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SUGAR MAPLE FINE ROOT RESPIRATION AND LONGEVITY ALONG A LATITUDINAL GRADIENT

By

ANDREW JAMES BURTON

A DISSERTATION

Submitted in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

(Forest Science -- Forest Ecology)

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This dissertation, "Sugar Maple Fine Root Respiration and Longevity Along a Latitudinal Gradient", is hereby approved in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in the field of Forest Science -- Forest Ecology.

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ABSTRACT

SUGAR MAPLE FINE ROOT RESPIRATION AND LONGEVITY ALONG A LATITUDINAL GRADIENT

By

Andrew J. Burton

Fine root ($\leq$ 1.0 mm diameter) respiration and longevity were studied in four sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood forests. The sites were located along a latitudinal temperature gradient and also differed in N availability. Root respiration was measured on excised roots as O_2 consumption at temperatures from 6 to 24 °C, and root longevity was assessed by direct observation using minirhizotrons. The experiments were conducted across multiple years which differed significantly in moisture availability.

Fine root respiration at the four sites was exponentially related to soil temperature (Q_{10} = 2.7) and linearly related to root N concentration and soil moisture availability. Temperature explained 90% of the variability in respiration. Differences among sites in respiration rates resulted from site-specific differences in N availability and root N concentration, and differences in soil moisture availability explained temporal variation within sites in respiration rate at a given temperature. Periodic moisture deficits were sufficient to cause declines of up to 17% in total growing season root respiration.

Root respiration increased significantly as the [CO_2] at which measurements were made was lowered, and was most sensitive to [CO_2] near and below normal soil concentrations

(<1500 $\mu l\ l^{-1}$). This suggests that estimates of root respiration made at or near atmospheric [CO_2] may overestimate actual rates occurring in the field. Sugar maple root respiration at atmospheric [CO_2] (350 $\mu l\ l^{-1}$) was about 139% of that at soil [CO_2] (1500 $\mu l\ l^{-1}$).

There were significant differences in surface root longevity among the sites, with median root lifespans for spring 1994 roots ranging from 278 to 397 days. The pattern across sites in root longevity corresponded to site differences in N availability, with longer root lifespans occurring where N availability was greater. It appears that roots are maintained as long as the benefit (nutrients) they provide outweighs the C cost of keeping them alive. It is hypothesized that greater root metabolic activity in nitrogen rich zones leads to greater carbohydrate allocation to the roots, and that reduction in root C sink strength, as local nutrients are depleted, provides the mechanism through which root lifespan is regulated.

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digitizing root images, and to Veronica Lessard for her help in analyzing root survival data.

During this research, many long days and nights were spent at field labs and research sites throughout the state of Michigan. I am indebted to the USDA Forest Service and the University of Michigan Biological Station for providing access to field laboratories, to the Forest Service and the Michigan DNR for making study locations available, and to the U.S. National Science Foundation and the USDA Forest Service Northern Global Change Program for helping fund this research. The fieldwork trips along the gradient typically consisted of ten consecutive 12 to 14 hour days. Despite this, my colleagues at the University of Michigan, Greg Zogg and David Rothstein, helped make these trips an enjoyable experience. If such efforts are required of me in the future, I sincerely hope that I will be blessed with similarly optimistic, hard-working, fun-loving, and borderline-psychotic comrades. Thanks guys.

Finally, I would like to thank my parents, Robert and Carol Burton, for their support and encouragement during all phases of my life and education. Without their love and encouragement, goals that I have achieved would remain unattainable dreams.

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INTRODUCTION

As researchers try to gain a better understanding of the possible impacts of global change on C cycling in forest ecosystems, increased attention is being focused on below-ground processes, which can consume greater than one half of annual net primary production in some forests (Fogel and Hunt 1979, Grier et al. 1981, Harris et al. 1977, Keyes and Grier 1981, Hendrick and Pregitzer 1993). Forests of the future may experience temperatures, moisture regimes, and N availabilities that differ significantly from those existing today (Pastor and Post 1988, Aber et al. 1989, Houghton and Woodwell 1989, Mintzer 1990). Each of these factors has the potential to impact root respiration and longevity and thus belowground C allocation. The desire to understand forest C balance for current and future climates has resulted in widespread efforts at constructing mechanistic, environmentally-regulated models of forest C cycles (Agren et al. 1991, Landsberg et al. 1991, Aber and Federer 1992). Fine roots are often an important component of these models, but limited information on the environmental controls of fine root respiration and longevity potentially lessens the utility of model predictions. Most models do increase root respiration rates as soil temperature increases (Billings et al. 1977, Cox 1975, Linder and Troeng 1982, Lawrence and Oechel 1983), and a few also account for the effects of root N concentration on respiration (Ryan et al. 1996). However, none adjust root respiration under drought conditions. The possibilities that root production and longevity may change as temperature, moisture, and N availability change also are seldom addressed. This lack of refinement regarding belowground processes does not reflect a lack of effort on the part of the model builders. Rather, it is indicative of our overall limited knowledge of the factors controlling

belowground processes and of the contradictions that occur in the information that does exist (e.g. both increasing and decreasing root longevity as N availability increases (Hendricks et al. 1993)).

In order to improve our understanding of the factors controlling fine root respiration and longevity, a series of experiments were conducted in sugar maple forests located along a latitudinal gradient in Michigan. The effects of temperature and N availability on fine roots ($\leq$ 1.0 mm diameter) were examined by studying four forests that differ in mean annual temperature and N availability. The experiments were conducted over three years of differing precipitation, allowing the effects of soil moisture to also be assessed. Objectives were: (1) to determine if differences existed among sites in respiration-temperature relationships and to assess whether such differences were related to variation among sites in local climate or N availability; (2) to determine if root respiration is influenced by soil moisture availability; (3) to develop predictive relationships for sugar maple root respiration based on soil temperature, root N concentration, and soil matric potential; and (4) to determine the relative influences of soil temperature and N availability on fine root longevity. The research also enabled the effects of soil moisture deficit on stand-level root respiration to be assessed and illustrated the potential errors that can occur if the effect of measurement [CO_2] on root respiration is not accounted for.

The links between N availability, fine root N concentration, and root respiration are described in Chapter I, as is the possibility that sugar maple populations at the sites may be genetically adapted to their local climate. Chapter II describes the reduction in

root respiration rate that occurs as measurement [CO_2] increases and the possible errors that can result when respiration is measured at a [CO_2] that differs significantly from typical soil [CO_2]. Chapter III uses multiple years of data to assess the combined effects of temperature, fine root N concentration and soil moisture availability on fine root respiration, and Chapter IV describes variation in fine root longevity among the sites and the relationship between fine root longevity and N availability.

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Chapter I

LATITUDINAL VARIATION IN SUGAR MAPLE FINE ROOT RESPIRATION

Abstract

A changing global climate may impact the respiration of fine roots. While many models adjust fine root respiration as temperature increases, the influence of soil nutrient availability and the possibility that root respiration may be adapted to local climate are often not addressed. Rates of fine root respiration were measured in four sugar maple (*Acer saccharum* Marsh.) forests located along a latitudinal gradient in Michigan. Root respiration was measured as O_2 consumption at temperatures ranging from 6 to 24 °C on excised fine root samples in early September, October and November of 1994. Root respiration increased exponentially with temperature with an average Q_{10} of 2.7; there were no differences in Q_{10} among sites. However, there were differences among sites in mean respiration rate at a given temperature. This site effect did not indicate ecotypic adaptation to local climate, but rather reflected fine root N concentration. Respiration at a given temperature was consistently higher in roots with higher N concentrations, and higher root N concentrations always occurred at sites having greater N mineralization rates. Results suggest increases in soil temperature could significantly alter root respiration C flux at these sites, as could changes in site N availability associated with chronic N deposition or altered N mineralization resulting from global climate change.

Introduction

As researchers try to gain a better understanding of the possible impacts of global change on C cycling in forest ecosystems, increasing attention is being focused on below-ground processes such as fine root respiration. Root respiration rates increase as soil temperatures increase (Billings et al. 1977; Cox 1975; Linder and Troeng 1982; Lawrence and Oechel 1983; Amthor 1984), and many ecosystem models alter C allocation to roots as climate warms (Aber et al. 1991; Bonan 1991; Ewel and Gholz 1991; Rastetter et al. 1991; Running and Gower 1991). Most models also adjust root respiration rates as soil temperatures increase under seasonal or climate warming scenarios. However, the possible existence of temperature races adapted to local climatic conditions is not addressed. Sowell and Spomer (1986) reported ecotypic variation in root respiration among elevational populations of *Abies lasiocarpa* [(Hook) Nutt.] and *Picea engelmannii* (Parry), with higher rates of root respiration occurring in roots from high elevation, cooler locations. If local populations of other tree species similarly are genetically adapted to local climate, estimates of respiration would have to be appropriately adjusted at different locations across a species range. Carbon allocation and utilization play an important role in the way some models predict species suitability and survival at a given location (Cohen and Pastor 1991); thus model predictions of changes in a species range could be in error. If temperature ecotypes exist, then elevated temperature (e.g. global warming) could conceivably result in stress and mortality across a species' entire range, rather than simply a shift in the northern and southern limits as many current models predict (Ledig 1992).

Plant tissue respiration also is influenced by the concentration of nutrients such as N (Jones et al. 1978; Ryan 1991a; Ryan et al. 1996). Greater respiration in high N tissues is due in part to maintenance respiration for protein repair and replacement (Penning de Vries 1975, Bouma et al. 1994). Additionally, cellular activity generally increases as tissue protein content increases (Ryan et al. 1996), and a significant percentage of root respiration can be associated with the uptake and assimilation of N (Bloom et al. 1992). Thus differences in N availability among sites and changes in N availability induced by N deposition (Aber et al. 1989) or global change (Pastor and Post 1988; Cohen and Pastor 1991) could significantly influence root respiration rates and ecosystem-level carbon allocation. Recent efforts at constructing forest C budgets have recognized this and incorporated both temperature and N concentration as a predictors of tissue maintenance respiration (Ryan 1991b, Ryan et al. 1996).

In order to assess factors influencing fine root respiration in northern hardwood forests, relationships between temperature and respiration were studied for excised fine roots from mature sugar maple (*Acer saccharum*, Marsh.) forests at four locations along a latitudinal gradient in Michigan. Objectives were to determine: (1) if differences existed among sites in respiration-temperature relationships, and (2) if such differences were related to variation among sites in local climate or nutrient availability. Since the goal was to examine differences among the sites, site-level sampling was conducted during the late summer and fall when root growth is low (Hendrick and Pregitzer 1993) and associated growth respiration would not be expected to contribute significantly to differences among the sites in observed respiration rates. Further information on factors influencing fine root

respiration at the sites was obtained by examining seedlings and soil ingrowth cores transplanted to common gardens the sites.

Materials and Methods

Study Sites

The four study sites are located along a 500 km latitudinal gradient in Michigan (Figure 1.1). Mean annual air temperature increases from 4.2 °C at Site A to 7.6 °C at Site D (Table 1.1). The summer soil temperature differential from Site A to Site D typically approaches 4 °C. Sites were selected to be similar in terms of age, stand structure, physiography, soil classification, basal area, and species composition (Burton et al. 1991).

The forests are second-growth northern hardwoods approximately 80 years in age, dominated by sugar maple and occurring on sandy, well-drained Spodosols (Table 1.1). The sites do differ in N availability determined using the buried bag technique (Table 1.1, Zogg et al. 1996). Nitrogen deposition also increases from north to south along the gradient (Table 1.1, MacDonald et al. 1992), but these differences are much less than those existing for net N mineralization (Table 1.1).

Site Comparison

At each site, three 30 x 30 m measurement plots, on which no destructive sampling was allowed, were established. Samples for root respiration were collected from 10 m wide buffer strips surrounding each plot. Buffer strips were divided into sixteen 10 x 10 m subplots and on each sampling date a soil core (10 cm deep, 5.4 cm diameter) was taken from the center of three randomly selected subplots. The three cores were transported to

Figure 1.1 Study site locations.

Table 1.1 Characteristics of four sugar maple forests in Michigan, USA.

	Site A	Site B	Site C	Site D
Site and Climate Characteristics				
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°51'	85°50'	86°09'
Elevation (m)	380	314	314	262
Mean annual precipitation (mm) [a]	870	830	810	850
Mean annual temperature [a] (°C)	4.2	5.2	5.8	7.6
N deposition (kg N ha^{-1}) [b]	6.8	9.1	11.7	11.8
N mineralization (μg N g $soil^{-1}$)[c]	52	82	85	57
Stand Characteristics (1992)				
Total basal area (m^2 ha^{-1})	32	30	30	30
Sugar maple basal area (%)	86.3	85.7	82.7	74.8
Overstory biomass (Mg ha^{-1})	261	261	274	234
Sugar maple biomass (%)	87.4	86.7	83.3	76.9
Overstory age	85	79	80	84
Soil Characteristics				
A+E horizons				
pH	4.6-5.1	4.6-5.3	4.4-4.5	4.3-5.2
Texture[d]	S-SL	S-LS	S-LS	S-LS
Bhs+Bs horizons				
pH	4.7-5.7	4.8-6.3	5.2-6.9	5.0-5.7
Texture	S-SL	S-LS	S-LS	S-LS
Classification[e]	AH,TH	AH,TH	AH,TH	EH

[a] 30 year means from National Oceanic and Atmospheric Administration (1983) records.

[b] Sum of wet plus dry NO_3-N and NH_4-N from MacDonald et al. (1992).

[c] Net N mineralization determined using the buried bag technique from Zogg et al. (1996).

[d] Range in soil texture: S=sand, LS=loamy sand, SL=sandy loam, SiL=silt loam, and SiCL=silty clay loam.

[e] Taxonomic Classification: AH=Alfic Haplorthods, TH=Typic Haplorthods, and EH=Entic Haplorthods.

nearby field labs (less than 1 hour travel time per site), and composited on a plot basis. All fine (≤1.0 mm), non-woody, live roots were sorted from each core and rinsed free of soil and organic matter with deionized water. Live roots were distinguished by white, cream, tan or brown coloration and a smooth appearance. Dead roots were dark brown or black in color, were brittle, and had frayed, rough edges.

Excess water was blotted from the root samples and four 0.5 g (fresh weight) subsamples were used for respiration measurement. Remaining roots were weighed then oven-dried (65 °C, 24 hr) for determination of fresh weight to dry weight conversions. Subsequently, these samples were ground and analyzed for N using a Carlo Erba NA 1500 Series II elemental analyzer (Fisons Instruments, Massachusetts, USA). Respiration subsamples were wrapped in moistened tissue paper and placed in temperature controlled O_2 consumption chambers (model LD 2/2 oxygen electrode, Hansatech Instruments Ltd., Norfolk, England) connected to constant temperature circulating water baths. Four complete respiration systems allowed the simultaneous determination of root respiration (as oxygen consumption) at four temperatures: 6, 18 and 24 °C; and ambient soil temperature (15 cm) existing at the site on the date of measurement. Samples were allowed to equilibrate to measurement temperature for approximately 20 min. Following this initial equilibration period, oxygen consumption rates remained constant for over 90 min and rates were measured for a minimum of 35 min. Respiration was calculated as nmol $O_2 \cdot$ g dry weight$^{-1} \cdot s^{-1}$. To ensure that all respiration samples were fresh, cores were collected from only one plot at a time and transported to the field lab for sorting and washing just prior to analysis. Time from collection to analysis was maintained at 3 h or

less. At no time were roots allowed to desiccate. Measuring root respiration along the entire gradient required 6 to 7 days. Root respiration was determined for three periods during late summer and fall of 1994: (1) 30 Aug - 05 Sep; (2) 05 Oct - 10 Oct; and (3) 03 Nov - 08 Nov; with measurements proceeding from south (Site D) to north (Site A) during each sampling trip.

Data from each site were pooled across sampling dates and differences in respiration among sites were tested using analysis of covariance, with site as the main factor and temperature as a covariate. The natural logarithm of respiration was used as the response variable since plant tissue respiration typically increases exponentially with temperature over the range studied in this experiment. Individual site regressions of ln respiration versus temperature were used to calculate site specific values of Q_{10}. Slopes of the individual regressions were compared using the method of Zar (1984). The combined influence of temperature and fine root N concentration on respiration was analyzed using a nonlinear regression model with fine root respiration assumed to be linearly related to N concentration and exponentially related to temperature.

Seedling and Root Ingrowth Core Experiments

At each site, 120 first year sugar maple seedlings were collected in intact PVC cores (7.5 cm diameter by 20 cm depth) in June 1993. Thirty randomly-selected seedlings originating from each site were transplanted in the intact cores to common gardens at all four sites. In the remainder of this report, the site from which a seedling was extracted will be referred to as "origin" while the site to which it was transplanted and subsequently grown will be referred to as "garden". All cores contained one seedling, with all other

living plants removed. The bottom of each core was covered with fiberglass screen to retain native soil but allow free drainage of precipitation. The screen permitted roots from the surrounding forest to penetrate from below, allowing the seedling cores to also function as root ingrowth cores. Only those cores in which the seedling had died due to herbivory or other natural causes were sampled as ingrowth cores.

In October, 1994, fine root respiration at 12 and 24 °C was determined for 4 seedlings of each origin located in common garden C. Fine root samples from the seedlings were small, typically about 0.1 g fresh weight. It was originally intended to measure root respiration for seedlings from all common gardens, but time limitations allowed only garden C to be sampled in 1994. The very small size of seedling roots contributed to the decision to delay further sampling of the seedlings beyond the time frame of this report.

In May, 1995, root respiration at 12 and 24 °C was determined for 6 ingrowth cores of each origin located in common garden A, and 5 ingrowth cores of each origin located in common garden B. Time constraints limited sampling to only two common gardens. Common gardens A and B were chosen due to their documented difference in N availability (Table 1) and their proximity to quality laboratory space. Respiration subsamples from the ingrowth cores were 0.5 g fresh weight.

Sample processing and respiration measurement for seedling and ingrowth roots were as outlined previously for the site comparison experiment. Following respiration measurement, all seedling and ingrowth roots were dried, ground and analyzed for N. Seedling root respiration data were analyzed using a two-factor (origin, temperature)

analysis of variance (ANOVA). Ingrowth core root respiration data were analyzed using a three-factor (origin, common garden, temperature) ANOVA.

Results

Patterns of Root Respiration Among Sites

Fine root respiration, measured as oxygen consumption, increased exponentially with temperature at all sites (Figure 1.2, Table 1.2), with an average Q_{10} of 2.7. There were no differences among sites in Q_{10} as evidenced by a lack of significant differences among slopes of the individual site regression lines in Table 2 ($P = 0.47$). However, there were significant differences among sites in respiration rate at a given temperature (Table 1.3), with respiration rates at Sites B and C generally higher than those at Sites A and D (Figure 1.2). The same pattern among sites occurred for fine root N concentration (Figure 1.3) and net N mineralization (Table 1.1). Rates of fine root respiration at 18 and 24 °C were positively correlated with both net N mineralization ($P < 0.05$) and root N

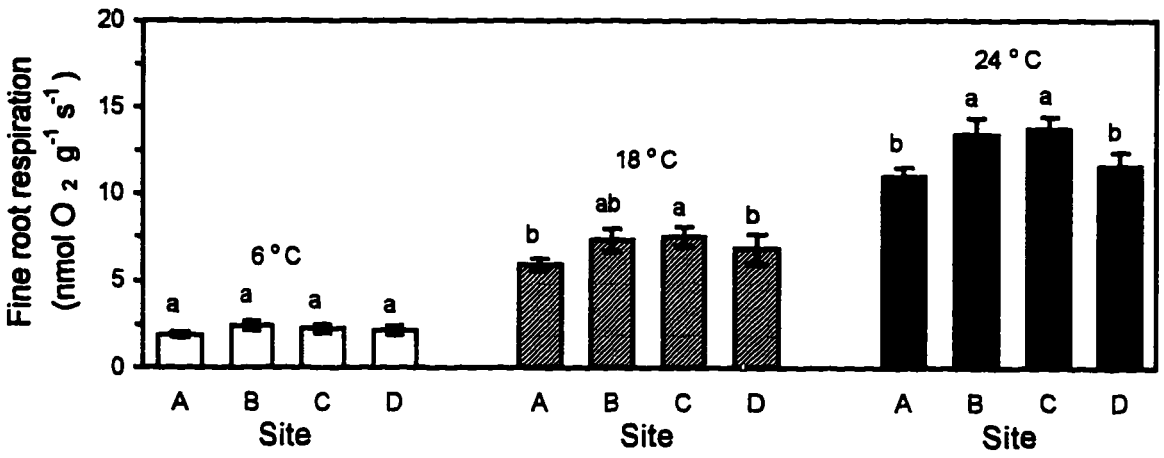

Figure 1.2 Mean root respiration at 6, 18 and 24 °C. Error bars indicate the standard error of the mean (n = 9). Bars within temperatures without common letters differ significantly at $P < 0.05$ (Tukey's HSD test).

