

US EPA ARCHIVE DOCUMENT

Effects of Elevated Atmospheric Carbon Dioxide Concentration and Temperature on Forests

Statement of the Problem

Concentrations of carbon dioxide (CO₂) and other trace gases have been increasing in the atmosphere due to human activity. By the 1980s, accumulating evidence suggested that increasing levels of these gases could produce higher global temperatures and changes in precipitation patterns. More information on how the biosphere controls atmospheric CO₂ was needed to understand the Earth's carbon cycle. Foremost, an understanding of source-sink relations between the atmosphere and the various components of the biosphere was needed. Consequently, research was undertaken to delineate the relations between atmospheric CO₂ concentrations, changes in global climate drivers, and responses of the soil-plant-atmosphere continuum (EPA 1993). The science questions governing the research were:

- What are the effects of elevated CO₂ and climate change on the growth and productivity of forest trees?
- Will elevated CO₂ and climate change alter the sequestration/exchange of carbon in the soil-plant-atmosphere continuum?
- What is the magnitude of these elevated CO₂ and climate change impacts and will they be widely distributed?

Approach

Research was conducted to investigate ecosystem responses to elevated atmospheric CO₂ and associated increases in atmospheric temperature over several years. NHEERL scientists built a state-of-the-science, sun-lit, controlled-environment chamber facility in which climatic and edaphic factors could be controlled and/or monitored during the multi-season exposure period (Tingey *et al.* 1996). A tree forest ecosystem was reconstructed in the chambers using Douglas-fir seedlings supported by one of its widely represented soil types (Rygiel *et al.* 2000). Climatic treatments were applied based on the natural, temporal variations in ambient climatic conditions found at the facility site, thus subjecting the reconstructed ecosystem to a realistic climatic profile (Tingey *et al.* 1996). Experimental treatments included increased levels of atmospheric CO₂ and elevated temperature (Olszyk and Tingey 1996).

Main Conclusions

Generally, the effects of increasing the atmospheric CO₂ concentration on the reconstructed Douglas-fir-soil ecosystem appear to have been limited by low nitrogen availability in the soil – a condition common in forest soils of the Pacific Northwest. This

result was supported by the Maine Biological Laboratory's General Ecosystem Model (GEM), used after completing the climate change experiment, to project longer-term and broader-scale consequences of climate change in Pacific Northwest Douglas-fir forests. Application of GEM to various sites in the western Cascades suggests that soil nitrogen is a primary constraint on changes in ecosystem carbon storage (McKane *et al.* 1997). For the nitrogen-poor montane site where the soil for the chamber experiment was obtained, the model predicts that total ecosystem carbon storage will increase by less than 10% during the next 100 years in response to projected increases in atmospheric CO₂ and temperature. In contrast, GEM predicts that carbon storage will increase by over 25% during the same period for a nitrogen-rich site in the western Cascades foothills.

Even though elevated atmospheric CO₂ increased photosynthetic rates (Lewis *et al.* 1999, Lewis *et al.* 2001), and while chlorophyll and carotenoid concentrations in the needles decreased under elevated CO₂ (Ormrod *et al.* 1999), the additional carbon acquired was not allocated to produce seedlings of greater biomass (Olszyk *et al.* 2003). Rather, it appears that the carbon was allocated to soil organisms which convert stored, unavailable forms of nutrients into available forms (Lin *et al.* 1999, Lin *et al.* 2001). These available forms can then be acquired by diverse and stable mycorrhizal fungi resident on the ephemeral, nutrient-absorbing fine roots (Rygiewicz *et al.* 2000, Hobbie *et al.* 2001).

While total carbon storage in the soil increased during the experiment, due to seedling growth and decomposition processes in the soil and litter layer, the amount of total stored carbon was not different among the climatic treatments. However, stable isotopic data suggest that a variable allocation of carbon into soil organic matter (SOM) of different qualities may have occurred, thus altering the long-term storage potential of the soil for carbon. In a related project on ponderosa pine, the effect of nitrogen to alter the seedlings' responses to atmospheric CO₂ concentration was clearly evident (Johnson *et al.* 2000), and reinforced the results found in the chamber study done on Douglas-fir. Taken collectively, these results indicate the overriding influence of the low nitrogen found in Pacific Northwest forests.

Projecting to larger scales, the responses of forest ecosystems to elevated CO₂ may be highly variable temporally and globally. In particular, the responses appear highly dependent on the quantity and availability of nutrient resources, and the capacity of nutrient acquisition processes relative to the increased amount of carbon available in the atmosphere. As the Douglas-fir study was run for only four growing seasons, it is uncertain if the observed responses to elevated CO₂ were transient, and eventually would change as ecosystem compartments continued to adjust to the altered ratios of available carbon to available nutrients.

Elevated temperature had a greater, and negative, impact on the seedlings than did the elevated CO₂ treatments. Elevated temperature directly and negatively affected the development and morphology of the seedlings. Seedlings grown under elevated temperatures had greater numbers of aborted and malformed buds, and abnormal

needle primordial tissue compared with seedlings in the ambient temperature treatments (Apple *et al.* 1998, Apple *et al.* 2000). In addition, the seedlings grown under the higher temperatures were shorter and more “bush-like” in morphology, thus hindering their ability to gain height (Olszyk *et al.* 1998a, Olszyk *et al.* 1998b). Elevated temperature delayed needle hardening in the fall, slowed dehardening in the spring and reduced the maximum hardness; rendering the trees less resistant to low temperatures (Guak *et al.* 1998).

Climate change will affect forested ecosystems differentially. While elevated temperature will most likely affect the growth of plant species directly, the effects on ecosystem structure and functioning may be more subtle to discern, but no less significant. Elevated temperature could lead to the replacement of sensitive species by more heat tolerant species. In the Pacific Northwest, the predominant lumber species, Douglas-fir, could experience abnormal growth patterns. But as Douglas-fir is a genetically diverse species, adaptation, either natural or managed, is likely. However, the cost to timber production is unknown.

Ecosystem effects of increasing levels of atmospheric CO₂ will depend on the nutrient status of specific forests. Increased forest production will occur where soils contain adequate nitrogen. In areas where nitrogen is limiting, elevated CO₂ levels will not increase the growth of trees -- even though photosynthesis may increase. Without sufficient nitrogen, the trees cannot use the additional CO₂ for growth. The additional carbon is used by soil organisms and respired to the atmosphere (Rygiewicz and Andersen 1994). In addition to contributing to CO₂ buildup in the atmosphere such changes in the soil foodweb, which controls nutrient availability for plants, could have long-term effects on ecosystem functioning.

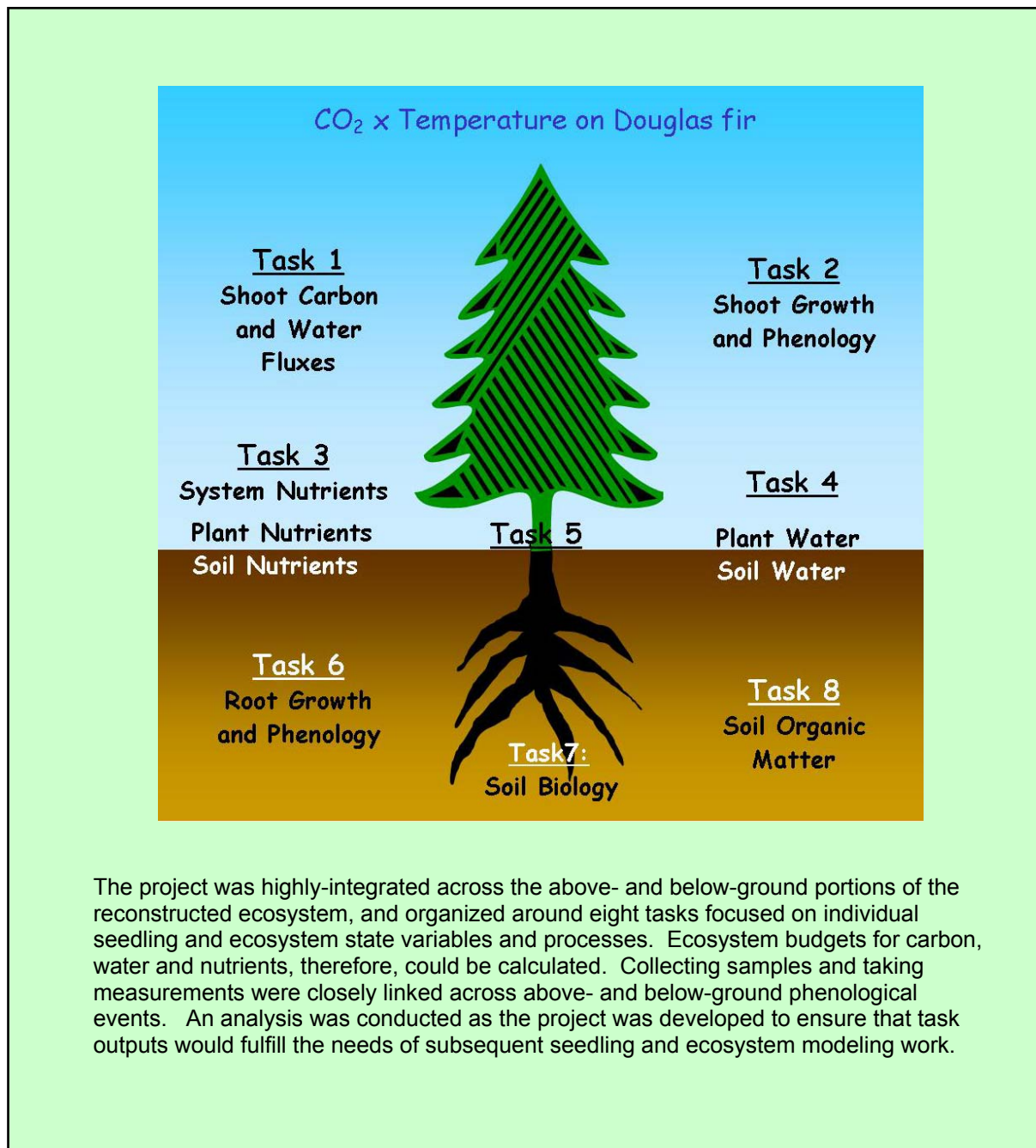
Experimental Approach to Study Seedling and Ecosystem Processes

