UNIVERSITÉ DU QUÉBEC À MONTRÉAL

EFFETS DE LA FERTILISATION À L'AZOTE SUR LA CROISSANCE, L'ARCHITECTURE RACINAIRE ET LE FLUX DE SÈVES DU PEUPLIER HYBRIDE

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EFFECT OF NITROGEN FERTILISATION ON HYBRID POPLAR GROWTH, ROOT ARCHITECTURE AND SAP FLOW

THESIS PRESENTED IN PARTIAL FULFILLMENT OF THE
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BY

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FOREWORD

This memoir presents the results of an experiment undertaken during the course of my Maîtrise en Sciences de l'Environnement commenced in September 2004. Permission was acquired to present my work in the English language and the results in the form of a scientific article. A French summary of my work is presented in the résumé. A general introduction precedes the scientific article in Chapter 1 and elaborates on the founding concepts of the experiment as well as the rational for undertaking the scientific inquiry. A general conclusion follows Chapter 1 and ties together the conclusions of the article with the founding concepts detailed in the general introduction. The second and third co-authors of the scientific article are Dr. Frank Berninger and Dr. Christian Messier. The article will be submitted during the fall of 2006.

I would like to thank my supervisor Dr. Frank Berninger for his generous support and patience, as well as my co-supervisor, Dr. Christian Messier, for his ability to challenge while simultaneously providing support and continuous encouragement. I would also like to extend a special thanks to Dr. Lluis Coll for his generous help, both in time and elbow grease, without which this project would have undoubtedly been much more challenging. Of course, this project would not have been possible without the help of several field and lab assistants (Janet Butcher, Natasha Caminsky, Charles-Etienne Cardella-Rinfret, Andrea Gauthier, Simon Laberge, Émilie R. Messier, Mathieu R. Messier, and Amélie Poitras-Larivière).

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ABSTRACT

The hybrid poplar is a preferred species in short-rotation plantations, as well as in other environmental projects, because it is one of the fastest growing trees in North America. In a sustainable development context, the poplar hybrid has the capacity of playing two roles: accelerated production of wood and forest conservation. Hybrid poplar plantations on a small territory could supply a large amount of wood that would otherwise be extracted from older, less productive sites. There is however a considerable debate on the legitimacy of using hybrid poplars in plantations, more so if such projects would require intensive use of fertilisation and irrigation.

Although the poplar has been a vastly studies species over the years, relatively little is known on the effect of fertilisation on the poplar's root system. We not only suspect that there is an effect, but that the form of nitrogen used (ammonium vs. nitrate) could have a specific effect on the root system hereby also affecting the efficiency of water transport in the root system.

Since hybrid poplars are known for their sensitivity to drought, it is pertinent in a plantation context, to understand how adding nitrogen-based fertiliser could affect the root system and its capacity to capture water from the soil.

This research has several objectives: describe the root architecture of hybrid poplars, determine how ammonium and nitrogen fertilisation affect root architecture, and in turn, if the resulting root architecture will affect water transport efficiency. The experiment was conducted in Montreal (Quebec) at McGill University's Macdonald Campus in an agricultural setting. Three-year old hybrid poplars (*Populus maximowiczii x P. balsamifera* 915313) were subjected to three different fertilisation treatments: i) 200kg/ha nitrate, ii) 200kg/ha ammonium and, iii) no fertilisation. Methods include sap flow measurements on individual roots and excavation of 18 whole-root systems. Results indicate that the fertilised trees grew significantly more than the control trees, but there were no significant differences in biomass allocation of aboveground and belowground parts. However, the results suggest significant differences in the fine to coarse root ratio between ammonium and nitrate treatments with nitrate-treated poplars showing the highest ratio, explaining perhaps why the nitrate-treated trees also have the highest sap flow rate. The study also revealed important and significant relations between the roots' proximal diameters and architectural parameters (i.e.: root length, link #, mass). The data could potentially be used in modeling poplar root systems. Because root response to fertilisation can vary between species, these results will help establish how poplar roots respond to varying forms of nitrogen fertilisation.

KEY WORDS: Hybrid poplar, root architecture, proximal root, sap flow.

RÉSUMÉ

Le peuplier hybride est l'essence préférée dans les plantations de ligniculture et autres projets environnementaux, car il est une des essences à plus grande croissance en Amérique du Nord. Dans un contexte d'aménagement forestier durable, le peuplier hybride pourrait avoir un double rôle : production accélérée de matière ligneuse et conservation de la forêt. Des plantations de peupliers hybrides dans une petite partie du territoire fourniraient une grande quantité de bois autrement extrait de sites plus anciens et moins productifs. En dépit de la vue apparemment optimiste des plantations de peuplier hybrides, il existe un débat considérable sur la légitimité d'utiliser le peuplier en forme de plantations pour subvenir à la demande de matière ligneuse, et surtout si cela nécessite l'utilisation de la fertilisation et de l'irrigation intensive.

Malgré le fait que le peuplier soit l'essence d'arbre le plus étudiée, peu est connu sur l'effet de la fertilisation sur le système racinaire, un élément important permettant une croissance rapide et assidue dans les plantations. On soupçonne que non seulement il existe un effet, mais qu'aussi la forme d'azote, soit le nitrate ou l'ammonium, pourrait avoir un dérangement particulier dans l'architecture racinaire engendrant des effets sur l'efficience du transport hydrique.

Étant donné que le peuplier est connu pour sa sensibilité à la sécheresse, il est donc pertinent, dans un contexte de plantation, de savoir comment l'ajout de fertilisant pourrait affecter le système racinaire et sa capacité de capter l'eau dans le sol.

Les objectifs de cette étude sont : 1) de décrire l'architecture du peuplier hybride, et 2) de déterminer comment la fertilisation à l'ammonium ou au nitrate influence l'architecture racinaire et l'efficacité de transport de l'eau. L'expérience a été réalisée au Campus Macdonald de l'université McGill à Montréal (Québec) en milieu agricole dans des plantations de trois ans de peuplier hybride (*Populus maximowiczii x P. balsamifera*) soumises à trois traitements différents : 1) 200 kg/ha de nitrate; 2) 200 kg/ha d'ammonium; et 3) aucune fertilisation. Des mesures de flux de sève ont été prises pour des racines individuelles et 18 systèmes racinaires complets ont été excavés. Les résultats démontrent une croissance significativement plus importante pour les arbres fertilisés, mais aucune différence significative dans l'allocation de biomasse aux parties aériennes ou souterraines. Par contre, les résultats suggèrent des différences significatives dans le ratio racines fines/grosses racines selon le traitement. Les peupliers traités au nitrate auraient ainsi le ratio le plus élevé. Ce qui explique un flux de sève significativement plus élevé pour les arbres traités au nitrate. Cette étude nous a permis de découvrir des relations importantes et significatives entre le diamètre proximal des racines et des paramètres architecturales (c.-à-d. longueur des racines, dynamique de ramification, poids). Ces données pourront éventuellement servir dans la modélisation de système racinaire. Étant donné que la réponse racinaire à la fertilisation peut varier en fonction de l'espèce étudiée, les résultats de cette étude aideront à déterminer comment les racines de peuplier répondent à différentes formes de fertilisation.

MOTS-CLÉS : Peuplier hybride, architecture racinaire, racine proximale, flux de sève.

INTRODUCTION

Poplars are the fastest growing commercial trees in North America and with the advent of vigorous hybrid crosses, growth under favourable conditions in northeastern America can average 1-3 cm in annual dbh increment and 1-2 m in height increase (Stanturf *et al.* 2001). Historically, interest in poplar culture has wavered with plantation objectives fluctuating between producing saw and timber wood products, pulpwood and/or for biofuels. In the recent past, advances in poplar science combined with mounting environmental awareness lead to alternative and ingenious applications of poplar plantations such as wind-protection barriers, erosion control, riparian buffers, phytoremediation, bioenergy, carbon sequestration, and urban plantings (Isebrands and Karnosky 2001). The use of fast-growing poplars in short-rotation plantations is also being considered in forest management regimes; a small portion of the territory under intense forest management would provide a large quantity of wood otherwise extracted from older less productive sites. The anticipated result is an increase in forest conservation and maintenance as well as an increase in forest product output. Despite the seemingly optimistic view of short-rotation plantations as a solution to forest management problems, the use of hybrid poplars is not without problems or risks.