Table 1.2 Individual site coefficients for regression of ln respiration (nmol O_2 g^{-1} s^{-1}) versus temperature (°C).

Site	n	Slope	Intercept	Standard error of estimate	r^2
A	36	0.097	0.057	0.210	0.91
B	36	0.096	0.266	0.265	0.86
C	36	0.107	0.041	0.251	0.90
D	36	0.096	0.119	0.260	0.87
All	144	0.099	0.120	0.255	0.88

Table 1.3 Analysis of covariance table for effects of site and temperature (covariate) on the natural logarithm of root respiration.

Source	df	SS	MS	F	*P* > F
Site	3	0.744	0.248	4.062	0.008
Temperature	1	67.477	67.477	1104.9	0.000
Error	139	8.489	0.061		

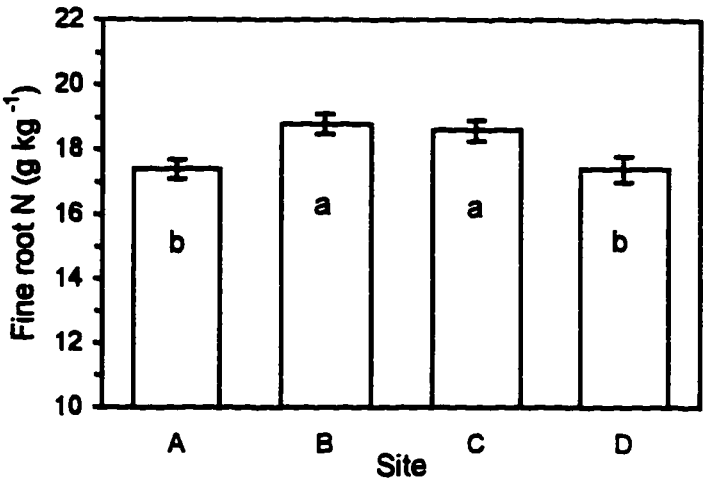

Figure 1.3. Mean site fine root N concentrations. Error bars indicate one standard error of the mean. Bars without common letters differ significantly at $P < 0.05$ (Tukey's HSD test).

concentration ($P < 0.10$). Fine root respiration was not correlated with mean annual site temperature. Fine root respiration across the sites was best predicted by a regression that included an exponential effect of temperature and a linear effect of fine root N concentration:

[1] $$Resp = 0.0622\ (N)\ e^{0.101\ (T)} \qquad (R^2 = 0.91)$$

where respiration is in nmol $O_2 \cdot g^{-1} \cdot s^{-1}$, N is in $g \cdot kg^{-1}$, and T is in degrees C.

Seedling and Root Ingrowth Cores

Root respiration in seedlings exhibited the same general pattern as the site comparison experiment, with respiration tending to be greater for seedlings of origins B and C (Table 1.4). The differences among seedling origins, however, were not significant ($P = 0.20$). There also were no significant differences among origins in seedling root N concentration (average N = 21 g kg^{-1}). Root ingrowth cores located at common garden B

also exhibited the pattern of higher root respiration in roots from cores of B and C origins (Table 1.4), but the differences again were not significant (P=0.20). In ingrowth cores from garden A, there were no significant differences among origins and the previously observed pattern, with higher rates in roots from soil cores of B and C origin, did not occur (Table 1.4).

Table 1.4 Fine root respiration in seedlings and ingrowth cores.

	Mean (S.E.) root respiration for origin			
Temperature	**A**	**B**	**C**	**D**
°C	---------- nmol O_2 g^{-1} s^{-1} ----------			
	Seedlings located at common garden C (n=4)			
12	4.6 (0.8)	5.4 (0.6)	5.2 (0.6)	3.8 (0.2)
24	15.1 (1.9)	17.5 (3.1)	18.0 (2.2)	14.9 (1.7)
	Root ingrowth cores located at common garden B (n=5)			
12	4.9 (0.7)	7.4 (1.0)	6.9 (1.6)	5.7 (1.1)
24	18.6 (2.0)	25.7 (5.2)	25.7 (1.9)	21.6 (1.2)
	Root ingrowth cores located at common garden A (n=6)			
12	3.8 (0.5)	3.8 (0.4)	3.4 (0.4)	4.2 (0.4)
24	13.8 (1.9)	13.2 (1.0)	11.6 (1.5)	13.9 (1.3)

Overall, mean fine root respiration rate in ingrowth cores from common garden A was significantly lower than that for cores from common garden B at both 12 and 24 °C (Figure 1.4). There was a similar difference among the two common gardens in mean fine root N concentration (Figure 1.4). Both fine root N concentration and temperature appear to be influencing respiration in the ingrowth core samples. When the mean N concentration for ingrowth roots from each common garden is used in eq [1], predicted root respiration rates at 12 and 24 °C are within about one standard error of the average values measured (Figure 1.4).

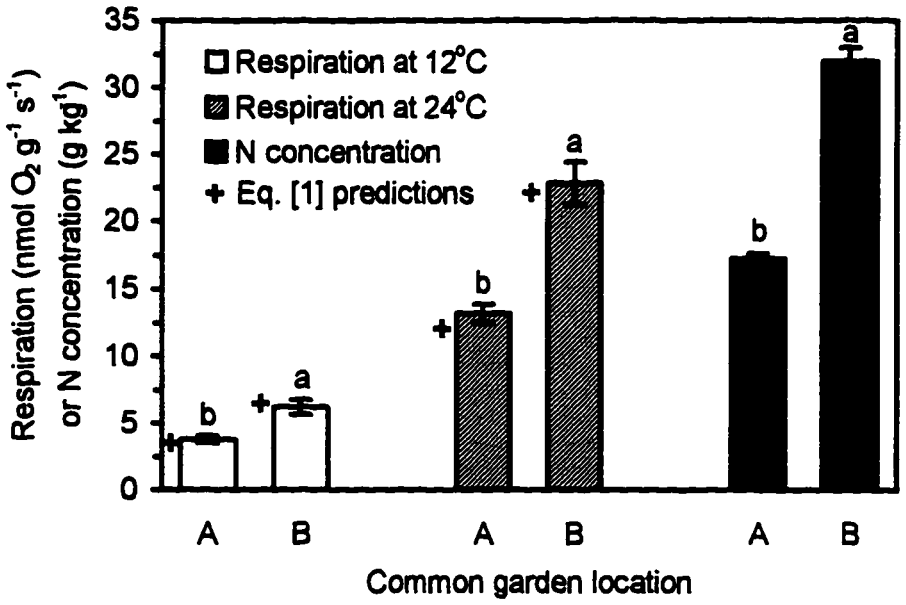

Figure 1.4 Mean fine root respiration rates and N concentrations for ingrowth cores located at common garden A (n = 24) and common garden B (n = 20). Common garden means are for all ingrowth cores sampled from the common garden, regardless of origin. Error bars indicate one standard error of the mean.

Discussion

When comparing results from this study to those of previous studies, several factors associated with methodologies need to be considered. This study reports values for O_2 consumption, while many other studies report values for CO_2 evolution. To directly compare such results, information on the respiratory quotient (ratio of CO_2 evolved to O_2 consumed; RQ) is needed. Using an IRGA hooked in line with the oxygen consumption chamber, an RQ of 0.8 was determined for the sites, with no significant differences existing among the four sites in RQ (A.J. Burton and G.P. Zogg, unpublished data). Carpenter and Mitchell (1980) previously reported an RQ of 0.92 for roots of sugar maple seedlings, and Edwards and Harris (1977) reported an RQ of 0.75 for roots in a mixed deciduous forest.

The CO_2 concentration at which measurements are made also can influence results. Qi et al. (1994) observed an exponential decrease in Douglas-fir root respiration as CO_2 increased from 130 to 7015 $\mu l\ l^{-1}$. The effect was most pronounced at CO_2 levels less than 2000 $\mu l\ l^{-1}$. For sugar maple, a 15% decrease in oxygen consumption was found as CO_2 concentration increased from 1000 to 3000 $\mu l\ l^{-1}$ (A.J. Burton and G.P. Zogg, unpublished). From 3000 to 25000 $\mu l\ l^{-1}$ CO_2, rates of root respiration remain essentially constant, with values always within 6% of the rate at 3000 $\mu l\ l^{-1}$ CO_2 (A.J. Burton and G.P. Zogg, unpublished). For the respiration measurements reported in this paper, average CO_2 concentrations in the sample cuvettes ranged from 3,900 $\mu l\ l^{-1}$ at 6 °C to 14,400 $\mu l\ l^{-1}$ at 24 °C, with no differences among sites. Soil CO_2 for northern hardwoods can range from near atmospheric to 19,000 $\mu l\ l^{-1}$ (Yavitt et al. 1995), and soil CO_2

concentrations of 600 to 2000 $\mu l\ l^{-1}$ have been measured at the four Michigan sites (G.P. Zogg, unpublished). Based on this, values for root respiration in this report should deviate no more than 20% from those that would be measured at actual soil CO_2 concentrations (ca. 1000 $\mu l\ l^{-1}$). Coincidentally, the bias induced by the high CO_2 concentrations in the oxygen consumption chambers is of the same magnitude and opposite sign as the adjustment needed to compensate for RQ. Due to these counteracting effects, rates of fine root respiratory CO_2 production at the sites are likely quite close to the reported values for O_2 consumption.

Observed rates of sugar maple fine root respiration (Figure 1.2) were well within the range reported for other deciduous species (Lawrence and Oechel 1983; Walters et al. 1993) and are slightly lower than rates previously reported for sugar maple. Walters et al. (1993) measured total root system respiration of 5 to 35 nmol $CO_2\ g^{-1}\ s^{-1}$ at 21.5 °C for sugar maple seedlings using an infra-red gas analyzer (IRGA). They found no effect on respiration of intact versus severed root systems. Reid and Strain (1994) used an IRGA to measure root respiration of sugar maple seedlings grown in root boxes and reported rates of 7 to 9 nmol $CO_2\ g^{-1}\ s^{-1}$ at 15 °C and 10 to 12 nmol $CO_2\ g^{-1}\ s^{-1}$ at 19 °C. Measurement of respiration at low CO_2 concentrations may have contributed to the higher rates of sugar maple root respiration observed in these studies. Fahey and Hughes (1994) used an IRGA to measure fine root respiration on freshly detached northern hardwood root mats and reported values of 8.0 nmol $CO_2\ g^{-1}\ s^{-1}$ in June when soil temperature was 12 °C and 5.2 nmol $CO_2\ g^{-1}\ s^{-1}$ in mid-August when soil temperature was 14 °C. Their August value is in better agreement with the results of this study, which also was conducted late in the growing season.

Fahey and Hughes (1994) ascribed the higher respiration rate in June, despite lower soil temperature, to respiration associated with rapid early season root growth. The respiration values determined in this study also likely contain a component of growth respiration in addition to maintenance respiration. However, since production of new roots at the four sites is minimal during the months sampled (Hendrick and Pregitzer 1993), maintenance respiration should represent the majority of measured O_2 consumption. Based on rates of fine root production presented by Hendrick and Pregitzer (1993) for September to October at Sites C and D, and a construction respiration cost of 0.25 g CO_2-C · g C^{-1} (Penning de Vries 1975), a growth respiration rate of 0.3 nmol CO_2 g^{-1} s^{-1} is estimated for the period during which the samples were taken. Using this approach, the maximum growth respiration estimated for any month at the sites is 1.1 nmol CO_2 g^{-1} s^{-1} in May when 26% of annual production occurs.

As expected, root respiration at the sites increased exponentially with temperature. The Q_{10} of 2.7 for root respiration is somewhat higher than the Q_{10} of 2 often reported for plant respiration (Amthor 1984; Lawrence and Oechel 1983; Ryan et al. 1996), but is well within the range reported for fine root respiration (2.7, Cox 1975; 2.1, Cropper and Gholz 1991; 1.5-3.0, Lawrence and Oechel 1983; 2.0, Sowell and Spomer 1986; 2.1, Zogg et al. 1996). Hypothesized regional increases in soil temperature (Botkin et al. 1989) would be expected to result in increased fine root respiration at all of the sites, but actual increases in the field might be less than predicted using the Q_{10} of 2.7, if acclimation to altered temperature regimes occurs. Acclimation to high temperatures can result in plant respiration rates lower than predicted (Amthor 1989), and acclimation to low temperatures has been shown to include increases in respiration rates (Billings 1974;

Chabot 1979). For sugar maple, Gunderson et al. (1995) reported acclimation in seedling foliar respiration, resulting in slightly lower respiration at high temperature for acclimated plants than predicted from ambient grown plants. The degree to which this phenomenon may affect sugar maple roots is unknown. Zogg et al. (1996) measured root respiration at field temperature (typically 8 to 14 °C) over the entire 1994 growing season at the same sites, and found less of an increase in respiration with temperature (Q_{10} = 2.1) than reported in this study. One possible explanation for the differences in observed Q_{10} is acclimation in root respiration as soil temperatures at a site change over the growing season.

Temperature was responsible for the majority of variation in fine root respiration observed in this study (r^2 = 0.88, Table 1.2); however, differences did exist among the sites in mean respiration rate at a given temperature. The pattern among sites (Figure 1.2) was not what one would expect if ecotypic variation related to climatic differences were responsible. If adaptation to local climate were responsible, one would expect to see highest rates of respiration at a given temperature occurring at the coolest site (Site A), with decreasing rates of respiration as one moved to the warmer, more southern sites. Such effects have been observed for several tree species (Sowell and Spomer 1986). Genetic differences in sugar maple do exist across the region encompassed by this study (Wood and Hanover 1980; J.P. Schoonover, pers. comm.), and provenance tests for sugar maple indicate the existence of temperature races (Kriebel and Gabriel 1969; Scanlon 1976), but the O_2 consumption results provide no evidence of genetic adaptation to local climate for root respiration. Gunderson et al. (1995) came to a similar conclusion regarding the influence of geographic source on foliar respiration of sugar maple

seedlings. Similarly, Ledig and Korbobo (1983) did not find any direct correspondence between seed source altitude and differences in temperature response functions for foliar dark respiration in sugar maple seedlings from an altitudinal gradient.

Site differences in N availability and fine root N concentration appear to be responsible for the observed differences among the four sites in respiration at a given temperature. Higher rates of respiration were consistently observed in roots having higher N concentration in both the site comparison and ingrowth core experiments, and the higher root N concentrations always occurred at sites having greater N mineralization rates. Zogg et al. (1996), working at the same sites, came to a similar conclusion. Clearly, root respiration C flux at these sites could be altered by hypothesized changes in N availability associated with chronic N deposition (Aber et al. 1989) or altered N mineralization resulting from global climate change (Pastor and Post 1988; Cohen and Pastor 1991).

An increase in respiration as N concentration increases has been documented for many types of plant tissue (Jones et al. 1978; Merino et al. 1982; Ryan 1991a), including roots (Ryan et al. 1996). In these studies maintenance respiration per unit N at 15 °C has ranged from 1.71 to 3.70 μmol CO_2 s^{-1} $(mcl\ N)^{-1}$ for foliage (Ryan 1991a, Ryan 1995, Ryan et al. 1996) and was reported as 2.6 μmol CO_2 s^{-1} $(mol\ N)^{-1}$ for fine roots (at 1500 μl l^{-1} CO_2, Ryan et al. 1996). Using equation [1], respiration per unit N at 15 °C for fine root samples from this study was 3.9 μmol O_2 s^{-1} $(mol\ N)^{-1}$.

No evidence of ecotypic variation in fine root respiration of sugar maple forests was found, but differences in respiration related to root N concentration did exist. A

simple predictive relationship based on temperature (Q_{10}) is therefore not appropriate for estimating fine root respiration across the stands studied. Such an equation does not predict the existing differences among the sites, resulting in errors of up to 10% in estimated root respiration rates for individual sites. The magnitude of error is even greater (up to 47%) for the high N concentration root samples from the ingrowth cores. For sugar maple forests, tissue N concentration should be considered when estimating fine root respiratory costs.

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Chapter II

EFFECT OF MEASUREMENT CO_2 CONCENTRATION ON SUGAR MAPLE ROOT RESPIRATION

Abstract

Accurate estimates of root respiration are crucial to predicting belowground C cycling in forest ecosystems. Inhibition of respiration has been reported as a short-term response of plant tissue to elevated measurement [CO_2]. This study sought to determine if measurement [CO_2] affected root respiration in samples from mature sugar maple (*Acer saccharum* Marsh.) forests and to assess possible errors associated with root respiration measurements made at [CO_2] lower than that typical of the soil atmosphere. Root respiration was measured as both CO_2 production and O_2 consumption on excised fine roots ($\leq$ 1.0 mm) at [CO_2] ranging from 350 $\mu l\ l^{-1}$ to > 20,000 $\mu l\ l^{-1}$. Root respiration was significantly affected by the [CO_2] at which measurements were made for both CO_2 production and O_2 consumption. Root respiration was most sensitive to [CO_2] near and below normal soil concentrations (< 1500 $\mu l\ l^{-1}$). Respiration rates changed little at [CO_2] above 3000 $\mu l\ l^{-1}$ and were essentially constant above 6000 $\mu l\ l^{-1}$ CO_2. These findings call into question estimates of root respiration made at or near atmospheric [CO_2], suggesting that they overestimate actual rates in the soil. In this study, sugar maple root respiration at atmospheric [CO_2] (350 $\mu l\ l^{-1}$) was about 139% of that at soil [CO_2]. Although the causal mechanism remains unknown, the increase in root respiration at low measurement [CO_2] is significant and should be accounted for when estimating or modeling root respiration. Until the direct effect of [CO_2] on root respiration is fully understood, it is

recommended that respiration be measured at [CO_2] representative of, or higher than, soil [CO_2]. In all cases, the [CO_2] at which measurements are made and [CO_2] typical of the soil atmosphere should be reported.

Introduction

Plant respiration is an important component of terrestrial C cycles (Landsberg 1986, Running and Gower 1991, Ryan 1991, Vogt 1991). In forest ecosystems, root respiration can represent 30 to 50 percent of total belowground C allocation (Ryan et al. 1996) and contributes from 30 to 60 percent of total soil CO_2 efflux (Edwards and Harris 1977, Ewel et al. 1987, Bowden et al. 1993, Haynes and Gower 1995, Ryan et al. 1996). Therefore, accurate estimates of root respiration are crucial to predicting responses of forest C cycling and productivity to climate change or elevated [CO_2].

Inhibition of respiration has been reported as both a long-term and short-term response of plant tissue to elevated [CO_2] (Amthor 1991). Altered respiration following long-term exposure to elevated [CO_2] can result from changes in growth rate, growth efficiency or tissue chemical composition (Amthor 1991, Bunce and Caulfield 1991, Wullschleger et al. 1992, 1994), and such changes may have important impacts on C cycling in a future, higher-[CO_2] world. Of more immediate concern, however, may be the short-term, reversible effects of elevated [CO_2], in which reductions in measured tissue respiration occur as the [CO_2] at which measurements are made is increased (Gale 1982, Gifford et al. 1985, Bunce 1990, Amthor et al. 1992). Such effects have recently been reported for roots of Douglas-fir seedlings (Qi et al. 1994). Whether this CO_2 effect also occurs for roots from mature trees and how universal it is across tree species is unknown. Still, these findings call into question estimates of belowground C allocation that are based in part on root respiration measured at or near atmospheric [CO_2] (350 $\mu l\ l^{-1}$), which is much lower than that typical of the soil environment.

This study sought to determine if measurement [CO_2] inhibits the respiration of fine roots ($\leq$1.0 mm) from mature sugar maple forests. Objectives were: (1) to determine the degree, if any, to which sugar maple root respiration declines as [CO_2] is increased; (2) to determine the [CO_2] range over which the effect occurs; (3) to determine if the effect is similar for both CO_2 production and O_2 consumption; and (4) to assess possible errors associated with root respiration measurements made at atmospheric [CO_2].

