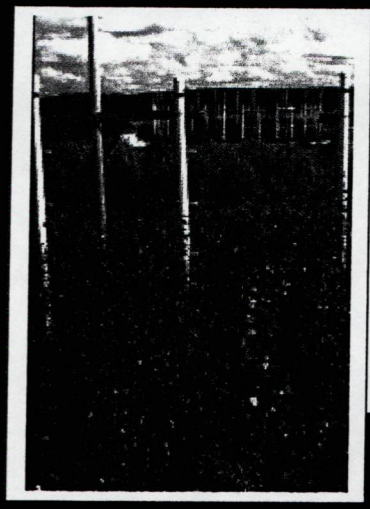
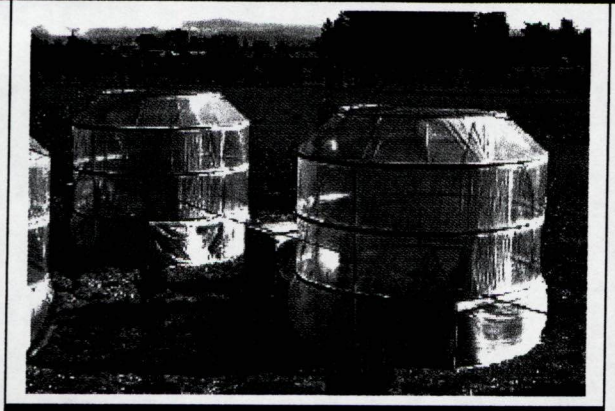
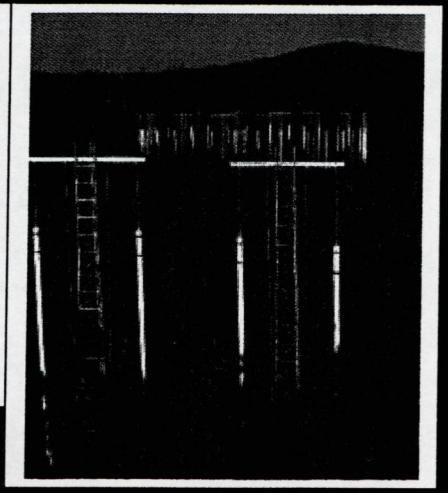
Title: *Tracing Carbon in Elevated CO_2 Experiments: A Workshop on Isotopic Analysis*

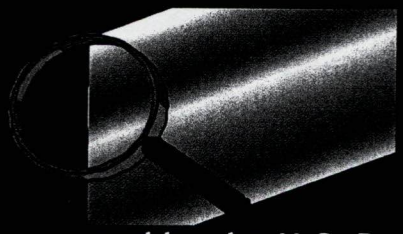






Tracing Carbon in Elevated CO_2 Experiments: A Workshop on Where the Carbon is Going

R. David Thomas Center, Duke University, Durham NC
October 18-21, 2001



Sponsored by the U.S. Dept. of Energy, with underwriting by Thermo-Finnigan

Duke University School of the Environment, University of Michigan School of Natural Resources and Environ., GCTE Focus-1 Office: Ecosystem Physiology and Global Change/University of Utah

GCTE

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Meeting agenda

Thursday Oct. 18
16:00-22:00 Participants arrive at RDU. Van pick-up and travel to Durham Hilton

18:40 Van pick-up at Durham Hilton for travel to Thomas Center

19:15 Welcome reception - University Room, Thomas Center
Meeting mission (Ellsworth)

Friday Oct. 19
7:10, 7:40 Van pick-up for travel to Thomas Center

7:30-8:30 Breakfast

Session 1: Introduction and Overview: The isotopic composition of ecosystems

8:30 Introduction and General Framework: the ecosystem carbon cycle (box model framework - Ellsworth)

9:00 Elevated CO_2 network update and role of ecosystem-atmosphere isotopic work (Pataki)

9:15 The isotopic composition of ecosystems and emerging issues in ecosystem isotope research (Ehleringer)

Session 1: *Isotopic fractionation in ecosystem processes*

10:00 The carbon isotope composition of soil (Leavitt)

10:30 The isotopic composition of ecosystem fluxes (Lin)

11:00-11:30 Break

11:30 Plant water sources and evapotranspiration (Pendall)

12:00 Isotopic tracers of nitrogen cycling (Evans)

12:30 Open Discussion

12:45-1:45 Lunch

14:00 Continued Discussion

14:30 Break

15:00 Field trip - Duke Forest and FACE array

17:00 Return to Durham Hilton

17:40 Travel to Thomas Center

18:00 Dinner

19:30 Mixer/Poster session + cash bar

Saturday Oct. 20

7:10, 7:40 Van pick-up for travel to Thomas Center

7:45-8:45 Breakfast

Session 2: *The application of isotopic tracers to study ecosystem C dynamics under high CO_2*

8:45 Carbon tracking of ecosystem pools and fluxes under elevated CO_2 (Gonzalez-Meler)

9:15 Tracing root and SOM dynamics at Duke FACE (Schlesinger)

9:45 Quantification of trace gas exchange between soils, forest canopies and the atmosphere using radon-222 (Martens)

10:15 Tracing new carbon under elevated CO_2 at ETH-FACE (van Kessel)

10:45-11:15 Break

11:15 If ^{13}C-fails as a tracer for the carbon distribution, is ^{14}C a possible solution? Experiences with natural CO_2 sources in Italy (Siegwolf)

11:45 Detection of the effects of elevated CO_2 on root biomass and contemporaneous responses of soil respiration and soil pCO_2 (King)

12:15 Open Discussion

12:30 Lunch

14:00 **Session 3:** ***Directed discussion of experimental design and protocol at elevated CO_2 sites*** (Ellsworth)

15:00 Break out groups for directed discussion

16:30 Plenary and open discussion

17:30 Adjourn; Optional visit to Duke Phytotron

19:00 Dinner

Sunday Oct. 21

7:10, 7:40 Van pick-up for travel to Thomas Center, participants check out of Hilton prior to leaving

7:45-8:45 Breakfast

8:45 Writing sessions in groups

10:30 Final plenary

11:00 Meeting adjourns, Participants depart

Tracing changes in ecosystem function under elevated CO_2

D.E. Pataki, D.S. Ellsworth, R.D. Evans, M. Gonzalez-Meler, J. King, S.W. Leavitt, G. Lin, R. Matamala, D.E. Pendall, R. Siegwolf, C. van Kessel, J.R. Ehleringer

Short abstract:

Isotopic tracers are providing answers about the fate of atmospheric carbon in the biosphere.

