# OZONE EFFECTS ON RED OAK ROOT DYNAMICS

by

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(ABSTRACT)

Many research projects concerning the possible deleterious effects of ozone on forest health have been conducted on individual tree species. The common goal of these projects has been to identify mechanisms of damage by ozone, and then extrapolate research results to forests. Results from seedling studies are used to parameterize process-based tree growth models which are used to project mature tree responses to different levels of ozone. This approach has been criticized because nothing is known about differences in seedling and mature-tree responses to ozone. Another problem is that few projects have examined the effects of ozone on belowground processes; therefore, very little data exists for parameterizing the models. In order to address the problem of scaling seedling results to mature trees, and increase our level of understanding of ozone effects on belowground processes, an ozone fumigation experiment on northern red oak seedlings and mature trees was conducted. It was hypothesized that carbon reallocation to replace foliage damaged by ozone would decrease fine-root production and turnover. The red oak trees and seedlings were fumigated for three years with three levels of ozone (subambient, ambient, and 2X ambient) in open-top chambers. After two seasons of exposure, 2X

ozone (0.082 ppm 7hr-mean conc.) reduced mature- tree cumulative net fine-root production and turnover by 31 and 41%, respectively, relative to ambient ozone (0.042 ppm 7hr-mean conc.). For the same time period, ozone had no effect on seedling cumulative fine-root turnover; fine-root production was 25% higher under ambient ozone relative to subambient and 2X ambient ozone. During the summer, 1994, mature tree BUE was reduced by 2X ozone. Decreased fine-root production, turnover, and BUE under 2X ozone for the mature trees indicates that ozone can alter the dynamics of belowground carbon allocation in mature red oak. Since the seedlings were not sensitive to ozone, use of seedling results for modelling purposes may underestimate mature tree responses to ozone.

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# CHAPTER I. INTRODUCTION

## STUDY RATIONALE

Increased atmospheric pollution resulting from industrialization, urbanization, and population growth may play a role in the decline of several forest ecosystems (McLaughlin, 1985). In several cases, reductions in tree growth rates have been correlated with increased levels of atmospheric pollutants (Norby and Kozlowski, 1981; McLaughlin et al., 1982; Puckett, 1982; Lorenc-Plucinska, 1984; Freer-Smith, 1985).

Analysis of global climate data suggests that concentrations of atmospheric gases known to impact forest health; i.e., ozone, methane, nitrous oxide, and others, will double within the next century (Keeling, 1984; Freidli et al., 1986). It follows then, that forest health may decline in the future. To ameliorate this potential problem, it has been suggested that tighter restrictions on industrial emissions are needed. However, before meaningful legislation can be developed, it is first necessary to understand how atmospheric pollutants interact with forests. The development of reliable predictive models that simulate tree responses to changing levels of atmospheric pollutants could be important tools used in the formulation of future emissions legislation.

In response to this need, quantitative models are continually being developed and refined as part of an overall effort to assess the impacts of atmospheric pollutants on forest ecosystems (Blake et al., 1990). The ability to quantify the amount and seasonal dynamics of carbon allocation is critical to understanding the mechanisms involved in a tree's ability to survive environmental stress (Edwards et al., 1977). Unfortunately, little is known about actual rates of carbon allocation, and thus it remains one of the least understood components of plant function (Agren, 1981). Moreover, available information concerning carbon allocation in plants has been largely derived from seedling studies (Weinstein and Beloin, 1990).

Plants obtain atmospheric carbon through photosynthesis (Barbour et al., 1987). Carbon fixed by photosynthesis is either stored as biomass or released during respiration to supply energy for metabolic processes (Barbour et al., 1987). Carbon used during respiration makes up a large portion of the total carbon fixed during photosynthesis (Pearcy et al., 1987). Respiration is not only vital for supplying energy for normal metabolic processes, but it is also critical for repairing damage caused by pollutants (Black, 1984). Unfortunately, pollution effects on respiration are not as well studied as pollution effects on photosynthesis (Black, 1984).

Studies of carbon allocation have focused largely on the aboveground portion of the tree, thus seriously limiting our understanding of whole-tree carbon allocation (Edwards and Harris, 1977). However, evidence suggests that roots do exhibit a response to pollutant stress (Hofstra et al., 1981). A reduction in root activity could in turn have a negative impact on the entire plant (Black, 1984). According to Cropper et al. (1985), the belowground portion of the plant represents a large percentage of biomass storage. Stored carbon is allocated to respiration for repairing foliage damaged by pollution. If the damage is severe enough then the normal carbon balance between shoots and roots will be altered, and overall plant growth could be affected (Treshow and Anderson, 1989).

Tree roots are normally associated with mycorrhizal fungi that increase the ability of the roots to uptake water and nutrients (Treshow and Anderson, 1989). If air pollution upsets the carbon balance in the roots, then the mycorrhizal fungi/root association may be affected, thereby disrupting overall plant growth. Trees with mycorrhizal associations have been found to fix more $CO_2$, allocate more carbon to the root systems, and lose more carbon through root respiration than nonmycorrhizal trees (Reid et al., 1983). Rapid turnover of mycorrhizal fungi can use a significant portion of net primary productivity (Vogt et al., 1982).

Fine-root turnover has been largely ignored as a component in belowground biomass production (Joslin and Henderson, 1987). Consequently, the role of fine-roots in net primary production of trees is poorly understood (McClaugherty et al., 1982). Fine-roots make up a large percentage of the belowground biomass and a significant portion of the net primary productivity (Harris et al., 1977). Therefore, fine-roots and their relationship to the carbon dynamics of trees must be understood in order to address questions concerning pollution damage (Aber et al., 1985).

Efforts to model stresses imposed by atmospheric pollutants are difficult because individual stresses may result in similar symptoms (Weinstein and Beloin, 1990). The metabolic activities of each organ in the tree, and its role in the overall balance of growth, must be understood if reliable predictive models are to be developed (Reynolds and Thornley, 1982).

Few models have been developed that depict carbon fixation and allocation in relation to environmental stresses (Hari et al., 1990). The relationship between physiological processes and tree structure has been difficult to model. If a whole tree model is to predict the effects of pollution, the structure of the model must reflect the effects of environmental change on growth processes (Bassow et al., 1990). Most tree-growth models rely on a simple carbon balance framework for allocating carbon to the various processes. Typically carbon is allocated through simple allocation ratios which tend to predict growth response fairly well (Mäkelä, 1990).

It has been hypothesized that by monitoring changes in tree physiological processes causes of forest decline can be identified (Edwards et al., 1977). Models involving pollution stress on trees are based on the hypothesis that pollution damage will cause changes in the carbon balance of trees (Weinstein and Beloin, 1990). Therefore, the capability of existing models to predict atmospheric pollution effects on trees can be improved through further understanding of the carbon balance of trees.

Current tree-growth models rely largely on data gathered from seedling studies (Weinstein and Beloin, 1990). The majority of these models were constructed based upon assumptions made regarding the relationship between seedlings and mature trees, without knowing whether the assumptions were totally valid. Consequently, this is a potential point of error in model development.

Attempts to study tree responses to pollution-induced stress are further confounded by time. In order to develop a successful model, the question of how trees respond to increased levels of pollutants over time must be addressed. The initial response of trees to any treatment is often chaotic. The chaotic response is initiated when the equilibrium of the tree with its environment is disturbed. A consequence of this is that the initial observations are not representative of the true response of trees to the treatment. The modeling effort is hampered during this phase because of the lack of confidence in the data. The ideal solution to this problem is to continue the study until the trees reach a new level of equilibrium under the respective treatments.

## OBJECTIVES

In order to address the potential impact of ozone on tree health, the atmospheric research program of the Tennessee Valley Authority (TVA) began a three-year ozone fumigation experiment on northern red oak (*Quercus rubra* L.). Northern red oak was chosen because it is a dominant and commercially important tree species in temperate hardwood forests of North America. The objectives of this research were to: (i) provide information for process models in order to predict the direct and indirect effects of ozone on tree health, (ii) address the important question of scaling seedling results to mature trees, and (iii) develop reliable field methods for measuring ozone impacts on tree health.

Meeting the above objectives required efforts from three areas of specialization. The modelling objective was addressed by David A. Weinstein et al., at Boyce Thompson Institute for Plant Research, located at Cornell University. In order to thoroughly study the effects of ozone on tree health, the field experimentation was divided into two areas of responsibility, aboveground and belowground. Lisa Samuelson and others studied the aboveground effects; the belowground effects were the focus of this thesis work.

The objective of the belowground study was to determine if ozone alters belowground carbon allocation in red oak trees and seedlings. In order to meet this objective it was necessary to improve existing methods for measuring belowground processes; therefore, an additional objective was to evaluate selected current belowground methods with the goal of improving these methods for this and future studies.

The specific objectives of this project were to:

**1.** Quantify ozone effects on net fine-root production and turnover for seedlings and mature trees,

**2.** Determine ozone effects on fine-root respiration and gross fine-root production for seedlings and mature trees,

**3.** Develop a more reliable method than the buried bag for measuring fine-root decomposition,

**4.** Compare the basal respiration and excised-root methods for measuring root respiration,

and the null hypotheses tested were:

**$H_O$ 1:** Ozone level has no effect on net fine-root production and turnover for seedlings and mature trees

**$H_O$ 2:** Ozone level has no effect on fine-root respiration and gross fine-root production for seedlings and mature trees

**$H_O$ 3:** The buried bag method does not underestimate fine-root decomposition

**$H_O$ 4:** Root respiration can be estimated using either the excised-root or the basal respiration methods

## RELATIONSHIP TO OTHER WORK

This project was a component of the Regional Ozone Vegetation Effects (ROVE) project developed by Dr. G. Edwards and Dr. M. Kelly of TVA. Other work conducted related to the aboveground portion of the tree. Information gathered from above and belowground measurements will be combined, with the goal of enhancing the predictive capability of a plant growth model, TREGRO, in relation to atmospheric pollution-induced stresses.

The ROVE project is a continuing effort sponsored by TVA and the Electric Power Research Institute (EPRI). The purpose of ROVE is to provide quantitative predictions of forest response to changing levels of ozone. The Utility Industry will use this information in order to properly evaluate present and future emissions standards. The project will interface with current and future studies supported by EPRI and other federal agencies.

The TREGRO model was developed as a part of the Response of Plants to Interacting Stresses (ROPIS) project. The goal of ROPIS and ROVE is to determine pathways of plant response to pollutants and identify changes in plant physiological processes that might be linked to pollution damage. This project attempts to identify pathways of plant response in the root system. The results of the project will be combined with other studies being undertaken as part of ROVE.

The Southern Global Change Program (SGCP) initiated at the federal level focuses on air pollution (primarily on elevated $CO_2$ and ozone) effects and changes in moisture and temperature patterns, and how these factors affect southern pine forests. SGCP parallels the goals of this research.

These programs, working together, will continue to contribute to development of reliable models that will describe and predict plant responses to changes in environmental conditions.

# CHAPTER II. LITERATURE REVIEW

## Ozone effects

### *Ozone effects on leaf physiology*

Ozone uptake is regulated by both the atmosphere-internal leaf ozone concentration gradient (Laisk et al., 1989) and stomatal conductance (Kozlowski et al., 1991). Once inside the mesophyll, ozone is either scrubbed by antioxidants (Chameides, 1989) or causes damage to photosynthetic machinery. The photosynthetic machinery is damaged through ozone oxidation of sulfhydryl and fatty acid groups (Heath, 1980), which affects membrane-bound photosynthetic processes (Mudd, 1984). In addition, ozone has been shown to lower foliar concentrations of simple and complex carbohydrates (Miller et al., 1969). Ozone's effect on photosynthetic processes and carbohydrate concentrations may explain why some studies have shown ozone reduces net photosynthesis in certain tree species (e.g. Barnes, 1972; Reich et al., 1986). However, tree photosynthetic response to ozone is species specific (Reich, 1987), with some species being unaffected (e.g. Seiler et al., 1994).

### *Ozone effects on carbon allocation*

Reduced photosynthetic rates are sometimes correlated with lower tree biomass. In a review of ozone literature, Reich (1987) found that photosynthesis and growth declined linearly in response to ozone. However, the relationship between photosynthesis and biomass production is not linear because a large portion of photosynthate is used for respiration and other metabolic processes. Photosynthate used in respiration and other processes could be reallocated to compensate for any potential stemwood growth loss from ozone. Several ozone fumigation studies have shown no negative stemwood growth response even though photosynthesis was reduced (Pye, 1988), indicating that carbon reallocation does occur.

Ozone effects on carbon allocation have been studied directly with $^{11}C$ and $^{14}C$ labeling. Loblolly pine (*Pinus taeda* L.) seedlings were exposed to 0.120 ppm ozone concentration for 12 weeks; using $^{11}C$ labeling, ozone reduced photosynthesis by 16%, phloem transport by 11%, phloem photosynthate concentration by 40%, and total carbon allocation belowground by 45% relative to a charcoal-filtered control (Spence et al., 1990). However, a $^{14}C$ study on the same species and similar ozone concentration showed no significant ozone effect on carbon allocation (Adams et al., 1990); the authors did report a trend of lower photosynthetic rates, greater respiration, and reduced belowground carbon allocation under elevated ozone. Even though ozone did alter patterns of carbon allocation in both studies, total seedling biomass was not affected. This supports the hypothesis that certain seedling species exposed to elevated ozone can maintain biomass production by altering patterns of carbon allocation.

The change in root:shoot ratio over time was measured in several ozone fumigation studies in order to determine how ozone might affect carbon allocation belowground. Reduced photosynthesis caused by stress generally increases the sink strength of shoots for carbon relative to the root sink strength (Cooley and Manning, 1987). Therefore, if ozone reduces photosynthetic rates, then the root:shoot ratio should decrease as carbon allocation belowground decreases. The resultant decrease in root biomass is perhaps the most sensitive indicator of ozone effects on trees (Kozlowski et al., 1991).

Woodbury et al. (1994), measured a 34% decrease in root:shoot ratio in hybrid *Populus* after four months of exposure to 0.079 ppm ozone concentration, relative to charcoal-filtered air. In a three-year study on *Populus tremuloides* Michx., ambient ozone (0.09 ppm) increased the root:shoot ratio by 20% relative to charcoal-filtered air (Wang et al., 1986). Ozone (0.10 ppm) had no effect on yellow-poplar (*Liriodendron tulipifera* L.) root:shoot ratio after six weeks of exposure (Chappelka et al., 1985). Elevated ozone (0.108 ppm) increased loblolly pine root:shoot ratios by 7% after eighteen weeks of exposure relative to subambient (0.032 ppm) ozone (Tjoelker and Luxmoore, 1991). Total seedling biomass was not affected by ozone in any of the above studies.

A reduction in root biomass, as observed by Woodbury et al. (1994), due to ozone could have positive or negative effects on whole-tree biomass. Carbon allocation belowground for production and maintenance of roots represents a significant portion of the net photosynthate available for growth (Kozlowski et al., 1991). Root growth is under both genetic and soil-site control; e.g., certain species develop large fibrous root systems regardless of soil-site conditions (Kramer, 1983). If the soil-site conditions are such that the tree does not require a large fibrous root system then the large quantity of carbon allocated belowground is not necessary, and could be used for aboveground growth. In this case, reduced carbon flow belowground due to ozone could be beneficial, as ozone would counteract the trees genetic program of producing root biomass.

The most popular scenario is that reduction of root biomass by ozone will negatively impact whole-tree biomass. In soils where water and/or nutrients are limiting, or very close to limiting, root exploitation of soil is critical to maintain tree growth. If ozone reduces root biomass, then the ability of the roots to exploit soil resources is diminished, potentially resulting in nutrient and/or water deficiences and reduced tree growth. However, Cooley and Manning (1987), suggested low soil nutrient status could increase root sink strength, potentially counteracting the effects of ozone on carbon allocation. This is supported by Tjoelker and Luxmoore (1991), who found that root:shoot ratios may have increased in response to nutrient deficiencies, thereby overriding the ozone effect.

## Net fine-root production and turnover

### *Ozone effects on net fine-root production and turnover*

Although the majority of ozone studies conducted on seedlings did quantify standing crops of roots, none attempted to quantify fine-root production and turnover. Fine-root production accounts for up to 70% of net primary production in forest trees

(Grier et al., 1981; Keyes and Grier, 1981), and therefore is an important component in whole-tree carbon budgets. Root turnover produces up to five times as much organic matter through decomposition than leaf litter (McLaugherty et al., 1982). The root turnover/leaf litter ratio varies among forest cover types. In general, this ratio is near 1 in hardwood forests, and typically exceeds 2 in softwood forests (Nadelhoffer and Raich, 1992). Why this ratio would differ between hardwoods and softwoods is unknown; however, one possible explanation is that hardwoods are more resource conservative than softwoods, which would explain their tendency to turn over less root biomass per unit litterfall.

The mechanisms behind root production and turnover are poorly understood (Gholz et al., 1986). Several attempts have been made to correlate production and turnover with soil variables (e.g. temperature and nutrient status). Santantonio and Santantonio (1987), found production and turnover to be out of phase with soil temperature, and production increased as site fertility increased. In a slash pine (*Pinus elliottii*) plantation, Gholz et al. (1986), found production decreased as site fertility decreased.

If ozone pollution decreases carbon allocation belowground, then fine-root production should decrease because less photosynthate is available for root growth. It is hypothesized that root turnover would also decrease as a carbon conserving strategy. If root turnover decreases, then the forest biogeochemical nutrient cycle would be altered; decreased organic matter input could reduce microbial mineralization of essential nutrients, thus affecting nutrient supply.

*Measurement of fine-root production and turnover*

Measuring fine-root production and turnover involves extracting soil core samples at selected intervals. Fine-roots usually have an even distribution in most forests (Hermann, 1977; Nambiar, 1983), therefore random sampling is sufficient to characterize root biomass (Bohm, 1979). The root biomass samples are separated into live and dead components to calculate production and turnover.

Several methods exist for determining production and turnover (Table II.1). The max-min method involves summing changes in live and dead root biomass. This method has been critized by several authors because it fails to account for the simultaneous occurrence of growth, death, and decomposition (Joslin and Henderson, 1987; Kurz and Kimmins, 1987; Publicover and Vogt, 1993). By only tracking changes in live and dead biomass, the max-min method underestimates production and turnover. The decision matrix method also involves summing increments and decrements in root biomass. With the decision matrix, positive changes in live biomass are considered production and positive changes in dead biomass are considered turnover. This method gives higher estimates than the max-min method (Kurz and Kimmins, 1987); however, the decision matrix is subject to the same criticisms.

**Table II.1.** Methods for determining fine-root production and turnover.

| Method | Reference |
| --- | --- |
| Max - min method | Harris et al., 1977 |
| Decision matrix method | Fairley and Alexander, 1985 |
| Budget method | Joslin and Henderson, 1987 |
| Compartment-flow model | Santantonio and Grace, 1987 |
| Nitrogen-budget method | Nadelhoffer et al., 1985 |
| Root starch-soil temperature method | Marshall and Waring, 1985 |

The budget method and compartment-flow models account for the simultaneous occurance of growth, death, and decomposition. These methods are based on the basic relationships

$$\mathrm{LFR}_{t+1} = \mathrm{LFR}_t + NP - T \qquad [1]$$

$$\mathrm{DFR}_{t+1} = \mathrm{DFR}_t + T - D \qquad [2]$$

where live fine-root biomass (LFR) at time ($t$ + 1) equals LFR at time ($t$) plus net fine-root production ($NP$) minus turnover ($T$), and dead fine-root biomass (DFR) at time ($t$ + 1) equals DFR at time ($t$) plus turnover ($T$) minus decomposition ($D$). Rearranging eq.1 to solve for net fine-root production gives

$$NP_j = \Delta\mathrm{LFR} + T_j \qquad [3]$$

where the subscript $j$ refers to the time interval $t$ to $t$ + 1. Rearranging eq.2 to solve for decomposition gives

$$D_j = \Delta\mathrm{DFR} + T_j \qquad [4]$$

The only unknown left is fine-root turnover ($T$) which is solved for using eq. 5 Santantonio and Grace (1987).

$$T_j = [\ k_j\, t_j\ \ (\mathrm{DFR}_{t+1} - \mathrm{DFR}_t \exp(-\,k_j\, t_j)\ ]\ /\ [\ 1 - \exp(-\,k_j\, t_j)\ ] \qquad [5]$$

where $k_j$ is a decay coefficient driven by soil temperature ($ST_j$) for time interval ($j$)

$$k_j = k_{\mathrm{ref}} \exp [\ q\ (\ ST_j - ST_{\mathrm{ref}}\ )\ ] \qquad [6]$$

where $q$ is a temperature coefficient determined from two reference (ref) rates of fine-root decomposition with different mean soil temperatures

$$q = [\ \ln k_1 - \ln k_2\ ]\ /\ (\ ST_1 - ST_2\ ) \qquad [7]$$

The budget method uses a virtually identical approach, and therefore will not be discussed.