Materials and Methods

Study Sites and Experimental Design

To investigate the effects of measurement [CO_2] on fine root respiration, a series of experiments examining CO_2 production and O_2 consumption was conducted. Fine root samples used in the experiments were collected from four sugar maple forests in Michigan (Table 2.1). The forests are second-growth northern hardwoods, approximately 85 years in age, dominated by sugar maple and occurring on sandy, well-drained Spodosols (Burton et al. 1991). Six 30 x 30 m study plots are located at each site (Zogg et al. 1996). Samples for root respiration measurements were collected from 10 m wide buffer strips surrounding each plot. For all experiments, soil cores (10 cm deep, 5.4 cm diameter) were collected from three random locations in each buffer. The cores were transported to nearby field labs (less than 1 hour travel time per site), and composited on a plot basis.

All fine ($\leq$1.0 mm), non-woody, live roots were sorted from each core and rinsed free of soil and organic matter with deionized water. Live roots were distinguished by white, cream, tan or brown coloration and a smooth appearance. Dead roots were dark

Table 2.1 Climatic and overstory characteristics of four sugar maple forests in Michigan, USA. Overstory data are for the year 1995.

	Site A	Site B	Site C	Site D
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°51'	85°50'	86°09'
Mean annual precipitation[a] (mm)	870	830	810	850
Mean annual temperature[a] (°C)	4.2	5.2	5.8	7.6
Total basal area (m^2 ha^{-1})	34	31	32	33
Sugar maple basal area (%)	86	86	83	75
Overstory age	88	82	83	87

[a] 30-year means from National Oceanic and Atmospheric Administration (1983) records.

brown or black in color, were brittle, and had frayed, rough edges. Root samples consisted primarily of intact small root mats consisting of many attached root segments. These small root mats typically contained two to five root orders and had total root lengths of < 50 to > 500 mm (V. Lessard, unpublished data). Care was taken to minimize damage to the roots, and fine roots were severed only when necessary for separation from coarse roots. Excess water was blotted from the root samples and 0.5 g (fresh weight) subsamples were wrapped in moistened tissue paper and used for respiration measurements. All respiration measurements reported in this paper were made at 24 °C and were completed within three hours of sample collection. Following respiration measurements root subsamples were oven-dried (65 °C, 24 h) for determination of dry weights.

Respiration as CO_2 Production

The effect of measurement [CO_2] on fine root CO_2 production was investigated by: (1) measuring CO_2 production in an open system using input [CO_2] of 350, 700, 1000 and 2000 $\mu l\ l^{-1}$; and (2) monitoring CO_2 production over time in a closed respiration system as [CO_2] increased from near atmospheric to > 5,000 $\mu l\ l^{-1}$. Carbon dioxide concentrations in these experiments were measured with an infra-red gas analyzer (IRGA; CIRAS-I portable gas analyzer, PP Systems, Haverhill, MA).

Measurement of CO_2 production at [CO_2] of 350, 700, 1000, and 2000 $\mu l\ l^{-1}$ was performed in September, 1995 using samples from all plots at each site. The IRGA was operated in an open-system configuration and programmed to provide the desired input [CO_2] to the sample chamber. At each concentration, respiration was allowed to achieve steady state before rates were recorded (approximately 20 minutes). For all samples, the 350 and 2000 $\mu l\ l^{-1}$ concentrations were the first two tested. The order in which these two concentrations were used was alternated from one sample to the next in order to assess reversibility of any observed CO_2 effect. Appropriate sample temperature was achieved by maintaining lab temperature at 24°C.

The highest input [CO_2] the IRGA could be programmed to provide was 2,000 $\mu l\ l^{-1}$. The effects on CO_2 production of measurement [CO_2] greater than 2,000 $\mu l\ l^{-1}$ were determined by monitoring CO_2 production in a closed system as [CO_2] increased from < 1000 $\mu l\ l^{-1}$ to > 5,000 $\mu l\ l^{-1}$. This experiment was conducted at Site B in May, 1995. Respiration subsamples were placed in a water-jacketed cuvette in a closed loop configuration with the IRGA. Samples were allowed to equilibrate to cuvette temperature

(24 °C) for 20 minutes, after which the system was closed and respiration was monitored for 50 to 60 minutes. Carbon dioxide concentrations were recorded every 5 minutes, allowing the effects of $[CO_2]$ from < 1000 μl l^{-1} to >5,000 μl l^{-1} to be determined. Initial $[CO_2]$ was typically around 700 μl l^{-1}. This was a result of room $[CO_2]$ of about 500 μl l^{-1} in the field lab and respiration that occurred between the time the system was closed and the initial reading was recorded.

Respiration as O_2 Consumption

The effect of measurement $[CO_2]$ on fine root O_2 consumption was investigated by: (1) monitoring O_2 consumption over time in a closed respiration system as $[CO_2]$ increased from < 1000 μl l^{-1} to > 20,000 μl l^{-1}, and (2) measuring O_2 consumption in a closed system at mean $[CO_2]$ of 14,000, 29,000 and 44,000 μl l^{-1}. Oxygen consumption was measured with temperature-controlled O_2 electrode cuvettes (model LD 2/2 oxygen electrode, Hansatech Instruments Ltd., Norfolk, England) connected to constant temperature circulating water baths (Burton et al. 1996, Zogg et al. 1996).

In September, 1995, respiration as O_2 consumption was measured in a closed cuvette as $[CO_2]$ increased from < 1000 μl l^{-1} to > 20,000 μl l^{-1}. This was done for all plots at all sites. Temperature in the field labs was maintained at 24 °C during this work to minimize the time needed for samples to equilibrate to measurement temperature. After root samples were placed in the cuvette, they were allowed to equilibrate for five minutes. The cuvettes were then purged with air and closed, and root respiration was followed for 50 to 60 minutes, during which cuvette $[CO_2]$ was allowed to increase, with O_2 concentrations recorded every five minutes. Initial $[CO_2]$ in the cuvettes was between

700 and 1000 $\mu l\ l^{-1}$ because of the injection of laboratory air at approximately 500 $\mu l\ l^{-1}$ CO_2, and respiration that occurred between the injection of the air and taking of the initial reading. The increase in $[CO_2]$ during this brief period was calculated by multiplying measured reduction in $[O_2]$ concentration (from atmospheric) by a respiration quotient of 0.8 (A.J. Burton and G.P. Zogg, unpublished data). The $[CO_2]$ in the cuvette at the beginning of each subsequent five-minute interval was estimated by adding a value equal to 0.8 times the reduction in $[O_2]$ that occurred during the previous five minutes.

The experiment in which O_2 consumption was measured in a closed system at mean $[CO_2]$ of 14,000, 29,000 and 44,000 $\mu l\ l^{-1}$ was conducted to investigate the effects of $[CO_2]$ concentration near or above the highest $[CO_2]$ that might occur in the soil (Yavitt et al. 1995, Amundson and Davidson 1990) or when using O_2 electrodes. The experiment was conducted at Site B in early October, 1994, and repeated at Site B in May, 1995. Fine root samples were placed in cuvettes which were then purged with ten cuvette volumes of certified gas standards containing 0, 15,000 or 30,000 $\mu l\ l^{-1}$ CO_2 and 21% O_2. After purging, the cuvettes were immediately closed. Samples were allowed to equilibrate to temperature (24 °C) for twenty minutes after which respiration was monitored for 40 to 50 minutes. Carbon dioxide accumulation during the equilibration and measurement periods resulted in mean measurement $[CO_2]$ in the closed cuvettes of 14,000, 29,000, and 44,000 $\mu l\ l^{-1}$ for the three treatments (values are based on initial $[CO_2]$, measured O_2 consumption, and a respiration quotient of 0.8).

Statistical Analyses

Analysis of variance (ANOVA) and repeated measures ANOVA (Wilkinson 1990) were used to compare root respiration rates recorded at different measurement [CO_2]. Where repeated measures ANOVA was used, the response functions for respiration were analyzed for linear, quadratic, and higher order effects of [CO_2] using orthogonal polynomials (Wilkinson 1990). In experiments where [CO_2] was allowed to increase over time for individual samples, data points were assigned to CO_2 concentration classes prior to repeated measures ANOVA. The concentration classes used were every 500 $\mu l\ l^{-1}$ for the experiment in which [CO_2] was allowed to increase from < 1000 $\mu l\ l^{-1}$ to >5000$\mu l\ l^{-1}$; and every 1000 $\mu l\ l^{-1}$ for the experiment in which [CO_2] in the oxygen electrode cuvette was allowed to increase from < 1000 $\mu l\ l^{-1}$ to >20,000 $\mu l\ l^{-1}$. The reversibility of the CO_2 effect was tested by a *t*-test in which the mean ratio of respiration at 350 $\mu l\ l^{-1}$ CO_2 to that at 2000 $\mu l\ l^{-1}$ was compared between samples that received the 350 $\mu l\ l^{-1}$ concentration first and those that received the 2000 $\mu l\ l^{-1}$ concentration first. To facilitate comparison of the CO_2 production and O_2 consumption data, results were also expressed as rates relative to the rate of respiration measured at 1000 $\mu l\ l^{-1}$ CO_2. Linear and non-linear regression (Wilkinson 1990) were used to examine the effect of [CO_2] on respiration for the resulting combined data set.

Results

Root respiration rate measured as CO_2 production was significantly affected by the [CO_2] at which measurements were made (Figures 2.1 and 2.2). Respiration rate measured at 350 $\mu l\ l^{-1}$ CO_2 was about 1.2 times the rate measured at 1000 $\mu l\ l^{-1}$ and 1.6 times greater

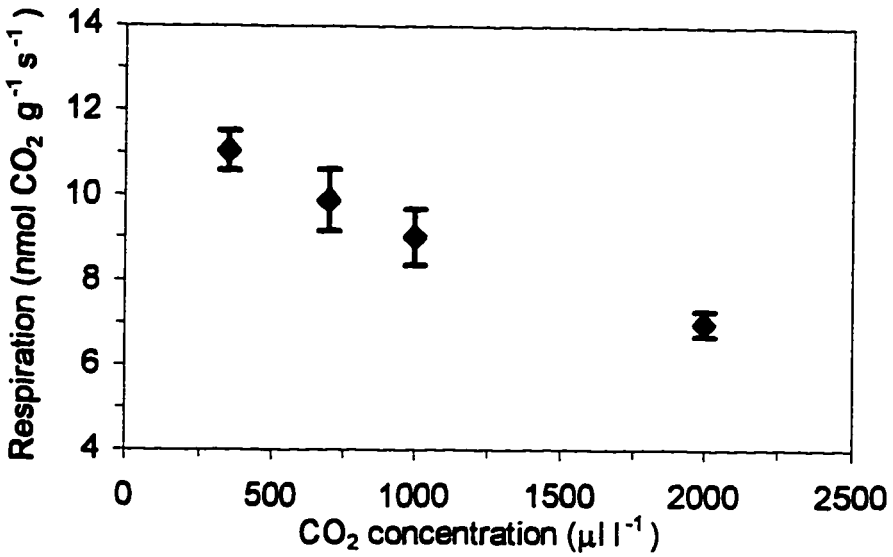

Figure 2.1 Respiration as CO_2 production at 350, 700, 1000 and 2000 µl l^{-1} CO_2 (all sites, September, 1995). Error bars indicate one standard error of the mean. The linear and quadratic effects of $[CO_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

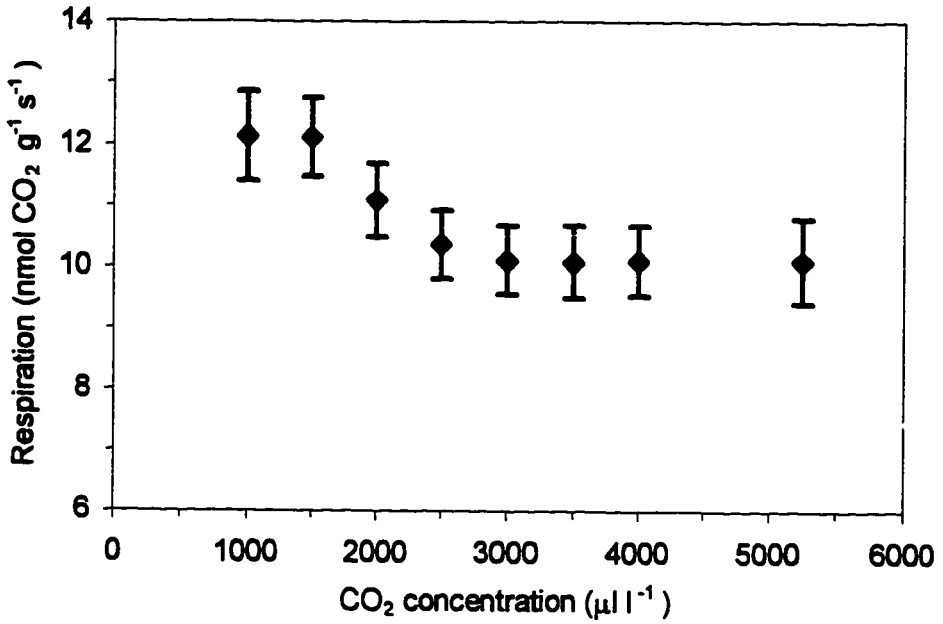

Figure 2.2 Respiration as CO_2 production at $[CO_2]$ from 1000 to > 5000 µl l^{-1} (Site B, May, 1995). Error bars indicate one standard error of the mean. The linear, quadratic and cubic effects of $[CO_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

than that measured at 2000 μl l^{-1} (Figure 2.1). As $[CO_2]$ was further increased, the decline in respiration rate was less noticeable, with rates changing little above 3000 μl l^{-1} (Figure 2.2). The effect of CO_2 on respiration was the same under increasing and decreasing $[CO_2]$. The ratio of respiration rate at 350 μl l^{-1} to that at 2000 μl l^{-1} was 1.57 under increasing $[CO_2]$ and 1.60 under decreasing $[CO_2]$ (no significant difference, P = 0.74).

Respiration rate measured as O_2 consumption also decreased with increasing measurement $[CO_2]$ (Figure 2.3). Respiration rates declined as CO_2 concentration increased to about 6000 μl l^{-1}, with most of the decline occurring at $[CO_2] \leq 3000$ μl l^{-1}. Above 6000 μl l^{-1} oxygen consumption rate was essentially constant (Figure 2.3).

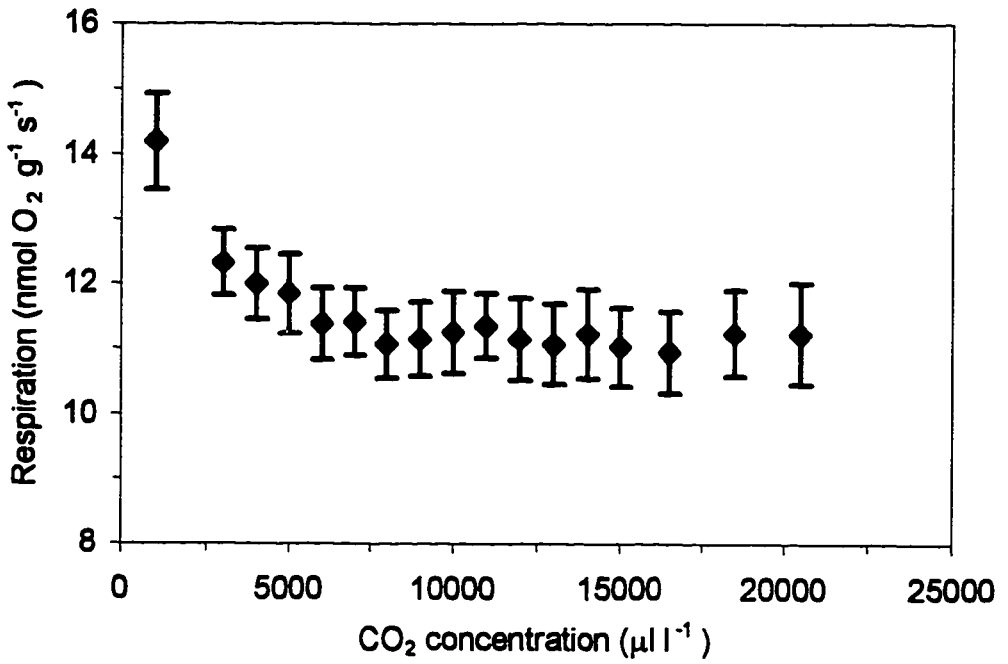

Figure 2.3 Respiration as O_2 consumption at $[CO_2]$ from 1,000 to > 20,000 μl l^{-1} (all sites, September, 1995). Error bars indicate one standard error of the mean. The linear, quadratic and cubic effects of $[CO_2]$ are significant at $P < 0.01$ (repeated measures ANOVA).

Increases in $[CO_2]$ to very high values (up to 44,000 $\mu l\ l^{-1}$) did not further reduce O_2 consumption rates (Figure 2.4).

Direct comparison of the effects of measurement $[CO_2]$ on O_2 consumption and CO_2 production is facilitated by expressing the observed respiration rates as percentages relative to rates at 1000 $\mu l\ l^{-1}$ (Figure 2.5). Rates of O_2 consumption and CO_2 evolution at 5000 $\mu l\ l^{-1}$ were both 83% of those observed at 1000 $\mu l\ l^{-1}$. As CO_2 concentrations were further increased to >20,000 $\mu l\ l^{-1}$, O_2 consumption declined to approximately 79% of the rate observed at 1000 $\mu l\ l^{-1}$. The data shown in Figure 2.5 can be modeled by the relationship:

$$R_{1000} = 78.5 + (17714/[CO_2]) \qquad R^2 = 0.89 \qquad (1)$$

where R_{1000} is respiration relative to that at 1000 $\mu l\ l^{-1}$, and $[CO_2]$ is measurement concentration in $\mu l\ l^{-1}$. Models with exponential functions had similar predictive ability to equation 1 for the range of data shown in Figure 2.5, but equation 1 was preferred as it predicts minimal additional decline in respiration at very high $[CO_2]$ (> 20,000 $\mu l\ l^{-1}$), in agreement with the results illustrated in Figure 2.4. Equation 1 can be rearranged to the following form which allows respiration rate (R_B) at a specified $[CO_2]$ to be estimated from the rate (R_A) measured at a different $[CO_2]$:

$$R_B = \frac{R_A\ (0.785 + 177 / [CO_2]_B)}{0.785 + 177 / [CO_2]_A} \qquad (2)$$

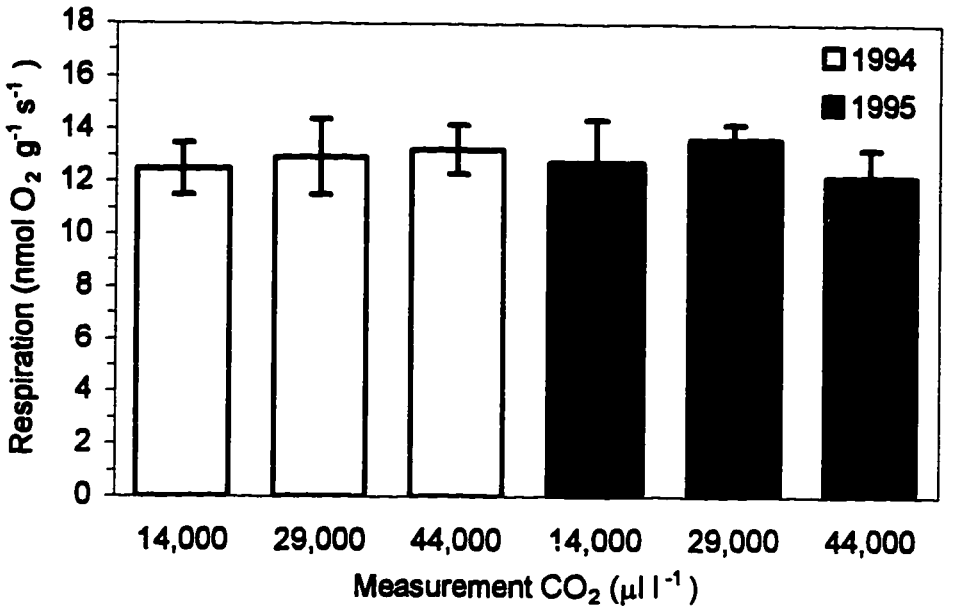

Figure 2.4 Mean root respiration at average measurement cuvette [CO_2] of 14,000, 29,000 and 44,000 µl l^{-1} (Site B). Error bars indicate one standard error of the mean. Respiration means within years are not significantly different ($P = 0.89$ in 1994; $P = 0.67$ in 1995).