Introduction

The responses of terrestrial ecosystems to rising atmospheric CO_2 concentrations are a critical uncertainty in our understanding of global change. Humans emitted 6 Gt/yr of carbon into the atmosphere from fossil fuel burning and cement production during the 1990's, yet only about half of this carbon accumulated in the atmosphere. The remainder was almost equally absorbed by the oceans and terrestrial ecosystems (IPCC 2001). The mechanisms underlying net carbon uptake by the terrestrial biosphere are unknown, but fertilization by elevated atmospheric CO_2 is commonly proposed (Schimel et al. 2000). While individual plants often respond to elevated CO_2 with increased photosynthetic

rates and greater biomass, net ecosystem carbon storage necessitates transfer of fixed carbon into long-lived ecosystem pools such as woody biomass and recalcitrant soil carbon (Körner 2002).

Ecosystem-scale experiments exposing a range of different plant communities to high CO_2 have been implemented to improve our understanding of whole-ecosystem responses to elevated atmospheric CO_2. These studies use technologies such as open and closed chambers, natural CO_2 springs, and Free-Air CO_2 Enrichment (FACE) to investigate potential changes to terrestrial ecosystem function under conditions of elevated CO_2 and are critical to our ability to determine the fate of carbon in the terrestrial biosphere. In addition, the direct effects of elevated CO_2 on photosynthesis may cause a number of indirect changes in ecosystem function. Plants may reduce water uptake under elevated CO_2 due to stomatal closure (Morison 1998). The exchange of both carbon and water with the atmosphere is closely related to the cycling of plant nutrients such nitrogen, which generally limits growth in temperate systems (Zak et al. 2000). In turn, changes in the balance of carbon and nitrogen often influence food quality for herbivores, which may propagate to changes in populations at different trophic levels (Lindroth 1996, Owensby et al. 1996).

The need to develop methods to quantify changes in relatively small-scale and short-term experiments presents challenges for the ecological community, but

also enormous opportunities. Measurements of C, H, O, and N isotopes have long been applied to various sub-disciplines of ecology as a unique tool that integrates physiological and physical processes over space and time (Box 1). With new developments in mass spectrometry, stable isotope analyses require very small samples of biomass, soil organic matter, or water to make inferences about plant physiology, biogeochemistry, and ecosystem function. This is of particular advantage in elevated CO_2 studies, where destructive measurements are limited to maintain the integrity of the experimental plots. In addition, many elevated CO_2 studies are isotopic tracer experiments by nature in that the experimental CO_2 source may contain a dual label of $^{13}C/^{12}C$ and $^{14}C/^{12}C$ that is distinct from the atmosphere. This label can be used to trace the fate of new carbon in the ecosystem under high CO_2 conditions to calculate the mean residence time of carbon in various carbon pools, which has long been a critical question in ecosystem ecology.

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

The ^{13}C label

The mean $\delta^{13}C$ of atmospheric CO_2 is currently about -8‰ (Keeling 2002). In contrast, CO_2 originating from geologically ancient carbon (eg, CO_2 from petroleum byproducts or CO_2 from wells) is relatively depleted in ^{13}C, with $\delta^{13}C$ values typically ranging from –27 to –45‰. In most elevated CO_2 experiments, the increase in CO_2 concentration is accomplished by mixing commercial CO_2 with ambient air. As a result, gradients of canopy CO_2 concentration, short-term fluctuations above and below the targeted concentration, and variations in $\delta^{13}C$ of the commercial CO_2 source may occur at various time scales. Periodic air sampling of the commercial CO_2 sources can provide important information about its isotopic composition. Subsequent odeling of CO_2 circulation in elevated CO_2 plots can provide quantitative estimates of average plant exposure to CO_2 and $^{13}CO_2$; however, sampling C_4 plants grown as controls may provide the best temporal integrator of CO_2 exposure (Pepin and Körner 2002, Box 2). C_4 plants utilize a photosynthetic pathway where initial fixation of carbon occurs via the enzyme PEP carboxylase. This enzyme has a much lower isotopic fractionation factor than Rubisco, the initial carbon-fixing enzyme for plants utilizing the more common C_3 pathway (Farquhar et al. 1989, O'Leary et al. 1981). Therefore, the isotopic composition of C_4 plants is less dynamic than C_3 plants, and is closely related to the isotopic composition and concentration of atmospheric CO_2.

At the Swiss canopy crane site, a mature temperate forest fumigated with CO_2 from tubing distributed throughout the canopy, C_4 plants have been placed in

pots throughout the canopy and are periodically sampled to calculate the effective atmospheric CO_2 concentration (Pepin and Körner 2002). This method agrees well with estimates from air sampling (Figure 1) and greatly reduces the need for frequent atmospheric modeling 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 stable carbon isotope label can then be used to follow the fate of experimental CO_2 into the ecosystem

The ^{14}C label

The commercial CO_2 derived from fossil sources contains no measurable ^{14}C, which has effectively all decayed away to ^{14}N (half life=5730 years). This provides a useful isotopic tracer that may show less temporal variation than the stable carbon isotope ratio of source CO_2. When the ^{14}C-free commercial CO_2 is added to CO_2 of the background air, ^{14}C concentrations are diluted in the elevated CO_2 plots. This signal is present in many of the previous and ongoing FACE experiments, but only one has attempted to take advantage of this signal (Leavitt et al., 1994) wherein results obtained from ^{14}C generally conformed to those from the ^{13}C tracer when used to follow the fate of new carbon into the ecosystem.

Natural abundance (background) ^{14}C also provides very useful information. Background ^{14}C is itself the remnant of an artificial tracer caused by

contamination of atmospheric CO_2 during above-ground hydrogen bomb test in the 1950's and 1960's. The "bomb spike" has provided a time marker centered in 1964, after which the atmospheric ^{14}C activity has declined as the pulse has been incorporated into the ocean, groundwater, plants and soils. Among other things, it has been very helpful in understanding carbon cycling in soils (eg, Dorr and Munnich, 1986; Jenkinson et al., 1992; Trumbore, 1993) and may be useful for measurements in ambient CO_2 plots.

Resolving the fate of elevated CO_2

Understanding the future distribution of atmospheric carbon in the terrestrial biosphere is a critical element in many elevated CO_2 experiments. Carbon fixed in photosynthesis can be transferred into numerous ecosystem pools (Figure 2). Will increased carbon fixed under elevated atmospheric carbon be transferred into long-lived pools, or will the ecosystem carbon cycle merely be accelerated, resulting in no long-term net gain of C? Tracing the fate of isotopically distinct carbon fixed under elevated CO_2 offers a unique means of resolving some of these uncertainties at the ecosystem scale.

