Effects of Elevated CO_2 and Nitrogen Fertilization on Fine Root Growth in seedling *Pinus ponderosa*

Statement of the Problem

In forest trees, less than 20% of the total biomass is belowground while more than 50% of the carbon acquired annually by plants may be allocated belowground (George & Marschner 1996). With rising atmospheric carbon dioxide (CO_2) the potential for effects of elevated CO_2 on forest trees is large. A number of studies have evaluated the effects of elevated CO_2 on plants (e.g., Ceulemans & Mousseau 1994; Curtis & Wang 1998, Norby 1994, Rogers *et al.* 1999, Taylor *et al.* 1994). Their general conclusion is that elevated CO_2 leads to increased photosynthesis and increased plant biomass, including increased root biomass. Will this increase in biomass lead to a greater proportion of the carbon being allocated belowground and potentially sequestered there?

Three science questions guided the study on ponderosa pine (*Pinus ponderosa* Dougl.):

- Will the size of the root systems of ponderosa pine seedlings increase to facilitate resource (water or nutrients) acquisition in response to elevated CO_2?
- Will ponderosa pine root systems be affected by the availability of nitrogen?
- Will the dynamics of ponderosa pine fine root production and mortality be affected by elevated CO_2 and/or nitrogen treatments?

Approach

Plants were exposed to CO_2 and nitrogen (N) fertilization in open-top field-exposure chambers located at the US Forest Service Institute of Forest Genetics near Placerville, CA. The experimental design was a replicated 3x3 factorial with 3 CO_2 levels (ambient air [~350 µmol mol-1]; ambient air + 175 µmol mol^{-1} and ambient air + 350 µmol mol^{-1}) and 3 levels of nitrogen (N) addition (0, 100 and 200 kg ha^{-1}); however, the 100 kg ha^{-1} N treatment at ambient + 175 µmol mol^{-1} CO_2 was omitted from the experimental design because of financial limitations. There were 3 replicates of each CO_2 and N treatment. The nitrogen was broadcast applied each March as ammonium sulfate. Soils were kept moist and relatively constant over the course of the study. Root images were collected from three minirhizotron tubes in each chamber every 2 months on S-VHS tape using a minirhizotron camera. The root images were analyzed using software that allows the user to measure the length and diameter of all roots and annotate mycorrhizae and fungal hyphae occurrence.

Main Conclusions

Fine roots explore soil for water and nutrients to support plant growth. Plants produce more or fewer fine roots as their resource needs change. A given species may produce more fine roots in nutrient poor soils than in nutrient rich soils. In arid environments plants typically produce more fine roots than in wetter environments. In this experiment the ponderosa pine trees – normally a dry forest species -- were well watered in order to examine the effects of elevated atmospheric carbon dioxide (CO_2) and nitrogen (N) on fine root dynamics.

Elevated CO_2 and N treatments both increased plant height, stem diameter and leaf area (Tingey et al. 1996, 1997). Elevated CO_2 resulted in significantly higher root biomass in the first 3 years and higher fine root turnover in the last 2 years. No significant N effects were noted for annual root biomass production, or turnover. Fine root nutrient cycling rates varied from 74 to 362 g m^{-2} yr^{-1} for C and 0.9 to 4.6 g m^{-2} yr^{-1} for N (Phillips et al, unpublished results).

Fine-root production and life span were strongly influenced by season and soil temperature (Johnson et al. 2000). Fine roots declined in importance with time and were replaced with mycorrhizae which continued to increase with time. This temporal pattern of root and shoot growth was not altered by providing additional CO_2 or N fertilization (Tingey et al. 1996).

Both CO_2 and N affected the fine roots, but the effects were independent and displayed contrasting effects. In this study, elevated CO_2 increased above and below ground plant growth because nitrogen was not limiting even in the unfertilized plots. Although elevated CO_2 increased fine root growth, it did not change the relationship between fine roots and needles indicating that elevated CO_2 did not increase the proportion of carbon allocated belowground (Tingey et al. 1996). Elevated CO_2 increased root lifespan but N decreased it (Johnson et al. 2000, Tingey et al. 1997). Initially, elevated N reduced the fine root area relative to the needle area, but this ratio was not altered by elevated CO_2 treatments.

