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“Effects of Elevated CO₂ on Root Dynamics and
Root Function in a Mojave Desert Ecosystem”

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Background

This research effort began as a collaborative proposal in 1996 between EPA's Western Ecology Division and the University of Nevada-Reno (UNR) for research at a Free-Air CO₂ Enrichment (FACE) facility that was being constructed at the Nevada Test Site (the Nevada Desert FACE Facility or NDFF). The Terrestrial Ecology and Global Change (TECO) program, administered by NSF, DOE, NASA, and USDA had issued a request for proposals that specifically invited collaborative proposals between federal research labs and universities. The collaborative proposal resulted in DOE funding a grant to the University of Nevada-Reno (Robert S. Nowak, Principal Investigator) and an interagency agreement to EPA. EPA was responsible for minirhizotron studies of root dynamics. UNR was responsible for studies of root physiology and resource acquisition, and routine collection of the minirhizotron images for analysis by EPA.

FACE Research at the Nevada Test Site

Study Site

The Nevada Desert FACE (Free Air CO₂ Enrichment) Facility is located at 36°49'N, 115°55' W in the Mojave Desert in southern Nevada at the U.S. Department of Energy's Nevada Test Site. The study site is situated at ~960 m elevation in the middle of a broad bajada (alluvial fan) along the southern end of Frenchman Flat. The vegetation of the site is characteristic of the northern Mojave Desert and is dominated by the xerophytic shrubs *Larrea tridentata* (creosote bush) and *Ambrosia dumosa* (bursage), among others.

Minirhizotrons

Transparent glass or plastic minirhizotron tubes are widely used to study root dynamics in experimental and natural systems. These tubes allow repeated observation of roots with specialized cameras, and provide a non-destructive means to track root production, growth, and turnover for extended periods of time. We studied fine root (<2

mm diameter) dynamics using minirhizotrons in the three elevated CO₂ (550 $\mu\text{mol mol}^{-1}$) and the three ambient CO₂ ($\sim 360 \mu\text{mol mol}^{-1}$) blower control FACE rings at the Nevada Desert FACE Facility. In each 25 m diameter ring, 28 minirhizotron tubes (44 mm O.D., 38 mm I.D.) were installed at a 30 degree angle from vertical, to a vertical depth of 1 m. Two tubes were installed under the center and edge of the canopies of each of four *Larrea tridentata* and four *Ambrosia dumosa* shrubs. In addition, twelve tubes were installed at systematic intervals along three transects to sample all other vegetation (Fig. 1).

Fig. 1 - Placement of minirhizotron tubes in plots

To minimize soil compaction and trampling of plants and the fragile cryptogamic soil crust, minirhizotron tube installation and all other research activities including minirhizotron video imaging in the FACE plots were conducted from platforms suspended above the ground. These platforms are suspended from a center-pivot, rotating catwalk, which is approximately 2 m above the ground (Fig. 2). The platform may be moved radially along the length of the catwalk to access the entire plot. The height of the platform is also adjustable in order to avoid damaging plants in its path. Minirhizotron installation in the six study rings took place in July-September 1997, shortly after completion of the suspended platforms.

Fig. 2 – Suspended platform above FACE plots, to avoid soil disturbance.

In typical minirhizotron installation techniques, either an auger or soil corer is used to make a hole into which the minirhizotron tube is inserted. However, gravel and cobbles in the soil at our study site prevented either augering or coring to a depth of 1 m, and the dry, sandy soil tended to collapse into the hole before the tube could be inserted. We devised a novel technique for installing minirhizotron tubes in sandy, rocky soils, with minimal soil disturbance (Phillips et al., 2000). The method involved using a pneumatic rock-drill mounted on a pneumatic screw-drive guidance system attached to the suspended platform (Fig. 3). A pipe driver was attached to the pneumatic rock-drill, which was used to drive a drill casing into the soil. We used steel drill casing (57 mm O.D., 49 mm I.D.) as a sleeve into which the polycarbonate minirhizotron tubes could be inserted. A hardened steel section of casing with beveled edges was screwed onto the end of the casing for cutting into the soil. When cobbles or gravel prevented the casing from penetrating any further, the pipe driver was removed from the rock-drill and a rock-drill bit (45 mm O.D. “star” bit) was inserted into the drill casing. The rock-drill bit was used to break up the material inside the drill casing and approximately 1-2 cm beyond the end of the casing. During this operation, compressed air was blown down through the drill shaft and bit, which lofted soil and drilling debris up the casing. A vacuum attached to a side vent on the drill casing pulled the lofted material out of the drill casing.