Plants grown under elevated CO_2 that contains a distinct tracer will incorporate the tracer into organic matter. Carbon inputs from the isotopically labeled plants to the soils can then 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 may FACE studies (Leavitt et al. 1994, 1996, 2001, Nitschelm et al. 1997, van Kessel et al. 2000, Schlesinger and Licheter 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 the experiment. Such isotopic tracers, whether originating from FACE experiments or from shifts of C_3 and C_4 vegetation, can then be further used to identify the specific physical or chemical organic carbon pools that new carbon has entered (eg. Jastrow et al. 1997). One commonly cited limitation of this method is that there is typically no similarly strong isotope labeling in the corresponding control plots, so that comparing the difference in results from the enriched and control CO_2 treatments is not straightforward. This can be overcome by using 1) small subplots of soils whose carbon isotopic composition was derived from C_4-plant growth (Ineson et al 1996, Cheng and Johnson 1998, Leavitt et al 2001), 2) small subplots within control plots exposed to pulsed pure $^{13}CO_2$ (Hungate et al. 1997, Leavitt et al. 2001), 3) labeled CO_2 for fumigation of whole-ambient treatments in chamber experiments (Lin et al. 1999, 2001), and 4) the small but quantifiable isotopic difference between the carbon in control plants and that in the local soil organic carbon (Nitschelm et al 1997).

Many elevated CO_2 experiments have shown that increased photosynthetic gain under elevated CO_2 may result in larger biomass than under ambient conditions (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 of understanding the significance of increased soil respiration is the partitioning between autotrophic (plant-derived) or heterotrophic (soil organism-derived) origin of the respired carbon. When plants are larger, a proportional increase in autotrophic respiration is anticipated due to increased root biomass. However, increased heterotrophic respiration may also be an indicator of an accelerated carbon cycle, in which increased plant biomass is quickly decomposed after deposition as litter or senesced roots. Traditional non-isotope field methods for partitioning total soil respiration components include removing aboveground vegetation, which may disturb the roots or the rhizosphere (Edwards and Norby 1999, Hogberg et al. 2001, Hanson 2000). Laboratory incubations of root-free soil might help determine heterotrophic responses to elevated CO_2 (e.g., Rice et al. 1994, Zak et al. 2000), but do not allow quantification of heterotrophic respiration fluxes *in situ*. Stable isotopes are increasingly used to partition soil fluxes using a variety of tracers in which growing plants attain an isotopic composition that is distinct from that of soil organic matter (Rochette and Flanagan 1997, Cheng and Johnson 1998, Lin et al. 1999 2001, Andrews et al. 1999, Pendall et al. 2001). It should be noted that *strict* separation of heterotrophic and autotrophic respiratory components is not

possible with a single isotopic label, as the microbial community immediately surrounding roots (the rhizosphere) may rapidly acquire the isotopic signature of labile root exudates. However, the substrate induced respiration method where C_4 sugar is applied to C_3 soils (Hogberg et al. 1996, Ekblad et al. 2001) provides good estimates for the partitioning between the heterotrophic and autotrophic respiration. Furthermore, the stable isotope method 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).

In the Duke FACE site in Durham, NC, soil respiration has increased in response to elevated CO_2 (Andrews and Schlesinger, 2001). In 1998, soil respiration increased by 220 g C/yr in the elevated CO_2 treatment. The heterotrophic component of this flux constituted an increase of 80 g C/yr, which is in good correspondence with the observation of Högberg et al. (2002). About 70% of the total increase in soil respiration in the elevated plots originated from carbon that was fixed since the beginning of the experiment, September 1996, according to the isotopic results. While the absence of an isotope tracer in the control plots prevents a comparable partitioning approach, these tracer results are a clear indication of a faster carbon cycle in response to elevated CO_2 that has implications for the potential for soil storage of atmospheric carbon.

With the use of ambient tracers, experiments using closed system mesocosms have found that elevated CO_2 actually stimulates decomposition of recently fixed carbon but suppresses decomposition of older SOM (Lin et al. 2001), particularly in the presence of high nutrient availability (Cheng and Johnson 1998, Cardon et al. 2001). In the Shortgrass Steppe Open Top Chamber (OTC) experiment in Colorado, an analogous C_3-C_4 disequilibrium between currently growing plants and SOM has allowed isotopic partitioning of soil respiration on three treatments: elevated and ambient chambers, as well as non-chambered control plots. In this experiment, elevated CO_2 resulted in a doubling of decomposition rates, but no change in root/rhizosphere respiration relative to ambient conditions (Pendall et al. 2002). In an OTC experiment on a California annual grassland, Hungate et al. (1997) found increases in both rhizosphere and heterotrophic respiration under 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. While 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, as well as 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

The mean residence time of carbon in various ecosystem pools is a critical uncertainty in several areas of carbon cycle research, particularly with regard to belowground fractions of biomass and soil organic matter (Randerson et al. 1998). In this regard, isotope analyses in elevated CO_2 experiments provide an opportunity for furthering 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 utilizing labeled CO_2.

At the Duke FACE experiment, soil CO_2 flux became isotopically depleted in ^{13}C in elevated CO_2 plots relative to ambient plots within one week of the initiation of CO_2 fumigation (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 photosynthate belowground, which likely influenced autotrophic respiration. Within one year of the initiation of fumigation, incubations of root-free soil showed isotopic depletion in the heterotrophic component of respiration. Yet after 32 days of incubation with no further carbon input, the ^{13}C-depleted respiration signal returned to values similar to soil from ambient plots. This indicated that the new carbon had been transferred to labile, short-lived soil organic matter pools that were rapidly exhausted in the absence of continuous inputs (Figure 3). These and other results support recent studies of natural abundance ^{13}C that show correlations between soil- and ecosystem-respired CO_2 in response to changes in environmental factors such as humidity and vapor pressure deficit (Eklbad and Högberg 2001, Bowling et al. 2002). These isotopic studies allow further delineation of the carbon cycle and provide strong evidence for rapid carbon cycling at the level of short-term photosynthesis and respiration. In addition, analyses of natural abundance radiocarbon in a number of ecosystems has indicated that the age of carbon in soil respiration is generally much younger than the age of bulk soil organic matter (Trumbore et al. 2000). Soil CO_2 flux appears to contain a large proportion of recently fixed carbon, both from live roots and from decomposition of recently deposited, labile organic matter.

Precise determination of root-turnover rates is difficult to obtain, 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-depleted isotope signature used for fumigation of CO_2 in FACE experiments can also be used to determine turnover rates of C 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 time course of a long-term CO_2 fumigation experiment (Table 2). Results from this isotopic approach have indicated that the mean residence time of roots in the Duke FACE experiment varied from 4 to 8-9 years depending on root size class and treatment. These root ages are much higher than previously estimated with other techniques (Matamala and Schlesinger, 2000). Yet the ^{13}C-based turnover rates 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, a critical discrepancy that merits further investigation due to the strong implications for ecosystem carbon balance. For example, at the Duke FACE site, a consequence of long mean residence times of live roots is that root turnover cannot explain the observed increase in soil respiration under elevated CO_2. Other rhizophere processes, such microbial decomposition of root exudates, increased mycorrhizal activity, and potential very fine root tip decomposition must be a significant component of the observed increase in respiration of new carbon at elevated CO_2.