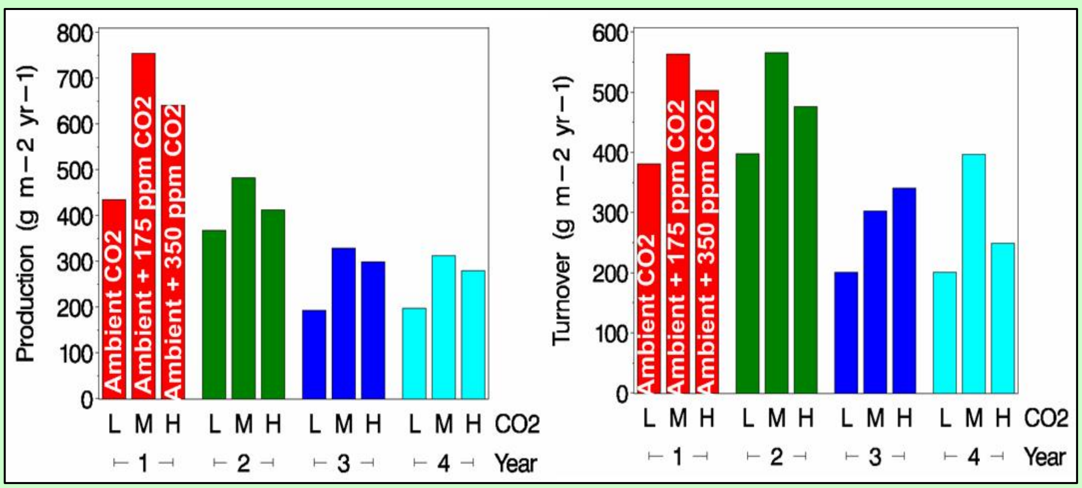
Soil exploration by the roots increased with elevated CO_2, but was unaffected when nitrogen was abundant as in the fertilized plots. Fine root production was increased by elevated CO_2 whereas N fertilization had no effect on fine root production (Tingey et al. 1996, 1997). This suggests that as the limiting resource, nitrogen in this case, increases the plants do not need to produce as many fine roots to explore more soil to acquire enough of the resource. In contrast, increased CO_2 resulted in more images containing roots suggesting more soil exploration by roots.

Elevated CO_2 increased mycorrhizal and fungal occurrence earlier than N fertilization, and elevated CO_2 increased C flux into mycorrhizae (Rygiewicz et al. 1997, Tingey *et al.* 1997). Higher levels of N resulted in increased mycorrhizal activity relative to fine root production. The amount of mycorrhizae relative to the amount of fine roots did not change with increased CO_2 – they both increased in the same proportion. However, N fertilization resulted in more mycorrhizae relative to roots. Under elevated CO_2 fine roots increased providing additional infection sites, and, consequently, mycorrhizal occurrence increased proportionally. In contrast, nitrogen fertilization increased root branching without increasing the amount of fine roots, thereby providing more site for mycorrhizal infection.

In summary, limiting resources determined the response of ponderosa pine root systems to changes in atmospheric CO_2 and soil nitrogen. As plants needed more nitrogen their roots explored more soil area to acquire this resource. Elevated CO_2 allowed the trees to take up more carbon for growth both above and below ground, but the amount allocated to the root system decreased as soil nitrogen become more available. Again, in this study water was not limiting and consequently did not affect the root system. With time mycorrhizae became more important to the root systems and effectively replaced the fine roots. Their abundance increased proportionally with fine roots under increased levels of CO_2, but increased in greater proportion than fine roots with higher levels of soil nitrogen.