In this manner, we installed 168 minirhizotrons in the six FACE rings, plus an additional 42 tubes in a nearby site for additional studies on the efficiency of minirhizotron sampling.

Fig. 3 – (1) Drill rig mounted on suspended platform. (2) Pneumatic rock-drill mounted on pneumatic screw-drive guide system. In this photo, the pipe driver is attached to the rock-drill and is being used to drive the drill casing into the ground. (3) A drill bit has been attached to the drill shaft and inserted down the drill casing to drill just beyond the end of the casing. Soil and rock debris lofted by compressed air blown through the drill shaft and bit are removed by a vacuum hose which attaches to the side vent seen on the top of the drill casing. (4) Minirhizotrons with insulated PVC caps. Note the minimal surface disturbance where the tubes were installed and the rockiness of the substrate. (5) Video image of roots several months after tube installation. (6) Minirhizotron tube excavated 1 year after installation. Note the good soil contact, the rockiness of the soil, and the ingrowth of roots around the tube. A white PVC pipe with numbered camera image positions has been inserted in the tube in this photo.

Root image collection

Using a specialized video camera, images were recorded from the 168 FACE ring minirhizotron tubes beginning in December, 1997. The camera was attached to a ratcheting mechanism which attached to the tube and allowed precise positioning of the camera at the same locations every sampling period [Johnson and Meyer (1998), Mechanical advancing handle that simplifies minirhizotron camera registration and image collection, *J. Environ. Qual.* 27: 710-714]. Images were collected every 4 cm of vertical depth in each tube, to a depth of 1 m. Forty-one sets of images were collected at 4 week intervals from December, 1997 through January, 2001. Degrading image quality, due to abrasion inside the minirhizotron tubes from repeated image collection over several years in a very dusty environment, necessitated termination of image collection at that time. Original 8mm video tapes from each collection are stored at the EPA lab in Corvallis, Oregon, and copies are stored at the University of Nevada, Reno.

Image processing

Images containing roots were converted to digital format and each root was traced, and the root type, length, and width were recorded in a database. Classification followed a scheme developed for this site. The ROOTS image processing software from Michigan State University was used for image analysis and data base development. This database allowed us to characterize fine root numbers, depth distribution, as well as production, mortality, and standing crop. Over 100,000 video images collected from December, 1997 through January, 2000 were analyzed; images after this time were too degraded for adequate analysis.

Quality Assurance

For all image analyses we used a system of computer programs written to check for 14 types of data errors and inconsistencies in the root database. As each new root data set was produced from an image collection, an iterative procedure of error checking and correction was followed until no more errors were identified in the data set. We also conducted Quality Assurance evaluations of the repeatability of data extraction from the minirhizotron root images for each image analyst each year. These were done by blind re-extraction of data from a previously processed set of images. Results indicated 95-99% completeness for data on root presence, excellent agreement on root classification, and sub-millimeter accuracy on length and width measurements. This validates the high quality of the quantitative data on root presence, length, width, and area. These and other procedures are described in more detail in Johnson et al. (2001).

Minirhizotron calibration and root spatial variability

We tested for but found no significant artifactual effects of the presence of minirhizotron tubes on fine root density. In each of three years, we extracted minirhizotron video images from 14 tubes located outside the FACE rings, and then excavated these tubes and collected soil samples along the top surface of the tubes (where

the video images were taken) and at various distances away from the tubes. Roots were extracted from the soil samples by wet sieving and they were dried and weighed. C and N analyses of these samples from previous years indicated that there was mineral contamination of the extracted roots, so ash-free dry weights were determined for the residual root samples from all years. Comparisons of the root biomass in the bulk soil samples with root length data from the associated minirhizotron root images showed good correlation. There was no indication of significant increases or decreases in root density immediately adjacent to the tube. These findings for a wet year (1998), a dry year (1999), and a more normal year (2000) give confidence in the use of minirhizotrons as a method for tracking root dynamics in this system.

