COTTONWOOD FINE-ROOT TURNOVER IN RESPONSE TO NITROGEN REGIMES IN A SHORT ROTATION PLANTATION STUDY

by

Christel C. Beno

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APPROVED BY THE GRADUATE COMMITTEE OF:

Dr. Jane Fischer Johnson, Committee Chair

Assistant Professor of Biology

Dr. Clive A. David

Professor of Forestry

Dr. Ronald F. Hensler

Professor of Soils

Abstract

Fine-roots are the most active and absorptive part of the root system. Roots respond to and are controlled in part by the environment. Environmental factors are often correlated with root dynamics including soil moisture, aeration, temperature, and nutrient supply. The mineral nutrient most frequently limiting to plant growth is nitrogen (N). Nitrogen availability is dependent on microbial activity and environmental conditions. Favorable conditions increase N availability and fine-roots take up the available N to meet plant demands. Plant nutrient demands in fast growing *Populus* spp. (poplar) can be high. Poplar plantations have the potential to absorb excess N in the environment and to be used as buffers along agricultural lands or used for remediation. The fast growth in poplars also has the potential to meet human needs. Correct manipulation of N fertilizer or site selection can induce rapid growth to meet wood resource demands for energy and industry and to sequester CO_2 and reduce green house gases. With so many possible uses for poplars and the potential to meet objectives by using N correctly, we tested cottonwood (*Populus deltoides*) fine-root response to various N fertilizer levels in a short rotation plantation. The plantation was maintained and studied for two years. Fine-root dynamics were controlled largely by temporal effects. Live-root standing crop increased 1.6 fold from the first through the second year. First year roots had longer life spans than second year roots. First year root production also responded to N treatment but no biological implications were found. Change in time with stand age and season had the strongest control over fine-root dynamics of this cottonwood stand under intensive management.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

BACKGROUND AND REVIEW

INTRODUCTION

Roots are vital to overall plant growth by absorbing nutrients, water and by providing anchorage for plants. Root biomass production exceeds aboveground productivity on a range of ecosystems (Caldwell, 1987). Root construction and maintenance influences both carbon (C) and nitrogen (N) consumption, while root death results in a partial return of nutrients to the soil (Eissenstat and Yanai, 1997). Roots consume large quantities of photosynthates and root biological processes have potential to limit overall plant growth. Therefore, in the past decade researchers and growers have placed more emphasis on root dynamics (Pregitzer and Friend, 1996). Since the 1990's, advances in minirhizotrons and associated computer technology has facilitated *in situ* observations of roots which allow scientists to assess root functions in plants and ecosystems more accurately.

Understanding the root systems of specific species and ecosystems will lead to better management especially under certain environmental conditions. Conditions of low N will limit plant growth. Managers can manipulate N by site selection and fertilizer applications to meet growth or biomass objectives. Objectives for pulp and energy can be met with fast growing *Populus* spp. (poplars). Managing N for poplars requires an understanding of the root system structure, function and physiology since root systems take up N to meet plant needs (Pregitzer and Friend, 1996). This chapter will review N effects on poplar roots. Roots are affected by many factors. Only factors more likely to be encountered in a poplar plantation are highlighted here.

ROOT SYSTEM FUNCTION

Root systems anchor plants into soil and are the primary organ for water and mineral nutrient uptake. Coarse woody roots anchor the plant while fine-roots are mainly responsible for water and nutrient uptake. Older, woody roots have inefficient nutrient uptake kinetics due to the C needed for maintenance (Yanai et al., 1995). In part, root structure and physiology are determined genetically. However, environmental conditions and internal C distribution also influence root growth and physiology. Root processes are interrelated with aboveground productivity. Leaves and roots have complementary functions in resource acquisition by exchanging photosynthates for mineral nutrients and water. In trees, photosynthesis occurs mainly in leaves. Photosynthates are transported via the assimilation stream in the phloem to roots where they are stored as starch in coarse roots or used for metabolic activity (Mohr and Schopfer, 1995). Mineral nutrients are acquired by roots through active and passive transport. Most nutrients move to the shoot via the transpiration stream either as an ion or as an assimilation product. Water uptake is driven by the water potential gradient from leaf to atmosphere, and is transduced along the water column to the root.

ROOT SYSTEM STRUCTURE

Root structure develops from branching patterns with lateral roots arising from the pericycle which is a single cell layer just below bound externally by the endodermis. Primary roots (root directly from the shoot base) are the source of the horizontal roots. Roots can continue branching up to the seventh order (Sutton and Tinnus, 1983). Poplars develop numerous horizontal roots between 5-20 cm below the soil surface which is a zone of high resource availability. Horizontal roots can extend as far as several times the height of the tree. Sinkers or vertical roots develop off horizontal roots and extend one to three meters deep (Pregitzer and Friend, 1996).

FINE-ROOTS

Fine-roots (<2mm diameter) are considered to be most active in absorption of water and mineral nutrients from soil. They have little role in storage or in support. In poplars, fine-roots are long, relatively unbranched, very thin, and do not undergo secondary thickening (Brundrett et al., 1990; Pregitzer and Friend, 1996). They exhibit determinant growth and extend only a few centimeters after emerging from lower-order laterals (Eissenstat and Yanai, 1997). They also are concentrated in well-aerated areas because the capacity of the soil to provide water and nutrients increased in these areas (Pregitzer and Friend, 1996). Fine-root life spans have been recorded for various forest trees ranging from 49 days for hybrid poplars (Hooker et al., 1995) to 340 days in sugar maple (*Acer sacchrum*) forests (Hendrick and Pregitzer, 1992a).

Fine-roots have associations with mycorrhiza. Ectomycorrhiza and sometimes vesicular-arbuscular mycorrhiza are associated with poplar fine-roots (Pregitzer and Friend, 1996). Poplar fine-roots infected with arbuscular mycorrhia have shorter life spans than uncolonized roots (Hooker et al., 1995). Infected poplar roots also have longer root length and greater root system branching (Hooker et al., 1992).

ABOVE- AND BELOW-GROUND ACTIVITY

Fine-root productivity is correlated with aboveground growth and physiology. Rapid root extension is correlated more with leaf area than with photosynthetic potential per unit leaf area (Rhodenbaugh and Pallardy, 1993). After canopy closure, fine-root standing crop also has been shown to remain stable (Vogt et al., 1987). Decreased crown area due to weather events or insect defoliation reduces leaf surface area, foliage retention time, and respiration. Loss of fine-roots is advantageous under crown stress because the photosynthates required for maintaining roots are reduced. In northern hardwood forests, fine-root production (length) was greatest in spring and summer, similar to seasonal trends of leaf production in deciduous forests (Hendrick and Pregitzer, 1996). Roots typically start growing before leaves emerge (Lyr and Hoffman, 1967). Without photosynthetic activities in spring, a leafless tree consumes large amounts of stored carbon to initiate root growth (Nguyen et al., 1991; Kozlowski, 1992).

SOURCE-SINK RELATIONSHIP

Above- and below-ground parts of a tree also demonstrate a source-sink relationship for carbohydrates. Carbon sources can be either photosynthesizing mature leaves or roots that store carbohydrates over winter. Sinks are nonphotosynthetic organs, such as in coarse roots, fruits, and immature leaves that need carbohydrates for growth, maintenance or storage (Taiz and Zeiger, 1998). Photosynthates are dispersed to the strongest sink determined by size and metabolic activity. Over 50% of the photosynthates produced are exported to the roots (Jurik et al., 1991). Root activity includes growth rate, meristem activity rate, and maintenance respiration rate. In addition, mycorhiza status (infection or non-infection), root age, root diameter and distance from tree bole influence the amount of carbohydrate diverted to roots (Vogt and Bloomfield, 1991).

NITROGEN IN PLANTS

Nitrogen is an important component of all tree organs. Nitrogen is a structural component of essential molecules such as amino acids, nucleic acids, and chlorophyll. Nitrogen concentration can affect tree growth, dry weights, N uptake and white root persistence (Mackie-Dawson et al., 1995). Root system productivity is dependent upon adequate mineral availability in the soil with N being one of the most limiting (Agren, 1985). Internal cycling or retranslocation of N in the plant meets most of the tree's N demands. Retention of prelitterfall N may be up to 70% in poplars through the dormant season (Pregizter et al., 1990).

Plants can acquire N from the soil in the form of ammonium (NH_4^+) ion or nitrate (NO_3^-). Ammonium is assimilated directly into amino acids in the roots. Nitrate is taken up from the soil and the preferred N form for many plant species including cottonwood (*Populus deltoides*) (Woolfolk et al., 1998). Nitrate is reduced to nitrite (NO_2^-) by the enzyme nitrate reductase in the shoots and roots of many plant species. Nitrite reductase further reduces NO_2^- to NH_4^+ that is finally assimilated into the amino acid glutamine. Glutamine is a precusor to many other essential amino acids (Taiz and Zeiger, 1998). Nitrogen assimilation consumes 25% of the energy expenditures of both roots and shoots, even though the element is less than 2% of the total dry weight of a plant (Bloom, 1997).

NITROGEN CYCLE

Nitrogen must be cycled in the environment to be available to plants. Nitrogen is made available to plants by mineralization and nitrification from various N pools. Most N is either found in the atmosphere as gaseous N_2 or bound in organic matter. Forms of N are found in several ecosystem pools: atmosphere, plant, soil organic matter, microbes and the soil solution. The cycling of N between these pools is dependent on the following processes: mineralization, immobilization, nitrification, N fixation, and denitritification. Mineralization is the transformation of organic N to inorganic N by decomposer organisms. Rates of mineralization are usually low in natural forest stands and vary considerably across different temperate ecosystems (Carlyle, 1986). Immobilization is the conversion of inorganic N to organic N in microbial or plant tissues (SSSA 1987). Grazing soil fauna can increase the release of N from immobilization by creating a more homogeneous distribution of decomposers and by exuding N-rich excretions. Availability of N to plants depends on the amount of N produced in net N mineralization which is the amount that mineralization exceeds immobilization. Nitrification is the conversion of NH_4^+ to NO_2^- and NO_3^-.

Bacteria use N from the atmosphere to make plant available N in the soil or in root nodules through nitrogen fixation. Denitrification by microorganisms returns gaseous N to the atmosphere. Nitrogen fixing microbes can change atmospheric N_2 directly with hydrogen into NH_4^+, which is a plant available form of N. Fixation rates for symbiotic bacteria (e.g., *Rhizobium* and *Frankia*) range from 100-200 kg N ha^{-1} y^{-1} and for non-symbiotic bacteria (i.e., cyanobacteria) rates are usually <5 kg N ha^{-1} y^{-1}

(Carlyle, 1986). Denitrification is a return of gaseous N to the atmosphere from NO_3^- reduction. The process is mediated largely by facultative anaerobic bacteria. Denitrification is influenced by the amount of NO_3^- and organic matter available, and environmental factors (e.g., Oxygen availability and temperature). Rates of denitrification are usually negligible in well-aerated soils. Nitrogen cycling and availability are dependent on decomposition and other microbial mediated processes as well as microorganism abundance and diversity (David, 1997).

CARBON CYCLE

Carbon is the basic component of all organic compounds and is cycled through the ecosystem. Carbon in organic matter is converted by microbes to inorganic carbon dioxide (CO_2). The largest, active source of C for living land organisms is CO_2 in the atmosphere. Plants reduce CO_2 from the atmosphere into carbohydrates during photosynthesis. Forests, soil, and litter sequester C. Because forests have greater productivity and life span compared to annual and herbaceous plants, they are the largest group of C consumers and sequestraters. Heterotrophic organisms break down plant material and respire C in the form of CO_2 back to the atmosphere (Smith, 1992). Carbon and mineral cycles are inherent to the functioning of ecosystems and integral to their structure (Barbour et al., 1987).

FINE-ROOTS IN THE ECOSYSTEM

Ecosystem N and C cycles are interrelated through plant inputs and outputs. Plant outputs include fine-root mortality that can represent a substantial C and nutrient flux in deciduous forest ecosystems (Cox et al., 1978; Joslin and Henderson, 1987; Hendrick and Pregitzer, 1992a; Burke and Raynal, 1994). Plant outputs involve net primary production (NPP). Total NPP can be 40-90% of fine-root production in temperate deciduous forest ecosystems (Fogel, 1985; Mackie-Dawson et al., 1995).