Although poplars are one the most studied species in tree science, there remains continued debate on the legitimacy of using poplar hybrids in short-rotation plantations to satisfy the increasing demand of wood resources. It is obvious that more research is required to supplement the knowledge base so that informed sustainable forest management and silviculture-related decisions can be made. Presently, the lack of information has prompted research in several fields ranging from forest management to the genetic research of the poplar genome. One area of investigation that requires more scrutiny concerns the effect of increased bioavailable soil nitrogen on the architecture of the root system and the potential effect that this may have on the hydraulic state of trees. The following text highlights the rationale for a study whose primary objective is to relate poplar root structure (or architecture) to the two resources most vital to roots and to the growth of the plant – water and nutrients. It will briefly touch on concepts related to root systems, more specifically the poplar root system, root architecture, and finally will review some of the literature on the links between fertilisation and root architecture and hydraulic dynamics.

Rationale

While poplars are fast-growing species, their successful establishment in a plantation depends on careful site selection, intensive tending, and the use of proven clones for the selected site (Stanturf *et al.* 2001). Poplars are vulnerable to drought stress and require unusually high amounts of nutrients to maintain growth. The two limiting growth factors, nutrients and water, known to limit yield in most agricultural systems, are most often provided by intensive irrigation and fertilisation (Lynch 1995). Applying these methods to the cultivation of poplars is recommended by several researchers (Stanturf *et al.* 2001), but little is known on how these methods affect the root system, the key plant organ associated with the acquisition of the two most essential and limiting soil resources, namely, water and nutrients. Few studies have addressed the hybrid poplar's root system response to fertilisation and water conduction. It is appropriate to study the use of fertilisation on poplar root architecture since it is intimately associated to water acquisition and water relations. Root system studies are fraught with difficulties and challenges due to the hidden nature of roots, however results stemming from such studies are often quite revealing.

The study of root systems

Roots account for a large portion of plant biomass and are often referred to as the "hidden half". Hidden in an opaque soil environment, the study of root systems is inherently complex. Furthermore, the considerable investment of labour necessary for a complete quantitative and qualitative analysis of root systems in their natural environment has limited the number of studies. However obscure, plant root systems have two primary functions for plant survival, these being the acquisition of resources from the soil, and anchorage (Atkinson 2000).

There are certain ambiguities as to what part of the root system functions in anchorage. Certainly, the mechanical characteristics of roots and soil, including the bond between individual roots and the soil matrix, play an important role in anchorage. According to Fitter (2002), the finely branched regions of the root system play little part in anchorage, leaving the proximal or central part of the root system the function of anchorage strength.

Stokes (2002) contends in a review of root anchorage that the tensile strength of the roots on the wayward side of the root plate accounts for more than 60% of the total anchorage, whereas the breaking of the roots underneath the soil-root plate account for less than 20%. This finding further reinforces Fitter's idea that the major function of the fine root system is resource acquisition and not anchorage, a function associated with the coarse roots and the proximal root system structure or heart of the root system.

Resource acquisition is intimately linked to soil exploration. Soil exploration and exploitation efficiency is linked to the branching pattern and distribution of roots in space and time (Fitter, 2002). More specifically, plants with higher specific root lengths have a greater capacity to conduct water per unit length in wet soil, and possibly a greater capacity for nutrient uptake (Eissenstat, 1992). The carbon cost of developing and maintaining these resource-acquiring organs must be balanced by their efficiency in absorbing water and nutrients (Persson, 1983). Assuming plant roots are seeking optimization in their efficiency of water and nutrient uptake, then environmental and genetic variation can influence efficiency strategies. Such were the results of a study where differences in fine-root soil exploitation strategies were found between conifers and deciduous trees of the boreal forest such that conifers invested in fewer coarse and presumably longer-lived roots compared to the highly branched and thinner roots found in shade-intolerant tree species (Bahaus and Messier, 1999).

There is scientific value in attempting to understand how root architecture influences the resource acquiring and transportation functions of the root system. Short-rotation forestry using hybrid poplars could be improved by understanding how nitrogen fertilisation could potentially alter root architecture and influence water uptake in the process—a worthy consideration since poplars are sensitive to drought conditions (Harvey, 1997).

The poplar root system

According to Dickmann *et al.* (2001), poplar root systems are especially complex because of the pronounced effects of genotype on root architecture which influences root size, number, structure and orientation. A poplar root system, established from a rooted hardwood cutting, can form between 30 and 50 first-order roots in the first year of growth. These roots vary in length from 40 cm to 1 metre and for 5-year old poplars; the lateral extension can reach 6 metres and a depth of 60 cm. It is believed that the roots that survive the first year of growth thicken by undergoing secondary growth and hence form the structural architecture of the root system. The development of the root system is highly dependent on the soil environment. Most coarse horizontal roots can extend several tree lengths away from the trees and are generally found between 5 and 20 cm below the soil surface. Sinker roots, deploying vertically downwards from the horizontal roots, can reach depths of 1 to 3 metres. Poplar trees have been found to have four to five orders of branching.

As is the case in most trees, poplar fine roots are concentrated near the ground surface, in the top 10 cm of the soil, and form extensive networks to capture water and nutrients. Poplar fine roots are usually infected with both ectomycorrhizae and endomycorrhizae. Little is known on the cost of maintaining these roots. The development of fine roots peaks twice during the growing season (Dickmann *et al.* 2001). The first occurrence is in the spring, soon after budbreak, and a smaller peak occurs in the fall.

Allocation of photosynthetically fixed carbon varies throughout the lifespan of poplar trees (seedling, mature, sylleptic) and changes significantly before and after bud set. Allocation is thought to be altered when nitrogen fertilisation is applied, and although it is known that poplar roots have a slight preference for ammonium (NH_4^+), little is known on how different forms of nitrogen affect the allocation of carbon, or the root architecture.

Fundamentals of root architecture

The complexity of root systems and the variability in root branching and distribution in soil space often results in destructive sampling and analysis techniques, and therefore designing a purposeful root study is fundamental to its success (Bengough *et al.* 2000). Caution must be taken to avoid misdirected studies. Moreover, in order to justify the laborious field excavation of any root system, their must be assurance that the data cannot be computed by simple allometric models (Atkinson 2000). In situations where data cannot easily be achieved this way or that more precise measurements are required, the measurements must clearly be linked to the problem in question. Therefore, the reasons for undertaking such a challenge must be well founded, and the methods for achieving the answers must pair off. The following text is an attempt to synthesize the present knowledge on root system architecture, focusing most intently on the available methods and approaches in root architecture characterisation and evaluation. This step is necessary to evaluate which method is most appropriate for the study of the poplar root architecture in a context where the objective is to determine how fertilisation affects poplar root architecture and how in turn this might affect root hydraulic relations.

A thorough review of the concepts of root architecture (Lynch, 1995) indicates that root architecture refers to the spatial configuration of the root system and implies that the shape of the configuration has functional significance. The author is careful to demarcate root architecture from the other widely used terms used to describe other aspects of root systems such as root morphology, root topology, and root distribution. Root morphology refers to the micro-study of the anatomical root structure related to cell and tissue organization, and this aspect is not traditionally a part of architectural considerations. Root topology and distribution – root branching and presence (rather than position) of roots in a grid – are both implied in root architecture. In this sense, root architecture encompasses these two terms but in addition includes the geometrical scaling of roots (Oppelt *et al,* 2001).

Jourdan and Rey (1997) offer a similar definition of root architecture based on the pioneers of architectural analysis of Hallé and Oldeman (1970). Although their working definition is based on observing root growth and to a certain extent branching pattern (topology), morphological differentiation at varying stages of development appears to be the focus of the architectural analysis, further contrasting Lynch's definition (1995).

The discrepancy in the definition of root architecture is further complicated by several other studies whose basic determinants of root architecture are topology and/or distribution (Arredondo and Johnson 1999, Bauer and Berntson 2001, Berntson 1997, Bouma *et al* 2001, Fitter 1986, 1987, Oppelt *et al* 2001, Rose 1983) or more recently, incorporate the complex principles of fractal geometry (Fitter and Strickland 1992, Moravek and Fiala 2004, Nielsen *et al* 1998, Oppelt *et al* 2000, Ozier-Lafontaine 1999, Salas *et al* 2004, Smith 2001, Spek and van Noordwijk 1994, Tatsumi *et al* 1989, van

Noordwijk *et al* 1994; van Noordwijk and Purnomosidhi 1995; van Noordwijk and Mulia 2002, Walk *et al* 2004).