The nitrogen budget and root starch methods are indirect methods for estimating production and turnover. These methods rely on hypothesized relationships that have not been validated. However, both methods are fairly rapid compared to the coring methods and may prove useful in the future.

*Fine-root decomposition*

Several methods exist for determining root decomposition (Table II.2). The decomposition rate obtained varies depending on soil climate, root diameter, and the method used. Intuitively, decomposition rates should also vary among species due to differences in root chemistry.

The effect of soil climate and diameter on root decomposition is explained by biogeochemical processes. Variation in decomposition rates among methods indicates some or all methods have artifacts. This is evident because if all methods captured the natural biogeochemical processes, then they would give the same decomposition rates, given that soil climate, diameter class, and species are the same.

The most popular method for measuring fine-root decomposition is the buried bag method. This method involves placing washed, air-dry, preweighed roots in a mesh bag and burying the bag in the soil. The bag is retrieved after a known interval and the remaining roots are weighed. The decomposition rate is then determined from the

**Table II.2.** Methods for determining root decomposition

| Method | Reference |
|---|---|
| Trench plot | Santantonio and Grace, 1987 |
| Buried bag | Fogel and Hunt, 1979 |
| Computational | Santantonio and Hermann, 1985 |
| Tethered root | Fahey et al., 1988 |

difference between initial and final root weight. Researchers have criticized this method because the bag may create unnatural conditions that may lead to unrealistic estimates of decomposition rates (Vogt and Persson, 1991). Criticisms are based on the facts that microorganisms are removed from root surfaces during sample preparation (Curl and Truelove, 1985) and the bag acts as a barrier to certain groups of decomposers (McClaugherty et al., 1982).

Root decomposition follows a linear decomposition pattern when the buried bag method is used (Gholz et al., 1986; Fahey et al., 1988; Ruark, 1993). Ruark (1993) reasoned that artificial drying within buried bags limits initial degradation of labile materials which results in the linear pattern. In contrast, *in situ* studies have shown that roots decompose in a nonlinear pattern. Decomposition is rapid initially, with a horizontal asymptote eventually being reached (Santantonio and Grace 1987; Ruark 1993). Rapid degradation of soluble carbohydrates followed by slow degradation of more recalcitrant materials results in the nonlinear shape. This process is generally defined by the differential equation

$$dC/dt = -kC \tag{8}$$

where, C is the change in biomass over time (t), and k is a rate constant (Paul and Clark, 1989).

In order to quantify fine root decomposition rates, measurement techniques must capture the natural dynamics of decomposition as shown in Eq. [8].

## Root and total soil respiration

*Ozone effects on root and total soil respiration*

The majority of ozone-effects studies attempt to quantify ozone effects on root biomass. However, few studies have measured root respiration due to inherent difficulties in developing reliable methodology. In a study by Edwards (1991), loblolly pine seedling root respiration was reduced 12% following exposure to 0.090 ppm ozone concentration for two growing seasons. Reduced root respiration was attributed to a shift in carbon allocation from roots to shoots. However, it is difficult to interpret respiration results without knowledge of ozone effects on production. For example, if ozone decreased root respiration but did not affect production, then the conclusion would be ozone increased belowground carbon use efficiency.

Edwards (1991) also measured a decrease in total soil respiration under high ozone. This response was attributed to reduced root respiration and reduced root exudation rates, with the reduction in exudation causing decreased microbial populations. The indirect effect of ozone on microbial populations emphasizes the need to quantify ozone impacts on the plant-soil system rather than just measuring tree physiology variables.

*Measurement of root and total soil respiration*

Three methods exist for measuring root respiration: *in situ*, excised root, and basal method. *In situ* measurements involve excavating live roots, placing them in a cuvette, and measuring the respiration rate with an infrared gas analyzer (IRGA) (Sisson, 1983; Cropper and Gholz, 1991). In the excised root method, soil core samples are taken and the roots are removed; root respiration is then measured either using the Warburg method (Steinbeck and McAlpine, 1966), or with an infrared gas analyzer. The basal method (Marshall and Perry, 1987) is based on the relationship

$$R_{\text{total soil}} = R_{\text{soil microbial activity}} + R_{\text{live root respiration}} \qquad [9]$$

where R is respiration (Raich and Nadelhoffer, 1989). A volume of soil is isolated with a trenched plot and all aboveground biomass is removed. Total soil respiration is measured at regular intervals. As the freshly severed root material decomposes, total soil respiration reaches a horizontal asymptote. The respiration rate at this asymptote represents soil microbial respiration or 'basal' respiration. Root respiration is obtained by subtracting the basal respiration rate from total soil respiration.

There are several methods employed in measuring soil respiration. Unfortunately, results usually differ depending on the method used (Cropper et al., 1985; Edwards and Harris, 1977). Recent studies have addressed these different methods in an attempt to standardize respiration measurements (Edwards, 1982; Raich et al., 1990).

Edwards (1982) compared two basic methods used in measuring soil respiration: static and dynamic. Static methods employ a chamber placed on the soil surface, with a $CO_2$ trap placed within. The chamber is sealed for a period of time, during which, $CO_2$ is absorbed into a medium (usually KOH, NaOH, or CaO). $CO_2$ absorption is determined through titration when either KOH or NaOH are used as the medium. For the CaO (soda lime) method, the difference in dry weight before and after placement in the chamber determines $CO_2$ absorption.

The dynamic method measures $CO_2$ with an infrared gas analyzer. Air is circulated through a chamber, and $CO_2$ released into the air is monitored by the IRGA (Cropper et al., 1985). Measurement techniques employing this method are considered to be more accurate than the static methods.

Several studies have compared the static methods to each other ( Edwards, 1982; Cropper et al., 1985). Cropper et al. (1985) found no significant difference between the static absorbants. However, Edwards (1982) found that KOH absorbed less $CO_2$ than soda-lime both in field and laboratory experiments. Edwards (1982) goes on to say that the absorption difference could be attributed to the normality of the KOH used.

The absorbants used in the static method yield significantly less $CO_2$ than the dynamic method (Cropper et al., 1985). Air movement through the chamber in the dynamic method appears to increase $CO_2$ evolution, resulting in higher values than for the static methods.

High variation exists using either the static or dynamic techniques due to the spatial heterogeneity of the forest floor (Edwards, 1982; Cropper et al., 1985; Raich et al., 1990). This variation can be minimized with proper choice of sample size.

The static methods are less sensitive than the dynamic method in detecting increases in $CO_2$ evolution resulting from higher temperatures (Edwards and Sollins, 1973). In spite of this drawback, the static methods, in particular the soda-lime method, remain attractive (Cropper et al., 1985). Integrated soil respiration rates can be obtained using the static methods, compared to dynamic methods which only capture point-in-time rates.

The soda-lime method has advantages over the other static methods. Edwards (1982) found that soda-lime absorbed more efficiently than KOH. Soda-lime can be stored on the shelf in glass bottles for months without loosing its absorption efficiency. Analysis of $CO_2$ absorption using the soda-lime method takes considerably less time when compared to the KOH method.

**Seedling versus mature tree comparisons**

Seedlings are normally used in ozone fumigation studies because of cost. However, the validity of extrapolating seedling responses to mature trees is questionable given that there are several physiological differences between seedlings and mature trees. The ratio of photosynthetically-active to inactive tissue decreases with age (Kramer and Kozlowski, 1979). Canopy microclimate changes as the canopy enlarges; this affects net radiation, wind speed, temperature, humidity, $CO_2$, and possibly ozone concentrations (Lee, 1978; Pye, 1988). Nutrient uptake and storage can affect availability as stands age (Bormann and Likens, 1979). All of these factors may act separately or together to cause trees to respond differently to ozone.

## Treatment effects over time

Forest trees exist in a state of dynamic equilibrium with their environment (Kozlowski et al., 1991). The system is dynamic because periodic disturbances affect the equilibrium condition (e.g. seasonality, insect and disease outbreaks). The second law of thermodynamics dictates that following a disturbance, the system will move towards a new equilibrium. Identifying the new equilibrium state of trees following disturbance is a primary goal of ozone pollution research.

When plants are exposed to different treatments, there are three basic phases of response over time (Fig. II.1). Prior to treatment, the trees are assumed to be in equilibrium (or homeostasis) with the environment. This homeostasis is characterized by fairly uniform yearly biomass cycles. When the ozone treatments are applied the initial equilibrium phase changes in response to the treatments. This is characterized as the disruption phase. The length of the disruption phase in years is unknown. In the conceptual model the disruption phase is assumed to be three years. The three-year time period was chosen for illustration purposes only. The point is that the equilibrium of the trees is disrupted by the treatments for some period of time.

The model depicts a scenario in which a new level of equilibrium is reached following the disruption phase. This new level of equilibrium is refered to as the post-treatment equilibrium level, and it is based on the second law of thermodynamics. Obviously, a myriad of possibilities exist for tree response to ozone. Other possible scenarios are no post-treatment equilibrium or complete system disfunction following exposure.

In order to meet the primary goal of this project, it was necessary to study how trees respond to different levels of ozone treatment over time. If a mechanistic model of belowground processes is to be developed, then the post-treatment equilibrium level must be identified, providing it exists.

The disruption phase is a chaotic initial response to the treatments. Consequently, developing a model based on observations gathered during this phase would yield a poor model. Models should be developed using the stable observations gathered during the post-treatment equilibrium level. Obviously, this requires a long-term commitment to the project (several years), and is beyond the scope of this research plan.

These ideas are presented in order to show what the long-term research objectives should be. This research focused on the disruption phase in the conceptual model. The goal of this project was to begin developing an understanding of tree response to ozone exposure, while keeping in mind that observations gathered during this phase might not represent the long-term response.

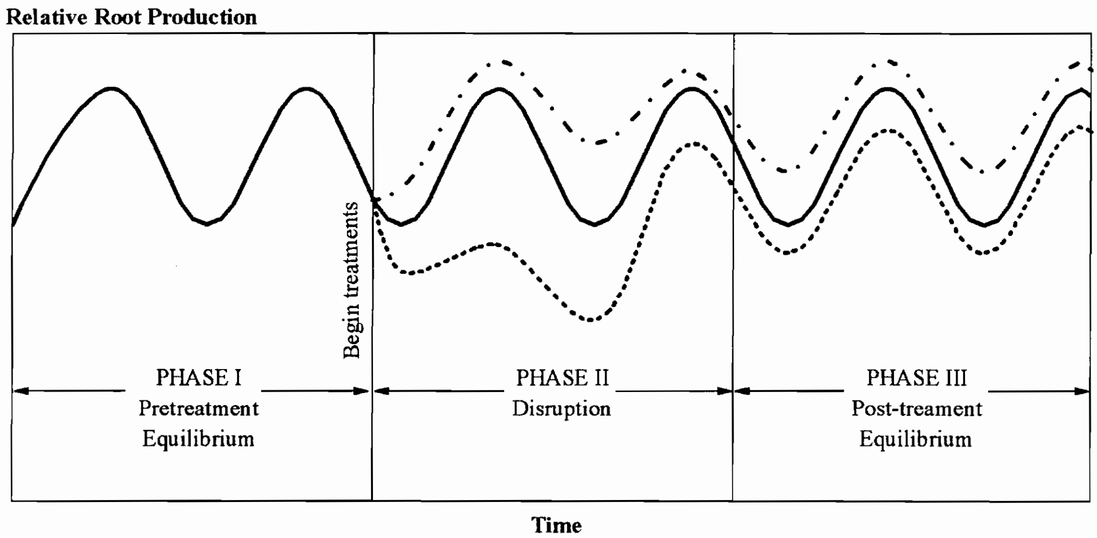


**Figure II.1.** Conceptual model of root biomass response to three different levels of ozone exposure. The figure shows three phases of response as a function of time. The curved lines represent ambient (solid), subambient (dash-dot-dash), and twice ambient (dashed) ozone exposure. This model assumes that a new level of equilibrium will be reached for the subambient and 2X ambient ozone treatments.

# CHAPTER III. MATERIALS AND METHODS

## Study design and layout

The field research facility was located in a northern red oak seed orchard on the TVA reservation in Norris, Tennessee. In the 1960's replicate cuttings were grafted to root stock at six by six meter spacing. These trees have since matured, and are now producing viable seed crops. Seeds from selected parent trees on the site were collected in 1990, and half-sib seedlings were grown for study. This situation was unique in that it allowed for the simultaneous study of mature trees and seedlings of genetically similar stock.

The facility consisted of eighteen open-top chambers that were adapted for studying ozone effects on mature trees and seedlings. Nine of the chambers enclose individual mature northern red oak trees; the remainder enclosed 30 two-year-old northern red oak seedlings each (Fig. III.1). The trees used in the study were selected at random from the orchard. Seedling chambers were located in areas not occupied by trees. The seedling and tree chambers were then blocked based on differences in solar radiation observed on the site. Three ozone treatments, subambient, ambient, and twice ambient, were then applied to each block, for a total of three blocks of three treatments each, for the trees and seedlings. The treatments were applied over three growing seasons, beginning in May, 1992, and ending in September, 1994.

The open-top chambers that enclosed the mature trees were 4.66 meters in

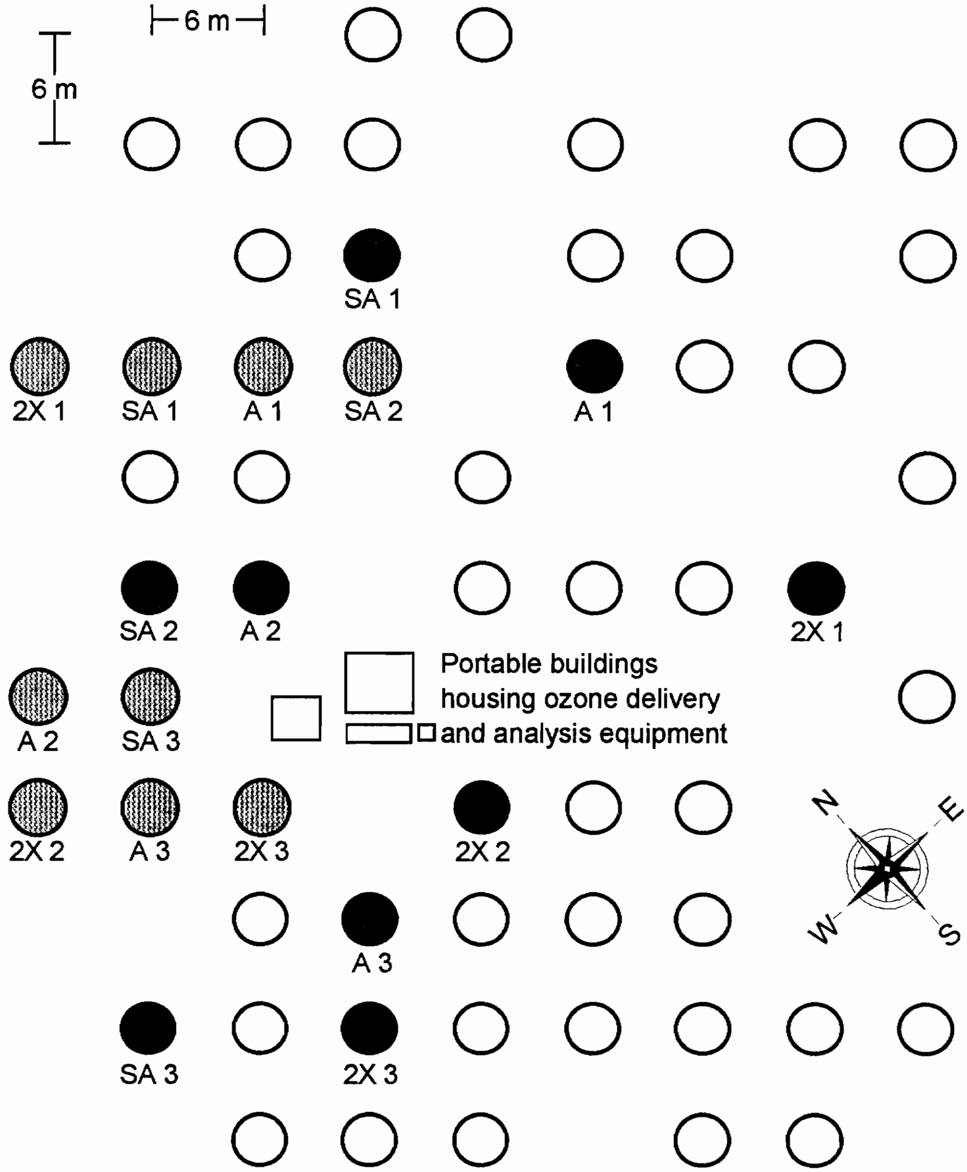


**Figure III.1** Diagram of the northern red oak seed orchard showing the spatial arrangement of the mature tree and seedling chambers. Open circles are unchambered mature trees, darkly shaded circles are chambered mature trees, and lightly shaded circles are the seedling chambers (30 seedlings per chamber). The codes under each chamber refer to treatment and block: SA, A, and 2X are subambient, ambient, and 2X ambient, respectively. The numbers 1, 2, and 3 refer to blocks 1,2, and 3, respectively.

diameter and 9.2 meters in height (Fig. III.2). The frame consisted of seven sections, including two plenum sections at the bottom and a frustrum at the top. The plenum sections were individually connected to a fan equipped with particulate filters. The fans were also capable of being equipped with charcoal filters for ozone exclusion. In order to avoid shading the tree, the two fans were stacked on top of each other on the north side of the chamber. The diameter of the opening at the top of the frustrum was 3.2 meters. The bottom plenum was equipped with two clips that allow entry on the south side of the chamber. The seedling chambers were 3.0 meters in diameter and 2.4 meters in height, and consisted of one plenum and a top section. As with the large chambers, the plenum was connected to a fan on the north side of the chamber.

Ozone levels within the chambers were continuously monitored by a TEII ozone analyzer housed in a nearby portable building. Air was drawn from the chambers, and delivered to the analyzer through heated Teflon sample lines. Computer software in the portable building compared ozone concentration in the chamber with ambient ozone concentration, the level of ozone delivered to the chamber was adjusted in order to maintain the desired treatment level. Ozone treatments began in the spring and terminated in the fall of each year. The target ozone levels were achieved and maintained throughout the study (Fig III.3), with the exception of a drop in the 2X ambient level for a three week period during the summer in 1994 (Fig III.3c).

Both the mature tree and seedling chambers were equipped with nozzles for artificial watering. Soil moisture content was monitored daily throughout the growing season with a time domain reflectometer (TDR). Soil moisture was returned to field capacity whenever the soil water potential fell below -0.2 to -0.5 MPa.

Soil temperature was recorded hourly at the 10cm depth in three chambers: two large chambers and one small chamber. Soil temperature did not differ significantly between the large and small chambers; however, the small chamber temperatures were consistently higher during the summer. Temperature ranged from 3.5 in January to 28 °C in July, with a mean temperature of 15.5 °C (Fig III.4).