Ratios of C:N in forested ecosystems influence patterns of mineralization and immobilization. High C:N results in immobilization of organic N in microbes. Fine-roots can have 2.0-2.8 times greater C:N than above-ground litter which represents 42% of annual C turnover for fine-roots in some ecosystems (Edward and Harris, 1977). Nitrogen release during decomposition of fine-roots may be 1.4-2.0 times larger than N release from above-ground sources (Henderson and Harris, 1975; Wells and Jorgensen, 1975; Carlyle, 1986). Soil N availability plays a key role in determining C fluxes of fine-root growth rates and fine-root mortality to soil. Fine-roots died at a faster rate when soil N was more available (Pregitzer et al., 1995).

ENVIRONMENTAL FACTORS INFLUENCE ROOT GROWTH

Oxygen availability

Oxygen availability, temperature, and fertility are soil environmental factors that influence root activity. The following sections will discuss each of these facts.

Roots are sensitive to the amount of oxygen in the soil, which varies due to water content and soil properties such as bulk density. Increased bulk density increases impedance, the physical resistance of soil to root growth, and decreases the aeration and gaseous pore size in soil. Root hydraulic conductance was reduced dramatically by low oxygen and high CO_2 concentrations in flooded *Populus trichocarpa* x *Populus deltoides* roots (Smit and Stachowiak, 1988). Hypoxia, the lack of oxygen, decreases root growth, stimulates the production of adventitious roots, and decreases whole plant growth (Pregitzer and Friend, 1996). Adventitious rooting and aerenchyma formation are morphological plasticity traits that allow poplars to survive under flooded conditions (Smit and Stachowiak, 1988). When young apple tree roots were submerged in water, all small to fine-roots died, but older woody roots survived (Childers and White, 1942). More oxygen is needed to produce and maintain fine-roots than to maintain an equal weight of existing woody roots, because fine-roots have high respiration rates that demand large amounts of oxygen (Head, 1973). Available oxygen increase nutrient up take efficiency to meet plant needs and management objectives.

Soil Temperature

Soil temperature affects root growth and morphology. Root productivity nearly ceases after leaf senescence. Carbohydrate reserves are metabolized during spring to fuel new growth in temperate regions. The cessation of root productivity in autumn is due both to lack of current photosynthate and low soil temperature. Lower temperatures reduce maintenance respiration costs of roots as growth ceases (Ryan, 1991). Also, root hydraulic conductance is influenced negatively by low temperatures since it reduces ion uptake (Kramer, 1983). As temperature decreases, root maintenance decreases (Ryan, 1991) but as temperature increases, root life span decreases (Eissenstat and Yanai, 1997). Hendrick and Pregitzer (1993b) observed marked differences between root productivity of two different ecosystems with different mean temperatures. Lobolly pine (*Pinus taeda*) roots will grow at a minimal temperature of 5°C, have optimum growth at 25°C, and are inhibited at temperature 35°C or above (Lyr and Hoffman, 1967). It is unresolved whether temperature correlations are due to phenotypic plasticity, ecoclinal, or genetic variation (Fahey and Hughes, 1994).

Soil Fertility

Under most conditions, plants have the ability to adapt and acquire nutrients strategically. Plant strategy theory suggests that if a patch (localized area of nutrients) is short lived, nutrient acquisition may not cover the costs of C used for root proliferation into the patch (Grime et al., 1991). However, on fertile sites, root systems are more active and dynamic than low fertility sites. Between internal and external conditions (e.g., plant nutrient status and environmental factors), roots either proliferate or senesce to optimize nutrient acquisition. Grime et al. (1991) predicted that with fertile soils root will exploit minerals by a dynamic root system in which individual root turnover is rapid. On infertile soils, roots will be less dynamic and functional throughout the year to intercept brief occurrences of minerals, because during the harsh conditions the cost of maintaining standing roots is less than the cost of building new ones (Yanai et al., 1995). Soil heterogeneity increases root competition between different tree root systems; whereas, in uniform sites more competition exists internally between roots and shoots (Eissenstat and Yanai, 1997).

Generally, root to shoot ratio should increase on poor sites and decrease on nutrient rich sites. For example, roots were 21% of the total biomass in high N two-year old apple trees and 26% in low N plants (Maggs, 1961). Two-year old *Ulmus americana* seedlings grown with high N had a root to shoot ratio of 0.28 compared to a ratio of 0.95

in trees grown with in low N (Walters and Reich, 1989). Nitrogen increases above-ground growth rates more than below-ground. Root to shoot ratios in poplars also have been shown to decrease with age (Shepperd and Smith, 1993). Due to genetic and environmental variation, root to shoot ratios also vary. This makes root to shoot numbers difficult to compare.

ROOT BIOMASS

Root biomass is hard to estimate because of methodological limitations. Root systems that have been excavated always leave an unknown portion in the ground. Fine-roots are delicate and thus hard to keep intact. Pot studies confine roots and usually are conducted with young seedlings. This leaves room for a large amount of error in calculating root biomass (Dickmann and Pregitzer, 1992). In fact, studies have shown fine-root mass to be 3 to 60% of the total root mass in poplars (Lyr and Hoffman, 1967; Pregizter et al., 1990). Total fine-root production in *Populus balsamifera* in Alaska averaged 4386 ± 322 kg ha^{-1} y^{-1} (Ruess et al., 1995). In Michigan, Hendrick and Pregitzer (1993a) found sites of northern hardwood ecosystems to produce 7300-8000 kg ha^{-1} y^{-1} of fine-root biomass. *Eucalyptus globulus* root to shoot biomass ratios were 0.18- 0.21 and *Populus* root to shoot ratios were 0.2-0.6 during the first year's of growth (Baker and Blackmon, 1977; Pereira and Landsberg, 1989; Dickmann and Pregitzer, 1992).

ROOT STUDY TECHNIQUES

Roots grow in the soil substrate making them difficult to study *in situ*. Past sampling methods were destructive, which included soil coring, root screens, and excavated blocks. Destructive methods are advantageous for determining biomass production especially at the ecosystem level (Bloomfield and Vogt, 1996). However, tedious labor is needed for soil coring and excavated blocks to remove roots from the soil and roots with diameters less than two mm can easily be missed (Vogt and Persson, 1991). Destructive techniques also sacrifice the ability to track roots over time in response to a treatment or certain environmental conditions or simultaneous measurement of root production, death and decomposition (Fogel, 1985; Santantonio and Grace, 1987; Kurz and Kimmins, 1995).

Recently, root science has progressed with advancements in minirhizotron (clear, plastic tubes) technology and associated computer software. Minirhizotrons are non-destructive and allow the repeated observation of fine-roots *in situ*. Non-destructive techniques provide data to calculate root growth and turnover rates. Minirhizotron assessment techniques have increased the understanding of root life span ecology which consequently has allowed root research to more accurately address questions concerning the dynamics of root growth and environmental interactions. Is fine-root life span increased in nutrient rich sites? Are leaf and fine-root life spans correlated? How much is root mortality under the control of the tree or are roots mainly lost to herbivory and parasitism? Minirhizotrons do have some disadvantages that include: 1) no biomass production estimates can be made, 2) the soil-tube interface may not be similar to the growing conditions in the bulk soil, and 3) high repetition of tubes is needed to represent the bulk soil (Mackie-Dawson and Atkinson, 1991; Hendrick and Pregitzer, 1992b).

FOUNDATIONS TO THIS STUDY

Fine-root turnover contributes to nutrient flows and pools in ecosystems. Fine-root uptake of N is critical to forest production as well as nutrient cycling. Improper N fertilization leads to forest degradation and N losses through leaching and volatilization. Understanding optimal root life span should do more than maximize nutrient uptake; it should maximize plant success in the environment (Eissenstat and Yanai, 1997). Interpreting fine-root physiology may enhance plantation establishment for riparian protection from agricultural runoff and bioremediation (cleansing of the environment with plants or microorganisms) of excess N in the environment.

Past studies of root response to N have had varied results (Eissenstat and Yanai, 1997). In mixed hardwood forests, root lengths and longevity increased with N availability (Pregitzer et al., 1993; Fahey and Hughes, 1994). However, high nutrient soils can also induce shorter fine-root life spans (Aber et al., 1985; Nadelhoffer et al., 1985). The apparent contradiction of results may be due to variation and limitations of methodology used to study roots (Eissenstat and Yanai, 1997). Minirhizotrons record the finest of roots and may not represent bulk soil if installed improperly. Destructive sampling gathers biomass data but may underestimate root production and mortality since both root processes can occur simultaneously. Therefore, a combination of minirhizotron and sequential soil coring provides a more comprehensive method for studying fine-root life span (Hendrick and Pregitzer, 1993a). Minirhizotrons and coring are favorable because production rates and life span information are derived from minirhizotron length data. Coring provides biomass estimates that are important for assessing C and nutrient budgets.

Fine-roots are an integral part of tree production and ecosystem cycling with N the most limiting nutrient to tree growth. The objectives of this study were to determine the rate of fine-root turnover of cottonwood (*Populus deltoides*) in response to N regimes. The study used one clone on a uniform site to control genetic and soil variation factors. Fine-root dynamics were monitored with both soil cores and minirhizotrons for a more extensive understanding of N effects on fine-root dynamics.

LITERATURE CITED

Aber J D, Melillo J M, Nadelhoffer K J, McClaugherty C A and Pastor J 1985 Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317-321.

Agren G I 1985 Limits to plant production. J. Theor. Biol. 113, 89-92.

Baker J B and Blackmon B G 1977 Biomass and nutrient accumulation in a cottonwood plantation - the first growing season. Soil Sci. Soc. Amer. J. 41, 632-636.

Barbour M G, Burk J H and Pitts W D 1987 Terrestrial plant ecology. The Benjamin/Cummings Publishing Company, Menlo Park, CA, 634 p.

Bloom A J 1997 Nitrogen as a limiting factor: Crop acquisition of ammonium and nitrate. *In* Ecology in Agriculture. Ed. L E Jackson. pp. 145-172. Academic Press, New York.

Bloomfield J and Vogt K 1996 Tree root turnover and senescence. *In* Plant Roots: The Hidden Half 2nd Edition, Revised, and Expanded. Eds. Y Waisel, A Eshel and U Kafkafi. pp. 363-381.

Brundrett M, Murase G and Kendrick B 1990 Comparative anatomy of roots and mycorrhizae of common Ontario trees. Can. J. Bot. 68, 551-578.

Burke M K and Raynal D J 1994 Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. Plant Soil 162, 135-146.

Caldwell M M 1987 Competition between root systems in natural communities. *In* Root Development and Function. Eds. P J Gregory, J V Lake and D A Rose. pp. 167-185. Cambridge University Press, Cambridge.

Carlyle J C 1986 Nitrogen cycling in forested ecosystems. For. Abs. 47, 307-336.

Childers N F and White D G 1942 Influence of submersion of roots on transpiration, apparent photosynthesis, and respiration of young apple trees. Plant Physiol. 17, 603-618.

Cox T L, Harris W F, Ausmus B S and Edwards N T 1978 The role of roots in biogeochemical cycles in an eastern deciduous forest. Pedobiologia 18, 264-271.

David C A 1997 Managing the invisible: Ecosystem management and macronutrient cycling. *In* Ecosystem Management: Applications for Sustainable Forest and Wildlife Resources. Eds. M S Boyce and A Haney. pp. 94-129. Yale University Press, New Haven.

Dickmann D I and Pregitzer K S 1992 The structure and dynamics of woody plant root systems. *In* Ecophysiology of Short Rotation Forest Crops. Eds. C P Mitchell, J B Ford-Robertson, T Hinckley and L Sennerby-Forsse. pp. 95-123. Elsevier Applied Science, New York.

Edward N T and Harris W F 1977 Carbon cycling in mixed deciduous forest floors. Ecology 58, 431-437.

Eissenstat D M and Yanai R D 1997 The ecology of root lifespan. *In* Advances in Ecological Research Vol. 27. Eds. M Begon and A H Fitter. pp. 1-60. Academic Press Limited, San Diego.

Fahey T J and Hughes J W 1994 Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, New Hampshire. J. Ecol. 82, 533-548.

Fogel R 1985 Roots as primary producers in below-ground ecosystems. *In* Ecological Interactions. Special Publication Series of the British Ecological Society No. 4. Eds. A H Fitter, E Atkinson, D J Read and M Usher. pp. 23-36. Blackwell Scientific Publications, Oxford.

Grime J P, Campbell B D, Mackey J M L and Crick J D 1991 Root plasticity, nitrogen capture and competitive ability. *In* Plant Root Growth: An Ecological Perspective. Special Publication series of the British Ecological Society No. 10. Ed. D Atkinson. pp. 381-397. Blackwell, Oxford.

