**PROGRESS REPORT
YEAR 2**

May 15, 1995

**PROGRAM FOR ECOSYSTEM RESEARCH
DEPARTMENT OF ENERGY**

**CHANGES IN THE FLUX OF CARBON BETWEEN PLANTS AND SOIL MICROORGANISMS
AT ELEVATED CO₂:
PHYSIOLOGICAL PROCESSES WITH ECOSYSTEM-LEVEL IMPLICATIONS**

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PROJECT SUMMARY

Our ability to interpret ecosystem response to elevated atmospheric CO₂ is contingent on understanding and integrating a complex of physiological and ecological processes. However, we have a limited understanding of the combined effects of changes in plant carbon (C) allocation, microbial activity, and nitrogen (N) dynamics on the long-term response of terrestrial ecosystems to elevated CO₂. Individually, these factors are potent modifiers of C and N dynamics, and an in depth understanding of their interactions should provide insight into ecosystem-level responses to global climate change.

With support from the Program for Ecosystem Research (PER), we are testing and refining a conceptual model depicting the influence of elevated atmospheric CO₂ on plant production, soil microorganisms, and the rate at which C and N are cycled in the plant-soil system (Fig. 1). It is based on the premise that above- and belowground plant production provide the primary link between the rising atmospheric CO₂ concentration and changes in the cycling of C and N within terrestrial ecosystems. Our previous experiments support the hypothesis that increased root growth can elicit a positive feedback response by microbial populations and N dynamics within the soil. Nevertheless, we lack a complete understanding of the exact mechanisms by which root production, soil C availability, microbial populations and net N mineralization changes under elevated CO₂.

Over the past year, we have made considerable progress toward understanding the extent to which atmospheric CO₂ and soil N availability modify the flow of C and N between plants and soil microorganisms. Plants are now well established in our open-top chamber system (Fig. 2) and have grown under experimental conditions for one field season.. Most importantly, we have gained several key insights into how atmospheric CO₂ and soil N availability modify the C allocation patterns of *Populus tremuloides* and, in turn, how changes in plant C allocation influence microbial activity in soil. Although we originally proposed conducting ¹⁴C and ¹⁵N labeling during the 1994 field season, we have delayed our initial isotope experiment until the mid-1995 field season. Plants in our low N availability treatment had relatively low leaf areas and simply could not assimilate enough ¹⁴CO₂ to reliably trace the flow of C between plants and soil microorganisms. During the 1995 field season, however, we will conduct two dual-isotope experiments to determine the extent to which atmospheric CO₂ and soil N availability influence the seasonal C allocation patterns of *Populus tremuloides*. By following the ¹⁴C and ¹⁵N in soil, we also will determine the extent to which changes in seasonal C allocation influence the activities of soil microorganisms.

ACCOMPLISHMENTS - YEAR 2

During the 1994 growing season, we collected a wide array of data on the response of plants and soil microorganisms to elevated atmospheric CO₂ and soil N availability. Specifically, we made periodic measurements of leaf area, photosynthesis, fine root production, fine root mortality, and fine root survivorship. Because the aspen genotypes in our experiment occur in the field, and because we know the field location of these genotypes, we compared the phenological development and photosynthetic rate of aspen genotypes under experimental and field conditions. Such an approach will allow us to determine the influence of experimental conditions (i.e., open-top chambers) on plant response, enabling us to better place our results in an ecosystem-relevant context.

Over the past year, we have initiated and are nearing the completion of detailed studies of how atmospheric CO₂ and soil N availability influence: A) the photosynthetic response of early- and late-senescent *Populus* clones, B) fine root dynamics, and C) soil N dynamics and microbial community composition.

A. Leaf Area, Photosynthesis, and Plant C Allocation

We began studying the photosynthetic response of early- and late-senescent *Populus* clones to CO₂ and soil N availability by non-destructively measuring leaf area throughout the growing season. Late-senescent clones rapidly accumulated leaf area over the growing season (Fig. 3). By September, they had a significantly greater total leaf area and leaf mass compared to the early-senescent clones (Fig. 3); that was true in all treatment combinations. Trees grown in high N soil had 3 to 4 times the leaf area and 2 to 2.5 times the leaf mass as those grown in low N soil. Atmospheric CO₂ concentration significantly affected specific leaf area. At high soil N availability, trees grown at elevated CO₂ had less leaf area, but greater leaf mass compared to those at ambient CO₂.