Topology

The topological approach, in the context of root architecture, was pioneered by Fitter (1986, 1987), and later evaluated, adopted or modified by several other researchers. Moving away from approaches that relied on developmental models (Rose 1983), Fitter described branching structures using a link-based system. Each link (a length of root between two nodes) is given its own identity. The link types such as internal or external help determine "magnitude". The "altitude" is the number of links in the longest path from and external link to the most basal link of the root system. The application of topology in root architecture studies has been associated with exploitation efficiency (Arredondo and Johnson 1999, Berntson 1994, Bouma *et al* 2001, Oppelt *et al* 2000) but it has been suggested that topological indices are inadequate for multi-scale or non-linear complex scaling – such as can be found in tree root systems (Berntson, 1997).

Fractal geometry

The use of fractal geometry in root studies is growing because it can lead to powerful indicators of the "space-filling" property of an object, which in root systems, equates with soil exploration efficiency. The fundamental properties of a fractal object include a degree of self-similarity across a range of spatial scales (resolutions), defined by a non-integer dimension known as fractal dimension (FD) (Halley *et al*, 2004). Self-similarity implies that an object can be decomposed into a finite number of reduced copies of itself (Prusinkiewicz 1998), resulting from the replicated iteration of elementary units (Sievänen *et al* 2000). A study, which measured topological indices as well as fractal dimensions for root systems, found that variations in FD could not be explained purely by topology (Oppelt *et al* 2001). The fractal index explains more than simply topology and geometry (Fitter and Strickland, 1992). It is a more encompassing measure, which, in combination with other indices, has the potential of being a simple yet telling index of architecture.

A variety of studies shows that fractal indices are related to genotypic and environmental influences (i.e.: nutrient and water availability, hybrid type, CO_2 level, etc). It would appear then that this would be a good method to link a synthetic, non-integer parameter to functional root properties. Nonetheless, there are certain problems associated with the use of fractals, the most important being the adherence of an object to self-similarity principles, and being of infinite length. In this strict sense, branching structures in plants are not entirely true fractal objects. Their structures are more or less self-similar and plant branching structures are only fractal within a limited range of scales (Godin 2000). In response to this, mixed fractals have emerged to reflect the different functional components of plant branching systems (Halley *et al.* 2004). Moreover, several methods have evolved in root studies and hence a debate has ensued on the methods and results of fractal studies to ascertain physiological functioning.

The use of the FD parameter to characterize poplar root architecture has potential use, but the approach previously described has been mostly applied to annual dicots and small root systems – trees on the other hand have coarse woody roots that make the aforementioned techniques inappropriate. It would be reasonable to assume that since coarse roots are responsible for anchorage and water and nutrient transport versus the fine root function of actual soil exploration for the uptake of water and nutrients, their architecture would be developmentally and structurally different. Therefore different methods would be necessary to accurately quantify the whole-plant root architecture. The pipe stem model (van Noordwijk *et al.* 1994), based on allometry and fractal branching principles, has often been used and modified by root scientists to study the root architecture of trees, especially in the field of agroforestry.

Pipe theory approach

The approach adopted by van Noordwijk *et al* (1994), based on pipe-stem model principles, uses non-destructive measurements of proximal root diameter to predict total root size developed and is thought to be a valuable tool to assess total root biomass (Drexhage *et al.* 1999). It is believed that the initial size of the diameter combined with knowledge on branching pattern is sufficient to predict total root length, root diameter distribution and specific root length (van Noordwijk *et al.* 1994). Ozier-Lafontaine *et al.* (1999) confirmed the usefulness of this approach and conclude that with proper calibration of the model, total root dry mass, total root length, diameters, as well as horizontal and vertical root system extension may be provided. Furthermore, it would appear that this approach combined with direct measurements of sap flow through individual roots could potentially bridge the gap between the characterisation of root architecture and functioning of roots in uptake of nutrients and water (van Noordwijk and Purnomosidhi 1995), however, applying the model to further our understanding of root functioning has seldom been done. Ong *et al* (1998) came close to achieving this link but stopped short in their comparison of the sap flow they measured in individual roots to the fractal characterisation of the coarse roots using the pipe stem model methods. They concluded that multiple methods are needed for total root system quantification and suggested that fractal methods be used to quantify coarse roots until the diameter declines to fine root, and then quantifying the fine root from the ratio of fine to coarser roots. Ozier-Lafontaine *et al.* (1999) also warn that root diameter requires special consideration because model coefficients vary with diameter size.

Architectural and hydraulic root response to fertilization

A poorly understood aspect of root architecture has been its plastic response to environmental variables (Lynch, 1995). Relatively few studies have attempted to understand the effects of fertilisation on both coarse root and fine root architecture and hence on the carbon allocation of these two fundamentally different root structures. Likewise, research focusing on the varying effects of nitrogen forms on growth is confusing at best. Nitrogen is a required nutritional element for plant growth and can naturally be found in different forms. However, information on how different forms of nitrogen affect root architecture is scarce. Furthermore, information on species' preference for an N-source is unclear. Of the 70 studies cited in Martinez-Louçao and Cruz (1999) where nitrate and ammonium fertilisation were compared in various plants, 21 plants supplemented with ammonium showed growth stimulation effects, 39 showed inhibition effects and the rest showed equal or slight improvement of growth.

More specific to trees, Bauer and Berntson (2001) have shown that for *Betula* and *Pinus*, plants grown with elevated levels of CO_2, ammonium-grown trees (NH_4^+) showed significant differences in biomass (57%). However, for both species, plants grown with nitrate as their sole source of N allocated more biomass to roots (53%). Although specific root length (SRL) did not vary according to nitrogen form, nitrate-grown plants had a significantly greater fine root diameter and root density than ammonium-grown plants. An experiment on *Populus deltoides* Bartr. ex Marsh (eastern cottonwood) confirms that the nitrogen form alters root architecture such that an increase in nitrate results in higher-order root development or branching (Woolfolk and Friend 2003). However, at 100% nitrate, higher-order root development was hindered. The authors conclude that when nitrogen is provided in a nitrate-dominating form, high root length density results and soil exploitation is facilitated.

Just as root architecture is affected by the N-form, there is reason to believe that nutrition can also affect water use. The relationship however between the nitrogen form and water is equally unclear. Poplars, known for their drought sensitivity, have been the subject of a few studies testing the effects of nutrition on water-use efficiency or drought resistance. In general, the studies showed that nitrogen fertilisation leads to higher water-use efficiency and xylem cavitation in poplar hybrids (Harvey & van den Driessche 1999, Rippulone *et al* 2004). There is little information on the effect of N-form (ammonium or nitrate) on water relations.

Hydraulic conductivity and root architecture

Hydraulic architecture refers to the design by which trees influence water movement from roots to leaves (Cruiziat *et al.*, 2002). The root system is the compartment classically associated with the uptake portion of the water pathway. The pipe-model has classically been used to understand the hydraulic design of plants (Enquist 2002) simply because the branching architecture of plants reflects the hydraulic architecture within, hence root architecture is synonymous with hydraulic architecture and clearly related to hydraulic conductance.

Smith and Roberts (2003) report a lack of data on root hydraulic conductivities, especially for field-grown plants at the scale of the root system. They measured the hydraulic conductance of severed roots at the base of the tree and related the measurements to corresponding root lengths of *Grevilla robusta* trees. For hydraulic measurements, high-pressure flow meters were used and for root lengths, a combination of fractal methods calibrated by root excavations was used. In their comparison to maize plants, no differences were noted in the hydraulic conductivity expressed per unit root length. The authors warn however that the resulting similarity cannot be generalized to other tropical or temperate systems.

There appears to be a valid association between root architecture and hydraulic conductivity but few studies have attempted to link both. Furthermore, work by Harvey and van den Driessche (1997, 1999) have shown that an increase in nitrogen fertilisation increases the tendency to xylem cavitations hence reducing hydraulic conductivity of nitrogen-fertilised trees, regrettably, measurements were restricted to leaves, stems and leaf : root ratios without any consideration for root architecture (branching, root length, etc.) or fine root to coarse root ratios. This area of study deserves further investigation.