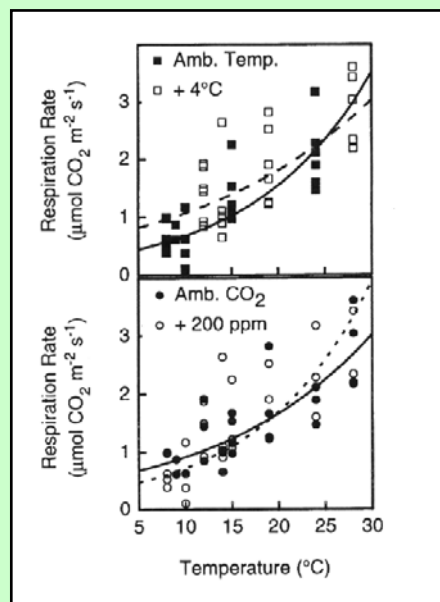
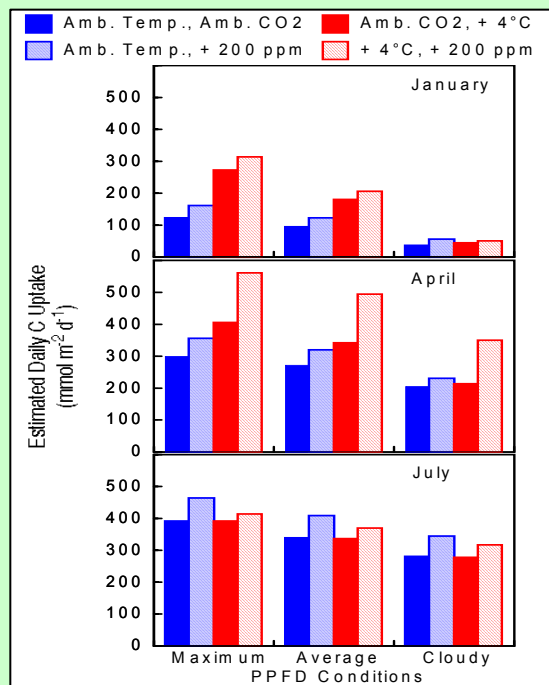


A 2 X 2 factorial treatment design was used: [ambient CO₂ and ambient + 200 ppm CO₂ (179 ppm achieved), ambient temperature, and ambient + 4 °C (3.8 °C achieved)]. Each of the four climatic conditions was replicated three times, which resulted in a total of 12 chambers being used for the experiment.

SPAR (Soil-Plant Atmosphere Research) chambers (1 x 2 m footprint) were used to simulate natural seasonal and diurnal changes in atmospheric [CO₂], air and soil temperatures, vapor pressure deficit (VPD), and soil moisture. Fourteen, two-year-old Douglas-fir seedlings were planted in each chamber in a natural, widely-represented, Cascade Mountains, high-elevation (1220 m) soil. The seedlings originated from open-pollinated seeds harvested from 5 low-elevation (300 to 460 m) seed zones in the Cascade and Coastal Mountain Ranges near Corvallis. Total N in the soil was < 0.1% (w/w), and NO₃⁻ and NH₄⁺ in soil solution were below detection limits (0.04 and 0.10 mg l⁻¹, respectively). Six cm of forest floor were placed on top of the soil. Climatic treatments were imposed for 4.5 growing seasons.

An Integrated Sampling Approach Was Designed to Track Carbon, Water and Nutrients through the Reconstructed Ecosystem



Elevated CO₂ Increased Photosynthesis but not Growth

Greater instantaneous photosynthetic rates (left panel) were observed under elevated CO₂ and temperature in the spring and winter. Some acclimation of photosynthetic rates to elevated CO₂ was evident as the exposure to climatic treatments progressed (data not shown). Even so, by the third and fourth growing seasons, elevated CO₂ increased net photosynthesis by an average of 21% across the two temperature treatments. The additional carbon acquired under elevated CO₂ was not released through increased “dark” respiration (respiration not associated with the process of photosynthesis).

The increased carbon uptake under elevated CO₂ and elevated temperature did not increase the final size of the seedlings. Moreover, elevated CO₂ had no other significant effects on whole seedling or individual seedling component biomass, % biomass allocation, or leaf area (not all data are shown). Other sinks for the additional carbon acquired are the continual production, death and decomposition of the ephemeral, nutrient-absorbing fine roots; the various organisms of the soil food web (both their biomass and respiration); and the soil organic matter (SOM). PPF is Photosynthetic Photon Flux Density, expressed as μmol photons m⁻² s⁻¹. (Lewis *et al.* 1999).

Under elevated temperature, compared with the ambient condition, shifts occurred only in the amounts of carbon allocated to needles and buds (Olszyk *et al.* 2003). Allocation of carbon related to the production, death and decomposition of the ephemeral, nutrient-absorbing, fine roots can not be determined from the final biomass of the seedlings as these roots were produced and decayed during the exposure period. The retrospective analysis of the allocation of carbon to produce these roots throughout the exposure is ongoing.

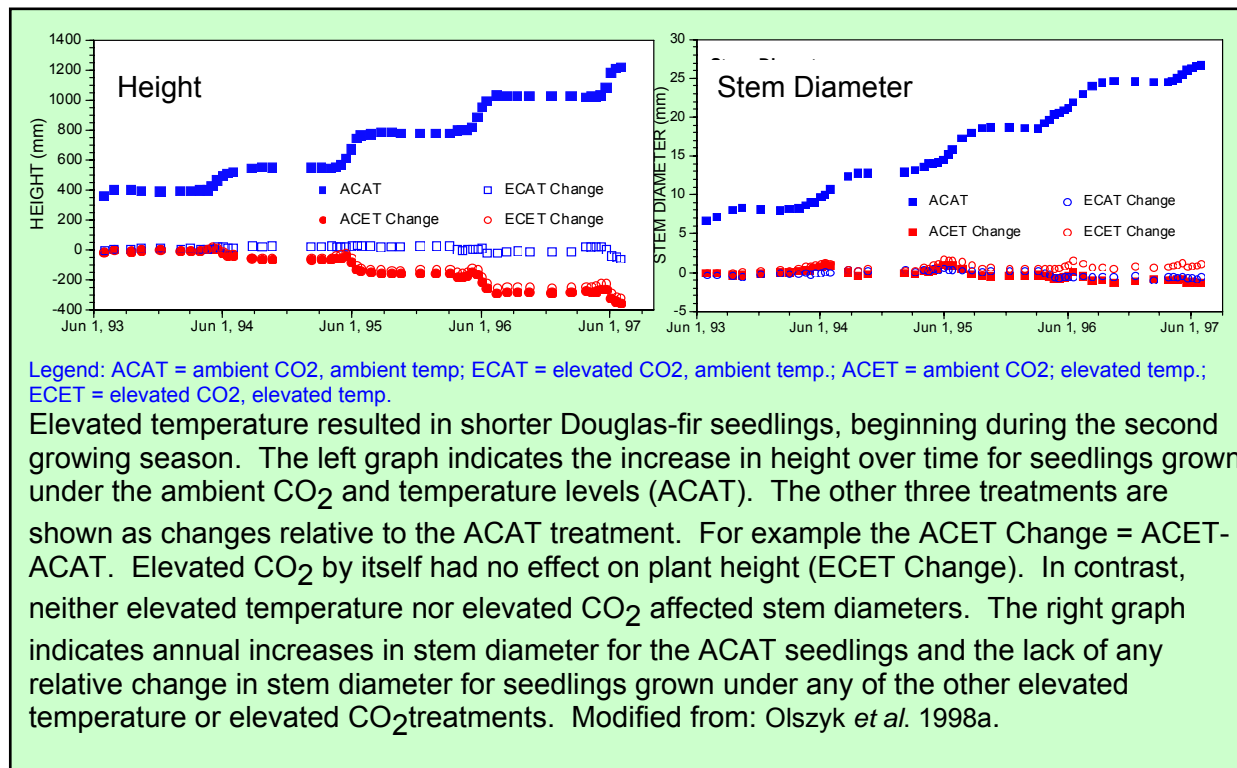
Elevated Temperature Affected Needle and Bud Growth



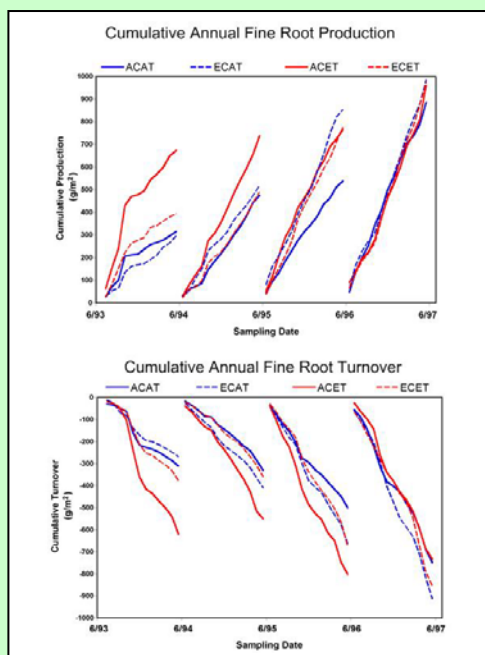
Douglas-fir buds (left panel) showed several signs of damage due to elevated temperature: (A) Dissected normal bud from the ambient climatic treatment; (B) Dissected abnormal bud from elevated temperature treatment with bud-scale-like needle primordial (arrow), and (C) convoluted bud scales (arrows); (D) Exterior of normal bud from ambient conditions; (E) Exterior of rosetted bud from elevated temperature treatment; (F) Normal buds from ambient conditions; (G) to (I) all are elevated temperature treatment showing - (G) rosetted abnormal buds, (H) a shoot with two small buds and reduced needles, and (I) a bud with reduced needles and elongated stalk originating from tree trunk. Scale = 1 mm. Source: Apple *et al.* 1998.

Under elevated temperature, a greater percentage of leader and branch buds opened early in the growing season (lower left panel). However, by the end of bud burst, a smaller total percentage of buds had opened under the higher temperatures. Needles produced under elevated temperature conditions were less able to withstand the colder temperature of winter (lower right panel). Indicated is the freezing temperatures at which 50% of the needles displayed visible signs of tissue damage (L_{50} °C). Source: Guak *et al.* 1998.