Carbon and nitrogen interactions

Ecosystem carbon and nitrogen cycles are closely correlated and isotope-based approaches can help elucidate changes occurring under elevated CO_2 conditions. Organic material in the form of roots, litter, and woody debris that is decomposed by the soil microbial community releases mineral nitrogen available for plant uptake (N mineralization). In temperate ecosystems, the availability of nitrogen is often a limiting factor for growth. In order 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 N), or increase their uptake of nitrogen. Additional sources of nitrogen include increased fixation by symbiotic or free living organisms that can utilize N from the atmosphere, greater uptake of existing mineral nitrogen, or increased rates of mineralization.

The number of possible alterations to the N 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 growth under elevated CO_2 can be sustained in the future. This question hinges in part on an understanding of carbon and nitrogen interactions. Natural abundance and artificially applied ^{15}N have long been utilized to examine this question in

ecology, and present considerable opportunities for understanding C-N interactions in elevated CO_2 experiments.

Lessons from natural abundance ^{15}N - The nitrogen isotope ratio ($\delta^{15}N$) is a very useful indicator of change in ecosystem N cycles. The $\delta^{15}N$ of plant-available nitrogen is a function of several variables, including nitrogen inputs into the ecosystem, the $\delta^{15}N$ 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 plant-available N and subsequently plant $\delta^{15}N$. Therefore, a significant shift in plant $\delta^{15}N$ can be a conclusive indicator of changes in the ecosystem N cycle long before small shifts in N content of different ecosystem components can be detected.

Recently, a large shift in plant $\delta^{15}N$ under simulated elevated CO_2 conditions has been observed at the Nevada Desert FACE Facility. Billings et al. (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 conditions (Figure 4). No significant differences were observed in root growth and water sources between the two treatments. An important observation was that plant $\delta^{15}N$ was actually greater than soil $\delta^{15}N$, suggesting enrichment of the plant-available N pool following mineralization.

This could be caused by an increase in gaseous N loss or by immobilization in microbial biomass, because both processes are accompanied by significant fractionation (Högberg 1997). No differences were observed in gaseous nitrogen loss, but soil respiration increased and inorganic N decreased under elevated CO_2. The shift in plant $\delta^{15}N$ and other supporting evidence strongly suggests an increase in microbial activity under elevated CO_2 associated with the greater inputs of organic substrate available for decomposition.

The numerous transformations that occur in the N cycle prohibit ^{15}N at natural abundance levels from being used as a tracer of N movement in ecosystems (Evans 2001, Robinson 2001). One exception is that ^{15}N discrimination is not observed during plant uptake of inorganic N at typical soil concentrations, so that whole-plant $\delta^{15}N$ will accurately trace that of the source if plants absorb NH_4^+ or NO_3^- (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 N to the host (Hobbie et al. 1999), but ^{15}N discrimination will not be observed if most of the nitrogen absorbed by the fungus is transferred to the host (Högberg et al. 1999).

Lessons from tracer ^{15}N – An alternative approach to the use of natural abundance N isotopes is to study N 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 10^3 to 10^4 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 plant nitrogen use efficiency or conversely, greater total N losses from the soil-plant system. Greater losses could occur following an increase of organic matter inputs into the soil. A number of studies have assessed how plant N use and total ^{15}N losses have been altered under elevated CO_2. Losses of applied ^{15}N were found to be slightly lower under elevated CO_2 in a microcosm study when the grass *Danthonia richardsonni* was grown under a high level of applied mineral N (Lutze and Gifford, 2000). Using ^{15}N-labelled fertilizer in a FACE experiment, elevated CO_2 concentration showed no significant effect on total ^{15}N losses (soil and plant) in *Lolium perenne* and *Trifolium repens* swards after 4 years of CO_2 enrichment (Hartwig et al. 2002). These studies suggest that the total N in the soil-plant system is not altered under elevated CO_2 in these ecosystems. Moreover, in the *L. 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-N dynamics were unaffected by prolonged elevated CO_2 exposure (Van groenigen et al. 2002).

Nitrogen fixation plays a key role in the overall N cycle and the total input in terrestrial ecosystems is estimated at around 100 Tg per year (Mosier et al. 2001). Estimates for N_2-fixing activity in the field are based on the dilution of ^{15}N in N_2-fixing legumes compared to non-N_2-fixing reference plants. Using the ^{15}N dilution approach, the percentage of N derived from N_2 fixation by *T. repens* grown under elevated CO_2 (FACE) conditions increased by 8 % over a three year period (Zanetti et al., 1996). Similar findings were reported for N_2-fixing diazotrophs in stands of a C_3 sedge and 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 N by the plant, available soil N 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 feedback to 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 changes in intrinsic water-use efficiency and 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, providing natural tracers that do not require artificial labeling (Figure 5). Unlike short-term measurements of leaf gas exchange and temperature, isotopic methods may integrate long-term changes in plant energy and water balance.

Lessons from C 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. $\Delta^{13}C$ takes into account variations in $\delta^{13}C$ of source air ($\delta^{13}C_{source}$, Box 1) in the formation of $\delta^{13}C_P$. This is an important distinction in elevated CO_2 studies, in which $\delta^{13}C_{source}$ is often significantly different from ambient. When effects of $\delta^{13}C_{source}$ are removed, $\Delta^{13}C_P$ is a function of the ratio of the partial pressures of CO_2 inside and outside the leaf (c_i/c_a, Box 2). By applying estimates of $\delta^{13}C_{source}$ and measurements of $\delta^{13}C_P$ in elevated CO_2 studies, we can assess the effects of

elevated CO_2 on c_i/c_a, i.e., 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 reviews by Morison 1985b and Drake et al. 1997), longer-term measurements using $\delta^{13}C_P$ to estimate assimilation-weighted c_i/c_a values have suggested a different conclusion. Lin et al. (In preparation) estimated c_i/c_a values from plants grown under elevated CO_2 from a variety of ecosystem facilities including chambers, micro- and meso-cosms, FACE, and CO_2 springs. They 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 changes in c_i/c_a are associated with increased water-use efficiency, or the ratio of carbon fixed to water lost (Farquhar et al. 1989). These ^{13}C measurements will not only help resolve uncertainty in long-term physiological changes in response to rising CO_2 concentrations, but will also improve parameterization of ecological models, for which c_i/c_a is often a critical variable (see Katul et al. 2002).

