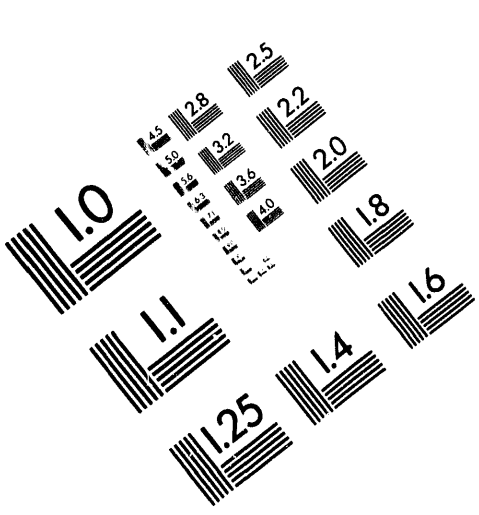

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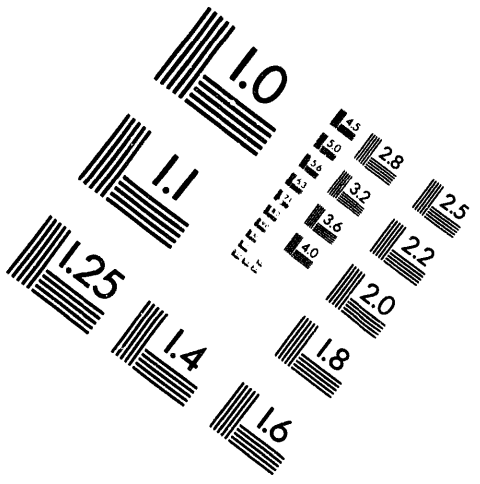