Results

Over 100,000 video images were analyzed and the appearance, growth, and the fate of 23,634 individual roots were tracked over time, totaling 125,679 root observations and measurements. The roots were very fine, with > 90% having a diameter < 0.3 mm (Fig. 4).

Fig. 4 – Fine root diameter distribution for all root observations during the course of the study (n=125,679).

Patterns of fine root length production, turnover, and standing crop were determined separately for tubes placed under *Larrea*, *Ambrosia*, and systematically placed along Transects in the FACE plots, and for the two CO₂ treatments at each of these locations. The observation periods 12/97-12/98 and 1/99-1/00 greatly contrasted in terms of precipitation. 1998 had about 3.5 times the normal annual rainfall, with profuse germination and growth of winter annuals. In contrast, 1999 had only about half the normal annual rainfall, with very little winter precipitation. This led to an almost complete absence of winter annual plant cover.

Fine root length production peaked in April or May of both years at all tube locations (Fig. 5). Peak fine root standing crop lagged about a month behind peak production. Peak turnover lagged 1-2 months behind peak standing crop, with maximum turnover during the hot summer months. Peak standing crop for 1999 was only about 60% of peak standing crop for 1998, reflecting poorer soil moisture conditions for root growth and the lack of annuals. There were no consistent differences among CO₂ treatments in fine root standing crop, production, or turnover under *Larrea* or *Ambrosia*. For Transect tubes, there were no consistent CO₂ treatment differences in fine root production or turnover; however, fine root standing crop was consistently lower in the elevated CO₂ treatment, with statistically significant differences in June-July 1998, and February-June and October 1999. Our University of Nevada collaborators (Nowak et al.) also found reduced root:shoot ratios for *Larrea* seedlings exposed to elevated CO₂ in greenhouse experiments. In addition, Nowak et al. [2001, Leaf conductance decreased under free-air CO₂ enrichment (FACE) for three perennials in the Nevada desert, New Phytologist 150: 449-458] found that elevated CO₂ caused decreased stomatal conductance in *Larrea* in the FACE plots. We speculate that higher water use efficiency associated elevated CO₂ and decreased conductance may allow sufficient water uptake with slightly smaller amounts of fine roots per unit biomass.

For tubes under *Larrea*, there was a tendency for a deepening of the root systems under elevated CO₂ with decreases in the 20-40 cm depth class and increases in the 40-60, 60-80, and 80-100 cm depth classes during the wet year of 1998 (Fig. 6). This trend did not persist into 1999, which was much drier.

Fig. 5. Standing crop, production, and turnover of fine root length in minirhizotron tubes installed under *Ambrosia* and *Larrea* shrubs, and along systematic transects in the FACE rings.

Fig. 6 – Distribution of fine root length by depth class for minirhizotron tubes under *Larrea* shrubs.

The results discussed above show the response of fine root length to CO₂ treatment. However, we have also developed a procedure for translating fine root lengths observed in 2-dimensional video images to fine root biomass per unit volume of soil (g m⁻³). First, specific root lengths (m g⁻¹ of root dry weight) were determined for fine roots in 0-0.3, 0.3-1, and 1-2 mm diameter classes from bulk soil samples. For the excavated tubes, the total root length, by diameter class, in all video frames was multiplied by the corresponding specific root length and summed to give biomass of all the fine roots observed. We assumed that all fine roots within some small depth of field from the minirhizotron were observed, so the total soil volume observed was the total frame area times depth of field. The value for this depth of field was determined by equating the fine root biomass density (g m⁻³) in the volumetric soil samples (taken along the upper surface of the tubes where video imaging is done) with that from the minirhizotron tubes. A depth of field of ~1 mm was found to approximately cross-calibrate the two methods of sampling in this way.