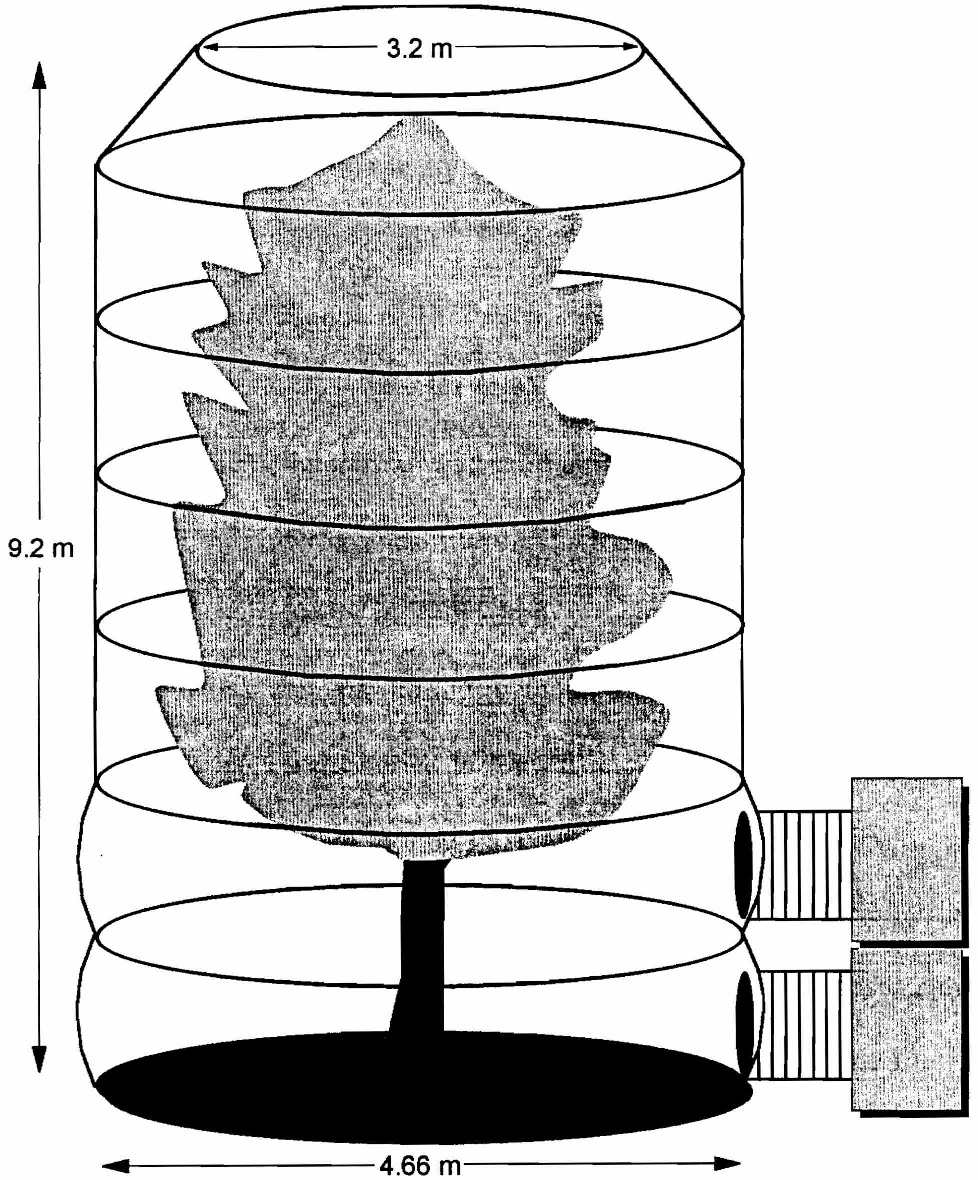


**Figure III.2** Drawing of large open-top fumigation chamber.

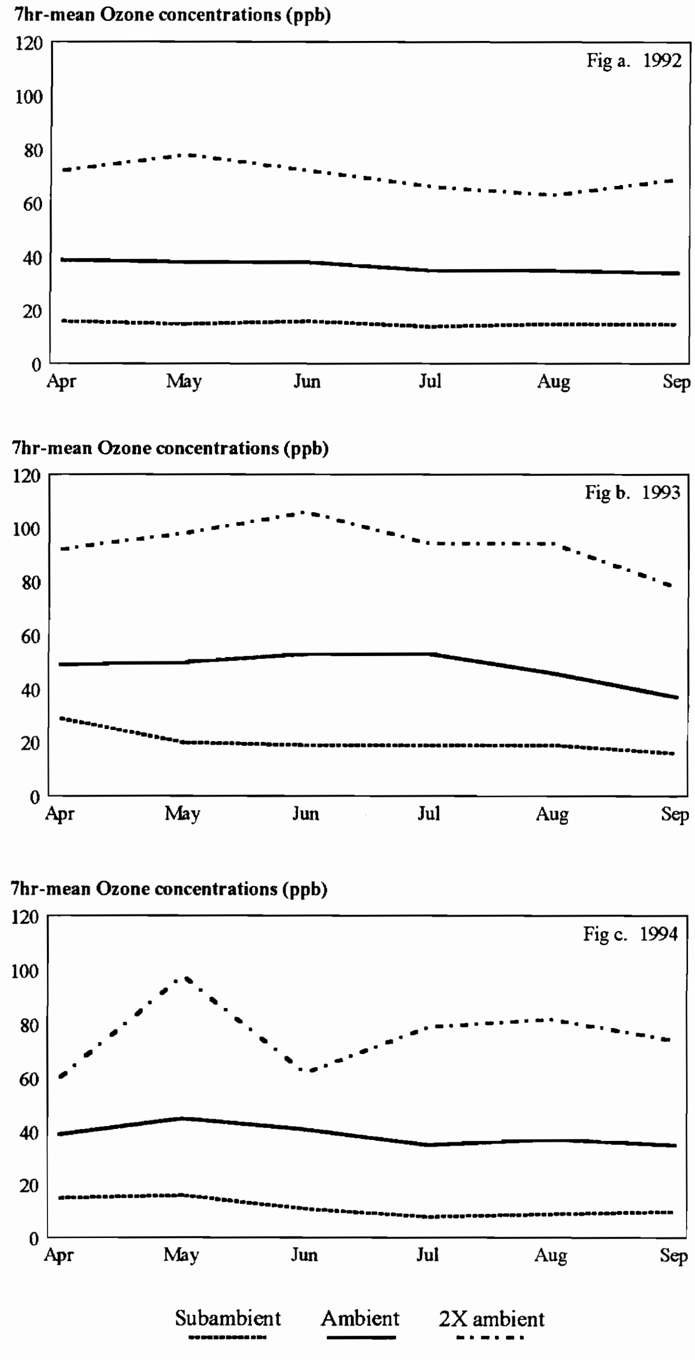


**Figure III.3** 7hr-mean monthly ozone concentrations (ppb) for three growing seasons. Seedling and mature tree concentrations are averaged by treatment: subambient (dashed line), ambient (solid line), 2X ambient (dash-dot-dash line).

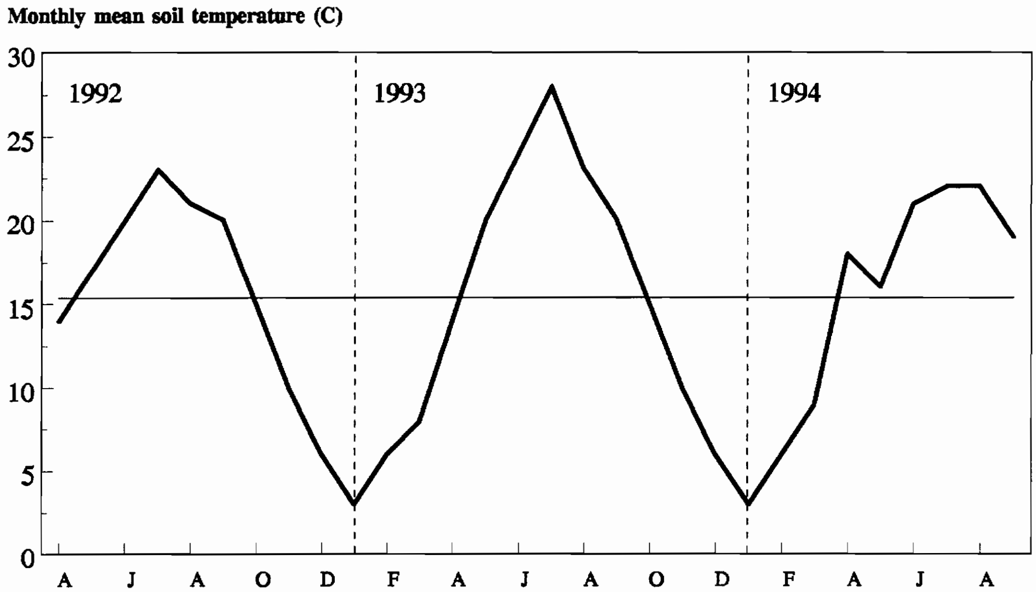


**Figure III.4** Monthly mean soil temperature (°C) for length of study. Temperature was automatically recorded hourly at 10cm depth in three chambers.

**General methods**

To test the hypothesis 'Ozone level has no effect on net fine-root production and turnover for seedlings and mature trees', the compartment-flow model (Santantonio and Grace, 1987) was used to calculate net fine-root production and turnover. The model required estimates of live fine-root biomass, dead fine-root biomass, and fine-root decomposition. Sequential coring was used to measure live and dead fine-root biomass. Soil samples were collected from four separate sections in each large chamber (Fig. III.5), and eight seedling pots per small chamber. Roots were washed from the soil cores, separated into two size classes (fine <2mm, and coarse >2mm), divided into live and dead, oven-dried and weighed. A complete description of the compartment-flow model and root sampling is given in chapter V. To estimate decomposition, an *in situ* sequential coring method was used. The decomposition rate was determined by fitting the results to a nonlinear decomposition equation. For a complete description of the *in situ* coring method, refer to chapter IV.

To test the hypothesis 'Ozone level has no effect on fine-root respiration and gross fine-root production for seedlings and mature trees', fine-root respiration was measured using the excised root method. During the summer, 1994, respiration was measured on live fine roots collected for biomass sampling. These samples were collected from previously undisturbed areas (Fig. III.5). Gross fine-root production was calculated by adding live fine-root respiration to net fine-root production. Complete descriptions of these methods are given in chapter V.

One of the objectives of this project was to develop a more reliable method for estimating fine-root decomposition. When we became involved in the project, the buried bag method was being used to estimate fine-root decomposition. However, a growing number of researchers feel that this method underestimates decomposition. Since our net fine-root production and turnover calculation required decomposition estimates, we felt is was necessary to develop a more reliable method for measuring decomposition. To test the hypothesis 'The buried bag method does not underestimate fine-root decomposition', a field study was conducted along with a comprehensive literature review. The purpose of the field study was to quantitatively compare the buried bag method with our *in situ* coring method. Buried bags and cores were placed in each chamber; the bags were buried in four different sections per chamber (Fig. III.5), and the cores were located around the edge of each chamber. The literature review was conducted in order to obtain root decomposition rates from a variety of sources. This literature-derived dataset was evaluated statistically to answer the question: is the buried bag method a reliable method for estimating fine-root decomposition? A complete description of the field methods and literature analysis is given in chapter IV.

Perhaps the greatest area of uncertainty with respect to belowground measurement techniques is root respiration. The variety of techniques, and lack of a sufficient number of studies to evaluate them, makes it difficult to choose a method. As a part of this project, we had the opportunity to compare the excised root and basal respiration methods. In the summer, 1994, all samples were collected from the undisturbed sections in each chamber (Fig. III.5). The *in situ* decomposition cores were used to measure basal respiration. Basal respiration was then compared to excised root respiration in order to test the hypothesis, 'Root respiration can be estimated using either the excised root or the basal respiration method'. A detailed description of these methods can be found in chapter VI.

**Experimental Design**

The main study design for the seedlings and trees was a randomized complete block design with three blocks of three treatments. While the seedling and tree designs were identical, the two studies were separate. Therefore, no statistical analysis could be conducted between the seedlings and trees; apparent treatment differences between seedlings and trees were qualitatively compared.

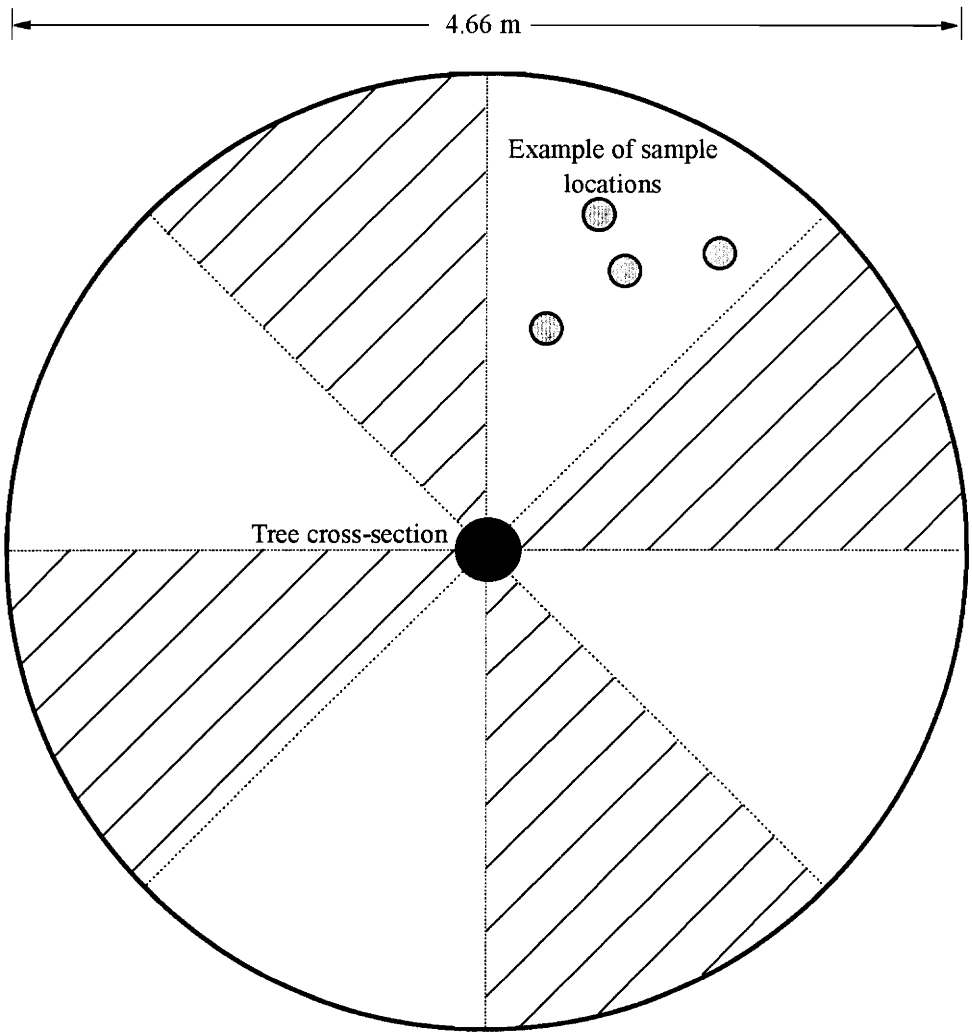


**Figure III.5** Soil surface of large fumigation chamber. The chamber floor was divided into eight sections. The summer 1994 samples were collected from the non-hatched sections. Hatched sections indicate where fine-root biomass and buried bag samples were collected during the first two years of fumigation.

# CHAPTER IV. COMPARISON OF BURIED BAG AND *IN SITU* METHODS FOR ESTIMATING FINE-ROOT DECOMPOSITION[1]


(ABSTRACT)

Fine root production and turnover calculations require reliable estimates of root decomposition rates. Traditionally, decomposition rates have been measured by burying known amounts of excavated roots contained in bags. This method has been criticized, however, because it is thought to underestimate decomposition. In response to this problem, an alternative method for measuring fine root decomposition was developed. The method used a sequential coring technique that allowed roots to decompose *in situ*. This method was evaluated against the buried bag method in an experiment conducted for two different seasons, fall and winter. In addition, a quantitative analysis of buried bag and *in situ* decomposition rates obtained from the literature was conducted. Results from the coring method closely fit ($r^2 \geq 0.87$) a first-order kinetics model $C_r = C_i \exp(-kt)$. The decomposition rate determined using the coring method (0.18 $k$ $mo^{-1}$) was three times greater than the buried bag method (0.06 $k$ $mo^{-1}$). Results from the literature analysis showed that the *in situ* method is a better estimator of decomposition rates than the buried bag method. Two artifacts of the buried bag method are that the rhizosphere is removed during root washing, and the roots are separated from the soil by the mesh bag. The barrier created by the bag could slow recolonization of the roots by rhizosphere organisms, and thus affect


[1]Manuscript submitted to Soil Science Society of America Journal in September 1994.

decomposition. The *in situ* method eliminates these artifacts, and more closely approximates the true decomposition rate.

## INTRODUCTION

Tree roots are an integral component in the carbon budgets of forest ecosystems. Root production has been estimated to account for between 8 and 67% of net primary production in forest trees (Grier et al., 1981; Keyes and Grier, 1981). This suggests that severe underestimates of carbon budgets will be obtained if the contribution from root production is ignored. Therefore, it is critical in any study of forest carbon budgets to quantify the role of root production.

In order to calculate production, root decomposition rates must be known (Joslin and Henderson, 1987; Santantonio and Grace, 1987; Publicover and Vogt, 1993). Several methods exist for determining root decomposition (Table IV.1). The decomposition rate obtained varies depending on soil climate, root diameter, and the method used. Intuitively, decomposition rates should also vary among species due to differences in root chemistry.

The effect of soil climate and diameter on root decomposition is explained by biogeochemical processes. Variation in decomposition rates among methods indicates some or all methods do not capture these processes. This is evident because if all methods captured the natural biogeochemical processes then they would give the same decomposition rates, given that soil climate, diameter class, and species are the same.

The most popular method for measuring fine root decomposition is the buried bag method. This method involves placing washed, air-dry, preweighed roots in a mesh bag and burying the bag in the soil. The bag is retrieved after a known interval and the remaining roots are weighed. The decomposition rate is then determined from the difference between initial and final root weight. Researchers have criticized this method because the bag may create unnatural conditions that may lead to decreased estimates of decomposition rates (Vogt and Persson, 1991). Criticisms are based on the facts that microorganisms are removed from root surfaces during sample preparation (Curl and Truelove, 1985) and the bag acts as a barrier to certain groups of decomposers (McClaugherty et al., 1982).

Root decomposition follows a linear decomposition pattern when the buried bag method is used (Gholz et al., 1986; Fahey et al., 1988; Ruark, 1993). Ruark (1993) reasoned that artificial drying within buried bags limits initial degradation of labile materials which results in the linear pattern. In contrast, *in situ* studies have shown that roots decompose in a nonlinear pattern. Decomposition is rapid initially, with a horizontal asymptote eventually being reached (Santantonio and Grace 1987; Ruark 1993). Rapid degradation of soluble carbohydrates followed by slow degradation of more recalcitrant materials results in the nonlinear shape. This process is generally defined by the differential equation

**Table IV.1.** Methods for determining root decomposition

| Method | Reference |
| --- | --- |
| Trench plot | Santantonio and Grace, 1987 |
| Buried bag | Fogel, 1979 |
| Computational | Santantonio and Hermann, 1985 |
| Tethered root | Fahey et al., 1988 |

$$dC/dt = -kC \qquad [1]$$

where, C is the change in biomass over time (t), and $k$ is a rate constant (Paul and Clark, 1989).

In order to quantify fine root decomposition rates, measurement techniques must capture the natural dynamics of decomposition as shown in Eq. [1]. Such methods will improve researchers' understanding of fine root dynamics by increasing the reliability of methods used for quantifying fine root dynamics. If the experimental objective is to quantify differences in intraspecies root decomposition rates, or differences in decomposition rates by diameter class, then the proper method must be used.

In response to this need, an alternative *in situ* method was developed and evaluated against the buried bag method. In addition, a comprehensive literature review on root decomposition studies was conducted. The objectives of this study were: (i) determine if the buried bag and *in situ* methods result in different decomposition rate estimates, and (ii) determine which method is better for studying differences in root decomposition between species and diameter classes.

## MATERIALS AND METHODS

### Background

This project evolved from a preexisting study of ozone effects on fine root dynamics. In the ozone-effects study, nine mature northern red oak (*Quercus rubra*

L.) trees were exposed to three levels of ozone; subambient, ambient, and twice ambient. The study began in May 1992 and is scheduled to be complete in December 1994. A detailed description of the study layout and experimental design was given by Edwards et al. (1994).

Originally, the buried bag method was used to determine fine root decomposition rates. The decomposition rates were used in the budget method (Joslin and Henderson, 1987) for estimating fine root production and turnover. After one year of measurements it was felt that the buried bag method yielded unrealistically low values. This feeling was based on a suspicion that the bags were excluding some organisms. Therefore, an attempt was made to develop a method that would result in more realistic estimates of fine-root decomposition. Measurements using the new method began in August 1993. Decomposition estimates using the buried bag method were continued in order to compare the methods statistically.