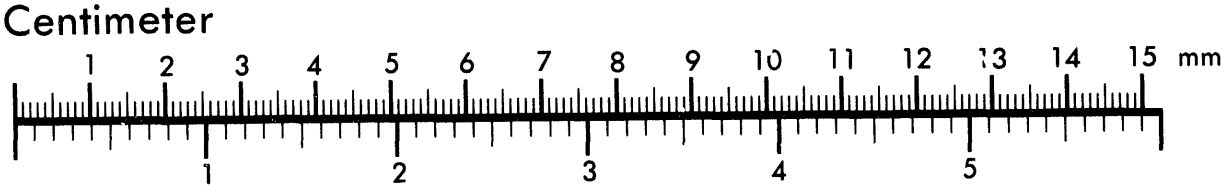

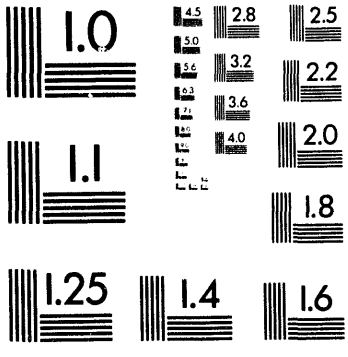

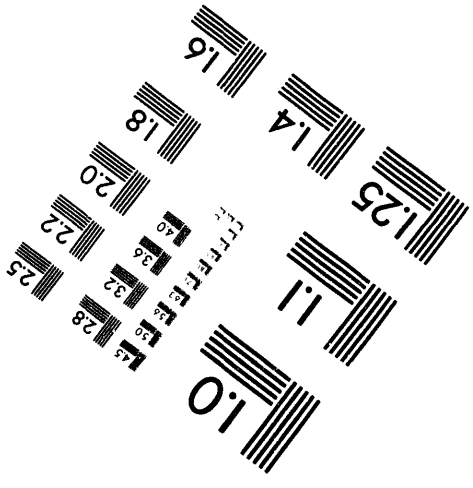

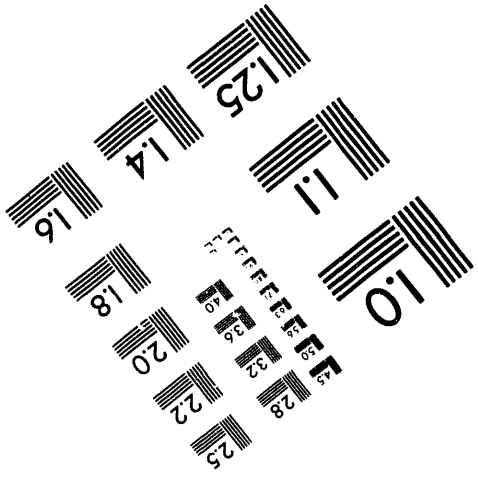

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Progress Report

May 15, 1994

Program for Ecosystem Research
Department of Energy

Changes in the Flux of Carbon Between Plants and Soil Microorganisms at Elevated CO_2: Physiological Processes with Ecosystem-Level Implications

Principal Investigators:

Donald R. Zak
School of Natural Resources and Environment
University of Michigan
Ann Arbor, Michigan 48109-1115
(313-763-4991)

Kurt S. Pregitzer
School of Forestry and Lake Superior Ecosystems Research Center
Michigan Technological University
Houghton, Michigan 49931

Project Overview

Our ability to interpret ecosystem response to elevated atmospheric CO_2 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 CO_2. 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 CO_2 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 CO_2 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 increase under elevated CO_2.

Our research is aimed at quantifying the physiological mechanisms leading to increased fine root production, microbial biomass and rates of N cycling at elevated atmospheric CO_2. More specifically, we will experimentally manipulate soil nitrogen availability and atmospheric CO_2 to understand how changes in plant resource availability influence the cycling of carbon and nitrogen between plants and soil microorganisms.

Accomplishments for Year 1

Project Staffing: We have been successful in recruiting several outstanding individuals who will work full time on our PER project over the next two years. Dr. Mark Kubiske, a physiological plant ecologist, has joined our project as a post-doctoral research associate and will work under the supervision of Dr. Kurt Pregitzer at Michigan Technological University. Dr. Kubiske is a recent Ph.D. from the Pennsylvania State University and will be responsible for root imaging, day-to-day maintenance, and isotope experiments. Carl Mikan and Jacqueline Courteau are Ph.D. students at the University of Michigan and will work under the supervision of Dr. Donald Zak. Carl Mikan recently received a M.S. in forest ecology from Pennsylvania State University and has a strong background in soil biology and tree physiology. He will assist with day-to-day operations and will be the lead person for the isotope experiments. Jacqueline Courteau received an undergraduate degree in biology/philosophy of science from Brown University; her interests are in the area of plant physiological ecology and she will study plant photosynthesis. Collectively, these individuals bring an array of expertise and interests that are well suited to our project.

Collection of Plant Material: At the suggestion of Dr. Clive Jorgensen, we have decided to use trembling aspen (*Populus tremuloides* Michx.) as an experimental organism, rather than *Populus euramericana* cv Eugenii as we had originally proposed. We are particularly fortunate to have phenological observations for a large number of trembling aspen clones on the University of Michigan Biological Station. Our phenological data include dates of flowering, leaf flushing, and leaf senescence for approximately 50 clones. We used these observations, some collected periodically since 1960, to select early- and late-senescing aspen genotypes. In October 1993, we collected the root systems of three early- and three late-senescing clones to propagate plant material for our experiment. There is approximately 3-4 weeks difference in autumnal senescence between these genotypes, a significant period of time given the 22 week growing season of northern Lower Michigan. Our rationale for using these clones was that genetic differences, like the duration of leaf display, should be an important factor influencing the annual net carbon gain of plants growing at elevated atmospheric CO_2. In turn, genotypic differences in net carbon gain should influence carbon inputs to soil and the amount of energy available for microbial metabolism.

