

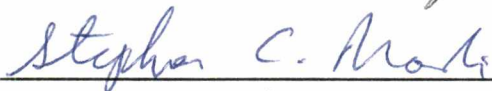
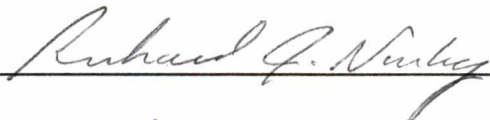
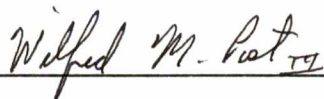
To the Graduate Council:

I am submitting herewith a dissertation written by Elizabeth Gerry O'Neill entitled "The Roles of Mycorrhizae, Soil Microbes, and Microbially-mediated Soil Processes In Forest Tree Responses to Rising Atmospheric CO₂." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Ecology.



Susan E. Riechert, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
And Dean of the Graduate School

**THE ROLES OF MYCORRHIZAE, SOIL MICROBES, AND
MICROBially-MEDIATED SOIL PROCESSES
IN FOREST TREE RESPONSES TO RISING ATMOSPHERIC CO₂**

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Elizabeth Gerry O'Neill

May, 1996

DEDICATION

This thesis is dedicated to my husband

Dr. Robert Vincent O'Neill

who embodies the mystery, the beauty and the laughter
in Science

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ABSTRACT

Carbon dioxide (CO₂) has been increasing exponentially in the earth's atmosphere since the mid-18th century. There is a need to understand the responses of the terrestrial component of the biosphere, including feedbacks from terrestrial systems to the atmosphere and vice versa. Understanding effects of elevated CO₂ on terrestrial systems includes an understanding of what constrains, or limits, responses of vegetation. Evaluating responses of soil processes and the organisms that mediate these processes may be second only to quantifying changes in photosynthetic potential in determining the status of future terrestrial ecosystems.

Increased productivity in forested ecosystems may be dependent upon microbially-mediated soil processes that increase nutrient availability in what are commonly nutrient-poor systems. Chemical transformations mediated by rhizosphere (root-associated) bacterial populations represent one mechanism by which soil nutrient availability might increase in a higher CO₂ world. However, in my studies, numbers of bacteria in the rhizospheres of yellow-poplar seedlings were not increased when seedlings were grown at twice-ambient CO₂. Selected microbial functional groups (phosphorus-dissolving bacteria, ammonium-oxidizing bacteria, and nitrite-oxidizing bacteria) in the rhizosphere also showed no response to a doubling of atmospheric CO₂.

A second mechanism proposed to increase nutrient uptake is stimulation of the mycorrhizal symbiosis, a ubiquitous relationship between plants and certain soil fungi. Mycorrhizal fungi receive the bulk of their metabolic and structural C from the plant host

and increase nutrient uptake by the host in return. Differential responses were seen between two major types of symbioses. Elevated CO₂ increased the number of ectomycorrhizal (ECM) root tips per unit fine root length in two forest tree species. However, no change in vesicular-arbuscular mycorrhizal (VAM) colonization was seen in yellow-poplar seedlings when grown in enriched CO₂. Because most deciduous temperate forests contain host plants of both ECM and VAM fungi, there is potential for CO₂ enrichment to change the competitive balance between plant species.

Nutrient availability in soils is ultimately dependent upon decomposition, or breakdown, of dead plant material (litter). Furthermore, rates of decomposition are sensitive to the chemistry of the materials being decomposed. There has been concern expressed that growth in elevated CO₂ might alter litter chemistry and retard decomposition, thus reducing nutrient availability. No changes in litter chemistry or decomposition rate were seen in either yellow-poplar or white oak litter when saplings were grown in the ground in open-topped chambers at two levels of CO₂ enrichment. This is in contrast to other studies, conducted in pots under artificial lighting, where litter chemistry was altered in such a way as to suggest dramatically decreased decomposition rates. We investigated the effect of pot culture on the chemistry of sugar maple litter produced under a combination of CO₂ enrichment and increased (+4°C) temperature treatments. Concentrations of nitrogen (N) were increased in litter produced in elevated temperatures, probably due to abnormal senescence (the process of leaf aging which culminates in leaf fall). However, there were no effects of CO₂ enrichment on litter chemistry, and little effect of pot growth.

CO₂ enrichment of forest tree species did not substantially alter soil microbial populations nor change plant litter chemistry. Mycorrhizal relationships were affected, however, in a manner that might lead to changes in tree species composition of forests. Forest ecosystems that are dominated by either ECM or VA-mycorrhizae (e.g., coniferous forests or tropical hardwood forests, respectively) might show little compositional change. Forest ecosystems comprised of mixed ECM and VAM host trees, however, could experience substantial changes in composition. This would have a greater impact on ecosystem level decomposition and nutrient cycling than will changes in leaf litter tissue chemistry.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.....	1
Elevated CO ₂ Effects on Vegetation.....	4
Rhizosphere Responses.....	4
Mycorrhizal Responses.....	9
Decomposition.....	10
II. ELEVATED ATMOSPHERIC CO ₂ EFFECTS ON SEEDLING GROWTH, NUTRIENT UPTAKE, AND RHIZOSPHERE BACTERIAL POPULATIONS OF <i>LIRIODENDRON TULIPIFERA</i> L.....	12
Abstract.....	12
Introduction.....	13
Methods and Materials.....	16
Results.....	20
Discussion.....	27
III. INCREASES IN MYCORRHIZAL COLONIZATION AND SEEDLING GROWTH IN <i>PINUS ECHINATA</i> AND <i>QUERCUS</i> <i>ALBA</i> IN AN ENRICHED CO ₂ ATMOSPHERE	33
Abstract.....	33
Introduction.....	34
Methods and Materials.....	36
Results.....	41
Discussion.....	48
IV. HIERARCHY THEORY AS A GUIDE TO MYCORRHIZAL RESEARCH ON LARGE-SCALE PROBLEMS.....	52
Abstract.....	52
Introduction.....	52

TABLE OF CONTENTS (continued)

	Hierarchy Theory Revisited.....	56
	A Clarifying Analogy.....	60
	A Practical Example.....	63
	Presence vs Function.....	67
	Summary.....	70
V.	LITTER QUALITY AND DECOMPOSITION RATES OF FOLIAR LITTER PRODUCED UNDER CO ₂ ENRICHMENT.....	72
	Introduction.....	72
	Litter Quality and the Decomposition Process.....	75
	CO ₂ Enrichment Effects on Litter Quality and Decomposition	80
	A Few Words to the Wise Decomposer.....	89
	Summary.....	96
VI.	CHANGES IN TISSUE CHEMISTRY OF SUGAR MAPLE LITTER WITH CO ₂ ENRICHMENT AND ELEVATED TEMPERATURE: RESPONSES IN POT-GROWN VS FIELD- GROWN SEEDLINGS.....	97
	Abstract.....	97
	Introduction.....	98
	Methods.....	101
	Results and Discussion.....	104
	Conclusions.....	114
VII.	DISCUSSION AND SUMMARY.....	115
	Rhizosphere Responses.....	115
	Mycorrhizal Responses.....	117
	Litter Quality and Decomposition.....	123
	Summary.....	125

TABLE OF CONTENTS (continued)

BIBLIOGRAPHY.....	126
VITA.....	145

LIST OF TABLES

TABLE		PAGE
II.1	Yellow-poplar seedling growth at two levels of atmospheric CO ₂	21
II.2	Nutrient content and concentrations of yellow-poplar seedlings following 24 weeks of exposure to ambient or elevated CO ₂	23
II.3	Numbers of total bacteria, phosphate-dissolving bacteria (PDB), ammonium-oxidizers (NH ₄ OX), and nitrite-oxidizers (NO ₂ OX) in the rhizosphere of yellow-poplars at the final harvest.....	26
III.1	Seedling physical parameters and mycorrhizal colonization in shortleaf pine (<i>P. echinata</i>) at two atmospheric concentrations of CO ₂	42
III.2	Proportions of mycorrhizal root tips, proportion of <i>Cenococcum</i> mycorrhizas, and dehydrogenase activity in rhizosphere soil of white oak.....	46
III.3	Physical parameters of white oak seedlings at two concentrations of atmospheric CO ₂	47
IV.1	Proportion of <i>Cenococcum graniforme</i> root tips (<i>C. graniforme</i> tips/100 mycorrhizal root tips) on white oak roots grown at two levels of atmospheric CO ₂	68
V.1	Nutrient ratios of litter produced at elevated CO ₂ for different species and with differing experimental approaches.....	81
V.2	Comparison of litter quality data from ambient treatments of elevated CO ₂ studies and reported values for the same species in the field.....	91
VI.1	Nutrient concentrations in foliar litter of sugar maple grown at two atmospheric CO ₂ concentrations and under two temperature regimes.....	105
VI.2	Green leaf nutrient concentrations in sugar maple in late summer, 1994.....	106
VI.3	ANOVA table for C concentrations in litter of sugar maple seedlings.....	107

LIST OF TABLES (continued)

VI.4	ANOVA table for N concentrations in litter of sugar maple seedlings.....	108
VI.5	ANOVA table for C:N ratios in litter of sugar maple seedlings.....	109
VI.6	Effects of CO ₂ enrichment and elevated temperature on specific leaf area (cm ² g ⁻¹) per plant.....	113

LIST OF FIGURES

Figure		Page
I.1	Conceptual diagram illustrating carbon (C) and nutrient (X, X') pathways in a plant soil system.....	2
I.2	Possible mechanisms hypothesized to alleviate nutrient limitations to forest tree growth in an enriched CO ₂ atmosphere..	5
I.3	Key to chapters including major organisms and processes covered in this thesis.....	8
II.1	Numbers of bacteria per g sample in nonrhizosphere soil and in the rhizospheres of yellow-poplars grown in ambient or elevated CO ₂ for 28 weeks.....	28
III.1	Proportion of mycorrhizal roots on white oak seedlings grown for 6 to 30 weeks at ambient or 700 µL L ⁻¹ CO ₂	44
IV.1	Conceptual diagram of the effect of moving from a higher hierarchical level to a lower one.....	57
IV.2	Percent VA-mycorrhizal root length in <i>L. tulipifera</i> (yellow-poplar) seedlings grown at either ambient (ca. 350 µg m ⁻³) or elevated (ca. 700 µg m ⁻³) CO ₂	65
V.1	Mass loss of yellow-poplar litter produced under three CO ₂ enrichment regimes.....	85
V.2	Changes in N concentrations in decomposing CO ₂ - enriched yellow-poplar litter residue through time.....	86
V.3	Changes in litter nutrient ratios with CO ₂ enrichment in field-grown plants vs. pot-grown plants.....	90
V.4	Differences in litter nutrient ratios between "ambient" (no CO ₂ enrichment) treatments in CO ₂ -effects studies and ratios reported in the literature for the same species under non-experimental conditions.....	93
VI.1	C:N ratios in sugar maple litter grown in pots or in the ground at two levels of atmospheric CO ₂ and two temperatures.....	111
VI.2	N concentrations in sugar maple litter grown in pots or in the ground at two levels of atmospheric CO ₂ and two temperatures...	112

LIST OF FIGURES (continued)

VII.1	Comparison of CO ₂ enrichment effects on ECM vs. VAM infection in field and environmental chamber studies.....	120
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CHAPTER I

INTRODUCTION

Carbon dioxide (CO₂) has been increasing exponentially in the earth's atmosphere since the mid-18th century. There is thus a need to understand the responses of the terrestrial component of the biosphere to CO₂ enrichment, including feedbacks from terrestrial systems to the atmosphere and vice versa. In predicting the effects of elevated CO₂ on terrestrial systems we will need to have an understanding of what constrains, or limits, responses of vegetation to CO₂ enrichment.

An increasing number of studies on effects of rising atmospheric CO₂ point to belowground systems as critical sources or sinks in global C cycling and as critical determinants of ecosystem response to climate change (Norby *et al.*, 1994). Forest ecosystems are constrained by the rates at which soil processes occur (particularly those involved in plant nutrient acquisition), and these processes are in turn constrained by rates of carbon input from the aboveground portion of the system (Fig I.1). Changes in C input to the soil, whether in the form of increased or chemically altered exudation from roots, through increased litter production, or through increased production and turnover of fine roots could have a dramatic effect on rates of belowground processes (Norby, 1994). Changes in chemical composition of either plant litter or fine roots as a result of atmospheric CO₂ enrichment could also affect process rates, especially rates of nutrient cycling (Strain and Bazzaz, 1983). All of these changes can affect productivity, diversity or activity of soil biota and potentially feedback to determine responses of ecosystems.

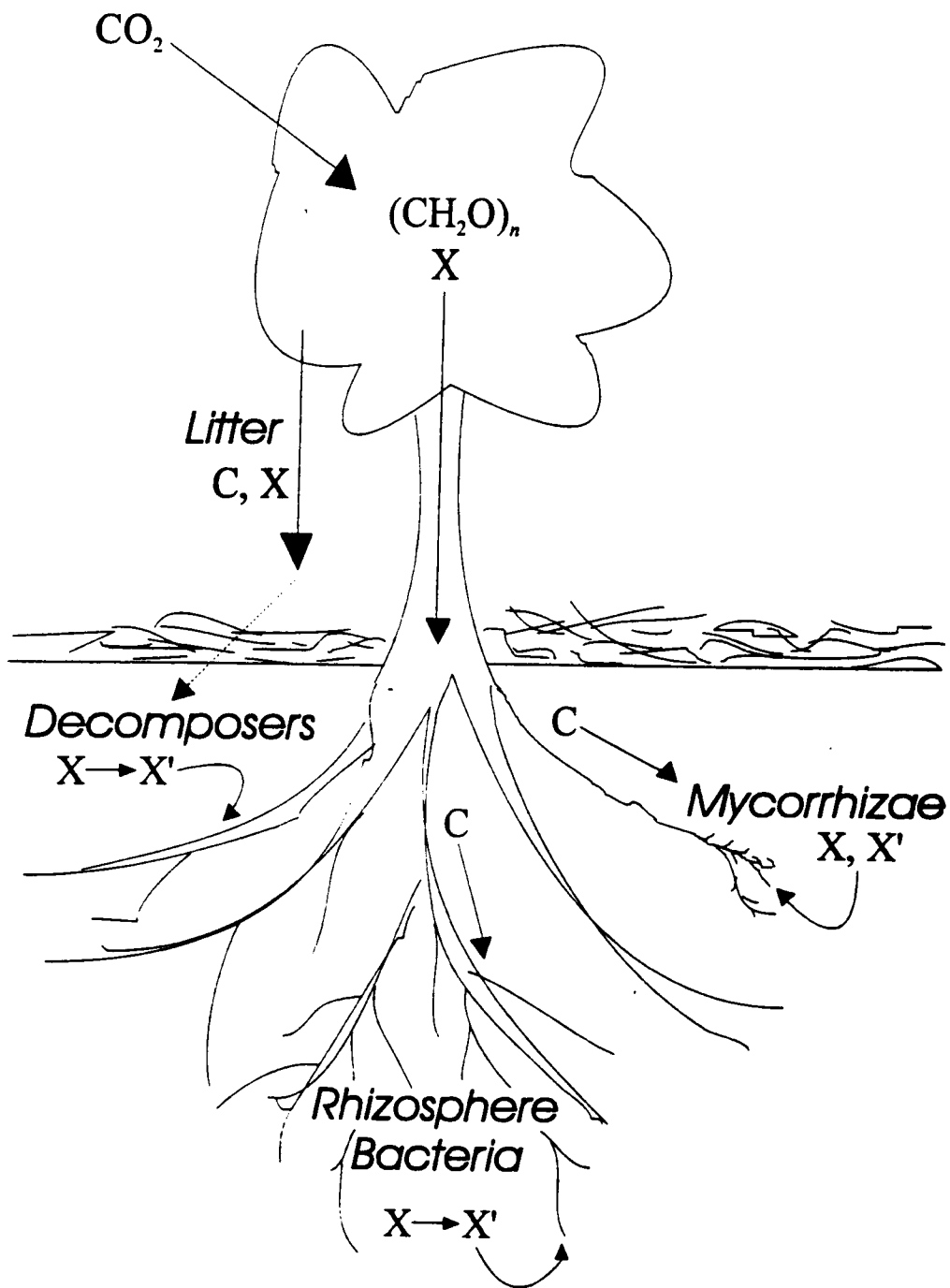


Figure I.1. Conceptual diagram illustrating carbon (C) and nutrient (X, X') pathways in a plant-soil system. Arrows indicate direction of transport. CO_2 , carbon dioxide; $(\text{CH}_2\text{O})_n$, carbohydrates; $X \rightarrow X'$, chemical transformation of nutrient X to nutrient X'.

The work reported in this dissertation investigates the extent to which particular groups of soil biota respond to indirect (i.e. plant-mediated) effects of CO₂ enrichment. With the exception of this introduction, the chapters are chronologically arranged (from 1987 through 1995) in the order in which the work was performed and the papers written. Chapters II through V are presented here in their published form. Much of the text in this introduction and in the summary chapter (Chapter VII) was published as an invited review (O'Neill, 1994) and has been modified to suit its function here.

The field of ecology has changed considerably in the past ten years, and this change is reflected in the dissertation. One major area of development has been in the level of sophistication required in the use of statistics. Journal editors require more detailed information on the statistical methods employed, allowing readers to make their own judgement on the validity of results rather than accepting on faith the conclusions of the authors. The simple statement that ANOVA was used for data analysis (Chapter II) is no longer sufficient. Furthermore, statistical tools for analysis of biological data that were in wide use in 1987 (e.g., Duncan's Multiple Range Test, Chapter II) have fallen into disfavor as researchers become more familiar with the limitations of particular techniques (Dye and Quinn 1989) and statisticians become more familiar with the reality of biological systems (Potvin and Roff 1993). I believe the conclusions reached in the earlier chapters of this dissertation are valid irrespective of the statistical methods used, however, I encourage readers to view them with a cautious and critical eye.

Elevated CO₂ Effects on Vegetation

Increased plant productivity as a result of CO₂ enrichment has been well-documented in agricultural systems where neither nutrients nor water place limitations on plant growth (Kimball 1983). Nutrient and water limitations in unmanaged ecosystems may well preclude biomass increases in forest tree species (Kramer 1981) unless these constraints are overcome by mechanisms that exploit the additional carbon available in an enriched CO₂ atmosphere (Fig. I.2). Luxmoore (1981) hypothesized that elevated CO₂ would increase photosynthesis and the resulting fixed carbon would be preferentially allocated to roots. An increase in available carbon in roots would enhance root processes, for example, increasing root growth and stimulating the growth and/or activity of symbiotic fungi, root-associated bacteria and nitrogen-fixing microorganisms. The result is corresponding increases in nutrient and/or water uptake. Luxmoore did not include responses of other groups of soil biota in his hypothesis. Subsequently, this hypothesis was expanded to include tree physiological adjustments in response to CO₂ enrichment that could alleviate nutrient limitations, such as increased nutrient use efficiency (Norby 1995; Johnson *et al.*, 1994).

Rhizosphere Responses

Stimulation of microbial nutrient transformations in the rhizosphere as a result of increased C input is one mechanism hypothesized by Luxmoore (1981) to increase nutrient availability. The rhizosphere is a qualitatively defined area of the soil in close proximity to fine roots that contains higher populations of some soil organisms than the

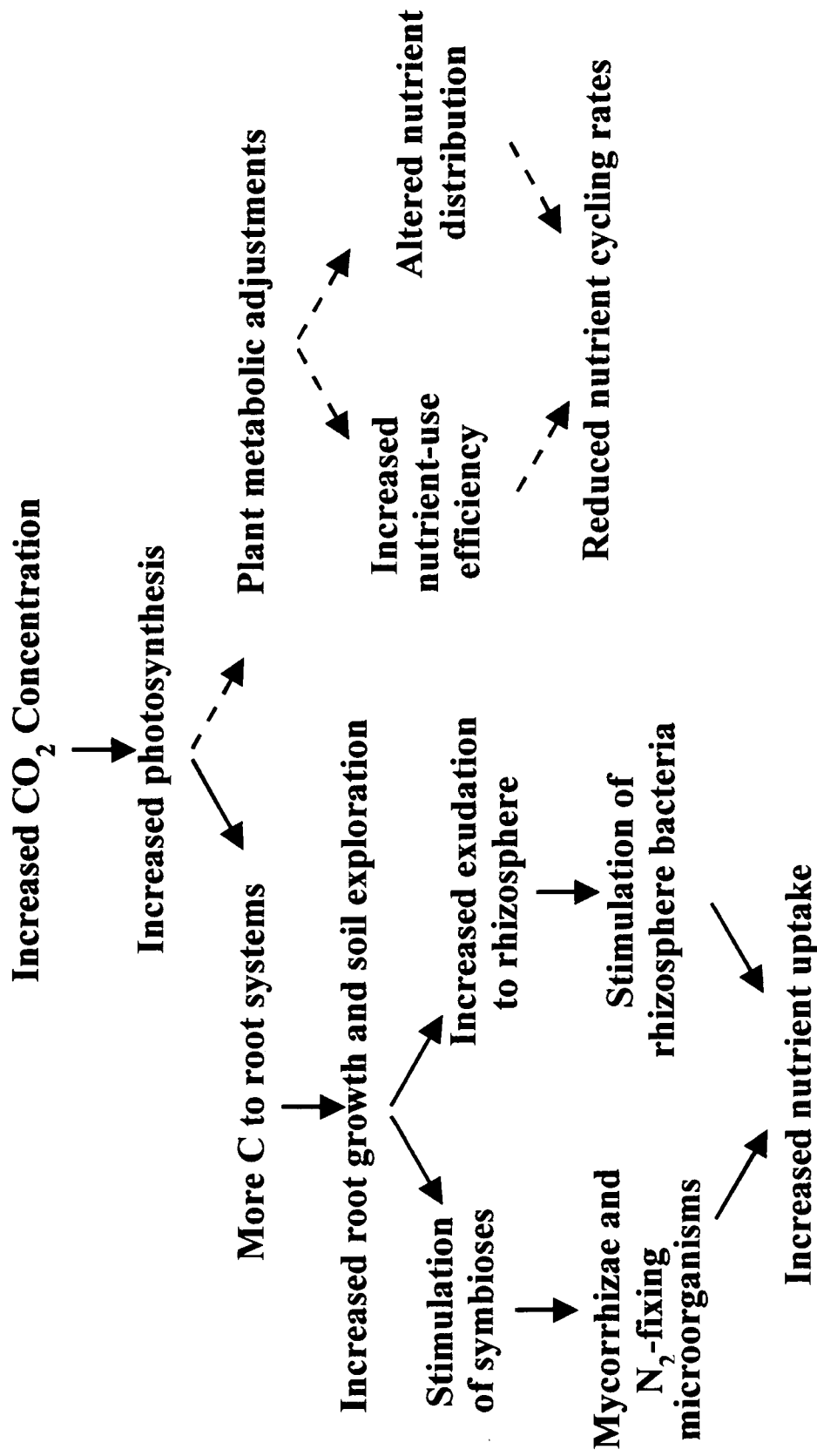


Fig. I.2. Possible mechanisms hypothesized to alleviate nutrient limitations to forest tree growth in an enriched CO₂ atmosphere. Pathways indicated by dotted lines are not covered in this dissertation. (Modified from Norby, *et al.*, 1995)

soil at large (Fig. 1.1). The rhizosphere exists primarily because of the deposition of easily assimilated organic materials from the plant root. If competition for C is the primary factor limiting soil microbial populations (Lockwood, 1981), environmental factors that influence root exudation, either through changes in exudation rate or chemical composition, could affect rhizosphere microbial populations. This has been demonstrated in the case of light and temperature changes (Fenwick, 1973; Rouatt and Katznelson, 1960; Rouatt *et al.*, 1963). It is reasonable to expect CO₂ effects as well. For a discussion of current information on exudation and fine root growth responses to CO₂ enrichment, see Norby (1994).

Zak *et al.* (1993) utilized an hypothesis of Clarholm (1985) to link elevated CO₂-induced increases in rhizosphere microbial populations to increases in N availability. According to Clarholm's hypothesis, as roots grow through the soil, soil microbe activity increases as a result of the pulse of exudate produced. Increased microbial respiration attracts bacteriophagic protozoans. As protozoans feeding on bacteria only assimilate 33% of bacterial N, the rest is excreted as cell wall or NH₄⁺ which can then be assimilated by plant roots and mycorrhizae (Clarholm, 1985; Fenchel, 1982). However, Robinson *et al.* (1989), and Griffiths and Robinson (1992) determined through the use of nitrogen balance models that, even taking bacterial grazing into account, root-induced nitrogen mineralization could not contribute substantially towards plant N acquisition. If these models accurately describe the relationship between root exudation and N mineralization, then an increase in N availability might not occur as a result of CO₂ enrichment unless exudate C-to-N ratios increase. Model predictions are driven strongly

by substrate C-to-N ratios; there is some evidence that these ratios may increase under CO₂ enrichment due to partitioning of C into nonstructural carbohydrates (Körner and Arnone, 1992).

In mycorrhizal plants, CO₂ effects would not necessarily be limited to the rhizosphere. Mycorrhizal hyphae are very efficient at redistributing recently-fixed C away from roots (Norton *et al.*, 1990). In fact, this efficiency is so great that it may be more correct to expand the rhizosphere concept through consideration of effects of CO₂ enrichment on the "mycorrhizosphere" (Rambelli, 1973). Ectomycorrhizal hyphae may extend as much as 2 m beyond the root surface and contain more than 120 lateral branches (Fogel, 1988). The increased surface area represented by this extraradical hyphal system may mean that mycorrhizae exert a much greater effect on the soil microbial biomass than do roots alone. In addition, rhizodeposition in mycorrhizal plants is greater than that produced by ecologically nonmycotrophic plants (Schwab *et al.*, 1984) or by rapidly growing nonmycorrhizal root tips of mycorrhizal plants (Norton *et al.*, 1990).