Effects of Elevated CO_2 on Root Production & Turnover

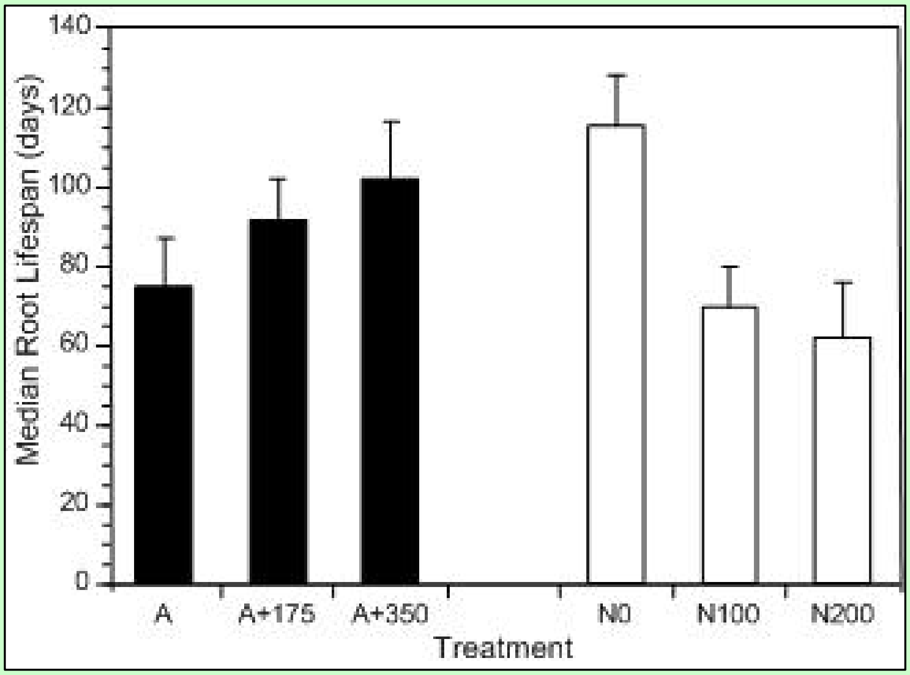
A 4-year study was conducted to determine the effects of atmospheric CO_2 and N-fertilization on *Pinus ponderosa* fine root ($\leq$2 mm) responses. Seedlings were grown in open-top chambers at 3 CO_2 levels (ambient, ambient+175 ppm, ambient+350 ppm) and 3 N-fertilization levels (0, 10, 20 $g{\cdot}m^{-2}{\cdot}yr^{-1}$). Length and width of individual roots were measured from minirhizotron video images collected bimonthly over 4 years. Biomass estimates were made by cross-calibrating with soil core root biomass. Neither CO_2 nor N-fertilization treatments affected root production and turnover seasonal patterns. Elevated CO_2 resulted in significantly higher biomass ($g{\cdot}m^{-2}$) in the first 3 years and higher turnover ($g{\cdot}m^{-2}{\cdot}yr^{-1}$) in the last 2 years. No significant N effects were noted for annual root biomass, production, or turnover. Fine root nutrient cycling rates varied from 74-362 $g{\cdot}m^{-2}{\cdot}yr^{-1}$ for C and 0.9-4.6 $g{\cdot}m^{-2}{\cdot}yr^{-1}$ for N. Higher turnover in elevated CO_2 was due to higher biomass rather than shorter life-span. Fine roots lived longer in elevated CO_2, and turnover relative to biomass was generally $\leq$ that in ambient CO_2, emphasizing the importance of root turnover definitions.



Effects of CO_2 and Nitrogen Fertilization of Fine Root Life Span

Ponderosa pine seedlings were grown at varying atmospheric CO_2 concentrations paired with varying concentrations of exogenously-applied nitrogen. Increasing CO_2 levels increased the lifespan of the ephemeral, nutrient-absorbing fine roots. Whereas, increasing the amount of applied nitrogen decreased root lifespan. These results illustrate: (1) the enhanced role of the ephemeral, nutrient-absorbing fine roots under elevated CO_2 to acquire nitrogen needed to balance the increased available CO_2, and (2) how the role of these roots was reduced as the available nitrogen increased.

Source: Johnson *et al*. 2000.



References Cited

Ceulemans R, Mousseau M. 1994. Effects of elevated atmospheric CO_2 on woody plants. *New Phytologist* 127: 425-446.