Head G C 1973 Shedding of roots. *In* Shedding of Plant Parts. Ed. T T Kozlowski. pp. 237-292. Academic Press, New York.

Henderson G S and Harris W F 1975 An ecosystem approach to the characterization of the nitrogen cycle in a deciduous forest watershed. *In* Forest Soils and Land Management. Eds. B Bernier and C H Wingate. pp. 179-193.

Hendrick R L and Pregitzer K S 1992a The demography of fine roots in a northern hardwood forest. Ecology 73, 1094-1104.

Hendrick R L and Pregitzer K S 1992b Spatial variation in tree root distribution and growth associated with minirhizotrons. Plant Soil 143, 283-288.

Hendrick R L and Pregitzer K S 1993a The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. Can. J. For. Res. 23, 2507-2520.

Hendrick R L and Pregitzer K S 1993b Patterns of fine root mortality in two sugar maple forests. Nature 361, 59-61.

Hendrick R L and Pregitzer K S 1996 Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. Ecology 84, 167-176.

Hooker J E, Black K E, Perry R L and Atkinson D 1995 Arbuscular mycorrhizal fungi induced alteration to root longevity of poplar. Plant Soil 172, 327-329.

Hooker J E, Munro M and Atkinson D 1992 Vesicular-arbuscular mycorrhizal fungi induced alteration in poplar root system morphology. Plant Soil 145, 207-214.

Joslin J D and Henderson G S 1987 Organic matter and nutrients associated with fine root turnover in a white oak stand. For. Sci. 33, 330-346.

Jurik T W, Briggs G M and Gates E M 1991 Soil respiration of five aspen stands in northern lower Michigan. Am. Midl. Nat. 126, 68-75.

Kozlowski T T 1992 Carbohydrate sources and sinks in woody plants. Bot. Rev. 58, 107-222.

Kramer P J 1983 Water Relations of Plants. Academic Press, New York, 489 p.

Kurz W A and Kimmins J P 1995 Water Relations of Plants and Soil. Academic Press, New York, 495 p.

Lyr H and Hoffman G 1967 Growth rates and growth periodicity of tree roots. Int. Rev. For. 2, 181-206.

Mackie-Dawson L A and Atkinson D 1991 Methodology for the study of roots in field experiments and the interpretation of results. *In* Plant Root Growth: An Ecological Perspective. Ed. D Atkinson. pp. 25-47. Blackwell Scientific Publications, Oxford.

Mackie-Dawson L A, Millard P and Proe M F 1995 The effect of nitrogen supply on root growth and development in sycamore and sitka spruce trees. Forestry 68, 107-117.

Maggs D H 1961 Changes in the amount and distribution of increment induced by contrasting watering, nitrogen, and environmental regimes. Ann. Bot. 25, 353-361.

Mohr F and Schopfer P 1995 Plant Physiology. Springer-Verlag, Berlin, 629 p.

Nadelhoffer K J, Aber J D and Melillo J M 1985 Fine roots, net primary production and nitrogen availability: a new hypothesis. Ecology 66, 1377-1390.

Nguyen P V, Dickmann D I, Pregitzer K S and Hendrick R L 1991 Late-season changes in allocation of starch and sugar to shoots, coarse roots and fine roots in two hybrid poplar clones. Tree Physiol. 7, 95-105.

Pereira J S and Landsberg J J 1989. Biomass production by fast growing trees. Eds. J S Pereira and J J Landsberg,. Proceedings of the NATO Advanced Research Workshop. Kluwer Academic Publishers, Obidos, Portugal.

Pregitzer K S and Friend A L 1996 The structure and function of *Populus* root systems. *In* Biology of *Populus* and Its Implication for Management and Conservation. Eds. R F Stettler, H D Bradshaw, P E Heilman and T M Hinckley. pp. 87-112. NRC Research Press, Ottawa.

Pregitzer K S, Hendrick R L and Fogel R 1993 The demography of fine roots respiration to patches of water and nitrogen. New Phytol. 125, 575-580.

Pregitzer K S, Zak D R, Curtis P S, Kubiske M E, Teeri J A and Vogel C S 1995 Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytol. 129, 579-585.

Pregizter K S, Dickmann D I, Hendrick R and Nguyen P V 1990 Whole-tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiol. 7, 79-93.

Rhodenbaugh E J and Pallardy S G 1993 Water stress, photosynthesis and early growth patterns of cuttings of three *Populus* clones. Tree Physiol. 13, 213-226.

Ruess R W, Van Cleve K, Yarie J and Viereck L A 1995 Contributions of fine root production and turnover to the carbon and nitrogen cycling in taiga forests of the Alaskan interior. Can. J. For. Res. 26, 1326-1336.

Ryan M G 1991 Effects of climate change on plant respiration. Ecolog. Appl. 82, 533-548.

Santantonio D and Grace J C 1987 Estimating fine root production and turnover from biomass and decomposition data: a compartment-flow model. Can. J. For. Res. 17, 900-908.

Shepperd W D and Smith F W 1993 The role of near-surface lateral roots in the life cycle of aspen in the central Rocky Mountains. For. Ecol. Manage. 61, 157-170.

Smit B and Stachowiak M 1988 Effects of hypoxia and elevated carbon dioxide concentration on water flux through *Populus* roots. Tree Physiol. 4, 153-165.

Smith R L 1992 Elements of Ecology. Harpercollins Publishers Inc., New York, 617 p.

SSSA 1987 Glossary of Soil Science Terms. Soil Science Society of America, Madison, WI, 44 p.

Sutton R F and Tinnus R W 1983 Root and root system terminology. Forest Monograph 24, 1-135.

Taiz L and Zeiger E 1998 Plant physiology. Sinauer Associates, Sunderland, MA, 792 p.

Vogt K A and Bloomfield J 1991 Tree root turnover and senescence. *In* Plant Roots: The Hidden Half. Eds. Y Waisel, A Eshel and U Kafkfi. pp. 287-306. Marcel Dekker, New York.

Vogt K A and Persson H 1991 Measuring growth and development of roots. *In* Techniques and Approaches in Forest Tree Ecophysiology. Eds. J P Lassoie and T H Hinckley. pp. 478-501. CRC Press, Boca Raton.

Vogt K A, Vogt D J, Moore E E, Fatuga B A, Redlin M R and Edmonds R L 1987 Conifer and angiosperm fine-root biomass in relation to stand age and site productivity in Douglas-fir forests. J. Ecol. 75, 857-870.

Walters M B and Reich P B 1989 Response of *Ulnus americana* seedling to varying nitrogen and water status. I. Photosynthesis and growth. Tree Physiol. 5, 159-172.

Wells C G and Jorgensen J R 1975 Nutrient cycling in loblolly pine plantations. *In* Forest Soils and Land Management. Proceedings of the Fourth American Forest Soils Conference, Quebec 1973. Eds. B Bernier and C H Wingate. pp. 137-158.

Woolfolk W T M, Friend A L, Mobley J A and Calhourn E A 1998 Growth response of cottonwood to varied NH4:NO3 ratio. *In* Soil Science Society of America, 1998 Annual Meeting Abstracts. pp. 304. ASA-CSSA-SSSA, Madison, WI.

Yanai R D, Fahey T J and Miller S L 1995 Efficiency of nutrient acquistion by fine roots and myccorrhizae. *In* Resource Physiology of Conifers: Acquistion, Allocation, and Utilization. Eds. W K Smith and T M Hinckley. pp. 75-103. Academic Press, San Diego.

CHAPTER 2

THE STUDY

ABSTRACT

Nitrogen (N) effects on fine-root dynamics were investigated in a cottonwood (*Populus deltoides*) short rotation plantation in Rhinelander, Wisconsin to estimate fine-root turnover at various N levels. Time-release fertilizer (17-6-12 plus micronutrients) was applied to plots at rates of 0, 50, 100, and 200 kg N ha^{-1} and replicated four times. Fine-root production, mortality, live-root standing crop, and life span were analyzed with monthly minirhizotron observations. Soil coring was used to measure fine-root biomass on three occasions during the two growing seasons. Results showed that fine-root dynamics were controlled by change in time and N had little to no influence over fine-root growth and death at this site. Live-root standing crop displayed a sigmoidal growth patterns in both years. Both years also had low mortality and roots with long life spans. Life span was influenced by the year of root initiation. The first year roots had life spans nearly twice as long as second year roots. Shorter life spans were also found in roots with diameters less than 0.5 mm. This study showed that cottonwood planted at close spacing on a sandy site has similar root dynamics on a range of N fertilizer levels. Root dynamics at this site are more controlled by stand age and change in season.

INTRODUCTION

Fine-root production has been shown to account for more than half of some forests annual net primary production (Keyes and Grier, 1981; Fogel, 1985; Gower et al., 1992). Determining belowground production helps to quantify ecosystem carbon (C) and nitrogen (N) budgets and improves forest management. Typically, fine-root production has been higher on sites with low available N compared with higher available N sites (Keyes and Grier, 1981; Alexander and Fairley, 1983; Axelsson and Axelsson, 1986; Gower et al., 1992). However, increased soil N availability increased the allocation of production to belowground biomass at several temperate forest sites (Nadelhoffer et al., 1985; Fahey and Hughes, 1994). Nitrogen effects on root dynamics are ambiguous and making comparisons among studies is difficult.

Understanding N effects on root growth may be confounded by stand age, species, N regime, and the research methods used (Price and Hendrick, 1998). Contrasting root results have been collected on 76 year old hardwoods (Fahey and Hughes, 1994) to 1.5 year old *Pinus ponderosa* (Tingey et al., 1997). Mature stand root production that increased in high N availability were measured using soil coring and destructive methods (Keyes and Grier, 1981; Alexander and Fairley, 1983; Axelsson and Axelsson, 1986; Gower et al., 1992) while increased root proliferation and decreased biomass was found with indirect nutrient budget techniques (Nadelhoffer et al., 1985). There are currently no reliable standard techniques available to evaluate various root study methods for accuracy (Hendricks et al., 1993). Destructive techniques allow measurement at one whereas, non-destructive techniques allow repeated measurements over time. Using a combination of techniques may lead to stronger conclusions about root dynamics and test the reliability of the methods. Both non-destructive and destructive techniques were used in this study to test the reliability of minirhizotrons and soil coring methods.

Our primary objective was to test cottonwood root dynamics in response to N regimes in a poplar plantation. Poplar plantation N management requires knowledge of root system dynamics to meet biomass production objectives efficiently and to protect the environment from excess N. This study applied a range of N levels to closely spaced and intensively managed cottonwood (*Populus deltoides* Bartr.) growing on a homogenous site. We hypothesized that increased N availability will increase cottonwood fine-root production and mortality at this site. More available N would allow for more growth, production. High N availability would also allow the plant to selectively senesce roots in zones of N depletion and initiate in areas of higher N availability. Therefore, higher mortality and turnover would occur in high N regimes. Consequently, we hypothesized low N availability would result in low production and mortality. The objectives were to determine fine-root production, mortality, live-root standing crop, and life spans with minirhizotrons and to estimate live fine-root biomass with soil cores and then to draw relationships and implications of fine-root response to N regimes.

METHODS

Study Site

The study site was a 0.5 ha plantation located in the Hugo Sauer Nursery at the North Central Forest Experiment Station in Rhinelander, Wisconsin (Oneida County, W89° 25', N45° 38'), USA. The nursery was established in the 1930's and was previously forested. The experimental site was fallow for 25 years prior to initiation of this experiment. The soil in the study area was a Croswell loamy-sand (Entic Haplorthod) (1993, Oneida County Soil Survey, USDA Soil Conservation Service). At 0-30 cm soil depth, pH was 5.4 and there was 81.8% sand, 3.9% clay, 2.4% C, and 0.14% N. The 30-60 cm depth had a pH of 5.6 and 88.5% sand, 3.1% clay, 0.5% C, and 0.03% N.

The climate in northern Wisconsin is continental with cold winters and warm summers. In Rhinelander, Wisconsin, temperatures average 20° C in July and -20° C in January. The growing season or freeze-free period usually is 80-100 days, and annual precipitation averages about 70 cm per year (1993, Oneida County Soil Survey, USDA Soil Conservation Service).

Plant Material

During the 1997 growing season, green stem cuttings of *Populus deltoides* Bartr. clone D-105 were propagated in a greenhouse and moved to a shade house where they set bud and over wintered. The containerized seedlings were hand-planted in the study site on May 1-2, 1998.