Chapter II reports effects of CO₂ enrichment on rhizosphere bacterial populations as well as on growth and mineral nutrition of the host plant, *Liriodendron tulipifera* L. (Fig I.3), which was reported in O'Neill *et al.*, 1987(a). Prior to this publication there had been little interest in the effects of elevated CO₂ on rhizosphere microbial populations. Interestingly, the question of effects on soil microbiota and the results in terms of nutrient cycling have become a hotly debated issue since that time (Holmes and Zak 1993; Zak 1993; Díaz, 1993).

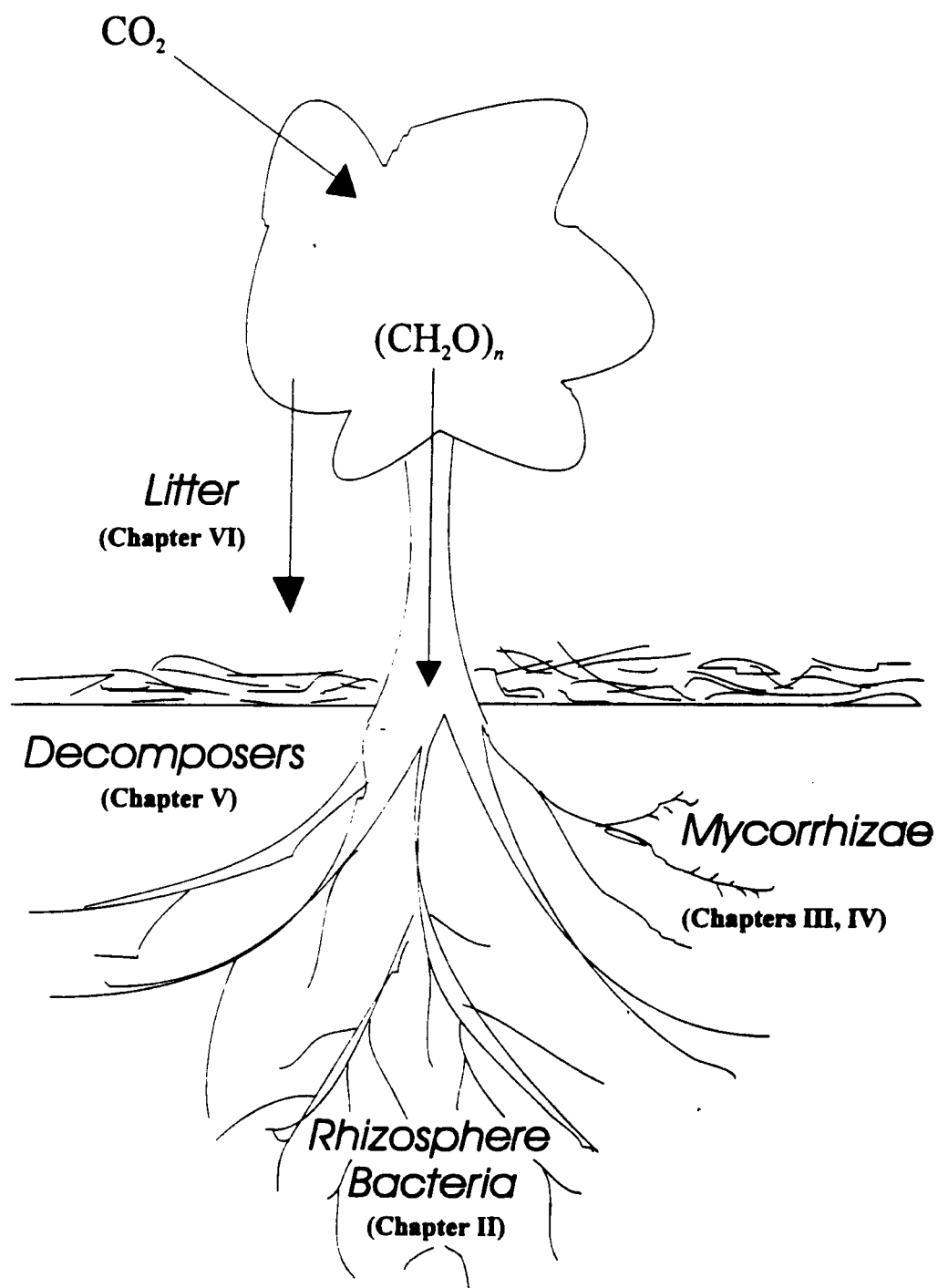


Figure I.3. Key to chapters including major organisms and processes covered in this thesis.

Mycorrhizal Responses

The concept of mycorrhizal benefit to host plant nutrient uptake is now generally accepted. In 1982, a workshop was held in Athens, Georgia to assess the state-of-the-science on potential effects of rising CO₂ on vegetation and to make recommendations for future research (Lemon, 1983). The working group on microbial effects identified the mycorrhizal symbiosis to be a key to plant nutrient acquisition and increased productivity in an enriched CO₂ world. However, mycorrhizae should be considered beyond their mere ability to enhance plant growth. They are frequently overlooked in questions of terrestrial C cycling, though mycorrhizae are the largest single consumer of net primary productivity (NPP) in terrestrial biomes (Allen, 1991). Although they may comprise only a small fraction of the standing organic matter pool at any one time, they are responsible for a major proportion of the annual flux of C to the soil. For example, while mycorrhizae and their associated fruiting structures comprised only 12 to 16.7% of the total soil organic matter in a second-growth Douglas fir forest, they were responsible for 53.5 to 89.5% of C flux to the soil. Litterfall only accounted for 10 to 10.4% of total C flux (Fogel and Hunt 1979, Fogel and Hunt 1983). Under elevated CO₂ conditions, increased translocation of carbon to roots could stimulate growth, activity or longevity of mycorrhizae and impact both plant nutrient uptake and C cycling (Fig I.1).

Most plant species are colonized by either ectomycorrhizal (ECM) or vesicular-arbuscular mycorrhizal (VAM) fungi. These two groups differ in their morphology, physiology, and host associations, and these differences may dictate the extent of their response to elevated CO₂. ECM fungi invest considerable plant-produced C in specialized

structures (mantles, fruiting bodies, rhizomorphs, and extensive extraradical mycelium). This demand for C may equate to heightened responsiveness (compared to VAM) to increased C supply.

Chapters III and IV deal with mycorrhizal responses to elevated CO₂ in very different ways (Fig. I.3). Chapter III is a traditional research paper on responses of ECM in two forest tree species (O'Neill *et al.*, 1987b). Chapter IV was published as an invited paper on theoretical considerations in mycorrhizal responses to air pollution and environmental change (O'Neill *et al.*, 1991). To some extent it resulted from a desire on my part to link my work with the larger world — to find a context in which to set small scale research during a time when large-scale scientific problems dominate the news.

Decomposition

Elevated CO₂ could also affect ecosystem nutrient cycling through indirect effects on decomposition processes, primarily those of leaf litter and fine roots. This might occur through changes in chemical composition ("quality") of litter (Melillo 1983; Fig I.1), or through changes in plant species composition of ecosystems (Ågren *et al.*, 1991).

The organisms responsible for plant residue decomposition are sensitive to the chemical composition of their substrate. Small changes in concentrations of C (particularly recalcitrant forms of C such as lignin), N and/or P can markedly change the breakdown rates of litter. Chemical changes observed in *green* (live) leaf tissue produced under CO₂ enrichment include increases in non-structural C (Körner and Arnone, 1992) and decreases in leaf N concentrations (Curtis *et al.*, 1989; Körner and Arnone, 1992;

Owensby *et al.*, 1993; Norby *et al.*, 1992), as well as changes in concentrations of secondary plant metabolites such as tannins (Johnson *et al.*, 1994; Lindroth and Kinney, 1993). These responses are remarkably similar across species. If these effects on green leaf nutrient composition are reflected in differences in foliar litter concentrations, decomposition rates could be reduced (Melillo, 1983). However, woody plants typically retranslocate nutrients from leaves to storage tissue prior to litterfall, leading to litter nutrient concentrations that are often very different from green leaf concentrations. Until recently, little data were available on senescent foliage produced under CO₂ enrichment, and still fewer data on leaves grown at variable CO₂ concentrations and allowed to senesce naturally in the field. Chapter V (Fig I.3) compares available data from pot and field studies on changes in leaf litter quality and decomposition with CO₂ enrichment. The result of this analysis led to the experiment reported in Chapter VI (Fig I.3), where leaf litter quality from potted and field-grown sugar maple seedlings, grown side-by-side in open-topped chambers under CO₂ enrichment and increased temperature, is evaluated.

CHAPTER II

ELEVATED ATMOSPHERIC CO₂ EFFECTS ON SEEDLING GROWTH, NUTRIENT UPTAKE, AND RHIZOSPHERE BACTERIAL POPULATIONS OF *LIRIODENDRON TULIPIFERA* L.

Abstract

Yellow poplar (*Liriodendron tulipifera* L.) seedlings were planted in unfertilized forest soil in boxes with a removable side panel and grown in atmospheres containing either ambient (367 $\mu\text{L L}^{-1}$) or elevated (692 $\mu\text{L L}^{-1}$) CO₂. Numbers of total bacteria, nitrifiers, and phosphate-dissolving bacteria in the rhizosphere and in non-rhizosphere soil were measured every 6 weeks for 24 weeks. Seedling growth and nutrient content were measured at a final whole-plant harvest. Root, leaf, and total dry weights were significantly greater, and specific leaf area was significantly less, in 692 $\mu\text{L L}^{-1}$ than in ambient CO₂. Uptake per gram plant dry weight of N, S, and B was lower at elevated CO₂, whereas uptake of P, K, Cu, Al and Fe was proportional to growth in both CO₂ treatments. Total uptake and uptake per g plant dry weight of Ca, Mg, Sr, Ba, Zn and Mn were not affected by CO₂ treatment. Bacterial populations differed due to CO₂ only at the final harvest, where there were significantly fewer nitrite-oxidizers and phosphate-dissolving bacteria in the rhizosphere of seedlings grown at 692 $\mu\text{L L}^{-1}$ CO₂.

Introduction

Climatic records from 1958 to the present show a clear pattern of increasing CO₂ in the atmosphere (Solomon *et al.*, 1985). A great deal of debate has centered about the possible dire consequences of the increase (Luther and Cess, 1985). Little actual work has been done on the effects of increasing CO₂ on natural vegetation, even though natural forest systems represent a significant potential sink for additional CO₂.

Increases in crop plant biomass as a result of exposure to elevated levels of atmospheric CO₂ are well documented (Kimball, 1983). However, agricultural systems are typically well-supplied with nutrients and water, unlike forests where availability of one or more nutrients and/or water may be limited. Unless mechanisms exist by which forest trees in infertile soil can increase uptake of, or decrease demand for, limiting nutrients, long-term growth increases will probably not occur (Kramer, 1983). Forest species could more effectively exploit nutrient pools at elevated CO₂ levels through increased root growth and soil exploration, through internal physiological adjustments that increase nutrient-use efficiency, or through changes in root microbial populations that are involved in nutrient transformations and plant nutrient uptake (Norby *et al.*, 1986 and Fig. 1.2).

One mechanism for maintaining nutrient status under elevated CO₂ would be an increase in root growth, resulting in exploration of a larger volume of soil and greater surface area for absorption. Increased growth of roots, especially fine roots, has been seen at elevated CO₂ in white oak, *Quercus alba* L. (Norby *et al.*, 1986). However, the maximum size of a tree's root system is influenced by genetic limits, climatic and

seasonal patterns, competition from other plants, and edaphic factors, so additional mechanisms might be necessary to insure sufficient nutrients to support increased growth. Internal physiological adjustments that improve the efficiency of use of nutrients, thus decreasing demand, would be one such mechanism.

The third mechanism hypothesized to increase nutrient acquisition relates to changes occurring in the rhizosphere - that part of the soil which is influenced by the root and which supports microbial populations that are greater in number and often different in composition than the soil at large. Interactions between rhizosphere flora and host plant occur, and can range from mutualistic symbioses (mycorrhizae and nitrogen-fixers) through more facultative associations, to parasitic or pathogenic relationships. Consequences to the host plant in terms of nutrient availability, disease resistance or susceptibility, and energy expenditure vary, and in most cases are not fully understood. Most of the microorganisms in the rhizosphere have in common some degree of dependence upon the root as a source of easily metabolized carbon compounds from root exudates, sloughed-off root cap cells, mucigel, or intact root cells. It is this microbial dependence upon products of photosynthesis combined with the ability to transform nutrients and increase plant nutrient uptake that would contribute to forest tree response to elevated CO₂.

Any environmental factor that affects photosynthesis can potentially affect rhizosphere function. For example, a direct relationship between photosynthesis and denitrification in the rhizosphere has been demonstrated; furthermore, differences in denitrification rates can be linked to differences in amino acid content of root exudates

(Scaglia *et al.*, 1985). Other microbial processes such as ammonification, nitrification, phosphate solubilization, and nitrogen fixation could be affected by changes in root exudation patterns resulting from increased CO₂ in the atmosphere. Rhizosphere populations have been shown to respond to light, temperature, and foliar fertilization (Rouatt and Katznelson, 1960; Rouatt *et al.*, 1963; Fenwick, 1973; Van Vuure and Delange, 1978; Vraný 1963), so there is reason to believe that elevated CO₂ would also have an effect.

In order to examine responses of rhizosphere populations to elevated atmospheric CO₂, we measured total numbers of bacteria in the rhizosphere and in non-rhizosphere soil. In addition, numbers of nitrifying and phosphate-dissolving bacteria (PDB) were determined, as these two groups were predicted to respond differently to changes in root exudate patterns. It is generally thought that nitrifying bacteria are not stimulated in the rhizosphere (Alexander, 1961), although increased numbers of ammonium-oxidizers were found in the rhizospheres of plants grown in coal mine spoils, a severely nutrient-limited system (Fresquez and Lindemann 1982). In contrast, bacteria capable of *in vitro* solubilization of insoluble phosphate are present in much greater numbers in proximity to roots (Katznelson *et al.*, 1962; Paul and Rao 1971). Several studies have shown increases in P uptake by plants grown in the presence of PDB (Azcón *et al.*, 1976; Raj *et al.*, 1981), although increased P nutrition observed with PDB could result from production of plant growth hormones by the bacteria rather than from solubilization of insoluble phosphates (Tinker and Sanders 1975).

In this paper we present results on seedling growth, nutrient uptake, and

rhizosphere bacterial populations in yellow poplars grown in ambient and elevated CO₂ atmospheres, and demonstrate that even in soils of apparent low nutrient availability, increased growth can occur with CO₂ enrichment.

Materials and Methods

Root observation boxes

In order to make repeated rhizosphere sampling possible, 16 root observation boxes, 40 x 30 x 10 cm, were constructed of a clear plastic material (Plexiglas). Three of four sides and the bottom (which was provided with drainage holes) were painted with flat black paint to exclude light. The fourth face was clear, for root observation, and removable, for rhizosphere sampling. Between harvests, this face was covered with black plastic. All boxes were canted at an angle of 22° towards the removable face to encourage root growth along this panel. Boxes were uniformly packed with nutrient-poor soil (Typic Dystrocrept, shaly silt loam A horizon) from a ridge-top site on the Oak Ridge Reservation. Soil was analyzed for chemical content before use and was considered to be deficient in total N (0.135%). Extractable ion concentrations were P, 7 µg g⁻¹; K, 111 µg g⁻¹; Ca, 1030 µg g⁻¹; and Mg, 99 µg g⁻¹. Eleven-week-old *Liriodendron tulipifera* L., started from seed in commercial potting medium and selected for uniform size, were planted in 12 of the 16 boxes. The remaining four boxes were left unplanted to provide a source of non-rhizosphere soil. Planted and unplanted boxes were randomly assigned to controlled environment chambers (26°C day, 10°C night, photosynthetic photon flux

density $450 \mu\text{mol m}^{-2} \text{s}^{-1}$, 16-h photoperiod). Chamber CO_2 levels were programmed to maintain either ambient or $700 \mu\text{L L}^{-1}$; actual mean CO_2 concentrations during the simulated day period for the duration of the experiment were $367 \pm 42.5 \mu\text{L L}^{-1}$ (ambient) and $692 \pm 37.2 \mu\text{L L}^{-1}$. The chamber monitoring and control system has been previously described (Norby *et al.*, 1984).

Seedling growth and nutrient status

After 24-weeks' exposure to either ambient or $692 \mu\text{L L}^{-1} \text{CO}_2$, seedlings were removed from the root boxes, and following rhizosphere sampling (see below), were separated into leaf, stem and root tissues. Leaves were counted and total leaf area was measured with a Li-Cor 1600 Area Meter. Premature leaf abscission occurred in both CO_2 treatments. Since we could not determine from which seedling individual leaves fell, abscised leaves were not included in determinations of dry weight, leaf area and nutrient content. Stem height was measured from the cotyledonary node to the base of the apical bud; stem diameter was measured at 5 cm intervals along this same length. Oven-dry weights on roots, stems and leaves were determined following drying at 75°C for two days. Plant tissues were ground in a Wiley mill, sent to the Soil Testing and Plant Analysis Laboratory at the University of Georgia and analyzed for N, S, P, K, Ca, Mg, Fe, Al, B, Cu, Zn, Sr and Ba.

Rhizosphere sampling

Samples for microbial assay were taken approximately every 6 weeks during the 24-week period, as well as at the final whole-plant harvest. On interim sampling dates, the front panel in each box was removed and approximately 0.5 g (fresh weight) of terminal feeder roots plus adhering soil (defined for this experiment as the "rhizosphere") were carefully removed and placed immediately in a tared vial of sterile distilled water. At the final harvest the 0.5 g rhizosphere sample for each seedling was a composite over the entire root system, and was processed in the same manner as those from interim samplings. On all sampling dates, non-rhizosphere soil was taken from the unplanted boxes after removal of the side panel and consisted of approximately 1 g (wet weight) of soil which was a composite from five sites within the soil block. Additional bulk soil and rhizosphere samples were taken at each harvest and weighed, oven-dried and reweighed to obtain a fresh-to-dry weight ratio. All 16 boxes were sampled on each sampling date.

Microbial assays

Vials containing roots or bulk soil were weighed immediately after sampling to determine fresh weight of sample. They were then emptied into sterile distilled water blanks containing a few drops each of Tween 80 and an anti-foaming emulsion and shaken for 10 min on a reciprocal shaker to disperse bacteria. The volume of blanks were precalculated so that additions of 0.5 g (fresh weight) root plus rhizosphere soil or 1.0 g (wet weight) bulk soil would result in a 10^{-2} dilution. A 19 ml aliquot of the resulting bacterial suspension was removed and fixed with 1 ml formalin for total bacteria counts

at a later time (Daley and Hobbie 1975).

Nitrifier populations were estimated by a Most-Probable-Number (MPN) method (Alexander and Clark 1965). Five 1 ml aliquots of each dilution in a dilution series were placed in liquid media containing either ammonium-N or nitrite-N and incubated at 23°C for 2 weeks. For three of the four sampling dates, a 10-fold dilution series was used for both ammonium- and nitrite-oxidizers. This series proved insensitive to the low numbers of nitrite-oxidizers present, so a two-fold series was used at the final harvest for this group only. The presence of NO_2^- ions in the cultures was determined by addition of Griess-Ilosvay reagent after incubation (Alexander and Clark 1965). MPN tables were used to estimate the numbers of nitrifiers per gram dry weight sample based on assay results (Fischer and Yates 1963).

Numbers of PDB per gram dry weight of sample were also estimated by MPN, using a modified multipoint inoculation technique (Kauri 1980). A glucose-amended soil extract agar with precipitated $\text{Ca}_3(\text{PO}_4)_2$ was used to culture the bacteria (Katznelson *et al.*, 1962). Cycloheximide was added (final concentration 40 $\mu\text{g ml}^{-1}$) to retard fungal growth. With this method, simultaneous inoculation of five replicate sites of five levels each in a dilution series produced a five-by-five grid of inoculated sites on each agar plate. Five replicate plates were prepared for each sample. After incubation for two weeks, the number of sites with PDB was identified by the presence of a clear halo surrounding the colony, indicating dissolution of $\text{Ca}_3(\text{PO}_4)_2$. A modified MPN table was used to estimate population levels (Kauri 1980; Fisher and Yates 1963).

Total numbers of bacteria per gram bulk soil or rhizosphere were determined by

staining 0.1 ml of bacterial suspension with acridine orange, concentrating the sample by filtration through an Irgalan Black-stained Nucleopore filter and counting under an epifluorescence microscope (Daley and Hobbie 1975). Fifteen fields on each of two replicate slides were counted. Bacterial populations were probably over-estimated using acridine orange, since this stain tends to adsorb to suspended clay particles, resulting in high levels of background fluorescence, which makes bacterial cells difficult to distinguish from particulates (Babiuk and Paul 1970). The formalin-fixed samples were counted approximately one week after harvest.

Data analysis

Seedling growth and nutrient content were analyzed using one-way ANOVA. Variances of bacterial data were not homogeneous, so data were log-transformed before analysis by ANOVA and Duncan's Multiple Range Test. Numbers of all bacterial groups measured in bulk soil were not significantly different due to CO₂ at any harvest, so these data were pooled within each harvest date.

Results

Seedling growth

After exposure for 24 weeks to elevated or ambient CO₂, seedlings grown at 692 $\mu\text{L L}^{-1}$ had greater leaf and root dry weights, and slightly greater cotyledonary node diameters (Table II.1). Total dry weight of plants in the enriched CO₂ atmosphere was

Table II.1. Yellow-poplar seedling growth at two levels of atmospheric CO₂. *P* is the probability that differences between means are not due to CO₂ treatment (n=6). Stem height was measured from cotyledonary node to base of apical bud. Stem diameter was measured 5 cm above cotyledonary node.

	CO ₂		Increase (%)	<i>P</i> =
	367 $\mu\text{l l}^{-1}$	692 $\mu\text{l l}^{-1}$		
Leaf number	35.0	41.5	18.6	>0.20
Leaf dry weight, g	11.6	19.6	69.0	0.06
Stem dry weight, g	9.8	13.4	36.7	0.11
Root dry weight, g	14.2	28.3	99.3	0.002
Total dry weight, g	35.6	61.4	72.5	0.015
Leaf area, cm ²	1770	2427	37.1	0.18
Leaf area ratio, cm ² g ⁻¹	48.5	39.5	-18.6	0.09
Specific leaf area, cm ² g ⁻¹	160	126	-21.2	0.02
Stem height, cm	58.5	62.8	7.4	>0.20
Stem diameter, cm	0.81	0.97	19.8	0.09
Stem density, g cm ⁻¹	0.97	0.88	-9.3	>0.20
Root-to-shoot ratio	0.71	0.94	32.4	0.13

173% that of plants grown at ambient levels; root dry weight was increased by 99%. Specific leaf area (leaf area per unit leaf dry weight) decreased 21% with elevated CO₂. The mean root-to-shoot ratio of seedlings grown at 692 $\mu\text{L L}^{-1}$ was 32.4% greater than seedlings grown at ambient CO₂, although this difference was only significant at $P=0.13$. There were no significant differences in leaf number, total leaf area, stem dry weight, height, or leaf area ratio (leaf area per gram total plant dry weight). Stem volume and density, calculated from weight, height and diameter data, were not different between the two treatments.

Seedlings in both CO₂ treatments became infested to varying degrees with a foliar fungal parasite, probably a powdery mildew, soon after being placed in the chambers. Repeated foliar applications of a broad-spectrum fungicide, chlorothalonil, failed to completely eradicate the pathogen. The exposed soil surface was covered during these treatments to minimize effects of the fungicide on soil microbial populations. There was no visual difference in severity of infection between seedlings grown in ambient or 692 $\mu\text{L L}^{-1}$ CO₂.

Nutrient uptake

There were three general patterns of whole-plant nutrient uptake in yellow-poplars exposed to the two levels of CO₂ (Table II.2). Seedlings were not analyzed for nutrient content when exposure to CO₂ was initiated, nor was nutrient content of abscised leaves determined (see Methods), so actual uptake by seedlings during the exposure could not be precisely calculated. Seedlings were selected for uniform size, however, so final net

Table II.2. Nutrient content and concentrations of yellow-poplar seedlings following 24 weeks of exposure to ambient or elevated CO₂. Categories were determined by CO₂ response (see text for details). *P* is the probability that there was no difference due to CO₂ concentration.

Category	Element	CO ₂ (μl l ⁻¹)		Increase (%)	P
		367	692		
Total nutrient content (mg) per plant					
I	N	544	636	16.9	>0.20
	S	76	102	34.2	0.13
	B	0.50	0.74	48.0	0.06
II	P	61.0	86.0	41.0	0.05
	K	479	790	64.9	0.02
	Cu	1.03	1.71	66.0	0.05
	Al	22.3	40.4	81.2	0.03
	Fe	9.8	18.7	90.8	0.006
III	Ca	258	401	55.4	0.06
	Mg	86.0	129.0	50.0	0.07
	Sr	0.80	1.20	50.0	0.07
	Ba	3.0	4.5	50.0	0.06
	Zn	2.3	3.8	65.2	0.09
	Mn	12.0	19.0	58.3	>0.20
Plant nutrient concentration (mg g ⁻¹ dry matter)					
I	N	15.4	10.3	-33.1	0.002
	S	2.14	1.67	-22.0	0.001
	B	0.014	0.012	-14.3	0.020
II	P	1.87	1.42	-24.1	0.10
	K	13.9	12.9	-7.2	>0.20
	Cu	0.030	0.027	-10.0	>0.20
	Al	0.672	0.646	-3.9	>0.20
	Fe	0.312	0.307	-1.6	>0.20

Table II.2 (continued)

Category	Element	CO ₂ (μl l ⁻¹)		Increase (%)	<i>P</i>
		367	692		
III	Ca	7.04	6.05	-14.1	0.12
	Mg	2.39	2.11	-11.7	0.08
	Sr	0.023	0.019	-17.4	0.11
	Ba	0.090	0.074	-17.8	>0.20
	Zn	0.068	0.060	-11.8	>0.20
	Mn	0.341	0.317	-7.0	>0.20

nutrient content was used as an estimate of relative uptake. N, S, and B (Category I) concentrations were significantly lower in seedlings grown at $692 \mu\text{L L}^{-1}$ than at ambient. Total plant content of these nutrients was not affected by CO_2 concentration, demonstrating increased growth without a concomitant increase in nutrient uptake for these elements. The second group, including P, K, Al, Cu, and Fe, were not different in concentration due to CO_2 , but were found in greater total amounts in seedlings from the elevated CO_2 treatment, indicating that uptake of these nutrients was proportional to C fixed. Category III elements Ca, Mg, Ba, Sr, Zn, and Mn showed no statistically significant differences in either concentration or total net content, although the seedling content of Ca, Mg, Ba, and Sr at $692 \mu\text{L L}^{-1}$ tended to be greater ($P \leq .07$) than content at ambient CO_2 , so these elements could be included with nutrients in the second group.