We stored the aspen root cuttings at 4 ^{o}C at the University of Michigan Matthaei Botanical Gardens until propagation began in early March 1994 . The root cuttings were placed on a mist bench (Fig 2) until sprouting occurred, usually within 2 to 4 weeks. Sprouts were excised from the root cutting, treated with indole butyric acid (rooting hormone), and placed in a small moist peat pot until a root system had developed (Fig. 3). After rooting, the individual ramets were placed on a greenhouse bench for 2 weeks and then transferred to a cold frame to acclimate to outdoor conditions. We have propagated approximately 100 ramets from each aspen clone, each of which will be graded for uniformity of leaf number and root development before planting. We will plant one ramet of each early-and late-senescing clone into each open-top chamber/root box on May 30, the beginning of the growing season on northern Lower Michigan.

Construction of Elevated CO_2 Facility: Over the past 9 months, we have made considerable progress in assembling our new CO_2 exposure facility. To date, we have constructed the open-top chambers (Fig. 4) and belowground root boxes containing minirhizotrons (Fig. 5), and have installed the entire experimental array open-top chambers/rootboxes (Fig. 6). At the suggestion of Dr. Clive Jorgensen, we also have included a nitrogen fertility treatment to investigate the interaction of elevated CO_2 and soil nitrogen availability on C and N cycling in the plant-soil system. As such, our experimental design now consists of factorial combinations of CO_2 (ambient and twice-ambient) and nitrogen availability (low and high) arranged in a randomized complete block design; each treatment is replicated twice in each of six blocks. We have experimentally modified soil nitrogen availability by combining organic-matter-poor native subsoil

with different proportions of the organic-matter-rich native surface soil. The low nitrogen availability treatment is a 1:5 ratio of surface to subsurface soil, and the high nitrogen availability treatment consists entirely of surface soil. We estimate the initial nitrogen mineralization rates of 45 ug N g^{-1} d^{-1} in the low nitrogen treatment and 345 ug N g^{-1} d^{-1} in the high fertility treatment. These values are ecologically relevant because they bracket rates measured in a wide array of Lake States forests. Moreover, this technique has worked well in the past and has produced large differences in plant growth related to rates of net nitrogen mineralization. Our array of open-top chambers/root boxes is now ready to receive plant material, and we expect no delays in the start of our experiment on May 30.

In addition to the construction and establishment of our experimental array, we also have ordered all materials and have assembled a new CO_2 dispensing system. Our new system allows us to more accurately monitor and control the atmospheric CO_2 concentration. We have refined the dispensing of CO_2 with the use of precision flow meters, upgraded our previous valving system bringing chamber air to the IRGA, and have more fully automated CO_2 monitoring with new control hardware and software. The system has been thoroughly tested and is ready for field installation. The system will be in place and operating on May 15, two weeks prior to planting, to insure that the dispensing system is accurately controlling and monitoring atmospheric CO_2. We do not foresee any problems with the CO_2 dispensing system that would delay the start of our field season.

^{14}C Labeling Cuvette: A critical aspect of our experiment is the *in situ* labeling of plants with $^{14}CO_2$. We have closely worked with the Radiation Control Service at the University of Michigan to insure that our experimental protocol meets with NRC standards. Over the past 9 months, we have constructed and rigorously tested our $^{14}CO_2$ labeling cuvette; it is working very well (Fig. 7). We are able to easily maintain ambient temperature within the cuvette, even on cloudless days when solar loading is high. Moreover, we are able to accurately monitor and adjust the CO_2 concentration within the cuvette to ambient and twice-ambient CO_2 concentrations, enabling us to label plants at their respective growth concentrations. We have conducted several trials using *Populus euramericana* in which we have scrubbed CO_2 from the chamber and have regenerated the atmosphere to 350 and 700 umol mol^{-1} using $NaH^{12}CO_3$. We are now ready to begin several preliminary experiments with *Populus tremuloides* to refine our $^{14}CO_2$ labeling technique before conducting *in situ* labeling at the end of this growing season. Data from these experiments will enable us to more accurately estimate the amount of time required to label plants in the field such that sufficient isotope has moved into microbial biomass and soil organic matter.