Study Design

A randomized complete block design was used which consisted of four blocks with four fertilizer levels. Slow release fertilizer (Sierra[1], 17-6-12, N-P-K, plus micronutrients (Mg, S, B, Cu, Fe, Mn, Mo, and Zn), three to four-month release Scotts, OH, US), was applied at rates of 0, 50, 100, or 200 kg N ha^{-1} in each block on May 5-7, 1998 and April 13, 1999. The fertilizer was applied with a calibrated spreader and then hand-raked into the soil. Each plot contained 196 trees (14 x 14) at 1 x 1 m square spacing. The measurement plot contained 64 trees (8 x 8) and was surrounded by three border rows. Plots were divided by 3-m alleyways for irrigation lines and tractor equipment. The entire site was enclosed by a 1.6 m tall 3-D electric deer fence.

In the summer of 1997, the site was prepared by spraying with glyphosate (N-(phosphonomethyl)glycine), tilling, and summer fallowing. In spring 1998, linuron ((3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea)) was applied at 1.12 kg ha^{-1} at planting to eliminate competing vegetation. Irrigation was used to supplement precipitation so that the plantation received at least 2.54 cm water each week throughout the study.

Strict weed control was implemented throughout the experiment's duration to avoid measurement of non-target species' roots. The site was tilled mechanically between the plots and glyphosate was applied directly to weeds within plots. To

[1] Mention of trade names does not constitute endorsement by the U.S. Department of Agriculture, University of Minnesota-Duluth's Natural Resources Research Institute, University of Mississippi, and the University of Wisconsin-Stevens Point.

eliminate any plot interaction, the lateral roots were severed by drawing a flat disk (45 cm radius) between plots in July of 1998 and 1999 and in spring 1999.

Air temperature (1 m) and soil temperature (15, 30 and 45 cm below the surface) were recorded every minute and averaged hourly with a CR-10 datalogger (Campbell Scientific, Inc., Logan, UT, USA). Rainfall and irrigation were recorded manually after each event. Soil moisture was monitored with soil tensiometers and kept at a minimum of -0.5 atm.

Minirhizotron Techniques and Analysis

Root dynamics were monitored with minirhizotrons (extruded, acrylic tubes of 5 cm I.D., 5.7 cm O.D. and 90 cm length to a vertical depth of 36 cm below soil surface). Eight tubes per plot were installed in each plot. Roots (< 2 mm diameter) growing along the upper surface of the tube were imaged with a microvideo camera (Bartz Technology, Santa Barbara, CA, USA) at high resolution (26 μm $pixel^{-1}$). The camera captured a 2.63 cm^2 image and 40 images were recorded per tube. Therefore, each tube had a total observed surface area of 105 cm^2. In 1998, images were recorded on June 1-2, July 6-7, August 3-4 and 30-31, September 25 and 27, October 24-25, and December 4-6 in 1998, and, in 1999, images were recorded on April 9-10, May 8-9, June 4-5, July 2-3 and 30-31, August 27-28, October 1 and 24-25, and November 20-21. Rootracker (Duke University, Durham, NC, USA) image analysis software was used to quantify the images. New roots were identified by image location on the tubes. Measurements included length, width and condition. Three root condition categories were used: 1) new, 2) previously observed and 3) missing. Due to the subjectivity in determining the condition of previously observed roots, only missing roots were considered to be dead. Also, only one technician analyzed the data and kept notes and calibration images to keep consistency in determining the condition of roots throughout the study.

Minirhizotron image data provided quantitative information on root production, mortality, live-root standing crop and life span. Root production was the cumulative length of newly observed roots. Root mortality was the cumulative length of roots that were missing. Standing live-root length was the difference between root production and root mortality.

Individual root life spans from the minirhizotrons are used in survival analyses to calculate survival functions and to test for treatment differences (Kalbfleisch and Prentice, 1980; Lee, 1992). Root survival time or life span was defined as the number of days between date of appearance and disappearance. The survival function is the probability of a root surviving to an observation date. It is appropriate to use survival analysis techniques with root data because data is right-censored roots; therefore, roots living past the final observation can be included in the analysis. Product-limit analysis was used for estimates of survival curves with 95% confidence intervals (SAS Institute Inc, 1989).

Destructive Techniques and Analysis

Root biomass was determined with soil cores. Eight random soil cores (5 cm diameter, 30 cm depth) from each plot were taken in October 1998, April 1999 and

October 1999. A Gillson root washer (Gillson's Variety Fabrication, Inc., Banzonia, MI, USA) was used to remove roots from cores (Smucker et al., 1982; Pallant et al., 1993) which were then stored in 20% methanol. Roots were separated into two size classes (<1 mm and greater or equal to 1 mm) for mass analysis.

Leaf N Analysis

Leaf N content was determined in leaf samples collected from the four center trees in each plot (July 1999). Samples from the upper and lower canopy from every plot were composited separately and analyzed for total N with a Carlo Erba mass analyzer. The average N concentrations are reported for the upper two thirds and lower third of the canopy.

Analysis

Parameters measured at several points in the plot, i.e. production, mortality, live-root length, and biomass were analyzed with a two-way repeated measures analysis, while those measured once per plot, i.e. N content, were analyzed using a one-way ANOVA (SAS Institute Inc, 1989). Least mean squares were used to separate leaf N means. Statistical results for production, mortality and standing crops were tested by the Wilks' Lambda, Pillai's Trace, Hotelling-Lawlley Trace and Roy's Greatest Root tests. Results are reported from the Wilks' Lambda test.

The log rank and Wilcoxon tests were used to determine differences between survival curves. Survival curves were stratified and tested for fertilizer treatment, year of appearance, and diameter using SAS LIFETEST procedure (SAS Institute Inc, 1989).

To determine the fine-root turnover rate, specific growth and death rates were calculated using the methods of Cheng et al. (1991). Specific growth rate ($mm\ mm^{-1}\ day^{-1}$) is fine-root production for a given observation period divided by days between observations and normalized by the average live-root length for that period. Specific death rate ($mm\ mm^{-1}\ day^{-1}$) is calculated similarly but mortality is used for the period rather than production. Fine-root turnover rate was the average of specific growth and specific death rates (Cheng et al., 1991, Appendix 1). Specific growth and death rates from the minirhizotrons were multiplied by soil core fine-root biomass to determine daily growth and death (Hendrick and Pregitzer, 1993) and belowground net primary production (Appendix 1).

RESULTS

Minirhizotron Fine-root Dynamics

Dynamics were influenced by seasonal trends and to a lesser extent fertilizer N. During both years, fine-root production, mortality and live-root standing crop were affected by time ($p < 0.0001$, Appendix 2). Greatest production rate occurred between July and September in 1998 and 1999 and reached a plateau after September both years (Figure 1A). There was little change in the curve (growth or death) from December to April (Figure 1A). Root production during the first growing season was half that of the second season. Mortality was also lower in the first year; it represented only about 9% of the total production in 1998 and 14% in 1999 (Figure 1A). During both years, peak mortality rate occurred in November (Figure 1A).

In November of both years, mortality rate was increasing and production rate was low. As a result, standing crop began to decrease in November 1998 and 1999 (Figure 1B). Live-root standing crop patterns were similar to production patterns because mortality was relatively low until late in the season. Both seasons did display sigmoidal growth patterns in the live-root standing crop (Figure 1B). The greatest mean 1998 live-root standing crop was in October at 4.7 ± 1.16 mm cm^{-2}. In 1999, the standing crop increased 1.6 fold from 1998 during the 1999 growing season to a maximum fine-root length density of 12.94 mm cm^{-2} in late August.

Fertilizer N treatments did not influence root growth and death as much as temporal effects. In the final 1998 observation, fine-root production was highest with the

200 N fertilizer rate and lowest with 50 N (Figure 1A). There were treatment differences in fine-root production means among the N treatments ($p = 0.07$). The highest morality rate tended to occur with the 200 N treatment in the final 1998 measurement (Figure 1A). As a result, 200 N had 54% more live-root length than 50 N treatment (Figure 1B). Differences were detected in means of live-root length means among the N treatments ($p = 0.05$).

The response trends to fertilizer N were not repeated in 1999. Although the 200 N treatment still had the greatest and 50 N the least mean fine-root production, the 0 N had the second greatest mean production followed by 100 N (Figure 1A). There were no significant differences among the N treatments for cumulative production and mortality. Live-root standing crop treatment means were different ($p = 0.09$). In 1999, the final 200 N live-root length density was 23% greater than 50 N, 14% greater than 100 N and 8% greater than 0 N (Figure 1B).

Minirhizotron Survival Analysis

Fine-root survival was affected by year of root initiation (Figure 2), N treatment (Table 1) and root diameter size (Figure 2). Roots initiated in 1998 had longer life spans compared to 1999 roots ($p < 0.0001$, Figure 2A). There were 5054 fine-roots initiated in 1998 across all N treatments and 44% of the fine-roots were alive at the end of the study. In 1999, 12,508 roots were initiated, with 55% still alive at the end of the study. Not all the roots died during the study, therefore, the survival curves were extrapolated past 0.5 probability of survival in order to determine the median life spans. The approximate median life span was 502 days in 1998 but only 260 days in 1999 (Table 1). During the first 113 days after initiation, roots from both years had similar survival patterns. During subsequent days, 1999 roots had shorter life spans (Figure 2A).

Fertilizer N influenced fine-root life span ($p < 0.0001$). At the 70% survival rate, the 200 N and 50 N treatments tended to have longer life spans (334 days) than the life span of 0 N and 100 N (278 days). By the end of the study (531 days) 42% were still living in 0 N, 50% in 50 N, 44% in 100 N, and 42% in 200 N treatments. Shortest median life span was 475 days in 200 N and longest in 50 N at 531 days (Table 1).

Fine-root life span was greater for roots with an initial diameter of 0.5-2 mm than roots less than 0.5 mm ($p < 0.0001$, Figure 2B). The median life span of small diameter roots was 447 days and 38% survived to the end of the study. In contrast, 74% percent of the large diameter roots were still alive at the end of the study and from an extrapolated curve had a median life span estimated at 1031 days.

Specific Growth, Death, and Turnover Rates

Specific daily growth rates were high during the mid to late summer but low in winter. Peak specific growth rates (SGR) were 1.9% day^{-1} in August 1998 and 1.8 % day^{-1} in July 1999 (Table 2). Minimal SGR were 0.05% day^{-1} in December 1998 and 0.04 % day^{-1} in November 1999 (Table 2). Specific death rates (SDR) reached 0.18% day^{-1} in August 1998 and 0.27% day^{-1} in May 1999 (Table 2). The SDR were lowest in December 1998 (0.07% day^{-1}) and April 1999 (0.02% day^{-1}, Table 2). As a result, percent turnover were greatest in August 1998 (1.03 % day^{-1}) and July 1999 (0.95%

day^{-1}) and the least (0.06 % day^{-1}) was observed in December 1998 and April 1999 (Table 2). The average replacement time in days was calculated by inverting turnover rate. Calculated turnover was shorter in 1998 (168 ± 43 days) than in 1999 (311 ± 95 days). Fertilizer N did not alter SGR, SDR or turnover.

Fine-root Biomass Cores

Despite consistent increases in live-root length observed in minirhizotron between 1998 and 1999 (Figure 1), similar seasonal patterns were not found in biomass (Figure 3). The root biomass treatment response differed among seasonal samples. In October 1998, the greatest root biomass was in 200 N (38.9 ± 0.61 g m^{-2}) and the least biomass was in 0 N (31.5 ± 3.67 g m^{-2}, $p = 0.025$, linear contrast). There were no differences among N treatments on the 1999 sampling dates.

Predicted biomass from the minirhizotron specific growth and death rates increased from the October 1998 to October 1999 coring dates (Figure 4). At each coring date, high N treatments were predicted to have the greatest biomass of all the treatments (Figure 4) and actual soil coring results did have greater mean biomass in high N treatments in October 1998 and April 1999 (Figure 3). Predicting biomass from minirhizotron measurements underestimated mass by 55% in October 1998 and overestimated 62% in October 1999 (Table 3). Only at the April 1999 sample did it estimate with >90% accuracy (Table 3).

Leaf N

Nitrogen had a positive effect on leaf N concentration. Control plots had 30% less leaf N concentration in the top two-thirds of the canopy and 13% less leaf N concentration in the lower third of the canopy than 50 N plots (Table 4, $p < 0.0001$). The 100 N treatment had the greatest mean leaf N concentration but was not different from 200 N treatment in the lower canopy (Table 4). Of the fertilized plots (50, 100, and 200 N), leaf N content was similar in the upper canopy (Table 4).