Rhizosphere bacteria

Variability of microbial measurements was extremely high between replicate seedlings, making detection of significant differences difficult. Effects of CO_2 concentration on rhizosphere populations were seen only at the final harvest (Table II.3). Comparing means of rhizosphere samples only (non-rhizosphere soil samples were not included for this analysis), both PDB and nitrite-oxidizers (NO_2OX) were significantly lower at $692 \mu\text{L L}^{-1} \text{CO}_2$ at this harvest. Numbers of total bacteria and ammonium-oxidizers (NH_4OX) were not different due to CO_2 , although they also tended to be lower at elevated CO_2 for this harvest.

A significant rhizosphere effect was seen at three out of four samplings for PDB

Table II.3. Numbers of total bacteria, phosphate-dissolving bacteria (PDB), ammonium-oxidizers (NH₄OX), and nitrite-oxidizers (NO₂OX) in the rhizosphere of yellow-poplars at the final harvest. Analysis for this table did not include data from nonrhizosphere soil samples. Values given below are means of six seedlings per CO₂ level and resulted from ANOVA performed on log-transformed data. *P* is the probability that differences between means are not due to CO₂ treatment.

CO ₂ (μl l ⁻¹)	Number per g dry weight sample			
	Total bacteria (x 10 ⁸)	PDB (x 10 ⁷)	NH ₄ OX (x 10 ³)	NO ₂ OX (x 10 ²)
367	5.6	3.0	3.1	8.3
692	4.7	1.5	1.1	4.6
<i>P</i> =	<0.20	0.05	>0.20	0.02

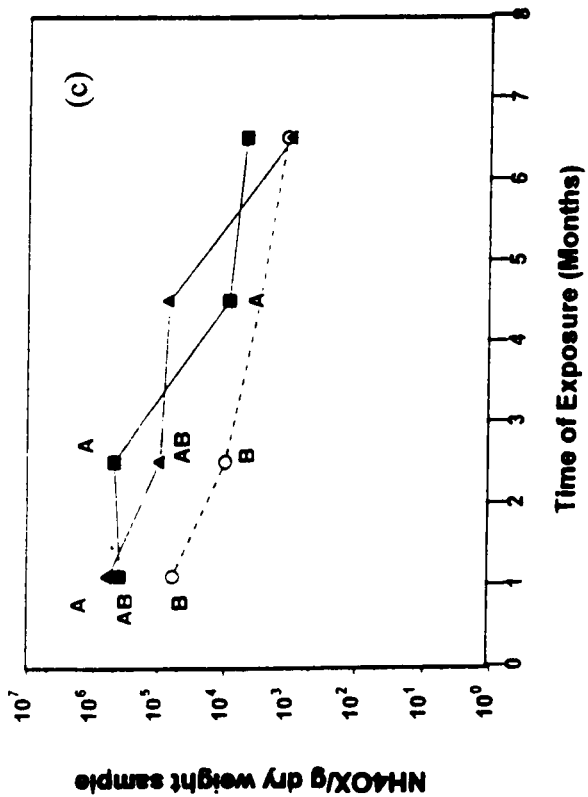
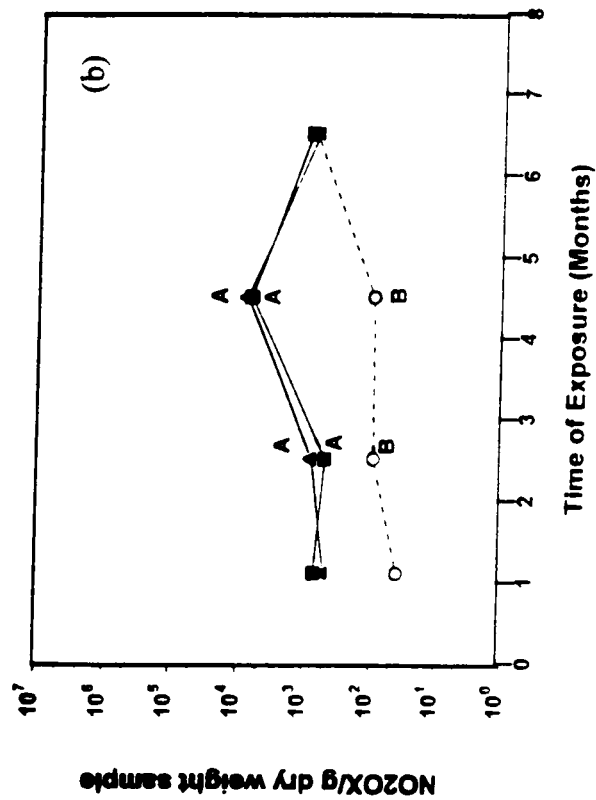
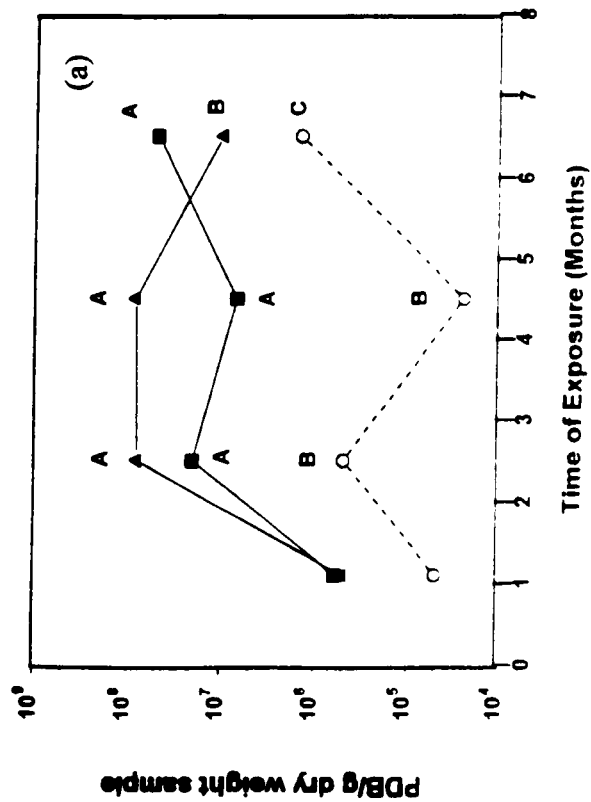
[Fig. II.1(a)] and at two of the four sampling dates for NO₂OX [Fig II.1(b)]. Rhizosphere populations were generally greater than populations in bulk soil for all groups measured, although again high variability tended to obscure differences.

Of all groups measured, only PDB maintained rhizosphere population levels significantly higher than bulk soil levels for the duration of the experiment [Fig. II.1(a)]. Other groups fell to near or below numbers for bulk soil by the final harvest. NH₄OX populations decreased steadily throughout the course of the experiment in both CO₂ treatments [Fig. II.1(c)]. NO₂OX, however, increased in number through the third sampling before declining [Fig. II.1(b)]. Total bacteria [Fig. II.1(d)] increased initially then declined sharply, with final rhizosphere population levels only 10% of those remaining in bulk soil, regardless of CO₂ concentration.

Discussion

Growth of yellow-poplar seedlings was clearly enhanced by exposure to elevated CO₂. Significant differences were seen only in dry weights, however, with no significant differences ($P \leq 0.05$) in height, stem diameter, leaf number or total leaf area. No measurements were made of root length, so differences in root dry weight could have been either a reflection of increased storage tissue or changes in numbers of fine roots. Yellow-poplar root systems seen through the clear panel of the observation boxes and at the final harvest of this study did not consistently appear to be more extensive in the seedlings grown at elevated CO₂, so increased storage of C may have been primarily responsible for the dry weight differences in roots.

Figure II.1. Numbers of bacteria per g sample in nonrhizosphere soil and in the rhizospheres of yellow-poplars grown in ambient or elevated CO₂ for 28 weeks. Means with the same letter within harvest are not different at $P \leq 0.05$. Harvests where no differences were observed are not lettered. (a) Phosphorus-dissolving bacteria; (b) Nitrite-oxidizing bacteria; (c) Ammonium-oxidizing bacteria; (d) Total bacteria. **Solid square**, yellow-poplar rhizosphere (367 $\mu\text{l l}^{-1}$ CO₂); **solid triangle**, yellow-poplar rhizosphere (692 $\mu\text{l l}^{-1}$ CO₂); **open circle**, nonrhizosphere soil.



The decrease in specific leaf area observed at 692 $\mu\text{L L}^{-1}$ indicates that excess carbon storage may also have occurred in leaves, although decreases in specific leaf area in elevated CO_2 atmospheres can also result from the addition of an extra layer of palisade cells (Thomas and Harvey 1983). Starch accumulation in leaves can result from an inability to utilize photosynthate when plants are deficient in N (Epstein 1972), and has been previously observed in herbaceous species grown in elevated CO_2 (Baker and Enoch 1983). Seedlings from both CO_2 treatments showed visible symptoms of N deficiency. A range of 1.9-3.0% leaf N (dry weight basis) has been considered to be in the "medium" range for *Liriodendron* (Leaf 1973). Using this criterion, both treatments (ambient leaf N = 1.70%; elevated leaf N = 1.26%, dry weight basis) would be classified deficient-to-low in N. However, in spite of an apparent deficiency in N, yellow-poplar seedlings were able to increase growth in response to enrichment with CO_2 , suggesting increased nitrogen-use efficiency, possibly through internal recycling of N from older leaves to actively growing tissue (Baker and Enoch 1983).

In contrast to N, S, and B, Category II elements were taken up in greater amounts at 692 $\mu\text{L L}^{-1}$, and, as tissue concentrations were not significantly lower than at ambient CO_2 , sufficient P, K, and other Category II nutrients must have been available for uptake. It is tempting to conclude that stimulation of PDB populations in the rhizosphere at elevated CO_2 may have contributed to the increased uptake of P, but a more likely explanation is that uptake was enhanced by increases in soil exploration by larger root systems. Increased mycorrhizal colonization could also have been a factor, as mycorrhizae have been shown to increase uptake of many of the nutrients in Categories II

and III (Gray and Gerdemann 1967; Harley and Smith 1983; Routien and Dawson 1943). Recent experiments in our laboratory have indicated that mycorrhizal colonization and development are stimulated by elevated CO₂ (O'Neill *et al.*, 1985).

Rhizosphere bacterial populations did not respond to conditions of elevated CO₂ as predicted. High variability between plants independent of CO₂ treatment made interpretation of rhizosphere results difficult. In addition, the presence of the foliar fungal parasite on seedlings from both CO₂ levels probably affected root exudation. Reduction in photosynthetic efficiency, increased leaf respiration and increased oxidation of leaf carbohydrates have been shown to occur with powdery mildew infestation and could be expected to affect availability of photosynthate for translocation and subsequent exudation (Allen 1942).

The trend towards decreases in rhizosphere populations through time is probably a function of decreased nutrient availability as competition with seedling roots increased. Reduced numbers of all bacterial groups at 692 µL L⁻¹ occurred by the final harvest, when the difference in root mass and presumably, the extent of soil exploration, was at a maximum. Supporting this conclusion is the observation that in bulk soil boxes, population levels either did not decline through time or declined less sharply than in boxes with seedlings. Competition for resources in the rhizosphere would be very different under dynamic field conditions than in the closed system represented by the root observation boxes.

In summary, increased biomass production in yellow-poplar seedlings was observed at elevated CO₂ in nutrient-poor forest soil, with or without an increase in

nutrient uptake. In this study, the ability to increase growth in response to CO₂ enrichment in soils with low nutrient availability was related primarily to increased soil exploration by larger root systems and improved nutrient-use efficiency. Contributions of rhizosphere bacteria to growth of forest trees under elevated CO₂ are still unclear; further research is needed to examine not just isolated rhizosphere bacterial responses, but also their response as part of the entire root-rhizosphere system, including mycorrhizae and non-symbiotic rhizosphere fungi.

CHAPTER III

INCREASES IN MYCORRHIZAL COLONIZATION AND SEEDLING GROWTH IN *PINUS ECHINATA* AND *QUERCUS ALBA* IN AN ENRICHED CO₂ ATMOSPHERE

Abstract

Forest tree biomass is hypothesized to increase in a CO₂-enriched atmosphere if mechanisms exist to ensure acquisition of limiting nutrients in forest soils. Investment of additional photosynthate produced at elevated CO₂ into mycorrhizal proliferation and root growth may provide one such mechanism. To test this hypothesis, mycorrhizal density and seedling biomass were measured in shortleaf pine (*Pinus echinata* Mill.) and white oak (*Quercus alba* L.) grown in controlled-environment chambers in unfertilized forest soil at ambient and 700 $\mu\text{L L}^{-1}$ CO₂. Mycorrhizal density was greater at elevated CO₂ in both species after 6 weeks of exposure; in white oak, the increased density persisted for 24 weeks. Root dry weight was increased 76% in *P. echinata* and 91% in *Q. alba* at 700 $\mu\text{L L}^{-1}$ CO₂; total seedling dry weight was increased by 66% and 56%, respectively. It is hypothesized that increased photosynthesis at elevated CO₂ offsets the carbon requirement for mycorrhizal establishment on shortleaf pine. Greater mycorrhizal density and enhanced first-year growth in both species may facilitate future nutrient acquisition, supporting further biomass increases in an enriched CO₂ atmosphere.

Introduction

Available data on atmospheric CO₂ concentrations indicate that levels have risen steadily over the past three decades (Gammon *et al.*, 1985) and are expected to continue to rise, although the magnitude of the increase is uncertain. Predicted concentrations of atmospheric CO₂ may affect global climate, sea level, biology, and human society. However, not all of the consequences of increased CO₂ are dire; productivity of natural vegetation may also increase with additional CO₂ input, if certain limitations can be overcome (Pearcy and Bjorkman, 1983; Oechel and Strain, 1985).

Herbaceous crop species have been shown to respond positively with increased atmospheric CO₂ (Kimball, 1983). Agricultural systems are usually well supplied with nutrients and water, unlike unmanaged forest systems where availability of some critical nutrients may be low. Although some discount the potential for a net increase in biomass of existing trees (Kramer, 1981), mechanisms may exist that would allow native species to fix increased amounts of CO₂ (Luxmoore, 1981). Initial work with woody species suggests that in some cases current limitations may be due to carbon availability rather than to low levels of nutrients such as nitrogen and phosphorus. In experiments conducted in controlled environments with increased atmospheric CO₂ concentrations, both Virginia pine (*Pinus virginiana* Mill.) and one-year-old white oak (*Quercus alba* L.) seedlings were shown to increase in dry weight when grown in enriched CO₂ atmospheres (Luxmoore *et al.*, 1986; Norby *et al.*, 1986). Growth also increased in yellow-poplar (*Liriodendron tulipifera* L.) exposed to 692 $\mu\text{L L}^{-1}$ CO₂ (E. G. O'Neill *et al.*, 1987). In all of these studies, patterns of uptake for some nutrients differed between seedlings grown

at elevated CO₂ and at ambient CO₂. For example, white oak and yellow-poplar seedlings showed increased growth at 692 µL L⁻¹ CO₂ without an increase in nitrogen uptake.

Phosphorus, on the other hand, was taken up proportionately to carbon fixed in these two studies, regardless of CO₂ concentration. One explanation for the ability of seedlings grown at elevated CO₂ to increase biomass while acquiring sufficient phosphorus from a soil considered "low" in available phosphorus would be enhancement of the mycorrhizal association by increased allocation of carbon to roots (Norby *et al.*, 1986).

Mycorrhizae enhance uptake of some nutrients (Harley and Smith 1983; Sanders *et al.*, 1975; Routien and Dawson 1943) and exert a beneficial effect on seedling growth and establishment. Their dependence upon products of photosynthesis is well-documented (Hacskeylo 1973; Harley and Smith 1983). Increased photosynthesis under conditions of CO₂ enrichment results in proportionately greater carbon allocation to roots (Norby *et al.*, 1986; O'Neill *et al.*, 1987), and might lead to increased initial infection by mycorrhizal fungi, to increased proliferation of the fungus following the initial stages of infection, or to a stimulation of mycorrhizal activity. Enhanced mycorrhizal function would satisfy part of the demand for additional nutrients under enriched CO₂ conditions.

Little experimental work has been done on mycorrhizal changes as a result of exposure of plants to elevated CO₂, but mycorrhizal responses to changes in light, temperature and foliar fertilization suggest that any factor that affects plant photosynthesis can affect the symbiosis (Mikola 1948; Hayman 1974; Theodorou and Bowen 1971). Increasing the density of mycorrhizal feeder roots, the biomass of extramatrical hyphae or the total number of mycorrhizal roots on a seedling would

enhance acquisition of relatively immobile nutrients because of greater surface area for uptake (Shemakhanova 1962).

In this paper, we report the results of two experiments that examined the responses of ectomycorrhizae in shortleaf pine (*Pinus echinata* Mill.) and white oak (*Quercus alba* L.) to elevated CO₂. Additionally, we measured seedling growth to see if the degree of mycorrhizal colonization was correlated with growth increases under conditions of elevated CO₂. Researchers have speculated that early mycorrhizal infection is not as beneficial to seedling growth in pine as it is to oaks (Marks *et al.*, 1982; Dixon *et al.*, 1984). Mycorrhizae are thought to be a carbon sink, especially during establishment of the symbiosis, which in oaks may be compensated for by the presence of large cotyledons (Dixon *et al.*, 1984; Shemakhanova 1962). In shortleaf pine, additional carbon fixed at high CO₂ could offset the mycorrhizal demand for photosynthate, resulting in larger, more vigorous 1st-year seedlings.

Methods and Materials

CO₂ monitoring and control system

Experiments were conducted in controlled environment chambers (26°C day, 10°C night, photosynthetic photon flux density 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 14-h photoperiod). Chamber CO₂ levels were programmed to maintain either ambient or 700 $\mu\text{L L}^{-1}$ CO₂; actual mean daytime CO₂ concentrations were $364.0 \pm 35.9 \mu\text{L L}^{-1}$ (ambient) and $682.8 \pm 48.5 \mu\text{L L}^{-1}$ during Experiment 1 and $365.4 \pm 32.9 \mu\text{L L}^{-1}$ (ambient) and $693.8 \pm 25.9 \mu\text{L L}^{-1}$ during Experiment 2.

L⁻¹ during Experiment 2. For simplicity, CO₂ levels throughout this paper will be referred to as 360 µL L⁻¹ (or ambient) and 700 µL L⁻¹. The chamber CO₂ monitoring and control system has been previously described (Norby *et al.*, 1984).

Experiment 1 (Shortleaf Pine)

Seeds of *P. echinata* were germinated in trays of vermiculite in a greenhouse. Six weeks later 60 seedlings that were similar in size and root development were transplanted to individual 945 ml pots filled with fresh, unsterilized soil (Typic Dystrocrept, shaly silt loam A horizon) from an upland hardwood site on the Oak Ridge National Environmental Research Park, Oak Ridge, Tennessee. Soil nutrient data can be found in Norby *et al.*, 1986. Half of the seedlings were randomly chosen for inoculation with a preparation of mycorrhizal spores [*Pisolithus tinctorius* (Pers.) Coker and Couch] adsorbed to the surface of vermiculite particles with water-soluble glue (Forest Industries). The remaining pots were similarly treated with vermiculite particles coated with glue, but free of spores. No attempt was made to maintain these latter plants as nonmycorrhizal; instead they were allowed to become infected by native inoculant present in the soil. In both cases, equal numbers of vermiculite particles were placed just below the soil surface and all pots watered thoroughly to dissolve glue and release spores into the soil-water solution.

Seedlings were randomly assigned to CO₂ treatment and harvest group. Twice a week, pots were returned to field capacity with tap water. No additional nutrients were provided to seedlings in either experiment.

Pines were harvested after 6 or 24 weeks in the chambers. At each harvest, dry weight of roots and shoots, diameter of stems at soil level, and height of seedling to the base of the apical bud were determined. In addition, at 24 weeks, secondary needles were removed from the shoot and weighed separately. All weights were taken after drying for two days at 75°C.

Mycorrhizal assessment was made with the aid of a dissecting microscope. At the first harvest, the length of all roots less than 1 mm in diameter and greater than 5 mm long were measured and the number of mycorrhizal short roots counted. At the final harvest, mycorrhizae were counted on first-order lateral systems to a total length of 200 cm and additionally, the ratio of mycorrhizal short roots to nonmycorrhizal short roots was determined. Data were expressed either as the number of mycorrhizal short roots per cm root length or as the proportion of short roots that were mycorrhizal (24 week harvest only).

Experiment 2 (White Oak)

Fresh *Quercus alba* L. acorns were obtained from the Tennessee Department of Forestry and stored moist at 4°C until used. Plastic pots, 10 x 10 x 35 cm long, tapering to the base and open at the bottom, were fitted with a nylon screen at the bottom and filled with soil from the same location as in Experiment 1. Three acorns were planted in each pot, and pots were randomly assigned to CO₂ treatment and harvest group. Chambers were rotated every 3 weeks. Germination took place at the assigned CO₂ level. For each pot, time zero was recorded as the date of emergence of the first "normal"

seedling, i.e., seedlings that were not stunted, multistemmed, or injured by larval feeding within the acorn. The remaining two seedlings (or acorns) within the pot were carefully removed with forceps as soon as this “normal” seedling was identified. Seedlings were allowed to become mycorrhizal from native inoculum present in soil. Watering regimes were as in Experiment 1; no fertilizer was provided.

Harvests were at 6, 12, 18, 24, and 30 weeks after emergence, with six replicate pots per CO₂ treatment in each harvest group. Tap and fine roots, stems, and leaves were dried for two days at 70°C and weighed. Dormant buds were removed at the final harvest and weighed separately. Stem diameter at soil level, height from soil level to the base of the apical bud and total leaf area were measured at all harvests.

At each harvest, root systems were excised from the shoot, washed free of soil, wrapped intact in moist paper towels and frozen in plastic bags for no more than 2 weeks until mycorrhizae could be counted. Rather than determine mycorrhizal density in terms of number of short roots per unit root length as in Experiment 1, we decided to measure the ratio of mycorrhizal root tips to nonmycorrhizal tips. As mycorrhizal infection was expected to result from a number of morphologically and physiologically different fungal species, counting individual tips was thought to be a more relevant reflection of mycorrhizal contribution to seedling response than counting numbers of short roots (Grand and Harvey 1982). Each root system was thawed in water at room temperature. Roots ≤ 2 mm in diameter were cut into segments approximately 1.25 cm long and placed in a water-filled Pyrex baking dish which had a 1.25 cm grid fixed to the bottom. Segments were spread as evenly as possible to cover the grid with a minimum of over-

lapping. With grid lines serving as coordinates, pairs of random numbers were generated and the root segment closest to the indicated coordinate intersection was removed to a petri dish for counting. Root tips were scored as mycorrhizal or nonmycorrhizal based on gross morphological characteristics as observed through a zoom stereomicroscope. Intact tips were counted in sequence along each segment until a total of 1000 root tips was reached. Mycorrhizal infection could then be quantified as a proportion and analyzed following arcsine transformation.

At the 18, 24 and 30 week harvests, soil was collected from each pot and analyzed for dehydrogenase activity using 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) reduction (Ingham *et al.*, 1985; Klein *et al.*, 1971). This assay is an index of activity of soil microorganisms; in this study, we wished to determine if it could be used as an indicator of the extent to which mycorrhizal hyphae had penetrated the soil. An attempt was made to obtain soil from areas in the root ball with actively growing roots, to minimize dilution effects resulting from differences in degree of soil exploration by roots at the two CO₂ levels. Soil was sieved to remove fine roots and stones, and three replicate 1-g samples tested. An additional 1-g sample from each pot was dried and reweighed to allow expression of results on a per gram dry weight soil basis.

Data Analysis

Data from Experiment 1 were analyzed using analysis of variance (randomized complete block design with 5 replicates per mycorrhizal treatment per CO₂ concentration at the first harvest and 10 replicates at the second). Variances of seedling growth

parameters were not homogeneous, so data were subjected to log-transformation before analysis.

Seedling growth data from Experiment 2 were analyzed using Student's t-test for comparison of means with CO₂ concentration as the only variable; in cases where variance was unequal, log-transformation was performed. Mycorrhizal data from Experiment 2 were analyzed using one-way ANOVA following arcsine transformation. Root and total seedling dry weights were regressed against mycorrhizal density (Experiment 1) and percent mycorrhizal short roots (experiment 2) to see if correlations could be found between seedling biomass and mycorrhizal response to elevated CO₂. In Experiment 2, percent mycorrhizal short roots was regressed against dehydrogenase activity ($\mu\text{g formazan g}^{-1} \text{ soil}$) for each harvest beginning at 16 weeks.