The principle objective of this study was to improve our knowledge on the effect of nitrogen fertilisation on poplar root architecture, more specifically, on the differences between nitrate and ammonium fertilisation. Linking form (architecture) to function was a secondary objective. We wanted to shed light on the relationship between root architecture and sap flow. Few studies have investigated this link between form and function at the root level. After carefully reviewing the different approaches to characterising root architecture, we opted for the use of measurements that are telling yet simple. To ensure knowledge transfer to non-experts of root science research, it is essential that the results and methods of root system studies have a certain degree of applicability in a field environment. Simple and effective methods that may help poplar culturists and farmers evaluate the health of their plantations is important to improve yield, and limit environmental degradation due to inappropriate use of fertilisation. Practical applicability and knowledge transferability were used as broad epistemological guides during the course of this research.

CHAPTER 1

EFFECT OF AMMONIUM AND NITRATE FERTILISATION ON HYBRID POPLAR GROWTH, ROOT ARCHITECTURE AND SAP FLOW

1.1 Résumé

Le peuplier hybride est l'espèce de prédilection au Canada pour les plantations à courte rotation. Cependant, les connaissances sont encore limitées quant aux effets de la fertilisation au nitrate ou à l'ammonium sur le système racinaire des peupliers hybrides. Il est probable que la fertilisation à l'azote induise des changements dans l'architecture racinaire et que ces effets varient selon la forme d'azote utilisée (ammonium ou nitrate). De ce fait, puisque le transport de l'eau est étroitement relié à l'architecture racinaire, il est raisonnable de supposer que son efficacité sera également affectée par la fertilisation à l'azote. Les objectifs de cette étude sont : 1) de décrire l'architecture du peuplier hybride, et 2) de déterminer comment la fertilisation à l'ammonium ou au nitrate influence l'architecture racinaire et l'efficacité de transport de l'eau. L'expérience a été réalisée au Campus Macdonald de l'université McGill à Montréal (Québec) en milieu agricole dans des plantations de trois ans de peuplier hybride (*Populus maximowiczii x P. balsamifera*) soumises à trois traitements différents : 1) 200 kg/ha de nitrate; 2) 200 kg/ha d'ammonium; et 3) aucune fertilisation. Des mesures de flux de sève ont été prises pour des racines individuelles et 18 systèmes racinaires complets ont été excavés. Les résultats démontrent une croissance significativement plus importante pour les arbres fertilisés, mais aucune différence significative dans l'allocation de biomasse aux parties aériennes ou souterraines. Par contre, les résultats suggèrent des différences significatives dans la ratio racines fines/grosses racines selon le traitement. Les peupliers traités au nitrate auraient ainsi le ratio le plus élevé. Ce qui explique un flux de sève significativement plus élevé pour les arbres traités au nitrate. Cette étude nous a permis de découvrir des relations importantes et significatives entre le diamètre proximal des racines et des paramètres architecturales (c.-à-d. longueur des racines, dynamique de ramification, poids). Ces données pourront éventuellement servir dans la modélisation de système racinaire. Étant donné que la réponse racinaire à la fertilisation peut varier en fonction de l'espèce étudiée, les résultats de cette étude aideront à déterminer comment les racines de peuplier répondent à différentes formes de fertilisation.

1.2 Abstract

Hybrid poplars are the preferred species in short-rotation plantations in Canada, however, little is known on how ammonium versus nitrate fertilisation affects the root system. Nitrogen-based fertilisation allegedly induces change in the root system architecture. It is suspected that varying forms of nitrogen, ammonium and nitrate, can also have different effects on architecture. Subsequently, since water transport is intimately linked with root architecture, we can expect that its efficiency will also be altered with nitrogen fertilisation. This research has several objectives: describe the root architecture of hybrid poplars, determine how ammonium and nitrogen fertilisation affect root architecture, and in turn, if the resulting root architecture will affect water transport efficiency. The experiment was conducted in Montreal (Quebec) at McGill University's Macdonald Campus in an agricultural setting. Three-year old hybrid poplars (*Populus maximowiczii x P. balsamifera* 915313) were subjected to three different fertilisation treatments: i) 200kg/ha nitrate, ii) 200kg/ha ammonium and, iii) no fertilisation. Methods include sap flow measurements on individual roots and excavation of 18 whole-root systems. Results indicate that the fertilised trees grew significantly more than the control trees, but there were no significant differences in biomass allocation of aboveground and belowground parts. However, the results suggest significant differences in the fine to coarse root ratio between ammonium and nitrate treatments with nitrate-treated poplars showing the highest ratio, explaining perhaps why the nitrate-treated trees also have the highest sap flow rate. The study also revealed important and significant relations between the roots' proximal diameters and architectural parameters (i.e.: root length, link #, mass). The data will could potentially be used in modeling poplar root systems. Because root response to fertilisation can vary between species, these results will help establish how poplar roots respond to varying forms of nitrogen fertilisation.

1.3 Introduction

Poplars are the fastest growing trees and are potentially the most versatile woody plants in North America. Consequently, they are often used in short-rotation plantations to supply the ever-increasing demand on wood and wood-related products. Furthermore, the use of highly performing hybrid poplars in poplar culture has lead to even more efficient and sustainable production (Riemenschneider *et al.,* 2001). Because of the poplars' fast growth potential, nitrogen—being the mineral element most often limiting plant growth and productivity—is required in substantial amounts to sustain growth and reduce rotation time (Dickmann *et al.,* 2001; Cooke *et al.*, 2005). Hence fertilisation use in poplar culture is increasing (van den Driessche *et al.*, 2003; Weih 2004). Although the *Populus* genus has been intensely studied in recent years (Ben Brahim *et al.*, 2000), few studies have explored the effects of fertilisation, more specifically of nitrogen fertilisation, on the poplar root system—the key plant organ associated with the acquisition of the two most essential and limiting soil resources, namely, water and nutrients.

Resource acquisition, be it for nutrients or water, is intimately linked with soil exploration and exploration efficiency, and hence with root architecture which refers to the spatial configuration of the root system (Lynch, 1995). The shape and configuration of the root system, such as its branching pattern and distribution, has functional significance (Fitter, 2002; Lynch, 1995). For example, plants with higher specific root lengths—one of many root architecture descriptors—were shown to have a greater capacity to conduct water per unit length in wet soil, and possibly a greater capacity for nutrient uptake (Eissenstat, 1992). Hence, studying root architecture is fundamental in understanding the effect of nitrogen fertilisation on the poplar root system.

The root system can acquire nitrogen in two forms: ammonium (NH_4+) or nitrate (NO_3-). Normally, plant species have preferences for either nitrogen form. According to Min *et al.* (2000), poplars are nitrogen generalists, capable of thriving in both high and low NH_4+ or NO_3- soil environments. Nonetheless, the physical, chemical and biological processes associated with the different forms of nitrogen are vastly different (Min *et al.,* 1999) and could potentially have different effects on root architecture. In fact, several studies have argued that NH_4+ versus NO_3- nitrogen supply does have a significant effect on tree roots. For one, the nitrogen-source was shown to be an important determinant of vertical root distribution in the soil (Fujimaki *et al.*, 2004), of root biomass (Bauer and Berntson, 2001), of fine root architecture (Woolfolk and Friend, 2003) and of vitality (Clemensson-Lindell and Persson, 1995).

Just as root architecture can be affected by the different nitrogen forms, there is reason to believe that the nitrogen form can also affect plant water relations. However the relationship between nitrogen form, root architecture and water relations is not clearly understood. Poplars, known for their drought sensitivity, have been the subject of a few studies testing the effects of fertilisation on water transport or drought resistance. However, information on the effect of nitrogen form (NH_4+ versus NO_3-) on water relations is lacking.

The primary aim of the present study was to investigate how nitrogen form (NH_4+ versus NO_3) affects poplar root architecture (form), and secondly, how this in turn affects sap flow (function) in the root system. In order to examine this link between form and function in the hybrid poplar root system, we adopted a multi-scale and holistic approach where the effects of nitrogen availability and nitrogen form were examined from a large scale (plant growth and allocation) to a fine scale (root dynamics and sap flow). Additionally, we investigated the effects of fertilisation on two different clones. We know from other studies that root architecture can vary according to soil and environmental conditions, and hence decided to take the exploration one step further by seeing if our results would be similar for two clones of the same hybrid. Practices in hybrid poplar culture would benefit from this knowledge.