Curtis PS, Wang X. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form and physiology. *Oecologia* 113: 299-313.

George E, Marschner H. 1996. Nutrient and water uptake by roots of forest trees. *Zeitschrift für Pflanzenernährung und Bodenkulture* 159: 11-21

Johnson, M.J., D.L. Phillips, D. T. Tingey and M.J Storm. 2000. Effects of elevated CO_2, N-fertilization and season on survival of ponderosa pine fine roots. Canadian Journal of Forest Research 30:220-228.

Norby RJ. 1994. Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide. *Plant and Soil* 165: 9-20.

Rygiewicz, P.T., M.G. Johnson, M.J. Storm, D.T. Tingey and L. Ganio. 1997. Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels. Plant and Soil 189:275-287.

Tingey, D.T., D.L. Phillips, M.G. Johnson, M.J. Storm and J.T. Ball. 1997. Effects of elevated CO_2 and N-fertilization on fine root dynamics and fungal growth in seedling *Pinus ponderosa.* Environmental Experimental Botany 37:73-83.

Tingey, D.T., M.G. Johnson, D.L. Phillips, D.W. Johnson and J.T. Ball. 1996 Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. Tree Physiology 16:905-914.

Annotated Bibliography of WED Research

Compton, J.E., L.S. Watrud, L.A. Porteous, and S. DeGrood. 2004. Response of Soil Microbial Biomass and Community Composition to Chronic Nitrogen Additions at Harvard Forest. Forest Ecology and Management 196:143-158.

Soil microbial communities may respond to anthropogenic increases in ecosystem nitrogen (N) availability, and their response may ultimately feedback on ecosystem carbon and N dynamics. We examined the long-term effects of chronic N additions on soil microbes by measuring soil microbial biomass, composition and substrate utilization patterns in pine and hardwood forests at the Harvard Forest Chronic N Amendment Study. Functional and structural genes for important N cycling processes were studied using DNA community profiles. In the O horizon soil of both stand types, N additions decreased microbial biomass as determined by chloroform fumigation-extraction. Utilization of N-containing substrates was much lower in N-treated pine soils than in the controls, indicating that N additions reduced potential microbial activity in the pine stand. Counts of fungi and bacteria as determined by direct and culture techniques were quite variable and did not show a clear response to N additions. Nitrogen additions, however, strongly influenced microbial community DNA profiles. The ammonium oxidizing gene *amoA* generally was found in high N treated soils, but not in control soils. The *nifH* gene for N_2-fixation was more difficult to amplify in the pine N treated soil than the controls, suggesting that the potential for nitrogen fixation was depressed after chronic N additions. Our findings indicate that chronic N additions decreased microbial biomass and altered DNA community profiles. These changes in microbial community structure and function may be an important component of the response of terrestrial ecosystems to increased N supply due to human activities.

Johnson, M. G., D. L. Phillips, D. T. Tingey, and M. J. Storm. 2000. Effects of elevated CO_2, N-fertilization, and season on survival of ponderosa pine fine roots. Canadian Journal of Forest Research 30:220-228.

Increasing carbon dioxide associated with global climate change may cause trees to live longer--at least their roots. EPA scientists in cooperation with scientists at the Desert Research Institute have completed a study on the effects of elevated carbon dioxide and nitrogen on the life span of Ponderosa pine fine roots. They found that fine roots of Ponderosa pines exposed to elevated atmospheric carbon dioxide lived longer. They used a minirhizotron camera system to observe the production and turnover of more than 3600 roots over 4 years and found that Ponderosa pine fine root production and survival were strongly influenced by season and seemed to be most strongly linked to soil temperature. Annual root production decreased with time, which suggests that root density reaches an optimal level that is likely constrained by site conditions. Assuming that longer-lived roots continue their resource acquisition functions, then elevated carbon dioxide may have the effect of extending the period of time that the root system can meet the resource demands of the shoot. Nitrogen fertilization decreased fine root life span. It seems that roots in resource-rich environments have shorter life spans than roots in resource-poor environments. Because uptake rates are lower in resource-poor soils, roots must function longer to meet the resource needs of the plant. Consequently, root life span is inversely related to the resource status of the soil.