Results

Experiment 1 (Shortleaf Pine)

At the 6-week harvest the number of mycorrhizal short roots per unit root length was twice as great at 700 $\mu\text{L L}^{-1}$ CO₂ as at 360 $\mu\text{L L}^{-1}$ CO₂ (Table III.1). In both CO₂ treatments, seedlings inoculated with *Pisolithus* had significantly more mycorrhizae than seedlings exposed only to native inoculum. However, after exposure for 24 weeks in the chambers, there was no longer any difference between CO₂ treatments either in numbers of mycorrhizal short roots per unit root length or in the proportion of short roots that were mycorrhizal. Inoculated and uninoculated seedlings were equally mycorrhizal at this

Table III.1. Seedling physical parameters and mycorrhizal colonization in shortleaf pine (*P. echinata*) at two atmospheric concentrations of CO₂. M = Inoculated with *P. tinctorius*; X = not inoculated. Inoculated and uninoculated seedlings were not different for any parameter at the 24-week harvest, and were pooled for analysis. For each parameter, means within harvest dates with the same letter are not significantly different at $P \leq 0.05$.[†]

	CO ₂ ($\mu\text{L L}^{-1}$)	6-week harvest		24-week harvest
		Inoculated	Not inoculated	(pooled)
Root weight, g	360	0.050±0.007 ^b	0.064±0.008 ^{ab}	0.46±0.02 ^c
	700	0.090±0.019 ^a	0.049±0.002 ^b	0.81±0.05 ^d
Shoot weight, g	360	0.055±0.001 ^a	0.078±0.008 ^a	0.86±0.04 ^c
	700	0.071±0.02 ^a	0.078±0.006 ^a	1.39±0.09 ^d
2° needle weight, g	360	—	—	0.05±0.009 ^c
	700	—	—	0.23±0.04 ^d
Total plant weight, g	360	0.105±0.008 ^b	0.142±0.015 ^{ab}	1.33±0.06 ^c
	700	0.161±0.027 ^a	0.127±0.007 ^b	2.20±0.13 ^d
Root:shoot	360	0.90±0.11 ^a	0.83±0.09 ^a	0.55±0.03 ^c
	700	2.55±1.6 ^a	0.64±0.05 ^a	0.61±0.04 ^c
Root length, cm	360	63.4 ± 3.9 ^a	110 ± 27 ^b	—
	700	98.8 ± 14.8 ^b	113 ± 9 ^b	—
Stem height, cm	360	5.98 ± 0.16 ^a	6.68±0.51 ^{ab}	10.0±0.38 ^c
	700	7.52 ± 0.28 ^b	6.58±0.21 ^{ab}	11.6±0.57 ^d
Stem diameter, mm	360	—	—	2.04±0.10 ^c
	700	—	—	2.38±0.10 ^d
Mycorrhizae/cm root	360	0.651±0.28 ^b	0.154 ± 0.043 ^b	4.16±0.17 ^a
	700	1.34±0.29 ^a	0.381±0.133 ^b	4.37±0.20 ^a
% mycorrhizal short roots	360	—	—	78.9±1.2 ^a
	700	—	—	78.6±0.99 ^a

[†]Values are means ± SE. Inoculated and uninoculated seedlings were not different for any parameter measured at the 24-week harvest, so data were pooled for analysis. For each parameter, means within harvest dates with the same letter are not significantly different at $P \leq 0.05$.

harvest. Some cross-contamination with *Pisolithus* was noted at both harvests, but uninoculated seedlings formed several other morphologically distinct mycorrhizas. Inoculation with *Pisolithus* combined with exposure to elevated CO₂ increased root weight, total plant weight, and seedling height as early as 6 weeks after initiation of CO₂ exposure (Table III.1). Only the CO₂ by mycorrhizal treatment interaction was significant; neither elevated CO₂ nor mycorrhizal treatment alone increased growth significantly at this harvest. However, by 24 weeks emergence most growth parameters measured were significantly greater owing to elevated CO₂. Total seedling dry mass was 66% greater in seedlings grown at 700 $\mu\text{L L}^{-1}$. Root dry mass was increased 76% by elevated CO₂, although root-to-shoot ratios were not affected. Secondary needles were evident at 700 $\mu\text{L L}^{-1}$ CO₂ several weeks before their appearance on seedlings grown at ambient and were nearly four times greater in dry weight at elevated CO₂ at 24 weeks.

Experiment 2. (White Oak)

As in the shortleaf pine study, mycorrhizal response to elevated CO₂ was evident before significant growth effects were seen (Figure III.1). Infection was sparse at the 6 week harvest, but was higher at 700 $\mu\text{L L}^{-1}$ CO₂ than at ambient ($P = 0.11$). From 12 to 24 weeks, mycorrhizal colonization was significantly greater on roots in the elevated CO₂ treatment. Again reflecting results from the pine study, colonization reached a maximum at 24 weeks and by 30 weeks was not significantly different due to CO₂ treatment. Many of the mycorrhizal tips seen at 30 weeks were apparently senescent and in some cases, were secondarily infected by a mycorrhizal fungus presumed to be *Cenococcum*

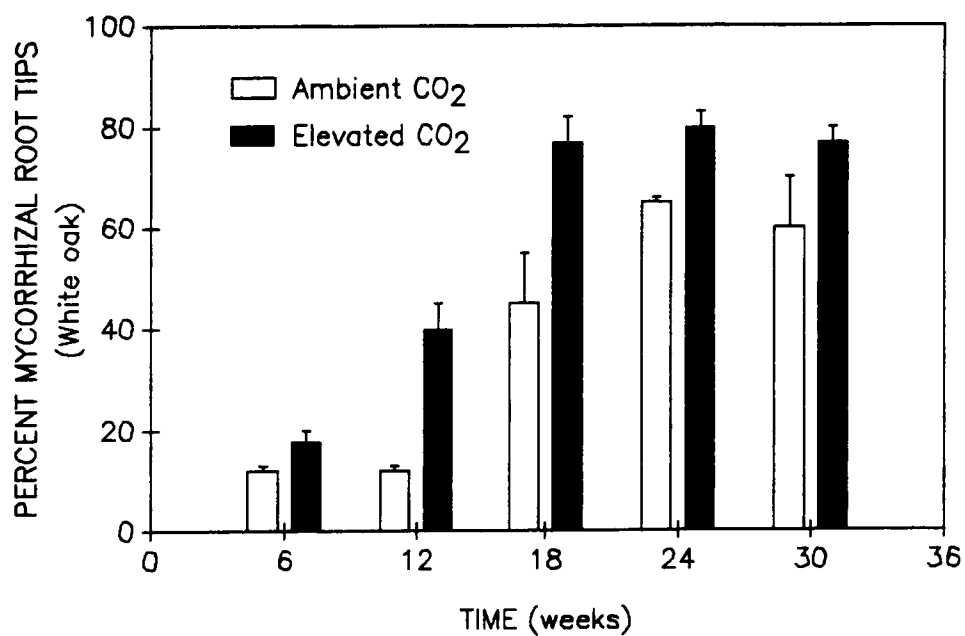


Figure III.1. Proportion of mycorrhizal roots on white oak seedlings grown for 6 to 30 weeks at ambient or 700 $\mu\text{L L}^{-1}$ CO₂. Each data point is a mean ($\pm$ S. E.) of six seedlings.

graniforme (Sow.) Ferd. et Winge. The percentage of mycorrhizal root tips colonized by *Cenococcum* was consistently greater at ambient CO₂ after 6 weeks (Table III.2), although due to high variability the differences generally were not statistically significant. No additional differences were noted in the occurrence of other mycorrhizas in the two CO₂ treatments.

Soil dehydrogenase activity, measured from 18 to 30 weeks (Table III.2), was correlated with mycorrhizal infection ($r = 0.85$). Activity was significantly higher at elevated CO₂, peaked in both treatments at 24 weeks, and was diminished and no longer significantly different between treatments by 30 weeks.

White oak seedlings grown in elevated CO₂ were larger than those in ambient CO₂ by 18 weeks after emergence (Table III.3). Total root weights, however, were significantly higher in seedlings grown at 700 $\mu\text{L L}^{-1}$ as early as the 12-week harvest. Initially, dry weights of fine roots (≤ 2 mm) were greater at elevated CO₂, with no significant difference in tap root mass. By 24 weeks, dry weights of tap roots were significantly greater at 700 $\mu\text{L L}^{-1}$ CO₂. At no time was there a difference in the proportion of total root mass represented by fine or tap roots due to CO₂ treatment. By 30 weeks postemergence, many seedlings in both treatments had begun a third flush or initiated growth at axillary buds, so variance of leaf parameters was high and precluded detection of significant differences in seedling growth. Dormant bud weights at 30 weeks in seedlings grown at 700 $\mu\text{L L}^{-1}$ were twice that of seedlings grown at ambient, suggesting a potential for continued growth increases in subsequent years (Norby *et al.*, 1986). Root-to-shoot ratios, leaf area, specific leaf area, and seedling height were either

Table III.2. Proportions of mycorrhizal root tips, proportion of *Cenococcum* mycorrhizas, and dehydrogenase activity in rhizosphere soil of white oak.

CO ₂ (μL L ⁻¹)	Harvest (weeks after emergence)				
	6	12	18	24	30
% Mycorrhizae (mycorrhizal root tips/100 root tips)					
360	10.9	12.2	50.2	70.1	62.4
700	19.0	40.8	77.4	84.4	76.0
Pr ₂ F	0.11	0.001	0.05	0.015	>0.20
<i>Cenococcum</i> (<i>Cenococcum</i> -colonized root tips/mycorrhizal root tips)					
360	30.0	67.8	21.8	21.7	17.6
700	36.5	26.8	8.7	9.0	15.2
Pr ₂ F	>0.20	0.004	>0.20	0.09	>0.20
Dehydrogenase activity (μg formazan/g dry weight sample)					
360	—	—	12.48	18.78	13.99
700	—	—	29.53	36.98	18.53
Pr ₂ F	—	—	0.001	0.015	>0.20

Table III.3. Physical parameters of white oak seedlings at two concentrations of atmospheric CO₂.

	CO ₂ ($\mu\text{L L}^{-1}$)	Harvest (weeks after emergence)				
		6	12	18	24	30
Leaf weight, g	360	1.16	1.51	1.45	1.27	1.85
	700	1.37	1.80	2.05*	2.37**	2.01
Stem weight, g	360	0.50	0.73	0.68	0.64	0.86
	700	0.53	0.81	1.05*	1.16**	1.01
Tap root weight, g	360	0.82	1.78	3.66	3.84	4.72
	700	1.20	2.53	5.36	8.42**	9.31
Fine root weight, g	360	0.16	0.28	0.84	0.54	0.77
	700	0.20	0.56**	1.34	2.55	1.71
Total weight, g	360	2.64	4.29	6.54	6.29	8.20
	700	3.30	5.71	9.73*	14.5**	14.0
Bud weight, g	360	—	—	—	—	0.018
	700	—	—	—	—	0.037
Root:Shoot	360	0.79	0.96	2.06	2.55	2.37
	700	0.80	1.22	2.29	3.03	3.31
Leaf area, cm ²	360	227	239	200	166	248
	700	242	269	239	293**	245
Specific leaf area, cm ² g ⁻¹	360	196	157	137	131	130
	700	179	152	118**	124	122
Diameter, mm	360	2.84	3.52	3.02	2.90	3.34
	700	2.90	3.82	3.97**	3.77**	3.55
Height, cm	360	24.0	26.2	24.5	21.6	22.1
	700	24.0	24.6	20.5	25.1	21.2

* Within harvest date, significantly different from value for 360 $\mu\text{L L}^{-1}$ at $P \leq 0.10$.

** Within harvest date, significantly different from value for 360 $\mu\text{L L}^{-1}$ at $P \leq 0.05$.

not significantly affected by CO₂ treatment or differed at only one harvest (Table III.3).

Discussion

In both experiments, mycorrhizal infection was enhanced by exposure of seedlings to elevated CO₂. Enrichment affected not only maximum mycorrhizal density but also rates of infection and proliferation. Differences in mycorrhizal colonization due to CO₂ were seen in both tree species before significant growth responses to CO₂ occurred, suggesting that, initially, additional carbon fixed under enriched conditions was being allocated to mycorrhizal biomass and respiration rather than tree growth. In the shortleaf pine experiment, CO₂ enrichment enhanced root growth at 6 weeks, but only in inoculated seedlings grown at 700 $\mu\text{l l}^{-1}$ CO₂. Roots in this group were 80% greater in dry weight than those grown in the ambient treatments. When growth responses due solely to CO₂ were seen, they also occurred first in roots. Even if mycorrhizae serve as a carbon sink during initial seedling growth, allocation of increased photosynthate fixed under elevated CO₂ to mycorrhizal biomass and then into fine root growth results in increased potential for nutrient and water uptake when trees become established.

Although it appeared from the shortleaf pine results that elevated CO₂ affected the rate of mycorrhizal establishment rather than the final intensity of infection, the white oak sampling through time suggests otherwise. By 24 weeks, white oaks at 700 $\mu\text{L L}^{-1}$ CO₂ still had a significantly higher proportion of mycorrhizal short root tips and higher soil dehydrogenase activity than did seedlings at ambient CO₂. By 30 weeks, however, decreased density of mycorrhizae, decreased dehydrogenase activity, and increased

variability between plants within both treatments suggests that fine roots and mycorrhizas were senescing, possibly due to prolonged pot culture. Pines at 24 weeks (of exposure to CO₂; seedling age actually equivalent to 30 weeks as in oaks) also exhibited symptoms of stress (e.g. chlorotic needles, senescent mycorrhizas, shriveled fine roots, and increased variability) so the apparent lack of difference in mycorrhizal density between CO₂ treatments at the final harvests was most probably the result of prolonged growth in containers.

The higher percentage of *Cenococcum* mycorrhizas at 360 $\mu\text{L L}^{-1}$ CO₂ through most of the white oak experiment and at the final harvests of both experiments has been observed in other elevated CO₂ studies in our laboratory (E. G. O'Neill and R. J. Norby, unpublished data). Work on *Cenococcum* by other researchers has shown it to be a poor competitor when conditions are favorable for other mycorrhizal species, but selected for when sub-optimal conditions exist, such as low light, drought, and extremes of nutrient supply (Mikola 1948; Worley and Hascskaylo 1959; Menge *et al.*, 1977). The recolonization of senesced mycorrhizas by this fungus has been observed previously (Mikola 1948) and may relate to the ability of the fungus to adopt a saprophytic mode of nutrition. *Cenococcum* is thought to be less efficient at phosphorus uptake (Langlois and Fortin 1984) and seedling growth enhancement (Marx *et al.*, 1978), but is capable of utilizing nitrate-N (Ho and Trappe 1980) and may confer some degree of drought resistance upon its host (Read *et al.*, 1977). In the current studies, there was a significant negative correlation between *Cenococcum* frequency (as a percentage of total mycorrhizal root tips) and total seedling dry weight in white oaks grown at 360 $\mu\text{L L}^{-1}$ CO₂ ($r = -0.75$

at 24 weeks and -0.65 at 30 weeks). At these same harvests, in the 700 $\mu\text{L L}^{-1}$ treatment, the correlation between *Cenococcum* and seedling dry weight was also negative, but not statistically significant. In this case, we hypothesize that the correlation does not result from *Cenococcum* effects, but instead reflects lower vigor of seedlings at 360 $\mu\text{L L}^{-1}$ grown for 24 to 30 weeks under conditions of moderate light and restrictive soil volume with low nutrient availability. Seedlings grown at elevated CO_2 might be more vigorous and therefore less likely to be infected by *Cenococcum*.

Seedling growth in both white oak and shortleaf pine was significantly enhanced by elevated CO_2 . Particularly important in terms of future growth potential are the increases in woody tissue weights (stem plus tap root) and dormant bud weights in the white oak experiment. At 700 $\mu\text{L L}^{-1}$ CO_2 , woody tissue dry weight was 85% greater, and dormant bud weights 105% greater than at 360 $\mu\text{L L}^{-1}$. At some harvests at elevated CO_2 , leaf dry weight and area were increased, and specific leaf area decreased. Consistently significant differences were not seen with these variables, since they were highly dependent upon what stage of development seedlings were in at the time of harvest.

Comparison of inoculated pine seedlings at the two CO_2 levels at the 6-week harvest supports the idea that additional carbon fixed at elevated CO_2 tends to offset the cost of mycorrhizal colonization. Inoculated seedlings grown at 360 $\mu\text{L L}^{-1}$ CO_2 had consistently lower dry weights, stem height and root length than uninoculated pines at the same CO_2 concentration and were lower than both inoculated and uninoculated seedlings grown at 700 $\mu\text{L L}^{-1}$ (Table III.1). Inoculated pines at elevated CO_2 , on the other hand, were apparently able to utilize additional photosynthate to support both seedling growth

and mycorrhizal establishment. This is suggested by significantly higher dry weights after 6 weeks and the earlier appearance of secondary needles on pines grown at $700 \mu\text{L L}^{-1}$ CO_2 . In shortleaf pine, significant correlation of dry weights and mycorrhizal density was only seen in the seedlings from the $700 \mu\text{L L}^{-1}$, 6-week harvest group ($r = 0.84$). However, in white oak, correlations of mycorrhizal infection with root and total dry weights were seen beginning at 12 weeks, and at most harvests, were significant ($P \leq 0.05$) at $700 \mu\text{L L}^{-1}$ CO_2 .

Clearly, for both species, longer term experiments are necessary to determine whether increases in biomass and mycorrhizal density observed in seedling experiments continue through subsequent growing seasons. Woody species should also be studied under conditions where growth is not restricted by container size or chamber effects and where seedlings are subjected to normal changes of season. Mycorrhizal populations may vary seasonally, so responses to long-term exposure to elevated CO_2 may differ from those observed in this series of experiments. Increased density of mycorrhizal short roots might not persist in subsequent growth periods, but we feel that the larger, more vigorous seedlings that result from enhanced mycorrhizal populations at elevated CO_2 during 1st-year growth could result in long-term increases in tree biomass even under low nutrient soil conditions.

CHAPTER IV

HIERARCHY THEORY AS A GUIDE TO MYCORRHIZAL RESEARCH ON LARGE-SCALE PROBLEMS

Abstract

Mycorrhizal researchers are faced with the dilemma of designing experiments on small-scale processes that contribute to the solution of large-scale problems, such as global change. Hierarchy Theory provides a paradigm that indicates the need for mycorrhizal research and suggests criteria that help to prioritize research objectives. In general, mycorrhizal research is justified whenever direct or indirect effects on mycorrhizal physiology or ecology form a critical constraint to large-scale system function. The paper attempts to extract the relevant concepts from the theory and provides a series of illustrative examples from mycorrhizal research.

Introduction

Physiological processes are the fundamental mechanisms underlying large-scale ecosystem behavior. This statement is indisputable, but it remains unclear exactly what needs to be understood about small-scale processes to understand or predict the larger scale. Equally important, it is often difficult to justify small-scale research to funding agencies that have adopted an holistic mentality forced upon them by large-scale problems.

Mycorrhizae clearly affect higher-level processes. Mycorrhizae benefit plant growth by several means, the most studied being an increase in the efficiency of phosphorus uptake. In enhancing plant growth, mycorrhizae influence ecosystem production. Therefore, it is logical to assume that mycorrhizae probably affect ecosystem behavior, although there are few cases where this has been clearly demonstrated. In a study involving ectomycorrhizal mat communities in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) stands, concentrations of biologically important minerals were greater in soils associated with ectomycorrhizal mats than in soil from non-mat areas within the same stand. This was attributed to calcium oxalate production by the mycorrhizal fungus *Hysterangium setchellii* (Fisher) [= *Hysterangium crassum* (Tul. and Tul.) Fisher, Sensu, Zeller and Dodge] (Entry *et al.*, 1987). These same mat soils exhibited elevated microbial biomass and increased levels of enzymes responsible for organic matter decomposition (Griffiths *et al.*, 1987). Evidence for the role of mycorrhizae and other soil biota in ecosystem function emphasizes the positive feedback nature of the system. Failure to revegetate an Oregon clearcut was attributed to loss of the soil microbial community (including mycorrhizal inoculum) followed by further revegetation failures leading to increased soil deterioration (Perry *et al.*, 1989). Disruption of the spacial distribution of soil biota was found to influence ecosystem properties even when species numbers and diversity were maintained (Allen & MacMahon, 1985).

However, except for a few examples such as these, little is known about mycorrhizal contribution to ecosystem function because most of the experimental

evidence deals with managed systems (i.e., reforestation sites, experimental plots or plants grown in environmental chambers). Available data on natural systems consist of measurements of current status, such as fruiting body production, spore densities or root tip counts. With almost a total lack of manipulative experimentation or long-term baseline data sets on unmanaged systems, there is no way to assess mycorrhizal contribution to ecosystem function nor any way to determine whether this function is disrupted by large scale, non-destructive perturbations, such as global climate change.

Even while attempts are made to demonstrate the criticality of mycorrhizal mechanisms at the ecosystem level, problems are appearing at even higher scales. Acid precipitation occurs at continental scales. Increasing atmospheric CO₂, disruption of the ozone layer and climate change are occurring at global scales. Is an understanding of mycorrhizal function critical to assessing the impacts of such large-scale changes?

Assuming the need for mycorrhizal research to address large-scale questions is established, exactly what research should be done remains unclear. Each new problem seems to require new and different approaches to mycorrhizal investigations. Although many large-scale questions have been addressed in recent decades, the information that was *needed* on small-scale processes has usually been very different from that which the plant physiologist, microbial ecologist, soil chemist or optimal foraging theorist wanted to *do* next. The criteria that determine the most important question to ask about mycorrhizae at the level of the mycorrhizae are different from the criteria that determine what information about mycorrhizae is needed to address the large-scale problems at hand. At the mycorrhizal level, the research with the greatest potential for incremental

breakthroughs in our understanding might involve biochemical investigations of enzymatic mechanisms for transport across the cell membrane. At higher levels, the most important investigations may involve spore dispersal patterns or survival rates in disturbed soils (Allen *et al.*, 1989).

This difference in small- and large-scale research priorities often causes problems in communication. Therefore, identifying a need for mycorrhizal research in solving large-scale problems is quite different from specifying exactly what is to be done. The purpose of this paper is not to identify areas of mycorrhizal research necessary to answer specific large-scale questions. Instead, a new paradigm is suggested - a way of viewing mycorrhizae in relation to higher-scale processes - that can be a guide to asking the right questions and prioritizing research.

How, then, does one go about asking the right question? And how are priorities determined for small-scale research in support of large-scale problems? The ultimate answer depends on the experience, insight and creative abilities of the individual scientist who sees something no one has previously noticed. Hierarchy Theory provides a set of principles or criteria on the basis of which hypotheses can be formulated and useful research can be designed; these criteria permit recognition of the "right question".

To proceed on this quixotic quest, aspects of Hierarchy Theory that seem relevant to the problem will be reviewed. Some common-sense principles from this review will be extracted and a series of examples presented to show how these principles might be applied to the process of prioritizing mycorrhizal research.

Hierarchy Theory Revisited

Hierarchy Theory begins with the empirical observation that complex systems are often arranged in hierarchies (Simon, 1962; Whyte *et al.*, 1969; Mesarovic *et al.*, 1970; Weiss, 1971; Pattee, 1973). This hierarchical organization seems particularly clear in ecosystems (Overton, 1975; Webster, 1979; Allen & Iltis, 1980; O'Neill & Waide, 1981; Allen & Starr, 1982). The concept of "levels of organization" has long been a part of our biological paradigm (O'Neill *et al.*, 1986): cells are organized into organs, organs into functional units (e.g. the digestive system), systems into organisms, and so on.

Hierarchy theory elaborates on this paradigm by noting that the levels in the hierarchy are distinguished by differences in rates. The lower levels (e.g. cells) function at rapid rates. Higher levels (e.g. organisms) are largely unconscious of this normal, lower-level activity and operate at slower rates. Still higher levels (e.g. the global environment) operate at such slow rates that they are regarded as constant background and are ignored.

When scientists study this hierarchical system, a level is chosen on which to concentrate. This focal level becomes the next most important factor of the theory. The level of interest defines the time and space scales of observation (Allen *et al.*, 1987). Specifying this level, from a hierarchical perspective, is crucial because it determines the resolution of the observations. By way of explanation, consider an observer, moving from the landscape level to the mycorrhizal level [Fig. VI.1(a-d)]. Looking down at the landscape, the observer is restricted by the relatively large temporal and spacial scale to a very coarse degree of resolution. As a result, little information can be gained about

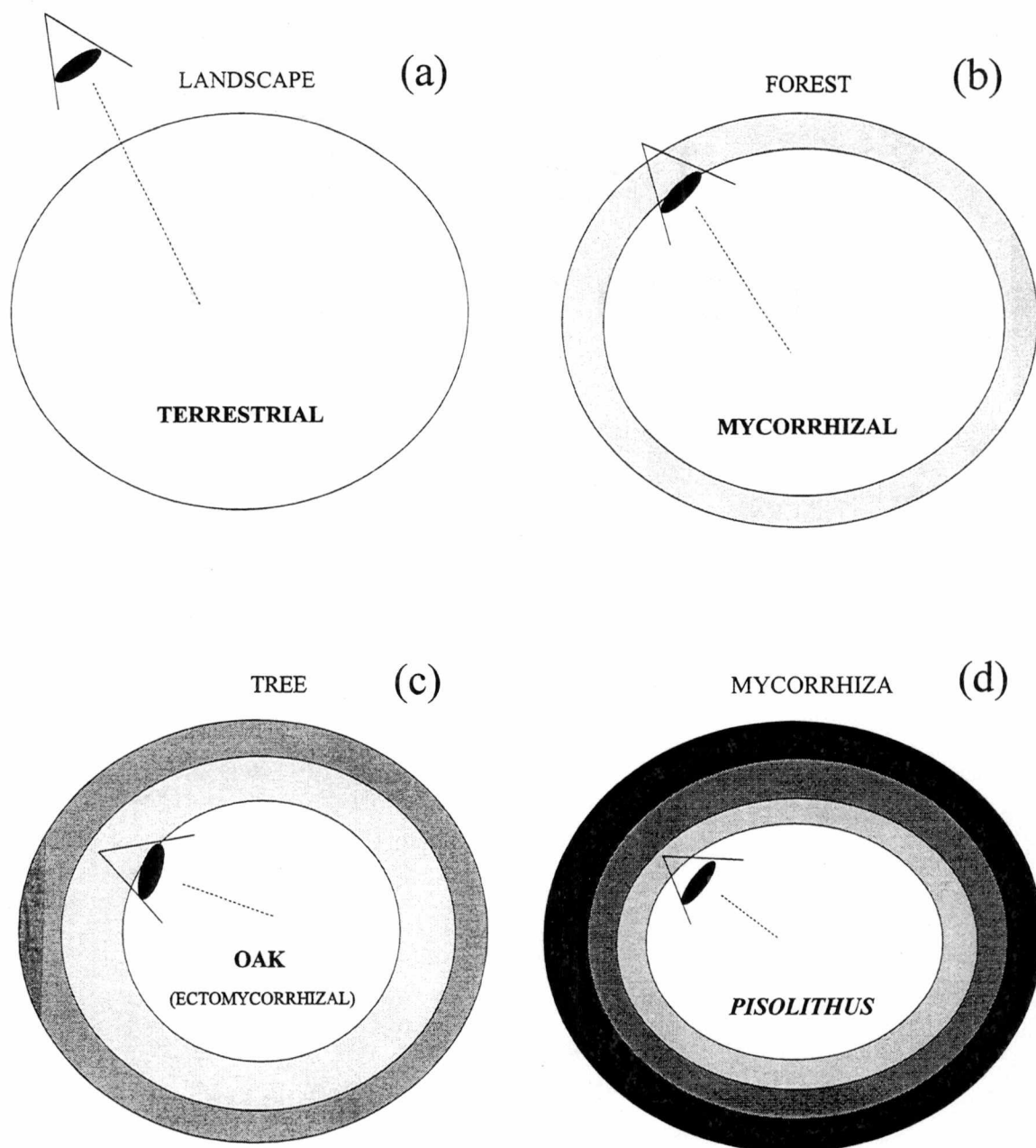


Figure IV.1. Conceptual diagram of the effect of moving from a higher hierarchical level to a lower one. The "eye" indicates the level, and the dotted line the view, of the observer (modified from Allen *et al.*, 1987). The text within each inner oval identifies what can be observed at that level. (a) Landscape level; (b) forest (ecosystem) level; (c) tree (organism) level; (d) mycorrhizal level.

mycorrhizae other than that arising from the distinction between terrestrial and aquatic systems [Fig. IV.1(a)]. If the focus narrows to a temperate forest ecosystem, the information available increases [Fig IV.1(b)]. The observer now knows that mycorrhizae are present as a component of the forest. But until a still lower level is observed, even the ecto- versus endomycorrhizal status of the trees cannot be predicted. Only at the single-tree scale of resolution is this information available. The observer may now determine that the tree is an oak, and therefore ectomycorrhizal, and can make certain inferences and predictions based on this knowledge [Fig. IV.1(c)]. At an even lower level of resolution, different species of ectomycorrhizal fungi might be distinguished [Fig. IV.1(d)]. Each change in level is accompanied by a change in the amount of information available and the kinds of observation used to measure the behavior at that level.