Seedling Size Was Negatively Affected by Elevated Temperature



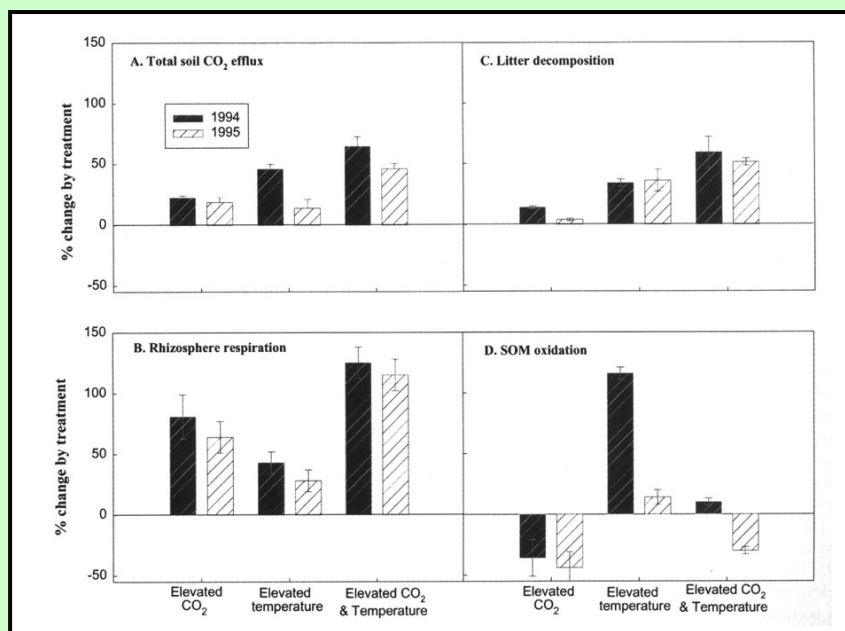
Elevated CO₂ and Temperature Altered Fine Root Distribution but not Production and Turnover



Fine roots play a key in the acquisition of water and nutrients needed to sustain growth. The growth of fine roots is coordinated with shoot growth so that the plant has sufficient resources.

The effects of elevated CO₂ and temperature on fine production and turnover were determined over a 4-year period. Elevated CO₂ and temperature altered fine root distribution; there were more fine roots deeper in the soil. There were no CO₂ effects on annual fine root production or turnover. During the first 2 years, elevated temperature (at ambient CO₂) increased fine root production, but there were no differences in the latter part of the experiment. Limited N availability likely minimized CO₂ response belowground as it did aboveground.

The Rhizosphere Responded to Elevated Atmospheric CO₂

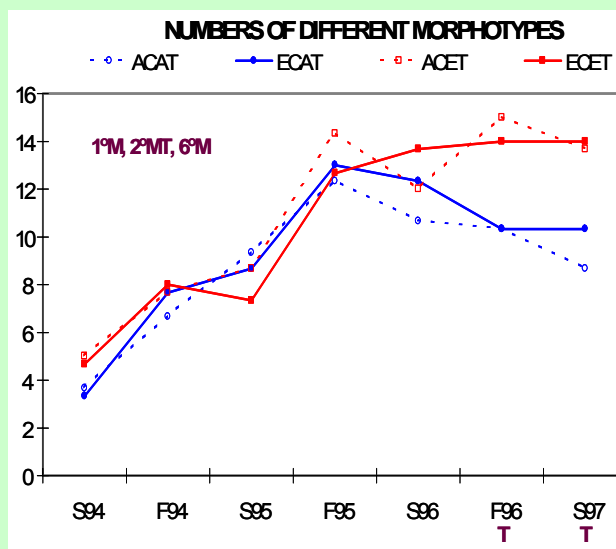
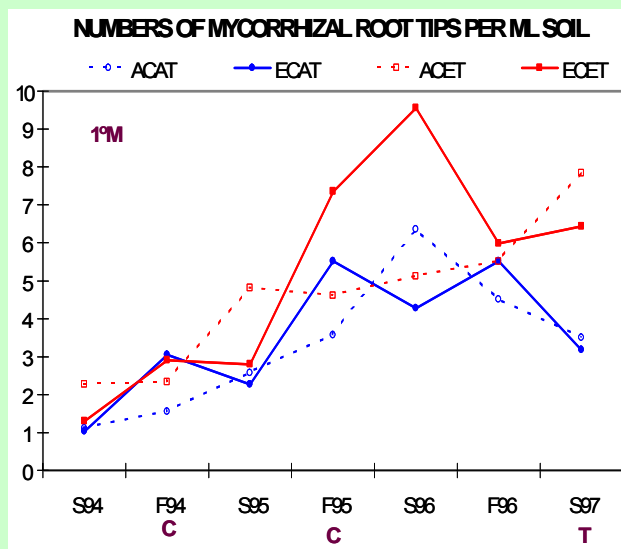


The amounts of carbon forming the total soil CO₂ efflux were mathematically partitioned into their source compartments within the soil. Since the atmospheric carbon delivered to the seedlings was depleted in ¹³C, it served as a tracer to analyze soil carbon dynamics. The dominant source of the soil CO₂ efflux in the soil-Douglas-fir ecosystem was the decomposition of the litter, followed by rhizosphere respiration (= root respiration + respiration of root-associated soil biota), and then from the oxidation of soil organic matter (SOM) (data not shown). Elevated CO₂ stimulated total soil respiration (Graph A).

Percent increase or decrease in the total flux of CO₂ released from the soil (A), and from its component sources (B, C, D) relative to respective CO₂ fluxes in the ambient climatic treatment.

Rhizosphere respiration was stimulated by elevated CO₂ and less so by temperature (Graph B). In contrast, litter decomposition was stimulated mostly by temperature (Graph C). The SOM response was highly variable (Graph D): from a decrease in oxidation under elevated CO₂, to an increased oxidation under elevated temperature; note that elevated CO₂ in the double elevated treatment countered the oxidation found in the elevated-temperature-only treatment. Differences in responses between 1994 and 1995 are attributed to the physical disruption done to the soil when it was transported from the Cascade Mountains to the chambers. The likelihood that the increased rhizosphere respiration was due to a transient, increased standing crop of the ephemeral, fine roots is still being analyzed.

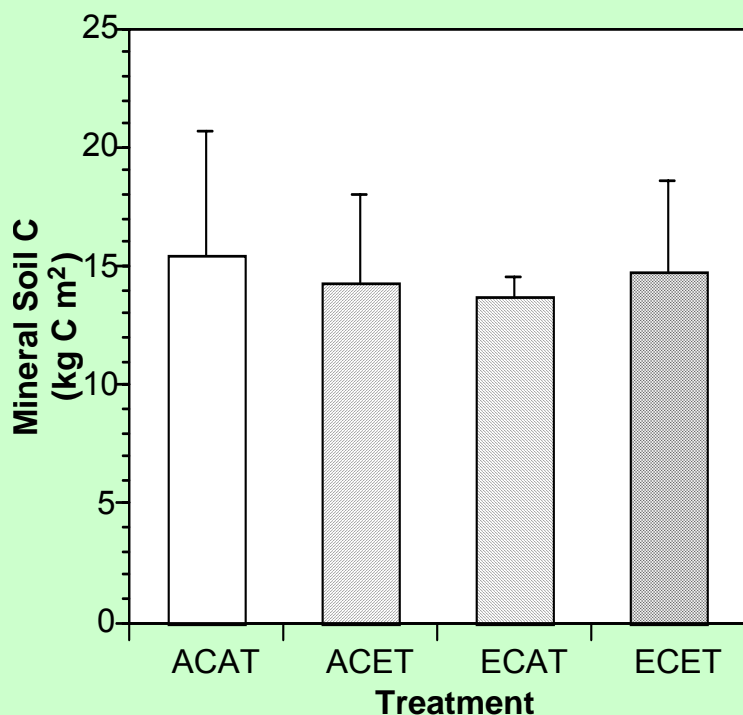
Carbon Delivered to Soil Foodweb to Explore Ecosystem for Nutrients



Legend: ACAT = ambient CO₂, ambient temp.; ECAT = elevated CO₂, ambient temp.; ACET = ambient CO₂; elevated temp.; ECET = elevated CO₂, elevated temp.

The increased soil CO₂ efflux attributed to rhizosphere respiration (= root respiration + respiration of root-associated soil biota) (previous sidebar) likely is due to increased standing crop of the ephemeral, fine roots and their decomposition, and/or the standing biomass, activity and decomposition of soil fungi. Coniferous forest ecosystems rely on free-living and symbiotic fungi (attached to the fine roots) to mobilize nutrients stored in the forest floor and soil, and to transport the mobilized nutrients to, and into, the plants to balance the carbon acquired aboveground. All low-nutrient terrestrial ecosystems increase their dependence on these fungi compared to higher nutrient conditions. The Douglas-fir seedlings formed high concentrations of mycorrhizal root tips in the low-nutrient soil (left graph), and exhibited a high degree of root colonization (nearly 100% of the root tips that developed were colonized, data not shown). The fungal community formed on the roots was highly-diverse and its structure was resistant to the climatic treatments (right graph). However, the most extensive portion of the symbiotic fungal biomass is the portion living at distance from the roots, and which explores the soil for nutrients, delivering them to the tree. Through the use of direct counting procedures and measuring the amounts of stable isotopes delivered into the soil foodweb, we anticipate identifying to which trophic structure(s) in the soil ecosystem the additional carbon acquired under elevated CO₂ was allocated, and then subsequently deposited as soil organic matter (SOM).