Lessons from O and H isotopes The isotopic composition of plant stem water reflects the isotopic composition of the plant's water source, as there is no fractionation against hydrogen (δD) and oxygen ($\delta^{18}O$) isotopes during plant water

uptake (White et al. 1985; Dawson and Ehleringer 1991) with the exception of several halophytes (Lin and Sternberg 1993). However, leaf water becomes isotopically enriched by 15-30‰ relative to stem water due to evaporative effects (Box 3). In the leaf, oxygen in CO_2 readily exchanges with the oxygen in liquid water, a rapid reaction in the presence of the plant enzyme carbonic anhydrase. Because plant leaves contain much more ^{18}O in water relative to ^{18}O in intercellular CO_2, the $\delta^{18}O$ of CO_2 takes on the $\delta^{18}O$ value of leaf water. Farquhar et al. (1993) and Farquhar and Lloyd (1997) 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) record the enriched $\delta^{18}O$ values of leaf water.

Models predicting the isotopic composition of leaf water and plant cellulose are shown in Box 3. These 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 more enriched in $\delta^{18}O$ if transpiration is reduced (equation 8), and even more so if this reduction causes an increase in leaf temperature and therefore an increase in the leaf saturation vapor pressure (equation 7). Conversely, reduced enrichment is expected if transpiration increases under elevated CO_2 due to increased plant size and rooting volume (equation 8).

Preliminary results from a recent FACE experiment (the Swiss Canopy Crane Project) showed that leaves of deciduous trees exposed to elevated CO_2 were enriched in $H_2{}^{18}O$ relative to those grown under ambient CO_2 (Siegwolf et al., unpublished data). However, the humidity, leaf 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 $H_2{}^{18}O$ according to equation 8. Cooper and Norby (1994) found species specific effects in $H_2{}^{18}O$, where *Quercus alba* showed no change under elevated CO_2, but *Liriodendron tulipifera* was ^{18}O-enriched in both leaf water and leaf cellulose (Figure 7). These results demonstrate that subtle changes in water and energy balance may occur in plants grown under elevated CO_2 that may be difficult to detect with traditional meteorological and physiological measurements. When integrated over time using isotopic approaches, these changes are more easily detected through effects on the isotopic composition of leaf water and organic components.

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, while water from deeper layers is more depleted. Since plants do not fractionate soil water in transpiration, stable isotope analyses provides a means of distinguishing soil evaporation and plant transpiration. The water vapor leaving the leaf is in

isotopic equilibrium with stem water. Therefore, soil evaporation and plant transpiration often have isotopically distinct values. This variation can provide a means of partitioning the components of evapotranspiration. In the Colorado OTC experiment, a perennial grassland, the oxygen isotope ratio of soil CO_2 was used as a proxy for $\delta^{18}O$ of soil water. Values in ambient chambers (AC) were more ^{18}O enriched than in elevated chambers (EC), which were similar to values in non-chambered (NC) plots (Ferretti et al. In review). An isotopic mass balance model showed that during an unusually dry growing season, transpiration in the EC treatment was higher than AC and lower than NC treatments. However, in an average year, transpiration was similar on all three treatments despite greater biomass growth in the EC treatments. Transpiration-use efficiency, or the amount of biomass produced per mm water transpired, was always greatest on EC and lowest on NC treatments. These results illustrate the utility of applying isotopic methods toward understanding changes in ecosystem water balance under elevated CO_2.

Trophic-level interactions

Elevated CO_2-induced changes in plant tissue composition and chemistry have important implications not only for carbon and nutrient cycles, but also for interactions between plants and communities of organisms at higher trophic levels. While studies of elevated CO_2 effects on fauna have been fairly limited, changes in leaf C:N and secondary compounds under high CO_2 have been shown

to change leaf consumption and growth rate of insect herbivores (Lindroth 1996a, 1996b). There is considerably less available information on the effects of high CO_2 on the chemistry of fine roots and root exudates, yet these changes have been postulated to have a strong influence on soil microbial community dynamics, and subsequently on the processes of nitrogen mineralization and immobilization that control plant N availability (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, Tieszen et al. 1983, Focken and Becker 1998). Therefore, stable carbon isotopes have been used extensively in a variety of terrestrial, aquatic, and marine food web studies (Rounick and Winterbourn 1986). In terrestrial ecosystems, natural abundance carbon isotopes have been used primarily to investigate C_3- versus C_4-derived food sources, as the isotopic end points of the potential foods are vastly different. However, the carbon isotope tracer in elevated CO_2 studies provides a unique opportunity to evaluate changes in feeding preference and diet in a high-CO_2 environment. CO_2 fumigation with a highly ^{13}C depleted source creates plant material of a unique signature that can be traced to higher trophic levels.

In a novel application of this idea, Cotrufo et al. (In prep.) collected litter from several FACE experiments with contrasting $\delta^{13}C$ values of approx. –27‰ in

ambient plots and –40‰ in elevated plots. Litter was then inoculated and incubated in 3 treatments: ambient only, elevated CO_2 only, and a 50-50 mixture of ambient and elevated CO_2 litter. 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 contained an intermediate $\delta^{13}C$ value (approx. 34‰) between $\delta^{13}C$ of CO_2 evolved from the individual ambient and elevated treatments. Therefore, in this study detritivores showed no preference for ambient over elevated CO_2 litter, in contrast to previous hypotheses based on the commonly observed increase in leaf C:N. These results were consistent with a recent meta-analysis finding 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 and secondary chemistry that affect herbivory.

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. Pulse ^{14}C labeling in an elevated CO_2 experiment on crops showed that high CO_2 caused reduced carbon allocation into the microbial carbon pool, likely due to a change in the quality of the carbon substrate (Patterson et al. 1996). It is also likely that alterations in the amount and composition of rhizodeposition can affect microbial community composition as well as biomass, but this has been difficult to assess. Recently, Radajewski et al. (2000) reported that metabolically distinct taxonomic groups could be identified by supplying an

isotopically enriched substrate of interest *in situ* and using density gradient centrifugation to separate the enriched DNA for sequence analysis. This technique holds a great deal of promise for evaluating shifts in microbial community composition in response to environmental perturbations such elevated CO_2. Thus, a variety of isotopic tracers can be applied to evaluating 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 in high-CO_2 experiments of terrestrial ecosystems. The additional CO_2 supplied in these experiments provides a very useful label that can be used to trace carbon flows and turnover rates in different pools, both under elevated CO_2 treatment conditions as well after completion of the long-term experiment when the supplemental CO_2 is turned off. In experiments where isotope ratio mass spectrometry is not available, archival of dry biomass and soil samples can provide opportunities for future isotope analysis of organic matter. Frozen leaf and soil samples, or if possible water extracted and stored in sealed head space vials, can provide valuable information regarding the effect of elevated CO_2 on the leaf water budget and possibly on the leaf energy balance. Linking these stable isotope

analyses with traditional approaches in elevated CO_2 ecosystem studies provides a unique opportunity for tracing short-term and long-term changes in the carbon cycle and its impacts on the nitrogen cycle, the water cycle, and trophic-level interactions.