One of the principles arising from this change in resolution with movement across scales is that ordinarily, effects on, or of, levels more than one step away from a level of interest cannot be measured directly. Signals are attenuated as they pass from one level to another. For example, mycorrhizae will not experience global temperature change directly, but indirectly via changes in soil temperature or plant response to air temperature. The soil and plant which comprise the mycorrhiza's higher-level environment have attenuated the temperature signal. Likewise, if the focal level is the forest, effects of mycorrhizal acid phosphatase production will be attenuated by several levels before they are "noticed" by the forest.

Selecting the proper level is especially important when attempting to relate organisms or processes operating at a small scale to those observed at the larger scale. It

is only natural, when interested in a particular organism, to focus on the level of that organism and look "upward" to the larger scales. However, to link mycorrhizal function to higher-level behavior, the focus may need to be higher than the mycorrhiza. A focus on the forest may be necessary, for example, to ask if and how mycorrhizae affect forest-level processes. These differences in level of interest and direction of observation, though subtle, are critical to defining research needs.

Once the level of interest is chosen, the scientist looks at the levels immediately above and below that level. Higher levels are perceived as operating so slowly that upper-level functions are constant. When studying an individual tree, the forest is assumed to be in equilibrium - a stable entity that serves as the context for the processes of interest. The higher level thus becomes the "environment."

Levels lower than the level observed become the "parts" whose functioning and interactions provide "mechanisms" that explain the phenomena of interest. For example, mycorrhizae are part of the mechanism that explains phosphorus uptake. Cell membrane transport and enzymatic activity are mechanisms explaining the functioning of the fungal cell. Signals from still lower levels, such as subatomic interactions, are so attenuated that they appear as background noise that can be ignored.

One of the principles that comes from looking at the world through hierarchical glasses is the concept of constraint. Operationally, higher levels impose boundary conditions or constraints on the processes of interest. A simplistic example is the pot in which an experimental plant is grown. Under most experimental conditions, plant roots are physically constrained by pot walls. In a natural system, environmental conditions

such as soil type or structure may limit root growth. Likewise, root growth may constrain mycorrhizal proliferation. Root and soil conditions form the higher-level environment for mycorrhizae and may set limits on the rates at which mycorrhizae can operate.

Lower levels in the hierarchy establish constraints in the sense that the "parts" must be present and operating normally. If some process, such as phosphorus fertilization, changes the permeability of the plant cell membrane, mycorrhizal function is immediately constrained. Mycorrhizae, in turn, may constrain plant growth. Obligate mycotrophs simply will not survive if the lower level mycorrhiza is not present.

This principle of upward constraint by lower-level components or processes is the key to the current discussion. Lower levels in the hierarchy constrain behavior of the higher levels. When lower-level entities are present and functioning normally, the process of interest can take place. However, lower levels can limit or even halt the processes of interest if they are absent or malfunctioning.

A Clarifying Analogy

The previous section established the fundamental principle that understanding mycorrhizal function is critical to addressing larger-scale problems whenever mycorrhizae constrain higher-level processes. A simple analogy is very helpful for placing this principle into context.

Consider the "large-scale" problem of training Olympic athletes. Under what circumstances should "small-scale" research be conducted on the functioning of the liver? Under most circumstances, the athletes are healthy; the liver is present and functioning

normally. While the athlete is healthy, training considerations involve the calories consumed, the hours slept, vascularization of the muscles, the balance between muscle development and recovery and so on. Most athletic trainers know nothing about detail liver function, yet the Olympic team continues to garner medals.

The trainer does not need to understand details of liver function; however, he must know that there are circumstances under which liver function becomes a limiting constraint on athletic performance. If the athlete consumes a toxic organic chemical, contracts hepatitis, or even consumes alcohol, suddenly the situation changes radically. Now, athletic performance is determined by the rate of liver function and not by the balance of aerobic and anaerobic training.

The relevance of the analogy is clear. When mycorrhizae are present and functioning normally, we need to know very little about the details of mycorrhizal processes. Indeed, for several decades, significant progress has been made on ecosystem energy flow and nutrient cycling, two higher-level processes that include mycorrhizae as lower-level components. Yet during this period, the vast majority of ecosystem ecologists were blissfully unaware of the existence of mycorrhizae (much less how to spell the word). However, increasing reports of failures to revegetate areas severely damaged by pollutants, forestry or mining led to a new awareness of the importance of the mycorrhizal symbiosis in natural systems (Berry & Marx, 1978; Perry *et al.*, 1989).

Here, a note of caution is appropriate: the argument should not be carried too far. The possibility of lower-level constraints cannot be used as an excuse for studying all possible lower levels. The Olympic Committee may be willing to fund a physician who

understands liver function, but is unlikely to finance molecular biologists, biochemists and subatomic physicists just in case the "quarks" do not function properly.

One additional point can be made by stretching the athletic training analogy. Not all lower-level processes have equal value in explaining a problem area. Under most circumstances, trainers do not need to know much about the liver, but they had better know something about digestion and nutrition. Basic food consumption is a lower-level process of immediate concern because dysfunction here has a greater potential to affect performance.

Similarly, a strong argument can be made for the importance of mycorrhizal processes in ecosystem function. The ecosystem is fundamentally a biogeochemical system in which persistent organic structures are formed within a surrounding inert geochemical matrix (O'Neill & Waide, 1981). If this definition for the ecosystem is valid, then the importance of mycorrhizal processes is obvious. Mycorrhizae form a fundamental link between the biotic portions of the ecosystem and the geochemical matrix. Signals, e.g. carbon and nutrient fluxes, pass rapidly between mycorrhizal fungus and plant. Mycorrhizae can be considered to be keystone mutualists because of the importance of the linkage and the rapid response time within which this positive feedback system operates (Gilbert, 1980; Odum & Biever, 1984). Even more significant, mycorrhizae occupy this same coupling position in all terrestrial ecosystems. Alterations in this linkage will fundamentally alter ecosystem behavior, regardless of whether one concern is with forest or desert, with North America or Siberia or with temperate or tropical systems. Mycorrhizae, therefore, can be seen as forming a critical link in a

system that is most often defined in terms of this linkage. Anything that affects the mycorrhizae can be expected to affect this fundamental linkage and can be expected to affect higher-level function.

To summarize, the athlete analogy serves to make several important points. Frequently, the solution of higher-level problems does not require detailed information about lower-level processes. Thus, global problems do not justify mycorrhizal research unless it can be established that mycorrhizal function will be altered. The need for mycorrhizal research hinges on establishing direct or indirect effects on the physical or physiological linkage between soil and plant. Once an effect is established, the implications for an upward constraint are also established, and mycorrhizal research attains a high priority.

A Practical Example

Now, how might this concept be applied to a large-scale problem? Carbon dioxide (CO₂) concentrations in the atmosphere have been increasing dramatically since the beginning of the industrial revolution (Gammon *et al.*, 1985). A change in carbon input to the atmosphere has implications on a global scale. Research on effects of elevated CO₂ could be conducted on many scales; a focus on forest tree responses was chosen because of the essential question of carbon storage in terrestrial ecosystems. Because forest soils are frequently nutrient-limiting, can an increase in forest tree biomass be predicted under CO₂ enrichment?

To ask the question from a hierarchical perspective is to ask what would constrain

forest tree responses to CO₂ enrichment. In unmanaged forest systems where soils are frequently nutrient-poor, mycorrhizae could constrain tree growth through their role in nutrient uptake. Increased C fixation and subsequent translocation to roots could benefit the mycorrhizal symbiosis, thus enlarging the constraint envelope and permitting greater nutrient uptake and tree growth.

To test the hypothesis, ectomycorrhizal (*Quercus alba* L. and *Pinus echinata* L.) and vesicular-arbuscular (V-A) mycorrhizal (*Liriodendron tulipifera* L.) tree seedlings were grown in nutrient-poor soil in controlled-environment chambers with varying levels of added CO₂ (O'Neill *et al.*, 1987; O'Neill & Norby, 1988). All three species grown at elevated CO₂ yielded greater biomass than their counterparts grown without additional CO₂. The percentage of root length infected by V-A mycorrhizae in *L. tulipifera* increased proportionately with root growth, with no differences in either timing or extent of colonization among CO₂ treatments (Fig. IV.2). This result is comparable to a teenage athlete on an enriched training diet; the liver continues to function normally as the body increases in size and strength. In this case, under conditions of elevated CO₂, V-A mycorrhizae functioned normally, albeit over a larger root system. This is also an example of downward (environmental) constraint by a higher level: the intensity of colonization by V-A mycorrhizae was apparently determined not by carbon supply, but by the availability of root tissue for infection.

When the two ectomycorrhizal species were exposed to elevated CO₂, again with indigenous mycorrhizae and nutrient-poor soil, results were somewhat different. As in the experiment described above, root systems increased in size. However, the rate of

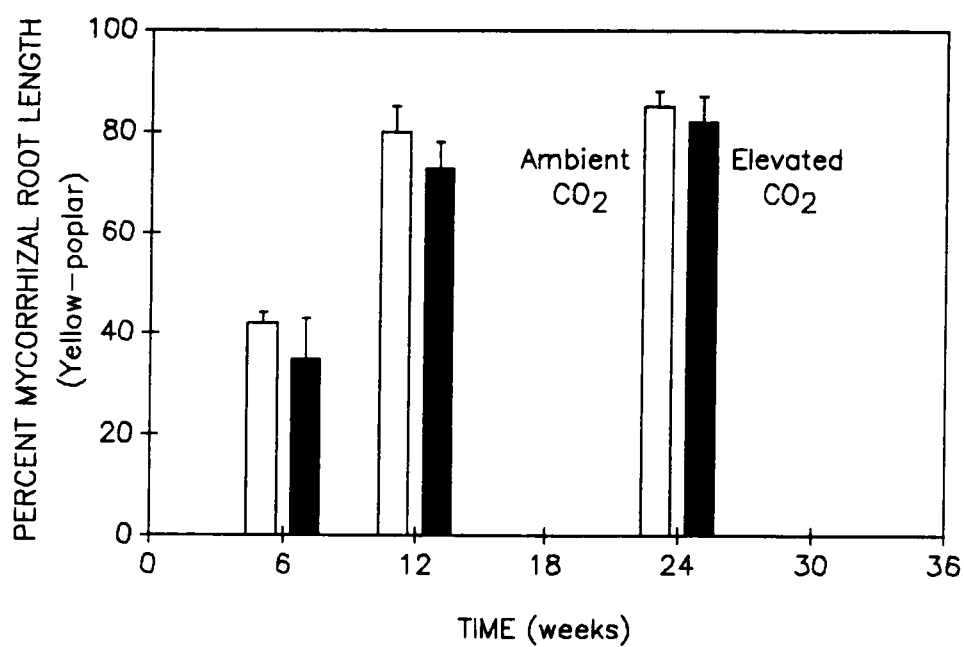


Figure IV.2. Percent VA-mycorrhizal root length in *L. tulipifera* (yellow-poplar) seedlings grown at either ambient (ca. $350 \mu\text{g m}^{-3}$) or elevated (ca. $700 \mu\text{g m}^{-3}$) CO₂. Error bars represent one standard error (O'Neill & Norby, 1988).

mycorrhization increased dramatically in seedlings grown at $700 \mu\text{g m}^{-3} \text{CO}_2$ (See Chapter III, Fig. III.1). From 6 through 24 weeks of growth, numbers of mycorrhizal root tips per centimeter of root length were significantly greater than those grown at ambient CO_2 .

The results of these initial experiments adequately answered the original question. Mycorrhizae increased either proportionately to, or greater than, plant growth. We can predict that in an enriched CO_2 atmosphere, mycorrhizal function will not constrain forest tree response. However, new and unexpected questions of constraint of higher level processes arise. What are the reasons for, and more importantly, the large-scale implications of, the observed differences in response between V-A mycorrhizae and ectomycorrhizae?

Does an increase in ectomycorrhizal infection relative to that of V-A mycorrhizae confer any competitive advantage on ectomycorrhizal tree species? Does more fungal tissue lead to greater nutrient acquisition (or water uptake or growth hormone production, for example)? If the answers to these questions are yes, changes in forest composition and species distribution could occur. Simple differences in "quantity" of mycorrhizae would constrain higher level processes.

How about the "quality" of fungal species infecting roots? Consider what might occur if fungal species differed in their ability to exploit increased carbon supply. Although no attempt was made in this series of experiments to identify mycorrhizae, it was noted that *Cenococcum graniforme* (Sow.) Ferd. et Winge, a ubiquitous, easily identified mycorrhiza, occurred significantly less frequently in elevated CO_2 treatments

(O'Neill *et al.*, 1987; Table IV.1). Other species shifts might be expected in a CO₂-enriched world. Evidence suggests that *C. graniforme* competes well with other fungi under low-light conditions (presumably, a carbon-availability response related to elevated CO₂) but is a poor scavenger for phosphorus (Mikola, 1948; Langlois & Fortin, 1984). On the positive side, *C. graniforme* may confer increased drought resistance on its host (Read *et al.*, 1977). Replacement of *C. graniforme* by other more efficient mycorrhizal species under CO₂ enrichment could benefit the host in nutrient-poor soils. This benefit could be offset, however, if local water availability decreased as a result of CO₂-induced global climate change. Differential sensitivity of individual fungal species to environmental perturbations combined with differences in host benefit would definitely have implications for higher-level behavior.

Presence vs Function

At this point, a distinction should be made between constraints imposed by mycorrhizal presence and those imposed by mycorrhizal function. Obviously, mycorrhizae cannot function without being present, but the converse is not necessarily true. Many studies have used infected root length as criteria for describing effects on the mycorrhizal symbiosis. However, the constraint exerted by the mycorrhiza may not be related to traditional measures of quantification. For example, Caldwell *et al.* (1985) measured competition for phosphorus between a shrub [*Artemesia tridentata* ssp. *vaseyana* (Rydb.) Beetle] and two competing grasses [*Agropyron desertorum* (Fisch. ex Link) Schult. and *Agropyron spicatum* (Pursh) Scribn. and Smith]. *Agropyron*

Table IV.1. Proportion of *Cenococcum graniforme* root tips (*C. graniforme* tips/100 mycorrhizal root tips) on white oak roots grown at two levels of atmospheric CO₂.†

CO ₂	Harvest (Weeks of Exposure)				
	6	12	18	24	30
360 µg m ⁻³	30.0	67.8	21.8	21.7	17.6
700 µg m ⁻³	36.5	26.8	8.7	9.0	15.2
Pr > F=	>0.20	0.004	>0.20	0.09	>0.20

† Modified from O'Neill *et al.*, 1987.

desertorum proved to be much more effective at competing for phosphorus against *A. tridentata* than did *A. spicata*. No differences in percentage root length occupied by mycorrhizal arbuscules or vesicles were observed between the two grasses. On the basis of these data, mycorrhizae did not seem to constrain the ability of the host to compete for phosphorus. However, the numbers of points at which mycorrhizal hyphae penetrated the roots were also quantified. Although *A. desertorum* did not differ in the extent of infection as traditionally measured, this species had twice as many penetration points per unit root with arbuscules as did *A. spicatum*. The potential for greater phosphorus uptake was related to numbers of penetration points rather than the extent of infection within the root.

These examples demonstrate several important points. First, a lower-level process (mycorrhizal function) can constrain higher-level behavior (competition) and, second, the presence of mycorrhizae, as traditionally determined, does not necessarily predict function of the symbiosis. Just as mycorrhizae, a lower level, can be seen as the interface between plant and soil, still lower levels, e.g. arbuscules, vesicles and penetration points, constrain the function of the mycorrhiza. Moreover, mycorrhizae can constrain higher-level behavior in ways other than their direct effect on plant vigor. More and more evidence indicates a mycorrhizal role in belowground structure, particularly in the role of hyphae in creating favorable environments for other processes to occur (Fogel, 1988; Perry *et al.*, 1989; Cromack *et al.*, 1988).

Summary

In a hierarchical system, lower levels affect higher levels through constraint. In addition, lower levels that serve as linkages between the abiotic and biotic parts of a system are especially important in large-scale behavior. Mycorrhizae represent one of these linkages, and therefore, if a large scale disturbance has the potential for affecting the function of the linkage, mycorrhizal research is not only justified, but necessary.

Once the necessity of mycorrhizal research is established, several points must be considered in designing the research. First, the questions asked must be very specific. Investigating "effects of" mycorrhizae suggests poor focus that does not adequately address subjects of interest.

The concept of constraint can suggest where mycorrhizae are likely to influence the behavior of higher levels. Large-scale behavior may be constrained by mycorrhizal processes such as spore dispersal mechanisms, interspecies competition or extraradical hyphal proliferation that have nothing to do with "percentage mycorrhizal root tips" or "centimeters of root occupied by mycorrhizal tissue." The belief that numbers of mycorrhizae fully describe their function is limiting and even worse, misleading.

Some experiments designed to investigate mycorrhizal mediation of plant response to climate change may yield results that reveal nothing new. However, a change in focus to a different level could uncover a mycorrhizal response that is a critical constraint to the system.

Finally, some large-scale questions do not require information about mycorrhizae, other than the assumption that they are present and functioning normally. Viewing a

system holistically does not mean that *no* component information is needed, but that not every last detail of mycorrhizal physiology, ecology and morphology is necessary to answer every question. By viewing systems as ordered, constraining and interacting levels instead of simple aggregations, the importance of small-scale processes such as the mycorrhizal symbiosis to large-scale problems can be demonstrated and the researcher can determine what information is necessary to understand mycorrhizal constraint when it occurs.

CHAPTER V

LITTER QUALITY AND DECOMPOSITION RATES OF FOLIAR LITTER PRODUCED UNDER CO₂ ENRICHMENT

Introduction

Decomposition of senesced plant material is one of two critical processes linking above- and below-ground components of nutrient cycles. As such, it is a key area of concern in understanding and predicting ecosystem responses to elevated atmospheric CO₂. Just as root acquisition of nutrients from soils represents the major pathway for nutrient movement from the soil to vegetation, decomposition serves as the major path of return to the soil. For any given ecosystem, a long-term shift in decomposition rates could alter nutrient cycling rates and potentially change the structure, function, and even the persistence of that ecosystem type within a given region. There is wide-spread concern that decomposition processes would be altered in an enriched-CO₂ world (Strain and Bazzaz, 1983; Melillo *et al.*, 1990; Schimel, 1993) . What is lacking presently is sufficient experimental data *at the ecosystem level* to determine whether these concerns have merit.

Elevated CO₂ in the atmosphere could have an impact on decomposition rates through several means: through changes in species composition of ecosystems, through direct effects on decomposer communities, or through changes in chemical composition of litter ("litter quality"). Changes in species composition of ecosystems resulting from

differential responses to elevated CO₂ may have the greatest impact on decomposition rates, as proportionate litter quality changes might occur (Ågren *et al.*, 1991; Pastor and Post, 1988). Amounts of carbon-based secondary metabolites and recalcitrant materials such as lignin vary among plant species. The relative contribution of differing plant species to an annual cohort of litter will in large part determine ecosystem-level decomposition dynamics. Communities composed of plant species that respond differently to CO₂ enrichment would be the most likely to experience changes in nutrient cycling rates. For example, physiological responses to elevated CO₂ and drought between loblolly pine (*Pinus taeda*) and sweetgum (*Liquidambar styraciflua*) led to a prediction that sweetgum would eventually displace loblolly pine where they co-occur (Tolley and Strain, 1985). Because these two species differ in litter quality, annual litter fall would presumably mirror changes in dominance within the system, and ecosystem decomposition and nutrient cycling would presumably change through time. Replacement of one species by the other might result not only from the relative response of the two species to elevated CO₂, but also, in this case, from an increase in nutrient cycling rates as the more recalcitrant litter of pine gave way to the relatively more decomposable sweetgum.

A second hypothesized effect of elevated CO₂ would be direct impacts to the decomposer community that result in reduced decomposition. However, although detritivores may be indirectly affected by CO₂-induced changes in litter palatability or nutritional quality, the strong concentration gradient that exists between soils and the atmosphere makes direct affects on soil biota unlikely (O'Neill, 1994).

Currently, the greatest area of concern lies in the possibility of changes in the chemical composition of plant litter. Increased C:N ratios of litter combined with increased amounts of soluble phenolics and structural compounds such as cellulose and lignin are hypothesized to be one consequence of growth at elevated CO₂ (Strain and Bazzaz, 1983; Melillo, 1983), and could reduce decomposition rates. Should rates be reduced, either absolute amounts or temporal patterns of availability of some nutrients (particularly N) would be altered, establishing a positive feedback loop that further lowers litter quality and availability of some nutrients (Pastor and Post, 1988).

This paper will emphasize those changes that result from vegetation responses to atmospheric CO₂ enrichment. However, changes in global climate that result from increased CO₂ in the atmosphere would also affect decomposition and nutrient cycling. Decomposition rates are dependent upon soil moisture and temperature, in addition to initial nutrient concentrations in the fallen litter (Pastor and Post, 1987). Changes in temperature and precipitation patterns could reduce decomposition rates, or even increase them in such a way as to offset decreased litter quality. Decreased stand-level transpiration rates could increase soil water potential (Eamus and Jarvis, 1989), again increasing or decreasing decomposition rates.

Thus, CO₂ effects on decomposition and nutrient cycling could be a complex interaction of plant litter quality, changes in ecosystem structure and alteration of temperature and moisture through climate change. This paper will consider elevated CO₂ effects on decomposition of leaf litter. Total decomposition in an ecosystem could also be affected by CO₂ fertilization through changes in the relative input to soil of foliar litter

compared to fine root and woody litter, or if there is a long-term change in the species composition of the ecosystem.

Two issues will be discussed: effects of CO₂ enrichment on foliar litter quality, and subsequent effects on decomposition rates. The focus will be primarily on N, for two reasons: 1) in many terrestrial ecosystems, N is the major nutrient limiting plant growth, and 2) experimental results from diverse ecosystem types have demonstrated that N concentrations are consistently reduced in green foliage produced at elevated CO₂ (Williams *et al.*, 1986; Curtis *et al.*, 1989; Norby *et al.*, 1992). We will also raise two important methodological questions. Much of the available data results from studies on plants grown in pots, often in environmental chambers. To what extent can we extrapolate from litter quality or decomposition data obtained in this manner to real ecosystems? And how do we resolve the issue of commonly-used "square-wave" experimental exposures that utilize rapid, single step CO₂ elevation to predict changes that will occur in nutrient cycling in ecosystems over decades of slow increase in CO₂ in the atmosphere?

Litter Quality and the Decomposition Process

Rates of litter decomposition have been correlated with initial nutrient concentrations in litter as well as the ratios of these nutrients to carbon compounds. For example, litter mass loss has been correlated with initial N concentrations (Taylor *et al.*, 1989), initial lignin concentrations (Aber *et al.*, 1990; Berg and McClaugherty, 1987), C:N ratios (Taylor *et al.*, 1989), or lignin:N ratios (Melillo *et al.*, 1982; Aber and Melillo,

1982; Melillo *et al.*, 1984). Predictions of CO₂-induced reductions in decomposition rates have been primarily based upon these observations.

However, in many cases where experimental results have led to the suggestion that decomposition rates will change under elevated CO₂, predictions have been based not upon concentrations in naturally-abscised foliage produced under CO₂ enrichment, but on concentrations in green leaves. Strain and Bazzaz (1983) discuss effects of changing tissue quality on both herbivores and decomposers, without making the distinction between green and senesced foliage concentrations. Woodward *et al.* (1991) state that rates of decomposition should decrease due to the "well established and associated increase in the C/N ratio of individual leaves." However, only two studies on leaf litter (see below), with conflicting results, are presented to support this contention.