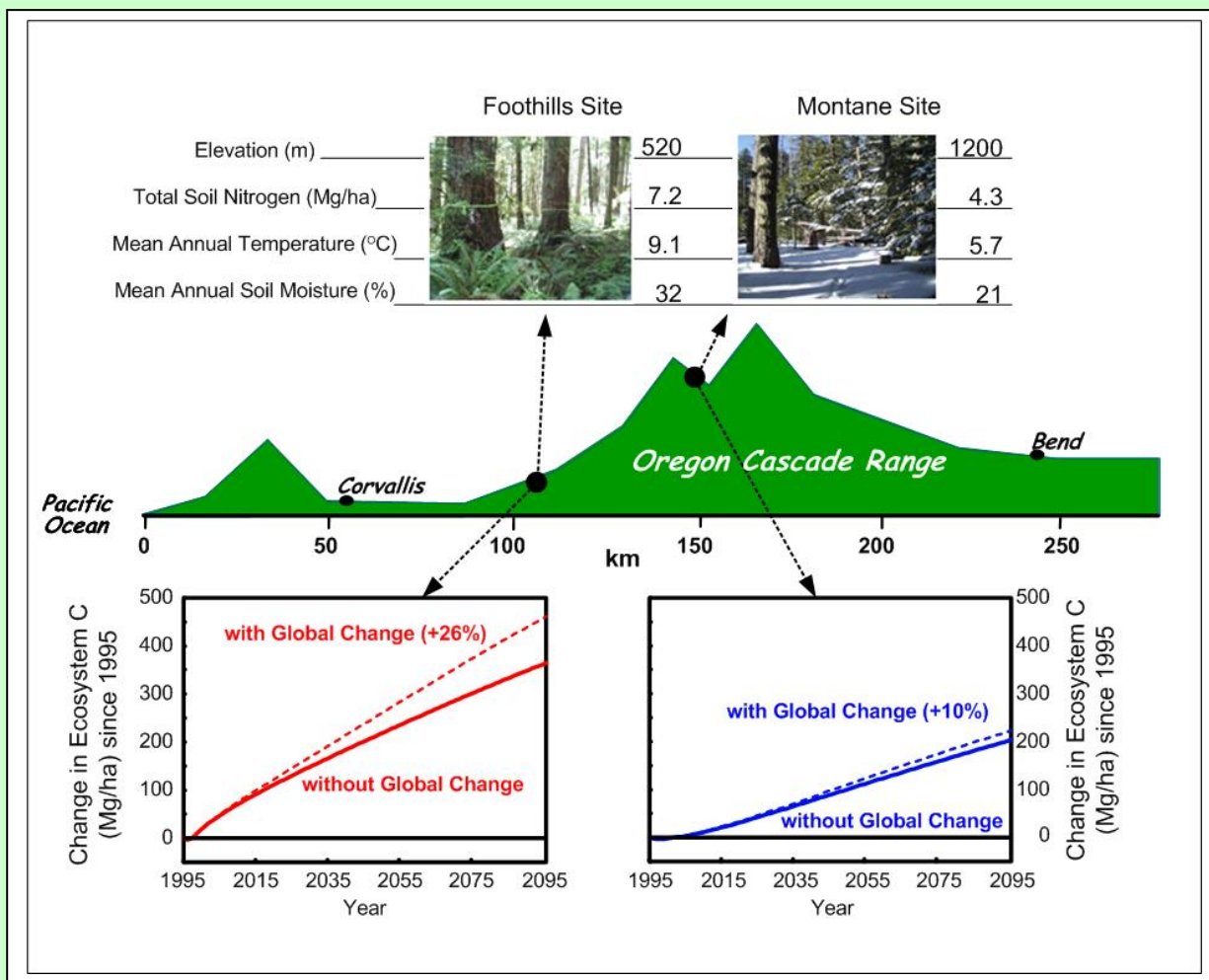
Soil Organic Matter May Be Depository for the Acquired Carbon



The amount of carbon stored in soil as organic matter (SOM) increased during the exposure, predictably as one might expect because of plant growth. However, none of the climatic treatments affected the total amount of carbon stored as SOM (graph at left). Soil ¹³C data suggest that SOM levels actually may have increased under elevated CO₂. As the total amount of SOM may not have been altered by the climatic treatments, the isotopic data suggest that certain quality fractions of SOM may have been differentially affected by the treatments. Thus the large total amount of SOM may be masking the more subtle responses in the individual quality fractions. Ultimately, the quality of SOM that is formed determines long-term storage of carbon in, and productivity of, forested ecosystems. SOM from the experiment has been separated into its quality fractions, and stable isotopic analyses are underway to address this aspect of the fate of the additionally-acquired carbon.

Soil Nitrogen Availability Constrained Carbon Storage in Response to Elevated CO₂ and Temperature

We used the General Ecosystem Model (GEM), a process-based model of terrestrial ecosystem biogeochemistry, to project longer-term and broader-scale consequences of increases in atmospheric CO₂ and temperature in Pacific Northwest Douglas-fir forests.



GEM was used to predict and analyze the effects of projected changes in CO₂, temperature & soil moisture on ecosystem carbon storage at the Foothills and Montane mature forest sites. The model was run with and without the projected changes in CO₂, temperature & soil moisture. All simulations started in 1995 with post-harvest conditions (90% of tree biomass removed).

Results illustrate that elevated CO₂ increases plant growth and net ecosystem C storage only when there are sufficient supplies of soil nitrogen as at the Foothills Site. A sensitivity analysis showed that CO₂ was much more important than temperature in increasing ecosystem C storage, and that elevated CO₂ increased storage of C in plants more than in soils.

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Olszyk, D., C. Wise, E. VanEss, M. Apple and D. Tingey. 1998b. Phenology and growth of shoots, needles, and buds of Douglas-fir seedlings with elevated CO₂ and (or) temperature. *Can. J. Bot.* 76:1991-2001.

Ormrod, D.P., V.M. Lesser, D.M. Olszyk and D.T. Tingey. 1999. Elevated temperature and carbon dioxide affect chlorophylls and carotenoids in Douglas-fir seedlings. *Int. J. Plant Sci.* 160:529-534.

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Rygielwicz, P.T., K.J. Martin and A.R. Tuininga. 2000. Morphotype community structure of ectomycorrhizas on Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings grown under elevated atmospheric CO₂ and temperature. *Oecologia* 124:299-308.

Tingey, D.T., B.D. McVeety, R. Waschmann, M.G. Johnson, D.L. Phillips, P.T. Rygielwicz and D.M. Olszyk. 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. *J. Environ. Quality* 25:614-625.

Annotated Bibliography of WED Research

Andersen, Christian P. and Paul T. Rygiewicz. 1991. Stress Interactions and Mycorrhizal Plant Response: Understanding Carbon Allocation Priorities. *Environmental Pollution* 73:217-244.

In this paper, a framework is presented for studying responses of mycorrhiza to external stresses, including possible feedback effects which are likely to occur. The authors review recent literature linking carbon allocation and host/fungal response under natural and anthropogenic stress, and present a conceptual model to discuss how carbon may be involved in singular and multiple stress interactions of mycorrhizal seedlings. Due to an integral role in metabolic processes, characterizing carbon allocation in controlled laboratory environments could be useful for understanding host/fungal responses to a variety of natural and anthropogenic stresses. Carbon allocation at the whole-plant level reflects an integrated response which links photosynthesis to growth and maintenance processes.

A root-mycocosm system is described which permits spatial separation of a portion of extramatrical hyphae growing in association with seedling roots. Using this system, it is shown that root/hyphal respiratory release of pulse-labeled ¹⁴C follows a sigmoidal pattern, with typical lag, exponential and saturation phases. Total respiratory release of ¹⁴C per mg root and the fraction respired of total ¹⁴C allocated to the root is greater in ponderosa pine inoculated with *Hebeloma crustuliniforme* than in noninoculated controls. Results illustrate the nature of information that can be obtained using this system. Current projects using the mycocosms include characterizing the dynamics of carbon allocation under ozone stress, and following the fate of organic pollutants. The authors believe that the system could be used to differentiate fungal- and host-mediated responses to a large number of other stresses and to study a variety of physiological processes in mycorrhizal plants.

Apple, Martha E., Melissa S. Lucash, David M. Olszyk, and David T. Tingey. 1998. Morphogenesis of Douglas-fir buds is altered at elevated temperature but not at elevated CO₂. *Environmental and Experimental Botany* 40:159-172.

Increases in atmospheric CO₂ and temperature are associated with global climate change. Scientists at the Western Ecology Division are investigating how these increases could affect the growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). This highly valued timber species is a dominant part of Pacific Northwest Ecosystems. During the four year experiment, seedlings were grown in sun-lit controlled environment chambers at ambient or elevated (+4° C above ambient) temperature, and at ambient or elevated (+200 ppm above ambient) CO₂. Elevated CO₂ had no effect on vegetative bud morphology, while the following unusual

morphological characteristics were found in the elevated temperature treatment: rosetted buds with reflexed and loosened outer scales, convoluted inner scales, clusters of small buds, needles elongating between scales, needle primordia with white, hyaline apical extensions, and buds with hardened scales inside of unbroken buds. Buds became rosetted in elevated temperature chambers after temperatures exceeded 40°C in July. It appears that rosettes form after long-term exposure to elevated temperature and after shorter periods of exposure to intense heat. Elevated temperature influences bud morphology and may therefore influence the overall branching structure of Douglas-fir. These morphological changes could not only compromise timber production, but they could also affect ecosystem processes in Pacific Northwest forests.

Apple, M. E., M. S. Lucash, D. L. Phillips, D. M. Olszyk, and D. T. Tingey. 1999. Internal temperature of Douglas-fir buds is altered at elevated temperature. *Environmental and Experimental Botany* 41:25-30.

Douglas-fir saplings were grown in sun-lit controlled environment chambers at ambient or elevated (+4° C above ambient) temperature from 1993 until 1997. In the fall of 1996 and the winter of 1997, we measured the internal temperatures of vegetative buds with thermocouple probes to explore the possibility that differences in energy balance contribute to the formation of abnormal buds (rosetted with reflexed and loosened scales) buds at elevated temperature. We compared temperatures of: 1) rosetted buds with those of normal buds in elevated temperature chambers, 2) buds at ambient and elevated temperature, and 3) buds and air in elevated temperature chambers. We found that buds from elevated temperature chambers had higher temperatures than those from ambient temperature chambers, and that abnormal buds had higher and earlier peak daily temperatures than normal buds. Bud temperature tended to be higher than air temperature late in the day but lower than air temperature at night. Elevated temperature may influence the temperature balance of buds and contribute to development of abnormal buds.