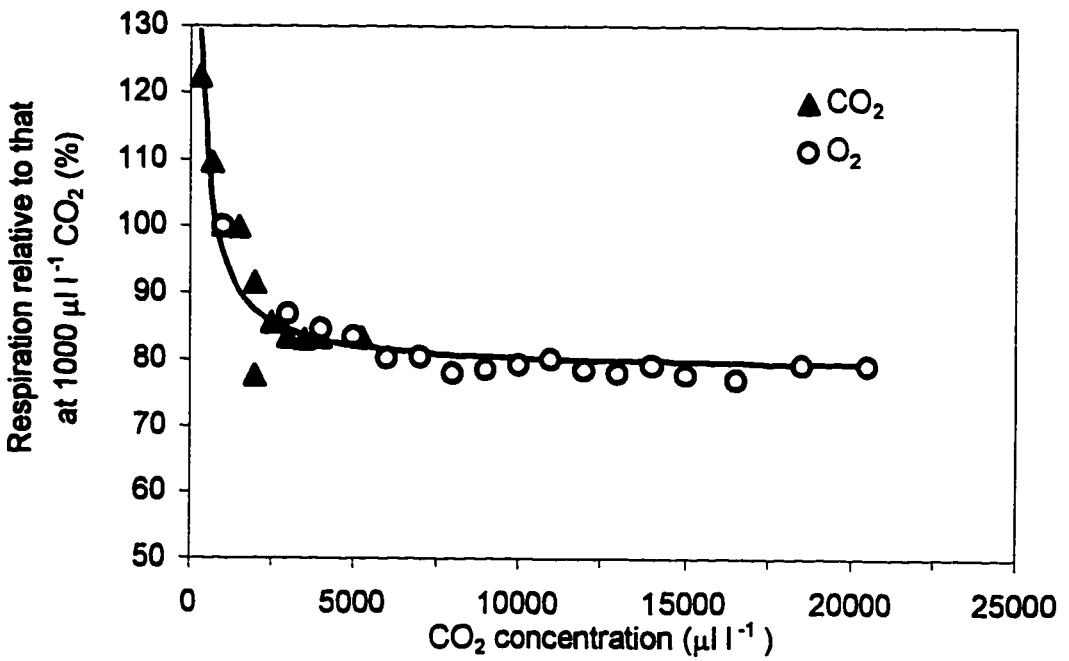

Figure 2.5 Oxygen consumption and CO_2 production expressed as rates relative to those occurring at a measurement [CO_2] of 1000 µl l^{-1}. The line displayed is that represented by equation 1.

Discussion

The reduction in respiration observed at high [CO_2] is an example of what Amthor (1991) termed a direct effect -- one that results from [CO_2] at the time of respiration measurement and is readily reversible. Such reductions have been reported previously for aboveground plant tissues (Reuveni and Gale 1985, Bunce 1990, Amthor 1991, Amthor et al. 1992) and for roots (Reuveni and Gale 1985, Palta and Nobel 1989, Nobel 1990), including the roots of trees (Qi et al. 1994, Ryan et al. 1996). The degree of direct reduction in respiration at high [CO_2] varies greatly among plants and tissues. Amthor et al. (1992) measured respiration in leaves of *Rumex crispus* (L.) at [CO_2] from 50 to 950 $\mu l\ l^{-1}$, and found that for every doubling of [CO_2], respiration declined by 25 to 30%. Respiration at 350 $\mu l\ l^{-1}$ CO_2 in leaves and plant shoots has been reported to be 1.1 to 2.0 times that at 700 $\mu l\ l^{-1}$ (Bunce 1990, El-Kohen et al. 1991, cited by Bunce 1994, Wullschleger et al. 1994). In contrast, Palta and Nobel (1989) found measurement [CO_2] between 350 and 2000 $\mu l\ l^{-1}$ did not affect respiration in *Agave deserti* roots, but that respiration was significantly reduced at [CO_2] above 2000 $\mu l\ l^{-1}$ and completely inhibited at 20,000 $\mu l\ l^{-1}$. Pheloung and Barlow (1981) found respiration in wheat apices was insensitive to [CO_2] from 300 to 5000 $\mu l\ l^{-1}$. Such findings have led to speculation that organs such as roots might be adapted to the range of [CO_2] that they normally experience and might be insensitive to [CO_2] at or below normal concentrations (Amthor 1991, Amthor et al. 1992). The results of this study and those of Qi et al. (1994) suggest that for tree species, the opposite is true -- root respiration is most sensitive to [CO_2] at and below normal soil values.

A median soil [CO_2] of 1,200 μl l^{-1} at has been measured at the four sites (G.P. Zogg, unpublished data from soil gas samples taken using a needle and syringe at 5 and 10 cm depths periodically during the 1995 growing season), similar to the value of 1,350 μl l^{-1} reported by Yavitt et al. (1995) for northern hardwoods in New York. Changes in respiration per unit change in [CO_2] were much greater at concentrations below these values than above them (Figure 2.5). Above 3,000 μl l^{-1}, further increases in [CO_2] resulted in very little change in respiration (Figure 2.5), suggesting it is probably better to view respiration as being artificially enhanced by low measurement [CO_2] rather than inhibited by high [CO_2].

Results from this study are for excised roots, and it is possible the sample excision and cleaning affected respiration rates to some degree. But it is doubtful that this had much impact on the observed [CO_2] effect, as Qi et al. (1994) observed similar results for intact, undisturbed roots of Douglas-fir seedlings. In their experiment, very large increases in respiration rate occurred at [CO_2] less than atmospheric and smaller changes in respiration rate occurred at [CO_2] above 1500 μl l^{-1}. These results are of concern because root respiration measurements are often made at or near the atmospheric [CO_2] of approximately 350 μl l^{-1} (Lawrence and Oechel 1983, Cropper and Gholz 1991, Ryan et al. 1996), potentially leading to errors in reported values. Ryan et al. (1996) estimated *Pinus radiata* root respiration at soil [CO_2] (about 1500 μl l^{-1}) to be only 23% of that measured at 400 μl l^{-1}. In this study, sugar maple root respiration at soil [CO_2] was about 72% of that at atmospheric [CO_2] (350 μl l^{-1}). These estimates may oversimplify the real world situation, as both root biomass and soil [CO_2] also vary with depth and time

(Yavitt et al. 1995, Hendrick and Pregitzer 1996); however, it is clear that respiration rates made near atmospheric [CO_2] overestimate actual field values.

The cause of lower plant tissue respiration rates at high [CO_2] is not well understood, but several theories have been proposed. Amthor (1991) suggested that direct inhibition of respiration by high [CO_2] might be caused by alteration of intracellular pH, inhibition of enzyme activity through the formation of carbamates, increased fixation of CO_2, or reduced consumption of respiratory products possibly caused by modification of membrane function. The data from this study suggest that CO_2 fixation is not the cause. Increased fixation of CO_2 into organic acids at high [CO_2] without a change in actual respiration would lead to a decrease in apparent CO_2 production, but this cannot explain the co-occurring decrease in O_2 consumption observed for the samples. Griffin et al. (1996) and Gifford et al. (1985) similarly found that rates of O_2 consumption and CO_2 production were both reduced by high [CO_2] in leaves.

In the experiments, changes in [CO_2] had a much greater influence on respiration at low [CO_2] than at high [CO_2] (Figure 2.5). This is expressed by the inverse relationship in equation 1, and is consistent with the type of relationship that might occur if pH changes in an unbuffered system or simple reactions such as carbamate formation were the cause. However, plant cytoplasmic pH appears to be well regulated (Smith and Raven 1979), making it unlikely that CO_2 could affect respiration through altered intracellular pH unless [CO_2] changes were very large (Bown 1985). The pH reduction of 0.35 units observed by Nobel (1990) in macerated root tissue exposed to 2% CO_2 for 10 h appears to be consistent with this prediction. Nobel (1990) found little change in root tissue pH

after 2 h of exposure to 2% CO_2, thus his results do not explain the reductions in respiration associated with much smaller changes in [CO_2] over relatively brief periods of time. In addition, the effects of high CO_2 on respiration have been observed in experiments in which pH was controlled (Amthor 1991).

Inhibition of enzyme activity and altered membrane function both remain as possible explanations for the observed effects of [CO_2] on respiration. Gonzalez-Meler et al. (1996) suggest that inhibition of respiratory enzymes, especially cytochrome *c* oxidase, is the basis for respiratory inhibition by high [CO_2]. Lorimer (1983) has argued that [CO_2] may regulate metabolism through carbamate formation (i.e., the reversible reaction between CO_2 and the amine group of an enzyme). For example, inhibited enzyme activity during the latter stages of oxidative decarboxylation could lead to a feedback mechanism in which respiration is reduced until cellular [CO_2] is lowered by diffusion. Membrane functions such as transport and maintenance of ion gradients could be affected by [CO_2] through interference with lipid function (localized CO_2 tension can make membranes "thinner") or through formation of carbamates with free amino groups of membrane proteins (Mitz 1979). Such changes could affect consumption of end products (e.g., ATP), reducing respiration through feedback control mechanisms (Amthor 1991).

Decreased activity of the alternative (cyanide resistant) pathway provides another possible explanation for reduced respiration at high [CO_2] (Amthor 1991, Qi et al. 1994). This non-phosphorylating pathway serves as a secondary pathway for excess respiratory substrate (Lambers 1980) and also may be important in the coarse control of carbohydrate metabolism (Day and Lambers 1983). When the alternative pathway is engaged, more

carbohydrate is expended per unit ATP and respiratory rates are potentially higher. If alternative pathway activity is reduced at elevated [CO_2], then reduced respiration could occur without a decrease in energy made available (Bunce 1990). Mechanisms through which [CO_2] might influence electron flow between the two pathways have not been definitively determined, but enzyme inhibition resulting from altered intracellular pH or carbamate formation has been suggested (Palet et al. 1991). Decreased respiration in plant tissues grown at elevated [CO_2] has been shown, in some cases, to be a result of decreased activity of the alternative pathway (Gifford et al. 1985). Such effects are a consequence of the [CO_2] history of a plant and are termed indirect effects (Amthor 1991). The mechanisms responsible for these indirect effects are not necessarily the same as those responsible for direct effects associated with [CO_2] at the time of respiration measurement (Amthor 1991). Qi et al. (1994) felt reduced alternative pathway activity was the most likely mechanism for the direct reduction in respiration they observed in Douglas-fir roots exposed to high [CO_2]. However, Reuveni et al. (1995) found that direct inhibition of respiration by high [CO_2] in duckweed (*Lemna gibba* L.) fronds was due primarily to reduced activity of the cytochrome pathway and found no support for the hypothesis that the alternative pathway was specifically suppressed. To date, no studies have tested for decreased alternative pathway activity in association with direct reduction of root respiration by high measurement [CO_2], but such experiments might prove illuminating.

Although the causal mechanism remains unknown, the significance of the reduction in root respiration as measurement [CO_2] increases cannot be denied. The results of this

study support the conclusion of Qi et al. (1994) that soil CO_2 should be accounted for when estimating respiration and that many earlier estimates of root respiration may require revision. The shape and magnitude of the [CO_2] effect observed in this study for excised sugar maple roots from mature forests generally appears similar to that reported by Qi et al. (1994) for intact root systems of Douglas-fir seedlings. How universal the effect is across tree species remains to be seen. Until the direct effect of [CO_2] on root respiration is fully understood, it is recommended that respiration be measured at [CO_2] representative of, or higher than, soil [CO_2]. Infra-red gas analyzers in which the input [CO_2] can be pre-programmed are one method of achieving this and show promise as a means of taking respiration measurements directly in the field. Such field measurements are needed to verify the applicability of results from controlled laboratory experiments to actual field situations. Unfortunately, input CO_2 of such IRGAs is typically limited to less than 2000 $\mu l\ l^{-1}$ at present, and, if an open system is used, the respiratory increase in [CO_2] is often small relative to the concentration of the input gas. Also, gas flow across the sample can potentially lead to desiccation. Oxygen electrodes can operate at much higher [CO_2] and require no gas flow across the sample, but the resulting data require conversion with an appropriate respiration quotient if they are to be used in constructing carbon budgets. For sugar maple, a respiration quotient of 0.8 has been measured (A.J. Burton and G.P. Zogg, unpublished data). In all cases, the use of CO_2-free air for respiration measurement should be avoided, and the [CO_2] at which measurements were made and typical of the soil atmosphere of the species studied should be reported.

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Chapter III

DROUGHT REDUCES ROOT RESPIRATION IN SUGAR MAPLE FORESTS

Abstract

Soil moisture deficits can reduce root respiration, but the effects have yet to be quantified at the stand level or included in models of forest C budgets. Fine root ($\leq$ 1.0 mm diameter) respiration was studied in four sugar maple forests for three growing seasons in order to assess the combined effects of temperature, N concentration, and soil moisture on respiratory rates. Fine root respiration at the four sites was exponentially related to soil temperature and linearly related to root N concentration and soil moisture availability. A majority of the variability in respiration rates in the data set was explained by temperature. Differences in soil moisture availability explained temporal variation within sites in respiration rate at a given temperature; whereas differences among sites in respiration rates resulted from site-specific differences in fine root N concentration. Periodic moisture deficits during 1995 and 1996 were sufficient to cause declines of up to 17% in total growing season root respiration at affected sites. Estimated reductions in respiration of up to 0.8 Mg C/ha represented a significant portion of annual aboveground woody biomass increment, arguing for the inclusion of soil moisture availability as a predictor of root respiration when modeling C allocation in forest ecosystems.

Introduction

Understanding the influence of environmental factors on the amount of C allocated belowground is critical if the effects of climate change on forest C balance are to be realistically modeled. Without such knowledge, tree growth and survival cannot be accurately forecast for future climates. Much of the C allocated belowground is lost to root respiration, which can consume from 8-52% of daily carbohydrate production from photosynthesis (Pate et al. 1979, Lambers 1987, Buwalda 1993, Lambers et al. 1996). Global change scenarios typically predict changes in soil temperature, N availability and moisture (Pastor and Post 1988, Aber et al. 1989, Houghton and Woodwell 1989, Mintzer 1990), all of which may impact root respiration and thus alter forest C balance. Advances have been made in understanding and modeling the effects of temperature and N concentration on root respiration (Lawrence and Oechel 1983, Ryan 1991b, Ryan et al. 1996, Burton et al. 1996, Zogg et al. 1996), and it also is known that soil moisture deficits can reduce root respiration (Vartanian and Chauveau 1986, Palta and Nobel 1989a,b, Hall et al. 1990, Gansert 1994). However, the effects of soil moisture have yet to be quantified at the forest stand level, and it is unclear how drought conditions might affect the response of root respiration to temperature and N.

Water stress can reduce photosynthetic C assimilation by plants (Bradford and Hsiao 1982, Hanson and Hitz 1982), and forest C models typically reduce gross primary productivity (GPP) accordingly during dry conditions (Running and Coughlan 1988, Ewel and Gholz 1991, Rastetter et al. 1991, Aber and Federer 1992, Nikolov and Fox 1994). Some models also alter the relative proportions of carbon allocated to leaves, stems and

roots (Running and Gower 1991), but none reduce root respiration as soil moisture deficits become severe. Predictions of tree growth and survival depend in part on the amount of C remaining after accounting for respiration (Running and Gower 1991, Aber and Federer 1992). If root respiration declines as soil moisture deficits occur, the resulting strain on C reserves will be less severe, possibly leading to a smaller reduction in growth and increased survival. A more complete understanding of the effects of soil moisture availability on root respiration is needed if such possibilities are to be assessed.

In 1994 fine root ($\leq$ 1.0 mm diameter) respiration was studied in four Michigan sugar maple (*Acer saccharum* Marsh.) forests and in order to quantify the effects of temperature and fine root N concentration on respiration in the absence of severe soil moisture deficits (Burton et al. 1996, Zogg et al. 1996). Measurements of fine root respiration were continued during 1995 and 1996, years in which soil moisture deficits of varying degrees occurred at the sites. These data made it possible to examine the combined effects of temperature, N concentration, and soil moisture on fine root respiration. Objectives were: (1) to determine if root respiration is influenced by soil moisture availability; (2) to develop predictive relationships for northern hardwood root respiration based on temperature, root N concentration, and soil matric tension; and (3) to assess the potential effects of moisture deficit on seasonal root respiration relative to stand level C budgets.

Methods

Root respiration was measured on excised fine roots collected from four sugar maple forests located along a 3° latitudinal transect in Michigan. The forests are second-

growth northern hardwoods, approximately 85 years in age, dominated by sugar maple and occurring on sandy, well-drained Spodosols (Burton et al. 1991, MacDonald et al. 1991, Randlett et al. 1992). Six 30 x 30 m study plots are located at each site. Three of these plots are control plots and the others have received annual fertilizer applications of 30 kg NO_3^--N/ha since 1994 (Zogg et al. 1996). To date, the NO_3^- additions have not altered root respiration or tissue N concentrations in the fertilized plots (Zogg et al. 1996, Zogg et al. 1997), so they will not be separated from the control plots in this report.

Samples for root respiration measurement were collected from 10 m wide buffer strips surrounding each plot. Soil cores (10 cm deep, 5.4 cm inner diameter) were collected from three random locations in each buffer. The cores were transported to nearby field labs (less than 1 hour travel time per site), and composited on a plot basis. All fine ($\leq$1.0 mm), non-woody, live roots were sorted from each core and rinsed free of soil and organic matter with deionized water. Live roots were distinguished by white, cream, tan or brown coloration and a smooth appearance. Dead roots were dark brown or black in color, were brittle, and had frayed, rough edges. Excess water was blotted from the root samples, and 0.5 g (fresh weight) subsamples were wrapped in moistened tissue paper and used for respiration measurements. Respiration was measured as O_2 consumption using temperature controlled O_2 electrodes (model LD 2/2 oxygen electrode, Hansatech Instruments Ltd., Norfolk, England) connected to constant temperature circulating water baths (Burton et al. 1996, Zogg et al. 1996). Three complete O_2 electrode systems were run simultaneously, allowing measurement of respiration at 6, 18 and 24 °C to be completed within three hours of sample collection. Following respiration measurements,

root subsamples were oven-dried (65 °C, 24 hr) for determination of dry weights, ground, and analyzed for N using an elemental analyzer (Carlo Erba NA 1500 Series II, Fisons Instruments, Massachusetts, USA).

Respiration measurements were made in early September, October, and November 1994; May, July, and September 1995; late June to early July, 1996, and September, 1996. Results presented as O_2 consumption in this report can be converted to CO_2 production using a respiratory quotient of 0.8 (A.J. Burton and G.P. Zogg, *unpublished data*). Root respiration is sensitive to the $[CO_2]$ at which measurements are made (Qi et al. 1994, Burton et al. 1997) and thus rates measured in the laboratory can differ from those that would occur at actual soil $[CO_2]$ in the field. For the four sugar maple sites, a correction factor of 1.16 is appropriate for adjusting values obtained at a measurement $[CO_2]$ (ca. 10,000 μL/L) to those that would occur at a soil $[CO_2]$ of 1,200 μL/L (Burton et al. 1997).

Soil matric tension at the sites was measured using gypsum blocks (Model 5201, Soilmoisture Equipment Corporation, Santa Barbara, CA). Single moisture blocks were located at depths of 15 and 75 cm at each plot. Soil temperature was measured using thermistors (Model ES-060-SW, Data Loggers Inc., Logan, UT) buried at 15 cm in each plot. Moisture blocks and thermistors were read every 30 min by Omnidata EasyLoggers (Models 824 and 925, Data Loggers Inc., Logan, UT), with average values recorded every 3 hours. Moisture block resistance readings (ohms) were converted to matric tension (MPa) using relationships developed in the lab from moisture blocks placed in

intact soil cores collected from the plots and equilibrated on soil moisture plates at tensions ranging from 0.01 to 1.5 MPa.

Analysis of variance (ANOVA) was used to compare root respiration among temperatures, sites and sampling periods (Wilkinson 1990). Since respiration is exponentially related to temperature, the natural log of respiration was used as the dependent variable in the ANOVA. Linear and non-linear regression (Wilkinson 1990) were used to assess the effects of temperature, root N concentration, and soil matric tension on respiration at the sites. Since soil moisture blocks can lag behind actual soil moisture, potential predictors for respiration included matric tension on the date of respiration measurement and during the first and second weeks following respiration measurement. Soil matric tension at 15 cm during the first week following respiration measurements was consistently most strongly correlated with observed respiration rates and thus was used as the indicator of soil moisture status in all regression analyses. Soil moisture data were indicative of the general moisture status of the study sites, not of the particular locations at which root samples were taken. Therefore, soil moisture data from individual plots were combined and site means ($n = 4$) for each sampling date ($n = 8$) and measurement temperature ($n = 3$) were used in the regression analyses (total $n = 96$).