A decrease in green leaf N concentration with elevated CO₂ has been observed in many CO₂-enrichment studies (Curtis *et al.*, 1989; Norby *et al.*, 1992). If green leaf concentrations were good indicators of litter concentrations, then a prediction of reduced litter quality under elevated CO₂ could be made with more confidence. However, in perennial plants, N concentrations usually differ between green and senesced foliage. In woody deciduous species, approximately one-half of the N content of green foliage is withdrawn from senescing tissue prior to leaf fall and retranslocated to rapidly growing tissues or stored in stem or roots until new growth is initiated (Chapin *et al.*, 1990; Cromack and Monk, 1975; Duvigneaud and Denaeyer-De Smet, 1970). Retranslocation of nutrients in coniferous species occurs before needles are shed, but may also occur seasonally in species where needles persist over multiple years (Nambiar and Fife, 1987).

Nitrogen is one of the primary nutrients remobilized, although other nutrients, as well as soluble carbon compounds, are withdrawn. The proportion of N retranslocated varies by species and may be correlated with N status of soil (Chapin and Kedrowski, 1983). If retranslocation results in N withdrawal prior to abscission, why doesn't a CO₂-induced decrease in green leaf N translate into lowered N concentrations in litter? The explanation may lie in allocation of leaf N to structural vs. soluble forms of N. If reductions in green leaf N reported in the literature are actually reductions in soluble N compounds (such as free amino acids, for example) and soluble N comprises the bulk of leaf N remobilized during abscission, then N concentrations in litter might be independent of the CO₂ concentration under which they were produced.

Recalcitrant C compounds frequently increase in concentration (% of dry mass) during senescence as other compounds are retranslocated. If N alone is translocated, with no corresponding change in non-nitrogenous C compounds, then remobilization could result in an even greater difference in C:N ratios under CO₂ enrichment than is seen in green leaves. Although there may be little retranslocation of carbonaceous materials during senescence (Chapin *et al.*, 1990), there is some evidence to the contrary: that movement of C occurs, from leaf to tree and vice versa. Horner *et al.* (1987) observed that the tannin content of Douglas-fir (*Pseudotsuga menziesii*) increased in foliage during senescence in closed canopy stands, and suggested active sequestration in litter may occur.

Litter decomposition occurs as the result of three interacting processes: 1) leaching of soluble compounds and physical fragmentation by abiotic forces such as

wind, 2) fragmentation (or comminution) by soil fauna and 3) microbial catabolism. These processes may be interdependent, as when leaf fragments are consumed by earthworms, then enriched and inoculated with bacteria and fungi during passage through the gut (Edwards and Fletcher, 1988). The first and third processes are heavily regulated by litter chemical composition; comminution and feeding by soil fauna may also be affected by litter chemistry and "palatability" to soil animals (Anderson, 1973). Because these processes occur simultaneously and/or sequentially, it is often difficult to predict ecosystem decomposition rates based simply on the chemistry of individual litter types (Blair *et al.*, 1990).

The most sensitive process with regard to potential effects of rising CO₂ is the chemical breakdown of litter by microorganisms. Nitrogen transformations are especially important, since in many of the world's ecosystems plant growth is limited by N availability. The decomposition of lignin and production of humic substances is also microbially mediated and results in the immobilization of at least a part of photosynthetically fixed C into long-lived soil C pools.

Several models have been proposed to explain decomposition processes (see Taylor and Parkinson, 1988, for a comprehensive listing of commonly-used models). The most widely accepted model, and possibly the most relevant for assessing CO₂ effects on litter decomposition is the two-phase, negative exponential model. Use of this model allows consideration of effects of CO₂-induced changes in both N and total C or lignin in litter. According to the two-phase model, initial N content of litter controls decomposition rates during early decay, while lignin (in substrates with high lignin

content) is the major control factor later (Taylor *et al.*, 1989). However, rates of mass loss during early stages may also depend upon total non-structural carbon (TNC) content of litter. If TNC concentrations are higher in litter produced at elevated CO₂ (which has been demonstrated in green leaves; Körner and Arnone, 1992) then rates of mass loss may initially be *higher* relative to litter produced at ambient CO₂, then lower in the latter stages of decay due to production of recalcitrant humic materials as nonstructural C is utilized by microbial decomposers. Reduced mass loss rates due to increased lignin or lignin-to-N ratios would also be manifested in the later stages of decomposition.

Mass loss rates may not be the most important parameter of decomposition with regard to ecosystem-level effects of elevated CO₂. The potential for changes in nutrient cycling rates, especially mineralization of N or P, is equally relevant to ecosystem response. During early stages of decomposition, two opposing N cycling processes are occurring: immobilization and mineralization. Nitrogen, frequently from exogenous sources, is immobilized by bacteria and fungi; at this stage immobilization greatly exceeds mineralization. Simultaneously, labile C compounds in the litter are utilized by the microbes and C:N ratios decline, approaching those of the decomposer microorganisms. Microbial C:N ratios generally range from 4-15 (Brady, 1990; Alexander, 1977), although ratios may vary with substrate nutrient content (Dowding, 1976). N mineralization eventually exceeds immobilization and N becomes available for further transformation and plant uptake. The point at which this switch from net immobilization to net mineralization occurs is called the "critical N concentration" (Pastor and Post, 1985), or "critical C:N ratio" (McClaugherty *et al.*, 1985), and is

dependent upon several factors: initial N concentration of litter, exogenous N availability, and the composition of the decomposer population, as well as temperature and moisture conditions. Changes in initial litter content of labile C as a result of CO₂ enrichment could also affect the point in time at which the critical N concentration is reached, because soluble nonstructural carbohydrates serve as the primary C source for microorganisms initially colonizing litter.

CO₂ Enrichment Effects on Litter Quality and Decomposition

What information do we have that supports the assumption that leaf litter quality or decomposition rates will change under elevated CO₂? Responses to date fall into one of two categories: elevated CO₂ either dramatically increases litter nutrient ratios and decreases N concentrations, or has little effect on either (Table V.1). At the ecosystem level, only herbaceous systems have been examined, although several autecological studies have been conducted on temperate deciduous forest species. Litter quality in tropical forest species has been studied in mixed assemblages in artificial model ecosystems. We are aware of no published information on litter quality responses to CO₂ enrichment in senescent conifer foliage, probably due to the long retention times of conifer needles on the tree. Some of the litter quality data presented here will be presented as "unpublished data" or as "personal communication". This is unfortunate, but necessitated because litter quality data is frequently not published until the decomposition study is concluded, several years from the initial CO₂ exposures.

In an open-top chamber study conducted on a natural salt-marsh, responses of

Table V.1. Nutrient ratios of litter produced at elevated CO₂ for different species and with differing experimental approaches. Values preceded by "~" are either recalculated from published data or were visually estimated from graph.

Species	CO ₂ Enrichment (μmol mol ⁻¹)				Reference
	C:N		Lignin:N		
	+0	+300	+0	+300	

POT STUDIES					
<i>Liquidambar styraciflua</i> L.			11.8‡	20.6‡	Melillo, 1983
<i>Castanea sativa</i> Mill.	40.4	75.0			Couteaux <i>et al.</i> , 1991
<i>Quercus alba</i> L.			5.7	4.8	Norby <i>et al.</i> , 1986
<i>Quercus alba</i> L.	(1989)		12.3	16.3	R. Norby, unpublished data
	(1990)		18.8	17.7	R. Norby, unpublished data
<i>Liriodendron tulipifera</i> L.			1.7 †	4.4 †	Boerner and Rebbbeck, 1993
<i>Fraxinus excelsior</i> L.	42.0	56.0	5.4	9.1	Cotrufo <i>et al.</i> , 1994
<i>Betula pubescens</i> Ehrh.	35.0	53.0	9.8	17.2	Cotrufo <i>et al.</i> , 1994
<i>Acer pseudoplatanus</i> L.	81.0	106.0	16.2	21.5	Cotrufo <i>et al.</i> , 1994
<i>Picea sitchensis</i> (Bong.) Carr.	18.0	18.0	4.6	6.0	Cotrufo <i>et al.</i> , 1994
FIELD STUDIES					
<i>Quercus alba</i> L.	(1989)		13.7	16.8	Norby <i>et al.</i> , 1995
	(1990)		14.2	15.7	Norby <i>et al.</i> , 1995
<i>Liriodendron tulipifera</i> L.	(1989)		16.3	16.6	Norby <i>et al.</i> , 1995
	(1990)		16.3	16.8	Norby <i>et al.</i> , 1995

Table V.1 (continued)

Species		CO ₂ Enrichment (μmol mol ⁻¹)				Reference
		C:N		Lignin:N		
		+0	+300	+0	+300	
<i>Scirpus olneyi</i>	(pure)	~82.0	~84.0			Curtis <i>et al.</i> , 1989
	(mixed)	~46.0	~50.0			Curtis <i>et al.</i> , 1989
<i>Andropogon gerardii</i>		102.4	95.5	37.9	35.9	Kemp <i>et al.</i> , 1994
<i>Sorghastrum nutans</i>		103.8	99.2	32.6	31.2	Kemp <i>et al.</i> , 1994
<i>Poa pratensis</i>		33.1	38.6	9.6	10.6	Kemp <i>et al.</i> , 1994

[†] CO₂ treatments were superimposed on an existing experiment exposing seedlings to twice-ambient levels of O₃

[‡] Structural C (lignin+cellulose):N

three communities to CO₂ were compared (Curtis *et al.*, 1989). Carbon:N ratios in green leaves of *Scirpus olneyi*, a C₃ sedge, were increased 20-40% at elevated CO₂ whether grown in a pure or mixed (with *Spartina patens*, a C₄ grass) stand. However, ratios of senescent leaves were not different. It was predicted that, on a seasonal basis, little effect on decomposition or N availability would occur as a result of CO₂ enrichment. Most measures of litter quality were also not different in senesced foliage from a tallgrass prairie exposed to twice-ambient elevated CO₂ in open-top chambers (Kemp *et al.*, in press; Owensby *et al.*, 1993). Although total N content was significantly reduced in mixed litter produced at elevated CO₂, no differences were seen in decomposition rate nor in final N concentrations in the litter after 2 years decomposition in the ambient field chambers. Changes in species composition at elevated CO₂ occurred, with C₃ forbs increasing while C₃ grasses declined and C₄ grasses remained constant (C. E. Owensby, pers. comm.). Differences were seen in species-specific decomposition rates, suggesting that changes in ecosystem species composition under CO₂ enrichment might alter ecosystem-level nutrient cycling (Kemp *et al.*, in press). In contrast, in a grassland microcosm experiment conducted at the Duke phytotron, C:N was increased in litter by CO₂ enrichment (H. W. Hunt, pers. comm.). In this study, which also examined interacting effects of CO₂ with changing temperature and moisture, soil cores were taken by driving steel irrigation pipes into the sod, then removing pipes with cores intact and transporting to the exposure facility. Although technically a "pot" study, use of intact cores permitted exposure of multiple species at the community level with minimal disturbance.

Estimated litter C:N ratios (estimated litter C = $0.45 \times$ litter biomass) were not different with a doubling of CO₂ in artificially-constructed model tropical ecosystems; C:N for ambient and elevated CO₂ treatments was 42 and 43.5, respectively (Körner and Arnone, 1992). Nitrogen return to the soil (g N m⁻²) was significantly greater at elevated than at ambient CO₂, due to significantly higher litter production at elevated CO₂.

Litter quality and mass loss rates were also not affected by CO₂ enrichment in yellow-poplar (*Liriodendron tulipifera* L.) in an open-top chamber study where sapling were grown in the ground and leaves were allowed to naturally senesce (E. G. O'Neill, unpublished data). Litter was collected as it abscised in the second year of growth at three levels of CO₂ (ambient air +0, 150, or 300 $\mu\text{mol mol}^{-1}$ CO₂). Green-leaf nitrogen concentrations were reduced by CO₂ enrichment (Norby *et al.*, 1992); however, no significant differences in lignin:N or C:N of litter were observed (Table V.1), although N concentrations were slightly reduced. Litter was placed in mesh bags and laid under the current year's litter in a mesic mixed hardwood stand. After 2 years of decomposition, total mass losses were not significantly different, with 39, 46 and 40% of the original mass remaining in the +0, +150 and +300 $\mu\text{mol mol}^{-1}$ treatments, respectively (Fig. V.1). Mass loss rate constants (k) were also not different. Nitrogen concentrations remained lower in the CO₂-enriched litter for the first year of decomposition (Fig. V.2). Maximum N concentrations (% original N) during the decomposition period, as well as the point in time where this maximum occurred, were not different. In a subsequent study utilizing yellow-poplar litter from the 1990 growing season, again, no differences in litter quality (Table V.1) or mass loss rates were observed after 2 years of decomposition.

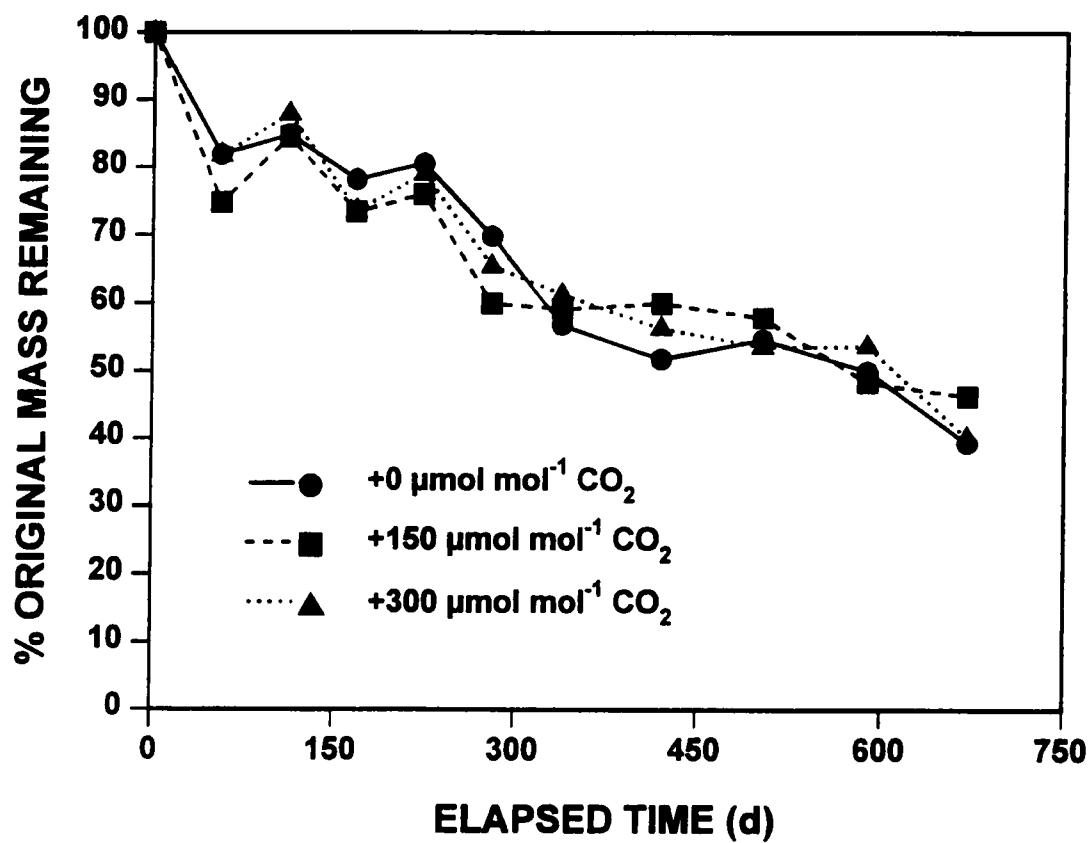


Figure V.1. Mass loss of yellow-poplar litter produced under three CO₂ enrichment regimes. Litter was decomposed in a mixed hardwood stand using litterbag techniques. Each data point reports a mean of 5 litterbags.

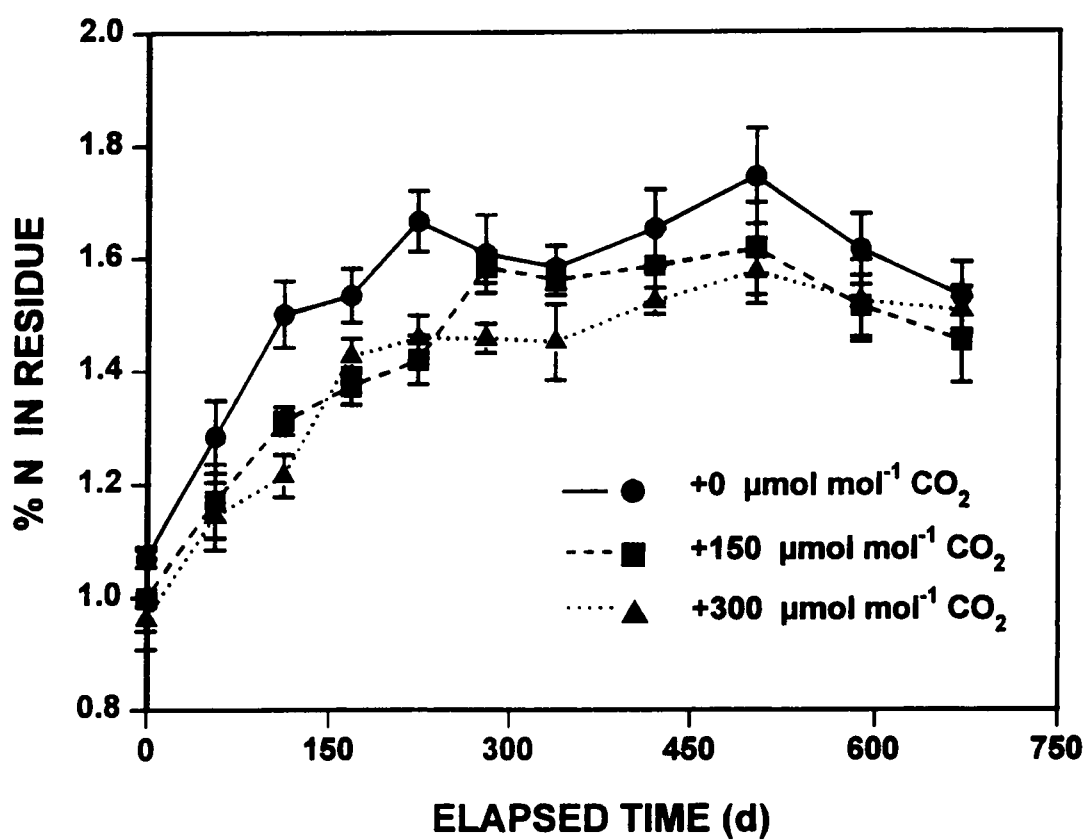


Figure V.2. Changes in N concentrations in decomposing CO₂- enriched yellow-poplar litter residue through time. Data points represent treatment means $\pm$ S. E. (n=5)

In contrast to work with natural or constructed ecosystems or with litter produced from field-grown saplings, evidence from autecological pot studies supports the hypothesis of reduced litter quality with CO₂ enrichment of the atmosphere. Nitrogen concentrations were decreased, and concentrations of soluble phenolics and structural C (cellulose and lignin) were increased when sweetgum (*Liquidambar styraciflua* L.) was grown in pots in open-top chambers at 935 $\mu\text{mol mol}^{-1}$ CO₂ for one growing season (Melillo, 1983). When elevated CO₂ (300 $\mu\text{mol mol}^{-1}$ CO₂) was added to a twice-ambient O₃ exposure of *Liriodendron tulipifera* L., N concentrations were reduced by 31% and lignin:N was increased two-and-a-half times relative to the O₃-only treatment (Boerner and Rebbbeck, 1993). Decomposition and first-year N loss rates were also reduced, although the decrease in N loss rate was correlated to structural changes in foliage, rather than to initial N concentrations. This study was also conducted on potted seedlings, in continuously-stirred tank reactors (CSTR's).

Increases in litter C:N and/or lignin:N with CO₂ enrichment were observed in pot-grown *Fraxinus excelsior* L., *Betula pubescens* Ehrh., *Acer pseudoplatanus* L. (Cotrufo *et al.*, 1994). In this same study, no changes in ratios occurred in a coniferous species, *Picea sitchensis* (Bong.) Carr., where needles were collected and dried prior to abscission. In all three deciduous species, where foliage abscised naturally, the changes in C:N and lignin:N were due both to reduced foliar N and increased lignin; N was not reduced in *Picea* needles. Both decay and respiration rates were reduced in the CO₂-enriched deciduous litter (although N mineralization rates were not) when the litter was decomposed in soil-free microcosm chambers. A similar study, utilizing micro-

lysimeters, observed temporal differences in N flux from CO₂-enriched ground litter of *Populus tremuloides* Michx., suggesting increased recalcitrance of residue compounds and reduced decomposition rates (D. W. Johnson and P. Henderson, pers. comm.). It should be noted that both of these studies examined decomposition in isolation from part or all of the soil decomposer community.

The role of soil fauna community complexity in breakdown of CO₂-enriched leaves was investigated in a microcosm approach by Coûteaux and co-workers (1991). Sweet chestnut (*Castanea sativa* Mill.) litter, obtained from seedlings grown in 25 l pots, was soaked in demineralized water to equalize soluble phenolic content, then sterilized and reinoculated with increasingly complex mixtures of decomposer fauna. Although initial N concentration was lower, and C:N higher, in litter produced at elevated CO₂, a reduction in mass loss rates and C mineralization over the 24-week experiment was seen only in the least complex assemblage (microflora plus added protozoans). In the more complex microcosms, total C loss was actually increased for CO₂-enriched litter. Decomposition was divisible into two distinct phases: the initial phase, where rates were dominated by initial litter quality factors and C mineralization was greatest in the control litter, and a latter phase, where the primary determinant of decomposition rate was the complexity of the decomposer community. Respiration rates were increased in the CO₂-enriched litter in the more complex communities.

A Few Words to the Wise Decomposer

The paucity of data on decomposition processes under CO₂ enrichment can be attributed to two related factors: 1) the persistence of the assumption that lignin:N and C:N will increase, so necessary measurements are not made, and 2) the scarcity of realistic exposure regimes to produce foliar litter and measure decomposition.

We must be cautious about forming hard-and-fast assumptions based solely on information gathered from artificial culture conditions. Nutrient ratios, on average, increased 46% (C:N) and 53% (lignin:N) in litter produced at elevated CO₂ when plant were grown in pots, but were not different due to CO₂ enrichment in any field studies (Table V.1; Fig. V.3). The range of mean values reported was also much greater for pot studies than for studies where unrestricted plants were grown in the ground. To assess how representative reported values of C:N or lignin:N were, we also compared ratios from the "ambient" treatment of these same studies to ratios reported in the literature for the same species. Kemp *et al.* (1994) included unchambered treatments as part of their experiment; ratios from this treatment were used as the basis for comparison. Results are given in Table V.2 and summarized in Figure V.4. In almost all cases where litter was gathered from plants grown in pots, regardless of pot size, "ambient" nutrient ratios are lower than those from the literature. In some cases the difference between "ambient" and literature values is greater than the increase due to CO₂ enrichment. In contrast, available data from "ambient" treatments from field studies correspond well with reported values for plants grown in the wild or in chamberless treatments as part of the experiment. The intent here is not to invalidate pot studies, but to urge caution in interpretation of results

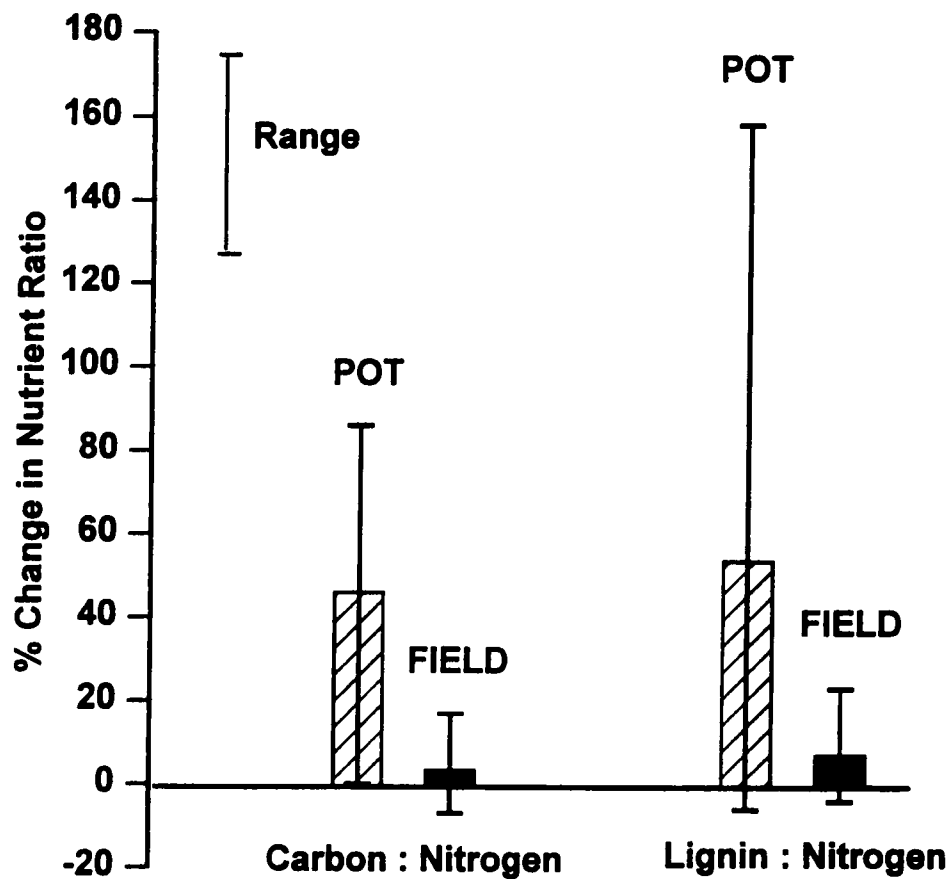


Figure V.3. Changes in litter nutrient ratios with CO₂ enrichment in field-grown plants vs. pot-grown plants. Data used to generate graph are presented in Table V.1.

Table V.2. Comparison of litter quality data from ambient treatments of elevated CO₂ studies and reported values for the same species in the field. AMB, Ambient CO₂ experimental treatment; FIELD, reported values from the field (or from unchambered controls where part of the experimental design).