Johnson, Mark G., David T. Tingey, Marjorie J. Storm and Donald L Phillips. 1995. Patterns of ponderosa pine fine root growth as affected by elevated CO_2: Initial field results. Plant Physiology 14:81-88.

The objective of this study is to determine whether the root growth of ponderosa pine *(Pinus ponderosa* Dougl. ex P. Laws. & C. Laws.) seedlings is affected by elevated atmospheric CO_2.

Seeds were sown in native soil (May 1991) in open-top field exposure chambers at Placerville, CA and exposed to either ambient CO_2 (-350 µmol mol^{-1}) or two levels of elevated CO_2 (525 and 700 µmol mol^{-1}). The root systems were monitored 5 times over a seven month period (October 1992 through April 1993) using a minirhizotron camera system which permitted repeated observations of the root/soil environment. The lengths and diameters of all roots observed were measured and roots were assigned to one of 4 classes: new, white, brown, decaying. Occupancy, the presence or absence of roots, fungal hyphae, and mycorrhizae, was expressed by the proportions of minirhizotron frames in which roots, fungal hyphae, or mycorrhizae occurred. Root surface area density was expressed as the proportion of the total video image area that was covered by roots of any class, as well as the proportion covered by roots in each class. The majority (-95%) of the roots observed were smaller than 2 mm in diameter. Brown roots occurred most frequently. Root occupancy was highest in the Bw horizon. Root occupancy and mean root surface area density were highest in the elevated CO_2 treatments early in the study Over the course of the study, however, root occupancy and mean root surface area density in the ambient treatment increased until they were similar to the levels observed in the elevated CO_2 treatments. Overall, our results indicate that elevated CO_2 provided an early stimulation of root proliferation, however, after 7 months this initial stimulation did not persist.

Rygiewicz, Paul T., Mark G. Johnson, Lisa Ganio, David T. Tingey, and Marjorie J. Storm. 1997. Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels. Plant and Soil, 275-87.

A significant unanswered question concerning the global carbon (C) cycle and climate change is the fate of the “missing” C. Speculation attributes storage of this C to one or more of several sinks. In terrestrial ecosystems, a major storage pool for C is the soil. Carbon fixed aboveground eventually enters the soil via root and rhizosphere processes. Mycorrhizae as C sinks in the rhizosphere, are among the first soil biota to receive C fixed aboveground, and thereby greatly influence C dynamics in soil ecosystems. One step in this C release is via fine root and mycorrhizal turnover. It is necessary to know the lifespan and seasonal occurrence of roots and mycorrhizae to determine C flux into the soil, and the capacity of the soil ecosystem to sequester C. Seasonal occurrence and the lifespan of ectomycorrhizae of ponderosa pine (*Pinus ponderosa* Laws) grown under three levels each of atmospheric CO_2 concentration and annual nitrogen (N) additions were followed on a two-month frequency for 18 months using minirhizotron tubes and camera. The frequency of mycorrhizal root tips increased with carbon dioxide concentration while the lifespan of mycorrhizal root tips was not affected by carbon dioxide or nitrogen additions. Previous research on fine roots without mycorrhizae concluded that increases in carbon dioxide and nitrogen shortened the lifespan of fine roots, i.e., increased the turnover rate and subsequent release of carbon dioxide into the atmosphere. This research shows that mycorrhizae appear to provide some protection or buffering against changes in the root environment. If trees increase their frequency of mycorrhizal symbiosis under elevated CO_2, effects of environmental conditions (nutrients, etc.) that influence root turnover and C storage may be lessened. A very large flux of C moves through the mycorrhizal symbionts into soil, and much of that C is readily transformed into compounds that ultimately enter the soil organic matter pool, or are released to the atmosphere via soil respiration. This is the first published report on mycorrhizal tip lifespan with respect to CO_2 and N, and suggests that results found for nonmycorrhizal tips should not be universally applied to tree root systems when estimating carbon flux. If scientists estimating biospheric feedbacks of carbon dioxide from global warming assume that all forests behave the same with regard to mycorrhizae and carbon processing, and that the effects of CO_2 and N found for nonmycorrhizal root tips are universal, then projections of carbon flux may be wrong.