Leaf-level photosynthesis (A) was measured on an upper crown leaf of each tree with an ADC LCA-2 portable photosynthesis system to follow C assimilation from early autumn through leaf senescence. Photosynthesis increased with elevated CO₂ in both N levels, and with high N in elevated but not ambient CO₂ (Figure 4). Decreased A in the elevated CO₂ treatments between 17 September and 2 October coincided with cessation of shoot expansion in the study trees, possibly reflecting decreased aboveground sink strength for photosynthate.

In general, early leaf-drop clones had higher photosynthesis per unit leaf area during September in elevated CO₂, but not in ambient CO₂, consistent with published trends relating A with leaf life-span (Reich et al. 1991, 1992). A general lack of clonal differences in timing of leaf senescence or in late-season A likely reflected a chamber effect on plant phenology, since the original field clones exhibited clear differences in the timing of leaf senescence. We expected that genotypic differences in autumnal leaf senescence would have influenced the duration of photosynthate production which may prove to be significant in a region with a relatively short growing season. However, our ability to detect such differences were apparently confounded by chamber effects which maintained an unseasonably warm microenvironment around the trees and interfered with their normal phenological patterns.

A preliminary, 2-point A/C_i (i.e., assimilation/intercellular CO₂ concentration) analysis was performed with an ADC LCA-3 analyzer to test for photosynthetic acclimation to elevated CO₂ in the high N trees. Contrary to expectations based on the theoretical effect of source-sink relations on photosynthetic capacity (Arp 1991, Stitt 1991), trees in high N soil exhibited photosynthetic down regulation at C_i of 350 and 695 prior to bud-set, but not after bud-set (Table 1). Nevertheless, post-budset trees in elevated CO₂ had higher leaf A at growth C_i than those in ambient CO₂, particularly in high N soil (Fig. 4; Table 1). This suggests that photosynthetic acclimation to elevated CO₂ may not always be an experimental artifact induced by restricted rooting volume or low nutrient availability (Thomas and Strain 1991, Stitt 1991) as neither condition was a factor in our high N root boxes.

Photosynthesis was also measured for every leaf along the vertical axis of the crown of one late-senescent clone to determine the effect of soil fertility and elevated CO₂ on the vertical distribution of

crown photosynthesis. Maximum leaf A ($A_{\max}$) was highest in elevated CO_2 and high N and the position of $A_{\max}$ tended to shift toward the apex of the crown following budset as shoot and leaf expansion ceased (Fig. 5). The position of $A_{\max}$ tended to be lower in the crown in high N trees, and higher in the crown in elevated CO_2 . Using the leaf area data, whole leaf A through the vertical axis of the trees was also estimated (Fig. 6). Preliminary analyses suggested that both elevated CO_2 and high soil N availability resulted in a greater contribution to whole tree C gain by the lower half of the crown. This is likely a result of altered crown N balance and crown architecture. Changes in crown architecture with elevated CO_2 have been reported previously (Reekie and Bazzaz 1989), but have not been related to tree C or N balance.

Elevated CO_2 is known to increase the photosynthetic quantum yield of C3 photosynthesis (Ehleringer and Björkman 1977, Kubiske and Pregitzer 1995). Lower crown leaves that generally photosynthesize in a light limited state should become more productive with elevated CO_2 possibly resulting in less N retranslocation from the lower crown during indeterminate growth (Field 1983). These fundamental changes in whole tree C assimilation patterns have important ramifications for ecosystem C balance. Leaves in the lower half of the crown tend to act as the source for belowground carbohydrate sinks (Dickson 1986, Wardlaw 1990, Coleman et al. 1995). We have demonstrated significantly greater belowground C allocation in *Populus* grown in elevated CO_2 , particularly in low soil fertility (Zak et al. 1993, Curtis et al. 1994, Pregitzer et al. 1995). Thus, ecosystem C allocation patterns may be driven by the response of foliage to atmospheric CO_2 and soil N availability.