DISCUSSION

Fine-root Growth, Death, and Standing Live-root Length

Temporal effects had the greatest control of fine-root dynamics in the minirhizotron observations. The seasonal growth patterns recorded in this study (Figure 1) and others (Majdi and Nylund, 1996; Majdi and Kangas, 1997; Price and Hendrick, 1998) are a typical sigmoid growth pattern that increase in spring and summer, plateau late summer and early fall, and then decrease fall and winter. Norway spruce (*Picea abies* L.) root production had a sigmoid growth pattern that increased from April to October, leveled off during winter then resumed production in March (Majdi and Nylund, 1996; Majdi and Kangas, 1997). The live-root standing crop of sweetgum (*Liquidambar styraciflua* L.) increased from March through September in the Middle Coastal Plain of Georgia (Price and Hendrick, 1998). In Wisconsin, pine (*Pinus resinosa* Ait.) and poplar (*Populus tristis* x *P. balsamifera* cv) had oscillatory patterns for live-root standing crop that increased and decreased around a consistent mean of live-root standing crop (Coleman et al., In Press).

Nitrogen level influenced root dynamics significantly in the first year (Figures 1 and 3). Statistical tests detected a significant difference between N levels in the production and live-root stand crop over time. This interaction is unclear and we cannot conclude any strong biological implications. Second year results also show little fine-root response to N (Figures 1 and 3). In general, the fine-roots in this study had little or no response to the applied fertilizer N in the minirhizotron observations or biomass cores.

Therefore, we do not accept our hypothesis that N will increase production and mortality for intensively managed cottonwood at this site. Past studies that reported no nutrient effect on root productivity concluded that the demands for C and N were similar among the treatments (Tingey et al., 1997; Price and Hendrick, 1998). Nitrogen did not change *Pinus ponderosa* fine-root length at yearly ages of two, three, and four in Placerviller, California (Tingey et al., 1997) nor sweetgum two years after coppicing (canopy closed during second year) in Georgia (Price and Hendrick, 1998), even though coppiced stands have an extensive root system. Price and Hendrick (1998) found that the soil N availability was similar throughout the site with fertilizer applications of 560 kg N ha^{-1} y^{-1} and 1120 kg ha^{-1} y^{-1}. In our study site, available N was not recorded but the trees were at adequate nutritional status for N (Table 4). Perhaps, non-limiting N on our site caused minimal root response, especially during the second year. The second year response was weaker than the first year response (Figures 1 and 3). There may have been greater inter-root competition in the second year since the site was more fully occupied and the canopy closed. The weak response to N in the second year could also represent a transitional stage for the stand between establishment and maturity. Establishing stands accumulate root length (Vogt et al., 1987; Tingey et al., 1997) and N would be in demand for the accumulating growth as seen in our coring results (Figure 3). Conversely, mature stands completely turnover the equivalent of their fine-root system (Fahey and Hughes, 1994; Coleman et al., 1996; Rytter and Rytter, 1998) and may be able to meet needs by remobilization. For poplars, N retranslocated the previous autumn can represent more than 50% of the N requirement (Bernier, 1984). Past studies would support our observations that first year stands accumulate root systems, and, during the second year, the root systems were in transitional stage and met nutrient demands by internal cycling.

The stand may also have reached optimum plant N levels in the second year. The N treatments may have increased N availability in this site beyond the N requirement. The estimated leaf N uptake in Table 4 shows that fertilized plots (50, 100, and 200 N) were different from the controls. Optimal N level for stand growth may be between the control and fertilized plots but further study would be needed. Adequate leaf N concentrations for hardwoods range from 1.5-3.0 % (Larcher, 1995). Sufficient leaf N levels for poplars have been reported between 2.5-3.4% (Ericsson et al., 1992).

Fine-root Longevity

Survival of roots also responded more to annual environmental and internal cycles than to N treatment. The low mortality (Figure 1A) and long life spans (Figure 2) suggests that the tree had adequate resources to maintain the ephemeral fine-root system, that low herbivory existed, and that other sinks for carbohydrates were not stronger than fine-root sinks. Life spans for a closed canopy stand of *Populus tristis* x *P. balsamifera* cv. were 149 days (Coleman et al., In Press) and *Populus* x *canadensis* cv. Beaupre grown in pots in a greenhouse had life spans of 35 days (Black et al., 1998). Our life spans were 2.6 to 10 times longer than those. The long life spans we observed would tend to maximize the efficiency of resource acquisition per unit C expended (Eissenstat and Yanai, 1997). Fertilizer N increased fine-root life spans and decreased mortality in 35 year old sitka spruce (Alexander and Fairley, 1983). High life spans and low mortality found from soil coring methods were suspected to originate from increased fine-root N uptake rates; therefore, fine-roots were cost effective sinks for C and were not shed (Alexander and Fairley, 1983). A coppiced sessile oak (*Quercus petraea*) stand sampled with soil cores also had fine-root mortality decrease and life spans increase when lime and gypsum were applied to improve nutrient availability (Bakker, 1999). Both Alexander and Fairley (1983) and Bakker (1999) hypothesize that the improved nutrient status of the plant affected the change in mortality and life span of fine-roots. The results of our study suggest that cottonwood was able to meet nutrient demands and effectively maintain the root system.

Cost-benefit analysis on root production and mortality predicts that low mortality occurs in young roots and peak mortality occurs at optimal life span (Eissenstat and Yanai, 1997). In our study, fine-root survival was affected strongly by phenological changes in stand development. Roots initiated in the first year had longer life spans than in the second year (Figure 2A). The first year's longer life span could represent mineral nutrient conservation in a new environment (Eissenstat and Yanai, 1997). The site was not exploited and resources were available to maintain the root system. The seedlings conserved resources in their new environment with low fine-root turnover. In the second year, established tree resources were shifted away from fine-root maintenance since establishment year fine-roots were still functional. Shorter functional life spans of fine-roots may have developed from more inter-root competition. Increased competition may have induced depletion zones in which roots would be senesced. It is hypothesized that if the third year of this study's cottonwood stand was observed fine-root lifespans may have been as short as those found in mature *Populus tristis* x *P. balsamifera* (Coleman et al., In Press) and *Populus* x *canadensis* (Black et al., 1998).

The survivorship analysis shows that fine-roots with a diameter less than 0.5 mm have a shorter life span. Other studies have shown that roots less than 0.5 mm compared to 0.5 – 2 mm diameter roots have different physiological response (Sword et al., 1996; Pregitzer et al., 1998; Price and Hendrick, 1998; Rytter and Rytter, 1998; Coleman et al., In Press). Coleman et al. (in press) found poplar (*Populus tristis* x *P. balsamifera* cv.) initial fine-root diameter was positively related to life span. Our findings of shorter life spans for roots less than 0.5 mm in diameter may be linked with feeder roots' physiological function.

Fine-root Biomass

Our biomass results showed that the fine-roots increase with increasing N in the establishment year of the stand and no treatment response was found in the second year (Figure 3). Our belowground net primary production (BNPP, Figure 3) contrasts from other studies because other studies were based on mature stands while this study used young seedlings (Keyes and Grier, 1981; McClaugherty and Aber, 1982; Gower et al., 1992; Ruess et al., 1995). Fifty year old Douglas-fir had an annual BNPP of 1540 – 4200 kg ha^{-1} (Gower et al., 1992) and 40 year old Douglas-fir had 4100 – 8100 kg ha^{-1} y^{-1} BNPP (Keyes and Grier, 1981). These results are about 40 times greater than our biomass results which makes comparisons difficult. With the young age of the stand, low BNPP would be expected.

Comparison of Methods

In this study, two different methods, minirhizotrons and soil cores, were used to quantify fine-root response to fertilizer N. Both techniques show a production response to N in the first year (Figure 1A and 3) and that N did not affect the roots in the second year. Since both methods give generally agreeable results, we accept that these trends are reliable.

While both destructive and non-destructive techniques measure unique root parameters and important results, current models still do not allow accurate prediction of net primary production from minirhizotrons or soil coring. Belowground net primary production estimates were different especially during the October of both years (Table 3). Soil coring can underestimate biomass production due to C losses not measured (eg., exudates, sloughing, herbivory) and also to simultaneous production and mortality that can occur between sampling (Vogt et al., 1998). Simultaneous growth and death can be monitored in minirhizotrons but temperature and moisture gradients along minirhizotron tubes could skew rooting density and root growth patterns on the tube's surface (Vogt et al., 1998). Therefore, we conclude that BNPP for our site was between the estimates generated from the minirhizotrons and the soil cores (Figures 3 and 4).

Conclusion

In summary, high N fertilizer by time interaction influenced production and live-root standing crop during the establishment year of cottonwood seedlings but no biological implication could be drawn between the treatment levels. Two-year old cottonwood fine-root systems had little or no response to N probably due to adequate N uptake at all treatment levels, including the control, and similar tree C and N demands among treatments. Second year trees also may have depended largely on remobilization to meet N needs.

Fine-root dynamics are controlled more by temporal effects with production peaking late summer to early fall and mortality peaking in early winter. Phenological changes controlled life spans in that roots initiated in the establishment year had longer life spans when the site was not exploited. The reduction in fine-root life span during the second year may be the result of the stand being in a transition and from resource allocation shifted from fine-roots to shoots. Root function also influenced root life span. Small diameter roots (less than 0.05 mm) are more active and had shorter life spans than larger roots. Life span or survival analyses in this study gave further insights on the controlling factors of root dynamics and the important role of temporal, phenological and functional factors. Nitrogen was not the controlling parameter of fine-root growth. Time, stand age, root function and the environment have stronger influence over fine-roots in this study. Further use of survival analyses for root survival will help answer questions about nutrient budgets and cycling to meet tree nutritional needs.

LITERATURE CITED

Aber J D, Melillo J M, Nadelhoffer K J, McClaugherty C A and Pastor J 1985 Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. Oecologia 66, 317-321.

Alexander I J and Fairley R I 1983 Effects of N fertilization on populations of fine roots and mycorrhizas in spruce humus. Plant Soil 71, 49-53.

Axelsson E and Axelsson B 1986 Changes in carbon allocation patterns in spruce and pine trees following irrigation and fertilization. Tree Physiol. 2, 189-204.

Bakker M R 1999 The effect of lime and gypsum applications on a sessile oak (*Quercus petraea* (M.) Liebl.) stand at La Crois-Scaille (French Ardennes) II. Fine root dynamics. Plant Soil 206, 109-121.

Bernier B 1984 Nutrient cycling in *Populus*: A literature review with implications in intensively-managed plantations. IEA/ENFOR Rep. 6. Canadian Forest Service, Ottawa, 46 p.

Black K E, Harbron C G, Franklin M, Atkinson D and Hooker J E 1998 Differences in root longevity of some tree species. Tree Physiol. 18, 259-264.

Cheng W X, Coleman D C and Box. J E, Jr. 1991 Measuring root turnover using the minirhizotron technique. Agr. Ecosyst. Environ. 34, 261-267.

Coleman M D, Dickson R E and Isebrands J G In Press Contrasting fine-root production, turnover and soil CO_2 efflux in pine and poplar plantations. Plant Soil.

Coleman M D, Dickson R E, Isebrands J G and Karnosky D F 1996 Root growth and physiology of potted and field-grown trembling aspen exposed to tropospheric ozone. Tree Physiol. 16, 145-152.

Eissenstat D M and Yanai R D 1997 The ecology of root lifespan. *In* Advances in Ecological Research Vol. 27. Eds. M Begon and A H Fitter. pp. 1-60. Academic Press Limited, San Diego.

Ericsson T, Rytter L and Linder S 1992 Nutritional dynamics and requirements of short rotation forests. *In* Ecophysiology of Short Rotation Forest Crops. Eds. C P Mitchell, J B Ford-Robertson, T Hinkley and L Sennerby-Forsse. pp. 35-65. Elsevier Applied Science, London.

Fahey T J and Hughes J W 1994 Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, New Hampshire. J. Ecol. 82, 533-548.

Fogel R 1985 Roots as primary producers in below-ground ecosystems. *In* Ecological Interactions. Special Publication Series of the British Ecological Society No. 4. Eds. A H Fitter, E Atkinson, D J Read and M Usher. pp. 23-36. Blackwell Scientific Publications, Oxford.

Gower S T, Vogt K A and Grier C C 1992 Carbon dynamics of Rocky Mountain Douglas-fir: influence of water and nutrient availability. Ecol. Monogr. 62(1), 43-65.