## Fine-Root Decomposition

### *Buried Bag Method*

Soil samples (30cm deep by 1m$^2$) were collected from the seed orchard. The sampling depth was equivalent to the sampling depth for the *in situ* method. Live fine roots <2mm diameter were separated from the soil and other organic matter by hand. The roots were then washed free of adhering soil and allowed to air-dry for several days. Twenty subsamples were then oven-dried at 65°C for 48 hours and weighed.

These samples were then ashed in a muffle furnace at 450°C for 24 hours and weighed. Forty-five 0.5g samples of the air-dried roots were placed in nylon mesh bags constructed of panty hose. The soil-free oven-dried weights of the samples in the bags were determined using the information gathered from the subamples. Five bags were buried 5cm deep in random locations[2] within each chamber. The location of each bag was marked with an orange ring. The bags were retrieved from the soil after four months. The bags were returned to the laboratory where the remaining roots were carefully removed and brushed free of soil. The final soil-free oven-dried weights were then determined as previously described.

The decomposition rate ($k$ mo$^{-1}$) was determined by chamber using the equation

$$-k = [\ \ln (Cr / Ci)\ ] / t \qquad [2]$$

where $Cr$ is final root biomass and $Ci$ is initial root biomass. This procedure was conducted for two intervals, fall (August 1993 to December 1993) and winter (December 1993 to April 1994).

*In situ Method*

Forty 5.1cm i.d. by 30cm long galvanized steel tubes (2in electrical conduit) were driven into the soil profile in each chamber using an electric jackhammer. In order to minimize the possibility of severing large roots, the tubes were located around the

[2]Locations were selected randomly from numbered grids superimposed on the chamber floors.

outer edge of each chamber. The pipes cut the roots and isolated them from the rest of the soil with a minimum of disturbance to the soil profile. This allowed the roots to decompose in their natural environment. Eight pipes per chamber were extracted immediately and the soil was removed.

Roots were separated from the soil using the hydropnuematic elutriation system (Smucker et al., 1982). The fine roots were then oven-dried and weighed. Soil-free oven-dried weights were determined using the procedure described in the buried bag section.

On the first of each month thereafter eight of the remaining pipes were removed from each chamber. The roots were extracted and weighed as previously described. This procedure was conducted over the same intervals as the buried bag method.

The mean weights of the within chamber monthly data were used to determine decomposition rates by chamber. The data were fit to a first order kinetic model integrated from Eq. [1]

$$C_r = C_i \exp(-kt) \qquad [3]$$

where, $C_r$ is fine root biomass remaining at time t (month), $C_i$ is initial fine root biomass, and $k$ is the rate constant ($mo^{-1}$). Nonlinear least squares regression was used to estimate the decomposition rates ($k$).

### Statistical Analysis

The effect of season (i.e., fall and winter) on decomposition rates ($k$) was evaluated with analysis of variance using the model,

$$k = \beta_0 + \beta_1 x_1 + \varepsilon, \qquad [4]$$

where $\beta_0$ and $\beta_1$ were unknown parameters, $\varepsilon$, was an error term, and $x_1$ was a dummy variable which equaled 1 if season was fall and 0 if season was winter. If the estimate of $\beta_1$ was not significantly different from zero, then there was no seasonal effect on decomposition. This analysis was conducted separately for each method using the individual chamber decomposition rates (n = 18 per method). If the seasonal decomposition rates were not significantly different, then the rates were pooled into one dataset for remaining analysis. The paired t-test (Ott, 1988) was used to test for significant differences between *in situ* and buried bag decomposition rate estimates.

**Methods Comparison**

A table of root decomposition rates was developed from values reported in the literature (Table IV.2). Sixteen tree species are represented along with a range of diameters and *k*-values. The *in situ* decomposition rates from this study were averaged, projected for one year (k $yr^{-1}$), and included in Table IV.2.

The table was treated as a data set for qualitative analysis of the methods' sensitivity to differences in species and diameter class. Species and diameter class were discussed in the Introduction as important variables that should affect decomposition rates. Our assumption is if the decomposition rate determined by a method does not change by species and diameter class, then the method is not capturing the true decomposition rate. Alternatively, if the decomposition rate determined by a method is related to species and diameter class, then the method is better at estimating the true decomposition rate.

This analysis was based on the relationship

$$k = \int ( T, S, D ), \tag{3}$$

where $k$ is a function of temperature ($T$), species ($S$), and diameter class ($D$). Soil moisture was not considered to be limiting decomposition in any of the studies, and was therefore excluded. The decomposition rates ($k$ yr$^{-1}$) were normalized for temperature using a $Q_{10}$ function of 2, and the equation

$$Q_{10} = (k_1/k_2)^{10/(T2 - T1)} \tag{4}$$

where $T_1$ was the temperature regime for which the decomposition rate, $k_1$, was determined, and $T_2$ is the temperature regime for which the rate constant, $k_2$, is desired. This equation was rearranged to solve for $k_2$

$$k_2 = k_1 \exp \{ \ln Q_{10}/[10/(T_2 - T_1)]\} \tag{5}$$

The average temperature (8.4°C) from all the studies was used for $T_2$. After normalizing for temperature, the decomposition rate, $k$, became a function of species and diameter class.

A wide variety of diameter classes were reported in the literature (Table IV.4). These classes were placed into one of five groups for qualitative analysis (Table IV.5). Roots should be separated into at least three classes (fine, medium, and coarse), because each class has different turnover and decay rates (Vogt et al., 1991). The two additional classes were defined because some authors treated all roots as one class. Tree species were grouped into one of five general cover types: (i) northern pines, (ii) spruce, (iii) northern hardwoods, (iv) mixed hardwoods, and (v) southern pines.

The trench plot method was included as an *in situ* method. The tethered root and computational methods were excluded because there was not sufficient data from these methods to include in the analysis.

## RESULTS AND DISCUSSION

The *in situ* coring method results closely fit the first-order kinetic model (r-square $\geq 0.87$) (Fig. IV.1), indicating that the method does capture at least a portion of the natural dynamics of decomposition. The standard errors were reasonable considering the naturally high variation in fine-root biomass (Ruark and Zarnoch, 1992); however, eight subsamples per chamber were required to obtain this low variation. The large number of samples required to control variation is a disadvantage when compared to the buried bag where fewer samples are required due to more control over variation. The fall and winter decomposition rates (0.21 and 0.16, respectively) were not significantly different ($p = 0.183$); however, the winter rate was slightly lower than the fall rate, which is what would be expected given the influence of soil temperature on decomposition. The fall and winter buried bag rates were essentially equal.

Since the fall and winter decomposition rates were not significantly different for either method, the within method fall and winter datasets were combined prior to conducting the paired t-test. The *in situ* coring decomposition rates were approximately three times greater than the buried bag rates (Table IV.4). Putting these rates in prespective, it would take approximately 1.5 years for 95% of the initial fine-root biomass to disappear using the *in situ* coring rates, and approximately 5 years for 95% of the initial fine-root biomass to disappear using the buried bag rates. Given that the soil on this site is a fine-loamy, mixed, thermic Cumulic Hapludoll (Samuelson and Edwards, 1993), five years is an unreasonably long period of time for 95% of the original root biomass to decompose. This result lead us to conclude that the buried bag method does have artifacts associated with it, causing the method to underestimate decomposition.

In general, the buried bag method results in lower decomposition rate estimates than the *in situ* method (Table IV.5). Little variation exists among the buried bag method decomposition rates with respect to cover type when compared to the *in situ* method decomposition rates. This indicates that the buried bag method is not related to differences in cover types. With the *in situ* method, the decomposition rate decreases with increasing proportion of coarse roots (Table IV.5); this response is supported by the results reported by Berg, 1984, Fahey et al., 1988, and Ruark, 1993 that show decomposition decreases with increasing root diameter. In contrast, the buried bag decomposition rates showed no trend of decreasing decomposition rate with increasing root diameter.

## CONCLUSIONS

Decomposition rates from our *in situ* method were the highest rates obtained from any method to date. However, the many factors that affect decomposition make it difficult to directly compare rates from other studies. Normalizing for temperature allowed a qualitative comparison of decomposition rates between methods. Assuming that decomposition rate is a function of species and diameter class, results of this analysis indicate that the *in situ* method is better than the buried bag method for estimating rates of root decomposition. It also suggests that cross-study comparisons are suspect when different methods of estimating decomposition are used.

**Table IV.2.** Annual root decomposition rates from several forest ecosystems.

| Location | Cover Type | Temp °C | Diameter Class (mm) | k† ($yr^{-1}$) | Method‡ | Reference |
|---|---|---|---|---|---|---|
| Belgium | *Picea abies* L. | 6 | < 1 | 1.19 | A | van Praag, 1988 |
| Belgium | *Picea abies* L. | 6 | 1-5 | 1.09 | A | van Praag, 1988 |
| New Zealand | *Pinus radiata* D. Don. | 11 | < 1 | 0.71 | A | Santantonio and Grace, 1987 |
| Sweden | *Pinus sylvestris* L. | 4 | 1-2 | 0.26 | B | Berg, 1984 |
| Sweden | *Pinus sylvestris* L. | 4 | 2-3 | 0.21 | B | Berg, 1984 |
| Sweden | *Pinus sylvestris* L. | 4 | 3-5 | 0.21 | B | Berg, 1984 |
| Sweden | *Pinus sylvestris* L. | 4 | 5-7 | 0.23 | B | Berg, 1984 |
| Sweden | *Pinus sylvestris* L. | 4 | 7-9 | 0.23 | B | Berg, 1984 |
| Sweden | *Pinus sylvestris* L. | 4 | 9-11 | 0.20 | B | Berg, 1984 |
| Estonia | *Picea abies* L. | 7 | < 1 | 1.19 | B | Ivask et al., 1991 |
| Virginia | Appalachian hardwoods | 3 | 0-0.5 | 0.63 | B | McClaugherty et al., 1984 |
| Virginia | Appalachian hardwoods | 3 | 0.5-3.0 | 0.77 | B | McClaugherty et al., 1984 |
| Wisconsin | *Acer saccharum* Marsh. | 0 | 0.5-3.0 | 0.82 | B | McClaugherty et al., 1984 |
| Wisconsin | *Pinus strobus* L. | 0 | 0.5-3.0 | 1.21 | B | McClaugherty et al., 1984 |
| Massachusetts | Mixed hardwoods | 0 | 0-0.5 | 0.35 | B | McClaugherty et al., 1984 |
| Massachusetts | Mixed hardwoods | 0 | 0.5-3.0 | 1.03 | B | McClaugherty et al., 1984 |
| Massachusetts | *Pinus resinosa* Ait. | 0 | 0-0.5 | 0.40 | B | McClaugherty et al., 1984 |
| Massachusetts | *Pinus resinosa* Ait. | 0 | 0.5-3.0 | 1.17 | B | McClaugherty et al., 1984 |
| Massachusetts | Mixed hardwoods | 8 | < 3 | 0.17 | B | McClaugherty et al., 1982 |
| Massachusetts | Mixed hardwoods | 8 | < 3 | 0.29 | B | McClaugherty et al., 1982 |
| Massachusetts | *Pinus resinosa* Ait. | 8 | < 3 | 0.26 | B | McClaugherty et al., 1982 |

| Location | Cover Type | Temp °C | Diameter Class (mm) | k† ($yr^{-1}$) | Method‡ | Reference |
|---|---|---|---|---|---|---|
| Massachusetts | *Pinus resinosa* Ait. | 8 | < 3 | 0.67 | B | McClaugherty et al., 1982 |
| New Hampshire | Northern hardwoods | 6 | < 2 | 0.32 | B | Fahey et al., 1988 |
| North Carolina | *Pinus taeda* L. | 15 | all | 0.38 | B | Ruark, 1993 |
| Missouri | Mixed hardwoods | 12 | < 5.0 | 0.34 | B | Joslin and Henderson, 1987 |
| Virginia | *Chamaecyparis thyoides* L. | 16 | 2-5 | 0.20 | B | Day and Megonigal, 1993 |
| Virginia | Maple-gum | 16 | 2-5 | 0.18 | B | Day and Megonigal, 1993 |
| Virginia | *Taxodium distichum* L. | 16 | 2-5 | 0.25 | B | Day and Megonigal, 1993 |
| Virginia | Mixed hardwoods | 16 | 2-5 | 0.29 | B | Day and Megonigal, 1993 |
| New Hampshire | *Acer saccharum* Marsh. | 6 | 2-10 | 0.11 | C | Fahey et al., 1988 |
| New Hampshire | *Fagus grandifolia* Ehrh. | 6 | 2-10 | 0.08 | C | Fahey et al., 1988 |
| New Hampshire | *Betula alleghaniensis* Britton | 6 | 2-10 | 0.07 | C | Fahey et al., 1988 |
| New Hampshire | *Picea rubens* Sarg. | 6 | 2-10 | 0.05 | C | Fahey et al., 1988 |
| Oregon | *Psuedotsuga menziesii* Mirb. | 12 | < 5 | > 2.26 | D | Santantonio and Hermann, 1985 |
| North Carolina | *Pinus taeda* L. | 25 | all | 0.18 | E | Ruark, 1993 |
| North Carolina | *Pinus taeda* L. | 15 | all | 0.23 | E | Ruark, 1993 |
| North Carolina | *Pinus taeda* L. | 25 | laterals | 0.34 | E | Ruark, 1993 |
| North Carolina | *Pinus taeda* L. | 15 | laterals | 0.53 | E | Ruark, 1993 |
| North Carolina | *Pinus taeda* L. | 15 | all | 0.36 | E | Ruark, 1993 |
| North Carolina | *Pinus taeda* L. | 15 | laterals | 0.58 | E | Ruark, 1993 |
| Tennessee | *Quercus rubra* L. | 16 | < 2 | 1.20 | E | This study |

† $k$ values have been normalized for temperature using a $Q_{10}$ of 2.
‡ Methods A = trench plot, B = buried bag, C = tethered roots, D = computational, E = *in situ*

**Table IV.3.** Diameter classes used in qualitative literature analysis.

| Diameter Class | Diameter (mm) |
|---|---|
| Fine | < 2 |
| Medium | 2 - 5 |
| Coarse | 5 - 11 |
| Fine - Medium | < 2 - 5 |
| Fine - Coarse | < 2 - 11 |

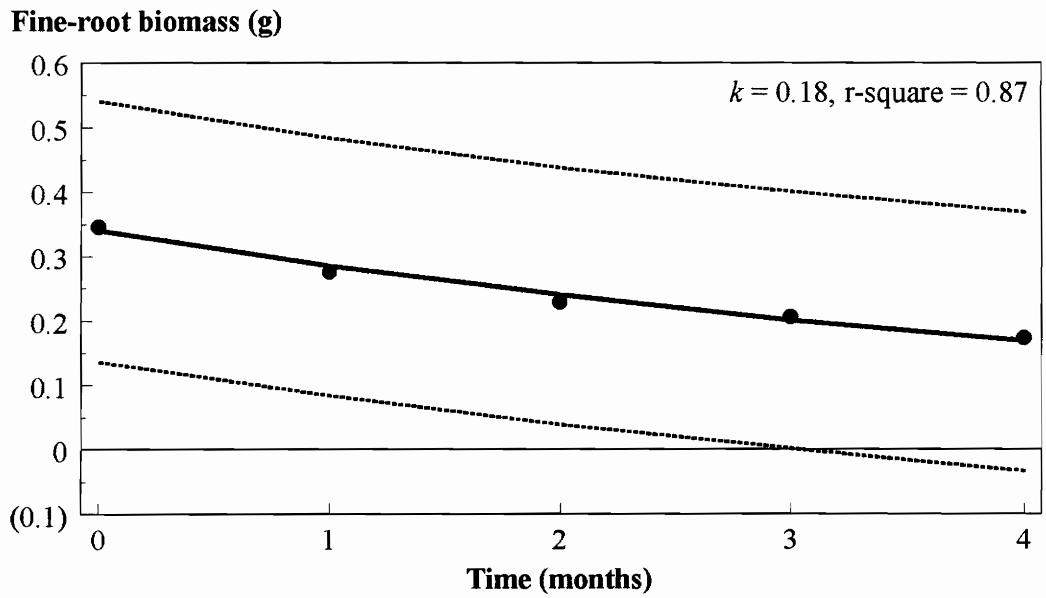


**Figure IV.1.** Results of *in situ* coring method showing nonlinear fit of data. Each point is the mean combining both sampling periods (n = 18 per point). The dashed lines represent upper and lower 95% confidence limits for the predicted values.

**Table IV.4.** Comparison between *in situ* and buried bag decomposition rates.

| Interval | Method: *In situ* | Method: Buried bag | p-value‡ |
|---|---|---|---|
| | ------- decomposition rate ($k$ $mo^{-1}$) ------- | | |
| Fall | 0.21 (0.03)§ | 0.06 (0.01) | |
| Winter | 0.16 (0.02) | 0.05 (0.02) | |
| p-value† | 0.183 | 0.683 | |
| Average | 0.18 (0.02) | 0.06 (0.01) | < 0.001 |

† Significance test between decomposition rates within methods using ANOVA and dummy variable (n = 18 per method).

‡ Significance test between average decomposition rates using the paired t-test (n = 36).

§ Values in parenthesis are standard errors.

**Table IV.5.** Summary table of mean decomposition rates by method, cover type, and diameter class.

| | Method | | | |
|---|---|---|---|---|
| | *In situ* | | Buried bag | |
| | $k$ yr$^{-1}$ | n | $k$ yr$^{-1}$ | n |
| Cover type | | | | |
| Mixed hardwoods | 1.53 | 1 | 0.41 | 11 |
| Spruce | 1.14 | 2 | 0.20 | 1 |
| Loblolly pine | 0.39 | 6 | 0.48 | 2 |
| Northern hardwoods | | | 0.57 | 2 |
| Northern pines | | | 0.46 | 11 |
| Diameter class | | | | |
| Fine | 1.15 | 3 | 0.36 | 6 |
| Fine - medium | 0.65 | 3 | 0.53 | 16 |
| Medium | | | 0.21 | 1 |
| Fine - coarse | 0.26 | 3 | 0.38 | 1 |
| Coarse | | | 0.22 | 3 |

## CHAPTER V. OZONE EFFECTS ON RED OAK ROOT DYNAMICS: SEEDLING VERSUS MATURE TREE COMPARISONS[1]

(ABSTRACT)

Previous research suggests that ozone pollution may cause forest decline; however, little is known about the mechanisms of damage, particularly with respect to belowground processes. It was hypothesized that carbon reallocation to replace tissues damaged by ozone would decrease fine-root production and turnover. Red oak seedlings and mature trees were exposed to three ozone treatments (subambient, ambient, 2X ambient) in open-top chambers. Net fine-root production and turnover were measured using the compartment-flow model. Mature-tree fine-root respiration was measured in summer 1994 in order to calculate gross fine-root production and belowground carbon use efficiency (BUE). After two seasons of exposure, 2X ozone (0.082 ppm 7hr-mean conc.) reduced mature tree cumulative net fine-root production and turnover by 31 and 41%, respectively, relative to ambient ozone (0.042 ppm 7hr-mean conc.). For the same time period, ozone had no effect on seedling cumulative fine-root turnover; fine-root production was 25% higher under ambient ozone, relative to subambient and 2X ambient ozone. During the summer, 1994, mature tree BUE was reduced by 2X ozone; we hypothesized that this response was due to increased root respiration for active nutrient uptake in order to meet increased foliar demand for nutrients. Decreased fine-root production, turnover, and BUE under 2X ozone for the

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mature trees indicates that ozone can alter the dynamics of belowground carbon allocation in mature red oak. Since the seedlings were not sensitive to ozone, use of seedling results for modelling purposes may underestimate mature-tree responses to ozone.

## INTRODUCTION

Increased atmospheric pollution resulting from industrialization, urbanization, and population growth may play a role in the decline of several forest ecosystems (McLaughlin, 1985). In several cases, reductions in tree growth rates have been correlated with increased levels of atmospheric pollutants (Norby and Kozlowski, 1981; McLaughlin et al., 1982; Puckett, 1982; Lorenc-Plucinska, 1984; Freer-Smith, 1985). Analysis of global climate data suggests that concentrations of atmospheric gases that are known to impact forest health; i.e., ozone, methane, nitrous oxide, and others, will double within the next century (Keeling, 1984; Freidli et al., 1986). Therefore, the prospect of forest decline should be a concern to the scientific community and the public at large.