Results

Fine root respiration increased with temperature and differed significantly among sites and sampling dates (Table 3.1). Significant interactions also occurred (Table 3.1), suggesting variation among sites and sampling dates in the factors controlling respiration. An examination of the patterns among sites in root respiration, root N concentration, and

Table 3.1 Analysis of variance of the effects of study site, temperature, and sampling period on fine root respiration in four Michigan sugar maple forests (n = 576).

Source	d.f.	MS	F - ratio	*P*
Site	3	0.88	16.7	0.000
Temperature	2	157.93	3019.4	0.000
Sampling period	7	0.95	18.2	0.000
Site x Temperature	6	0.04	0.8	0.606
Site x Sampling period	21	0.18	3.5	0.000
Temperature x Sampling period	14	0.15	2.9	0.000
Site x Temperature x Sampling period	42	0.09	1.7	0.008
Error	480	0.05		

soil moisture at each sampling date (Figure 3.1) helps explain these interactions. During sampling periods when moisture deficits did not occur (Figures 3.1A, 3.1B and 3.1E), differences among sites in root respiration followed patterns among sites in fine root N concentration, with Sites B and C having higher root respiration and higher root N concentration than Sites A and D. In these non-drought sampling periods, there were always significant correlations between fine root N concentration and respiration at 24°C ($P < 0.01$) and 18°C ($P < 0.05$), with weaker correlations at 6°C ($P < 0.10$). When drought occurred at one or more of the sites (Figures 3.1C, 3.1D, and 3.1F), N concentration and respiration were only occasionally correlated.

The occurrence of drought caused respiration rates at individual sites to be reduced to levels lower than those that would be predicted from fine root N concentration and temperature (Figures 3.1C, 3.1D, and 3.1F). Within sites, respiration at 24°C was

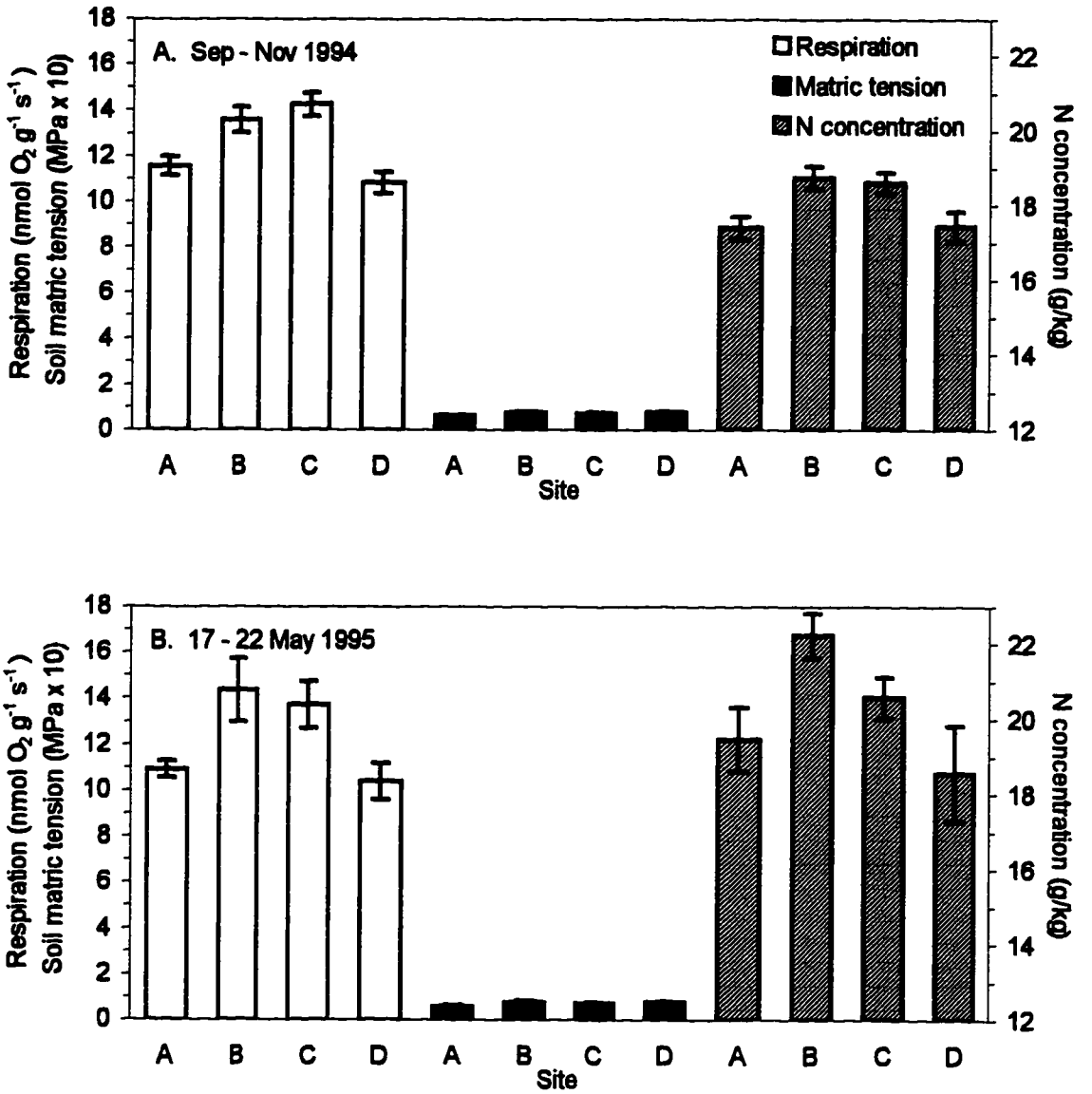

Figure 3.1 Fine root respiration (24°C), soil matric tension, and fine root N concentration in four Michigan sugar maple forests during six sampling periods. Error bars indicate one standard error of the mean. The Sep - Nov 1994 period (A) represents means across three separate samplings conducted during the same season in 1994.

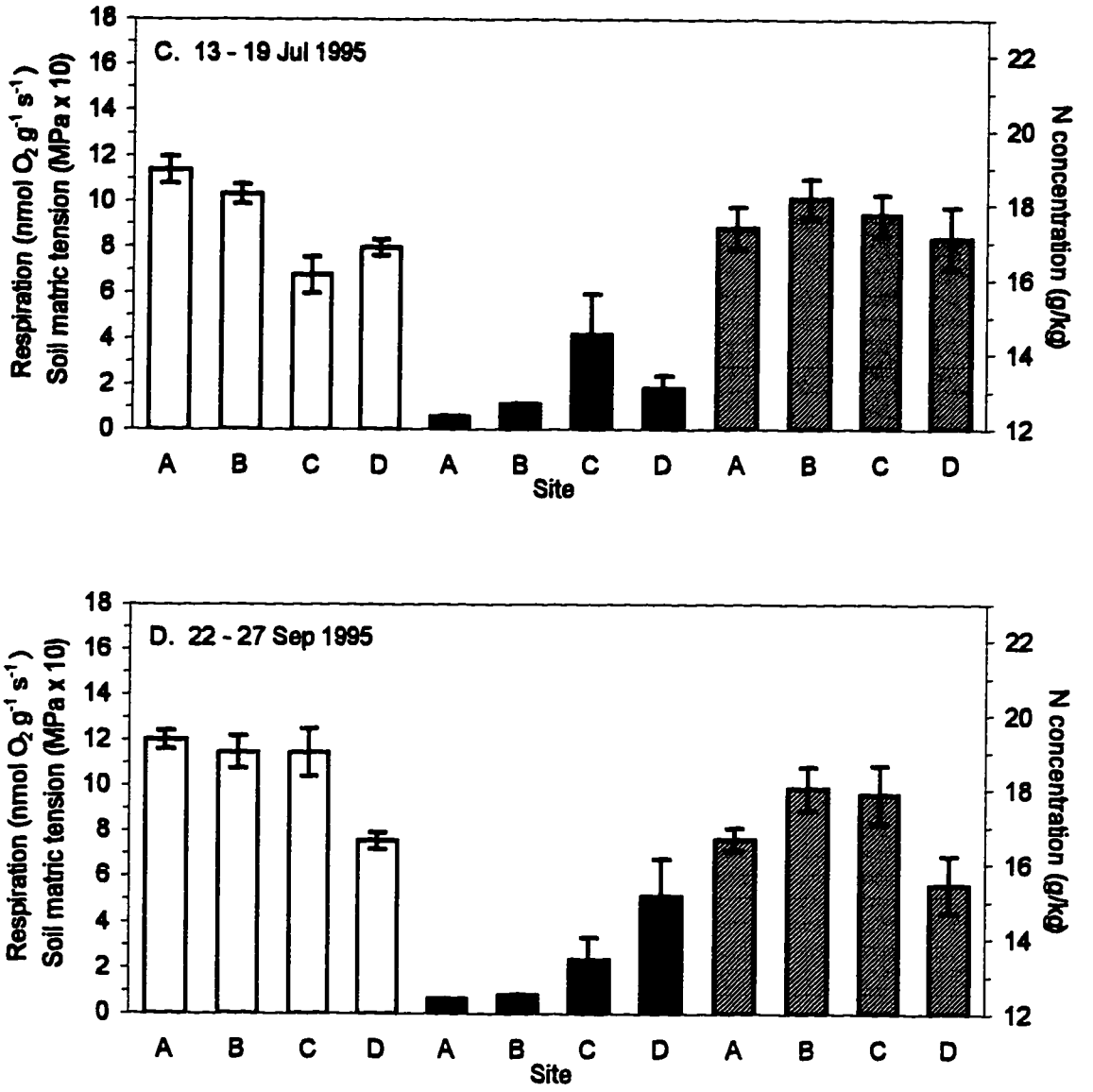

Figure 3.1 (continued)

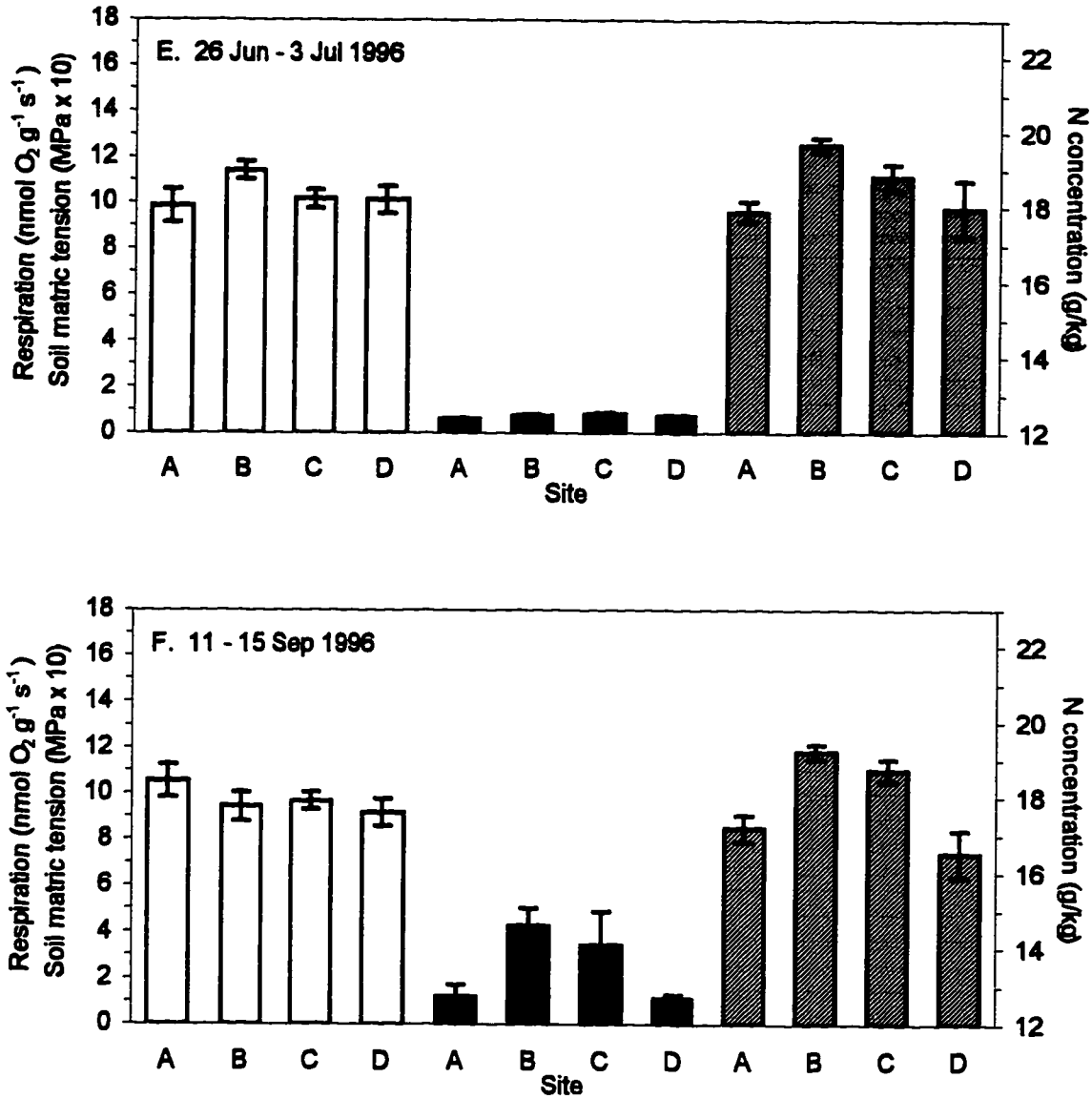

Figure 3.1 (continued)

negatively correlated with soil matric tension ($P < 0.05$) for sites which experienced drought (Sites B, C and D), but not for Site A, which experienced no moisture deficits during the three years of study. Interactions of site with sampling period in Table 3.1 reflect the fact that moisture deficits of similar intensity did not occur simultaneously at all sites.

Overall, there were no differences among sites in the effect of temperature on respiration (non-significant site x temperature interaction, Table 3.1). Based on mean respiration rates at 6 and 24°C, the average Q_{10} across sites was 2.6. The interaction between time period and temperature (Table 3.1) suggests that the rate of increase in respiration with temperature (Q_{10}) was not the same for all time periods. It appears that this effect may be in part a consequence of moisture deficit, as the Q_{10} for respiration was between 2.5 and 3.0 for all sites and time periods except for Sites C and D in July, 1995 and Site D in September, 1995. These sites were experiencing strong moisture deficits during these times (Figures 3.1C and 3.1D), and had Q_{10}'s for respiration of 2.1 to 2.2. However, not all sites experiencing moisture deficit had lower Q_{10} values. Moisture deficits at Sites B and C in September, 1996 (Figure 3.1F) did not result in lowered Q_{10} for respiration ($Q_{10} = 2.7$ for both sites).

Temperature, root N concentration, and soil matric tension were all significant predictors ($P < 0.05$) of root respiration in linear regression analyses. However, the best model for predicting fine root respiration was a non-linear model in which respiration was linearly related to N concentration and soil matric tension, and exponentially related to temperature:

$$R = [0.063\ (N) - 0.670\ (M)]\ e^{0.098\ (T)} \quad (R^2 = 0.93, P < 0.001, SEE = 1.08) \quad (1)$$

where R is respiration in nmol O_2 g^{-1} s^{-1}, N is root nitrogen concentration in g/kg, M is soil matric tension in MPa, and T is temperature in °C.

It is possible that the moisture effect does not become important until soil matric tension reaches a certain threshold. Piecewise regression models were used to explore this possibility, but resulted in virtually no change in the predictive strength of the regression, and suggested a threshold matric tension of 0.08 MPa, which is only slightly higher than values typcially recorded for moist soils during the dormant season. A lack of data over the entire continuum from moist to dry soils in the current data set may have limited its utility for determining an exact threshold.

Discussion

The observed decline in root respiration with increasing soil moisture deficit is consistent with reports that gradually applied moisture stress can cause reduced respiration in plant tissues (Wilson et al. 1980, Amthor and McCree 1990). In roots, such effects have been observed in herbaceous species (Vartanian and Chauveau 1986, Hall et al. 1990), desert succulents (Palta and Nobel 1989a,b), and tree saplings (Gansert 1994). The periodic moisture deficits which occurred during 1995 and 1996 at Sites B, C and D were sufficient to cause overall declines in total growing season root respiration. For example, at Site C estimated fine root respiration for June through September, 1995 is 17% less when soil moisture deficits are accounted for than when they are not (Figure 3.2).

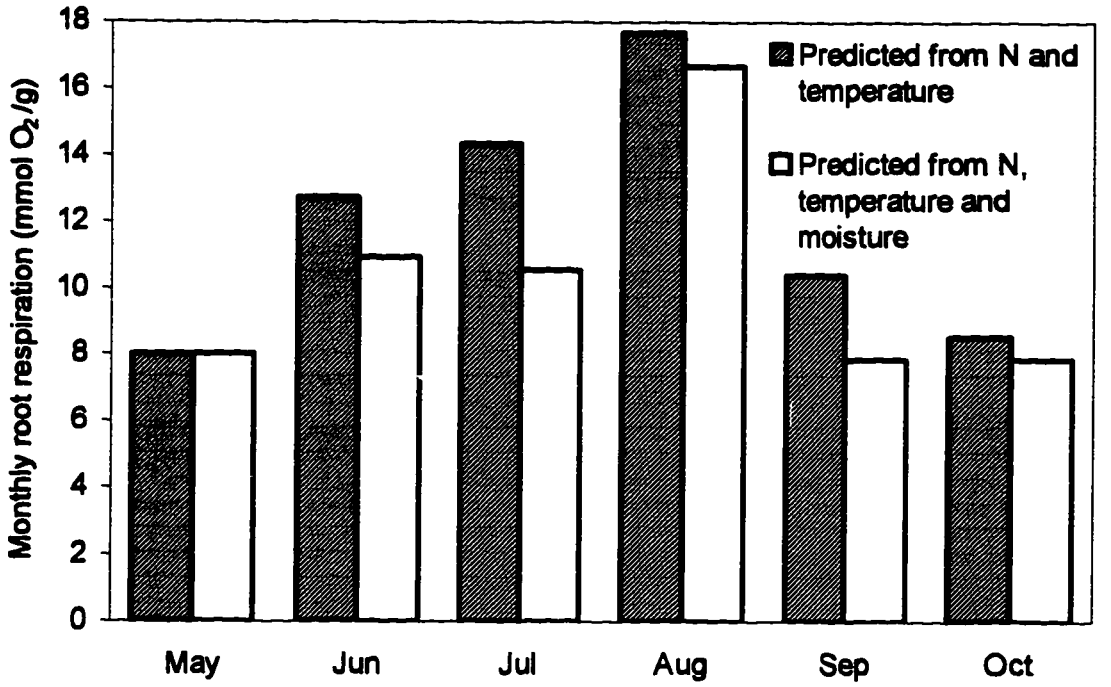

Figure 3.2 Estimates of monthly fine root respiration at Site C with and without accounting for soil moisture deficits. Equation (1) was used to derive respiration estimates. A soil matric tension typical of moist conditions (0.07 MPa) was used for all months when estimating respiration without accounting for soil moisture effects.

It could be questioned whether the declines in respiration measured on excised, rinsed roots are representative of those actually occurring in the field under drought conditions. It is suspected that the laboratory procedure, in which roots were rehydrated during rinsing, resulted in measured respiration rates that were the same or slightly higher than those occurring in the field during dry conditions. Others have reported a return to normal respiration rates within 24 to 36 hours after rewetting severely dehydrated roots (Vartanian and Chauveau 1986, Palta and Nobel 1989a,b), but these returns toward normal did not begin for several hours after rehydration. Respiration measurements for

the sugar maple sites were completed within about 3 hours of sample collection. Additionally, it should be noted that total soil respiration at the four study sites declined markedly during the same dry periods for which reduced root respiration rates were measured (Zogg et al. 1997). This would indicate that root respiration, microbial respiration, or both were impacted by soil moisture deficits. The respiration rates measured during non-drought periods are similar to those reported for freshly detached root mats of northern hardwoods in the field (Fahey and Hughes 1994), and no differences in respiration have been found for attached and detached roots of small sugar maple seedlings from the sites (A.J. Burton and G.P. Zogg, *unpublished data*).