Acknowledgements

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Box 1 – Isotopes in ecology

Isotopes are atoms of element that differ in the number of neutrons. Heavier isotopes form stronger chemical bonds than lighter ones, 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 that between vapor and liquid water. These properties provide important information about physical and biochemical processes and are the basis for utilizing stable isotopes in ecological applications. In addition, the radioactive isotopes such ^{14}C provide a another tracer, particularly useful means of dating biological material. Commonly used isotopes in ecology are given in Table 1.

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
	^{14}C	$<10^{-10}$
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037

	^{18}O	0.204

Table 1. Isotopes of common elements and their natural abundances. All are stable with the exception of ^{14}C.

Isotopic composition is often expressed as a ratio between the heavier to the light isotope. Absolute ratios are 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:

$$\delta = (R_{sample}/R_{standard} - 1) * 1000 \text{ ‰} \qquad (1)$$

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

Isotope ratios are expressed relative to common international standards, such as the Pee Dee Belemnite formation (PDB), a standard for carbon 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:

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

To calculate Δ from δ:

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

In contrast, ^{14}C is expressed as an "activity" (concentration). Using conventional radiocarbon (gas proportional counting or liquid scintillation) or accelerator mass spectrometer (AMS) ^{14}C dating methods, the ^{14}C activity is measured and expressed as percent modern carbon (pmC) or fraction modern carbon (fmC or F), in which by convention (Stuiver and Polach, 1977) "modern carbon" activity is the background activity in 1950. 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‰.

Box 2 – The Farquhar model of photosynthetic discrimination

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

$$\Delta^{13}C_P = a + (b - a)*c_i/c_a \qquad (5)$$

where c_i/c_a is the ratio of intercellular to ambient CO_2 partial pressure, a is the fractionation associated with diffusion (4.4‰), and b is the net enzymatic fractionation associated with carboxlyation (27‰). This equation can also be expressed with regard to the isotopic composition of biomass if the isotopic composition of the air is known (Figure 6). In this case, 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.

For C_4 plants, we must separate fractionation factors for Rubsico and 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^- "leaks" out of bundle sheath cells back into the stomatal cavity. These effects were quantified by Farquhar (1983):

$$\Delta^{13}C_P = a + (b_4 + b_3\phi - a)*c_i/c_a \qquad (6)$$

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 1989). In this case, variability in $\delta^{13}C_P$, which is related to $\Delta^{13}C_P$ according to equation (3), can be interpreted as function of δ_{source} (see Box 1).

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 due to 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):

$$\delta_{LS} = \delta_s + \varepsilon_{eq} + \varepsilon_k + (\delta_a - \delta_s - \varepsilon_k) * e_a/e_i \qquad (7)$$

where δ stands for $\delta^{18}O$, the subscript a is ambient water vapor, s is source water, ε_{eq} is the equilibrium fractionation, which is temperature dependent (9.2‰ at 25°C, Majoube, 1971), ε_k is the kinetic fractionation of water, which depends on the molecular diffusion of $H_2{}^{18}O$ 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 & 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 1979; Sternberg 1989; Yakir 1992;). However, the isotopic signal from the leaf cellulose is not

transferred as a one-to-one ratio into tree-ring cellulose (Saurer et al. 1997b). Equation 7 may be combined with a mechanistic model for this isotopic signal transfer developed by Roden et al (2000) to interpret tree-ring cellulose values.

While equation (7) is useful to predict 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 1989; Luo and Sternberg 1992; Yakir et al. 1994; Helliker and Ehleringer 2000). Some of this maybe caused by patchiness (Terashima et al 1989), leaf compartmentalization (Yakir et al. 1993), and the distance from the site of evaporations at the cell walls (Farquhar & 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 &Lloyd proposed: (equation according to Yakir, 1998):

$$\delta_{LW} = \delta_s + (\delta_{Ls} - \delta_s) * (1 - e^{-P}) / P \qquad (8)$$

where δ_{LW} is the $\delta^{18}O$ bulk leaf water, δ_{Ls} in equation (7) and P is the Péclet number. P is defined as $P = E^*L / C_w^*D$, where E is the transpiration rate (mol $m^{-2} s^{-1}$), L (m) the effective maximal mixing path length, C_w the molar concentration of water (mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water ($m^2 s^{-1}$). An alternative

approach to estimating $\dot{E}$ to determine δ_{LW} has been proposed by Roden and Ehleringer (1999), who apply best-fit empirical coefficients.

Table 2. Mean residence times of fine roots in the Duke Forest FACE site estimated using ^{13}C and ^{14}C isotope tracers. 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). R. Matamala, M.A. Gonzalez-Meler and W. Schlesinger, unpublished results.

Fine Root Class	Ambient	Elevated
< 1 mm	4-5 y	4.2 y
1-2 mm	6-7 y	5.9 y
> 2 mm	8-9 y	6.0 y

Figure Captions

Figure 1. Determination of experimental 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. From [ ].

Figure 2. Schematic of ecosystem carbon transfer through various pools with examples of the carbon isotope ratio (□^{13}C) of carbon pools from elevated CO2 experiments. Data from [ ].

Figure 3. The carbon isotope ratio ($\delta^{13}C$) of soil CO_2 flux during incubation of soil from the Duke Forest FACE experiment. From Andrews et al. 1999.

Figure 4. The nitrogen isotope ratio ($\delta^{15}N$) of leaves of *Ambrosia dumosa* (Amdu), *Krameria erecta* (Krer), *Pleuraphis ridiga* (Plri), and *Larrea tridentata* (Latr) at the Nevada Desert FACE Facility. Modified from Billings et al. 2002.

Figure 5. Isolines of the oxygen isotope ratio ($\delta^{18}O$) in precipitation (relative to v-SMOW) in the United States. From Kendall and Copien 2001.

Figure 6. Carbon dioxide and water vapor exchange from plant stomata to the atmosphere as a function of internal stomatal concentration (c_i) and atmospheric concentration (c_a).

Figure 7. Oxygen isotope ratios of *Liriodendron tulipifera* (relative to v-SMOW) grown under elevated and ambient CO_2 concentrations. Data from Cooper and Norby 1994.