Planned Activities

During the 1994 growing season, we will be collecting a wide array of data on the response of plants and soil microorganisms to elevated atmospheric CO_2 and soil nitrogen availability. Specifically, we will be make 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 will compare the phenological development and photosynthetic rate of aspen genotypes under experimental and field conditions. This will enable us to determine the influence of experimental conditions (i.e., open-top chambers) on plant response, enabling us to place our results in an ecosystem-relevent context.

Our largest task for the 1994 field season is our dual labeling experiment with ^{14}C and ^{15}N 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, will well prepare us for this task. Following the addition of ^{14}C and ^{15}N to the

experimental units, we will continuously monitor soil respiration to determine when ^{14}C 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 intend to analyze this data prior the start of the next field season and prepare several manuscripts summarizing our results.

Publications

Pregitzer, K.S., D.R. Zak, P.S. Curtis, M.E. Kubiske, J.A. Teeri, and C.S. Vogel. 1994. Atmospheric CO_2, soil nitrogen, and fine root turnover. Science *in review*

Kubiske, M.E., and K.S. Pregitzer. 1994. Effect of elevated CO_2 and light availability on the photosynthetic light response of trees of contrasting shade tolerance. Tree Physiology *in review*

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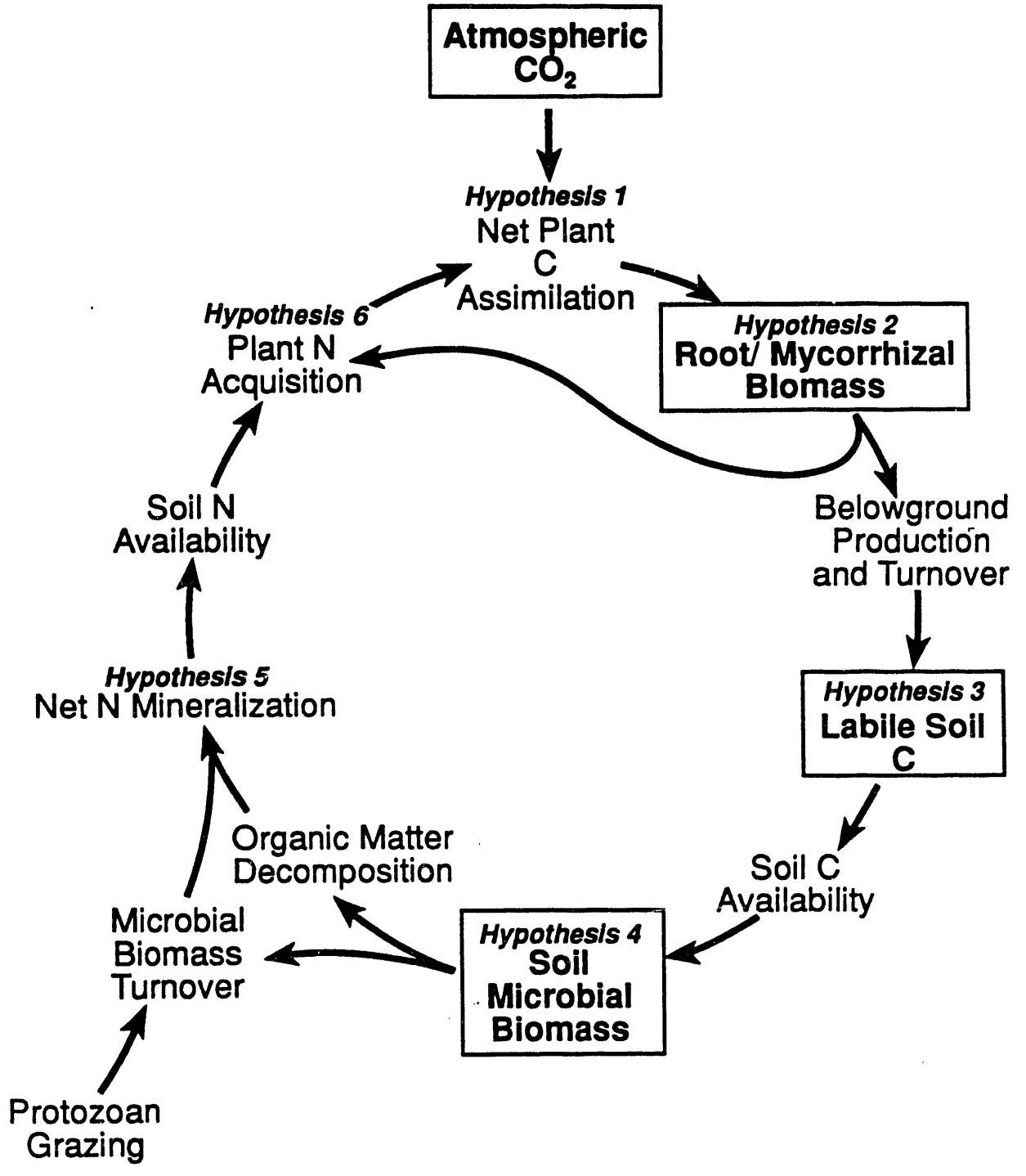