Apple, Martha E., David M. Olszyk, Douglas P. Ormrod, James Lewis, Darlene Southworth, and David T. Tingey. 2000. Morphology and stomatal function of Douglas-fir needles exposed to climate change: elevated CO₂ and/or temperature. *International Journal of Plant Sciences* 161:127-132.

Climate change may impact the productivity of conifer trees by influencing needle morphology and function. to test the responses of needles to climatic variables, Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco saplings were grown in sun-lit controlled environment chambers at ambient or elevated CO₂ (+200 ppm above ambient) and at ambient or elevated temperature (+4EC above ambient). Needle characteristics evaluated included size (length, width, area), stomatal density (stomata/mm²), percent stomatal occlusion, and the quality of epicuticular wax. Needle function was evaluated as d loss of water through transpiration and as stomatal conductance to water vapor. Elevated CO₂ did not affect any needle parameters: either

in terms of size, stomatal density, epicuticular wax, or needle function. In contrast, with elevated temperature needles were longer, had less finely granular epicuticular wax, and increased transpiration and stomatal conductance rates. These results indicate that elevated temperatures, but not elevated CO₂ associated with climate change may influence Douglas-fir needle structure and function, and hence, tree productivity.

Börner, T., M.G. Johnson, P.T. Rygielwicz, D.T. Tingey and G.D. Jarrell. 1996. A two-probe method for measuring water content of thin forest floor litter layers using time domain reflectometry. Soil Technology 9: 199-207.

Few methods exist that allow non-destructive in situ measurement of the water content of forest floor litter layers (Oa, Oe, and Oi horizons). Continuous non-destructive measurement is needed in studies of ecosystem processes because of the relationship between physical structure of the litter and the biological and chemical processes that take place therein. We developed a method using time domain reflectometry (TDR) to monitor water content in a coniferous forest floor litter layer. Litter and mineral soil horizons were reconstructed in test beds in which TDR probes were placed and measurements taken using a range of litter and mineral soil water contents. Two probes are necessary when litter thickness is less than the spatial sensitivity (6 to 8 cm) of the TDR probes; one probe placed in the mineral soil and another one at the interface of the litter and mineral soil. Using this arrangement of TDR probes and simple mathematical relationships, the volumetric water content of forest litter can be estimated continuously. When the results of the two-probe method are compared to volumetric water content of forest litter obtained by gravimetric means there is a strong positive linear relationship between the two measured values of litter water content ($r^2 = 0.93$). The two-probe method, however, underestimates litter water at low water contents and overestimates it at high water contents. This error has at least three components: (1) TDR instrument error, (2) errors in estimating volumetric water content from gravimetric data, and (3) using a TDR calibration curve not specific for high organic matter litter layer material. Calibrating the instrument for this specific condition should improve the overall estimate of the litter layer water content.

Cordoba, A.S., de Mondonca, M.M., Sturmer, S.L., Rygielwicz, P.T. 2001. Diversity of Arbuscular Mycorrhizal Fungi Along a Sand Dune Stabilization Gradient: A Case Study at Praia de Joaquina on the Ilha de Santa Catarina, South Brazil. Mycoscience 42: 379-387.

Species diversity of arbuscular mycorrhizal fungi (AMF) was assessed along a dunes stabilization gradient (embryonic dune, foredune and fixed dune) at Praia da Joaquina (Joaquina Beach), Ilha de Santa Catarina. These dunes served as a case study to assess whether diversity and mycorrhizal inoculum potential increase along the gradient. Ten soil samples were collected from each stage, pooled, and then six, 100g soil sub-samples taken to identify and enumerate spores. Twelve AMF species were detected, and all three families in Glomales were represented. Gigasporaceae species

dominated in the embryonic dune while Glomaceae species dominated in the fixed dune. Total spore numbers and richness increased as the dunes became more stabilized. However, indices of Margalef, Simpson and Shannon reached maximal values at different stages suggesting that species abundance distributions were different at each stage. In both embryonic and fixed dunes, species abundance data fit the broken stick model while in the foredune, the log series model best described the data. Mycorrhizal inoculum potential followed spore numbers and increased along the gradient suggesting that spores are important in initiating root colonization in this system. Relationships between edaphic factors and functional roles of Glomales families as determinants of AMF distribution are discussed.

Guak, Sunghee, David M. Olszyk, Leslie H. Fuchigami, and David T. Tingey. 1998. Effects of elevated CO₂ and temperature on cold hardiness and spring bud burst in Douglas-fir (*Pseudotsuga menziesii*). *Tree Physiology* 18:671-679.

Physiological adaptations of woody plants to stress could be significantly affected by climatic change, i.e., increasing atmospheric CO₂ concentration and air temperature. Cold hardiness was evaluated for Douglas-fir (*Pseudotsuga menziesii*) seedlings grown in semi-closed, sun lit chambers with ambient or ambient plus 200 $\mu\text{mol mol}^{-1}$ CO₂ and ambient or ambient plus 4°C air temperature. Needles were sampled on five dates from October 1995 to April 1996. Needles were frozen to a range of temperatures and rated for visible injury (browning). Cold hardiness was determined as the temperature for 50% injury "LT50". Elevated temperature delayed the times of both cold hardening of the trees in the fall and dehardening in the spring. At maximum cold hardiness (mid-January), elevated temperature trees were significantly less hardy compared to ambient temperature trees. Elevated CO₂ decreased cold hardiness compared to ambient CO₂ during both cold hardening and dehardening. The time of initial bud burst was affected by temperature treatment, but at the elevated temperature bud burst was erratic and terminal shoot growth poor compared to the ambient temperature possible due to disturbed dormancy and unsatisfied chilling requirements. Thus, in areas with currently mild winters such as western Oregon, climatic warming may disturb the physiological processes of dormancy and cold hardiness development; and lack of adequate chilling may affecting normal bud burst and subsequent vigorous shoot growth.

Hobbie, Eric. A., Jillian Gregg, David M. Olszyk, Paul T. Rygielwicz and David T. Tingey. 2002. Effects of climate change on labile and structural carbon in Douglas-fir needles as estimated by $\delta^{13}\text{C}$ and C_{area} measurements. *Global Change Biology* 8:1072-1084.

Models of photosynthesis, respiration, and export predict that foliar labile carbon (C) should increase with elevated CO₂ but decrease with elevated temperature.

Sugars, starch, and protein can be compared between treatments, but these compounds make up only a fraction of the total labile pool. Moreover, it is difficult to assess the turnover of labile carbon between years for evergreen foliage. Here, we combined changes in foliar C_{area} (C concentration on an areal basis) as needles aged with changes in foliar isotopic composition ($\delta^{13}\text{C}$) caused by inputs of ¹³C-depleted CO₂ to estimate labile and structural C in needles of different ages in a four-year, closed-chamber mesocosm experiment in which Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were exposed to elevated temperature (ambient + 3.5°C) and CO₂ (ambient + 179 ppm). Declines in $\delta^{13}\text{C}$ of needle cohorts as they aged indicated incorporation of newly fixed labile or structural carbon. The $\delta^{13}\text{C}$ calculations showed that new C was 41 ± 2% and 28 ± 3% of total needle carbon in second- and third-year needles, respectively, with higher proportions of new C in elevated than ambient CO₂ chambers (e.g. 42 ± 2% vs. 37 ± 6%, respectively, for second-year needles). Relative to ambient CO₂, elevated CO₂ increased labile C in both first- and second-year needles. Relative to ambient temperature, elevated temperature diminished labile C in second-year needles but not in first-year needles, perhaps because of differences in sink strength between the two needle age classes. We hypothesize that plant-soil feedbacks on nitrogen supply contributed to higher photosynthetic rates under elevated temperatures that partly compensated for higher turnover rates of labile C. Strong positive correlations between labile C and sugar concentrations suggested that labile C was primarily determined by carbohydrates. Labile C was negatively correlated with concentrations of cellulose and protein. Elevated temperature increased foliar %C, possibly due to a shift of labile constituents from low %C carbohydrates to relatively high %C protein. Decreased sugar concentrations and increased nitrogen concentrations with elevated temperature were consistent with this explanation. Because foliar constituents that vary in isotopic signature also vary in concentrations with leaf age or environmental conditions, inferences of c_i/c_a values from $\delta^{13}\text{C}$ of bulk leaf tissue should be done cautiously. Tracing of ¹³C through foliar carbon pools may provide new insight into foliar C constituents and turnover.

Hobbie E.A., M.G. Johnson, P.T. Rygielwicz, D.T. Tingey, D.M. Olszyk 2004. Isotopic estimates of new carbon inputs into litter and soils in a four-year climate change experiment with Douglas-fir. *Plant and Soil* 259: 331-343.

Soil carbon is a major reservoir of terrestrial carbon and a potential sink of atmospheric CO₂. Therefore, numerous studies have attempted to quantify soil carbon responses to environmental factors such as global warming, elevated CO₂, or ecosystem management.

Changes in soil carbon can be based on soil ¹³C/¹²C ratios, however, there are problems in interpreting the results based on current methodology. We present a modified method to quantify the effects of global climate change on plant inputs of carbon to soil that accounts for isotopic fractionation between biotic inputs and new soil organic matter. In a four-year study, the effects of elevated CO₂ and temperature were

determined for reconstructed Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] ecosystems. The ¹³C patterns in litter and mineral soil horizons were measured and compared to $\delta^{13}\text{C}$ patterns in needles, fine roots, and coarse roots. The ¹³C patterns clearly indicated the proportion of new carbon added to each soil layer which was 7-9% for the top litter layers, 13-15% for the top mineral soil (A) horizon, and 4% for the lower (B2 and C) soil horizons. However, under the nitrogen-limited growth conditions used in this study, neither elevated CO₂ nor temperature affected the soil carbon sequestration patterns. The isotopic enrichment of newly incorporated soil carbon relative to plant inputs was about 2‰. This enrichment must be accounted for when using shifts in soil $\delta^{13}\text{C}$ to calculate inputs of plant carbon into the soil, and has probably resulted in significant underestimates of new soil carbon inputs in prior global change studies that assumed no isotopic fractionation between biotic inputs and newly incorporated soil carbon.

Hobbie, E.A., D.M. Olszyk, P.T. Rygielwicz, D.T. Tingey and M.G. Johnson. 2001. Foliar nitrogen concentrations and natural abundance of ¹⁵N suggest nitrogen allocation patterns of Douglas-fir and mycorrhizal fungi during development in elevated carbon dioxide concentration and temperature. *Tree Physiology* 21:1113-1122.