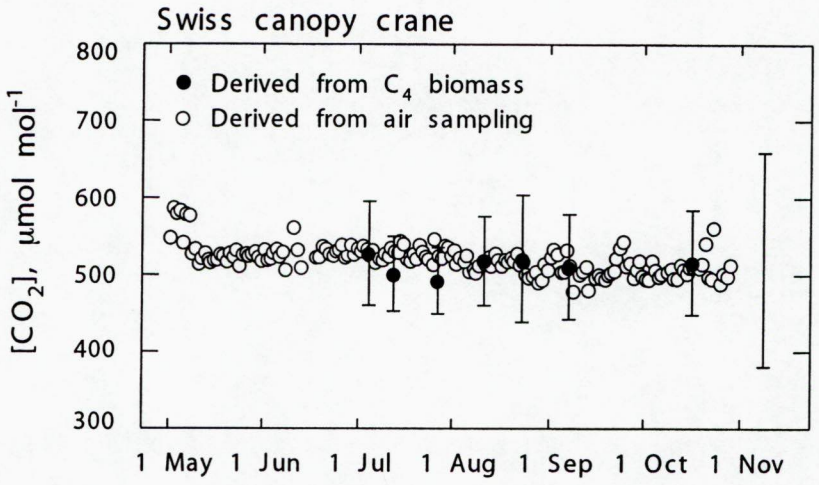
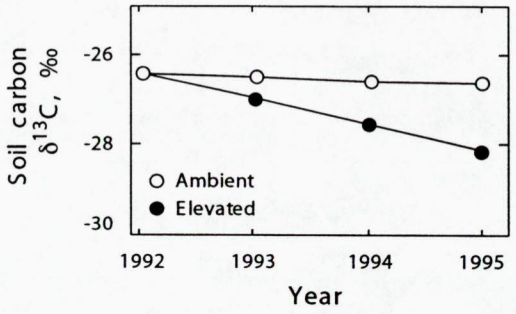
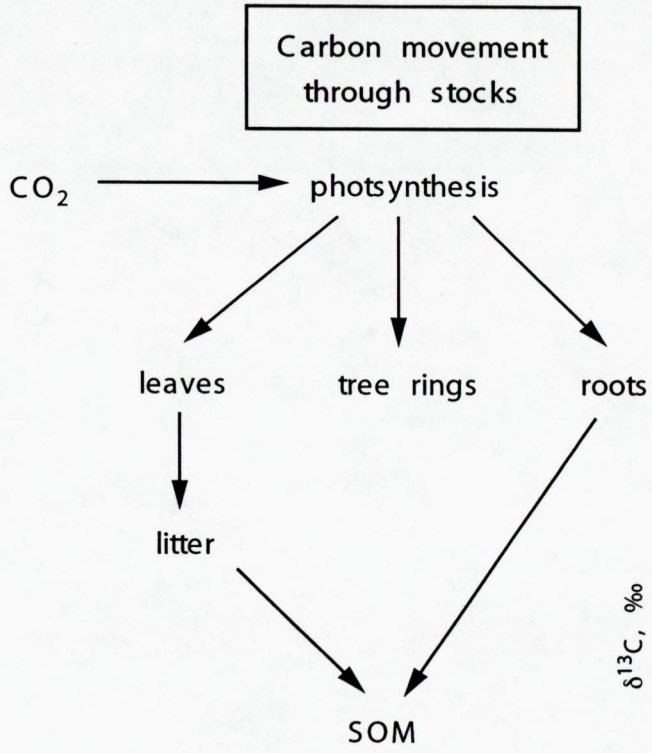

Swiss canopy crane
Derived from C_4 biomass
Derived from air sampling
$[CO_2]$, µmol mol^{-1}
800
700
600
500
400
300
1 May
1 Jun
1 Jul
1 Aug
1 Sep
1 Oct
1 Nov

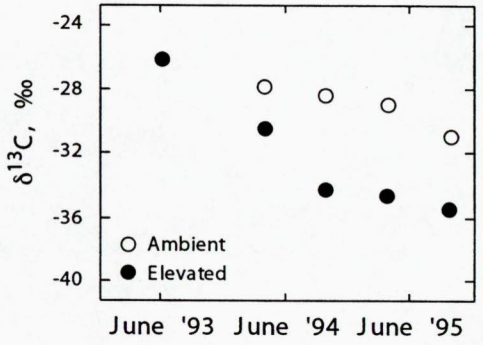
Figure 1 .01





Leaf $\delta^{13}C_P$

Site	Elevated CO_2	Ambient CO_2
Oak Ridge	xxx	xxx
Duke	xxx	xxx
Corvallis	xxx	xxx



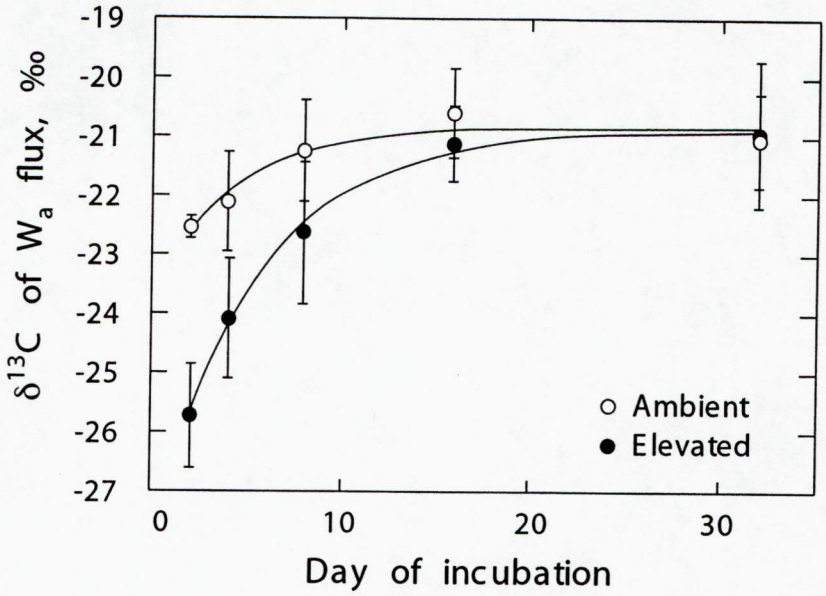

-19
-20
-21
-22
-23
-24
-25
-26
-27
δ13C of Wa flux, ‰
0
10
20
30
Day of incubation
Ambient
Elevated