Figure 1. A conceptual model depicting the response of plants and soil microorganisms to elevated atmospheric CO_2 and soil nitrogen availability. Our model predicts a positive feedback between the atmospheric CO_2 concentration and rates of belowground plant production. We predict that increased inputs of C to soil from higher rates of fine root turnover at elevated CO_2 should increase the flow of nitrogen through microbial biomass, facilitating an increase in rates of net nitrogen mineralization.

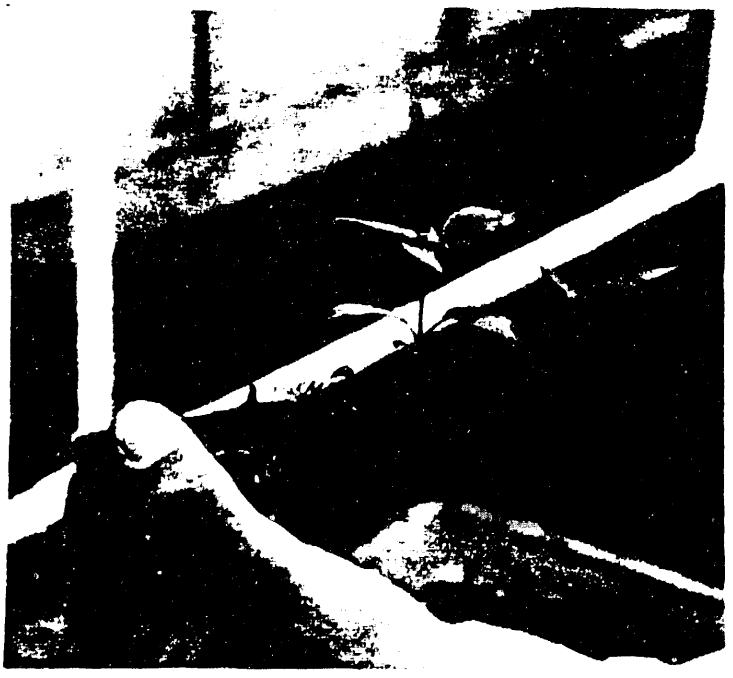

Figure 2. A root cutting from an early-senescing *Populus tremuloides* genotype collected at the University of Michigan Biological Station. Pictured are several sprouts that originated from single root cutting approximately 24 cm in length.

Figure 3. An aspen ramet propagated from sprouts borne from a root cutting. After a sprout has been excised from a root cutting, it is treated with indole butyric acid to initiate root development and placed in a moist peat pot. After an outdoor acclimation period, we will plant one ramet of each genotype in each open-top chamber/root box.