Pseudotsuga menziesii (Mirb.) Franco (Douglas-fir) seedlings were grown in a 2 x 2 factorial design in enclosed mesocosms at ambient temperature or 3.5 °C above ambient, and at ambient CO₂ concentration [CO₂] or 179 ppm above ambient. Two additional mesocosms were maintained as open controls. We measured the extent of mycorrhizal infection, foliar nitrogen (N) concentrations on both a weight basis (%N) and area basis (N_{area}), and foliar $\delta^{15}\text{N}$ signatures (¹⁵N/¹⁴N ratios) from summer 1993 through summer 1997. Mycorrhizal fungi had colonized nearly all root tips across all treatments by spring 1994. Elevated [CO₂] lowered foliar %N but did not affect N_{area}, whereas elevated temperature increased both foliar %N and N_{area}. Foliar $\delta^{15}\text{N}$ was initially -1‰ and dropped by the final harvest to between -4 and -5‰ in the enclosed mesocosms, probably because of transfer of isotopically depleted N from mycorrhizal fungi. Based on the similarity in foliar $\delta^{15}\text{N}$ among treatments, we conclude that mycorrhizal fungi had similar N allocation patterns across CO₂ and temperature treatments. We combined isotopic and N_{area} data for 1993-94 to calculate fluxes of N for second- and third-year needles. Yearly N influxes were higher in second-year needles than in third-year needles (about 160 and 50% of initial leaf N, respectively), indicating greater sink strength in the younger needles. Influxes of N in second-year needles increased in response to elevated temperature, suggesting increased N supply from soil relative to plant N demands. In the elevated temperature treatments, N effluxes from third-year needles were higher in seedlings in elevated [CO₂] than in ambient [CO₂], probably because of increased N allocation below ground. We conclude that N allocation patterns shifted in response to the elevated temperature and [CO₂] treatments in the seedlings but not in their fungal symbionts.

Hobbie, E.A., Watrud, L.S., Maggard, S., Shiroyama, T., Rygielwicz, P.T. 2003. Carbohydrate Use by Litter and Soil Fungi Assessed Through Stable Isotopes and BIOLOG[®] Assays. Soil Biology & Biochemistry 35: 303-311.

Soil fungi are integral to decomposition in forests, yet identification of probable functional roles of different taxa is problematic. Here, we compared carbohydrate assimilation patterns derived from stable isotope analyses on cultures with those produced from cultures on Biolog[®] SP-F plates for 12 taxa of soil- and litter-inhabiting saprophytic fungi isolated from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) ecosystems. To determine the relative assimilation of malt extract versus sucrose by ¹³C stable isotope analyses, we cultured fungi with malt extract plus either C₃- or C₄-derived sucrose as carbon sources. *Rhodotorula graminis* and *Fusarium oxysporum* assimilated the highest proportion of sucrose, a *Mortierella* isolate and the unidentified sterile isolate FPC 341 assimilated the lowest proportion of sucrose, and the remainder of the cultures assimilated similar and intermediate proportions of sucrose. We then used Biolog SF-P plates to determine the metabolic activity of the fungi on eight carbohydrates similar to those present in the isotopic study: glucose, fructose, galactose, maltose, sucrose, cellobiose, lactose, and glycogen. In general, metabolic activity was greatest on maltose and glucose and lowest on fructose. Two of the isolates (*Aspergillus flavus* and *F. oxysporum*) had higher metabolic activity on the glucose-containing disaccharide cellobiose than on glucose, strongly suggesting preferential uptake of cellobiose compared to glucose and suggesting the potential ability to use cellulose. The high metabolic activity of these cultures on galactose, a primary constituent of hemicellulose, also suggested cellulolytic capabilities. With metabolic activity normalized among cultures, the *Mortierella* isolate and the unidentified sterile isolate FPC 341 had the lowest metabolic activity on sucrose, results generally consistent with assimilation patterns calculated isotopically. Low metabolic activities of *R. graminis* and *F. oxysporum* on maltose in Biolog assays were qualitatively consistent with isotopic results. The small assimilation of maltose in these two cultures when sucrose was also present suggested that sucrose inhibited maltose uptake. Assimilation of sucrose as calculated isotopically was correlated with the ratio of sucrose : maltose assimilation as calculated from Biolog assays ($r^2=0.45$, $p=0.0145$, $n=12$). These results indicate that stable isotope studies and Biolog methodologies may provide complementary information to characterize functional roles of fungi in forest litter and soil.

Hobbie, E.A., D.T. Tingey, P.T. Rygielwicz, M.G. Johnson, D.M. Olszyk 2002. Contributions of current year photosynthate to fine roots estimated using a ¹³C-depleted CO₂ source. Plant and Soil 247: 233-242.

The quantification of root turnover is necessary for a complete understanding of plant carbon (C) budgets. A variety of techniques for quantification have been developed, including sequential coring, root in-growth cores, minirhizotron methods,

nitrogen (N) budget methods, and C flux methods. We present an additional method to distinguish current- from prior-year allocation of carbon (C) to roots in global change experiments using changes in ¹³C resulting from application of tank-derived CO₂. In a four-year study examining effects of elevated CO₂ and temperature on reconstructed Douglas-fir (*Pseudotsuga mensiezii*) ecosystems, ¹³C patterns of fine roots and foliage were measured yearly. Native soil of low nitrogen (N) content was used, so plant N supply relied on natural soil N processes. Regression analyses showed that 75% of fine root C originated from current-year photosynthate, with no effects of elevated CO₂ or temperature under these N-limited conditions. The method is useful as an independent measure of the contribution of current-year photosynthate to root C and could be used to improve estimates of root C budgets with concurrent measurements of root C pools. We calculated an isotopic enrichment of root C relative to foliar C of 2‰. This enrichment agrees with prior measurements of the enrichment of heterotrophic versus autotrophic plant tissues and must be accounted for when using shifts in foliar ¹³C to calculate inputs of plant C into the soil. This enrichment is probably a contributing factor to the progressive enrichment in ¹³C with increasing depth in soil profiles.

Homann, Peter S., Robert B. McKane and Phillip Sollins. 2000. Belowground processes in forest-ecosystem biogeochemical simulation models. *Forest Ecology and Management* 138:3-18.

Numerical simulation models of forest ecosystems synthesize a broad array of concepts from tree physiology, community ecology, hydrology, soil physics, soil chemistry and soil microbiology. Most current models are directed toward assessing natural processes or existing conditions, nutrient losses influenced by atmospheric deposition, C and N dynamics related to climate variation, and impacts of management activities. They have been applied mostly at the stand or plot scale, but regional and global applications are expanding. Commonly included belowground processes are nutrient uptake by roots, root respiration, root growth and death, microbial respiration, microbial mineralization and immobilization of nutrients, nitrification, denitrification, water transport, solute transport, cation exchange, anion sorption, mineral weathering and solution equilibration. Models differ considerably with respect to which processes and associated chemical forms are included, and how environmental and other factors influence process rates. Recent models demonstrated substantial discrepancies between model output and observations for both model verification and validation. The normalized mean absolute error between model output and observations of soil solution solute concentrations, solid phase characteristics, and process rates ranged from 0 to >1000%. There were considerable differences among outputs from models applied to the same situation, with process rates differing by as much as a factor of 4, and changes in chemical masses differing in both direction and magnitude. These discrepancies are attributed to differences in model structure, specific equations relating process rates to environmental factors, calibration procedures, and uncertainty of observations. Substantial improvement in the capability of models to reproduce

observed trends is required for models to be generally applicable in public-policy decisions. Approaches that may contribute to improvement include modularity to allow easy alteration and comparison of individual equations and process formulations; hierarchical structure to allow selection of level of detail, depending on availability of data for calibration and driving variables; enhanced documentation of all phases of model development, calibration, and evaluation; and continued coordination with experimental studies.

Lewis, J. D., M. Lucash, D. Olszyk, and D. T. Tingey. 2001. Seasonal patterns of photosynthesis in Douglas fir seedlings during the third and fourth year of exposure to elevated CO₂ and temperature. *Plant, Cell and Environment* 24:539-548.

Increases in atmospheric CO₂ and associated global warming may interact to affect on the rate of uptake of CO₂ into plants through the processes of photosynthesis, could affect overall tree productivity. Thus, we examined the interactive effects of elevated CO₂ and temperature on seasonal patterns of photosynthesis in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Seedlings were grown in sunlit chambers controlled to track either ambient (~ 400 ppm) CO₂ or ambient + 200 ppm CO₂, and either ambient temperature or ambient + 4 °C. Light-saturated net photosynthetic rates were measured approximately monthly over a 21-month period. Elevated CO₂ increased net photosynthetic rates 21% on average across temperature treatments during both the 1996 hydrologic year, the third year of exposure, and the 1997 hydrologic year. Elevated mean annual temperature increased net photosynthetic rates 33% on average across CO₂ treatments during both years. Seasonal temperature changes also affected net photosynthetic rates. Across treatments, net photosynthetic rates were highest in the spring and fall, and lowest in July-August and December-January. Seasonal increases in temperature were not correlated with increases in the relative photosynthetic response to elevated CO₂. Seasonal shifts in the photosynthetic temperature optimum reduced temperature effects on the relative response to elevated CO₂. These results suggest that the effects of elevated CO₂ on net photosynthetic rates in Douglas-fir are largely independent of temperature.

Lewis, J.D., M. Lucash, D.M. Olszyk and D.T. Tingey. 2002. Stomatal responses of Douglas-fir seedlings to elevated carbon dioxide and temperature during the third and fourth years of exposure. *Plant, Cell and Environment* 25:1411-1421.