B. Fine-Root Dynamics

During the 1994 field season, we recorded video images of developing *Populus* root systems on two-week intervals. Images were collected for the early- and late-senescing clones growing in each of our atmospheric CO_2 and soil N availability treatment combinations. We devoted this past winter to digitizing these images, the first step in calculating rates of fine root production and mortality. This is a very labor intensive task, because each video image must be viewed and the roots must be digitized by hand. We collected thousand of images over the past field season, and it will take several more months before we are able to calculate rates of fine root production and mortality. We will continue collecting video images this field season and will continue to process those already digitized. We anticipate that fine root data from the 1995 field season will be complete by mid-summer 1996.

C. Soil N Dynamics and Microbial Community Composition

We hypothesized that greater belowground plant production under elevated atmospheric CO_2 could alter soil C availability and increase the flow of N within the soil system. In laboratory experiments, we used ^{15}N to trace the flow of NH_4^+ and NO_3^- in the soils of *Populus tremuloides* grown under two levels of atmospheric CO_2 (ambient and twice-ambient) and two levels of soil N availability (low and high). Gross and net rates of N mineralization as well as the N content of microbial biomass were not significantly influenced by atmospheric CO_2 (Table 2), but all displayed large differences related to soil N availability (data not shown). Gross rates of nitrification differed significantly at ambient ($205 \text{ ug g}^{-1} \text{ d}^{-1}$) and twice-ambient ($301 \text{ ug g}^{-1} \text{ d}^{-1}$) CO_2 . However, greater rates of gross nitrification under twice-ambient CO_2 were offset by a significant increase in NO_3^- immobilization, producing equivalent rates of net nitrification at ambient and twice-ambient CO_2 . In short, we found no evidence to suggest that greater

rates of belowground plant production under elevated atmospheric CO₂ will slow rates of N transformations in soil.

We used soil from our ¹⁵N experiment to gain insight into the influence of atmospheric CO₂ and soil N availability on the composition of microbial communities in soil. The active and total biomass of soil bacteria, fungi and protozoans were determined by direct count, and we used phospholipid fatty acid analysis to confirm these results. This work has been conducted in collaboration with Dr. Elaine Ingham (Oregon State University) and Dr. David White (University of Tennessee) and it is now nearing completion. Our preliminary results suggest that soil N availability has a substantial influence on microbial community composition, but that atmospheric CO₂ does not.

PLANNED ACTIVITIES - YEAR 2

Our largest task for the 1995 field season is our dual labeling experiment with ¹⁴C and ¹⁵N to trace the fluxes of C and N between plants and soil microorganisms. We are confident that the preliminary work already conducted, in combination with further refinement of our technique with additional trials, has well prepare us for this task. We will conduct our dual-isotope experiment during mid-growing season and again prior to leaf fall in mid-September. By modifying our original plan, we will be able to determine how atmospheric CO₂ and soil N availability influence the seasonal C allocation patterns of *Populus tremuloides*. By following the label in the soil, we also will be able to sensitively determine the extent to which changes in plant C allocation influence the activity of soil microorganisms. Following the addition of ¹⁴C and ¹⁵N to the experimental units, we will continuously monitor soil respiration to determine when ¹⁴C has moved from the plants into the soil. At that time, we will harvest the experiment and determine the distribution of isotope in plant tissue, microbial biomass, and soil organic matter pools. We will be collaborating with Dr. David White to determine the extent to which greater fine root production at elevated CO₂ influences the composition of microbial communities in soil. Our experiment provides a unique opportunity to follow the flow of plant-derived ¹⁴C into fatty-acid phospholipids, which can be used to determine the abundance of soil bacteria, actinomycetes, and fungi.

In addition to our labeling experiment, we will continue to measure photosynthesis, leaf area, and fine root production and mortality throughout the 1995 growing season. Now that our experimental plants are well established, we also will begin comparing rates of photosynthesis between genetically-identical individuals growing under experimental conditions and those that naturally occur in the field. We plant to spend this winter analyzing data and preparing manuscripts for publication.