From the largest scale, we examined the effect of fertilisation treatment on overall growth and allocation. We hypothesize that at the whole-tree scale, the fertilisation treatments will have a significant and positive impact on overall growth and that ample availability of nutrients in the soil of fertilised trees will result in significantly less investment (relative to aboveground biomass) in their root structure compared to non-fertilised trees. At an intermediate scale, we examined the effect of treatment on large individual roots (from the proximal area to the tip) to see if the treatments would affect coarse root architecture. We predict that fertilized-tree roots will have less mass, links and will generally be shorter in length than non-fertilised trees. Taking a closer look at the individual roots by dissecting them into root orders will help us determine if the treatments influence the coarse-root system architecture. At a finer scale, investment in resource-acquiring structures (fine roots) will be higher in non-fertilised trees resulting in a higher fine-root to coarse root ratio than in fertilised trees. Since poplars have been noted as having a *slight* preference for ammonium (Dickmann *et al.,* 2001) and because ammonium is less mobile in the soil, we predict that trees fertilised with ammonium will have lower fine-root to coarse root ratios than trees fertilized with nitrate. In short, we believe that having an ample supply of nutrients, all other conditions being equal, would lead to less-complex, less-branched and least costly root systems. For our secondary objective, we wanted to link root architecture (form) to sap flow (function). Here, we predict that sap flow variation will be explained, in part, by the presence of fine roots such that sap flow will increase with increasing fine root to coarse root ratio since the surface of fine roots is the location of water uptake. Increases in sap flow potential would reduce the risk of drought since the trees would be more capable of taking in water when environmental conditions and growth required it.

Finally, this study indirectly addresses an environmental issue since it deals with the question of nitrate use in poplar plantations. There is growing concern that an increase in fertilisation would lead to an increase in ground water contamination since nitrate easily leaches into the soil (Dinnes *et al* 2002). Using an ammonium-based fertiliser rather than a nitrate one could prove beneficial by preventing leaching. The results of this study could potentially shed light on questions concerning the use of fertilisation in poplar plantations. Understanding the effects of nitrogen-form on plant growth would lead to better environmental management of poplar plantations.

1.4. Material and methods

1.4.1 Study Site

The study was conducted in Montréal, Québec at McGill University's Macdonald Campus (45°25' N lat., 73°56'W long., elevation 39 m.). The mean annual temperature is 6°C, 20.9° in July and -10.4° in January. The mean annual precipitation is 920 mm, 90.1 mm in July and 70.4 mm in January.

1.4.2 Plant material, plantation establishment, and field design

In June of 2004, two hybrid poplar clones (*P.maximowiczii* x *balsamifera*), numbered 913311 and 913313 by the Québec Ministère des ressources naturelles (further referred to as clone 311 and 313), were planted on predominantly sandy agricultural soil whose previous vegetation cover was a corn crop. The two adjacent fields (approximately 20 meters to either side) were used for soybean and corn crops. The open site, approximately half a hectare (50 meters x 50 meters), was chosen for its homogenous, deep sandy soils and its high drainage capacity to facilitate root system excavation.

We randomly hand-planted 64 trees from both the 311 and the 313 clonal types (128 in total) in a grid pattern in plots of four trees of the same clonal type, resulting in 16 plots per clonal type. Each plot was randomly divided into four fertilisation treatment groups ensuring that these groups were equally divided by clonal type resulting in 4 plots per clonal type per treatment,16 trees per clonal type per treatment (Table 1.1 for summary).

Prior to planting, the trees were measured for height, diameter at 40cm from root collar, initial root length, and a qualitative assessment of the state of the roots was taken. Each tree was marked with nail polish 40 cm above the root collar for future growth measurements.

Table 1.1 Summary of plantation design and tree assignment to treatments.

Clonal type	Total # trees)	Total # Plots (4 trees/plot)	Plot/Trees per treatment		
			Control	Nitrate	Ammonium
311	64	16	4/16	4/16	4/16
313	64	16	4/16	4/16	4/16

1.4.3 Fertilisation treatments

An original experimental design included four fertilisation treatment types: control, nitrate fertilisation (NO_3^-), ammonium fertilisation (NH_4^+) and, mixed nitrate-ammonium fertilisation (NO_3^- and NH_4^+). However, because of the intensive labour required to excavate root systems and the time investment required to make fine architectural measurements, not all treatment types were used for this specific experiment. Efforts focused on establishing if differences existed between the two nitrogen forms, therefore the mixed nitrate-ammonium fertilisation (NO_3^- and NH_4^+) was not used. A parallel project on canopy architecture will make use of this treatment. For the sake of clarity, only the details pertaining to this experiment will be explained.

Fertiliser was applied three times during the two growing seasons (2004: June 26th, July 16th and August 6th; 2005: June 3rd, June 24th, July 15th) totalling 200kg/ha of nitrogen per year. The quantity of fertilisation is not the same for all treatments because the absolute quantity of nitrogen in each type of fertilizer was different. Calculations were made to insure that each treatment received the same amount of nitrogen. For the nitrate treatments, 3 applications of 138 grams of powdered potassium nitrate (13.7%-N) were applied manually at the base of the tree (1 metre radius), totalling 200kg/ha by the end of the growing season. For the ammonium treatments, 3 applications of 90 grams of powdered ammonium sulphate (21%-N) was applied manually at the base of the tree (1 metre radius), totalling 200kg/ha by the end of the growing season. Fertilisation was applied three times per growing season at equal intervals and trees were given time to establish themselves and show signs of growth prior to the initial treatment. To reduce competition for nutrients with other plants, we used systemic herbicide (RoundUp®) combined with manual removal of vegetative competition near the base of the trees.

1.4.4 Ongoing field measurement

Throughout the 2004 and 2005 growing seasons, we recorded growth in diameter at 40cm above root collar (previously marked before planting) and height. We measured nutrient ion supply using Western Ag Plant Root Simulator (PRS)™ probes (Western Ag. Innovations, Saskatoon, SK, Canada).

During both 2004 and 2005, the Western Ag PRS™ probes were used to monitor the nitrate and ammonium ion supply. In 2004, the probes were buried for two consecutive 4-week periods. In 2005, because the results neared maximum ion detection capacity, the probes were buried for 3-week periods.

During the summer of 2004, the ratio of ammonium to nitrate ions was low in the ammonium treatment; measurements of available ammonium ions revealed a low presence of ammonium. Suspecting effects of nitrification, we applied a nitrification inhibitor, dicyandiamide (DCD), to maintain higher levels of available ammonium in the soil. DCD application during the 2005 season increased nearly 15-fold the available ammonium to nitrate ratio for the ammonium treatment (Table 1.2).

1.4.5 Excavations and tree biomass

At the end of the 2005 growing season, we randomly selected 18 trees of the 313 clones for whole-system root excavations (6 from each treatment type: control, nitrate and ammonium). Time and labour being a limitation, we narrowed our excavations to one clone to insure we would have enough subjects for statistical analysis. The excavation process began August 1st 2005 and ended with the last excavation on September 2nd 2005. Whole root excavations were limited to roots greater than 2mm. The roots were

Table 1.2. PRS™-Probe Supply Rate (mg/10cm2/burial period) and NH^4 to NO^3 ratio in three treatments for 2004 and 2005

	Control Treatment			Nitrate Treatment			Ammonium Treatment		
Year	NO^3	NH^4	NH4:NO3	NO^3	NH^4	NH4:NO3	NO^3	NH^4	NH4:NO3
2004	163	24	0.0248 *a*	1345	3	0.0023 *a*	797	117	0.2163 *b*
2005	64	5	0.1562 *c*	234	5	0.0244 *c*	147	358	3.2366 *d*

a - *d* Values followed by the same latter in a row or column are not significantly different (*Tukey-Kramer,* $P < 0.05$)

excavated by hand using various digging tools and great care was taken to maintain the integrity of the root system architecture. Roots that plunged perpendicularly into the soil for more than 50cm were severed, tagged with flagging, and later excavated using a shovel once the root system was removed from its growth site. The severed roots were kept for biomass measurements. With a team of three to four persons, most trees took an entire day to excavate. A few of the larger trees took more than a day and therefore the excavated root system was placed in large plastic bags overnight with water to maintain moisture and prevent root desiccation.

Once excavated, the root systems were severed from the stem at the root collar. The trees were separated into the following plant parts for biomass analysis: leaves, branches, stem, and root system. From each excavated root system, we randomly chose and severed two roots from the root stump for root architecture analysis. These will be referred to as proximal roots (See section 2.6). Plant parts were brought to the lab where the root systems were carefully washed. Because of the high sand content of the soil, the root systems were easily cleaned and roots larger than 2mm were easily conserved. Plant parts were oven dried at 60°C until constant mass and weighed to the nearest 0.01 g.