The 17% decline in root respiration illustrated in Figure 3.2 represents only a small portion of annual GPP for these sites, but it still has an important impact on the overall C budget of a stand. Given a fine root biomass of about 7 Mg/ha at Site C (Hendrick and Pregitzer 1993), and using the conversions from O_2 consumption to CO_2 production described in the Methods section, the reduction in respiration illustrated in Figure 3.2 represents about 0.8 Mg C/ha over the growing season. This is a significant proportion of the 2 Mg C/ha in aboveground woody biomass increment measured at Site C in 1995 (A.J. Burton, *unpublished data*). Moreover, this value is slightly higher than the 0.5 Mg C/ha in aboveground woody increment measured at the site in 1989, a very dry year following a year of extremely heavy seed production (D.D. Reed, *unpublished data*). Carbon balance models are of little utility if they cannot accurately predict annual aboveground increment. Thus, an error of 0.8 Mg C/ha associated with ignoring reduced respiration during soil moisture deficits is potentially very significant. In models which use stored C reserves to

assess a tree's likelihood of surviving stress events, such an error could result in erroneous estimates of both growth and survival.

For plant tissues in general, it is felt that a majority of the reduction in total respiration during water stress results from decreased construction respiration (Hanson and Hitz 1982). However, this does not appear to be the case for the tree roots used in this study. It is known that root elongation decreases during periods of moisture stress (Teskey and Hinckley 1981, Kuhns et al. 1985), but reduced root growth alone cannot account for the declines in respiration observed during the more severe periods of soil moisture deficit at the sites. For example, using a mean fine root production rate for Site C of 0.14 g g^{-1} mo^{-1} between June and September (calculated from the data of Hendrick and Pregitzer 1993), a construction respiration cost of 0.25 g CO_2-C · g C^{-1} (Penning de Vries 1975, Ryan 1991a), and a root C concentration of 50% (A.J. Burton, *unpublished data*), monthly growth respiration is estimated to be 1.44 mmol CO_2-C / g (1.55 mmol O_2 / g under laboratory measurement conditions). Total elimination of this level of growth respiration is insufficient to explain the monthly declines in respiration occurring at Site C in June, July, and September of 1995 (1.8 to 3.8 mmol O_2 / g , Figure 3.2). Much of the observed reduction in respiration must be due to decreases in either maintenance respiration or respiration associated with ion uptake and transport processes.

Lower maintenance respiration could result from reduced overall metabolic activity (e.g. protein synthesis) associated with cell dessication (Amthor and McCree 1990). Low soil moisture content can decrease ion movement toward and uptake by plant root systems (Bradford and Hsiao 1982, Jungk 1996) and can inhibit the rate at which nutrients are

made available through mineralization (Dickinson and Pugh 1974). Short-term reductions in these processes during dry periods could lead to temporarily reduced respiratory requirements for ion uptake and nutrient transport. In roots of non-woody species, ion uptake can represent a large portion of non-growth respiration (Veen 1980, Lambers 1987, van der Werf et al. 1988, Poorter et al. 1991, Lambers et al. 1996). Consequently, much of the reduction in root respiration during periods of low soil moisture in such plants could be due to reduced ion uptake. Estimates of ion-uptake respiration for woody species are sparse, but it appears ion-uptake respiration is a much lower proportion of total respiration (Buwalda 1993). Thus, it is likely that reduced respiration for ion uptake and nutrient transport is less important than reduced maintenacne respiration in explaining the reduction in total respiration observed for sugar maple roots at low soil moisture contents.

An interesting observation during the study was the lower respiratory Q_{10} at Sites C and D during periods of moisture deficit in 1995. This reduction in Q_{10} may be indicative of differential effects of drought on the various components of total respiration. For instance, there could be a certain portion of maintenance respiration (e.g. basal respiration, Marshall and Perry 1986, Amthor 1989) that is less dependent on temperature than either growth or ion-uptake respiration and that must occur even during periods of drought. If this were the case, soil moisture deficits would reduce both total respiration and Q_{10} as observed in 1995. Such a mechanism would be advantageous to the plant in that root tissues would remain viable during drought, while the C lost to respiration would be limited during excessively warm periods that often accompany moisture deficits.

Differences in soil moisture availability explained much of the temporal variation in respiration rate at a given temperature within sites, whereas differences among sites in respiration rates were due to site differences in fine root N concentration. During non-drought sampling periods in this study, the highest respiration rates always occurred in fine roots having the highest N concentrations (Figure 3.1). The cross-site relationship between fine root N and respiration is in agreement with data previously reported for these sites (Zogg et al. 1996, Burton et al. 1996). In contrast to between-site differences in root N concentration, seasonal differences within sites in root N concentration were not reflected by corresponding changes in respiration rate. When only non-drought measurement periods are compared, there were no clear seasonal differences in respiration rate within sites despite clear seasonal trends in fine root N concentration (Figure 3.3). The seasonal pattern in root N concentration within sites was characterized by high values in the spring, declining to lower levels in late summer and early fall, followed by slight increases in November. This pattern suggests storage and allocation of N for root growth, with the highest root N in the spring resulting from high levels of stored N just prior to rapid root and foliar growth. Overwinter storage of soluble (non-protein) N has been reported for roots of several tree species (Tromp 1983, Millard and Proe 1991, 1992). As stored N is allocated to new growth in the spring and root biomass increases, root N concentration should decline, as occurred from May through October at the sites. If changes in the amount of stored N are responsible for the pattern illustrated in Figure 3.3, and if respiration is controlled by the non-stored (primarily protein) portion of N, then

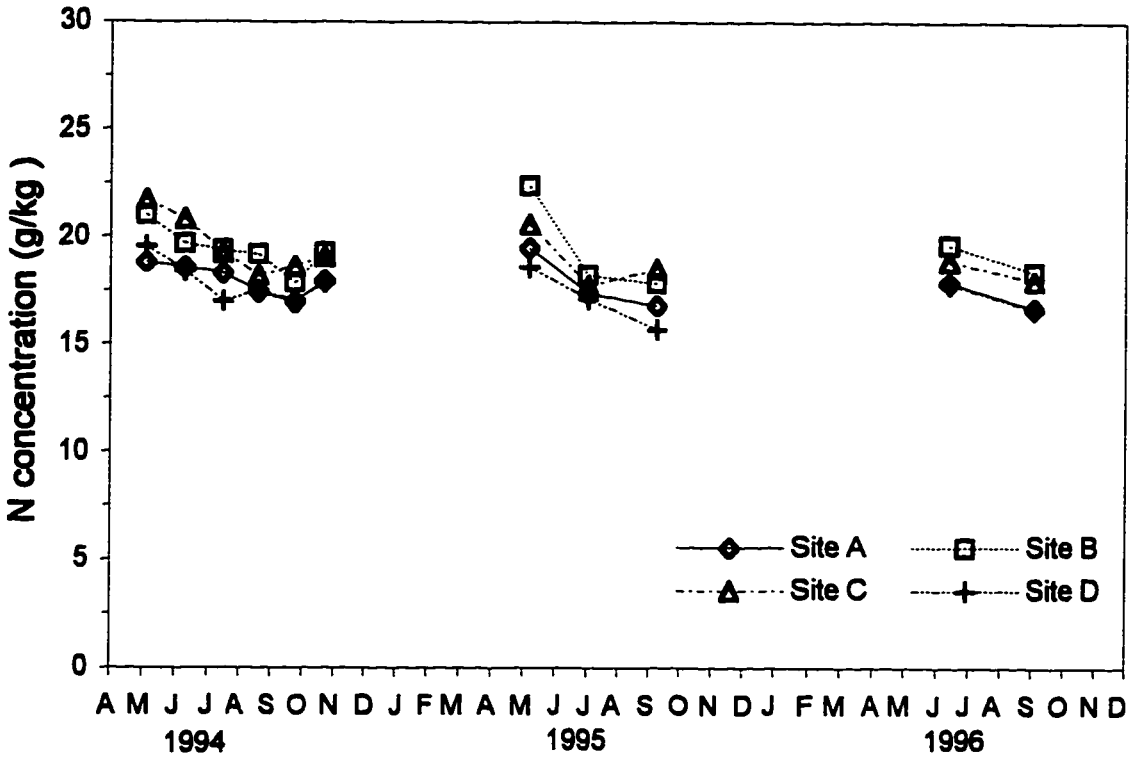

Figure 3.3 Seasonal patterns in fine root N concentration for 1994, 1995, and 1996. Fine root N data for May, June and July 1994 are those reported by Zogg et al. (1996).

respiration within sites should be independent of the seasonal changes in N concentration associated with N storage and remobilization. This is in agreement with the relatively consistent respiration rates observed across seasons in non-drought time periods, and with the lack of temporal correlations within sites between N concentration and respiration.

Temperature, N concentration and soil moisture availability were all significant predictors of fine root respiration in the sugar maple forests studied. Although temperature alone was sufficient to explain a majority (90%) of the observed variability in respiration, root N concentration and soil moisture potential explained significant portions

of the remaining variation among sites and sampling periods, respectively. Many ecosystem process models estimate root respiration based solely on soil temperature (Running and Gower 1991), and only a few recent efforts at modeling fine root respiration have included both temperature and N concentration as predictors (Ryan 1991b, Ryan et al. 1996). To date, none have included soil moisture. The results of this study suggest that soil moisture potentials $\leq$ -0.2 MPa can significantly reduce fine root respiration in sugar maple forests. Over a growing season, soil moisture deficits can be sufficient to reduce root repiration by amounts that are significant relative to the amount of C allocated to aboveground biomass increment. These findings argue for a better understanding of the influence of soil moisture on root respiration and for the inclusion of soil moisture availability as a predictor of root respiration as physiologically-based models of forest C allocation become more advanced.

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Chapter IV

HIGH NITROGEN AVAILABILITY INCREASES FINE ROOT LONGEVITY IN SUGAR MAPLE FORESTS

Abstract

Fine root biomass turnover has a strong influence on the belowground allocation of C and nutrients in forest ecosystems. Minirhizotrons were used to observe fine root ($\leq$ 1 mm) production and longevity over two growing seasons in four sugar maple-dominated northern hardwood forests located along a latitudinal temperature gradient. The sites also differed in N availability, allowing the relative importance of temperature and N availability in controlling fine root lifespans to be assessed. For surface roots (0-10 cm deep), significant differences in root longevity existed among the sites, with median root lifespans for spring 1994 root cohorts ranging from 278 to 397 days. The pattern across sites in root longevity did not follow the north to south temperature gradient, but rather corresponded to site differences in N availability, with longer average root lifespans occurring where N availability was greater. This suggests the possibility that roots are maintained as long as the benefit (nutrients) they provide outweighs the C cost of keeping them alive. It is proposed that greater metabolic activity of roots in nitrogen rich zones leads to greater carbohydrate allocation to the roots, and that reduction in root C sink strength as local nutrients are depleted provides the mechanism through which root lifespan is regulated.

Introduction

Fine root longevity has a strong influence on belowground allocation of C and nutrients in forest ecosystems (Gholz et al. 1985, Nadelhoffer et al. 1985, Joslin and Henderson 1987, Arthur and Fahey 1992, Hendrick and Pregitzer 1993a). As such, there is a great deal of interest in understanding how soil environmental factors such as temperature and N availability affect root lifespan. Some evidence exists that higher temperatures decrease root lifespan (Self et al. 1995), and root longevity appears to be greater in species from cold environments (Eissenstat and Yanai 1997). Improved N availability has been shown to decrease fine root production and biomass at the stand level (Haynes and Gower 1995), and evidence exists for both increased (Keyes and Grier 1981, Vogt et al. 1986, Pregitzer et al. 1993) and decreased (Aber et al. 1985, Nadelhoffer et al. 1985, Pregitzer et al. 1995) fine root lifespans in more fertile soils. This contradictory evidence has led to the development of a variety of hypotheses to explain root longevity (Nadelhoffer et al. 1985, Hendricks et al. 1993, Eissenstat and Yanai 1997), but direct observational tests of these hypotheses in mature forest ecosystems are needed.

Hendrick and Pregitzer (1993b) examined fine root longevity in two sugar maple-dominated northern hardwood forests in Michigan and found shorter root lifespans at the more southern site. They speculated that warmer soil temperatures at the southern site might be the cause. This paper reports the results of an expansion of that study to include a total of four sites along a latitudinal, temperature gradient. Recent work has documented that differences in N availability also exist among the sites (Zogg et al. 1996). In order to assess the relative importance of temperature and N availability on fine

root lifespans at the sites, minirhizotrons were used to observe fine root production and longevity over two growing seasons. Objectives of the study were: (1) to determine if differences existed among the four sites in root longevity; (2) to assess whether such differences corresponded to differences among sites in temperature or N availability; (3) to define seasonal patterns of fine root production; and (4) to compare fine root production and longevity in surface (0 - 10 cm depth) and subsurface (40 -50 cm depth) soils.

Methods

Fine root production and longevity were studied in four sugar maple-dominated northern hardwood forests located along a 3° latitudinal transect in Michigan (Table 4.1). The forests are second-growth northern hardwoods, approximately 85 years in age, dominated by sugar maple, and occurring on sandy, well-drained Spodosols (Burton et al. 1991, MacDonald et al. 1991, Randlett et al. 1992). Mean annual air temperature increases by about 3 °C from north to south (Site A to Site D) along the latitudinal gradient, and differences among the sites in N availability and fine root N concentration have been documented (Zogg et al. 1996, Burton et al. 1996), with higher N availability and fine root N concentrations occurring at Sites B and C (Table 4.1). Since moisture availability also has the potential to impact fine root dynamics, soil matric potential was monitored at the sites using gypsum blocks (Model 5201, Soilmoisture Equipment Corporation, Santa Barbara, CA). Three moisture blocks were located at a depth of 15 cm at each site, with data read by Omnidata EasyLoggers (Model 824, Data Loggers Inc., Logan, UT) every 30 minutes and average values recorded every 3 hours.

Table 4.1 Selected characteristics of four sugar maple forests in Michigan, USA. Overstory data are from the year 1995. Site means for N mineralization, root N concentration, and root respiration followed by a different letter are significantly different at the 0.05 level of probability.

	Site A	Site B	Site C	Site D
Latitude (N)	46°52'	45°33'	44°23'	43°40'
Longitude (W)	88°53'	84°51'	85°50'	86°09'
Mean annual precipitation[a] (mm)	870	830	810	850
Mean annual temperature[a] (°C)	4.2	5.2	5.8	7.6
Total basal area (m^2 ha^{-1})	34	31	32	33
Sugar maple basal area (%)	86	86	83	75
Overstory age	88	82	83	87
N mineralization[b] (μg N g $soil^{-1}$)	52b	82a	85a	57b
Fine root N concentration[c] (mg g^{-1})	17.4b	18.8a	18.6a	17.4b
Fine root respiration[c] (nmol O_2 g^{-1} s^{-1} at 24 °C)	11.0b	13.4a	13.8a	11.6b

[a] 30-year means from National Oceanic and Atmospheric Administration (1983) records.

[b] Data from Zogg et al. (1996).

[c] Data from Burton et al. (1996).

The fine root production and longevity data reported in this paper were collected from three 30 x 30 m study plots located at each site (originally installed in 1987). In the summer of 1993, five clear polybutyrate minirhizotron tubes (2 m long x 5.08 cm inside diameter) were installed at each plot at a 45° angle to the soil surface to vertical depth of > 70 cm. Numbered, rectangular image frames (0.9 x 1.3 cm) were scribed every 0.9 cm along a transect on the exterior surface of each minirhizotron tube prior to installation. The image frames were oriented upward during tube installation and enabled the videotaping of the same locations within each minirhizotron at all sampling dates. To

prevent inadvertent rotation of the minirhizotron tubes, the exposed tops were tied with surgical tubing to two adjacent 0.5 m lengths of polyvinyl chloride (PVC) pipe (1.25 cm diameter, installed in the soil to greater than 1/2 of their length). The aboveground portions of the tubes were painted black to prevent light penetration and then repainted white to minimize heat load.

Video images were collected from the minirhizotron tubes at approximate five week intervals from mid-May through early November in 1994, and at five to six week intervals from mid-April to mid-November in 1995. All sites were sampled at all dates, with the exception of Site A in April, 1995, which was inaccessible due to deep snow cover. Image collection proceeded from south (Site D) to north (Site A) and typically was completed within 7 days. The sampling periods used to generate the data reported in this paper were: May 17 - 24, June 19 - 27, July 26 - 30, August 30 - September 5, October 4 - 10, and November 2 - 7 in 1994; and April 13-23, May 15 - 22, June 28 - July 5, August 7 - 12, September 21 - 25, and November 14 - 20 in 1995. All video images were recorded on Hi-8 mm videotape using a model BTC 1.125 Minirhizotron Research Color Camera (Bartz Technology Co., Santa Barbara, CA).

A PC-based, interactive image analysis system (ROOTS, Hendrick and Pregitzer 1992a, 1993a) was used to analyze root images. Videotaped minirhizotron images were projected onto a computer monitor, and the image from each frame was temporarily "captured" on-screen by ROOTS. For the first image collection date, the lengths and diameters of all roots were traced to the nearest 0.01 mm; and each root was given an identification number, classified as living or dead (based upon color and consistency in the

image), and designated as white or brown. All measurements and classifications were written to a dBASE III+ file (Ashton-Tate, Torrance, CA) by ROOTS. The tracings were saved in separate disk files. For all subsequent image sets, the tracing from the previous date were overlain on the new image, allowing previously existing roots to be identified. These were then retraced and designated as white, brown, dead, or missing. New roots also were traced, given identification numbers, and designated as new. Roots that were "missing" and did not reappear at any subsequent image collection date were assumed to have died. Complete records were kept for all roots, even after they were classified as dead or missing. By using ROOTS to match tracings with identification numbers, the fates of individual roots or units of root length were followed from initiation until death (Hendrick and Pregitzer 1992a,b).

Fine root lifespans at the sites were assessed by studying the survival of contemporaneously produced root cohorts from each site. Cohorts consisted of all roots $\leq$ 1 mm in diameter produced between any two sampling dates at a site (mean diameter of the roots studied was actually 0.31 mm). Cohorts from both the surface soil (top 10 cm) and subsurface soil (40 - 50 cm below the soil surface) were identified and tracked. Data from all fifteen tubes at each sites were analyzed for the 40 - 50 cm depth. For the 0 - 10 cm depth, much higher root densities and constraints on the amount of time and labor available for digitizing images necessitated the use of only 9 tubes per site (three from each plot). For both depths, the survival of cohorts produced within each sampling interval was determined for each subsequent sampling date, and survival was expressed as a fraction of both initial cohort root number and initial cohort root length still surviving. Monthly estimates of new fine root production at the sites were made by assuming

production was constant for each day in the time interval between consecutive imaging dates. Estimates of daily production were then summed for each month.

Fine root survival functions (for root number) and median root lifespans were determined using the life table method of failure-time analysis (Fox 1993), which is appropriate for right censored data (Lee 1992). Differences among sites in root survival were tested using the Wilcoxon multiple comparison described by Fox (1993).

Results

For surface soils, differences in fine root longevity existed among the four study sites (Figure 4.1), with fine root survival over time at Sites B and C equal to or greater than that at Sites A and D for almost every cohort (Figure 4.1, Table 4.2). This pattern across sites in fine root survival does not correspond to the north to south temperature gradient, but rather reflects site differences in N availability and fine root N concentration (Table 4.1), with longer average root lifespans occurring where N availability was greater. Median root lifespans for the spring 1994 root cohorts were 278, 357, 397, and 291 days for Sites A, B, C, and D, respectively.