PUBLICATIONS AND PRESENTATIONS - YEAR 2

A. Published Manuscripts

- Zak, D.R., D. Ringelberg, K.S. Pregitzer, D.L. Randlett, D.W. White, and P.S. Curtis. 1995. Soil microbial communities beneath *Populus grandidentata* Michx. growing at elevated atmospheric CO₂. Ecological Applications *in press*.
- Pregitzer, K.S., D.R. Zak, P.S. Curtis, M.E. Kubiske, J.A. Teeri, and C.S. Vogel. 1995. Atmospheric CO₂, soil nitrogen and fine root turnover. New Phytologist *in press*.
- Kubiske, M.E., and K.S. Pregitzer. 1995. Effects of elevated CO₂ and light availability on the photosynthetic light response of trees of contrasting shade tolerance. Tree Physiology *in press*.

B. Manuscripts in Review

- Randlett, D.L., D.R. Zak, and K.S. Pregitzer. 1995. Elevated atmospheric CO₂ and leaf litter chemistry: influences on microbial respiration and net N mineralization. Oecologia *in review*.

C. Manuscripts in Preparation

- Kubiske, M.E., C. Mikan, D.R. Zak, K.S. Pregitzer, and J.A. Teeri. 1995. Fundamental changes in canopy photosynthesis in response to atmospheric CO₂ and soil N availability. Nature *in preparation*.
- Kubiske, M.E. C. Mikan, J. Courteau, D.R. Zak, K.S. Pregitzer, and J.A. Teeri. 1995. Late-season carbon assimilation of four *Populus* clones in relation to CO₂ and soil fertility. Canadian Journal of Forest Research *in preparation*
- Mikan, C., D.R. Zak, and D.L. Randlett. 1995. Elevated atmospheric CO₂, soil microorganisms and the flow of N within the soil system. Ecological Applications *in preparation*.
- Mikan, C., D.R. Zak, D. Ringleberg, and D. White. 1995. Soil microbial communities beneath *Populus tremuloides*: the influence of atmospheric CO₂ and soil N availability. Oecologia *in preparation*.
- Ringelberg, D., J.O. Stair, R.J. Norby, D.R. Zak, and E.G. O'Neill. 1995 Increased microbial biomass without a shift in community structure in white oak with exposure to elevated atmospheric CO₂. Global Change Biology *in preparation*.

D. Presentations

- Zak, D.R., and K.S. Pregitzer. 1994. Responses of soil microorganisms to changes in plant production with rising atmospheric CO₂. Annual Meetings of the Ecological Society of America, Knoxville, TN (August 1994).

- Randlett, D.L., D.R. Zak, and K.S. Pregitzer. 1994. Elevated CO₂ and leaf litter chemistry: changes in microbial respiration and net N mineralization. Annual Meetings of the Ecological Society of America, Knoxville, TN (August 1994).
- Kubiske, M.E., C. Mikan, J. Courteau, D.R. Zak, K.S. Pregitzer, and J.A. Teeri. 1995. Late-season carbon assimilation of four *Populus* clones in relation to CO₂ and soil fertility. Annual Meetings of the Ecological Society of America, Snowbird, UT (August 1995).
- Mikan, C., D.R. Zak, and D.L. Randlett. 1995. Elevated atmospheric CO₂, soil microorganisms and the flow of N within the soil system. Annual Meetings of the Ecological Society of America, Snowbird, UT (August 1995).

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Zak, D. R., K. S. Pregitzer, P. S. Curtis, J. A. Teeri, R. Fogel and D. L. Randlett. 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil* 151: 105-117.

Table 1. Mean ($\pm$ se) photosynthesis rates of *Populus tremuloides* clones with early and late leaf drop phenology grown in elevated and ambient CO₂ (growth C_a) and measured at ambient and elevated internal CO₂ (measurement C_i). Measurements were conducted prior to bud set (26 August - 1 September) and after bud set (29 September - 3 October). Means within a row or column of each measurement time (pre or post bud set) with the same letter are not significantly different ($P > 0.05$). Measurements on high soil fertility plants only.