1.4.6 Proximal roots

Prior to oven drying for biomass, we analysed the proximal roots in the lab. Each link (segment between branching events) was separated by order using Fitter's (1986) and Rose's (1983) topological scheme. Starting from the initial or mother link (order = 1), the first branching event divides the link into two or more links. The subsequent link with the largest diameter maintains the root order of the mother link (in this case, order = 1). The other links become order 2. Each link was followed and the ordering scheme was repeated until we reached a root tip (Figure 1.1).

Once all the link orders were identified, they were measured for length, and cut. The numbers of links per order were counted. The links were then oven-dried at 60°C until constant mass and subsequently weighed to the nearest 0.001g.

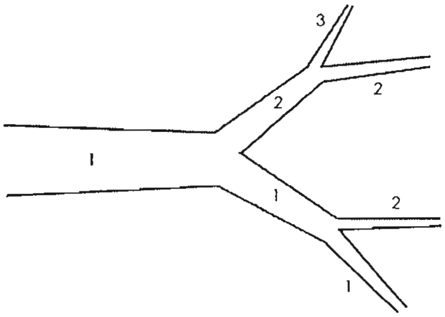

Figure 1.1 Schematic representation of the hybrid poplar root order used for architectural analysis.

1.4.7 Sap flow and fine roots

For this segment of the experiment, being limited by equipment constraints, we randomly selected 18 trees from each clonal type (311 and 313), six from each treatment type (control, nitrate and ammonium). Individual root sap flow and fine root measurements were taken at the end of the 2005 growing season. From each of the trees, we randomly selected two roots for sap flow and fine root measurements for a total of 72 roots. Because the trees selected from the 313 clone were the same as those for the whole-root excavation and that sap flow measurements require intact root systems, the data was collected at least one week prior to the total excavation of the root systems. Because of the high number of roots to be monitored for sap flow, measurements had to be staggered over a 6-week period starting July 25th. Approximately 12 roots were monitored simultaneously for seven consecutive days before being removed and reinstalled on the next series of randomly chosen roots.

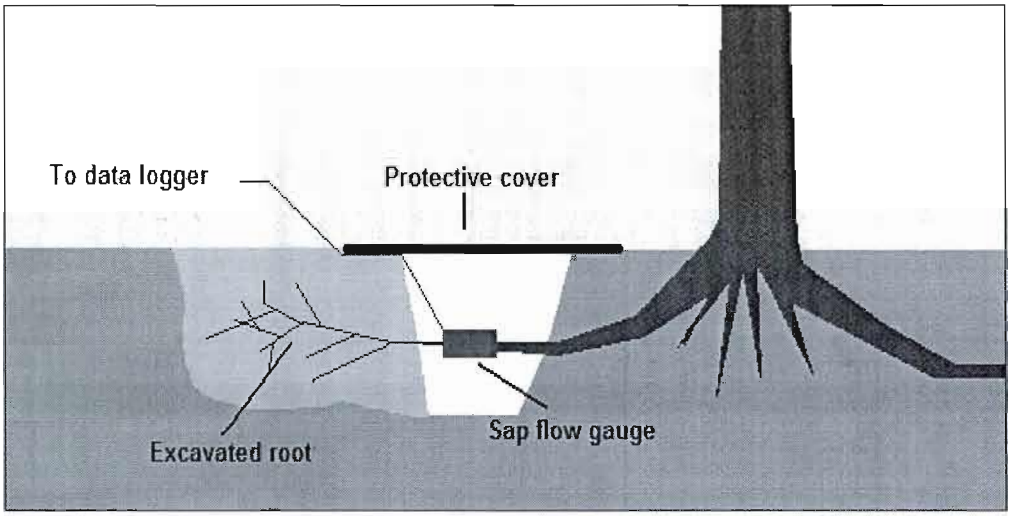

Figure 1.2 Installation of the sap flow gauges in the field (adapted from Coners and Leuschner 2002)

Using the Dynagage Flow32 Sap Flow system and Dynagage SGA5 microsensors (Dynamax Inc., Houston, TX, USA), we adapted Coners and Leuschner's (2002) field protocol to our experimental design (Figure 1.2). The protocol required partial root excavations about 1-2 metres away from the base of the tree stems to expose suberized roots of approximately 4-5 mm in diameter. A small pit was dug around the root to allow easy installation of the gauges. The root diameter was noted before the installation of the sensors. Installation of the SGA5 microsensors followed established Dynamax procedures (Dynamax F32 Manual, 2005). The important difference being that the gauges were installed on individual roots rather than aboveground stems. After installation, the hole was filled with insulating packing material to not only prevent soil from falling back into the pit and damaging the gauges, but also to reduce temperature fluctuations. A foam board covered the pit opening to further protect the gauges and diminish temperature fluctuations. The validity of measuring individual root sap flow using heat ratio method sensors has been confirmed by a study evaluating methods of measuring sap flow in roots of woody plants (Burgess *et al.*, 2000).

When sap flow monitoring was complete, each root was carefully excavated by hand from the point where the gauge was installed to the finest of root tips. The roots were kept intact and fresh in a sealed bag and sent to the laboratory in a cooler for further analysis using WinRHIZO Pro v. 2005b software and an LC4800-II scanner, an optical-scanner-based image analysis system (Regent Instrument Inc., Montréal, QC, Canada). Once at the lab, the sap flow roots were gently washed using a fine mesh protection at the bottom of the sink to retrieve severed fine roots. The roots had to be cut into pieces prior to scanning so that they could be immersed in water on the root trays provided with the WinRHIZO system. The roots were scanned at the recommended 400 dpi on the LC4800-II scanner, which employs two lighting systems thus increasing the measuring accuracy. Once scanned, the software was able to provide us with the following information: average diameter, total length, length by diameter class, total volume, volume by diameter class, total surface area, surface area by diameter class, and total number of tips. Once scanning was completed, the roots were separated into fine roots (<2mm) and coarse root (>2mm) using digital callipers. Coarse and fine roots were then oven dried at 60°C until constant mass and measured to the nearest 0.001 g.

1.4.8 Calculating sap flow

The Dynagage Flow32 Sap Flow system was set up to continuously monitor sap flow in the microsensors every 30 s. Every 5 minutes, the average values were stored in the Dynamax Flow32 Sap Flow Monitor (data logger). The stem heat balance (SHB) method developed by Sakuratani (1981) was used to determine the sap flow in the individual hybrid poplar roots. The method assumes that the sap flow has reached a steady state and requires a constant energy input from the heater within the sensors. The temperature gradient measured upstream and downstream from the heater within the sensor is used to calculate the flow using energy balance principles. Falling within the recommended range of 3.5 – 5 V., the SGA5 sensor heater input voltage was maintained at 3.8 V.

We calculated sap flow in g h^{-1} (grams per hour) from 6 am to 9 pm for seven consecutive days. For every tree, a weekly mean daily g h^{-1} and mean max g h^{-1} was calculated. Because of technical problems in certain weeks, some weekly means are not based on seven full days of monitoring. Roots were rejected if they did not have a minimum of four days of monitoring or if calculated sap flow revealed problems associated with the technical installation of the gauges (i.e.: extreme high values, extreme fluctuations, etc). A high rate of failure of sap flow measurements due to such problems is typical. These were easily identifiable by graphing flow versus time. In total, 21 of the 72 roots were rejected leaving 51 reliable measurements of sap flow. One root was excluded from the analysis because the root was only partially excavated and therefore the data unreliable.

1.4.9 Statistical analysis

Tree growth was tested using a repeated measures analysis using the PROC MIX module of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Other analyses were performed using JMP 5 (SAS Institute Inc., Cary, NC, USA). ANOVAs and GLMs (General Linear Models) were used to test the effect of treatments on proximal root and fine root architecture and on sap flow. Regressions were used to establish relationships between root architecture parameters and trees size diameters. Details are included in the results section. For every analysis, the significance level was considered when P was less than 0.05.

1.5 RESULTS

1.5.1 Growth

At planting time, the mean initial diameter (40cm above root collar) and mean stem height of the 311 clone was 11.17 ± 1.59 mm and 174.93 ± 24.25 cm. For the 313 clone, the mean initial diameter (40cm above root collar) and mean stem height was 11.42 ± 1.52 mm and 184.31 ± 36.94 cm. Neither clone had branches or coarse roots, and the buds had not yet opened when planted in June 2004.