Of the primary pollutants, ozone perhaps has the greatest impact on the health of forest trees (Reich, 1987). Ozone pollution is everywhere in the industrialized world; within a region, ozone concentrations in rural forested areas can exceed concentrations in urban areas (Meagher et al., 1987). Little work has been published concerning ozone impacts on natural forests; however, in one well documented case in southern

California, ozone was shown to be the cause of forest ecosystem decline (Miller, 1984). The degrading effects of ozone on seedling health have been well documented in controlled studies (Schafer and Heagle, 1989; Adams et al., 1990; Spence et al., 1990; Anderson et al., 1991; Tjoelker and Luxmoore, 1991; Edwards et al., 1991abc), and several review articles have been published (eg. Cooley and Manning, 1987; Reich, 1987; Pye, 1988). Specifically, ozone has been shown to decrease photosynthetic rates, decrease stomatal conductance, alter patterns of carbon allocation, and change root:shoot ratios for several species of tree seedlings.

Results from several of these ozone exposure studies on seedlings have been used to parameterize and quantify mechanistic tree growth models. These models are used for predicting long-term mature tree responses to ozone pollution (eg. Weinstein and Beloin, 1990). The validity of extrapolating seedling responses to mature trees is questionable given that there are several physiological differences between seedlings and mature trees. Seedlings and mature trees differ in percent photosynthetically active tissue, carbon allocation patterns, and their ability to exploit soil resources (eg. water and nutrients)(Pye, 1988). In order to properly parameterize tree growth models, differences between seedling and mature-tree responses to ozone must be quantified.

Although a significant amount of work has been done on seedling responses to ozone, very few studies have attempted to quantify the effects of ozone on belowground processes. Carbon allocation belowground for fine-root production can account for up to 70% of net primary production in forest trees (Grier et al., 1981;

Keyes and Grier, 1981). Ozone can reduce root:shoot ratios (eg. Woodbury et al., 1994), indicating that ozone affects belowground carbon allocation; this response is most likely explained by reallocation of carbon aboveground to replace foliage damaged by ozone (Pye, 1988). If ozone reduces belowground carbon allocation, then fine-root production should decrease; this could negatively impact tree growth because less root surface area would be available for nutrient and water uptake.

Traditionally, fine-root production studies have only included biomass production (ie. net production). Few studies have attempted to quantify gross fine-root production, or net production plus carbon respired. Gross fine root production is the value of interest because it represents total carbon allocated belowground for production of fine roots. The ability to partition carbon between respiration and biomass would greatly increase our understanding of ozone effects on whole tree carbon budgets, as well as provide much needed data for parameterizing mechanistic models. Inherent difficulties in measuring fine-root respiration have prevented researchers from quantifying this component in the past. However, if the objective is to determine ozone effects on whole tree processes, then fine-root respiration must be quantified.

In order to further our understanding of ozone impacts on tree health, a long-term (3yr) ozone fumigation experiment on mature trees and seedlings was conducted. The objectives of this experiment were: (i) to quantify ozone effects on net fine-root production and turnover, (ii) determine ozone effects on gross fine-root production and belowground carbon use efficiency, and (iii) identify similarities and differences between seedling and mature tree belowground responses to ozone. In addition, an overall objective was to provide experimental data for use in improving forest decline predictions generated by process-based models.

## MATERIALS AND METHODS

### Study site

The study was conducted in a thirty-year-old northern red oak (*Quercus rubra* L.) seed orchard located on the Tennessee Valley Authority Reservation in Norris, TN. Nine trees were selected at random and enclosed in large open-top chambers. Three fumigation treatments were applied to the chambers; subambient, ambient, and twice ambient ozone concentrations, in a randomized complete block design consisting of three blocks of three treatments. Blocking was done to account for variation in solar radiation observed on the site. A detailed description of the ozone delivery and monitoring system was given by Edwards et al., 1994.

Thirty two-year-old potted seedlings grown from acorns from the mature trees were placed in one of nine small open-top chambers. The seedling chambers were subjected to the same ozone treatments as the mature trees using the same experimental design.

The ozone fumigation experiment began in April 1992 and finished in September 1994, thus covering three growing seasons. The treatments were only applied during the foliar growing season each year, and were shut down in the winter.

## Fine-root production and turnover

The compartment-flow model (Santantonio and Grace, 1987) was used to calculate net fine-root (<2mm) production and turnover. The model uses a differential equation to calculate fine-root turnover based on direct measurements of the standing crops of live (LFR) and dead fine roots (DFR) and the decomposition rate ($k$). This method is based on the following basic relationships

$$\mathrm{LFR}_{t+1} = \mathrm{LFR}_t + NP - T \qquad [1]$$

$$\mathrm{DFR}_{t+1} = \mathrm{DFR}_t + T - D \qquad [2]$$

where $NP$ is net production, $T$ is turnover, and $D$ is decomposition. Rearranging eq.1 to solve for net fine-root production gives

$$NP_j = \Delta\mathrm{LFR} + T_j \qquad [3]$$

where the subscript $j$ refers to the time interval $t$ to $t + 1$. Rearranging eq.2 to solve for decomposition gives

$$D_j = \Delta\mathrm{DFR} + T_j. \qquad [4]$$

The only unknown left is fine-root turnover ($T$) which is solved for using the Santantonio and Grace (1987) equation,

$$T_j = [\ k_j\ t_j\ (\mathrm{DFR}_{t+1} - \mathrm{DFR}_t \exp(-\ k_j\ t_j\ )\ ]\ /\ [\ 1 - \exp(\ -\ k_j\ t_j\ )\ ], \qquad [5]$$

where $k_j$ is a decay coefficient driven by soil temperature ($ST_j$) for time interval ($j$):

$$k_j = k_{\mathrm{ref}} \exp[\ q\ (\ ST_j - ST_{\mathrm{ref}}\ )\ ], \qquad [6]$$

where $q$ is a temperature coefficient determined from two reference rates of fine root decomposition with different mean soil temperatures:

$$q = [\ \ln k_1 - \ln k_2\ ]\ /\ (\ ST_1 - ST_2\ ). \qquad [7]$$

Therefore, in order to calculate net fine-root production and fine-root turnover, it was necessary to measure live and dead fine-root biomass, fine-root decomposition, and soil temperature.

**Fine-root biomass**

Live and dead fine-root biomass were determined using sequential coring. Sixteen soil samples (3.8cm diameter by 30.5cm deep) were randomly removed from each large chamber and composited. Two (1.9cm diameter by 30.5cm deep) soil samples were removed from eight seedling pots per small chamber and composited. Soil samples were taken from both the large and small chambers at several points in time throughout the study.

Roots were separated from the soil using the hydropneumatic elutriation system (Smucker et al., 1982). Live and dead roots were distinguished based on the visual and mechanical criteria of Santantonio and Hermann (1985). The live and dead roots were divided into coarse (>2mm) and fine (<2mm) root size classes. The samples were oven-dried at 65°C for 48hr and weighed. Sample weights were then corrected for adhering soil by ashing them at 500°C and weighing the remaining material; this weight was subtracted from the oven-dried weight to obtain soil-free oven-dried weight.

**Fine-root decomposition**

Fine-root decomposition was determined for fall and winter, in 1993, using an *in situ* coring technique. Eighty 5.1cm by 30cm long galvanized steel tubes (2-inch electrical conduit) were driven into the soil profile in each chamber using an electric jackhammer. The pipes cut the roots and isolated them from the rest of the soil with a minimum of disturbance to the soil profile; this allowed the roots to decompose under natural soil conditions. Eight pipes per chamber were extracted and the soil was removed.

Roots were separated from the soil using the hydropnuematic elutriation system. The roots were divided into the two size classes and soil-free oven-dried weights were determined as previously described. On the first of each month thereafter, eight of the remaining soil columns were removed from each chamber. The roots were extracted and weighed as previously described.

The data were then fit to a first-order kinetic model

$$FR_r = FR_i \exp(-k\,t) \qquad [8]$$

where $FR_r$ is fine-root biomass remaining at time $t$ (mo), $FR_i$ is initial fine-root biomass, and $k$ is the decomposition rate constant ($mo^{-1}$). Soil temperature was automatically recorded in the large and small chambers on an hourly basis; using these data, mean soil temperature for each time interval was determined. The temperature coefficient $q$ was then calculated using eq.7.

**Root respiration and gross fine-root production**

Live fine-root respiration (*u*mol $CO_2$ $m^{-2}$ $s^{-1}$) was measured 3 times in conjunction with fine-root biomass sampling during the 1994 growing season. A modified infrared gas analyzer (IRGA) (LI-6200, Li-Cor, Inc., Lincoln, NE, USA) technique was used to measure $CO_2$ efflux from excised live fine roots. The live fine roots from the soil samples collected for determining fine-root biomass were used to measure root respiration. Soil samples were removed from each chamber and placed in glass baking dishes. Live fine roots were immediately hand separated from the soil samples and cleaned of adhering soil with deionized water. The roots were patted dry with paper towel and placed in a cuvette attached to the IRGA for $CO_2$ measurements. Total time elapsed from removing the samples to completing the IRGA measurements never exceeded 10min. We assumed that root respiration would not change significantly in this short period of time.

After measuring live fine-root respiration, the samples were dried at 65°C for determination of oven-dried weights. Live fine-root respiration rates were then converted from *u*mol $CO_2$ $m^{-2}$ $s^{-1}$ to mg $CO_2$ g-root$^{-1}$ day$^{-1}$; this was based on the assumption that root respiration remained constant.

Live fine-root respiration (*LFRR*) was calculated for each sampling interval for summer 1994 based on the assumption that ΔLFR and Δ*LFRR* were linear between sampling dates. Average live fine-root biomass ($(LFR_t + LFR_{t+1}) / 2$) was determined and multiplied by the average *LFRR* ($(LFRR_t + LFRR_{t+1}) / 2$) for the same interval. Gross fine-root production ($GP$) was then calculated as

$$GP_j = NP_j + LFRR_j \qquad [9]$$

with $LFRR$ only including the carbon component of respiration.

An evaluation of the effect of ozone on belowground carbon use efficiency ($BUE$) was conducted.

$$BUE_j = NP_j \ / \ GP_j \qquad [10]$$

where $BUE$ equals the ratio of net fine-root production ($NP$) to gross fine root production ($GP$). The $BUE$ is an index of the plants' efficiency at producing fine root biomass; ie., a plant with a high $BUE$ would be producing fine-root biomass at a lower respiration cost than a plant with a low $BUE$.

**Total soil respiration**

Total soil respiration was measured in conjunction with root respiration using an identical IRGA coupled with a soil respiration chamber (LI-6000-09, Li-Cor, Inc., Lincoln, NE, USA). Two soil respiration measurements were taken from four locations in each chamber; fine-root samples were removed from the same locations for biomass and respiration measurements.

After placing the soil respiration chamber in contact with the soil surface, the IRGA was turned on and the $CO_2$ in the chamber was drawn below ambient. The $CO_2$ level was monitored for several seconds until the system came into equilibrium; at this point, a 30 second reading was taken. If the reading did not bracket the ambient $CO_2$

level, then successive readings were taken until the ambient level was bracketed; also if the bracket was skewed, then new readings were taken. This procedure minimized any potential errors associated with chamber leakage.

### Statistical analysis

All belowground data were averaged by chamber, and the chamber was treated as the experimental unit. Two-way analysis of variance (ANOVA) with three blocks and three ozone treatments was used to test the effect of ozone on within-year net fine-root production and turnover, and cumulative net fine-root production and turnover. ANOVA was also used to test for ozone effects on gross fine-root production and belowground use efficiency for summer 1994. In all analyses, when treatment effects were significant ($\alpha$=0.10), Duncan's multiple range test was used for means separation.

## RESULTS AND DISCUSSION

### Fine-root production and turnover

Ozone treatments did not affect mature tree net fine-root production or turnover in 1992, the first year of exposure (Fig V.1a). 2X ozone reduced net fine-root production of mature trees in the spring by 47% in 1993, relative to ambient ozone. In 1992, net fine-root production and turnover were highest under 2X ozone for the seedlings (Fig V.1b). Seedling net fine-root production and turnover under subambient ozone were out of phase in 1993 compared to the other treatments. Mature tree net fine-root production peaked in early spring, and seedling net fine-root production peaked in mid-summer.

Hogsett et al. (1989) found that ozone can reduce root growth more than shoot growth following one season of exposure, indicating that ozone can reduce belowground carbon allocation. This was supported by Anderson et al. (1991), who exposed ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) seedlings to 122 and 169 ppm-h ozone dose for one growing season; spring root starch reserves were reduced by 43%, resulting in an approximate decrease of 60% in spring root growth. Therefore, the reduction in root production of mature red oak in spring under 2X ozone observed in this study was most likely a result of lower levels of stored carbohydrates. The lack of synchrony in root production and turnover among treatments indicates that ozone altered patterns of carbon allocation in the seedlings.

Following two growing seasons of exposure, 2X ozone (0.082 ppm 7hr-mean conc.) reduced mature-tree cumulative net fine-root production and turnover by 31% and 41%, respectively, relative to ambient ozone (0.043 ppm 7hr-mean conc.) (Fig V.2a). Seedling cumulative net fine-root production (350 g $m^{-2}$ ) was highest under ambient ozone, compared to subambient (0.018 ppm 7hr-mean conc.) and 2X ambient ozone (230 and 280 g $m^{-2}$, respectively) (Fig V.2b). Ozone did not affect cumulative seedling fine-root turnover. Net fine-root production occurred into the winter for the seedlings, and ceased in the fall for the mature trees. The seedlings and trees produced similar total amounts of fine-root biomass (275 g $m^{-2}$); however, the mature trees turned over 36% more biomass than the seedlings.

Corraborating our results, Hanson et al. (1994), found that the mature red oak tree photosynthetic rates and leaf conductance rates were reduced by approximately 50% by the 2X ambient treatment, yet seedling photosynthesis and conductance were unaffected. Furthermore, Samuelson and Edwards (1993), found 2X ozone reduced carboxylation efficiency and leaf dry weight of the mature trees, but the seedlings again remained unaffected. A separate study found that ozone (0.079 ppm 7hr-mean conc.) did not affect northern red oak seedling growth compared to a charcoal-filtered control (0.024 ppm 7hr-mean conc.) (Reich et al., 1986). This agrees with the results from our study which was conducted with similar ozone concentrations and same age northern red oak seedling. Seedling lack of sensitivity to ozone compared with mature trees may be explained by canopy leaf position (Samuelson and Edwards, 1993), differences in foliar anatomy and stomatal response (Hanson et al., 1994), or acclimation ability.

The ratios of cumulative net fine-root production to cumulative fine-root turnover were compared (Table V.1). This ratio indicates if the tree is: a) aggrading roots, $P/T > 1$, b) steady state, $P/T = 1$, or c) loosing roots, $P/T < 1$. It was hypothesized that higher ozone exposures would result in higher P/T ratios given that total production was less with higher exposure (i.e., greater root retention). However, ozone had no significant effect on the P/T ratios of either trees or seedlings. The mature trees had lower P/T ratios than the seedlings, indicating that red oak seedlings retain a greater percentage of carbon in live fine-root biomass than mature trees.

**Fine-root and soil respiration**

Ozone exposure had no significant effect on mature-tree fine-root respiration or total soil respiration during the summer, 1994 (Table V.2). This finding contrasts with data reported by Edwards (1991) for loblolly pine (*Pinus taeda* L.) trees that showed a 12% reduction in root respiration rates in response to elevated ozone; this supports the hypothesis that species may differ in their response to ozone. Fine-root respiration ranged from 30 to 45% of total soil respiration; this agrees well with other studies showing that root respiration is approximately 40% of total soil respiration in oak forests (Paul and Clark, 1989). The results from our study showed no clear relationship between fine-root respiration and total soil respiration (Table V.2); this finding emphasizes the need to use caution when attempting to quantify belowground tree response to ozone based solely on total soil respiration measurements.

**Primary production and carbon use efficiency**

In the summer 1994, net fine-root production was greatest under ambient ozone (166 g $m^{-2}$, 0.039 ppm 7hr-mean conc.), compared to subambient (96 g $m^{-2}$, 0.012 ppm 7hr-mean conc.) and 2X ambient (122 g $m^{-2}$, 0.076 ppm 7hr-mean conc.)(Table V.3). The reduction in net fine-root production under 2X ozone observed in summer, 1994, is consistent with the results through 1993; however, the reduction in net fine-root production under subambient ozone for summer 1994 is in contrast with the cumulative effects through 1993. Research with annual crops has shown that plants can mitigate ozone damage by increasing the amount of carbon allocated to foliage production (Oshima et al., 1979); therefore, the reduction in net fine-root production observed under 2X ozone is most likely explained by reallocation of carbon from belowground to aboveground in order to replace foliage damaged by ozone. We feel that the negative effect of subambient ozone on net fine-root production is an artifact of charcoal-filtering; an explanation for this is offered below under the subheading 'Procedural Findings'.

The mature trees under ambient ozone had the highest belowground use efficiency (Table V.3), indicating that these trees are respiring less carbon per unit of biomass produced than either the subambient or 2X ambient trees. Lower belowground use efficiency under 2X ozone indicates that 2X ozone reduced the efficiency of fine-root biomass production. Increased respiration relative to production under 2X ozone could be a direct function of ozone damage to foliage. Foliage controls active root uptake of nutrients through regulating the flow of carbon used for root respiration; in addition, hormones synthesized in foliage play a significant role in regulating nutrient uptake (Läuchli, 1984). An explanation for the increased proportion of root respiration under 2X ozone is that replacement of foliage damaged by ozone increased the aboveground demand for nutrients. In order to meet this demand, hormones synthesized by foliage signaled the roots to increase active uptake of nutrients required to rebuild foliage; in order for active uptake to increase, a greater proportion of carbon allocated belowground was used for respiration. Another possible explanation for increased respiration under 2X ozone is that the reduction in carbon supplied belowground resulted in decreased root vigor, making the roots more susceptible to attack; respiration may have increased in order to repair root tissues damaged by attacking organisms.

Respiration accounted for approximately 40% of gross fine-root production for the subambient and 2X ambient trees; the 40% respiration value is commonly used in process models that simulate tree response to ozone (eg. Weinstein and Beloin, 1990). Our results indicate that under ambient ozone levels, red oak tree roots respire approximately 30% of the carbon allocated for fine-root production, thus it appears that the 40% respiration value used in process models overestimates root respiration. Overestimating root respiration would cause process models to underestimate net fine-root production.

**Procedural findings**

We hypothesized that the subambient ozone treatment would positively impact belowground processes relative to the ambient ozone treatment; therefore, the negative response of all measured belowground parameters for the subambient treatment was unexpected. Other studies have reported growth stimulation in response to low concentrations of ozone (Kress and Skelly, 1982); however, these seemingly contradictory responses have yet to be explained. One possible explanation for the lower subambient values, compared to ambient values, is that charcoal-filtering negatively affected air quality in the process of filtering out ambient ozone. For example, nitrogen dioxide, a compound that would be excluded by charcoal-filtering, may negate the deleterious effects of ozone on tree growth (Kress, 1979). Therefore, the use of charcoal-filters to reduce ozone levels below ambient may serve to confound ozone fumigation experiments, thus leading to uninterpretable results. Another possible explanation is the trees are acclimated to ambient ozone, and reducing ozone below this level causes damage.

The results of this study underscore the importance of, and problems with, multiple growing season experiments with treatments based on fluctuating ambient air quality. Ozone did affect production and turnover for both the seedlings and mature trees at several points in time (Fig V.1); however, cumulative fine-root turnover for seedlings was not affected by elevated levels of ozone (Fig V.2). If this study were terminated after one growing season, then we would have concluded that 2X ozone increased seedling net fine-root production and turnover, and ozone has no effect on mature tree net fine-root production and turnover. This observation emphasizes the importance of using repeated measurements for adequate lengths of time, and using a number of response variables to ascertain the effects of air quality on plants.