Two major components of climate change, increasing atmospheric [CO₂] and increasing temperature, may substantially alter the effects of water availability to plants through effects on the rate of water loss from leaves. To determine the interactive effects of elevated [CO₂] and temperature on seasonal patterns of water loss from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, we measured needle stomatal conductance, transpiration and instantaneous transpiration efficiency (ITE;

moles CO₂ assimilated per mole H₂O transpired). The seedlings were grown in sunlit chambers at either ambient CO₂ or ambient + 180, mol mol⁻¹ CO₂, and at ambient temperature or ambient + 3.5 °C. Needle gas exchange at the growth conditions was measured approximately monthly over 21 months. Across the study period, growth in elevated [CO₂] decreased transpiration rates an average of 12% and increased ITE an average of 46%. The absolute reduction of transpiration rates associated with elevated [CO₂] significantly increased with seasonal increases in vapour pressure deficit (VPD). Growth in elevated temperature increased transpiration rates an average of 37%, and did not affect ITE. Combined, growth in elevated [CO₂] and elevated temperature increased transpiration rates an average of 19% compared to growth in ambient conditions. Stomatal sensitivity to VPD did not significantly vary between CO₂ or temperature treatments. This study suggested that climate change may substantially alter needle-level water loss and water use efficiency of Douglas-fir, but will not change stomatal sensitivity to VPD.

Lewis, J.D., D. Olszyk and D.T. Tingey. 1999. Seasonal patterns of photosynthetic light response in Douglas-fir seedlings subjected to elevated atmospheric CO₂ and temperature. *Tree Physiology* 19:243-252.

Increases in atmospheric CO₂ concentration and temperature are predicted to increase the light response of photosynthesis by increasing light-saturated photosynthetic rates and apparent quantum yields. We examined the interactive effects of elevated atmospheric CO₂ concentration and temperature on the light response of photosynthesis in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Seedlings were grown in sunlit chambers controlled to track either ambient (-400 ppm) CO₂ or ambient + 200 ppm CO₂, at ambient temperature or ambient +4°C. Photosynthetic light response curves were measured over an 18-month period beginning 32 months after treatments were initiated. Light-response curves were measured at the growth CO₂ concentration, and were used to calculate the light-saturated rate of photosynthesis, light compensation point, quantum yield and respiration rate. Elevated CO₂ increased apparent quantum yields during two of five measurement periods, but did not significantly affect lightsaturated net photosynthetic rates, light compensation points or respiration rates. Elevated temperature increased all parameters. There were no significant interactions between CO₂ concentration and temperature. We conclude that down-regulation of photosynthesis occurred in the elevated CO₂ treatments such that carbon uptake at a given irradiance was similar across CO₂ treatments. In contrast, increasing temperature may substantially increase carbon uptake rates in Douglas-fir, assuming other environmental factors do not limit photosynthesis; however, it is not clear whether the increased carbon uptake will increase growth rates or be offset by increased carbon efflux through respiration.

Lin, Guanghui, J.R. Ehleringer, P.T. Rygielwicz, M.K. Johnson and D.T. Tingey. 1999. Elevated CO₂ and temperature impacts on different components of soil CO₂ efflux in Douglas-fir terracosms. *Global Change Biology*, 5:157-68.

Forests may be net sinks or sources of atmospheric CO₂, thereby mitigating or contributing to anthropogenic sources of CO₂. Increasing atmospheric [CO₂] or temperature stimulates soil respiration. However, no in situ techniques exist to partition respiration unambiguously into its sources [root respiration, and decomposition of litter and soil organic matter (SOM)]. The partitioning is possible using stable isotopes of carbon and oxygen. Soil respiration was partitioned while Douglas-fir seedlings were grown under four climate treatments. Under all treatments, litter decomposition contributed the most to respiration, followed by root respiration and then SOM decomposition. We show for the first time that increased respiration rates under climate treatments result from varying responses of the sources. The combined treatment of elevated [CO₂] and temperature enhanced respiration the most, and elevated [CO₂] alone the least. Respiration under elevated temperature was intermediate. Our results strongly suggest that, unless carbon influx to litter or SOM increases to offset enhanced carbon release from these sources, predicted global climate change may decrease long-term carbon storage in forest floors and soils.

Lin, Guanghui, Paul T. Rygielwicz, James R. Ehleringer, Mark G. Johnson, and David T. Tingey. 2001. Time-dependent responses of soil CO₂ efflux to elevated atmospheric [CO₂] and temperature in experimental forest mesocosms. *Plant and Soil* 229:259-270.

In order to balance terrestrial ecosystem carbon budgets under altered climates, it is necessary to know if soils will be net sources or sinks of carbon. Simply measuring the total amount of CO₂ released from soils (soil CO₂ efflux) has yielded conclusive evidence to address this question and numerous other questions concerning terrestrial ecosystem function since soil CO₂ efflux results from innumerable, interacting processes. One approach that may improve the reliability of using soil CO₂ efflux is to attribute the total efflux to its component sources. In general, soil CO₂ efflux results from respiration of plant roots and soil biota. Using a dual isotope method we developed previously, coupled with mixing models, we partitioned the efflux into the source components of 1) respiration from roots + rhizosphere (rhizosphere respiration is from organisms directly dependent on carbon substrates released from plant roots), 2) respiration from organisms decomposing the litter layer, and 3) respiration of organisms decomposing the soil organic matter (SOM). The partitioning was done for the Douglas-fir/soil system installed in the mesocosms at WED and subjected to four climatic treatments involving atmospheric CO₂ concentrations and temperatures. We partitioned the efflux for a two-year period to evaluate the stability of the sources to respond to the climate treatments. Total efflux was increased by elevated CO₂ or

elevated temperature in both years, but the enhancement was much less in the second year. Rhizosphere respiration generally increased less in the climate treatments in the second year compared with the first year. Respiration due to litter decomposition also tended to increase less under elevated CO₂ in year two but there was no difference in the response to elevated temperature between the two years. In contrast, respiration due to SOM oxidation showed similar responses under elevated CO₂ in the two years but substantially less SOM oxidation occurred under elevated temperature in year two. Our results indicate that the plant/soil system responded rapidly but not consistently through time to the climate treatments. Some of the temporally-varying responses may have been due to the transient nature of physiological processes, while other variations may reflect effects of antecedent soil disturbance caused when the ecosystem was constructed in the mesocosms. Our results strongly indicate the need to conduct long-term (multi-year) projects of ecosystems in order to obtain reliable measures of their function when they are subjected to environmental stresses.

Olszyk, D.M., M.G. Johnson, D.T. Tingey, P.T. Rygielwicz, C. Wise, E. VanEss, A. Benson, M.J. Storm and R. King. 2003. Whole-seedling biomass allocation, leaf area, and tissue chemistry for Douglas-fir exposed to elevated CO₂ and temperature for 4 years. *Can. J. For. Res.* 33: 269-278.

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were grown under ambient or elevated (ambient + 180 $\mu\text{mol}\mu\text{mol}^{-1}$) CO₂ and ambient or elevated (ambient + 3.5°C) temperature in outdoor, sunlit chambers with a field soil. After 4 years, seedlings were harvested and measured for leaf area, leaf, fine root (<1 mm diameter), and structural (buds, branches, stems, main root, and lateral roots >1 mm in diameter) dry masses, and leaf and fine root C/N ratio, percent sugar, and percent cellulose. Elevated CO₂ did not affect biomass production or allocation for any plant organ but increased specific leaf mass, leaf C/N ratio, and percent sugar and decreased the ratio of leaf area to structural weight and leaf percent cellulose. Elevated temperature tended to reduce biomass allocation to leaves and leaf sugar concentration. Fine root percent sugar tended to increase with elevated temperature but only at elevated CO₂. Therefore, for Douglas-fir seedlings growing under naturally limiting soil moisture and nutrition conditions, elevated CO₂ and temperature may have little impact on biomass or leaf area except for reduced specific leaf mass with elevated CO₂ and reduced biomass allocation to leaves with elevated temperature. However, both elevated CO₂ and temperature may alter leaf chemistry.

Olszyk, David, Claudia Wise, Erica VanEss, Martha Apple and David Tingey. 1998. Phenology and growth of shoots, needles, and buds of Douglas-fir seedlings with elevated CO₂ and/or temperature. *Canadian Journal of Botany* 76:1991-2001.

Increased atmospheric carbon dioxide and associated global warming may affect tree growth, but impacts of these combined stresses are largely unknown, especially over multiple growing seasons. Corvallis scientists studied the effects of elevated atmospheric carbon dioxide and elevated temperature associated with predicted global warming on Douglas-fir. Seedlings were grown for three full growing seasons in outdoor sun-lit chambers which maintained diel and seasonal variation in climate. Elevated carbon dioxide had no impact on overall phenology and growth of terminal shoots, needles, or buds. In contrast, elevated temperature affected phenology and growth compared to ambient temperature, i.e., main-flushes occurred slightly earlier in the spring, overall shoot and needle growth rates were higher earlier during the season, final terminal shoot length was reduced, and final needle length was either reduced, increased, or unchanged depending on season. The lammas-flush was delayed and/or decreased at elevated temperature. Leading terminal bud break and growth occurred earlier, resting winter bud length was reduced, and bud width tended to increase with elevated temperature. Thus, at least during seedling growth, elevated temperatures associated with global warming may reduce both main and lammas-flush growth, thereby altering tree productivity; whereas elevated carbon dioxide does not affect growth at either the current or elevated temperature.

Olszyk, David, Claudia Wise, Erica Van Ess, and David Tingey. 1998. Elevated temperature but not elevated CO₂ affects stem diameter and height of Douglas-fir seedlings: results over three growing seasons. Canadian Journal of Forest Research 28:1046-1054.

Global climatic changes may produce dramatic changes in forest productivity over the next century, but data are lacking to evaluate potential impacts of key aspects of global change, elevated temperature and CO₂, on tree growth. Thus at Corvallis the EPA is carrying out a long term study on the response of Douglas-fir [*Pseudotsuga menziessii* (Mirb.) Franco] trees to elevated CO₂ (+200 $\mu\text{mol mol}^{-1}$) and/or elevated temperature (+4 C). Seedlings were grown for three complete growing seasons in outdoor, sun-lit chambers. To simulate Oregon field growing conditions, trees received a wet-dry season cycle of soil moisture and relied on soil biological processes for nutrients. Elevated temperature advanced the date of initiation of shoot growth during each growing season: stem diameter and height began to increase earlier and stopped increasing earlier compared to trees grown at ambient temperature. At the end of the three seasons, elevated temperature resulted in significantly shorter trees at the elevated compared to the ambient temperature trees; but temperature had no effect stem diameters. Elevated CO₂ had no effect on either stem diameter or height at any time and there was no evidence for any CO₂ x temperature interactions. Thus, at least during early growth under field-like soil moisture and fertility conditions, elevated temperatures associated with global warming may reduce shoot height, but not necessarily stem diameter, suggesting a shift in allocation of above-ground biomass from canopy to stems with implications for competition during seedling establishment and for modeling tree growth. In contrast, elevated CO₂ may not affect at least early seedling shoot growth as measured by stem diameter or height.