For roots deeper in the soil profile (40 - 50 cm), no clear pattern existed among the sites in ranking of fine root longevity (Figure 4.2, Table 4.2). Within sites, deep root cohorts generally did not show the gradual mortality over time found in surface roots. Rather, they tended to have little or no mortality for some period of time, followed by a short period of rapid mortality (Figure 4.2). This pattern in part, might reflect low root production (Figure 4.3) and small cohort size at the 40 - 50 cm depth. The small cohort

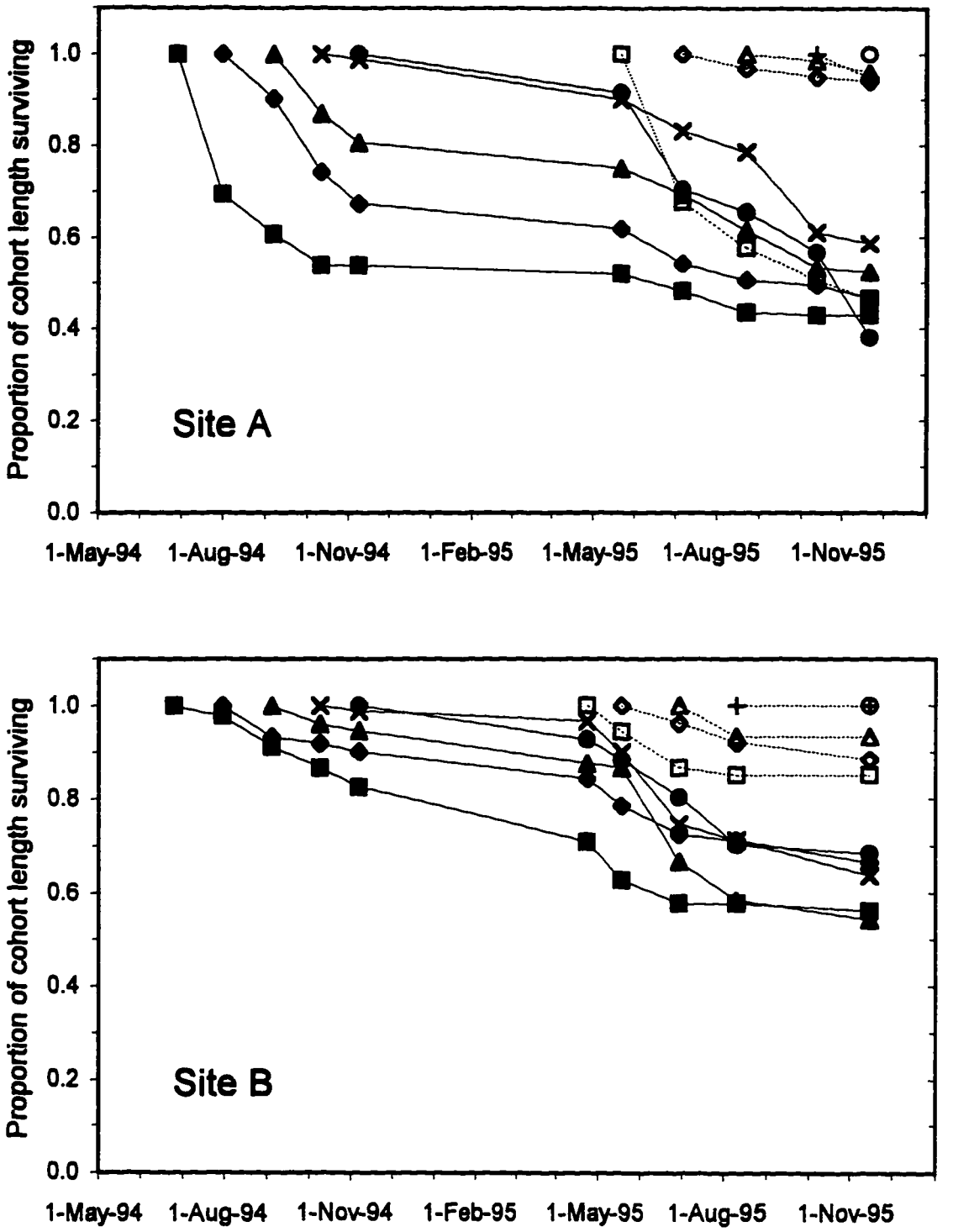
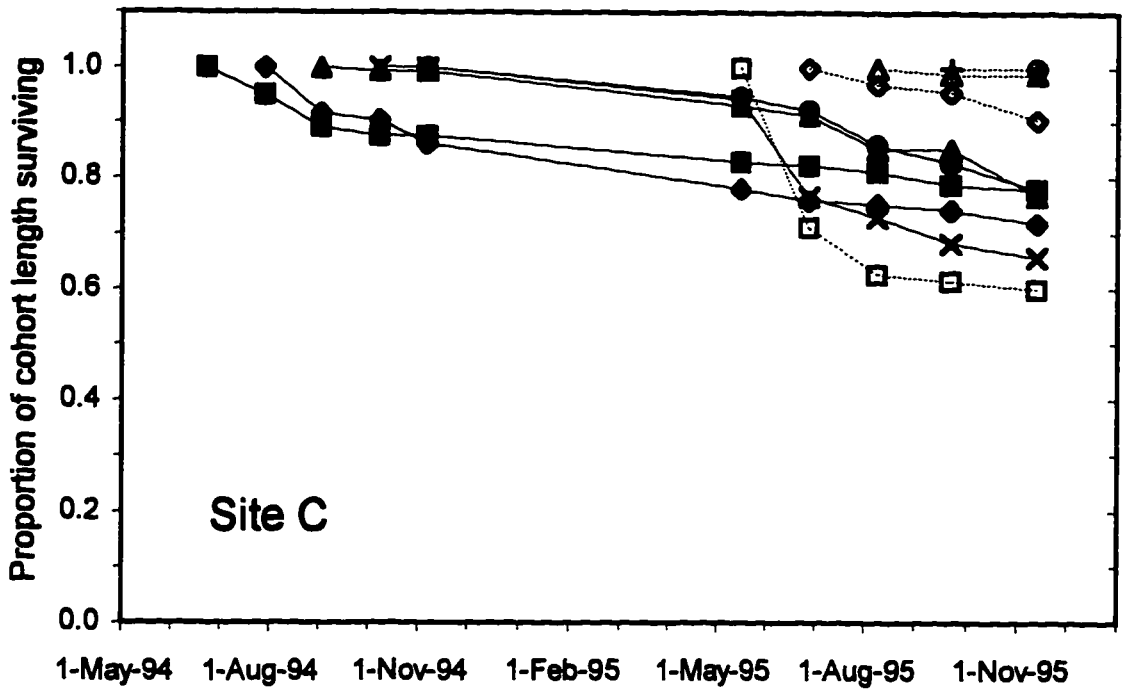


Figure 4.1 Length survival curves for cohorts of surface soil (0 - 10 cm deep) fine roots from four Michigan sugar maple forests. Cohort sizes ranged from 16 to 100 roots (mean = 47, standard deviation = 25).



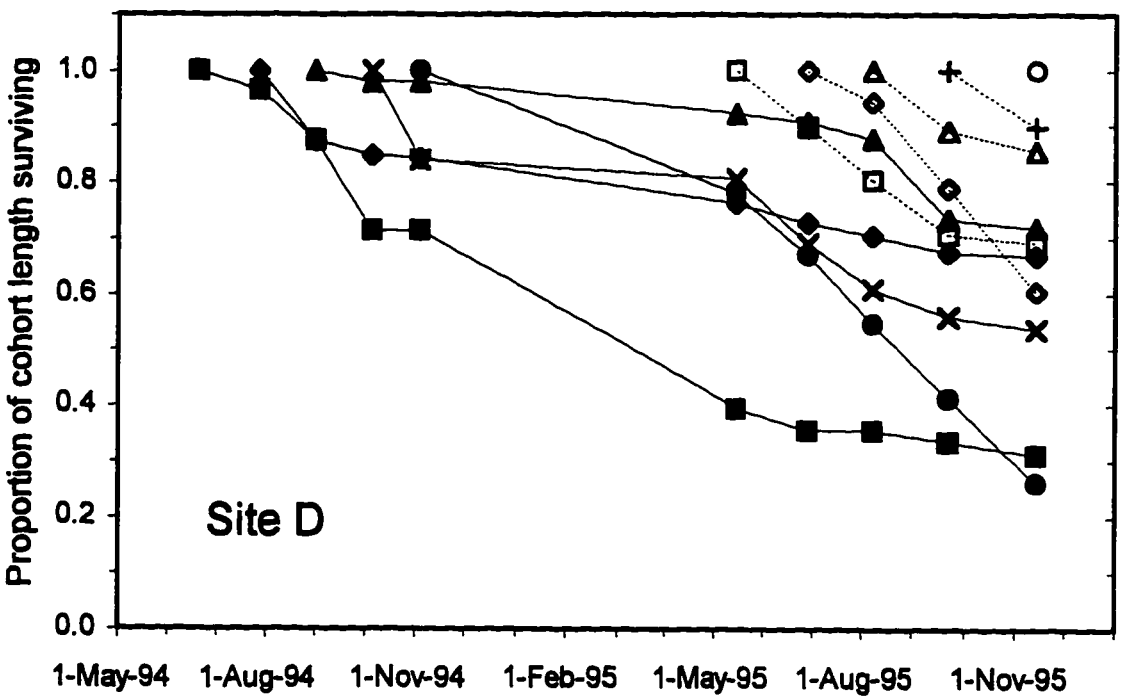


Figure 4.1 (continued)

Table 4.2 Percent of cohort root number surviving at one year for 1994 root cohorts, or at the end of the 1995 growing season for 1995 cohorts. Within observation dates, survival percentages for surface soil cohorts followed by different letters are significantly different at the 0.05 level of probability.

Date first observed	Site A	Site B	Site C	Site D
	----------Proportion surviving ----------			
	Surface soil (0 - 10 cm) cohorts			
June 19-27, 1994	0.48b	0.63ab	0.82a	0.36b
July 26-30, 1994	0.50b	0.73ab	0.75a	0.70ab
August 30 - September 5, 1994	0.62c	0.68bc	0.85a	0.73b
October 4-10, 1994	0.61	0.71	0.68	0.56
November 2-7, 1994	0.38b	0.68a	0.78a	0.26b
May 15-22, 1995	0.46b	0.85a	0.66ab	0.65ab
June 28 - July 5, 1995	0.94	0.89	0.91	0.60
	Subsurface soil (40 - 50 cm) cohorts			
June 19-27, 1994	ND[a]	0.53	0.65	0.39
July 26-30, 1994	ND	0.46	0.64	0.97
August 30 - September 5, 1994	ND	0.92	1.00	ND
October 4-10, 1994	ND	ND	ND	0.30
November 2-7, 1994	0.65	0.84	0.68	ND
May 15-22, 1995	ND	ND	ND	1.00
June 28 - July 5, 1995	1.00	1.00	0.97	ND

[a] Not determined due to no new roots or a cohort size of fewer than 10 roots.

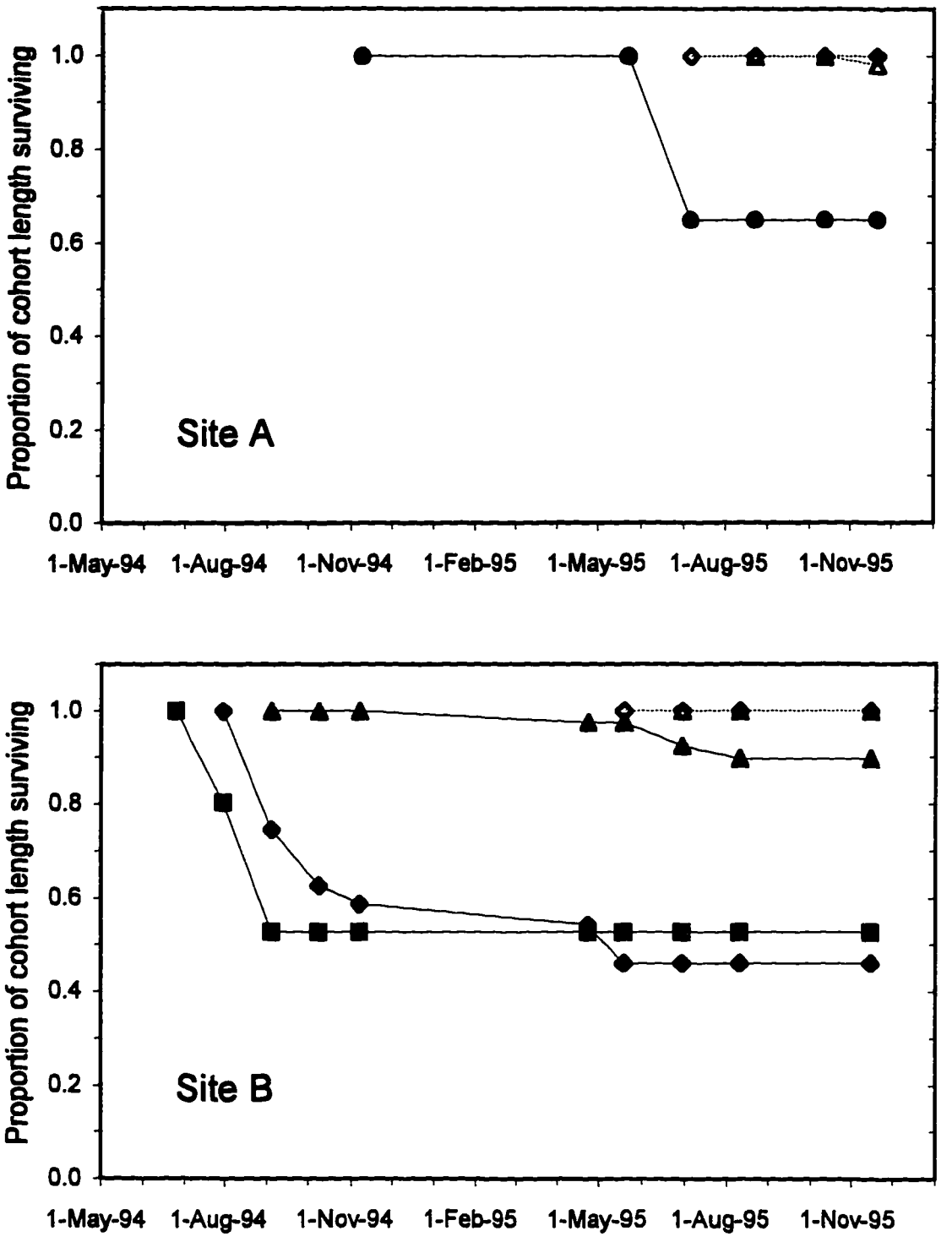
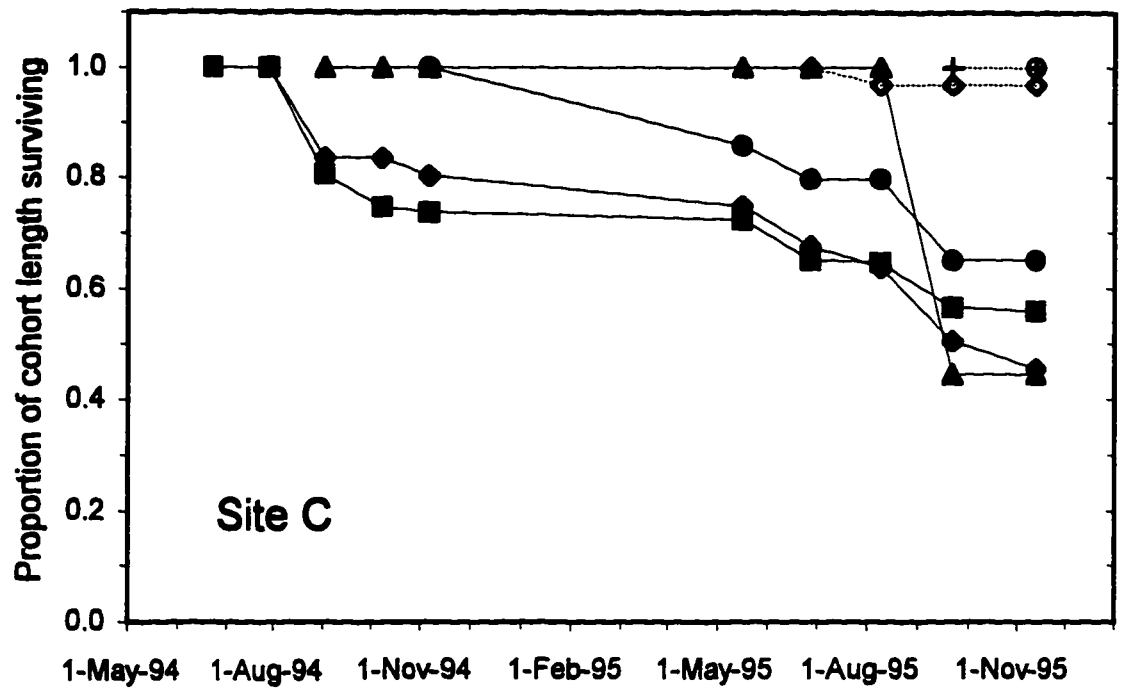


Figure 4.2 Length survival curves for cohorts of subsurface soil (40 - 50 cm deep) fine roots from four Michigan sugar maple forests. Only cohorts with a minimum of 10 roots are shown.



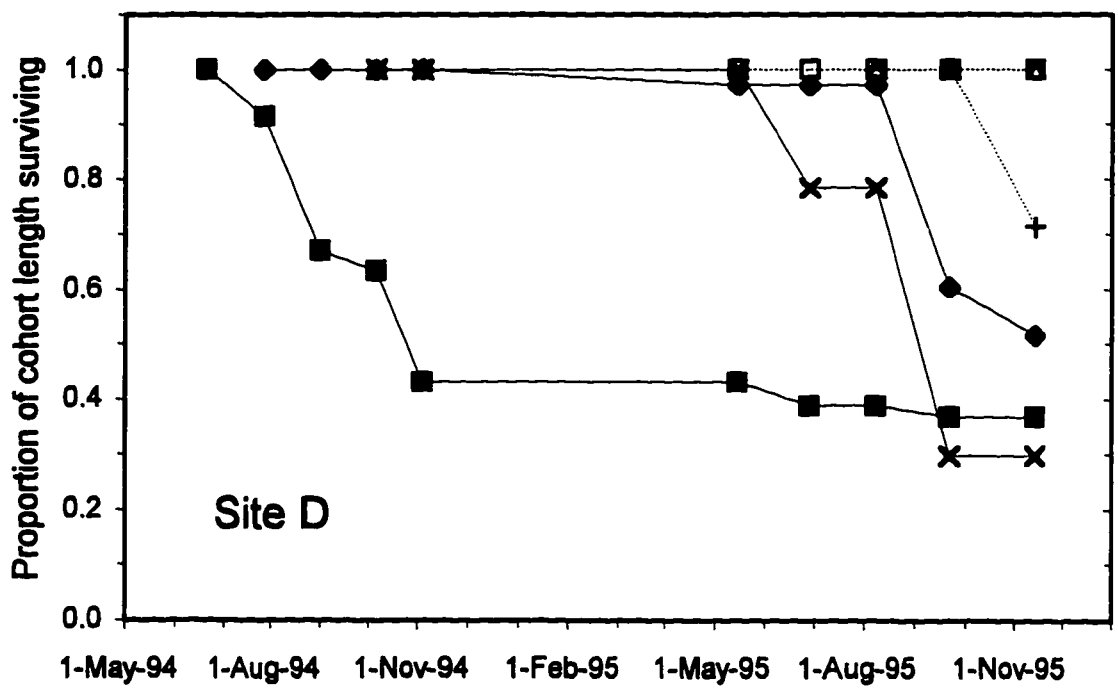


Figure 4.2 (continued)

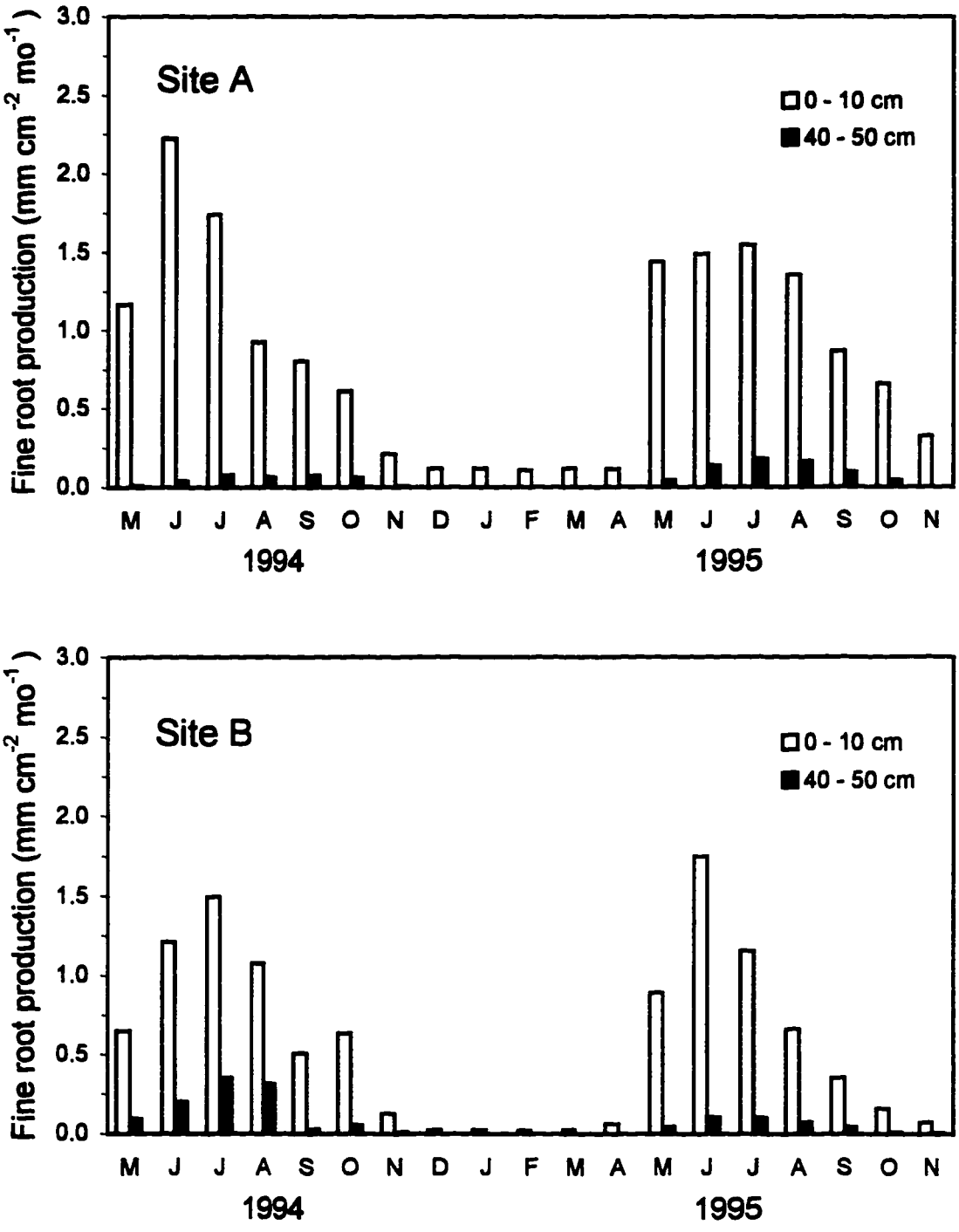


Figure 4.3 Monthly fine root production for 1994 and 1995 for four Michigan sugar maple forests.