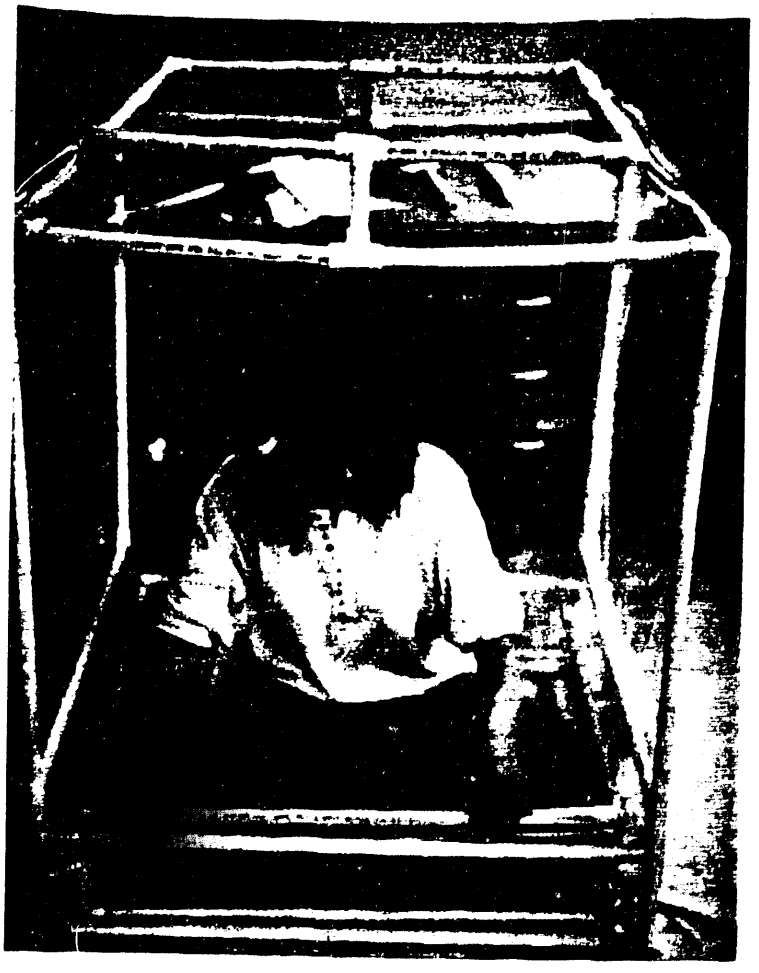

Figure 4. Construction of open-top chambers used to elevate atmospheric CO_2.

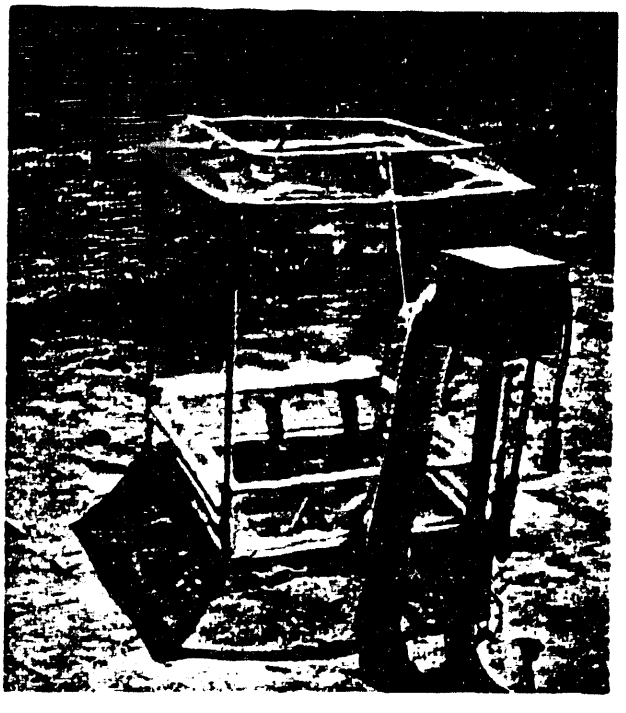

Figure 5. Open-top chamber installed on a belowground open-bottom root box. Plant root systems are contained within the root box enabling us to monitor fine root production and mortality with the minirhizotron camera. The minirhizotron tubes are the white tubes extending from the soil surface inside the open-top chamber.