Species	C:N		Lignin:N		References [‡]
	AMB	FIELD	AMB	FIELD	
POT STUDIES					
<i>L. styraciflua</i> L.			11.2	52.2	Melillo, 1983
<i>C. sativa</i> Mill.	40.4	63.2			Couteaux <i>et al.</i> , 1991
<i>Q. alba</i> L.			5.7	17.2	Norby <i>et al.</i> , 1986
<i>Q. alba</i> L.	(1989)		12.3	17.2	R. Norby, unpublished data
	(1990)		18.8	17.2	R. Norby, unpublished data
<i>L. tulipifera</i> L. [†]			1.7	14.6	Boerner and Rebbbeck, 1993
<i>F. excelsior</i> L.	42.0	30.5			Cotrufo <i>et al.</i> , 1994
<i>B. pubescens</i> Ehrh.			9.8	34.6	Cotrufo <i>et al.</i> , 1994
<i>A. pseudoplatanus</i> L.	81.0	18.1			Cotrufo <i>et al.</i> , 1994
<i>P. sitchensis</i> (Bong.) Carr.	18	80.0			Cotrufo <i>et al.</i> , 1994
FIELD STUDIES					
<i>Q. alba</i> L.	(1990)		13.7	17.2	Norby <i>et al.</i> , 1995
	(1991)		14.2	17.2	Norby <i>et al.</i> , 1995
<i>L. tulipifera</i> L.	(1989)		16.6	14.6	Norby <i>et al.</i> , 1995
	(1990)		16.3	14.6	Norby <i>et al.</i> , 1995
<i>A. gerardii</i>			102.4	72.8	Kemp <i>et al.</i> , 1994

Table V.2 (continued)

Species	C:N		Lignin:N		References [‡]
	AMB	FIELD	AMB	FIELD	
<i>S. nutans</i>	32.6	36.3	103.8	105.0	Kemp <i>et al.</i> , 1994
<i>P. pratensis</i>	9.6	8.7	33.1	28.0	Kemp <i>et al.</i> , 1994

[†] CO₂ treatments were superimposed on an existing experiment exposing seedlings to twice-ambient levels of O₃

[‡]Field data references: *L. styraciflua*, E. G. O'Neill and R. J. Norby, unpublished data; *C. sativa*, Anderson, 1973; *Q. alba*, *L. tulipifera*, Cromack and Monk, 1975; *F. excelsior*, *B. pubescens*, *A. pseudoplatanus*, Bockock, 1964; *P. sitchensis*, Hayes, 1965; *A. gerardii*, *S. nutans*, *P. pratensis*, Kemp *et al.*, 1994.

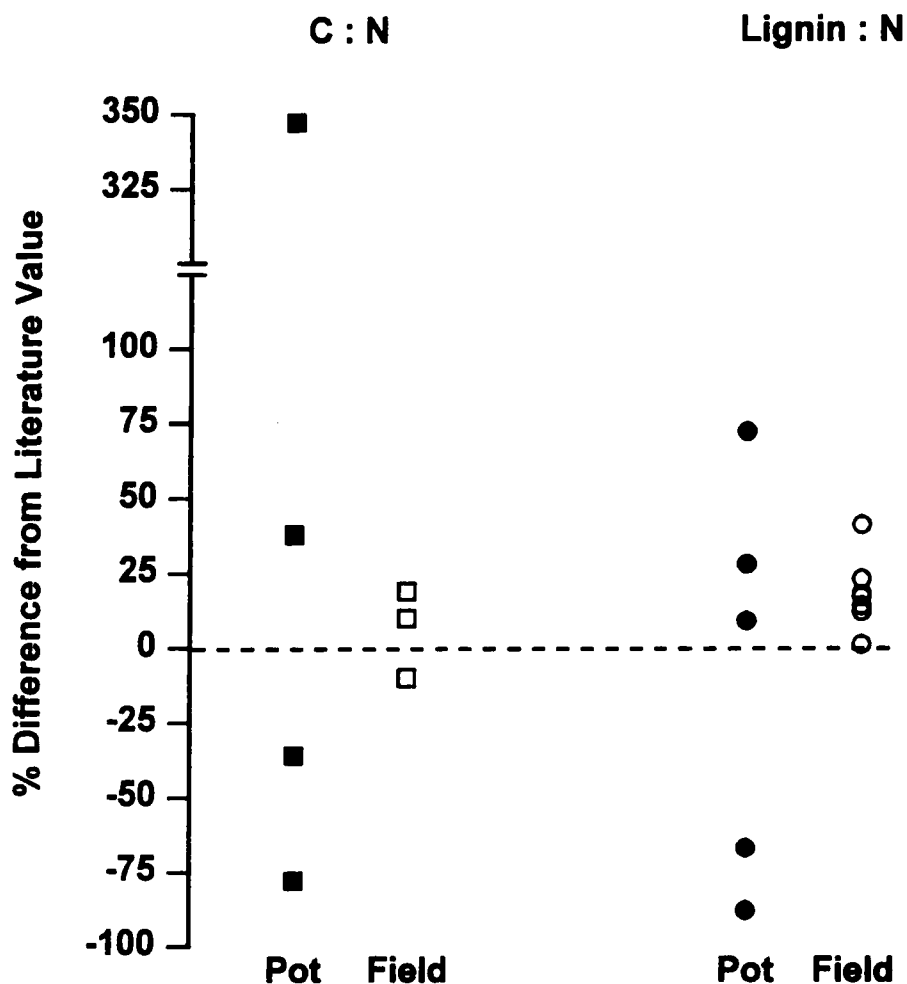


Figure V.4. Differences in litter nutrient ratios between "ambient" (no CO₂ enrichment) treatments in CO₂-effects studies and ratios reported in the literature for the same species under non-experimental conditions. In cases where unchambered treatments were included in the design, these values were used for comparison against "ambient" treatments. Data used to generate figure are presented in Table V.2.

and extrapolation to the real world. Results suggest that plants exposed in artificial environments may differ from field-grown plants in either N content or in C partitioning and these differences may confound responses to CO₂ enrichment. Lignin biosynthesis, for example, may be related to light quality or mineral nutrition (Anderson and Beardall, 1991; Waring *et al.*, 1985). Whether grown in open-top chambers or environmental cabinets, potted plants may behave differently from unconstrained, field-grown seedlings due to root restriction (Thomas and Strain, 1991) although differences may be due to available nutrients, rather than pot size or shape (McConnaughay *et al.*, 1993). Pot studies can be invaluable for identification and understanding of mechanisms; but care must be taken when culture or exposure conditions have the potential to interact with CO₂ to affect response (Strain and Thomas, 1992).

Likewise, if measurement of decomposition at the ecosystem level is the goal, then researchers must take care not to exclude processes or biota responsible for changes occurring during decomposition. Experiments that do not include exposure to natural and complete decomposer communities, for example, cannot be easily extrapolated to the field (Woodward *et al.*, 1991; Woodward, 1992). Assessing the potential for changes in decomposition with rising CO₂ only has meaning if it can be extrapolated to the scale of ecosystems. Lignin:N ratios of leaves produced at elevated CO₂ can be measured at the whole plant level. However, to assess effects of CO₂ enrichment on decomposition, we must produce litter *and* decompose it in a CO₂-enriched ecosystem. Although data exists to suggest either decreased or unaltered litter quality with CO₂ enrichment, no data have been produced where decomposition has occurred under elevated CO₂ to test the

hypothesis that decomposition rates will change.

We may also introduce error into predictions of ecosystem response if we rely solely on single-species decomposition studies. Although mass loss rates averaged across several co-occurring plant species and derived from single-species experiments may be comparable to field values, other components of the process may not compare as well. For example, use of mixed litter bags for decomposition may give a more accurate estimate of N process rates than averaging across single species bags. Differences in N flux from litter bags were observed when single species bags were compared to mixed species bags (Blair *et al.*, 1990).

Finally, the limitations of "square wave" exposure systems must be considered when extrapolating from experimental results to predictions about future ecosystem behavior under elevated CO₂. At the ecosystem level, a rapid, single step increase in CO₂ ("square-wave" exposure) is likely to produce different physiological responses than would be observed in ecosystems exposed to slowly increasing CO₂ levels (Eamus and Jarvis, 1989). In fact, what we may be measuring is the response of an ecosystem in the process of adjustment, rather than an ecosystem in dynamic equilibration. In addition, if litter quality declines slowly as CO₂ increases, and available soil N decreases as a result, then trees experiencing twice-ambient CO₂ in the real world will experience it under a different range of soil nutrient conditions than those employed currently in research (Woodward, 1992). Present experiments are useful for identifying mechanisms, but are limited in their utility as predictors of future ecosystem response.

The questions raised above lead inevitably to the conclusion that ecosystem

processes such as decomposition and nutrient cycling must be studied *at the ecosystem level*. In some cases, as with grasslands and other short-stature ecosystems, this has been or is being done. The development of large-scale exposure systems, such as Free-Air CO₂ Exposure (FACE) facilities, offers opportunities for measurement of ecosystem-level decomposition. However, for some systems, although information can be gained by pot studies, by constructed ecosystems, or by planting multiple individuals of like or unlike species within open-topped chambers, ultimately we will encounter important questions that can only be answered with large-scale and long-term experiments.

Summary

Elevated CO₂ may impact ecosystems through its effect on nutrient cycling processes. Decomposition represents a critical process in nutrient cycling, and is especially vulnerable to disturbance through changes in nutrient content or nutrient ratios in litter. Evidence to date is inconclusive, but field results from several ecosystems suggest that rates of decay (and nutrient cycling) will be unaffected by CO₂ enrichment.

Contradictory results in current work appear to be related to experimental approach and scale of observation, e. g., pot versus field studies and single- versus mixed-species decomposition experiments. In order to determine the potential for CO₂-induced changes in decomposition rates to affect ecosystems, research must be conducted at the ecosystem level. This has been accomplished for some ecosystems, but is still lacking for forests, where large size and long life spans of component organisms make large-scale, long-term research as difficult as it is necessary.

CHAPTER VI

CHANGES IN TISSUE CHEMISTRY OF SUGAR MAPLE LITTER WITH CO₂ ENRICHMENT AND ELEVATED TEMPERATURE: RESPONSES IN POT-GROWN VS FIELD-GROWN SEEDLINGS

Abstract

There is widespread concern that decomposition rates will be reduced in an enriched-CO₂ world, resulting in changes in nutrient cycling dynamics. Experimental observations of changes in litter quality – i.e. reduced N concentrations and increased lignin:N or C:N ratios – underlie this concern. Many of these observations have been made on plants grown in pots, often under artificial lighting conditions. In order to test whether cultural conditions may confound results in litter decomposition studies, we compared senesced litter from potted and non-potted sugar maple (*Acer saccharum* L.) grown side-by-side in open-topped chambers and exposed to factorial combinations of CO₂ and temperature. Litter from potted seedlings contained lower concentrations of C than did litter from unconstrained seedlings. However, neither N nor C:N changed when plants were grown in pots. There were no interactions between “pot” and either CO₂ or temperature. CO₂ had no effect on nutrient concentrations of sugar maple litter. Elevated temperature, however, increased N concentrations and decreased C:N in all seedlings. Delayed senescence and incomplete retranslocation of nitrogen at higher temperatures was the most likely reason for the increased N. We cannot conclude from this study that

utilizing potted seedlings for studies on litter chemistry confounds responses to exposure to either elevated CO₂ or increasing temperature.

Introduction

Nutrient concentrations in green foliage varies among tree species, and can differ within a species as they are affected by soil fertility, moisture availability, phenology, pathology, and herbivory. Growth under atmospheric CO₂ enrichment, with or without supplemental fertilization, commonly reduces green leaf nitrogen concentrations (Koch and Mooney 1996). Concentrations of carbon compounds such as cellulose, lignin, soluble carbohydrates and polyphenolics may also change with increased CO₂ (Lindroth, 1996). These responses usually occur regardless of whether plants are grown in the ground (Norby *et al.*, 1992; Johnson *et al.*, 1996) or under more artificial conditions (Curtis *et al.*, 1996; Körner and Arnone, 1992; Williams *et al.*, 1986). In naturally abscised plant litter, however, nutrient concentrations are more stable to environmental or cultural factors because soluble nutrients are withdrawn from leaves prior to abscission and conserved in woody tissue.

There is some evidence of environmental influence on litter tissue chemistry. For example, litter N concentrations in Ponderosa and Jeffrey pine (*Pinus ponderosa* Laws. and *Pinus jeffreyi* Grev. & Balf, respectively) varied along an O₃ gradient in the San Bernadino (CA) mountains and were 54% greater on sites with high levels of pollution than on less polluted sites (Fenn and Dunn, 1989). Decomposition rates were positively correlated with N content of litter. Premature needle loss as a result of O₃ exposure was

offered as a probable cause for the differences in N concentration. Premature abscission due to cold stress was also thought to result in higher litter N concentrations in *Populus tremuloides* Michx. (Taylor and Parkinson, 1988). Annual variation in lignin and cellulose were also reported. In a recent paper, Findlay and colleagues (1996) also reported O₃-induced effects on abscission and litter nutrient concentrations, with litter N content higher in damaged leaves than in controls. Unlike Fenn and Dunn (1989), however, Findlay showed N to be negatively correlated with decomposition rate. They hypothesize that although N content was higher in O₃-damaged litter, "bound" phenolics which tend to complex nitrogen were also higher. Mite herbivory resulted in a similar response, whereas ultraviolet radiation (UV-B) exposure (which does not physically disrupt leaves) did not. In a recent study on the Oak Ridge Reservation, I found that soil water availability was inversely related to N concentrations and positively related to C:N ratios in dogwood (*Cornus florida* L.), maple (*Acer* spp.), and chestnut oak (*Quercus prinus* L.). Again, responses may be due to aberrant retranslocation, as litter from sites with reduced water input abscised earlier in the season and contained the highest amounts of N (E. G. O'Neill, unpublished data).

Staaf (1987) found no variation in N and lignin concentrations in European beech (*Fagus sylvatica* L.) litter from three mature beech forests with varying soil types and fertility. Waring *et al.* (1985) observed decreases in lignin and concomitant increases in foliar N in the green foliage of willow (*Salix aquatica* Smith) grown in high nutrient hydroponic solutions under low light. Although it would probably be inaccurate to draw conclusions from the latter study regarding effects of light intensity or fertilization on

litter because of the differences between green and senescent foliage, the relative changes in lignin content could be expected to persist through senescence.

There is widespread concern that decomposition rates will be reduced in an enriched-CO₂ world, resulting in changes in nutrient cycling dynamics. Experimental observations of changes in litter chemistry – i.e. reduced N concentrations and increased lignin:N or C:N ratios – underlie this concern (See Chapter V). However, many of these observations have been made on plants grown in pots of varied soil volumes, often under artificial lighting conditions. In all studies to date utilizing pot-grown plants and artificial lighting, litter N declines and C:N or lignin:N ratios increase with CO₂ enrichment (O'Neill and Norby, 1996).

Studies where plants were grown directly in the ground under natural lighting have produced no such changes (Curtis *et al*, 1989; Kemp *et al*, 1994; O'Neill and Norby, 1996, Randlett *et al*, 1996). In addition, when C:N ratios of the "ambient" treatment in all studies were compared to literature values for the same species, field studies yielded ratios most comparable to unmanipulated plants (O'Neill and Norby, 1996). The range of values reported was also lower in field studies than in pot studies. Although the total number of observations on litter chemistry responses to CO₂ enrichment is small, the marked difference between results from pot studies vs field studies warrants investigation. If responses to elevated CO₂ or any environmental treatment are confounded by growth under artificial conditions (e. g., in pots or under artificial illumination) then conclusions drawn from these studies should be viewed with caution.

The work described in the present paper was conducted as part of a larger

experiment on Temperature and CO₂ Interactions in Trees (*TACIT*) initiated at the Oak Ridge Global Change Field Research Facility in 1994. We tested the hypothesis that seedling growth in pots results in abnormal litter tissue chemical composition. This could lead to incorrect assumptions about the response of forest trees to environmental change, specifically to CO₂ enrichment and temperature increase.

Methods

TACIT site description and treatment conditions

TACIT is located on an old-field site on the Oak Ridge National Environmental Research Park in Oak Ridge, Tennessee. Soils at the site are Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudult). In Spring, 1994, one-year-old seedlings of sugar maple (*Acer saccharum* L.) and red maple (*Acer rubrum* L.) were planted in the ground in standard USDA open-topped chambers (3 m diameter x 2.4 m height). Six seedlings of each species were established within the chambers. Two additional seedlings of sugar maple were planted as described below and used for both soil respiration studies and litter quality comparisons. The chambers were covered with 77% shade cloth providing 23% ambient daylight conditions, approximating lighting conditions in a forest gap. Weed control was achieved through the use of water-penetrable mulch cloth, and no supplemental water or fertilizer was provided during the experimental period.

Seedlings were grown under factorial combinations of two atmospheric CO₂ levels (ambient air or ambient air + 30 Pa CO₂) and two temperature levels (outside

ambient temperature or ambient + 4°C). Pure CO₂ was injected into the airstream of one-half of the chambers to establish CO₂ enrichment. Temperature control was achieved by heating or cooling (through evaporative coolers) air in each open-top chamber to its respective set point just prior to the air entering the chamber. Elevated temperature chambers averaged 3.5°C above ambient chambers from May 1994 through December, 1994 (Norby *et al*, submitted). Soil temperatures measured at 10 cm depth were approximately 2°C higher in elevated temperature chambers than in ambient temperature chambers, but did not vary horizontally across the chamber. Three blocks of four chambers were maintained, with each of the four factorial treatment combinations of CO₂ and temperature randomly assigned to a chamber within each block.

In order to measure elevated CO₂ and temperature effects on soil CO₂ efflux and litter quality, a subset of seedlings in each chamber was planted as follows. At four equally-spaced points within a chamber, soil was excavated and four 20 L pots (28 cm x 35 cm) with bottoms removed were sunk in the resulting holes to their rims. Soil from each hole was carefully re-packed in the pots to the original bulk density and vertical profile. Sugar maple seedlings were planted in two randomly selected pots per chamber; the remaining two pots were left unplanted to serve as controls for the soil efflux measurements.

Litter collection

Litter samples from sugar maple seedlings grown in the ground and in the pots were used for assessment of tissue composition responses to treatment. Abscised litter

was collected from individual seedlings in the chambers from September 30 to November 28, 1994. Following a hard freeze during the last week in November which effectively ended senescence, all remaining leaves were removed from the seedlings. Leaf area was measured with a LI-3000 area meter (Li-Cor, Inc., Lincoln, NE, USA), then oven-dried at 65°C for two days and weighed. Specific leaf area (m²/g) was determined on a total plant basis. Litter was pooled by plant and ground to a medium (40 mesh) powder in a Wiley mill.

Carbon and nitrogen analyses

After re-drying the ground litter overnight at 65°C, samples were analyzed for total carbon and nitrogen by combustion in a Carlo-Erba CN Analyzer (Milan, Italy) using atropine as the internal standard and NBS apple leaves (National Bureau of Standards, Washington, DC) as an external standard.

Data analysis

Data were analyzed by split-plot ANOVA using a mixed model, with POT ("No Pot," n=6 per chamber; "Pot," n=2 per chamber) as the random factor, and chamber as the experimental unit. Eight plants were excluded from the analysis due to stem compression resulting from the use of stem-flow gauges in another study on these seedlings.

Results and Discussion

Influence of POT

No significant interactions were seen between POT and any combination of temperature and CO₂ for any nutrient or measure of litter quality. POT alone had little effect on litter quality with the exception of litter C concentration (Table VI.1). Carbon concentrations in litter from potted seedlings were significantly lower than in litter from unconstrained seedlings ($P=0.003$) for all CO₂ and temperature treatments. Nitrogen concentrations were not different in litter from pot-grown plants, compared to plants grown in the ground. Growth in pots tended to reduce C:N of litter ($p=0.093$).

Influence of CO₂

Enrichment with CO₂ did not significantly alter any measure of litter quality (Table VI.1). Lowered N concentrations and increased C:N ratios were observed in green leaves late in the growing season (pre-senescence), but this did not persist through senescence (Table VI.2).

Influence of temperature

Chamber temperature was the main factor in litter quality changes (Tables VI.3-VI.5). Carbon was reduced in litter produced in the high temperature chambers (Table VI.1). This observed decrease may have little biological significance *per se*. Reductions in C were small (1.6% averaged across all treatments), and total C is not usually

Table VI.1. Nutrient concentrations in foliar litter of sugar maple grown at two atmospheric CO₂ concentrations and under two temperature regimes. (Mean $\pm$ s.d.)

		CO ₂ Concentration			
		Ambient + 0 Pa		Ambient + 30 Pa	
		Temperature	No Pot	Pot	No Pot
Carbon	Ambient	44.19 ± 0.52	43.89 ± 1.2	44.11 ± 0.26	43.42 ± 0.26
	Ambient + 4° C	44.26 ± 0.62	42.19 ± 1.4	43.55 ± 0.80	42.80 ± 0.74
Nitrogen	Ambient	0.72 ± .05	0.75 ± 0.08	0.79 ± 0.08	0.70 ± 0.12
	Ambient + 4° C	1.01 ± 0.04	1.01 ± 0.16	0.92 ± 0.16	0.91 ± 0.05
C:N Ratio	Ambient	62.91 ± 2.9	60.43 ± 4.7	59.15 ± 7.6	65.51 ± 12.3
	Ambient + 4° C	47.34 ± 2.8	43.68 ± 9.0	48.72 ± 8.7	47.46 ± 3.2

Table VI.2. Green leaf nutrient concentrations in sugar maple in-late summer, 1994. Data represent concentrations in leaf discs collected and pooled across chambers within treatment. Owing to the absence of replication in sampling the discs, no statistical analysis was possible.

Treatment	Nutrient composition		
	% N	% C	C:N
Ambient CO ₂ , Ambient Temperature	1.26	44.07	34.98
Ambient CO ₂ , Elevated Temperature	1.43	44.25	30.94
Elevated CO ₂ , Ambient Temperature	1.18	44.11	37.38
Elevated CO ₂ , Elevated Temperature	1.05	44.43	42.31

Table VI.3. ANOVA table for C concentrations in litter of sugar maple seedlings.

Source	d.f.	SSE	F	Pr>F
Block	2	10.457	4.07	0.022
CO ₂	1	0.360	0.328	0.59
Temp	1	6.523	5.935	0.051
CO ₂ *Temp	1	0.174	0.158	0.70
Whole-plot error	6	6.594		
Pot	1	12.003	9.349	0.003
Pot*whole-plot treatment	3	6.177	1.604	0.20
Pot*Blk and Pot*Blk*Whole-plot treatment	8	8.225	0.801	0.60
Error	58	74.463		
Total	81	131.125		

Table VI.4. ANOVA table for N concentrations in litter of sugar maple seedlings.

Source	d.f.	SSE	F	Pr≥F
Block	2	0.076	.900	0.41
CO ₂	1	0.019	0.818	0.40
Temp	1	0.673	29.002	0.002
CO ₂ *Temp	1	0.038	1.654	0.25
Whole-plot error	6	0.139		
Pot	1	0.009	0.203	0.65
Pot*whole-plot treatment	3	0.022	0.170	0.92
Pot*Blk and Pot*Blk*Whole-plot treatment	8	0.157	0.462	0.88
Error	58	2.469		
Total	81	4.174		

Table VI.5. ANOVA table for C:N ratios in litter of sugar maple-seedlings.

Source	d.f.	SSE	F	Pr≥F
Block	2	349.76	1.37	0.26
CO ₂	1	34.799	0.250	0.63
Temp	1	3070.3	22.081	0.003
CO ₂ *Temp	1	12.162	0.087	0.78
Whole-plot error	6	834.30		
Pot	1	0.909	0.007	0.093
Pot*whole-plot treatment	3	206.94	0.566	0.64
Pot*Blk and Pot*Blk*Whole-plot treatment	8	629.52	0.646	0.74
Error	58	7408.3		
Total	81	14622.3		

correlated with decomposition rate. Unless changes occurred in the concentrations of individual C compounds (lignin vs soluble C, for example), one would not expect to see significant impacts to decomposition processes. On the other hand, the ratio of C to other chemical constituents of litter has been inversely correlated with decomposition rates. Temperature altered C:N ratio in litter ($P=0.003$), with C:N ratios 25% lower, on average, in plants grown at elevated temperature (Fig. VI.1).

Litter N was increased by 30% overall in the high temperature treatments (Fig VI.2; $P=0.002$). The relative increase was greatest under CO_2 enrichment, however, there were no interactions of temperature and CO_2 . Specific leaf area (leaf area/unit leaf biomass) declined in the elevated temperature treatments (Table VI.6) which might indicate increased uptake of N by these seedlings.