Overall, the treatment had a significant effect on diameter growth ($P < .0001$) and clonal types had different reactions to fertilisation treatments. The 311 clone reacted positively and significantly to the ammonium and nitrate treatments whereas the 313 clone had no significant reaction to either of the fertilisation treatments. The nitrate treatment for the 311 clone had the greatest growth of any other treatment x clone category (Figure 1.3). However, growth was not significantly greater than in the 311 ammonium-treated trees.

1.5.2 Biomass and allocation

Biomass and allocation measurements were restricted to the 313 clone. The results reflect the previous results on growth where fertilisation had no effect on the biomass allocation of the 313 clone. Mass ratios were similar across treatments (Figure 1.4). On average for all treatments, root biomass accounted for 23% of the total biomass whereas leaves accounted for 26% of the biomass.

Similarly, when total biomass, leaf biomass, and root biomass were plotted as a function of diameter, treatment had no significant effect on the strongly significant positive linear relationships (Figures 1.5, 1.6, 1.7). Likewise, treatment had no significant effect on the root to shoot ratios plotted as a function of diameter. Here the relationship was a negative linear relationship. As diameter increased, less biomass was allocated to roots versus to shoots (Figure 1.8).

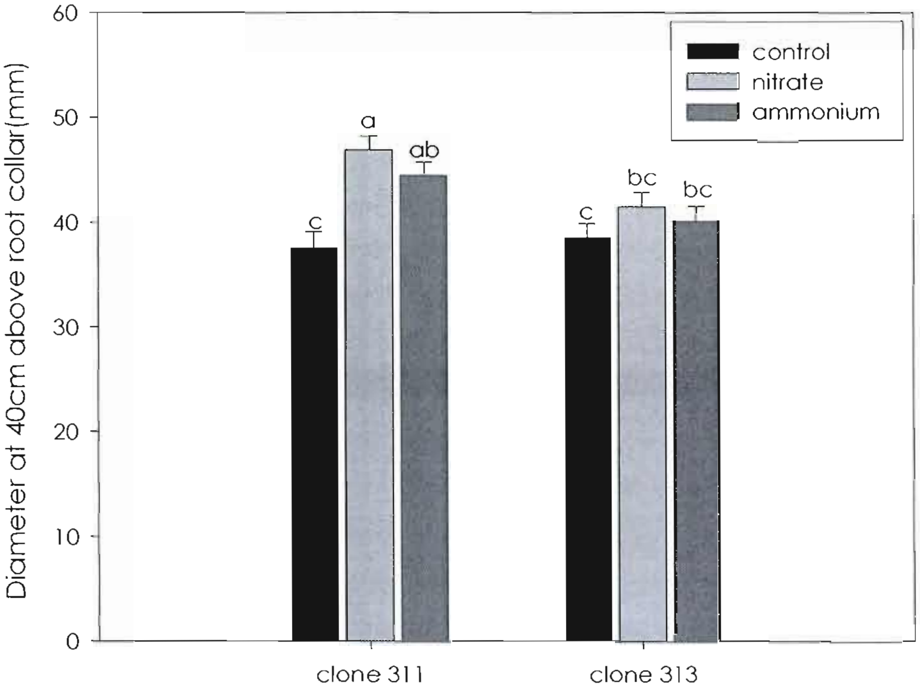

Figure 1.3 Mean diameter growth (mm) taken 40cm above the root collar for clonal type and treatment (2004 and 2005 season). Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Each bar is the mean ± SE. Treatment bars with the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$).

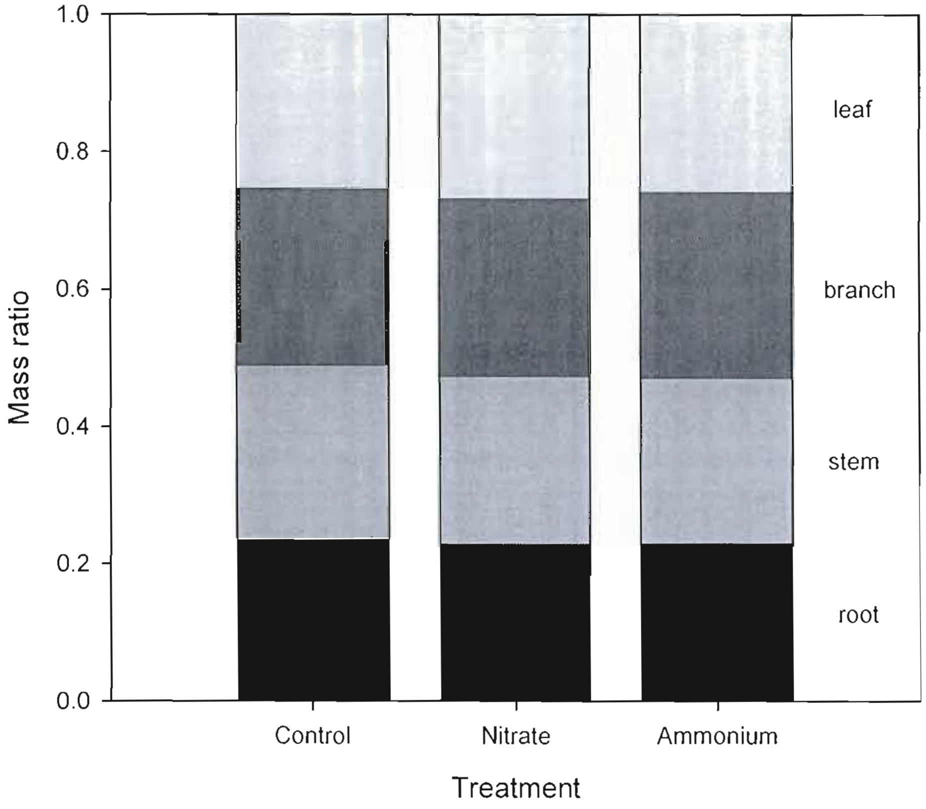

Figure 1.4 Mass ratios (plant part biomass/total biomass) of clone 313 trees excavated in August 2005. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Each bar represents the mean of 6 trees. No significant difference was found across treatments.

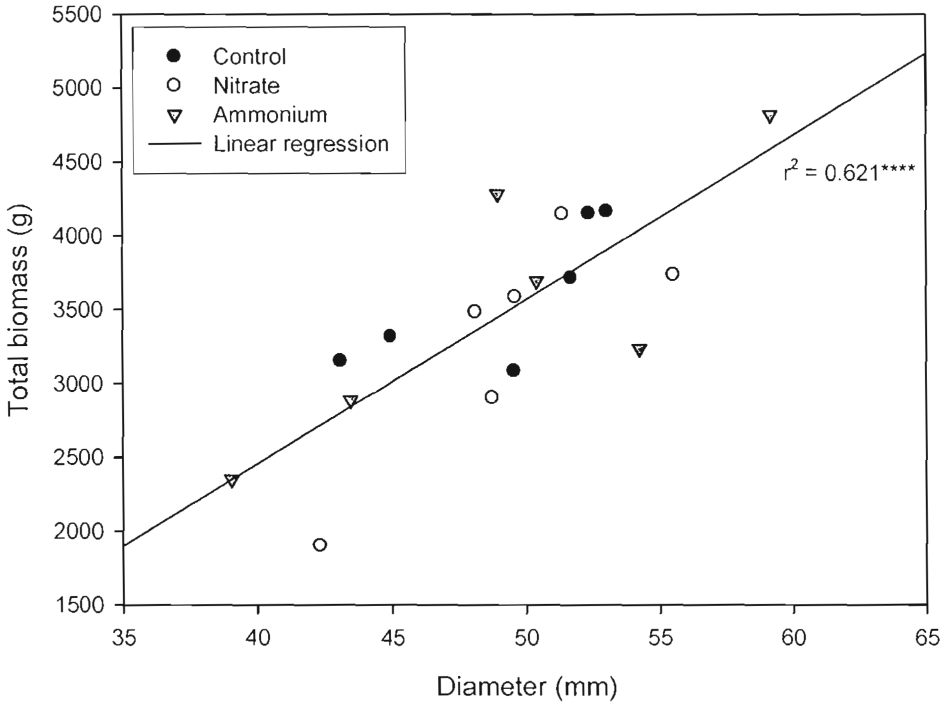

Figure 1.5 Total biomass of clone 313 trees as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression ($P < 0.0001$) for combined treatments is shown since no significant difference is found between treatments.