This procedure has several advantages. First, it eliminates non-standard units such as mm/frame or mm/tube which have characterized many minirhizotron studies, and which are difficult to relate to other measurements in ecosystem studies. Second, it cross-calibrates minirhizotron root observations with root biomass densities from soil cores. Third, it allows reporting not only of root production, turnover, and standing crop in length units, but biomass units as well. Fourth, this can be combined with determinations of fine root C and N concentrations to give estimates of the rate of C and N cycling through fine root turnover. These methodological advances were described in detail in two journal papers, Tingey et al. (2000) and Johnson et al. (2001).

Our University of Nevada collaborators (Nowak et al.) harvested fine roots in both ambient and elevated CO₂ FACE plots in 2000 and 2001. Their data indicate higher specific root lengths (m/g dry weight) for roots < 0.3 mm in diameter for both *Ambrosia* and *Larrea* under elevated CO₂ treatments, approximately double those for the ambient CO₂ treatment. High specific root length is often associated with lower construction cost, increased hydraulic conductivity, and other properties in many species. Several studies with other species have reported the opposite trend of decreasing specific root length with elevated CO₂ exposure (Tingey et al. 2000). If these specific root length values are used to convert fine root length to fine root biomass as described above, then the roughly equal amount of fine root length production, turnover, and standing crop in the two CO₂ treatments translates to substantially lower fine root biomass production, turnover, and standing crop in the elevated CO₂ treatment. This result is rather unexpected since many elevated CO₂ studies have shown increases in belowground biomass allocation. However, our University of Nevada collaborators (Nowak et al.) also found reduced root:shoot ratios for *Larrea* seedlings exposed to elevated CO₂ in greenhouse experiments.

We found no significant difference in fine root C concentrations (~45%) or N concentrations (~1%) among the CO₂ treatments for either *Larrea* or *Ambrosia*. Coupling this with the lower rate of fine root biomass turnover under elevated CO₂ would indicate lower rates of C and N cycling through fine root mortality. Again, this is a surprising result since many elevated CO₂ studies report increases in root biomass and turnover, and thus imply increases in C and N cycling rates through fine roots.

Fine Root Dynamics Research at Other Sites

Cost savings from the necessary curtailment of image collection and processing due to image quality degradation freed up some Subaward funds for related tasks within the same scope of work. After receiving approval from the DOE Project Officer, similar minirhizotron image collection and analysis tasks were undertaken at two additional sites as described below.

Terrestrial Ecophysiological Research Area (TERA) facility, Corvallis, OR – A 3-year experimental study on the effects of elevated CO₂ and ozone on ponderosa pine seedlings was conducted in sunlight environmentally controlled mesocosms in Corvallis, OR from 1998-2001. As part of that study, minirhizotron images were collected every 4

weeks from horizontal tubes at 4 soil depths in the mesocosms. Because of lack of funds at the termination of the experiment, minirhizotron images could only be analyzed to determine fine root dynamics responses to CO₂ and ozone at the 3 shallowest soil depths, precluding any examination of root responses in deeper horizons. The additional DOE funds redirected to this task (~\$8K) enabled analysis at the deeper horizons for a more complete picture of root responses to CO₂ and ozone treatments and their interactions, including inputs to soil organic C pools from fine root turnover. Approximately 10,000 images were analyzed (each individual root classified, digitized, and data stored in a data base). In conjunction with other scientists involved in this project, publications are being prepared on the interactive effects of CO₂ and ozone on fine root standing crop biomass, production, turnover, life span, and rates of C and N cycling through fine root turnover. DOE support of this effort will be acknowledged in all resulting publications.

Metolius Research Natural Area, Camp Sherman, OR – A related field study on ponderosa pine trees of different ages is taking place in the Oregon Cascades at the Metolius Research Natural Area administered by the US Forest Service. This site is also the subject of intensive study on ecosystem CO₂ fluxes by Oregon State University (Bev Law, PI) as part of the Ameriflux network funded by DOE. Minirhizotrons have been in place at this site since 1998, and images were collected under EPA funding during the growing seasons of 1999, 2000, and 2001. Termination of funding for the fine root dynamics portion of this study precluded collection and analysis of a 4th growing season of minirhizotron images to quantify ponderosa pine fine root standing crop biomass, production, turnover, and life span. The additional DOE funds redirected for this task (~\$17K) allowed this effort to continue for a 4th year (2002). This will provide both a field study comparison of fine root dynamics for the mesocosm seedling study described above, and also provide valuable information for the OSU Ameriflux effort on belowground systems in different age stands, including rates of C cycling through fine root turnover. Approximately 20,000 minirhizotron images were collected during five sampling periods in the 2002 growing season. The images have all been analyzed (each individual root classified, digitized, and data stored in a data base). In conjunction with other scientists involved in this project, publications are being prepared on these results. DOE support of this effort will be acknowledged in all resulting publications.