Hendrick R L and Pregitzer K S 1992 The demography of fine roots in a northern hardwood forest. Ecology 73(3), 1094-1104.

Hendrick R L and Pregitzer K S 1993 The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. Can. J. For. Res. 23, 2507-2520.

Hendricks J J, Nadelhoffer K J and Aber J D 1993 Assessing the role of fine roots in carbon and nutrient cycling. Tree 8(5), 174-178.

Kalbfleisch J D and Prentice R L 1980 The statistical analysis of failure time data. John Wiley & Sons, Inc, New York

Keyes M R and Grier C C 1981 Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can J. For. Res. 11, 599-605.

Kubiske M E, Pregitzer K S, Zak D R and Mikan C J 1998 Growth and C allocation of *Populus tremuloides* genotype in response to atmospheric CO_2 and soil N availability. New Phytol. 140, 251-260.

Larcher W 1995 Physiological Plant Ecology. Spring-Verlag, Heidelberg, Germany, 506 p.

Lee E T 1992 Statistical Methods for Survival Data Analysis. John Wiley & Sons, Inc., New York, 482 p.

Majdi H and Kangas P 1997 Demography of fine roots in response to nutrient applications in a Norway spruce stand in southwestern Sweden. Ecoscience 4(2), 199-205.

Majdi H and Nylund J E 1996 Does liquid fertilization affect fine root dynamics and lifespan of mycorrhizal short roots? Plant Soil 185, 305-309.

McClaugherty C A and Aber J D 1982 The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. Ecology 63(3), 1481-1490.

Nadelhoffer K J, Aber J D and Melillo J M 1985 Fine roots, net primary production and nitrogen availability: a new hypothesis. Ecol. 66(4), 1377-1390.

Pallant E, Holmgren R A, Schuler G E, McCracken K L and Drbal B 1993 Using a fine root extraction device to quantify small diameter core roots (>.025 mm) in field soils. Plant Soil 153, 273-279.

Pregitzer K S, Laskowski M J, Burton A J, Lessard V C and Zak D R 1998 Variation in sugar maple respiration with root diameter and soil depth. Tree Physiol. 18, 665-670.

Pregitzer K S, Zak D R, Curtis P S, Kubiske M E, Teeri J A and Vogel C S 1995 Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytol. 129, 579-585.

Price J S and Hendrick R L 1998 Fine root length production, mortality and standing crop dynamics in an intensively managed sweetgum (*Liquidambar styraciflua* L.) coppice. Plant Soil 205, 193-201.

Raich J W and Nadelhoffer K J 1989 Belowground carbon allocation in forest ecosystems: Global trends. Ecology 70(5), 1346-1354.

Ruess R W, Van Cleve K, Yarie J and Viereck L A 1995 Contributions of fine root production and turnover to the carbon and nitrogen cycling in taiga forests of the Alaskan interior. Can. J. For. Res. 26, 1326-1336.

Rytter R-M and Rytter L 1998 Growth, decay, and turnover rates of fine roots of basket willows. Can. J. For. Res. 28, 893-902.

SAS Institute Inc 1989 SAS/STAT Users Guide, 6, 4th Ed. SAS Institute Inc, Cary, NC, USA. 846 p.

Smucker A J M, McBurney S L and Srivastava A K 1982 Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74, 500-503.

Sutton R F and Tinnus R W 1983 Root and root system terminology. Forest Monograph 24, 1-135.

Sword M A, Gravatt D A, Faulkner P L and Chambers J L 1996 Seasonal branching and fine root growth of juvenile loblolly pine five growing seasons after fertilization. Tree Physiol. 16, 899-904.

Teskey 1981 Influence of temperature and water potential on root growth of white oak. Physiol. Plant. 52, 363-369.

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

Vogt K A, Vogt D J and Bloomfield J 1998 Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200, 71-89.

Vogt K A, Vogt D J, Moore E E, Fatuga B A, Redlin M R and Edmonds R L 1987 Conifer and angiosperm fine-root biomass in relation to stand age and site productivity in Douglas-fir forests. J. Ecol. 75, 857-870.

Zogg G P, Zak D R, Burton A J and Pregitzer K S 1996 Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. Tree Physiol. 16, 719-725.

	Median Life Span (days)
Treatment	
0 kg N ha^{-1}	481
50 kg N ha^{-1}	531
100 kg N ha^{-1}	502
200 kg N ha^{-1}	475
Year of Initiation	
1998	502
1999	260

Table 1. Median life spans for each N treatment and each year. Median life span was determined by extrapolating the survival curves past 0.5 survival probability if more than half of the roots were still alive at the end of the study.

1998 Sampling Dates	6-Jul-98	3-Aug-98	30-Aug-98	27-Sep-98	24-Oct-98	4-Dec-98
SGR	1.55%	1.87%	1.87%	1.08%	0.15%	0.05%
SDR	0.00%	0.19%	0.17%	0.14%	0.10%	0.07%
Turnover Index	0.78%	1.03%	1.01%	0.60%	0.13%	0.06%

1999 Sampling Dates	9-Apr-99	8-May-99	4-Jun-99	2-Jul-99	30-Jul-99	27-Aug-99	1-Oct-99	24-Oct-99	20-Nov-99
SGR	0.10%	0.52%	0.72%	1.80%	0.87%	0.29%	0.09%	0.04%	0.04%
SDR	0.02%	0.27%	0.10%	0.10%	0.14%	0.16%	0.13%	0.11%	0.36%
Turnover Index	0.06%	0.37%	0.41%	0.95%	0.51%	0.21%	0.10%	0.08%	0.20%

Table 2. Average specific growth rate (SGR), specific death rate (SDR) and Turnover Index. Percentages represent mm mm^{-1} day and calculated according to Cheng et al. (1991) methods. SGR is the production normalized by the average live-root standing crop for that period. SDR is similar to SGR by mortality is normalized by live-root standing crop. Turnover Index is the average of SGR and SDR.

Biomass	Oct-98	Apr-99 (g m-2)	Oct-99
predicted	20.28	35.58	89.76
actual	31.48	37.58	34.59
% difference	55	6	62

Table 3. Fine-root biomass estimates for the control from "actual" field results and "predicted" biomass from specific growth and death rates at each of the coring dates. Predicted biomass was derived by subtracting the initial biomass x specific death rate from the initial biomass x specific growth rate.

	Leaf N Content (%)	
Treatment	Position 1	Position 2
0	2.22 (0.14)[a]	2.0 (0.11)[a]
50	3.28 (0.03)[b]	2.3 (0.03)[c]
100	3.34 (0.12)[b]	2.6 (0.09)[d]
200	3.21 (0.07)[b]	2.7 (0.09)[d]

Table 4. Average Leaf N concentration at each treatment level and canopy position in July 1999. Position 1 is the upper two thirds of the canopy and position 2 is the lower third of the canopy. Numbers in parenthesis are standard errors of the mean. Statistically different (<0.05) treatments are marked with different letters.

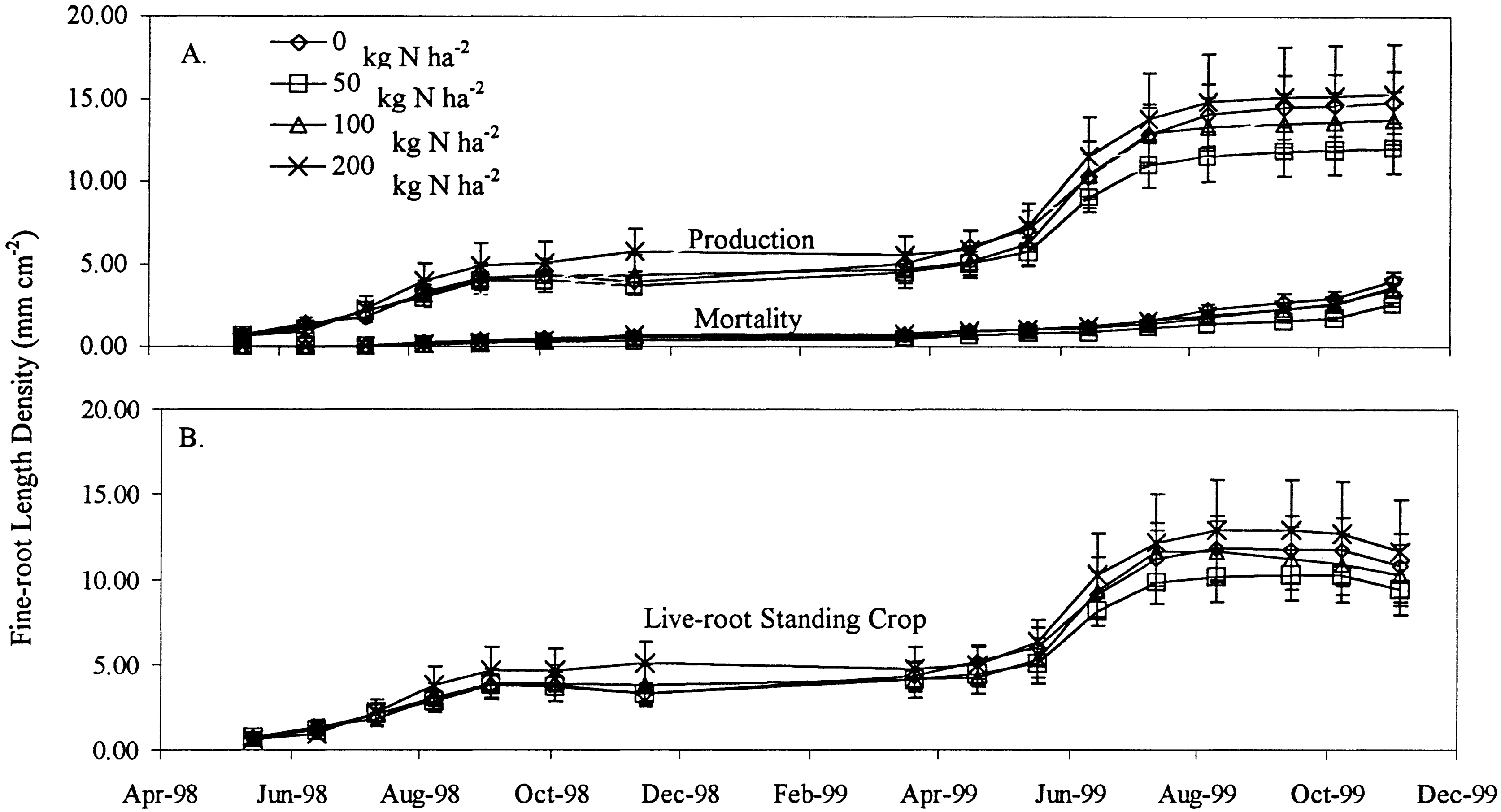

Figure 1. Cottonwood seedlings' fine-root (0 - 2 mm diameter) cumulative production, cumulative mortality (A) and resulting live-root standing crop (B) by N treatment from 1998 to 1999. Each data point represents the mean fine-root length density (mm cm^{-2}) of four replicates that were observed 16 times in minirhizotrons beginning on June 7, 1998 and ending November 20, 1999. Vertical bars indicate the standard error of the mean for each N treatment at each observation date.