Ormrod, D. P., V. G. Lesser, D. M. Olszyk, and D. T. Tingey. 1999. Elevated Temperature and carbon dioxide affect chlorophylls and carotenoids in Douglas-fir seedlings. International Journal of Plant Science 160:529-534.

Increased atmospheric carbon dioxide and associated global warming may affect tree growth, but the physiological mechanisms responsible for such changes are uncertain. Pigment analyses are widely used to evaluate plant vigor. When combined with needle sampling, pigment analyses provide rapid, inexpensive, and non-destructive data on plant stress. Additionally, plant pigments are readily measured using aircraft and satellite sensors. Corvallis scientists studied the effects of elevated atmospheric carbon dioxide and air temperature on needle pigments of Douglas-fir trees. Elevated carbon dioxide reduced pigment concentrations in current year needles. In contrast, elevated temperature was associated with increases in pigments concentrations in both current and previous year needles. Needle pigments were found to be responsive to temperature stress and enhanced atmospheric carbon dioxide. Consequently, needle and leaf pigments could provide a useful indicator of climate-related ecosystem stress.

Rygiewicz, Paul T. and Christian P. Andersen. 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. Nature 369:58-60.

Plants and soils are a critically important element in the global carbon-energy equation. It is estimated that in forest ecosystems over two-thirds of the carbon is contained in soils and peat deposits'. Despite the importance of forest soils in the global carbon cycle, fluxes of carbon associated with fundamental processes and soil functional groups are inadequately quantified, limiting our understanding of carbon movement and sequestration in soils. We report here the direct measurement of carbon in and through all major pools of a mycorrhizal (fungus-root) coniferous seedling (a complete carbon budget). The mycorrhizal symbiont reduces overall retention of carbon in the plant-fungus symbiosis by increasing carbon in roots and below-ground respiration and reducing its retention and release above ground. Below ground, mycorrhizal plants shifted allocation of carbon to pools that are rapidly turned over, primarily to fine roots and fungal hyphae, and host root and fungal respiration. Mycorrhizae alter the size of below-ground carbon pools, the quality and, therefore, the retention time of carbon below ground. Our data indicate that if elevated atmospheric CO₂ and altered climate stressors alter mycorrhizal colonization in forests, the role of forests in sequestering carbon could be altered.

Rygiewicz, Paul T., Kendall J. Martin and Amy R. Tuininga. 2000. Morphotype community structure of ectomycorrhizas on Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings grown under elevated atmospheric CO₂ and temperature. Oecologia, 124:299-308.

Mycorrhizas, a symbiosis between the roots of plants and fungi, alter the carbon economy and nutrient uptake capabilities of plants, their regeneration, and the nutrient cycling and sustainability of ecosystems. During 1993-1997, scientists assessed how

climate change stresses affected the abundance of the symbiosis, and the diversity of the fungi forming the symbiosis under altered climate conditions. The individual and interactive effects of elevated atmospheric CO₂ and temperature were assessed [ambient atmospheric CO₂ concentration, elevated CO₂ (200 ppm above ambient), ambient temperature, and elevated temperature (4 °C above ambient)]. In 1993, two-year-old Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings were planted in environment-tracking chambers (terracosms) containing reconstructed, native forest soil. We categorized the ectomycorrhizal (ECM) root tips into morphotypes using their gross morphological traits. A highly diverse and stable community of ectomycorrhizas was established in the terracosms (a total of 40 morphotypes was encountered during the experiment). When we considered the morphotype community in its entirety, we did not find large changes in its diversity (Simpson's index) due to climate treatments. While some morphotypes were negatively affected seasonally by higher temperatures (*Rhizopogon* spp. group), others (*Cenococcum* sp.) seemed to thrive. Underlying the dominant patterns of change in diversity, the subdominant populations responded slightly differently. Community diversity increased at a greater rate for all subdominant populations than the rate of increase of diversity over time when dominant populations were included in the community. Overall, disturbance by climate change seems to affect the symbiosis differentially, with the level of the symbiosis primarily affected by CO₂ and the proportions of individual fungal species forming the symbiosis primarily affected by temperature. Such results have implications on whether this obligate symbiosis can be maintained as the geographic distribution of trees species changes as future climate is altered.

Rygiewicz, P.T., K.J. Martin and A.R. Tuininga. 1997. Global Climate Change and Diversity of Mycorrhizae *in Progress in Microbial Ecology*, Martins, M.T., M.I.Z. Sato, J.M. Tiedje, L.C. N .Hagler, J. Döbereiner and P.S. Sanchez, editors. Pp 91-98.

We discuss elevated CO₂ and temperature effects on ectomycorrhizal diversity during the first part of a 3-4 year exposure using Douglas-fir seedlings. Ectomycorrhizae (ECM) are sorted into morphotypes by gross morphology. Number of ECM tips and number of morphotypes increased as exposure progressed indicating adjustment from nursery to native soil. Treatments may affect numbers of tips in the *Rhizopogon* morphotypes differentially by season. Simpson's index changed by season and was affected by temperature. Morphotype diversity as the exposure continues may affect dominance of the *Rhizopogon* sp. group. Treatment effects on specific root length, and ECM tip and morphotype numbers did not correspond so this aspect of C allocation may not influence colonization.

Tingey, David T., John A. Laurence, James A. Weber, Joseph Greene, William E. Hogsett, Sandra Brown and E. Henry Lee. 2001. Elevated CO₂ and temperature alter the response of *Pinus ponderosa* to ozone: a simulation analysis. *Ecological Applications* 11:1412-1424.

We investigated the potential impact of projected future temperature and CO₂ concentrations in combination with tropospheric O₃ on the annual biomass increment of *Pinus ponderosa* Doug. ex Laws. TREGRO, a process-based whole-tree growth model in which trees experienced a seasonal drought, was used to study the interactions of CO₂, temperature, and O₃ on tree growth along a latitudinal gradient in California, Oregon, and Washington, USA. The annual biomass increment increased in proportion to CO₂ concentration, although the magnitude varied among sites. Increasing air temperature (+ 1.3°C) increased growth at most sites. Elevated CO₂ increased the temperature optimum for growth at four sites and decreased it at two sites. The annual biomass increment decreased with increasing O₃ exposure. The differences in O₃ effects among sites were primarily controlled by differences in precipitation. Although increasing CO₂ can reduce the O₃ impact, it does not eliminate the impact of O₃. Elevated CO₂ would enhance tree growth more if O₃ exposures were reduced, especially in the more polluted sites. The greatest benefit for tree growth would come from reducing O₃ exposures in the most polluted sites, but we must also consider locations that have high inherent O₃ sensitivity because of their mesic conditions. Limiting the increase of O₃ levels in those areas will also increase tree growth.

Tingey, David T., Bruce D. McVeety, Ron Waschmann, Mark G. Johnson, Donald L. Phillips, Paul T. Rygielwicz, and David M. Olszyk. 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. *J. Environ. Qual.* 25:614-625.

A new environmental-tracking, sun-lit controlled-environmental facility (terracosm) that can control and manipulate climatic and edaphic factors while maintaining natural environmental variability was developed to study the effects of environmental stresses on a model ecosystem.

Tingey, David T., Donald L. Phillips, and Mark G. Johnson. 2000. Elevated CO₂ and conifer roots: effects on growth, life span and turnover. *New Phytologist* 147:87-103

WED scientists completed a review of the effects of elevated CO₂ on conifer roots for an invited presentation at an International Symposium, "Root

Dynamics and Global Change: An Ecosystem Perspective" sponsored by New Phytologist and Global Change and Terrestrial Ecology (GCTE). The review concluded. That elevated CO₂ increases root growth and fine (diameter 2 mm) root growth across a range of species and experimental conditions. However, there is no clear evidence that elevated CO₂ changes the proportion of C allocated to roots, measured as either the root/shoot ratio or the fine root/needle ratio. Elevated CO₂ tends to increase mycorrhizal infection, colonization and the number of mycorrhizae and extramatrical hyphae, supporting its key role in aiding the plant to more intensively exploit soil resources. Only two studies have determined the effects of elevated CO₂ on conifer fine root life span and there is no clear trend. Although data are limited, elevated CO₂ increases the absolute fine root turnover rates in conifers. However, the standing crop root biomass is also higher, and the effect of elevated CO₂ on relative turnover rates (turnover/biomass) runs the gamut from an increase to a decrease. This review provides important data for assessing the ecological consequences of elevated CO₂ on coniferous forests.

Tingey, D.T., D.L. Phillips, and M.G. Johnson. 2003. Optimizing minirhizotron sample frequency for an evergreen and deciduous tree species. *New Phytologist* 157: 155-161.

Data on the production and turnover of fine roots are needed to parameterize plant growth models and to assess the impacts of stressors on ecosystems. Increasingly minirhizotrons are being used in natural ecosystems to determine fine root production and turnover, as they provide a nondestructive, *in situ* method for studying fine root dynamics. WED scientists recently completed a study to determine how image collection frequency influences estimates of fine root production and turnover for an evergreen (*Pseudotsuga menziesii* [Mirb.] Franco) and a deciduous (*Tilia cordata* Mill.) tree. Because it is costly to collect and analyze root images it is desirable to minimize the number of images collected. However, if the sampling interval is too long, fine roots can appear and disappear between samplings, leading to underestimates of production and turnover. For example, if a sampling interval of 8 weeks is used, 24 and 35% of the fine root production in *P. menziesii* and *T. cordata*, respectively, is not measured compared to the 0.5 week interval. Fine root turnover displays the same sensitivity to sample frequency as production. The conclusion that sampling frequency influences estimates of fine root production and turnover applies not only to the minirhizotron method but also to sequential coring and in-growth cores, methods that also rely on periodic sampling to estimate production and turnover. These findings will lead to improved estimates of fine root production and turnover and will assist in developing better models and risk assessment procedures to determine the impacts of stressors on vegetation.