## CONCLUSIONS

The results of this study showed that doubling the ambient ozone concentration from 0.043 ppm (7hr-mean) to 0.082 ppm (7hr-mean) reduced net fine-root production and turnover in mature northern red oak trees. This indicated that carbon allocation patterns were altered by elevated ozone, and probably more carbon was being allocated aboveground than belowground. In addition, the 1994 data suggested that ozone affects the efficiency of carbon utilization (BUE) in mature trees; the data also suggest that root respiration under 2X ozone may have increased in response to higher nutrient demands for foliage production. The lack of a seedling response to 2X ozone indicates that red oak seedlings are not as sensitive to elevated ozone as mature trees. The implication of this result is that extrapolating seedling results to mature trees for modelling purposes may underestimate mature tree responses to ozone.

**Table V.1.** Ozone effects on mature tree and seedling cummulative net fine-root production, turnover, and the production/turnover ratio (P/T). Values within columns followed by different letters are significantly different at alpha = 0.10.

| | Production ($g\ m^{-2}$) | Turnover ($g\ m^{-2}$) | P/T |
|---|---|---|---|
| Mature trees | | | |
| Subambient | 241a | 161ab | 1.5 |
| Ambient | 261a | 206a | 1.3 |
| 2X ambient | 183b | 118b | 1.6 |
| Seedlings | | | |
| Subambient | 234b | 111 | 2.1 |
| Ambient | 348a | 110 | 3.3 |
| 2X ambient | 277ab | 106 | 2.6 |

**Table V.2.** Root biomass, root respiration, and total soil respiration rates for mature northern red oak trees exposed to subambient, ambient, and 2X ambient ozone levels during the summer 1994.

| Treatment | Fine-root biomass ($g\ m^{-2}$) | $CO_2$ efflux rates ($mg\ m^{-2}\ hr^{-1}$) Fine-roots | $CO_2$ efflux rates ($mg\ m^{-2}\ hr^{-1}$) Total soil |
|---|---|---|---|
| May | | | |
| Subambient | 115 | 81 | 193 |
| Ambient | 133 | 97 | 219 |
| 2X ambient | 97 | 98 | 266 |
| July | | | |
| Subambient | 157 | 136 | 536 |
| Ambient | 177 | 125 | 539 |
| 2X ambient | 138 | 192 | 417 |
| September | | | |
| Subambient | 184 | 106 | 345 |
| Ambient | 270 | 146 | 278 |
| 2X ambient | 198 | 170 | 293 |

**Table V.3.** Ozone effects on mature tree net fine-root production (NP), gross fine-root production (GP), and belowground use efficiency (BUE) for summer 1994. Values within columns followed by different letters are significantly different at alpha = 0.10.

| Treatment | NP ($g\ m^{-2}$) | GP ($g\ m^{-2}$) | BUE |
|---|---|---|---|
| Subambient | 96a | 154b | 0.62a |
| Ambient | 166b | 230a | 0.73b |
| 2X ambient | 122a | 209ab | 0.58a |

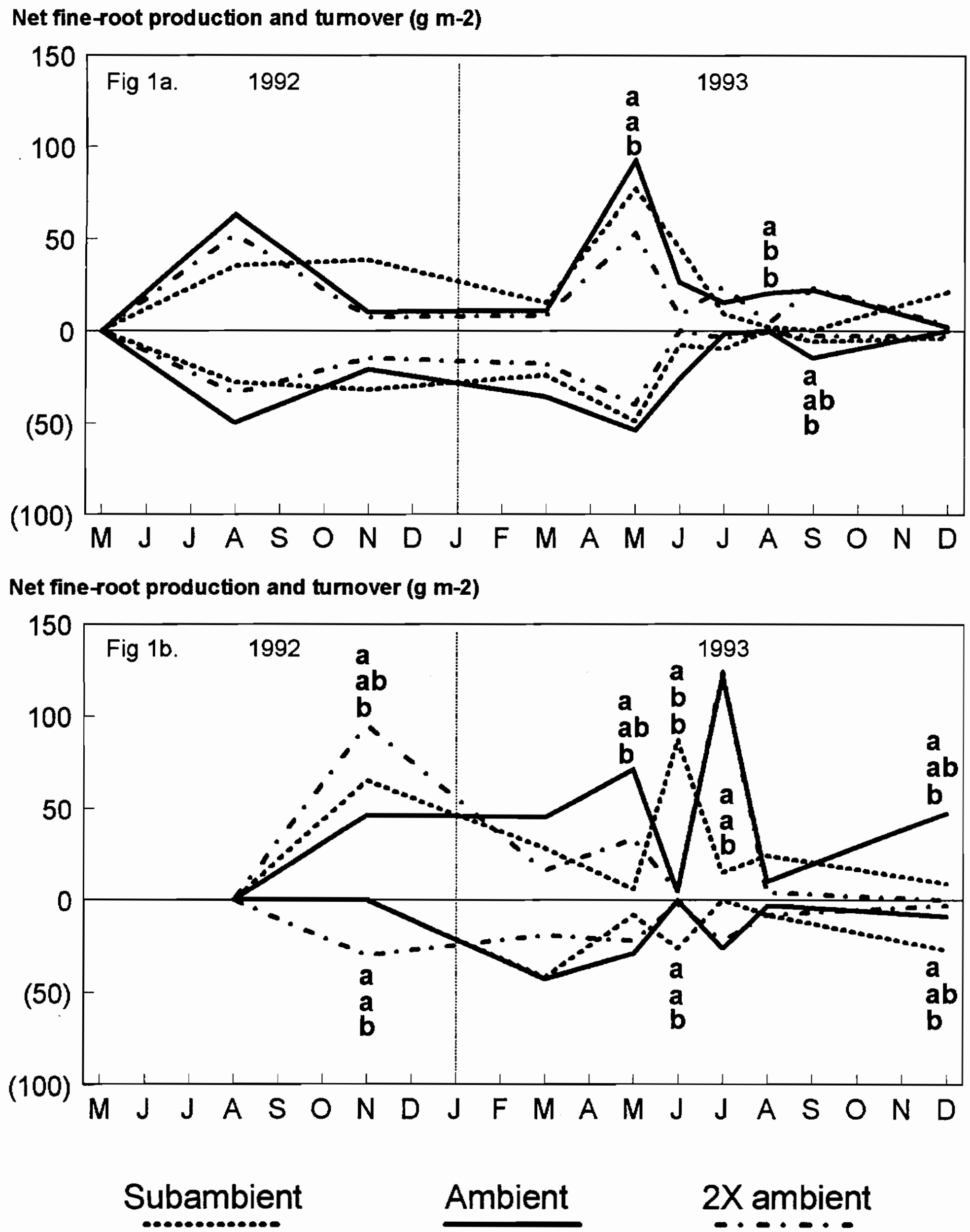


**Figure V.1.** Periodic net fine-root production and turnover for the 32 year-old trees (Fig 1a.) and 2 year-old seedlings (Fig 1b.). Production is shown above the zero line and turnover is shown below the zero line. Production and turnover values within months followed by different letters are significantly different at alpha = 0.10.

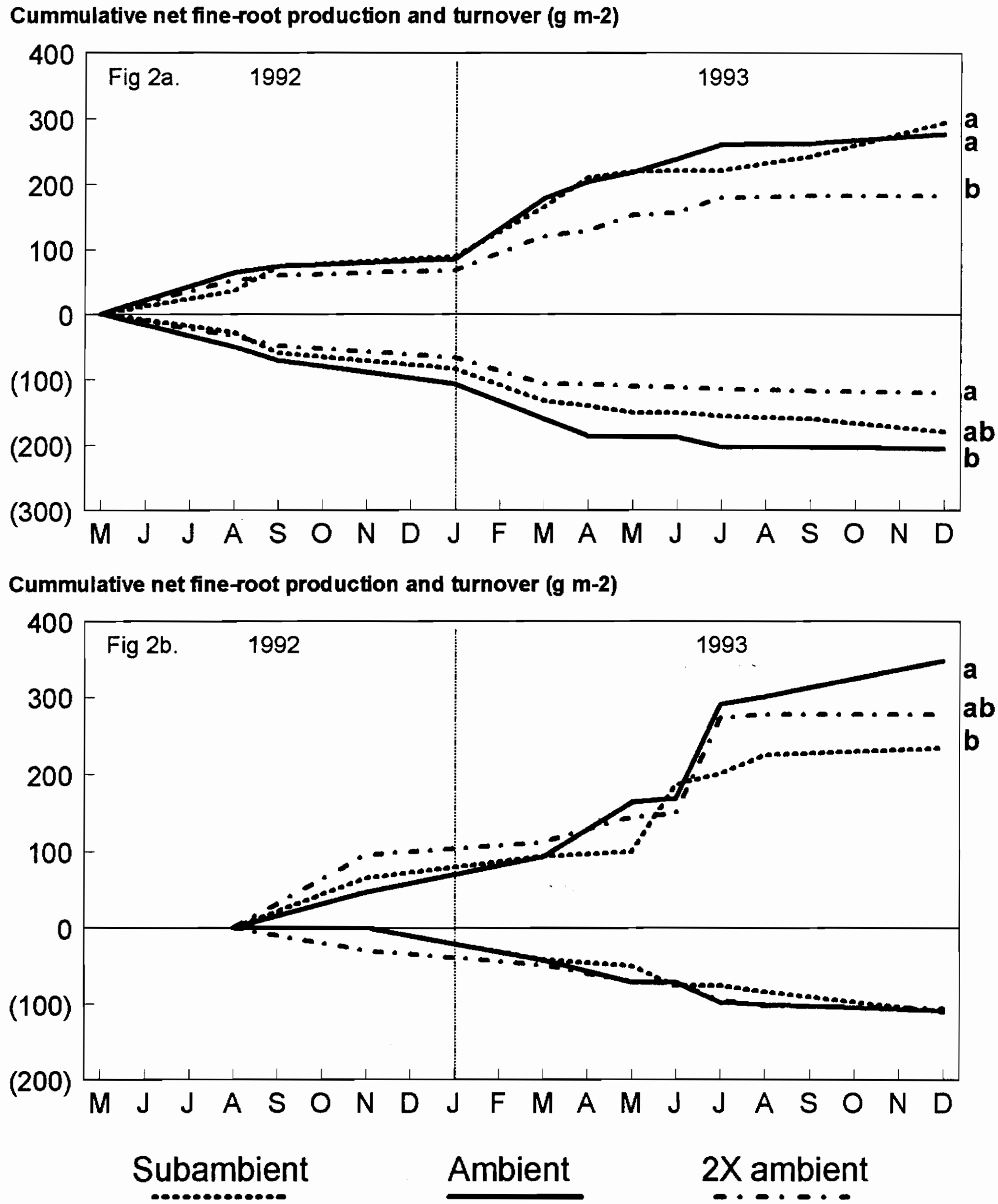


**Figure V.2.** Cummulative net fine-root production and turnover for the 32 year-old trees (Fig 2a.) and two year-old seedlings (Fig 2b.). Production is shown above the zero line and turnover is shown below the zero line. Cummulative production and turnover values followed by different letters are significantly different at alpha = 0.10.

# CHAPTER VI. FRACTIONATING TOTAL SOIL RESPIRATION INTO ROOT, RHIZO-MICROBIAL, AND NON-RHIZO-MICROBIAL RESPIRATION COMPONENTS[1]


(ABSTRACT)

Total soil respiration can be separated into three components: root, rhizo-microbial, and non-rhizo-microbial respiration. Fractionating respiration into these components is important because they are functionally different with respect to their carbon source. Unfortunately, it is difficult to measure these components, and confidence in existing methods is not high. Using a combination of the basal and excised-root methods, an effort was made to separate the components of soil respiration in a controlled study. We hypothesized that combining the basal and excised-root respiration methods would allow us to quantify three components of soil respiration: root respiration ($R_{root}$), rhizo-microbial respiration ($R_{rhizo}$), and non-rhizo-microbial respiration ($R_{micro}$). Root respiration determined by the basal method was approximately one-third greater than root respiration determined by the excised-root method (52 versus 32% of total soil respiration, respectively). In order to explain this discrepancy, a decomposition model for the root carbon fractions (easily decomposable, slowly decomposable, and recalcitrant) was constructed using literature-derived data. Results from the model run showed that the easily and slowly decomposable carbon fractions disappeared approximately 3 months after the basal measurements began. Since these carbon fractions contained the rhizo-microbial



[1]Manuscript submitted to Soil Biology and Biochemistry in March 1995.

carbon source (ie. root exudates), rhizo-microbial respiration ceased, and respiration measured after 3 months was from non-rhizo-microbes ($R_{micro}$); this indicated that the basal method actually measured non-rhizo-microbial respiration. This allowed us to fractionate the components of soil respiration based on the relationship: total soil respiration ($R_{total}$) = $R_{root}$ + $R_{rhizo}$ + $R_{micro}$; subtracting $R_{root\ (excised\text{-}root\ method)}$ and $R_{micro\ (basal\ method)}$ from $R_{total}$ gave us an estimate of rhizo-microbial respiration; and the contribution of these three components to $R_{total}$: $R_{root}$ = 32%, $R_{rhizo}$ = 20%, and $R_{micro}$ = 48%. These results are important because they suggest a way that soil respiration can be separated into at least three functionally different components, and they show that rhizo-microbial respiration may represent a significant sink for photosynthetically-fixed carbon.

## INTRODUCTION

The interest in quantifying soil respiration in ecological studies is growing. Soil respiration measurements are used for developing ecosystem carbon budgets (Edwards, 1982), determining mineralization rates (Kelly and Strickland, 1984), and as an index of root respiration (Raich et al., 1990). However, soil respiration cannot be used to determine mineralization or root respiration without prior knowledge of the contribution of each of these components to soil respiration.

Soil respiration is generally divided into two compartments, root respiration and microbial respiration (Raich and Nadelhoffer, 1989) (Fig VI.1A). We suggest that the microbial respiration compartment be further divided into two functionally different compartments (a three compartment model) based on the carbon source used as the energy substrate (Fig VI.1C). Rhizo-microbial respiration (Cheng et al., 1993) is associated with microorganisms utilizing root exudates as their primary carbon source, while, non-rhizo-microbes use the soil organic matter pool as their carbon source. In making this distinction, our focus is not on specific populations of microoganisms, but rather on the carbon source used for energy; i.e., we realize that certain species of microorganisms may mineralize either rhizosphere or non-rhizosphere derived organic matter.

Several studies have quantified the carbon cost of mycorrhizae (Reid et al., 1983; Marshall and Perry, 1987); however, in most forest-carbon budget studies, the rhizo-microbial component of belowground processes has been largely ignored. Researchers have focused on carbon loss from tree root systems via fine-root turnover and root respiration, and have not accounted for carbon loss through root exudation (carbon source for rhizo-microorganisms). This lack of accounting is due to inherent difficulties in separating root and rhizo-microbial respiration (Helal and Sauerbeck, 1991). Recent evidence suggests that root exudation rates may be up to 100 times greater than previously thought (Cheng et al., 1993), suggesting that root exudation may represent a significant and unaccounted for loss of photosynthetically-fixed carbon. This emphasizes the need for continued research into the interaction between rhizo-microorganisms and root exudates, as well as the need to develop reliable methods to measure these variables in forests.

The two compartment soil respiration model (Fig VI.1A and 1B) has been the basis for most methods developed to measure root and microbial respiration in forests. Three general methods exist for measuring root respiration: *in situ*, excised-root, and the basal method. *In situ* measurements involve excavating live roots without severing them, placing them in a cuvette, and measuring the respiration rate with an infrared gas analyzer (IRGA) (Sisson, 1983; Cropper and Gholz, 1991). In the excised-root method, soil core samples are taken and the roots are removed; root respiration is then measured either using the Warburg method (Steinbeck and McAlpine, 1966), or with an IRGA. Microbial respiration can then be calculated by subtracting root respiration from total soil respiration (Fig VI.1A). The basal method (Marshall and Perry, 1987) uses the two compartment soil respiration model directly. A volume of soil is isolated with a trenched plot and all aboveground biomass is removed. Total soil respiration is measured at regular intervals. As the freshly-severed root material decomposes, total soil respiration decreases, eventually reaching a horizontal asymptote. The respiration rate at this asymptote represents soil microbial respiration or 'basal' respiration. Root respiration is then obtained by subtracting the basal respiration rate from total soil respiration (Fig VI.1B).

Each of these methods have potential artifacts associated with them: the *in situ* method assumes that the cuvette does not alter environmental conditions that in part regulate respiration; the excised-root method assumes that severing the roots prior to inserting them into the IRGA does not change the respiration rate; and the basal method assumes that killing the roots will not affect microbial respiration. Considering the potential artifacts associated with each method, and the feasibility of measuring multiple samples, we believe that the excised-root method is the most reliable and practical for measuring root respiration. This contention is based on a study that showed that leaf gas-exchange does not change significantly for at least 15min after being severed from the stem (Samuelson and Edwards, 1993), indicating that root respiration should not change significantly if the measurements are made soon after severing.

A study was conducted in which the excised-root and basal respiration methods were compared. It was hypothesized that the basal method would overestimate root respiration through elimination of the rhizo-microbial respiration compartment. It was further hypothesized that the basal method could be used in conjunction with the excised-root method to partition respiration among three compartments (Fig VI.1C): root respiration ($R_{root}$) determined by the excised-root method, non-rhizo-microbial respiration ($R_{micro}$) determined by the basal respiration method, and rhizo-microbial respiration ($R_{rhizo}$) determined by subtracting the sum of $R_{root}$ and $R_{micro}$ from total soil respiration ($R_{total}$).

## MATERIALS AND METHODS

### Study layout

This project was a subset of an ozone-effects study on fine-root dynamics. In the ozone-effects study, nine mature northern red oak (*Quercus rubra* L.) trees, contained in open-top chambers, were exposed to different levels of ozone. The ozone study began in May 1992 and ended in October 1994. A detailed description of the study layout and experimental design was given by Edwards et al. (1994).

The respiration study was conducted during the summer of 1994. The basal and excised- root respiration methods were paired within each of the nine mature tree chambers, and each chamber was considered an experimental unit.

### Respiration Measurements

Total soil and excised-root respiration measurements were taken at the beginning of June using the methods described by Kelting et al. (in review). Basal respiration was determined using a modified trench plot technique. In each chamber, 40 steel cylinders (5.1 cm i.d. by 30 cm long) were driven into the soil profile using an electric jackhammer. Total soil respiration was then measured monthly, for five months, from eight cores per chamber using an infrared gas analyzer (LI-6200, Li-Cor, Inc., Lincoln, NE, USA) equipped with a soil respiration chamber (LI-6000-09, Li-Cor, Inc., Lincoln, NE, USA). Soil temperature was measured simultaneously with respiration. In order to insure that microbial activity was not limited by water, the cores were artificially watered in order to maintain the soil water potential above -0.5 MPa.

**Data analysis**

Prior to data analysis, the raw respiration data were normalized to the mean soil temperature, 19.8°C, using the $Q_{10}$ function. We varied the $Q_{10}$ value from 1.7 to 2.3, and observed no significant effect on the respiration data. This lack of sensitivity to the $Q_{10}$ values was due to the narrow range in soil temperature; the high was 22°C in August, and the low was 15°C in October. Based on this experimentation, we decided to use a $Q_{10}$ of 2; this value is frequently used to characterize soil microbial respiration (e.g. Zak et al. 1993). The temperature-normalized monthly data were then averaged by chamber, and standard errors were calculated based on these chamber averages (n=9).

**RESULTS AND DISCUSSION**

Root respiration determined via the excised-root method (0.962 mg $CO_2$ g-root$^{-1}$ hr$^{-1}$) was within the 0.4 to 3.8 mg $CO_2$ g-root$^{-1}$ hr$^{-1}$ range reported by Ledig et al. (1976). On an area basis, $R_{root}$ was 32% of $R_{total}$ using the excised-root measurements (Table VI.1). The basal respiration results suggest a horizontal asymptote after three months of decomposition (Fig VI.2); however, there were insufficient data points to fit a nonlinear equation, and the shape of the curve suggests something other than first-order kinetics decomposition. If we assume that the September rate represents the basal rate, then $R_{root}$ by the basal method would be approximately 52% of $R_{total}$ (Fig VI.1B), approximately one-third more than the $R_{root}$ % determined using the excised-root method. However, we argue that basal respiration measured at this point does not reflect the level of microbial activity when live roots are present, because depletion of rhizosphere-available carbon reduced the microbial population. The implication of this argument is that the basal method overestimates $R_{root}$ by the respiration amount associated with the rhizo-microbial population. If this argument is true, then rhizo-microbial respiration ($R_{rhizo}$) could be determined using the basal respiration method combined with an estimate of $R_{root}$ using the excised-root method:

$$R_{total} - R_{micro\ (by\ basal\ method)} = R_{root} + R_{rhizo} \quad [1]$$

$$R_{root} + R_{rhizo} - R_{root\ (by\ excised\text{-}root\ method)} = R_{rhizo}. \quad [2]$$

By combining these methods, respiration for the three components in this study was $R_{root}$ = 32%, $R_{rhizo}$ = 20%, and $R_{micro}$ = 48% of total soil respiration (Fig VI.1C).