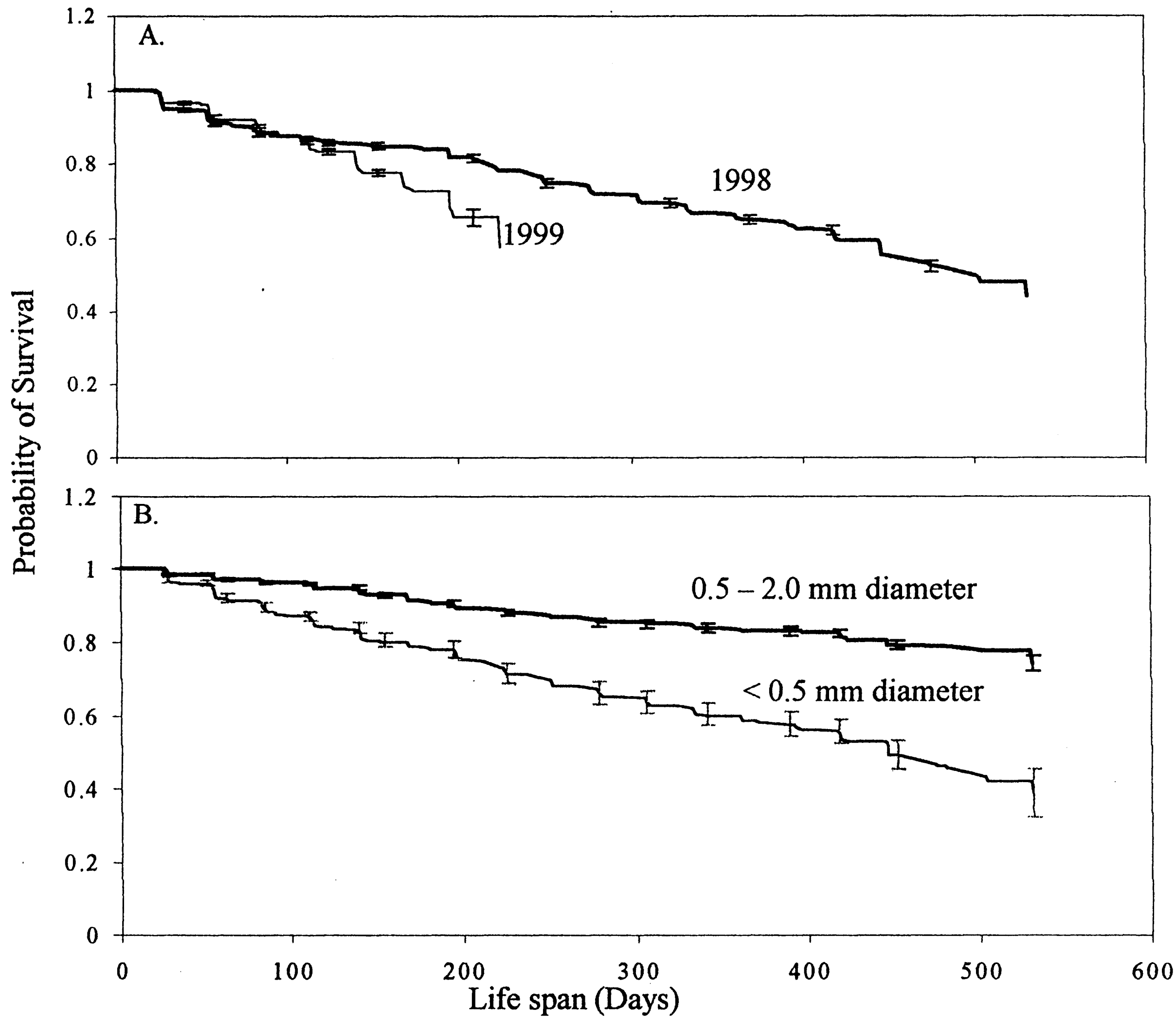

Figure 2. Fine-root survival curve estimates as observed through minirhizotrons. Day 0 represents the first observation during the study that a root appeared. As time passes the percentage of roots surviving decreases as determined with SAS product-limit analysis. Vertical bars indicate a 95% confidence interval. A. Fine-root survival for roots initiated in 1998 and in 1999. B. Fine-root survival by diameter class (<0.5 mm and 0.5 - 2 mm).

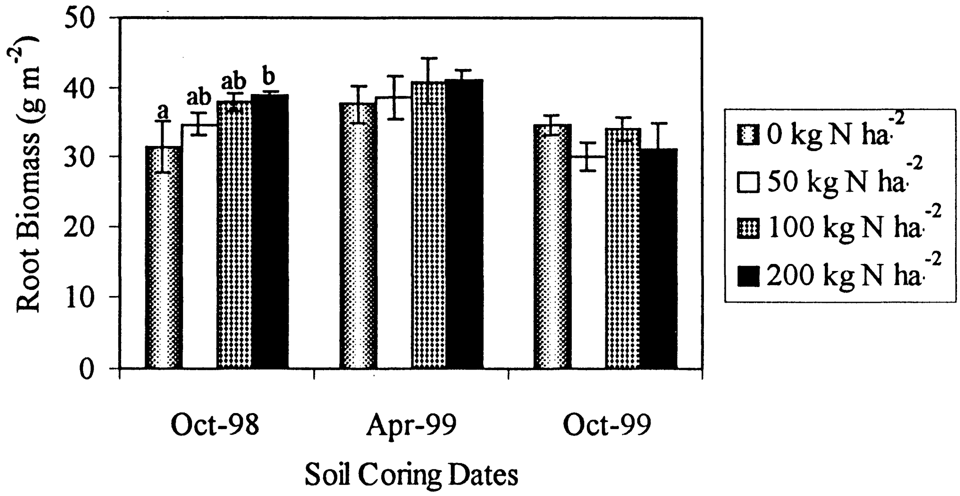

Figure 3. Average fine-root biomass from soil cores collected in October 1998, April 1999 and October 1999 in each of the N treatments. Each date had four replications per N treatment. N treatments with different letters in October 1998 are significantly different from each other ($p < 0.05$, Tukey's Studentized Range Test). Vertical bars are the standard error of the mean.

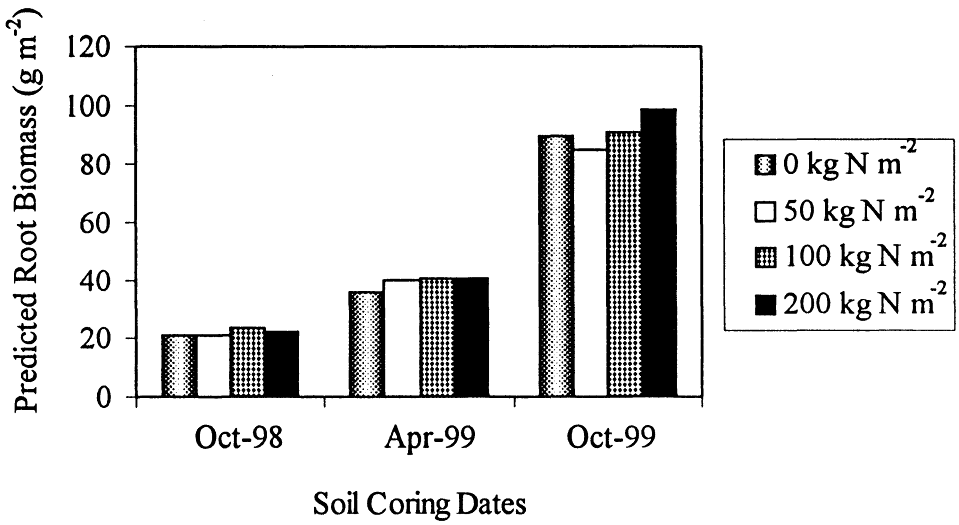

Figure 4. Fine-root biomass for each of the coring dates, predicted by specific growth rate x initial biomass minus specific death rates x initial biomass.

THESIS SUMMARY

Root systems are essential to water and nutrient acquisition as well as ecosystem nutrient cycling. Nitrogen (N) is an important nutrient to plant growth and metabolism. The objectives of this research are to study fine-root dynamics in response to N regimes. Few studies have investigated fine-root response to N. Past results have shown both increased and decreased longevity in N enriched sites. This contradiction may be partly due to the different methods used in prior studies. The use of soil coring and minirhizotrons, fine-root activity can be assessed accurately over time. Both of these methods were used in this study to investigate cottonwood (*Populus deltoides*) fine-root response to various fertilizer (N) levels. The planted trees were maintained according to poplar plantation silvicultural practices. We found N influenced fine-root dynamics very little in the first and second years of the plantation. Roots initiated in the first year lived longer than second year roots. The first year represented an establishing stand. By the second year the canopy closed and competition increased among the trees. The shorter life spans observed in the second year stand could represent a stand in transition to maturity. Future studies should consider stand age and test fine-root dynamics from establishment to maturity to confirm if our second year results do represent a transitional stage.

These results imply that establishing cottonwood can be grown on a range of N availability sites. If increased bole size is the objective of a management plan, this study shows that different N fertilization levels will not divert resources to fine-root turnover. We also found that turnover increased with stand age. Therefore, plantation managers can expect that establishing cottonwood will put more resources into fine-root turnover with age.

APPENDIX 1

CALCULATIONS

Minirhizotron data can be used to determine daily growth rates and belowground net primary production based on specific growth rates (SGR) and specific death rates (SDR). Those calculations will be shown in this appendix in detail and follow established methods by Cheng et al. (1991).

The following example datasets, dataPRD and dataMRT, were collected from minirhizotrons on four successive observation dates (Table 1). Production (Prod) is the increase in length density (mm cm^{-2}) since the last observation (Table 1). Mortality is the decrease or disappearance of length density (mm cm^{-2}) since the last observation (Table 1).

Table 1. Minirhizotron data (mm cm^{-2}) for dates 1-4.

Data: **dataPRD**

Date	1	2	3	4
Treatment	Prod1	Prod2	Prod3	Prod4
0	89.56	51.73	9.32	2.91
50	49.75	57.03	4.59	2.74
100	61.12	57.32	10.64	2.83
200	99.17	57.46	8.04	1.96

Data: **dataMRT**

Date	1	2	3	4
Treatment	Mort1	Mort2	Mort3	Mort4
0	-12.99	-7.74	-4.91	-7.76
50	-2.25	-1.76	-3.31	-2.74
100	-3.70	-6.50	-5.44	-5.87
200	-6.38	-8.30	-7.77	-17.07

Calculating specific growth and death rates

The first step to calculate specific growth and death rates is to determine cumulative growth (CProd) and death (CMort). Initial measurements are the same as the original data on the first date because there is no previous measurement. However, on the second measurement, the increase of length density in the second measurement plus the length density of the previous date would equal the cumulative growth, CProd (Equation 1, Example 1, Table 2). Cumulative mortality (CMort) is calculated similar to production (Equation 1, Example 1, Table 2).

Equation 1: $CPROD_j = \sum (CPROD_{j-1} + PROD_j)$

$$CMORT_j = \sum (CMORT_{j-1} + MORT_j)$$

Example 1: For Treatment 0 on Date 3,

$$CPROD3 = (CPROD2 + PROD3)$$

$$CPROD3 = 141.28 + 9.32$$

$$CPROD3 = 150.60 \text{ mm cm}^{-2}$$

$$CMORT3 = (CMORT2 + MORT3)$$

$$CMORT3 = -20.73 + -4.91$$

$$CMORT3 = -25.64 \text{ mm cm}^{-2}$$

Table 2. Cumulative production (CProd) and cumulative mortality (CMort) calculated from the raw data in Table 1. Units = mm cm^{-2}.

Data: **dataCPRD**

Date	1	2	3	4
Treatment	CProd1	CProd2	CProd3	CProd4
0	89.56	141.28	150.60	153.52
50	49.75	106.77	111.37	114.11
100	61.12	118.44	129.09	131.92
200	99.17	156.63	164.68	166.64

Data: **dataCMRT**

Date	1	2	3	4
Treatment	CMort1	CMort2	CMort3	CMort4
0	-12.99	-20.73	-25.64	-33.40
50	-2.25	-4.01	-7.32	-10.06
100	-3.70	-10.20	-15.64	-21.51
200	-6.38	-14.67	-22.44	-39.51

Next, the live-root standing crop must be determined for each date. Cumulative production plus the cumulative mortality results in the live-root standing crop (Crop) (Equation 2, Example 2, Table 3).

Equation 2: $CROP_j = CPROD_j + CMORT_j$

Example 2: For Treatment 0 on Date 3,

CROP3 = CPROD3 + CMORT3

CROP3 = 150.60 + -25.64

CROP3 = 124.96 mm cm^{-2}

Table 3. Live-root standing crop (Crop) derived from Table 2. Units = mm cm^{-2}.

Data: **dataCROP**

Date	1	2	3	4
Treatment	Crop1	Crop2	Crop3	Crop4
0	76.56	120.55	124.96	120.12
50	47.49	102.77	104.05	104.05
100	57.42	108.24	113.44	110.41
200	92.80	141.96	142.24	127.13

Finally, specific growth rate (SGR) is determined by dividing the cumulative growth by the number of days since the last observation and then dividing again by the average live-root standing crop since the last observation (Equation 3, Example 3, Table 4). Specific death rates (SDR) are calculated similarly (Equation 3, Example 3, Table 4).

Equation 3: $SGR_j = (CPROD_j/(DATE_j - DATE_{j-1})) / ((CROP_j + CROP_{j-1})/2)$

$$SDR_j = (CMORT_j/(DATE_j - DATE_{j-1})) / ((CROP_j + CROP_{j-1})/2)$$

Example 3: For Treatment 0 on Date 3, assume 30 days passed between Date 2 and Date 3:

$$SGR3 = (CPROD3/(DATE3 - DATE2)) / ((CROP2 + CROP3)/2)$$

$$SGR3 = (150.60/30) / ((120.55 + 124.96)/2)$$

$$SGR3 = 0.0438 \text{ day}^{-1}$$

$$SDR3 = (CMORT3//(DATE3 - DATE2)) / ((CROP2 + CROP3)/2)$$

$$SDR3 = (25.64/30) / ((120.55 + 124.96)/2)$$

$$SDR3 = -0.0060 \text{ day}^{-1}$$

Table 4. Specific growth rates (SGR) and specific death rates (SDR) derived from Table 2 and Table 3. Units = day^{-1}.