Figure 6. Establishing the array of open-top chambers/root boxes at the University of Michigan Biological Station. Our experiment consists of 32 experimental units arranged in a randomized block design. We are maintaining twice ambient CO_2 concentrations in 16 boxes with the use of our newly constructed CO_2 dispensing system. Nitrogen availability treatments consisted of mixing native subsoil and native surface soil in different proportions. Low fertility treatment is a 1:5 surface to subsurface soil mixture, and the high fertility treatment consists entirely of surface soil. Soils for both treatments were homogenized with a cement mixer prior to filling each box by hand.

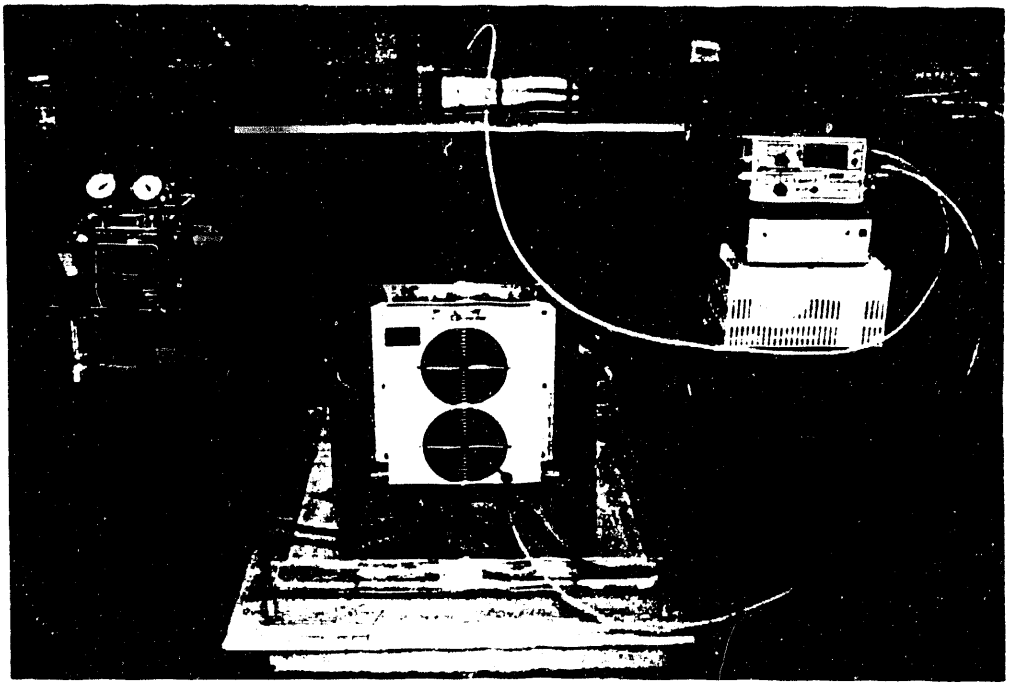

Figure 7. ^{14}C labeling cuvette designed to label *Populus tremuloides* growing at ambient and twice-ambient atmospheric CO_2 in the field. The labeling cuvette is temperature controlled with a solid-state electronic cooler that tracks the ambient temperature. Carbon dioxide concentrations within the cuvette are monitored with the use of an infra-red gas analyzer. The cuvette is also equipped with a loop that allows us to remove the CO_2 from the atmosphere within the chamber via a soda-lime trap and regenerate the atmosphere with $^{14}CO_2$. The $^{14}CO_2$ is produced by adding $NaH^{14}CO_3$ to HCl contained in a gas generating vessel.

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