Splining a curve through the data from the basal respiration experiment revealed some of the dynamics of soil respiration that appear to support the validity of the three compartment model (Fig VI.2). The three compartment soil respiration model (Fig VI.1C) was superimposed on Figure VI.2, in order to place the decomposition dynamics in the perspective of the model. Total soil respiration is depicted at 470 mg $CO_2$ $m^{-2}$ $hr^{-1}$ just prior to severing roots. The initial drop in soil respiration measured on the cores was attributed to a rapid drop in live-root respiration when the roots were severed. An increase in respiration occurred from July to August. Tree root exudation has been shown to occur for up to 79 days after severing the roots (Lopushinsky, 1980); therefore, a portion of the respiration that occurred from July to August is most likely from continued rhizo-microbial decomposition of root exudates. Increased respiration during this period probably resulted from an increase in the available carbon supply via root death; additions of available forms of organic matter stimulate microbial activity (eg. Katznelson and Rouatt, 1957). The reduction in respiration from August to September is attributed to a decreasing available carbon supply in the rhizosphere. The relatively level period from September to October indicates that rhizo-microbial activity has ceased, and the remaining respiration is attributed to non-rhizo-microbes using carbon supplied by more recalcitrant materials.

These interpretations of the data in Figure VI.2 are further corroborated by literature-derived data. A tree-root decomposition curve was constructed using published decomposition rates for easily decomposable (eg. proteins), slowly decomposable (eg. hemicellulose), and recalcitrant (eg. lignin) litter (0.2, 0.08, and 0.01 $k$ day$^{-1}$, respectively (Paul and Clark, 1989)), and average carbon fractions reported for woody tree roots (31% easily decomposable, 33% slowly decomposable, and 36% recalcitrant) (Fahey et al., 1988). A first-order kinetics model was then used to plot the decomposition curve for root biomass obtained in this study (Fig VI.3):

$$C_r = C_1 \exp^{-kt} + C_2 \exp^{-kt} + C_3 \exp^{-kt}, \qquad [3]$$

where root biomass remaining ($C_r$) equals the percent easily decomposable ($C_1$), slowly decomposable ($C_2$), and recalcitrant ($C_3$) carbon, with each component multiplied by their respective rate constant ($k$) times time (t).

Results from the model run showed that the easily decomposable carbon fraction ($C_1$) would disappear in about 25 days (Fig VI.3). This is the fraction that would contain the root exudates, thus it is expected that the root exudates would decompose within the first month. The slowly decomposable carbon fraction ($C_2$) disappeared in 55 days, leaving only the recalcitrant ($C_3$) fraction to provide energy for microbial activity. Microbial activity has been shown to peak at the point when the slowly decomposable fraction is depleted, with activity slowing when the recalcitrant fraction is left (Paul and Clark, 1989).

Some caution is prudent in applying these modelling results to our respiration results because it has been shown that roots can remain viable for some time after being artificially severed (Publicover and Vogt, 1993). However, the negative impact of severing the roots on soil respiration (Fig VI.2) indicates that root death began very soon after severing. Therefore, we assumed that the decomposition process began within the first month (i.e., June). The August peak in soil respiration (Fig VI.2) corresponds with the depletion of the slowly decomposable carbon fraction (Fig VI.3). Decreased soil respiration from this point is attributed to the remaining recalcitrant lignin fraction. Microbial activity should continue declining because the recalcitrant fraction is the only remaining carbon fraction. This modelling exercise helps explain the observed dynamics of the decomposition curve in Figure VI.2, and further corroborates our contention that our proposed method for partitioning a three-compartment respiration model has merit.

## CONCLUSIONS

The results of this study provide strong evidence to support the use of a combination of the excised-root respiration method and the basal respiration method to estimate three components of soil respiration. In this experiment, $R_{rhizo}$ accounted for 20% of $R_{total}$. These results are significant because the carbon source for this respiration component is functionally different from that of the other two components. This fact has largely been ignored in the past. Furthermore, partitioning this respiration component is important because it will alternatively be attributed in a two compartment model to root respiration or microbial respiration depending on which sampling methodology is used. The excised-root method alone combines $R_{rhizo}$ with $R_{micro}$, while the basal method alone combines $R_{rhizo}$ with $R_{root}$. The implication of this is that use of basal method $R_{root}$ values in forest carbon budget studies would overestimate the root respiration flux. However, the basal method can be used to estimate total belowground carbon allocation (i.e., $R_{rhizo}$ + $R_{root}$), which, in some studies, may be the more important value. Although further experimentation is needed to further quantify and test this hypothesis under other conditions and with other species, the test of the decomposition dynamics hypothesis using literature-derived data further corroborates our contention that rhizo-microbial respiration plays a significant and functionally-distinct role in overall soil respiration.

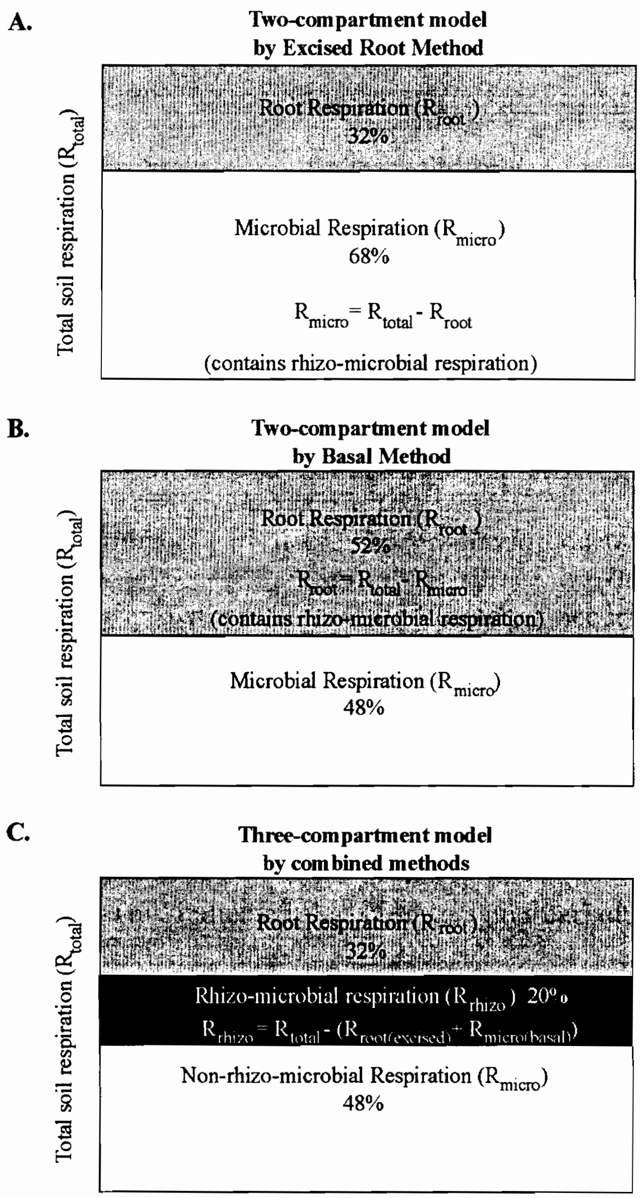


**Figure VI.1.** Comparisons of a two-compartment respiration model derived by the excised-root method (Fig 1A), a two-compartment model derived by the basal method (Fig 1B), and a three-compartment model derived by a combination of the two methods (Fig 1C).

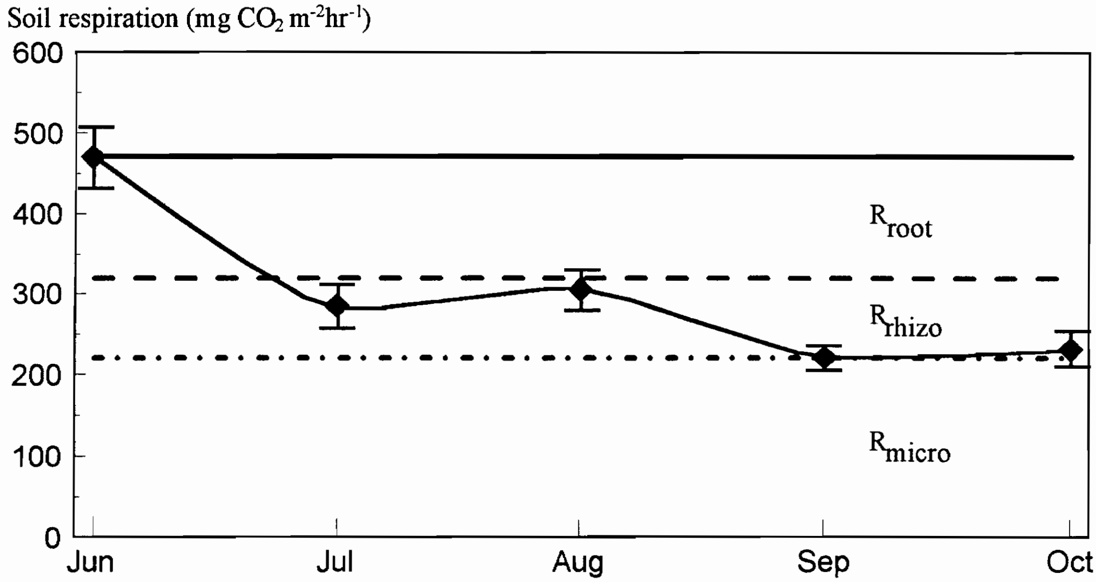


**Figure VI.2.** Monthly total soil respiration measured from the *in situ* basal method cores during the summer 1994. Each sample point is the mean of nine chambers, and a spline curve connects the points. Initial total soil respiration (solid line), total soil respiration minus root respiration calculated via excised-root method (dashed line), and 'basal' microbial respiration (dash-dot line) are superimposed on the graph.

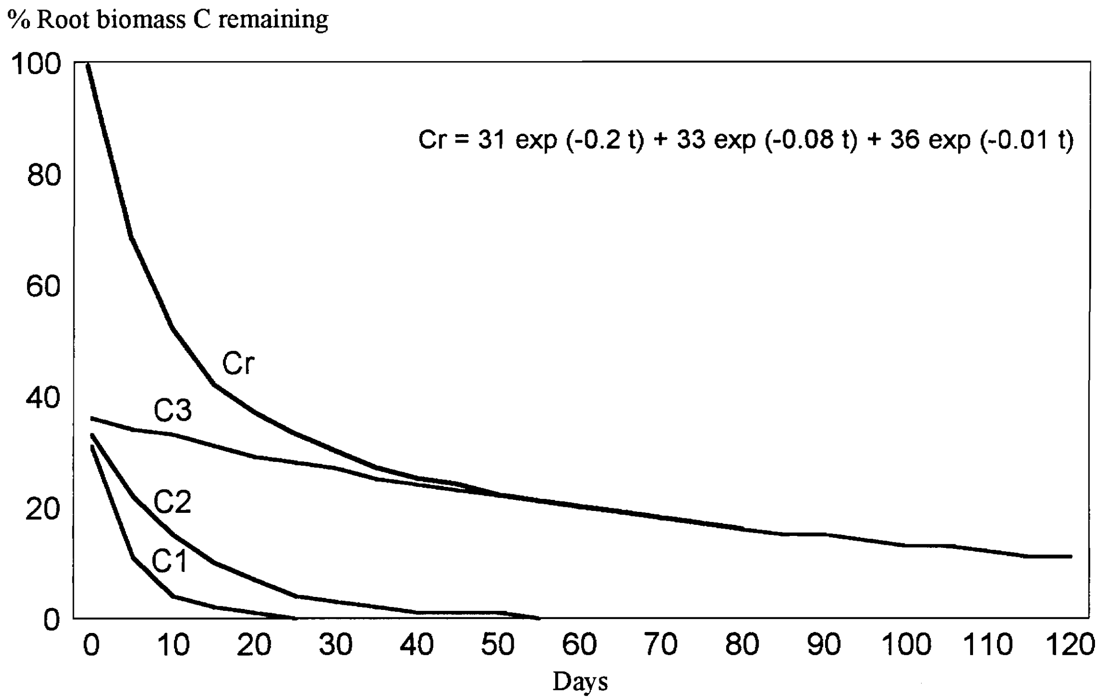


**Figure VI.3.** Decomposition of root biomass using a first-order kinetic model and literature- derived rate constants and carbon fractions. Cr is root biomass decomposition, C1 is decomposition of the easily decomposable fraction, C2 is decomposition of the slowly decomposable fraction, and C3 is decomposition of the recalcitrate fraction.

**Table VI.1.** Root biomass, excised-root respiration, and total soil respiration measured in June 1994. The respiration results were normalized to 19.8°C using a $Q_{10}$ function of 2. n = 9.

| | Root Respiration | | Total Soil Respiration |
|---|---|---|---|
| Root biomass | Biomass basis | Area basis | Area basis |
| ($g\ m^{-2}$) | ($mg\ CO_2\ g\text{-}root^{-1}\ hr^{-1}$) | ($mg\ CO_2\ m^{-2}\ hr^{-1}$) | ($mg\ CO_2\ m^{-2}\ hr^{-1}$) |
| 157 | 0.962 | 151 | 470 |

## SUMMARY

The objectives of this research were to determine how ozone affects northern red oak belowground carbon dynamics, and to identify differences in seedling and mature tree responses to ozone. Previous studies have shown that ozone can damage the photosynthetic mechanism. We hypothesized that this damage would increase the proportion of carbon supplied aboveground for repair of tissues damaged by ozone; it was further hypothesized that this carbon reallocation would result in a decrease in fine-root production and turnover; with decreased production occurring because of reduced carbon available for growth, and decreased turnover occurring as a carbon conserving strategy.

Doubling the ambient ozone level from 0.042 ppm (7hr-mean conc.) to 0.080 ppm (7hr-mean conc.) reduced mature tree cumulative fine-root production and turnover by 31 and 41%, respectively. This indicated that belowground carbon allocation was reduced by 2X ambient ozone. In contrast, elevated ozone did not affect seedling fine-root production and turnover. These results were supported by Samuelson and Edwards (1993) and Hanson et al. (1994), who both found that photosynthetic processes of the seedlings in this study were not sensitive to ozone, yet elevated ozone did negatively impact mature tree photosynthetic processes. Combining the aboveground and belowground results, we concluded that the mature trees were more sensitive to ozone than the seedlings. An additional conclusion was that use of seedlings for parameterizing models may lead to underestimates in the mature tree

response to ozone.

The summer 1994 study showed that 2X ozone reduced the belowground efficiency of carbon utilization in the mature trees, indicating that greater quantities of carbon were respired to produced and maintain fine-root biomass. We hypothesized this respiration response was due to increased active nutrient uptake to replace foliage damaged by ozone. Measurements of total soil respiration during this same period yielded no ozone effect on total soil respiration; this result led us to conclude that total soil respiration by itself is not a good indicator of root activity.

Additional work that evolved from this project resulted in improving current methods for measuring belowground processes. The substudy comparing the buried bag and *in situ* root decomposition methods showed that the buried bag method underestimates decomposition. This result was important because it showed that if the buried bag method is used, fine-root production and turnover will be underestimated. During the summer, 1994, we made a serious attempt at separating root from total soil respiration. To accomplish this, the excised-root and basal respiration methods were used simultaneously. Results from this study showed that total soil respiration could be partitioned into root, rhizo-microbial, and non-rhizo-microbial respiration, when these methods are used in concert. The ability to partition respiration in this way will allow researchers to quantify the amount of carbon lost through root respiration, root exudation, and non-rhizosphere microbial respiration. This study also showed that rhizo-microbial respiration is a significant sink for photosynthetically fixed carbon.

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# APPENDIX A.

## Environmental data

**Average monthly ozone concentrations**

Average monthly ozone concentrations

| Year | Month | Subambient | Ambient | 2X ambient |
|---|---|---|---|---|
| | | 7hr-Mean Ozone Concentrations (ppb) | | |
| 1992 | Apr | 16 | 39 | 72 |
| | May | 15 | 38 | 78 |
| | Jun | 16 | 38 | 72 |
| | Jul | 14 | 35 | 66 |
| | Aug | 15 | 35 | 63 |
| | Sep | 15 | 34 | 69 |
| | Oct | 14 | 30 | 62 |
| 1993 | Apr | 28 | 50 | 92 |
| | May | 20 | 51 | 97 |
| | Jun | 20 | 52 | 103 |
| | Jul | 20 | 53 | 93 |
| | Aug | 21 | 47 | 94 |
| | Sep | 19 | 42 | 80 |
| 1994 | Apr | 13 | 41 | 64 |
| | May | 17 | 46 | 94 |
| | Jun | 11 | 42 | 60 |
| | Jul | 8 | 37 | 85 |
| | Aug | 10 | 40 | 88 |
| | Sep | 10 | 41 | 84 |

## Soil temperature data

| Year | Month | Soil temp (C) |
|---|---|---|
| 1992 | Apr | 14 |
| | May | 17 |
| | Jun | 20 |
| | Jul | 23 |
| | Aug | 21 |
| | Sep | 20 |
| | Oct | 15 |
| | Nov | 10 |
| | Dec | 6 |
| 1993 | Jan | 3 |
| | Feb | 6 |
| | Mar | 8 |
| | Apr | 14 |
| | May | 20 |
| | Jun | 24 |
| | Jul | 28 |
| | Aug | 23 |
| | Sep | 20 |
| | Oct | 15 |
| | Nov | 10 |
| | Dec | 6 |
| 1994 | Jan | 3 |
| | Feb | 6 |
| | Mar | 9 |
| | Apr | 18 |
| | May | 16 |
| | Jun | 21 |
| | Jul | 22 |
| | Aug | 22 |
| | Sep | 19 |

## APPENDIX B.

### Fine-root biomass data

Explanation of chamber codes for following pages.

| CHAMBER | BLOCK | TREATMENT |
|---|---|---|
| 10A | 3 | subambient |
| 10C | 3 | twice ambient |
| 9C | 3 | ambient |
| 8D | 2 | twice ambient |
| 6A | 2 | subambient |
| 6B | 2 | ambient |
| 3C | 1 | subambient |
| 4E | 1 | ambient |
| 6G | 1 | twice ambient |
| 4A | 1 | subambient |
| 4B | 1 | ambient |
| 4C | 2 | subambient |
| 4X | 1 | twice ambient |
| 7A | 3 | subambient |
| 7X | 2 | ambient |
| 8A | 3 | ambient |
| 8B | 3 | twice ambient |
| 8X | 2 | twice ambient |