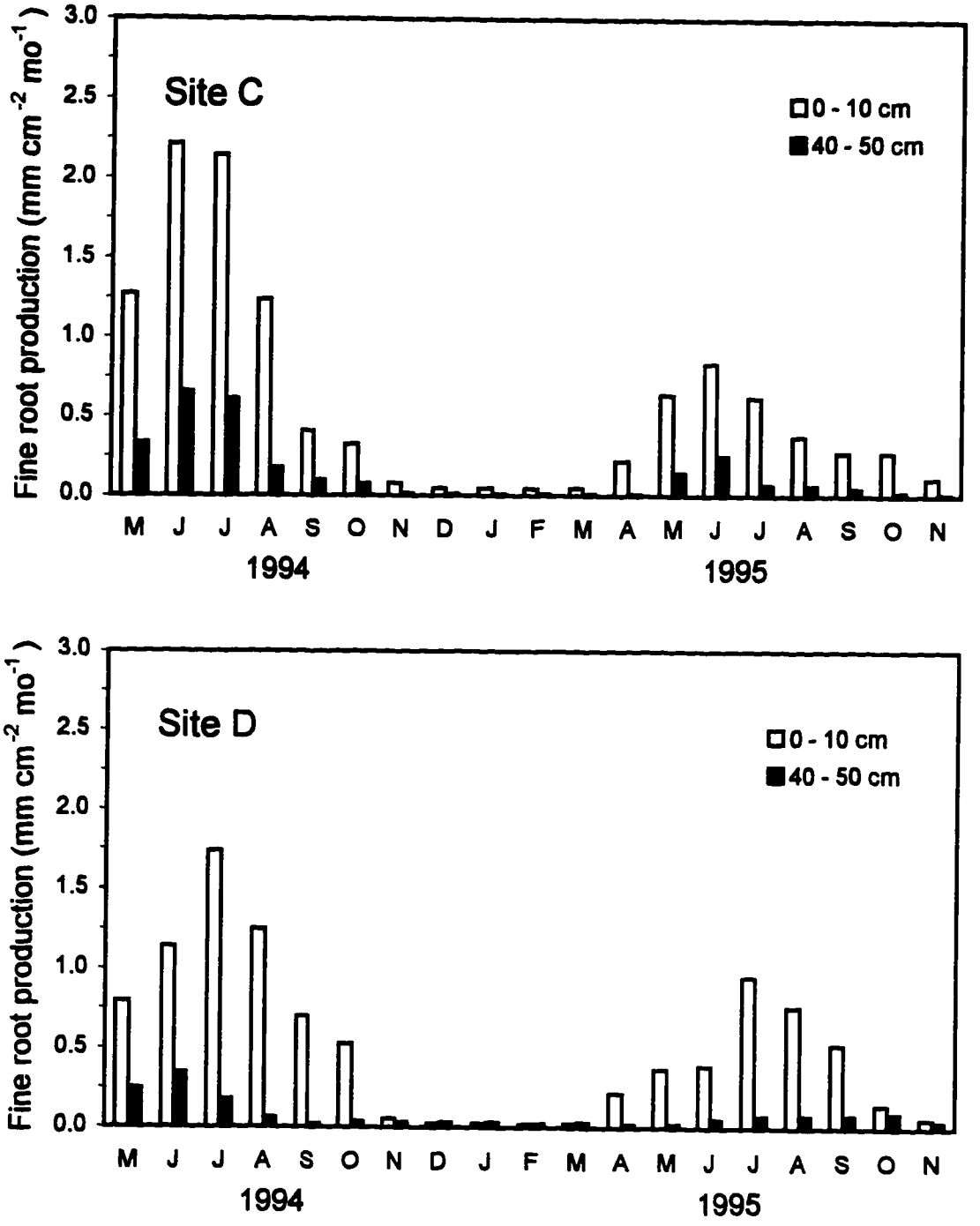


Figure 4.3 (continued)

size caused the loss of only a few roots to greatly impact the fraction of the cohort surviving.

Fine root production in both surface and subsurface soils occurred over the entire growing season at all sites, but tended to be slightly greater during the first half of the growing season (Figure 4.3). In both years, peak monthly production always occurred in either June or July, and 40% of annual surface fine root production had occurred by the end of June (one and a half to two months after leaf out). Averaged across sites, fine root production in the surface soil (0 - 10 cm) was nine times greater than at the 40 - 50 cm depth (Figure 4.3). The difference among the depths was most extreme at Site A which had sixteen times more root production in surface soil than at 40 - 50 cm.

Annual surface fine root production was similar in 1994 and 1995 at Sites A and B, but was lower in 1995 than in 1994 at Sites C and D (Figure 4.3). Lower levels of root production at Sites C and D in 1995 corresponded with severe soil moisture deficits occurring at those sites throughout the 1995 growing season (Figure 4.4).

Discussion

Fine Root Longevity in Surface Soil Roots

Root lifespans observed for surface soil (0-10 cm) roots in this study are near the midpoint of values estimated for forest ecosystems based on turnover of biomass and nutrients (Fogel 1983, Nadelhoffer et al. 1985, Ewel and Gholz 1991) and are at the upper end of fine root lifespans reported from direct observation studies (Eissenstat and Yanai, 1997). Root longevity for the early season 1994 cohorts was similar to that reported

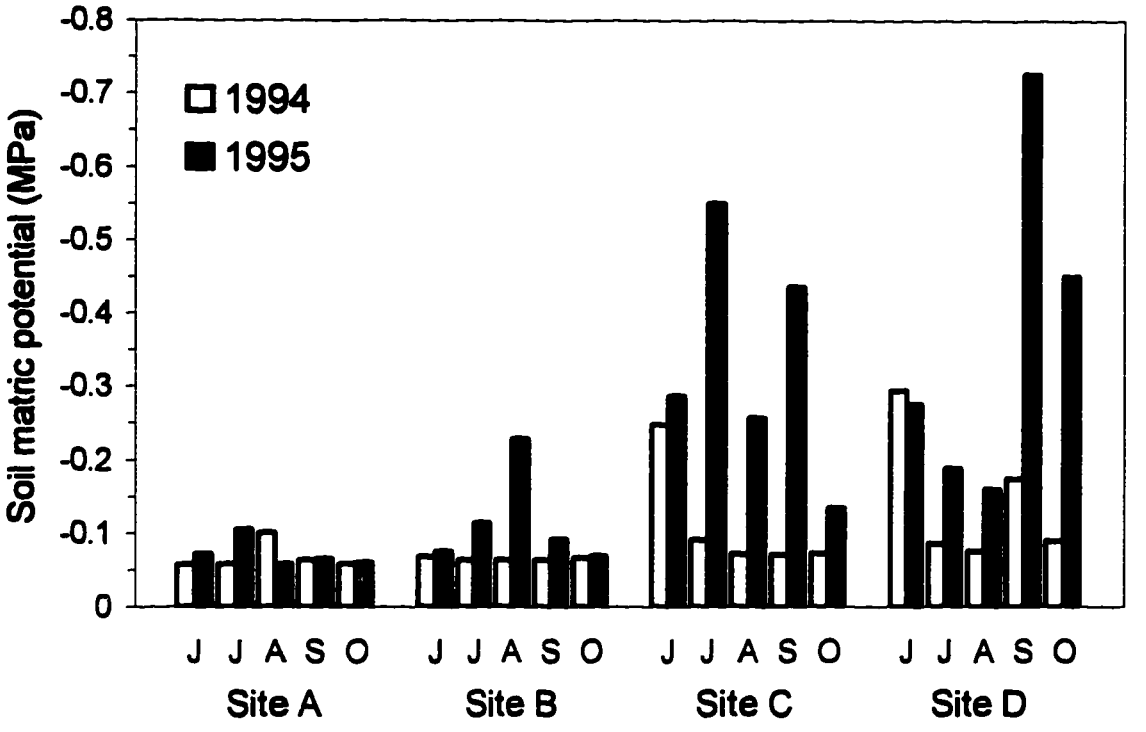


Figure 4.4 Monthly soil matric potential for 1994 and 1995 for four Michigan sugar maple forests.

by Hendrick and Pregitzer (1992a) for an early season cohort at Site C in 1989 (median of 340 d), and slightly longer than root lifespans reported by Fahey and Hughes (1994) for late spring cohorts in northern hardwoods in New Hampshire. Hendrick and Pregitzer (1993a) previously examined fine root longevity at two of the study sites used in this research and found differences between Sites C and D that were similar to those observed in the current study. They suggested that more rapid root death at the southern site (Site D) might be associated with warmer soil temperatures at the site. The current data set, which includes two additional, progressively cooler sites, suggests that temperature does

not explain differences among the sites in root lifespan. Rather, it appears that differences among sites in root longevity are related to variation in N availability.

Estimates of fine root turnover derived from root biomass and nutrient budgets have suggested both increased (Keyes and Grier 1981, Vogt et al. 1986) and decreased (Aber et al. 1985, Nadelhoffer et al. 1985) fine root lifespans in more fertile soils. In direct observational studies utilizing a single species or forest type, root lifespans have been shown to be both positively (Keyes and Grier 1981, Pregitzer et al. 1993) and negatively (Pregitzer et al. 1995) related to N availability. These findings have led to the generation of several competing hypotheses regarding the effect of N availability on root longevity and the mechanisms by which N availability might control root longevity (Raich and Nadelhoffer 1989, Hendricks et al. 1993, Eissenstat and Yanai 1997).

The finding that fine root longevity is greater where N availability is higher suggests that roots are maintained as long as the benefit (nutrients) they provide outweighs the C cost of keeping them alive. Eissenstat and Yanai (1997) used simulation modeling and cost-benefit analysis to estimate root lifespans that maximized root efficiency in terms of nutrient acquired per unit C expended for root construction and maintenance. Their analyses suggested that roots located in nutrient-rich patches should live longer. This agrees with the results of the nutrient patch studies conducted by Pregitzer et al. (1993) and Fahey and Hughes (1994) in hardwood forests and appears to be consistent with the findings for the four northern hardwood forests . The soils at the four sites can be viewed as a mixture of patches of low and high nutrient availability, with the high N availability sites having a greater frequency of high N patches. At the high N

sites, a larger percentage of the roots observed would be in high N zones where root lifespans are longer, leading to greater average root lifespan.

Previous studies at the four sites have consistently measured higher root respiration rates (at a given temperature) at Sites B and C than at Sites A and D (Table 4.1, Burton et al. 1996, Zogg et al. 1996, Burton et al. 1997), indicating that root metabolic activity is greater in N rich zones. Faster depletion of the cytosolic sugar pool in metabolically more active roots has been shown to result in increased carbohydrate allocation to the roots (Minchin et al. 1994), suggesting the possibility that the C sink strength of roots may play a role in regulating their lifespan. Under such a mechanism, root activity would remain high until local nutrients were depleted. At that time, root activity would decline, lowering sink strength, ultimately leading to cessation of C supply and root death. Greater time to nutrient depletion in nutrient-rich patches would result in to greater root lifespans, such as those observed in the surface soils of the high N availability sites (Sites B and C).

The above mechanism appears to explain the pattern in root longevity observed at the four sites, but certainly does not resolve the controversy regarding the effects of N availability on root longevity. Some of the contradicting evidence regarding N effects on root lifespan may be the result of some experiments studying a single forest type (or species) at different N availabilities, whereas other studies examined a variety of different forest types, each adapted to sites of different fertility. It is possible that average root lifespan in species adapted to nutrient poor environments is longer than that in species adapted to nutrient rich environments as hypothesized by Nadelhoffer et al. (1985),

Eissenstat (1992), and Grime (1994), while higher N availability within a single species or forest type might lead to longer root lifespans as observed in this study and by Pregitzer et al. (1993), Fahey and Hughes (1994), and Keyes and Grier (1981). If this is true, one would expect lower average root activity (respiration) in roots of N-poor sites, permitting long root lifespans despite low nutrient gain per unit time (Eissenstat and Yanai 1997). Direct observation of root longevity coupled with measurements of root respiration may help resolve the controversy. Ideally, such experiments will be conducted both along natural N availability gradients in single forest types and across forest types adapted to sites of differing N availability. Experiments in which N availability is artificially altered might also prove useful, but caution should be used in interpreting results for stands that may not be in equilibrium with the "new" N availability. Nitrogen fertilization of mature forests often leads to a reduction in total root biomass (Vogt et al. 1990, Haynes and Gower 1995). For this new "equilibrium" root biomass to be achieved, root production must decrease (Vogt et al. 1990, Gower et al. 1992), and/or average root lifespan must temporarily shorten. Until the forest has adapted to improved N availability and a quasi steady-state root biomass is achieved, estimates of root lifespan may not accurately reflect those that would occur under a similar, natural level of N availability.

Fine Root Longevity in Subsurface Soil

The pattern among sites in surface root longevity was not apparent for cohorts from the 40 - 50 cm soil depth. For these deeper roots, longevity patterns among sites varied considerably among cohort initiation times (Table 4.2). This may be due in part to the very small size of the cohorts observed, especially at Site A. Nitrogen availability has

not been measured for the 40 - 50 cm soil depth, so its relationship to the observed root lifespans is unknown. It can be assumed that N availability at 40-50 cm is less than at 0-10 cm. Using the same cost-benefit analogy used for surface roots, then either lifespans of the deeper roots should be shorter or the maintenance cost per unit time for the roots should be less (Eissenstat and Yanai 1997). Subsurface root lifespans were not clearly less than those for surface roots, but much lower rates of root respiration have been found in fine roots from the 40-50 cm depth than in surface roots (M.J. Laskowski and A.J. Burton, unpublished data), in agreement with the hypotheses. It also is possible that the deeper fine roots function more for water uptake than nutrient acquisition. In that case, the benefit they provide needs to be assessed differently than for surface roots when attempting to predict longevity.

Seasonality of Root Production

The finding of continuous root production over the entire growing season, with more than 50% of root production occurring in the first half of the growing season (by mid-July), is consistent with numerous previous observations for sugar maple (Morrow 1950) and northern hardwood forests (Fahey and Hughes 1994, Burke and Raynal 1994, Hendrick and Pregitzer 1996, 1997). Hendrick and Pregitzer (1996) found a higher proportion of root growth early in the season at deeper depths (> 50 cm), something which occurred only occasionally at 40-50 cm in the current study. They also reported circumstantial evidence that episodic deep (50 - 100 cm) root production was related to periods of high water demand. In the current study, no increase in root production was observed at the 40-50 cm depth during the periods of severe moisture deficit at Sites C

and D in 1995. Instead, a decrease in root production occurred at both 0-10 cm and 40-50 cm during the drought periods (Figure 4.3). This is consistent with previous reports of reduced root initiation and elongation in dry soils (Morrow 1950, Teskey and Hinckley 1981, Kuhns et al. 1985).

There was no evidence of enhanced or episodic root mortality at Sites C and D in response to high soil moisture deficits in the middle of the 1995 growing season. However, fine root respiration decreased during drought periods in that year (Burton et al. 1997). These findings provide additional support for Eissenstat and Yanai's (1997) cost-benefit approach to assessing root longevity, as their analyses suggested that shedding roots in dry soil may not be necessary for root efficiency if reductions in maintenance respiration occurred to match reductions in nutrient uptake.

The controversy regarding the effects of N availability on root longevity is not likely to be resolved any time soon, and it is possible that no single theory can explain the effects of N availability across all species. Trees such as aspen and sugar maple differ greatly in their above-ground strategies for resource acquisition, so one must question whether it is reasonable to expect them to respond similarly belowground. The results of this study do provide support for the cost-benefit approach to determining root lifespan (Eissenstat 1992, Eissenstat and Yanai 1997), and it is suggested that changes in root C sink strength as nutrients are depleted may provide the mechanism through which root lifespan is regulated.

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Chapter V

SUMMARY AND RECOMMENDATIONS

Differences among the four study sites in fine root respiration and root longevity consistently reflected the pattern among sites in N availability, rather than differences in mean annual temperature. These results should prove helpful in assessing how C allocation in temperate deciduous forests will respond to altered N availability resulting from chronic N deposition or N fertilization. Although soil temperature was not responsible for between site differences in inherent root respiration capacity, it still explained the vast majority of variability observed in the root respiration data set. Indeed, an average Q_{10} of 2.7 for root respiration indicates a strong dependence on temperature at all sites, suggesting that raising mean annual temperature by only a few degrees has the potential to greatly increase C allocated to root respiration. Attempts to predict northern hardwood C cycling for future climates clearly need to take the effects of altered temperature, soil moisture, and N availability into account, as all impacted root respiration and the latter two both influenced root production.

An interesting finding of the experiments is the apparent compensatory responses of root respiration and root longevity to increasing N availability. Higher C allocation associated with faster root turnover (shorter lifespans) at the two low N sites (A and D) is at least partially offset by lower rates of root respiration per unit root mass. To fully assess such compensatory responses, the effect of N availability on fine root biomass also needs to be considered. Preliminary estimates for the sites (A.J. Burton, unpublished data)

suggest that root biomass may be higher at the low N sites (A and D). This would be in agreement with the effects of N availability on root biomass documented in the current literature. If these preliminary estimates hold true, total belowground C allocation at the low N sites will be higher than that at the high N sites. Interestingly, tree height and aboveground biomass are lower at the low N sites, suggesting optimization of above- and belowground C allocation to obtain resources in a balanced way.

The studies detailed in this dissertation have improved our understanding of the factors controlling root respiration and root longevity in northern hardwood forests, but the generality of the relationships still needs to be assessed. The observed effects of root N concentration and temperature on respiration are quite consistent with recent reports in the literature. However, there is virtually no available evidence against which to assess the universality of the finding of reduced respiration at both the root and stand level during periods of severe moisture deficit. Since future climates are expected to include not only higher temperatures but also more year-to-year variability in moisture supply, this is potentially is an important trait that may allow trees to survive conditions that current C models would predict to cause mortality.

The finding that root lifespans were longer where N availability was higher will likely add to the controversy regarding the influence of N on root longevity. Viewing the root longevity - N availability relationship as a cost-benefit process served to explain the results observed for the four sites and will hopefully lead to additional studies that will help resolve the controversy. Experiments, such as those outlined in Chapter IV, which examine the effects of N availability on root respiration and root longevity, both within

and among forest types, are sorely needed. It is strongly suggested that root longevity in such experiments be determined using direct observations to avoid some of the potential problems associated with the assumptions on which C and nutrient budget methods are based. The hypotheses that greater metabolic activity of roots in nitrogen rich zones leads to greater carbohydrate allocation to those roots, and that reduction in root C sink strength as local nutrients are depleted provides the mechanism through which root lifespan is regulated, also need to be experimentally evaluated.