Figure 3 .01

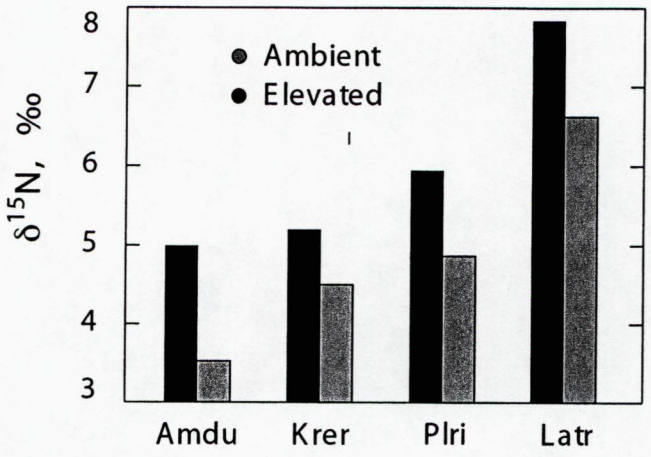
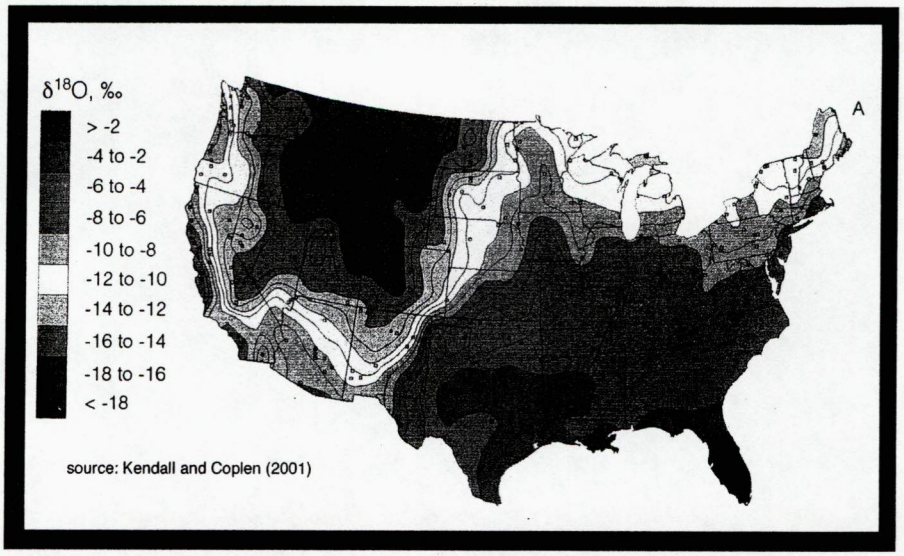
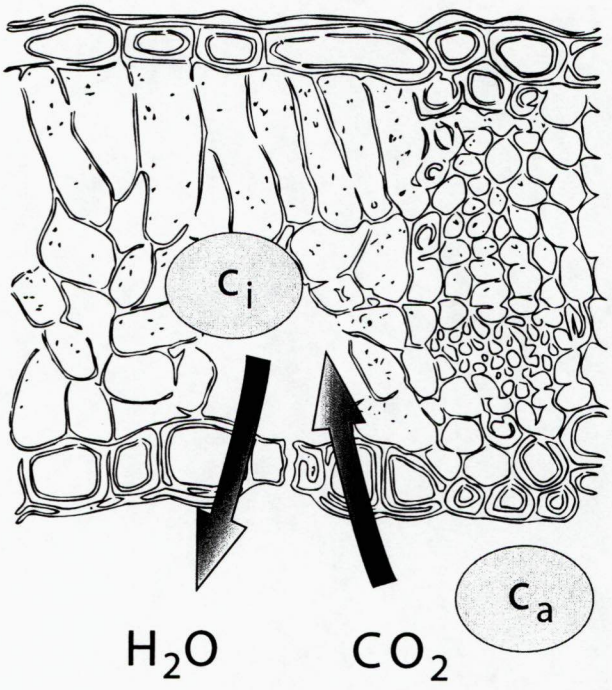
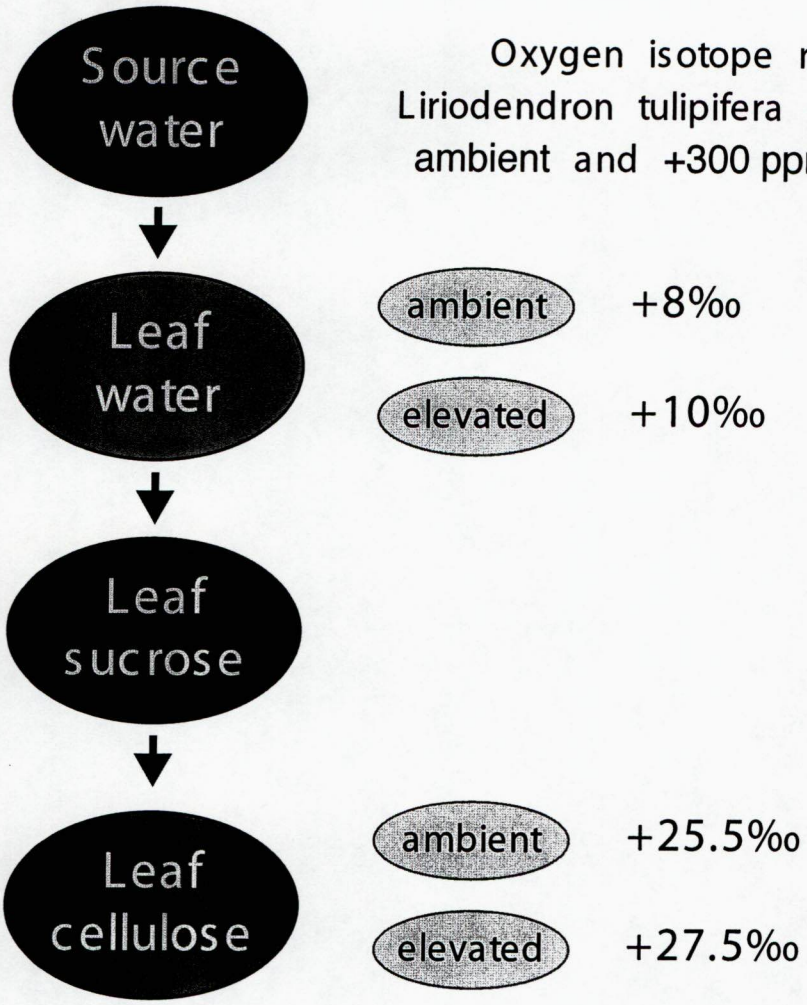


Figure 4 .01


δ18O, ‰
> -2
-4 to -2
-6 to -4
-8 to -6
-10 to -8
-12 to -10
-14 to -12
-16 to -14
-18 to -16
< -18
A
source: Kendall and Coplen (2001)


c_i
c_a
H_2O
CO_2


Source water
Leaf water
Leaf sucrose
Leaf cellulose
Oxygen isotope ratios of Liriodendron tulipifera grown under ambient and +300 ppm treatment
ambient
+8‰
elevated
+10‰
ambient
+25.5‰
elevated
+27.5‰