**Mature-tree fine-root biomass data**

| Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) | Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) |
|---|---|---|---|---|---|---|---|
| 51992 | 10A | 77 | 51 | 62193 | 4E | 113 | 153 |
| 51992 | 10C | 53 | 35 | 62193 | 6A | 75 | 37 |
| 51992 | 3C | 50 | 33 | 62193 | 6B | 103 | 25 |
| 51992 | 4E | 125 | 83 | 62193 | 6G | 43 | 48 |
| 51992 | 6A | 57 | 37 | 62193 | 8D | 47 | 38 |
| 51992 | 6B | 38 | 26 | 62193 | 9C | 42 | 65 |
| 51992 | 6G | 45 | 30 | 80493 | 10A | 81 | 35 |
| 51992 | 8D | 52 | 34 | 80493 | 10C | 133 | 17 |
| 51992 | 9C | 97 | 65 | 80493 | 3C | 87 | 31 |
| 81592 | 10A | 51 | 34 | 80493 | 4E | 135 | 39 |
| 81592 | 10C | 78 | 52 | 80493 | 6A | 90 | 27 |
| 81592 | 3C | 62 | 41 | 80493 | 6B | 60 | 26 |
| 81592 | 4E | 124 | 83 | 80493 | 6G | 32 | 21 |
| 81592 | 6A | 75 | 50 | 80493 | 8D | 49 | 41 |
| 81592 | 6B | 92 | 61 | 80493 | 9C | 67 | 30 |
| 81592 | 6G | 27 | 18 | 90193 | 10A | 79 | 11 |
| 81592 | 8D | 84 | 56 | 90193 | 10C | 79 | 15 |
| 81592 | 9C | 82 | 55 | 90193 | 3C | 76 | 8 |
| 112392 | 10A | 65 | 46 | 90193 | 4E | 169 | 20 |
| 112392 | 10C | 87 | 37 | 90193 | 6A | 95 | 17 |
| 112392 | 3C | 32 | 18 | 90193 | 6B | 81 | 15 |
| 112392 | 4E | 137 | 56 | 90193 | 6G | 41 | 19 |
| 112392 | 6A | 77 | 75 | 90193 | 8D | 45 | 30 |
| 112392 | 6B | 59 | 51 | 90193 | 9C | 72 | 24 |
| 112392 | 6G | 22 | 17 | 100193 | 10A | 65 | 12 |
| 112392 | 8D | 49 | 53 | 100193 | 10C | 102 | 12 |
| 112392 | 9C | 57 | 54 | 100193 | 3C | 54 | 14 |
| 32893 | 10A | 36 | 44 | 100193 | 4E | 128 | 32 |
| 32893 | 10C | 62 | 27 | 100193 | 6A | 83 | 19 |
| 32893 | 3C | 46 | 32 | 100193 | 6B | 78 | 16 |
| 32893 | 4E | 96 | 78 | 100193 | 6G | 37 | 15 |
| 32893 | 6A | 54 | 53 | 100193 | 8D | 83 | 31 |
| 32893 | 6B | 47 | 42 | 100193 | 9C | 115 | 40 |

| Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) | Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) |
|---|---|---|---|---|---|---|---|
| 32893 | 9C | 32 | 46 | 120393 | 3C | 105 | 15 |
| 52693 | 10A | 9 | 77 | 120393 | 4E | 134 | 17 |
| 52693 | 10C | 84 | 45 | 120393 | 6A | 84 | 9 |
| 52693 | 3C | 63 | 44 | 120393 | 6B | 75 | 6 |
| 52693 | 4E | 104 | 116 | 120393 | 6G | 39 | 16 |
| 52693 | 6A | 147 | 87 | 120393 | 8D | 57 | 23 |
| 52693 | 6B | 110 | 62 | 120393 | 9C | 85 | 19 |
| 52693 | 6G | 23 | 64 | 40194 | 10A | 91 | 12 |
| 52693 | 8D | 42 | 55 | 40194 | 10C | 71 | 10 |
| 52693 | 9C | 75 | 69 | 40194 | 3C | 145 | 36 |
| 62193 | 10A | 104 | 12 | 40194 | 4E | 79 | 6 |
| 62193 | 10C | 75 | 33 | 40194 | 6A | 113 | 15 |
| 62193 | 3C | 81 | 56 | 40194 | 6B | 111 | 8 |
| 40194 | 6G | 39 | 5 | 51694 | 8D | 70 | 10 |
| 40194 | 8D | 30 | 10 | 51694 | 9C | 81 | 16 |
| 40194 | 9C | 33 | 13 | 70194 | 10A | 150 | 7 |
| 51694 | 10A | 89 | 14 | 70194 | 10C | 180 | 12 |
| 51694 | 10C | 169 | 15 | 70194 | 3C | 144 | 11 |
| 51694 | 3C | 100 | 9 | 70194 | 4E | 285 | 29 |
| 51694 | 4E | 216 | 24 | 70194 | 6A | 177 | 19 |
| 51694 | 6A | 155 | 20 | 70194 | 6B | 133 | 11 |
| 51694 | 6B | 101 | 9 | 70194 | 6G | 124 | 28 |
| 51694 | 6G | 53 | 15 | 70194 | 8D | 110 | 19 |
| 90794 | 6A | 215 | 29 | 70194 | 9C | 114 | 28 |
| 90794 | 6B | 240 | 26 | 90794 | 10A | 146 | 35 |
| 90794 | 6G | 127 | 19 | 90794 | 10C | 285 | 19 |
| 90794 | 8D | 181 | 26 | 90794 | 3C | 192 | 15 |
| 90794 | 9C | 182 | 32 | 90794 | 4E | 390 | 27 |
| 32893 | 6G | 22 | 27 | 120393 | 10A | 62 | 13 |
| 32893 | 8D | 26 | 42 | 120393 | 10C | 101 | 7 |

## Seedling fine-root biomass data

| Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) |
|---|---|---|---|
| 81592 | 4A | 35 | 159 |
| 81592 | 4B | 41 | 108 |
| 81592 | 4C | 49 | 147 |
| 81592 | 4X | 19 | 0 |
| 81592 | 7A | 50 | 216 |
| 81592 | 7X | 43 | 110 |
| 81592 | 8A | 41 | 75 |
| 81592 | 8B | 26 | 0 |
| 81592 | 8X | 30 | 0 |
| 112392 | 4A | 121 | 27 |
| 112392 | 4B | 60 | 0 |
| 112392 | 4C | 102 | 30 |
| 112392 | 4X | 100 | 16 |
| 112392 | 7A | 104 | 33 |
| 112392 | 7X | 113 | 18 |
| 112392 | 8A | 91 | 24 |
| 112392 | 8B | 77 | 23 |
| 112392 | 8X | 93 | 29 |
| 32893 | 4A | 80 | 43 |
| 32893 | 4B | 71 | 52 |
| 32893 | 4C | 79 | 69 |
| 32893 | 4X | 101 | 31 |
| 32893 | 7A | 122 | 31 |
| 32893 | 7X | 74 | 22 |
| 32893 | 8A | 103 | 45 |
| 32893 | 8B | 62 | 11 |
| 32893 | 8X | 84 | 35 |
| 52793 | 4A | 80 | 0 |
| 52793 | 4B | 92 | 52 |
| 52793 | 4C | 98 | 24 |
| 52793 | 4X | 93 | 43 |
| 52793 | 7A | 87 | 41 |
| 52793 | 7X | 172 | 53 |
| 52793 | 8A | 108 | 47 |
| 52793 | 8B | 77 | 33 |
| 80493 | 4X | 144 | 19 |
| 80493 | 7A | 189 | 0 |
| 80493 | 7X | 185 | 45 |
| 80493 | 8A | 134 | 45 |
| 80493 | 8B | 124 | 22 |
| 80493 | 8X | 164 | 38 |
| 90393 | 4A | 142 | 0 |
| 90393 | 4B | 179 | 25 |
| 90393 | 4C | 196 | 26 |
| 90393 | 4X | 136 | 32 |
| 90393 | 7A | 147 | 20 |
| 90393 | 7X | 81 | 27 |
| 90393 | 8A | 117 | 12 |
| 90393 | 8B | 101 | 22 |
| 90393 | 8X | 129 | 20 |
| 120393 | 4A | 138 | 25 |
| 120393 | 4B | 120 | 29 |
| 120393 | 4C | 95 | 17 |
| 120393 | 4X | 88 | 0 |
| 120393 | 7A | 88 | 46 |
| 120393 | 7X | 148 | 10 |
| 120393 | 8A | 186 | 12 |
| 120393 | 8B | 53 | 0 |
| 120393 | 8X | 89 | 19 |
| 51694 | 4A | 226 | 0 |
| 51694 | 4B | 356 | 0 |
| 51694 | 4C | 178 | 0 |
| 51694 | 4X | 212 | 11 |
| 51694 | 7A | 405 | 0 |
| 51694 | 7X | 535 | 0 |
| 51694 | 8A | 313 | 0 |
| 51694 | 8B | 226 | 0 |
| 51694 | 8X | 335 | 0 |
| 70194 | 4A | 256 | 24 |
| 70194 | 4B | 690 | 17 |

| Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) |
|---|---|---|---|
| 90794 | 8A | 476 | 0 |
| 90794 | 8B | 724 | 0 |
| 90794 | 8X | 851 | 0 |
| 90794 | 7A | 683 | 0 |
| 90794 | 7X | 742 | 105 |
| 52793 | 8X | 110 | 31 |
| 62193 | 4A | 149 | 32 |
| 62193 | 4B | 108 | 33 |
| 62193 | 4C | 155 | 56 |
| 62193 | 4X | 103 | 39 |
| 62193 | 7A | 145 | 19 |
| 62193 | 7X | 81 | 26 |
| 62193 | 8A | 121 | 46 |
| 62193 | 8B | 100 | 27 |
| 62193 | 8X | 24 | 0 |

| Month/ Year | Chamber | Live (g m-2) | Dead (g m-2) |
|---|---|---|---|
| 80493 | 4B | 159 | 21 |
| 80493 | 4C | 151 | 39 |
| 70194 | 4C | 665 | 1 |
| 70194 | 4X | 555 | 12 |
| 70194 | 7A | 550 | 49 |
| 70194 | 7X | 354 | 0 |
| 70194 | 8A | 312 | 23 |
| 70194 | 8B | 514 | 17 |
| 70194 | 8X | 448 | 25 |
| 90794 | 4A | 602 | 0 |
| 90794 | 4B | 923 | 61 |
| 90794 | 4C | 961 | 0 |
| 90794 | 4X | 961 | 40 |
| 80493 | 4A | 115 | 18 |

# APPENDIX C.

## Fine-root decomposition data

## *In situ* **decomposition data**

| Interval | Harvest | Chamber | Biomass (g m-2) |
|---|---|---|---|
| 80193 | 0 | 10A | 0.398 |
| 80193 | 0 | 10C | 0.464 |
| 80193 | 0 | 3C | 0.193 |
| 80193 | 0 | 4E | 0.563 |
| 80193 | 0 | 6A | 0.389 |
| 80193 | 0 | 6B | 0.383 |
| 80193 | 0 | 6G | 0.189 |
| 80193 | 0 | 8D | 0.284 |
| 80193 | 0 | 9C | 0.334 |
| 80193 | 1 | 10A | 0.233 |
| 80193 | 1 | 10C | 0.238 |
| 80193 | 1 | 3C | 0.227 |
| 80193 | 1 | 4E | 0.402 |
| 80193 | 1 | 6A | 0.378 |
| 80193 | 1 | 6B | 0.245 |
| 80193 | 1 | 6G | 0.131 |
| 80193 | 1 | 8D | 0.129 |
| 80193 | 1 | 9C | 0.179 |
| 80193 | 2 | 10A | 0.124 |
| 80193 | 2 | 10C | 0.365 |
| 80193 | 2 | 3C | 0.240 |
| 80193 | 2 | 4E | 0.339 |
| 80193 | 2 | 6A | 0.273 |
| 80193 | 2 | 6B | 0.300 |
| 80193 | 2 | 6G | 0.123 |
| 80193 | 2 | 8D | 0.207 |
| 80193 | 2 | 9C | 0.249 |
| 80193 | 3 | 10A | 0.168 |
| 80193 | 3 | 10C | 0.176 |
| 80193 | 3 | 3C | 0.195 |
| 80193 | 3 | 4E | 0.344 |
| 80193 | 3 | 6A | 0.299 |
| 80193 | 3 | 6B | 0.198 |

| Interval | Harvest | Chamber | Biomass (g m-2) |
|---|---|---|---|
| 80193 | 3 | 9C | 0.139 |
| 80193 | 4 | 10A | 0.111 |
| 80193 | 4 | 10C | 0.169 |
| 80193 | 4 | 3C | 0.161 |
| 80193 | 4 | 4E | 0.366 |
| 80193 | 4 | 6A | 0.162 |
| 80193 | 4 | 6B | 0.130 |
| 80193 | 4 | 6G | 0.087 |
| 80193 | 4 | 8D | 0.082 |
| 80193 | 4 | 9C | 0.132 |
| 120193 | 0 | 10A | 0.446 |
| 120193 | 0 | 10C | 0.350 |
| 120193 | 0 | 3C | 0.308 |
| 120193 | 0 | 4E | 0.642 |
| 120193 | 0 | 6A | 0.293 |
| 120193 | 0 | 6B | 0.277 |
| 120193 | 0 | 6G | 0.282 |
| 120193 | 0 | 8D | 0.206 |
| 120193 | 0 | 9C | 0.246 |
| 120193 | 1 | 10A | 0.366 |
| 120193 | 1 | 10C | 0.363 |
| 120193 | 1 | 3C | 0.280 |
| 120193 | 1 | 4E | 0.597 |
| 120193 | 1 | 6A | 0.286 |
| 120193 | 1 | 6B | 0.245 |
| 120193 | 1 | 6G | 0.246 |
| 120193 | 1 | 8D | 0.134 |
| 120193 | 1 | 9C | 0.262 |
| 120193 | 2 | 10A | 0.188 |
| 120193 | 2 | 10C | 0.355 |
| 120193 | 2 | 3C | 0.206 |
| 120193 | 2 | 4E | 0.387 |
| 120193 | 2 | 6A | 0.218 |

| Interval | Harvest | Chamber | Biomass (g m-2) |
|---|---|---|---|
| 120193 | 2 | 6B | 0.159 |
| 120193 | 2 | 6G | 0.173 |
| 80193 | 3 | 6G | 0.088 |
| 80193 | 3 | 8D | 0.113 |
| 120193 | 2 | 8D | 0.120 |
| 120193 | 2 | 9C | 0.119 |
| 120193 | 3 | 10A | 0.201 |
| 120193 | 3 | 10C | 0.328 |
| 120193 | 3 | 3C | 0.156 |
| 120193 | 3 | 4E | 0.368 |
| 120193 | 3 | 6A | 0.270 |
| 120193 | 3 | 6B | 0.222 |
| 120193 | 3 | 6G | 0.128 |
| 120193 | 3 | 8D | 0.154 |
| 120193 | 3 | 9C | 0.154 |
| 120193 | 4 | 10A | 0.212 |
| 120193 | 4 | 10C | 0.170 |
| 120193 | 4 | 3C | 0.167 |
| 120193 | 4 | 4E | 0.422 |
| 120193 | 4 | 6A | 0.203 |
| 120193 | 4 | 6B | 0.129 |
| 120193 | 4 | 6G | 0.102 |
| 120193 | 4 | 8D | 0.142 |
| 120193 | 4 | 9C | 0.185 |
| 120193 | 4 | 9C | 0.185 |

## Buried bag decomposition data

| Interval | Chamber | decomp rate (k mo-1) |
|---|---|---|
| 80193 | 10A | 0.05 |
| 80193 | 10C | 0.05 |
| 80193 | 3C | 0.04 |
| 80193 | 4E | 0.06 |
| 80193 | 6A | 0.04 |
| 80193 | 6B | 0.05 |
| 80193 | 6G | 0.09 |
| 80193 | 8D | 0.09 |
| 80193 | 9C | 0.10 |
| 120193 | 10A | 0.14 |
| 120193 | 10C | 0.12 |
| 120193 | 3C | 0.14 |
| 120193 | 4E | 0.01 |
| 120193 | 6A | 0.02 |
| 120193 | 6B | 0.02 |
| 120193 | 6G | 0.01 |
| 120193 | 8D | 0.01 |
| 120193 | 9C | 0.02 |

# APPENDIX D.

## Respiration data

## Mature-tree respiration data

| Date | Chamber | fine-root respiration (mg g root-1 hr-1) | total soil respiration (mg m-2 hr-1) |
|---|---|---|---|
| 52594 | 10A | 1.00 | 178.20 |
| 52594 | 10C | 0.85 | 346.10 |
| 52594 | 3C | 0.40 | 196.61 |
| 52594 | 4E | 0.67 | 377.98 |
| 52594 | 6A | 0.73 | 203.74 |
| 52594 | 6B | 0.75 | 148.90 |
| 52594 | 6G | 1.65 | 176.42 |
| 52594 | 8D | 0.94 | 274.63 |
| 52594 | 9C | 0.89 | 129.89 |
| 70694 | 10A | 1.09 | 362.14 |
| 70694 | 10C | 1.30 | 424.71 |
| 70694 | 3C | 0.85 | 498.37 |
| 70694 | 4E | 0.65 | 882.11 |
| 70694 | 6A | 0.69 | 747.05 |
| 70694 | 6B | 0.95 | 421.34 |
| 70694 | 6G | 0.98 | 429.07 |
| 70694 | 8D | 2.03 | 399.17 |
| 70694 | 9C | 0.55 | 315.02 |
| 90994 | 10A | 0.57 | 335.02 |
| 90994 | 10C | 0.46 | 388.67 |
| 90994 | 3C | 0.67 | 383.13 |
| 90994 | 4E | 0.68 | 373.23 |
| 90994 | 6A | 0.49 | 318.19 |
| 90994 | 6B | 0.52 | 206.32 |
| 90994 | 6G | 2.52 | 250.27 |
| 90994 | 8D | 0.32 | 241.36 |
| 90994 | 9C | 0.26 | 255.22 |

## Seedling respiration data

| Date | Chamber | fine-root respiration (mg g root-1 hr-1) | total soil respiration (mg m-2 hr-1) |
|---|---|---|---|
| 51694 | 4A | 1.10 | 161.37 |
| 51694 | 4B | 1.12 | 162.56 |
| 51694 | 4C | 1.66 | 192.46 |
| 51694 | 4X | 1.06 | 187.14 |
| 51694 | 7A | 1.10 | 200.18 |
| 51694 | 7X | 1.14 | 236.81 |
| 51694 | 8A | 0.85 | 152.06 |
| 51694 | 8B | 2.69 | 212.26 |
| 51694 | 8X | 0.55 | 124.54 |
| 70194 | 4A | 1.15 | 211.46 |
| 70194 | 4B | 0.91 | 275.22 |
| 70194 | 4C | 1.57 | 369.07 |
| 70194 | 4X | 0.98 | 436.39 |
| 70194 | 7A | 1.08 | 402.93 |
| 70194 | 7X | 1.18 | 325.71 |
| 70194 | 8A | 1.49 | 231.66 |
| 70194 | 8B | 0.94 | 380.16 |
| 70194 | 8X | 3.12 | 191.47 |
| 90794 | 4A | 0.95 | 598.95 |
| 90794 | 4B | 0.17 | 460.15 |
| 90794 | 4C | 0.32 | 989.01 |
| 90794 | 4X | 0.68 | 642.51 |
| 90794 | 7A | 1.05 | 609.84 |
| 90794 | 7X | 0.72 | 579.55 |
| 90794 | 8A | 1.35 | 341.95 |
| 90794 | 8B | 0.73 | 848.43 |
| 90794 | 8X | 0.47 | 464.90 |

## Basal respiration data

| Month | Chamber | Respiratio |
|---|---|---|
| June | 10A | 3.56 |
| June | 10C | 3.71 |
| June | 3C | 3.73 |
| June | 4E | 4.77 |
| June | 6A | 7.47 |
| June | 6B | 4.34 |
| June | 6G | 3.83 |
| June | 8D | 4.87 |
| June | 9C | 4.28 |
| July | 10A | 1.52 |
| July | 10C | 2.70 |
| July | 3C | 2.09 |
| July | 4E | 2.85 |
| July | 6A | 3.15 |
| July | 6B | 4.50 |
| July | 6G | 3.27 |
| July | 8D | 3.67 |
| July | 9C | 2.68 |
| August | 10A | 2.60 |
| August | 10C | 1.69 |
| August | 3C | 2.09 |
| August | 4E | 1.62 |
| August | 6A | 2.59 |
| August | 6B | 2.05 |
| August | 6G | 1.69 |
| August | 8D | 1.78 |
| August | 9C | 1.24 |
| Septembe | 10A | 1.48 |
| Septembe | 10C | 1.14 |
| Septembe | 3C | 1.36 |
| Septembe | 4E | 0.95 |
| Septembe | 6A | 1.44 |
| Septembe | 6B | 1.11 |
| Septembe | 6G | 0.85 |
| Septembe | 8D | 1.19 |
| Septembe | 9C | 0.90 |
| October | 10A | 1.25 |
| October | 10C | 1.17 |
| October | 3C | 1.31 |
| October | 4E | 0.76 |
| October | 6A | 1.01 |
| October | 6B | 0.83 |
| October | 6G | 0.44 |
| October | 8D | 1.19 |
| October | 9C | 1.02 |

# VITA

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