Growth C _a	Measurement C _i	Pre-Bud Set		Post-Bud Set	
		Early	Late	Early	Late
350	350	24.7 $\pm$ 1.6 a	20.6 $\pm$ 1.4 d	25.3 $\pm$ 2.1 a	23.8 $\pm$ 1.8 c
711	350	21.7 $\pm$ 1.5 c	17.9 $\pm$ 1.7 b	24.7 $\pm$ 4.4 a	18.6 $\pm$ 1.5 c
350	695	34.3 $\pm$ 2.4 b	28.0 $\pm$ 1.5 c	38.3 $\pm$ 3.4 b	34.9 $\pm$ 2.6 d
711	695	31.7 $\pm$ 1.9 d	23.2 $\pm$ 2.7 a	31.5 $\pm$ 3.7 b	29.0 $\pm$ 2.4 d

Table 2. Rates of gross and net nitrogen transformations at ambient and twice-ambient atmospheric CO₂. We found no significant differences in microbial biomass N or rates of N transformation in the soil of *P. tremuloides* growing at ambient and twice-ambient atmospheric CO₂. Values are treatment means $\pm$ one standard deviation; means with the same letter are not significantly different at $\alpha = 0.05$.

POOL or PROCESS	<u>Ambient CO₂</u>	<u>Elevated CO₂</u>
Microbial Biomass N (ug N g ⁻¹)	2.78 $\pm$ 0.393a	2.83 $\pm$ 0.417a
Gross mineralization (ug N g ⁻¹ d ⁻¹)	0.303 $\pm$ 0.0431a	0.306 $\pm$ 0.0343a
Gross nitrification (ug N g ⁻¹ d ⁻¹)	0.205 $\pm$ 0.0375a	0.301 $\pm$ 0.0560b
Gross N immobilization (ug N g ⁻¹ d ⁻¹)	0.089 $\pm$.0295a	0.097 $\pm$ 0.0229a
Gross NO ₃ ⁻ immobilization (ug N g ⁻¹ d ⁻¹)	-0.007 $\pm$ 0.0259a	0.069 $\pm$ 0.0257b
Net N mineralization (ug N g ⁻¹ d ⁻¹)	0.214 $\pm$ 0.0320a	0.208 $\pm$ 0.0357a
Net nitrification (ug N g ⁻¹ d ⁻¹)	0.212 $\pm$ 0.0310a	0.232 $\pm$ 0.0419a

Figure 1. A conceptual model depicting the response of plants and soil microorganisms to rising atmospheric CO₂ and changes in soil N availability. Our model predicts a positive feedback between the atmospheric CO₂ concentration and rates of belowground plant production. We predict that higher rates of fine root turnover at elevated CO₂ should increase the flow of nitrogen through microbial biomass.

Figure 2. An oblique areal view of the experimental array of open-top chambers (A), an oblique view of the experimental array taken from the ground (B), and a view inside an open-top chamber following planting (C). In panel C, the white tubes projecting from the ground are minirhizotrons which are used to capture video images of the developing *Populus* root systems.

Figure 3. The leaf area and leaf mass of *Populus tremuloides* clones of differing leaf drop phenology grown in two levels of atmospheric CO₂ and two levels of soil N availability.

Figure 4. Late-season photosynthetic rate (A) of an upper crown leaf of *Populus tremuloides* clones of differing leaf drop phenology grown in two levels of atmospheric CO₂ and two levels of soil N availability.

Figure 5. Maximum leaf photosynthetic rate (upper panel) and the relative crown position of the leaf exhibiting the maximum rate (lower panel, 100 = apex) for a single *Populus tremuloides* clown grown in two levels of atmospheric CO_2 and two levels of soil N availability.

Figure 6. Vertical distribution of whole leaf photosynthesis for a single clone of *Populus tremuloides* ($n = 4$) growing two levels of atmospheric CO₂ and soil N availability. Budset occurred between 18 September and 1 October, 1994 in all trees.