We believe, however, the most likely explanation for the observed changes in litter N is an indirect response to increased temperature via delayed senescence, rather than increased uptake. Increased uptake would be reflected in green leaf nitrogen concentrations (Table VI.2), which were apparently not different between ambient and elevated temperature chambers (although these data cannot be tested statistically). Abnormal senescence would not require that initial leaf N concentrations be different, only that either the rate or chemistry of N remobilization differ with temperature. Rates of N remobilization and translocation were not directly measured as the leaves senesced. However, leaves in the high temperature treatments had reduced rates of chlorophyll loss and abscised later than those in the ambient temperature treatments (Hartz and Norby, 1995). The point in time where 50% of leaves had abscised was 8 days later, on average,

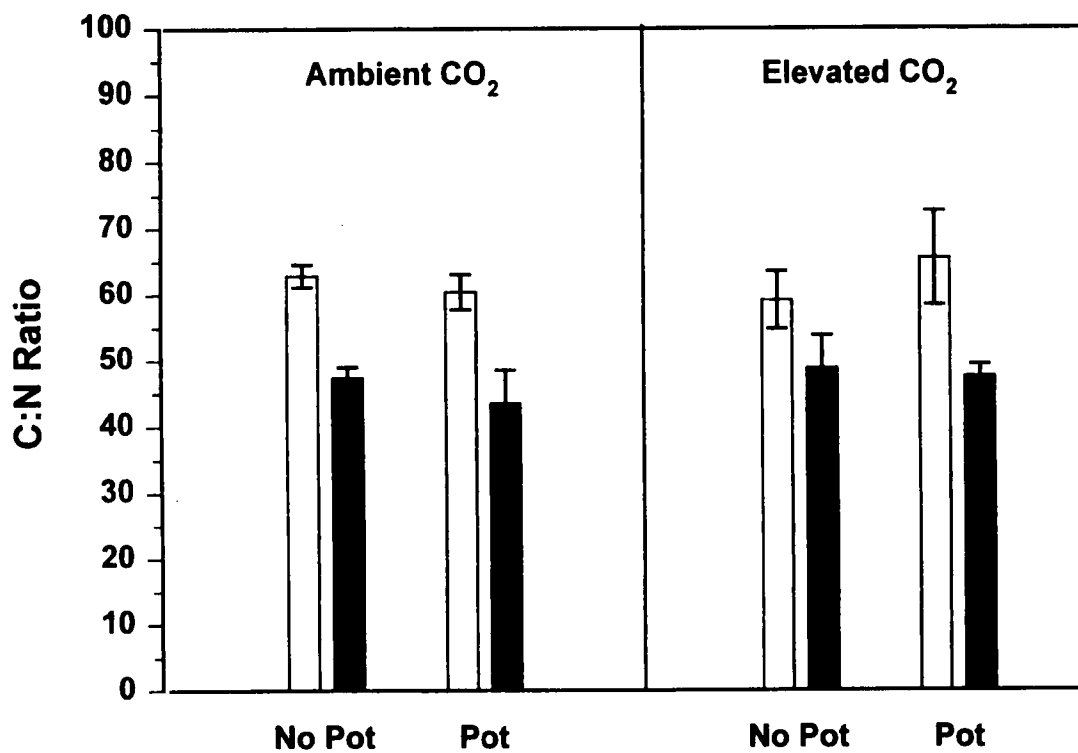


Figure VI.1. C:N ratios in sugar maple litter grown in pots or in the ground at two levels of atmospheric CO₂ and two temperatures. **Open bars**, ambient temperature; **Solid bars**, ambient temperature + 4°C. Bars represent means ± 1 S.E.

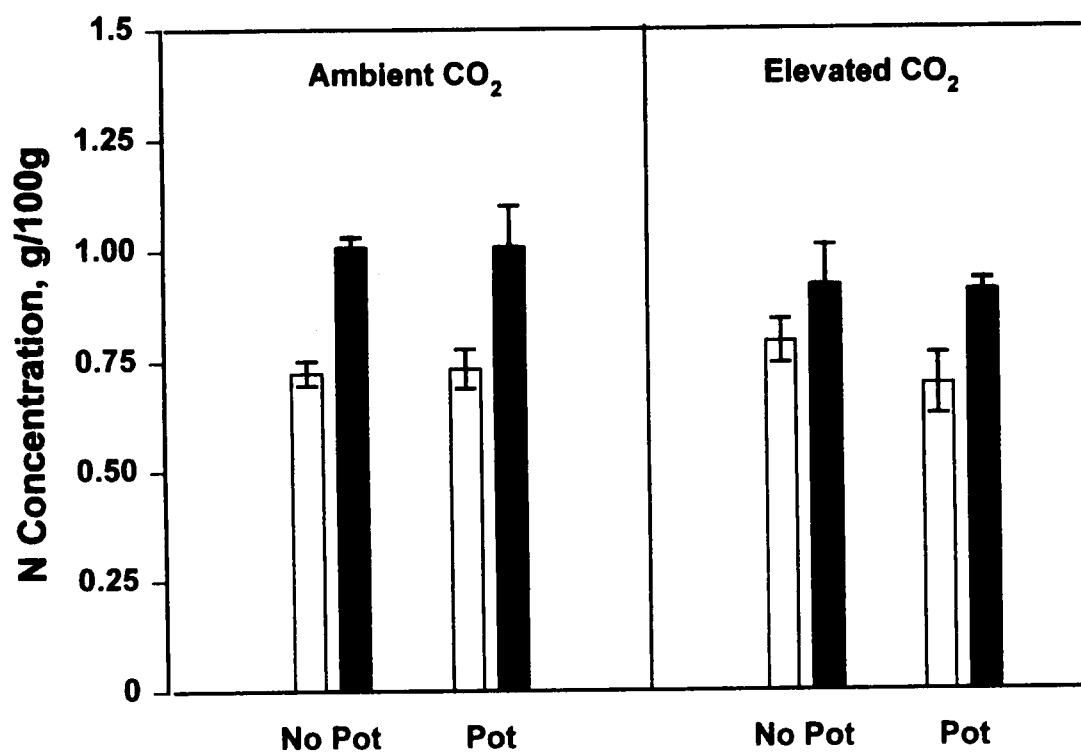


Figure VI.2. N concentrations in sugar maple litter grown in pots or in the ground at two levels of atmospheric CO₂ and two temperatures. **Open bars**, ambient temperature; **Solid bars**, ambient temperature + 4°C. Bars represent means ± 1 S.E.

Table VI.6. Effects of CO₂ enrichment and elevated temperature-on specific leaf area (cm² g⁻¹) per plant (data represent a mean of three chambers, with Pot and No Pot treatments pooled, $\pm$ 1 s.d.).

Temperature	CO ₂ Concentration	
	Ambient	Ambient + 30 Pa CO ₂
Ambient	168.21 $\pm$ 8.98	153.79 $\pm$ 10.5
Ambient +4°C	143.50 $\pm$ 13.0	144.96 $\pm$ 17.7

for the +4°C treatment . During the final week of November, all senescence was effectively stopped by several nights of temperatures below freezing. If retranslocation of N (and other nutrients) in the elevated temperature chambers was incomplete at that time, the resulting litter would have higher concentrations of N.

Conclusions

We cannot conclude from this study that utilizing potted seedlings for studies on litter chemistry confounds responses to exposure to either elevated CO₂ or increasing temperature. This does not necessarily vindicate litter quality studies where litter was collected from plants grown under artificial conditions. First, these were small seedlings in relatively large pots and were most certainly not root bound. If plant C and N dynamics are sensitive to root restriction (Thomas and Strain, 1991; Gunderson *et al.*, 1993), then pot size relative to plant size could be a critical confounding factor in response to environmental change. Second, pot studies are almost always conducted under artificial lighting conditions. Light, rather than the presence of the pot, could be the explanation for the differences in response to CO₂ seen in the literature.

Pot studies can provide valuable information about responses of plants to changing environmental conditions. They are inappropriate when the response of interest is sensitive to experimental protocol. The difficulty lies in determining which responses are sensitive and which are not. The concern over potential changes in decomposability of litter produced under changing climatic conditions. However, conclusions drawn from plants grown in pots, with artificial light or in artificial soils must be viewed with caution.

CHAPTER VII

DISCUSSION AND SUMMARY

Rhizosphere Responses

Microbial populations in the rhizospheres of yellow-poplar did not increase in response to elevated CO₂ as hypothesized. There was a strong trend towards higher numbers of PDB in the rhizosphere of seedlings grown at elevated CO₂, suggesting the potential for enhanced phosphorus availability with CO₂ enrichment. Numbers of nitrifying bacteria (NH₄OX and NO₂OX) also did not increase; populations of the latter, in fact, were lower at the final harvest in the rhizosphere of CO₂-enriched plants. Numbers of total bacteria in the rhizosphere did not increase with CO₂ enrichment in my studies.

To some degree, results from the present study conflict with those of other researchers, though Whipps (1985) also found no change in bacterial numbers (using dilution plate counts) at elevated CO₂ in wheat rhizospheres. Higher numbers of rhizosphere bacteria (quantified with fluorescein isothiocyanate direct counts) and increased total microbial respiratory activity (measured by dehydrogenase assay) were found in field-grown cotton exposed to elevated CO₂ (Rogers *et al.*, 1992). In a later study, similar results were obtained (Runion *et al.*, 1994). These researchers also assessed the potential for infestation by a common root pathogen (*Rhizoctonia solani*) and although there was a trend towards higher infestation potential at elevated CO₂, a

bioassay for these damping-off pathogens did not substantiate the trend.

Zak *et al.* (1993) provided additional evidence that elevated CO₂ may increase microbial biomass/activity not only in the rhizosphere, but also in nonrhizosphere soil. For both rhizosphere and bulk soil of *Populus grandidentata* Michx. grown in elevated or ambient CO₂ in open-top chambers, microbial biomass C (measured by chloroform fumigation) was greatest at elevated CO₂. Labile or available C increased in the rhizosphere with CO₂ enrichment, but not in nonrhizosphere soil. Nitrogen availability was also hypothesized in Zak's study to increase as a result of increased root exudation. In short-term laboratory incubations, net N mineralization was greater in nonrhizosphere soil from the elevated CO₂ treatments than in the ambient treatment. However, available N was not increased significantly in either bulk or rhizosphere soil by CO₂ enrichment.

Why are there conflicting results? Rhizosphere responses could vary with plant species, community type, or soils (Zak *et al.*, 1992). Differences in methodology may also lead to opposing conclusions. Chloroform fumigation and the dehydrogenase assay measure total microbial (bacterial plus fungal) biomass and activity, respectively, whereas direct counting does not distinguish between living and dead cells and accounts only for changes in the bacterial component of the microflora. Results may not conflict, for example, if there were a preferential stimulation of the fungal community that increased total activity or microbial biomass, but was not reflected in bacterial direct counts. Little attention has been paid to community structure in the rhizosphere and how this may change under the influence of rising CO₂.

Differences in nutrient availability in the rhizosphere may also constrain

responses. In a review of potential effects of elevated CO₂ on C-dynamics in soil, van Veen *et al.* (1991) summarized work of several researchers who showed that more C is incorporated into microbial biomass under conditions of relatively high soil nitrogen status than in low N soils. They concluded that C availability is not the only rate-limiting factor for rhizosphere microbial growth.

Finally, responses to elevated CO₂ may manifest themselves through process rates, rather than effects on population size. Holmes and Zak (1993) investigated seasonal changes in soil microbial biomass and net N mineralization rates and found that biomass remained relatively constant through time though microbial turnover rates increased in response to variable C input. Increased turnover rates in soil microbes as a result of increased root or hyphal exudation under CO₂ enrichment could enhance mineralization and nutrient availability, as nutrients are released to the soil matrix when bacterial cells lyse after death. Increases in microbial population size, on the other hand, could lead to increased microbial immobilization, decreased nutrient availability and constrain plant response to elevated CO₂ (Díaz *et al.*, 1993).

Mycorrhizal Responses

Total plant mycorrhization increased with elevated CO₂ for both types of symbioses examined. VA-mycorrhizal yellow-poplar (*Liriodendron tulipifera* L.) grown at three atmospheric CO₂ concentrations showed no difference in the proportion of root length colonized. It would be incorrect to say there was no response to elevated CO₂, however. In this experiment, total fine root length increased dramatically in plants grown

at elevated CO₂, resulting in more mycorrhizal tissue (and presumably greater nutrient acquisition) per plant. Although VAM have not been examined under CO₂ enrichment in other woody species, several researchers report results from exposures of grass species to elevated CO₂. In a serpentine grassland exposed to elevated CO₂ in open-top chambers and sampled at monthly intervals over a two-year period, VAM colonization was not increased (Whitbeck, 1993). Mycorrhizal infection by VAM also did not increase in cotton roots exposed to elevated CO₂ in a Free-Air CO₂ Enrichment (FACE) experiment (Rogers *et al.*, 1994). In contrast, Monz *et al.* (1994) provide evidence that the responses of VAM to CO₂ enrichment are not uniform. In their study, VAM colonization in *Bouteloua gracilis* (a C₄ grass) increased at elevated CO₂ but there was no increase in *Pascopyrum smithii* (a C₃ grass). The work of Monz and co-workers is also notable in that interactive effects of CO₂ enrichment, temperature and water availability on VAM were also demonstrated. The combination of elevated CO₂, elevated temperature, and decreased precipitation reduced VAM colonization in *P. smithii* but increased colonization on *B. gracilis*; the latter effect was primarily due to elevated CO₂.

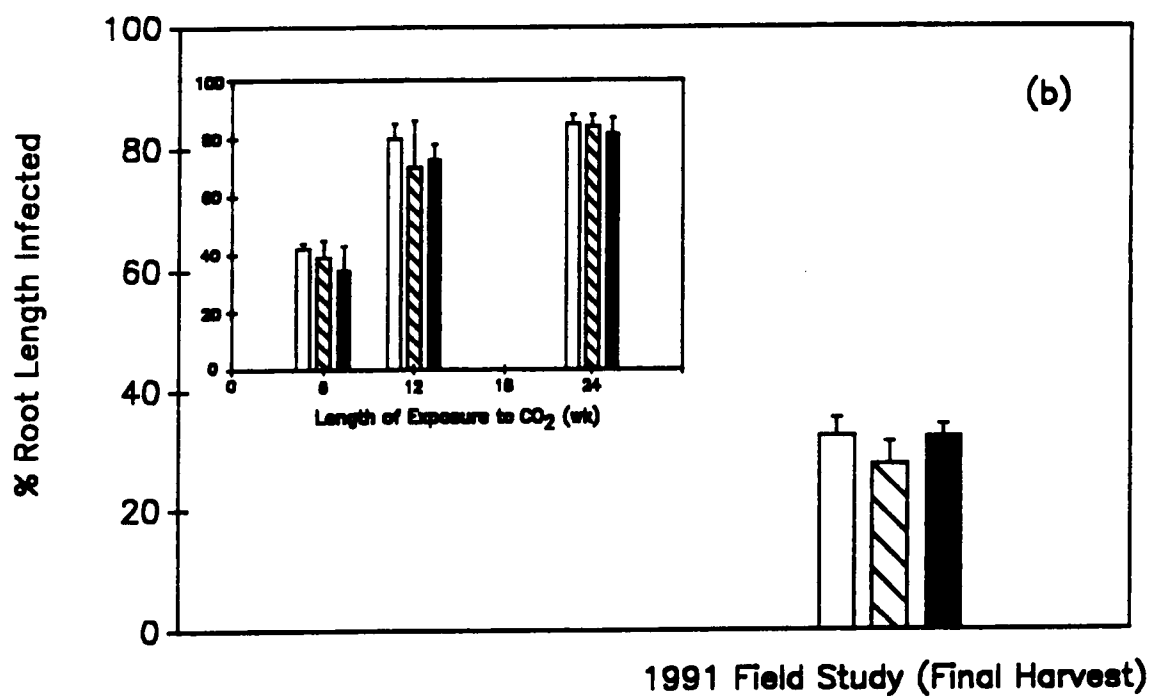
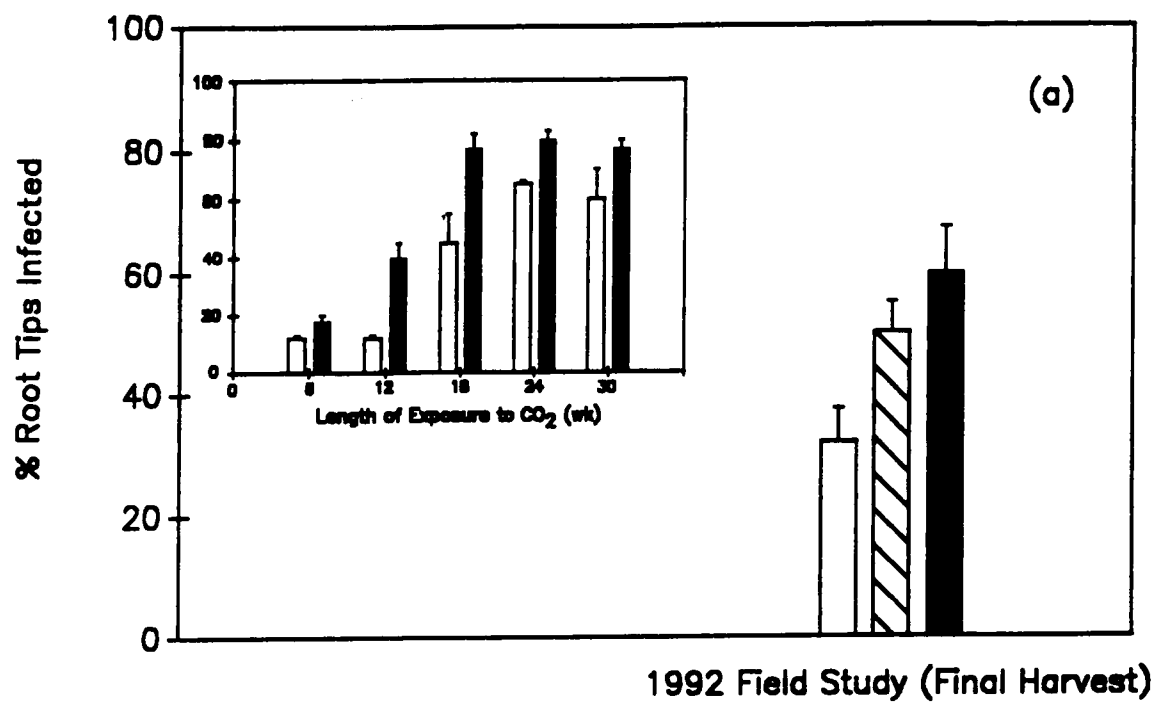
In the present study, ECM fungi exhibited greater colonization per unit root length/mass at elevated CO₂ than at current atmospheric levels. CO₂ supplied at twice-ambient levels was shown to result in both higher numbers of mycorrhizal short root tips (proportion of total fine root tips) and a more rapid rate of colonization in white oak and shortleaf pine. Exposure to elevated CO₂ also appeared to offset the temporary growth lag frequently seen in tree seedlings during the early stages of mycorrhizal establishment. When seedlings were grown at ambient CO₂ for 6 weeks, the total biomass of plants

inoculated with the mycorrhizal fungus *Pisolithus tinctorius* (Pt)-was reduced compared to uninoculated seedlings. However, Pt-inoculated seedlings grown at $700 \mu\text{mol mol}^{-1}$ CO_2 exhibited a 27% biomass *increase* over uninoculated seedlings. In a related experiment by Lewis and colleagues (1992; 1994), root colonization by *P. tinctorius* was also higher under elevated CO_2 on one population of loblolly pine (*Pinus taeda* L.), but not on another. Biomass of these seedlings in a low P treatment was greater at elevated CO_2 only in the population of seedlings that experienced enhanced colonization.

Nutrient concentrations were not determined in all of the present experiments, so there was no way to determine how the differences in response related to seedling nutrient uptake. Responses of both VAM and ECM were confirmed in the field, when mycorrhizal colonization late in the growing season was assessed on white oaks and yellow-poplars grown in the ground in open-top chambers at three concentrations of atmospheric CO_2 (Fig. VII.1). Again, proportional infection of root length was increased by CO_2 enrichment in white oak but unchanged in yellow-poplar and both species had increased fine root biomass at their respective final harvests (Norby, 1994).

Effects of CO_2 enrichment on the mycorrhizal symbiosis may not simply be related to colonization rates. In fact, undisturbed ecosystems may be "saturated" with regard to mycorrhizae. Decades of plant growth under steadily increasing CO_2 levels may result in differences in fungal species composition, rather than changes in percent infection or colonization rates. Little is known about interactions among differing species of mycorrhiza, although mycorrhizal diversity is extremely high. Although mycorrhizae have been long regarded as functionally redundant in ecosystems, evidence from single

Figure VII.1 Comparison of CO₂ enrichment effects on ECM vs. VAM infection in field and environmental chamber studies. Primary figures are results obtained at final harvest of field grown saplings exposed to three levels of atmospheric CO₂ for four years in open-top chambers. Inserts are data obtained from one-year-old seedlings in environmental chamber pot studies (O'Neill *et al.*, 1987; O'Neill *et al.*, 1991; see also Chapters III and IV). Legend: **Open bars**, Ambient air + 0 $\mu\text{mol mol}^{-1}$ CO₂; **Cross-hatched bars**, ambient + 150 $\mu\text{mol mol}^{-1}$ CO₂; **Solid bars**, ambient + 300 $\mu\text{mol mol}^{-1}$ CO₂. **a)** Ectomycorrhizal white oaks. Data represent percentage of total root tips that were mycorrhizal. **b)** VA-mycorrhizal yellow-poplar. Data represent proportion of fine root length that was mycorrhizal.



species plant-fungus tests in controlled studies seems to indicate otherwise. The different host-fungus combinations are morphologically and physiologically different. They respond differently to experimental treatment, and colonization may result in varied benefit to the host plant. Environmental factors may influence the diversity of species upon a single host and this could in turn feed back to affect host plant response. In the experiment reported in Chapter III, one ECM species (*Cenococcum graniforme*) was abundant on white oak seedlings grown at ambient CO₂, but consistently under-represented (on many seedlings absent altogether) on seedlings grown at elevated CO₂. Impacts on key mycorrhizal species could constrain the response of the host tree to other, interacting stresses. *Cenococcum*, for example, is hypothesized to confer increased drought resistance on its host. Loss of this species in a high CO₂ atmosphere might reduce the ability of forest tree species to adjust to reduced precipitation. A highly diverse mycoflora may be more important to the ability of a plant to adjust to elevated CO₂ and climate change than simply a threshold biomass. There is an urgent need to examine interactive effects of varied fungal species, in tests with simultaneous colonization by multiple fungi, on different hosts and in different soils (Bledsoe, 1992).

An additional area requiring study includes the relationship between mycorrhizal propagule dispersion rates and their roles in ecosystem migration and species composition (Allen, 1987; Allen *et al.*, 1989; O'Neill *et al.*, 1991).

Mycorrhizae can structure communities by making resources more available to

some plants than others (Allen, 1991; Caldwell *et al.*, 1985). Ecosystem structure may change under elevated CO₂ and climate change if fungal species dominance changes or if rates of propagule dispersal cannot keep pace with host species migration.

Litter Quality and Decomposition

Much of the belowground work to date has focused on organisms, that is, responses of rhizosphere microorganisms and symbionts that affect responses of individual plants. Although it is clear that root symbionts and rhizosphere bacteria represent a first-order response to rising CO₂, responses of organisms that are less intimately associated with roots may also be an integral part of ecosystem response. In fact, it is not until the focus shifts away from the root that ecosystem-level effects can be discerned.

Litter decomposition rates, integral to nutrient availability and nutrient cycling rates, are partially dependent upon the chemical composition of the litter, though there is debate over which nutrients or indices of litter quality are most predictive of decomposition rates. Due to increased C fixation and reduced N concentrations of green leaves observed at elevated CO₂ a prevailing assumption has been that litter quality will decline, and nutrient cycling slowed as a result. The analysis in Chapter IV casts doubt on these assumptions. Most studies conducted under extremely artificial growing conditions (i.e., using pots and artificial lighting) have confirmed predictions of declining litter quality. However,

no studies where plants were grown in the ground, under natural sunlight, have demonstrated any changes in either litter quality or decomposition rates of litter produced under CO₂ enrichment. Growth in pots does not appear to be the underlying reason for this difference, at least when the ratio of pot size to plant size is large. Further examination of the influence of light quality on litter chemical composition is needed.

All studies conducted to date on decomposition and CO₂ enrichment have examined dynamics of single species. Decomposition rates could also be affected by CO₂ enrichment if plant species composition changes within the ecosystem. Plant species composition has a profound effect upon overall decomposition rates (Kelly and Beauchamp, 1987). Shifts in species composition as a result of long-term exposure of the ecosystem to rising CO₂ may alter *ecosystem* litter quality and change decomposition rates. Although short-term mass loss rates of mixed-species litter may not differ from those of its component species, other parameters, such as N dynamics, C mineralization rates, and long-term mass loss rates may. Blair *et al.* (1990) found no difference in mass loss when comparing single- and mixed-species litter. However, single-species litter responses proved to be a poor predictor of mixed-litter response for bacterial, fungal and nematode densities. Estimates of N flux based on single-species data resulted in underestimates of N release and overestimates of accumulated N relative to observed responses of mixed litter. Ecosystem-level effects of elevated CO₂ on decomposition should be based upon mixed-litter results, preferably over a time

scale long enough to include lignin decomposition dynamics.

Summary

Responses of rhizosphere and nonrhizosphere microorganisms to elevated CO₂ remain unclear. Microbial results to date suggest that metabolic activity (measured as changes in process rates) is stimulated by root C input, rather than population size (measured by cell or colony counts). Mycorrhizal colonization per unit root length in ECM species increases with CO₂ enrichment whereas in VAM species it does not, suggesting that ECM host plants might gain a competitive edge over VAM hosts in a higher CO₂ world. Preliminary data on foliar litter decomposition suggests that neither nutrient ratios nor decomposition rates will be affected by rising CO₂. This is another important area that may be better understood as the number of longer term studies with more realistic CO₂ exposures increase. Evidence continues to mount that C fixation increases with CO₂ enrichment and that the bulk of this C enters the belowground component of ecosystems. The global fate and effects of this additional C may affect all hierarchical levels, from organisms to ecosystems, and will be largely determined by responses of soil biota.

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VITA

Elizabeth Gerry O'Neill was born in Providence, Rhode Island in 1946. As the daughter of a career Naval officer, she attended 18 different schools before graduating from McLean High School in McLean, Virginia. She attended the University of Illinois from 1964-1966 and worked as a laboratory technician for then-doctoral student Robert O'Neill. They were married in 1967 and following Robert's graduation, moved to Oak Ridge, Tennessee where she worked as a licensed Practical Nurse at the Oak Ridge Methodist Medical Center. In 1975, she entered the University of Tennessee and joined the Environmental Sciences Division (ESD) at Oak Ridge National Laboratory as a laboratory technician. She received her Bachelor of Arts in Botany in 1988.

Her involvement in elevated CO₂ effects research began in 1983, when she was asked by Dr. Robert Luxmoore to investigate mycorrhizal responses to CO₂ enrichment. In 1987 she was awarded ESD's first Distinguished Technical Achievement Award and became a Research Associate in 1988. She has been active in the mycorrhizal and soil ecology research communities, serving as Treasurer of the U. S. Soil Ecology Society from 1989-1993. In 1994, she organized an international workshop on "Belowground Responses to Rising Atmospheric CO₂" which resulted in a special issue of the journal *Plant and Soil*. She has authored 27 open-literature publications (including four invited papers and three book chapters) and is Principal Investigator or Co-Principal Investigator on 4 major grants. Her fascination with the ecology of soil biota and their relation to aboveground ecosystems remains unabated.