Software development and evaluation

This multi-year root dynamics study involved the analysis of approximately 100,000 minirhizotron video images, including over 23,000 individual roots, each of which was observed multiple times. The magnitude of this analysis and data base construction effort brought to light several inadequacies of the root image processing software at the time. The ROOTS software that we used since 1992, along with many other researchers, was in need of updating for the following reasons:

1. First, the software had some idiosyncrasies, with apparent program calls to computer BIOS versions which were no longer used, which made it unusable on computers less than about 5 years old. As these older systems became unusable,

- they could not be replaced with newer (and faster) computers because of the incompatibility problem.
2. Second, as individual roots disappeared, the software required that the analyst continue to denote position markers for them on the screen. While a continuous record of presence and absence for each root is desirable in the database, this created needless processing time for the image analyst. This was especially critical in multi-year studies such as this project, where the number of disappeared roots exceeds the number of roots present after some time has elapsed.
 3. Additional deficiencies, such as the lack of support for modern generic image file formats such as JPEG, operation as a DOS rather than Windows application, and others also limited the continued usability of the earlier ROOTS software.”

Under this Interagency Agreement we contracted with Michigan State University to revise and upgrade this software. Development of a new version of the ROOTS software has been completed. A number of new features have also been added which should substantially improve the ability of the belowground research community to carry out minirhizotron studies of root dynamics. Further information can be found at <http://roots.rsgis.msu.edu>.

Several new software packages specifically designed for minirhizotron image analysis of roots have appeared over the last few years. We did a comparative evaluation of the features and usability of five of these software packages: ROOTS, RooTracker, WinRhizoTron, RootView, and RMS (Root Measurement System). This will be written up as a report on the evaluation to submit to the Technological Tools section of the Bulletin of the Ecological Society of America, or other suitable forum.

Presentations

Phillips, D., M. Johnson, D. Tingey, C. Biggart, R. Nowak, and J. Newsom (1998). Minirhizotron installation in rocky, sandy soils minimizing soil disturbance. Presentation at Ecological Society of America meeting, Baltimore, MD.

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Andersen, C.P., D.L. Phillips, P.T. Rygielwicz and M.J. Storm. 2001. Patterns of root growth, turnover, and distribution in different aged ponderosa pine stands. IUFRO Conference "*Linking the Complexity of Forest Canopies to ecosystem and Landscape Function*", Sisters, Oregon, July 2001.

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Phillips, D.L., D.T. Tingey, M.G. Johnson, C.E. Catricala, and T.L. Hoyman. (2002). Effects of elevated CO₂ on fine root dynamics in a Mojave Desert ecosystem: a FACE study. Presentation at Ecological Society of America meeting, Tucson, AZ.

Nowak, R.S., D.L. Phillips, D.T. Tingey, and M.G. Johnson (2004). Effects of elevated CO₂ on root growth and physiology for desert plants. Upcoming presentation at International Workshop on Short- and Long-Term Effects of Elevated Atmospheric CO₂ on Managed Ecosystems, Ascona, Switzerland.

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Phillips, D.L., et al. Effects of elevated CO₂ on fine root dynamics in a Mojave Desert ecosystem. In preparation.

Andersen, C.P., D.L. Phillips, P.T. Rygielwicz, and M.J. Storm. Seasonal patterns of fine root production and turnover in ponderosa pine stands of different ages. In preparation.

Other

MSU ROOTs Tracer 2. Michigan State University, Center for Remote Sensing,
<http://roots.rsgis.msu.edu>