Tingey, David T., Mark G. Johnson, Donald L. Phillips, Dale W. Johnson and J. Timothy Ball. 1996. Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. Tree Physiology 16:905-914.

We monitored effects of elevated CO_2 and N fertilization on shoot and fine root growth of *Pinus pondrosa* Dougl. ex P. Laws. and C. Laws. grown in native soil in open-top field-exposure chambers at Placerville, CA, over a 2-year period. The experimental design was a replicated 3 x 3 factorial with the center treatment missing; plants were exposed to ambient (-365 µmol mol^{-1}) air or ambient air plus either 175 or 350 µmol mol^{-1} CO_2 in combination with one of three rates of N addition (0, 100 or 200 kg ha^{-1} year^{-1}). All CO_2 by N interactions were nonsignificant. Both the CO_2 and N treatments increased plant height, stem diameter and leaf area index (LAI). Elevated CO_2 increased fine root area density and the occurrence of mycorrhizae, whereas N fertilization increased coarse root area density but had no effect on fine root area density. Spring flushes of shoot height and diameter growth were initiated concurrently with the increase in new root area density but height and diameter growth reached their maxima before that of fine roots. The temporal patterns of root and shoot growth were not altered by providing additional CO_2 or N. Greatest root loss occurred in the summer, immediately following the period of greatest new fine root growth. Elevated N initially reduced the fine root area density/LAI ratio independently of CO_2 treatment, indicating that the relationship between fine roots and needles was not changed by CO_2 exposure.

Tingey, David T., Mark G. Johnson, Donald L. Phillips and Marjorie J. Storm. 1995. Effects of elevated CO_2 and nitrogen on ponderosa pine fine roots and associated fungal components. Journal of Biogeography 22:281-287.

The effects of CO_2 and nitrogen treatments on ponderosa pine *(Pinus ponderosa* Dougl. ex P. Laws. & C. Laws.) fine roots and associated fungal structures were monitored for a year (October 1992 to October 1993) using a minirhizotron camera system. The trees were grown in native soil in open-top field-exposure chambers at Placerville, CA and exposed to ambient (- 350 µmol mol^{-1}) air or ambient air plus either 175 or 350 µmol mol^{-1}) CO_2 and three levels of nitrogen addition (0, 100 and 200 kg ha^{-1}); however, the 100 kg ha^{-1} N treatment at ambient plus 175 µmol mol^{-1} CO_2 treatment was omitted from the experimental design. Roots were classified as new, white, brown, decaying or missing and their lengths and diameters measured. The occurrence of mycorrhizae and fungal hyphae was also recorded. The majority (> 90%) of roots observed were smaller than 2 mm and the mean diameter decreased during the study. None of the root parameters measured showed a significant response to elevated CO_2. The elevated CO_2 treatments consistently showed an increase in root area density averaging 50% larger compared to ambient CO_2, but this response was not statistically significant due to the high spatial variability of root distribution. Only new root area density showed a significant nitrogen response. The most new roots were initiated between April and June and the highest level of root loss occurred between June and August. The occurrence of mycorrhizae and fungal hyphae increased in response to CO_2 treatment but not the nitrogen. Their highest levels of occurrence were during August and October 93.

Tingey, David T., Donald L. Phillips, Mark G. Johnson, Marjorie J. Storm, and J. Timothy Ball. 1997. Effects of elevated CO_2 and N-fertilization on fine root growth and mortality of *Pinus ponderosa*. Environmental and Experimental Botany 37: 3-83.