Data: **dataSGR**

Date	1	2	3	4
Treatment	SGR1	SGR2	SGR3	SGR4
0	n/a	0.0478	0.0438	0.0447
50	n/a	0.0508	0.0385	0.0392
100	n/a	0.0511	0.0416	0.0421
200	n/a	0.0477	0.0414	0.0442

Data: **dataSDR**

Date	1	2	3	4
Treatment	SDR1	SDR2	SDR3	SDR4
0	n/a	-0.0047	-0.0060	-0.0075
50	n/a	-0.0011	-0.0014	-0.0025
100	n/a	-0.0016	-0.0033	-0.0050
200	n/a	-0.0019	-0.0037	-0.0060

Calculating Turnover Index

The turnover index is calculated by the average of the SGR and –(SDR) (Equation 4, Example 4, Table 5).

Equation 4. $TI_j = (SGR_j + -SDR_j) / 2$

Example 4. For Treatment 0 on Date 3,

$TI3 = (SGR3 + -SDR3) / 2$

$TI3 = (0.0438 + -(-0.0060) / 2$

$TI3 = 0.0025\ day^{-1}$

Table 5. Turnover index (TI) derived from Table 4. Units = day^{-1}.

Date: **dataTI**

Date	1	2	3	4
Treatment	TI 1	TI 2	TI 3	TI 4
0	n/a	0.0117	0.0025	0.0011
50	n/a	0.0141	0.0011	0.0010
100	n/a	0.0132	0.0028	0.0013
200	n/a	0.0097	0.0021	0.0013

Predicting Belowground Net Primary Production

To predict belowground net primary production, the initial fine-root biomass must be known. The initial biomass or most recent predicted biomass is multiplied with the SGR and SDR of the measurement date and the two resulting calculations are added together. After the addition, the resulting number is the change in biomass for one day and must be multiplied by the numbers of days since the last date. The change in growth since the last measurement is added with the biomass of the last date for a predicted standing biomass for the current measurement date (Equation 5, Example 5, Table 6).

Equation 5. $BNPP_j = [((BNPP_{j-1} * SGR_j) + (BNPP_{j-1} * SDR_j)) * (DATE_j - DATE_{j-1})] + BNPP_{j-1}$

Example 5. For Treatment 0 on Date 3, assume there is 30 days between measurement dates.

BNPP3 = [((BNPP2 * SGR3) + (BNPP2 * SDR3)) * (DATE3 – DATE2)]] + BNPP2

BNPP3 = [((7105.3 * 0.0438) + (7105.3 * -0.0060)) * 30) + 7105.3

BNPP3 = [(13.75 + -11.42) * 30] + 7105.3

BNPP3 = 7203.6 kg ha^{-1}

Table 6. Belowground net primary production (BNPP) derived from Table 4. Initial biomass collected in the field at Date 1 was 5000 kg ha^{-1}. Units = kg ha^{-1}.

Data: **dataBNPP**

Date	1	2	3	4
Treatment	BNPP1	BNPP2	BNPP3	BNPP4
0	n/a	7105.3	7203.6	7078.0
50	n/a	8905.9	9167.7	9114.0
100	n/a	8468.1	8807.4	8587.2
200	n/a	7331.5	7317.4	6979.5

LITERATURE CITED

Cheng W X, Coleman D C and Box. J E, Jr. 1991 Measuring root turnover using the minirhizotron technique. Agr. Ecosyst. Environ. 34, 261-267.

APPENDIX 2.
STATISTICAL OUTPUTS

Statistical outputs from the general linear model (SAS Institute Inc, 1989):

A. Fine-root Production in 1998

Applied to Orthogonal Components
Test for Sphericity: Mauchly's Criterion = 0.0005034

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=1 M=2 N=25

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.1788474	39.7918	6	52	0.0001
Pillai's Trace	0.8211526	39.7918	6	52	0.0001
Hotelling-Lawley Trace	4.5913585	39.7918	6	52	0.0001
Roy's Greatest Root	4.5913585	39.7918	6	52	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=3 M=1 N=25

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.6084471	1.5743	18	147.5635	0.0737
Pillai's Trace	0.4410397	1.5512	18	162	0.0789
Hotelling-Lawley Trace	0.5647618	1.5897	18	152	0.069
Roy's Greatest Root	0.383526	3.4517	6	54	0.0059

B. Fine-root Mortality in 1998

Applied to Orthogonal Components
Sphericity test not performed due to determinant equal to zero.

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=1 M=1.5 N=25.5

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.3700217	18.047	5	53	0.0001
Pillai's Trace	0.6299783	18.047	5	53	0.0001
Hotelling-Lawley Trace	1.702544	18.047	5	53	0.0001
Roy's Greatest Root	1.702544	18.047	5	53	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=3 M=0.5 N=25.5

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.7272822	1.1959	15	146.7111	0.281
Pillai's Trace	0.2980784	1.2135	15	165	0.2661
Hotelling-Lawley Trace	0.3409021	1.1742	15	155	0.2973
Roy's Greatest Root	0.1814035	1.9954	5	55	0.0937

C. **Fine-root Standing Crop in 1998**

Applied to Orthogonal Components
Test for Sphericity: Mauchly's Criterion = 0.0006055

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

	H=Type III SS and CP Matrix for time				
	E= Error SS and CP Matrix		S=1	M=2	N=25
Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.1780326	40.0136	6	52	0.0001
Pillai's Trace	0.8219674	40.0136	6	52	0.0001
Hotelling-Lawley Trace	4.6169484	40.0136	6	52	0.0001
Roy's Greatest Root	4.6169484	40.0136	6	52	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

	H=Type III SS and CP Matrix for time				
	E= Error SS and CP Matrix	S=3		M=1	N=25
Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.5902375	1.6798	18	147.5635	0.0489
Pillai's Trace	0.4548	1.6082	18	162	0.0633
Hotelling-Lawley Trace	0.6198681	1.7448	18	152	0.0373
Roy's Greatest Root	0.476377	4.2874	6	54	0.0013

D. Fine-root Production in 1999

Applied to Orthogonal Components
Test for Sphericity: Mauchly's Criterion = 1.247 E-10

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=1 M=3 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.2081004	23.7836	8	50	0.0001
Pillai's Trace	0.7918996	23.7836	8	50	0.0001
Hotelling-Lawley Trace	3.8053729	23.7836	8	50	0.0001
Roy's Greatest Root	3.8053729	23.7836	8	50	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=3 M=2 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.694782	0.8117	24	145.6164	0.7176
Pillai's Trace	0.340321	0.8317	24	156	0.6924
Hotelling-Lawley Trace	0.3905392	0.7919	24	146	0.7423
Roy's Greatest Root	0.1985184	1.2904	8	52	0.269

E. Fine-root Mortality in 1999

Applied to Orthogonal Components
Test for Sphericity: Mauchly's Criterion = 1.4989 E-7

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=1 M=3 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.1823425	28.0262	8	50	0.0001
Pillai's Trace	0.8176575	28.0262	8	50	0.0001
Hotelling-Lawley Trace	4.4841857	28.0262	8	50	0.0001
Roy's Greatest Root	4.4841857	28.0262	8	50	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=3 M=2 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.6742019	0.8834	24	145.6164	0.6239
Pillai's Trace	0.3599789	0.8863	24	156	0.6202
Hotelling-Lawley Trace	0.4338634	0.8798	24	146	0.6287
Roy's Greatest Root	0.2783977	1.8096	8	52	0.0963

F. Fine-root Standing Crop in 1999

Applied to Orthogonal Components
Test for Sphericity: Mauchly's Criterion = 1.05 E-8

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=1 M=3 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.2367376	20.1508	8	50	0.0001
Pillai's Trace	0.7632236	20.1508	8	50	0.0001
Hotelling-Lawley Trace	3.2240854	20.1508	8	50	0.0001
Roy's Greatest Root	3.2240854	20.1508	8	50	0.0001

Manova Test Criterion and Exact F Statistics for hypothesis of no TIME*TREATMENT Effect

H=Type III SS and CP Matrix for time
E= Error SS and CP Matrix S=3 M=2 N=24

Statistic	Value	F	Num DF	Den DF	Pr>F
Wilks' Lambda	0.5364594	1.4532	24	145.6164	0.0929
Pillai's Trace	0.5528864	1.4686	24	156	0.0855
Hotelling-Lawley Trace	0.7064221	1.4325	24	146	0.1015
Roy's Greatest Root	0.6752275	2.3889	8	52	0.0283

H. Root Biomass in October 1998

General Linear Models Procedure

Source	df	Type III SS	F Value	Pr >F
Treatment	3	0.00592336	3.36	0.0213

Contrast	df	Contrast SS	F Value	Pr>F
Fertilizer linear	1	0.0046079	31.59	0.0003
Fertilizer quadratic	1	0.00131144	8.99	0.015
Fertilizer cubic	1	0.00000402	0.03	0.8718

Tukey's Studentized Range (HSD) Test

Alpha= 0.05 df= 112 MSE= 0.000587

Critical Value of Studentized Range= 3.688

Minimum Significant Difference= 0.0158

Tukey Grouping	Mean	N	Treatment
A	0.067497	32	200
A			
AB	0.065853	32	100
AB			
AB	0.060206	32	50
B			
B	0.050103	32	0

G. Root Biomass in April 1999

General Linear Models Procedure

Source	df	Type III SS	F Value	Pr >F
Treatment	3	0.00045685	0.28	0.8401

Contrast	df	Contrast SS	F Value	Pr>F
Fertilizer linear	1	0.00025563	0.56	0.4737
Fertilizer quadratic	1	0.00004406	0.10	0.7633
Fertilizer cubic	1	0.00008104	0.18	0.6836

I. Root Biomass in October 1999

General Linear Models Procedure

Source	df	Type III SS	F Value	Pr >F
Treatment	3	0.00136779	1.33	0.2675
Contrast	df	Contrast SS	F Value	Pr>F
Fertilizer linear	1	0.00018498	0.45	0.5188
Fertilizer quadratic	1	0.00001665	0.04	0.8448
Fertilizer cubic	1	0.00117446	2.86	0.1249

J. Fine-root Diameter Size Survival

Diameter Size	Total	Failed	Censored	% Censored
Less than 0.5 mm	16764	4570	12194	72.7392
Greater than 0.5 mm	798	108	690	86.4662
TOTAL	17562	4678	12884	73.3629

Test of Equality over Strata

Test	Chi-Square	df	Pr> Chi-Square
Log-Rank	145.6882	1	0.0001
Wilcoxon	98.7245	1	0.0001
-2log (LR)	175.8891	1	0.0001

K. Fine-root Survival by Year

Year	Total	Failed	Censored	% Censored
1998	5054	2336	2718	53.7792
1999	12508	2342	10166	81.276
TOTAL	17562	4678	12884	73.3629

Test of Equality over Strata

Test	Chi-Square	df	Pr> Chi-Square
Log-Rank	155.3898	1	0.0001
Wilcoxon	49.3566	1	0.0001
-2log (LR)	49.3394	1	0.0001

L. Fine-root Survival by Treatment

Treatment	Total	Failed	Censored	% Censored
0	4592	1288	3304	71.9512
50	3702	899	2803	75.7158
100	4237	1193	3044	71.8433
200	5031	1298	3733	74.2
TOTAL	17562	4678	12884	73.3629

Test of Equality over Strata

Test	Chi-Square	df	Pr> Chi-Square
Log-Rank	41.5529	3	0.0001
Wilcoxon	52.3316	3	0.0001
-2log (LR)	39.5365	3	0.0001

M. Leaf N Concentration

General Linear Models Procedure

Source	df	Type III SS	F Value	Pr >F
Treatment	3	4.16438438	37.35	<0.0001
Position	1	2.91007813	78.3	<0.0001

Contrast	df	Contrast SS	F Value	Pr>F
Fertilizer linear	1	1.19002585	74.73	<0.0001
Fertilizer quadratic	1	0.84945819	53.34	<0.0001
Fertilizer cubic	1	0.0427097	2.68	0.1274