The effects of elevated CO_2 and N-fertilization on shoot and fine root growth of *Pinus ponderosa* Dougl. ex P. Laws. C. Laws. grown in native soil in open-top field-exposure chambers at Placerville, CA were monitored for a two-year period using minirhizotrons. The experimental design was a replicated 3 x 3 factorial with the center treatment missing; plants were exposed to ambient (~365 μmol mol^{-1}) air or ambient air plus either 175 or 350 μmol mol^{-1} CO_2 and 3 levels of N addition (0, 100 and 200 kg ha^{-1} yr^{-1}). The CO_2 by N interactions were not significant. By the second year, elevated CO_2 increased fine root occurrence, root length, root intensity and rooting depth compared to ambient CO_2 while N fertilization had no effect. Fine root mortality was increased by N fertilization but was reduced in elevated CO_2. Highest mortality occurred during summer and the lowest during winter. Elevated CO_2 initially increased mycorrhizal and fungal occurrence. Spring flushes of shoot height and diameter growth were initiated concurrently with the increase in new fine root growth. Initially, elevated N decreased the fine root length/LAI ratio but the effect did not continue. The fine root length/LAI ratio was similar among CO_2 treatments, indicating that the relationship between fine roots and needles was not changed by CO_2 exposure.

Vose, J. M., K. J. Elliott, D. W. Johnson, D. T. Tingey, and M. Johnson. 1997. Soil respiration response to two years of elevated CO_2 and N fertilization in ponderosa pine (*Pinus ponderosa* Doug. ex Laws.). Plant and Soil 190:19-28.

Scientists at WED recently completed a cooperative study with colleagues from the USFS and Desert Research Institute to determine the effects of elevated CO_2 and N fertilization on Ponderosa pine and associated rhizopshere processes. Elevated CO_2 treatment significantly increased the evolution of CO_2 from the soil; rates increased in relation to the CO_2 exposure level. The increased rates of soil CO_2 efflux indicate that rhizosphere processes such as root respiration or fungal respiration (i.e., decomposition) are increased by elevated CO_2. The soil CO_2 efflux rates were linearly related to the occurrence of fungi in the soil but unrelated to the occurrence of fine roots showing the fungal processes in the rhizosphere were the major biological factors controlling soil CO_2 efflux.

Vose, James M., Katherine J. Elliott, Dale W. Johnson, Roger F. Walker, Mark G. Johnson and David T. Tingey. 1995. Effects of elevated CO_2 and N fertilization on soil respiration from ponderosa pine *(Pinus ponderosa)* in open-top chambers. Canadian Journal of Forest Research 25:1243-1251.

We measured growing season soil CO_2 evolution under elevated atmospheric CO_2 and soil nitrogen (N) additions. Our objectives were to determine treatment effects, quantify seasonal variation, and determine regulating mechanisms. Elevated CO_2 treatments were applied in opentop chambers containing 3-year-old ponderosa pine *(Pinus ponderosa* Dougl. ex Laws.) seedlings. Nitrogen applications were made annually in early spring. The experimental design was a replicated factorial combination of CO_2 (ambient, + 175, and + 350 µL L^{-1} CO_2) and N (0, 10, and 20 g.m^{-2} N as ammonium sulfate). Soils were irrigated to maintain soil moisture at >25%. Soil CO_2 evolution was measured over diurnal periods (20-22 h) in April, June, and October 1993 using a flow-through, infrared gas analyzer measurement system. To examine regulating mechanisms, we linked our results with other studies measuring root biomass with destructive sampling and root studies using minirhizotron techniques. Significantly higher soil CO_2 evolution was observed in the elevated CO_2 treatments in April and October; N effects were not significant. In October, integrated daily values for CO_2 evolution ranged from 3.73 to 15.68 g CO_2 m^{-2} day^{-1} for the ambient CO_2 + 0 N and 525 µL L^{-1} CO_2 + 20 g.m^{-2} N, respectively. Soil CO_2 flux among treatments was correlated with coarse root biomass ($r^2 = 0.40$; $p > F = 0.0380$), indicating that at least some of the variation observed among treatments was related to variation in root respiration. Across all sample periods and treatments, there was a significant correlation ($r^2 = 0.63$; $p > F = 0.0001$) between soil CO_2 evolution and percent fungal hyphae observed in minirhizotron tubes. Hence, some of the seasonal and treatment variation was also related to differences in heterotrophic activity.