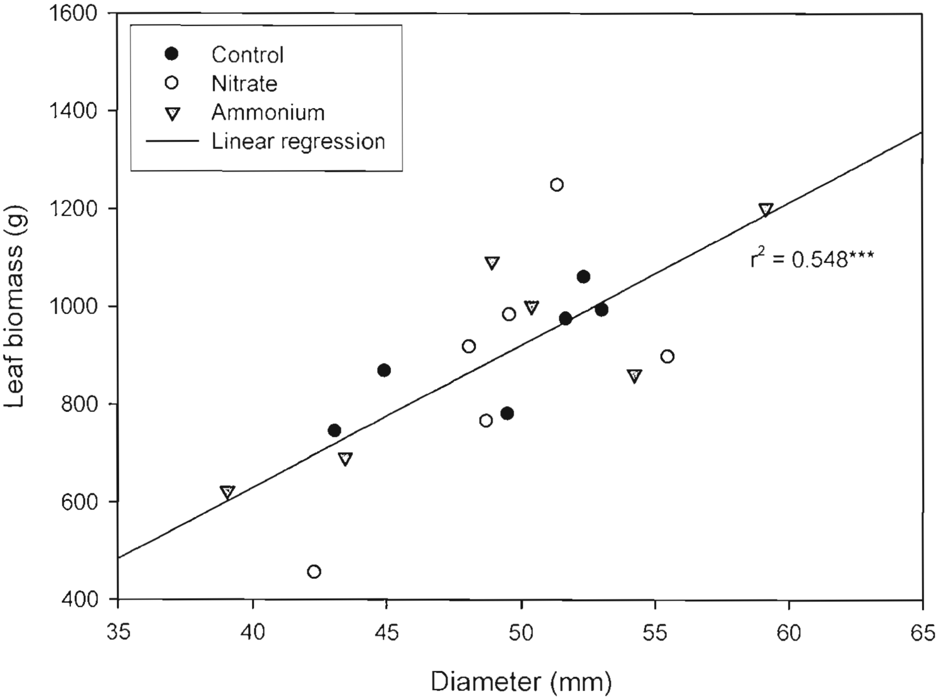

Figure 1.6 Leaf biomass of clone 313 trees as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control and 200 kg N ha^{-1} year^{-1} of nitrate and 200 kg N ha^{-1} year^{-1} of ammonium. Least-squared linear regression ($P < 0.001$) for combined treatments is shown since no significant difference was found between treatments.

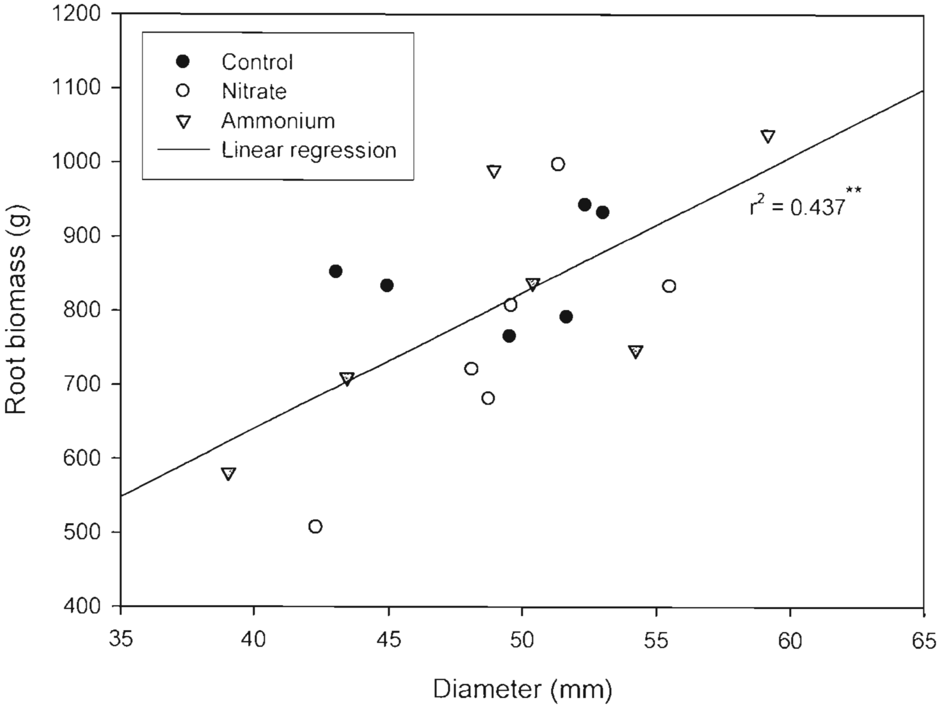

Figure 1.7 Root biomass of clone 313 as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control and 200 kg N ha^{-1} year^{-1} of nitrate and 200 kg N ha^{-1} year^{-1} of ammonium. Least-squared linear regression ($P < 0.01$) for combined treatments is shown since no significant difference was found between treatments.

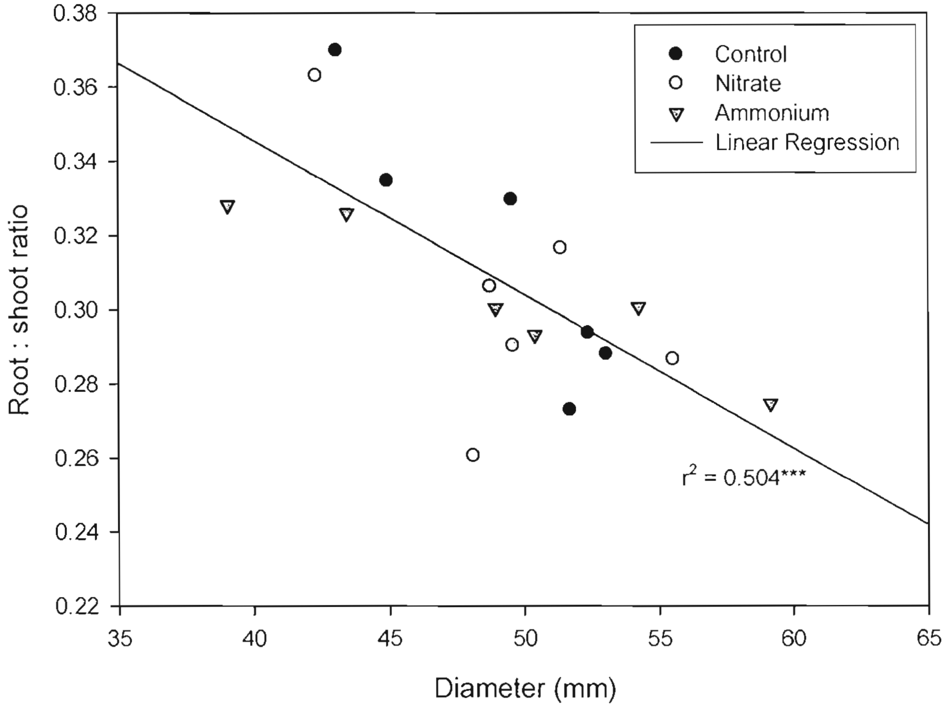

Figure 1.8 Root:shoot ratio of clone 313 as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control and 200 kg N ha^{-1} year^{-1} of nitrate and 200 kg N ha^{-1} year^{-1} of ammonium. Least-squared linear regression (*P* < 0.001) for combined treatments is shown since no significant difference was found between treatments.

1.5.3 Proximal Roots

Proximal root diameters (ranging between 10.2 and 43.1 mm) were found to be significantly and linearly correlated to total root length ($R^2 = 0.621$, $P < 0.0001$), mass ($R^2 = 0.795$, $P < 0.0001$) and link number ($R^2 = 0.663$, $P < 0.0001$) (Figures 1.9, 1.10, 1.11). ANOVAs testing the effect of treatment on total root length, root mass and root link number revealed no significant effect. Similarly, ANOVAs revealed that treatment had no significant effect on total root length, mass or link # when the parameters were standardized by their proximal diameters. However, even though not statistically different, nitrate-treated trees had consistently lower values across treatments such that for mass per proximal diameter, the nitrate treatment had mean values approximately 24% lower than the control and 26% lower than the ammonium treatment (Figure. 1.12). For link # per proximal diameter, the mean nitrate values were 25% lower than the control and 27% lower than the ammonium (Figure. 1.13). For total root length, this trend did not hold. The mean values for total length per proximal diameter were only 5% and 6% lower for the control and ammonium treatments respectively (Figure. 1.14).

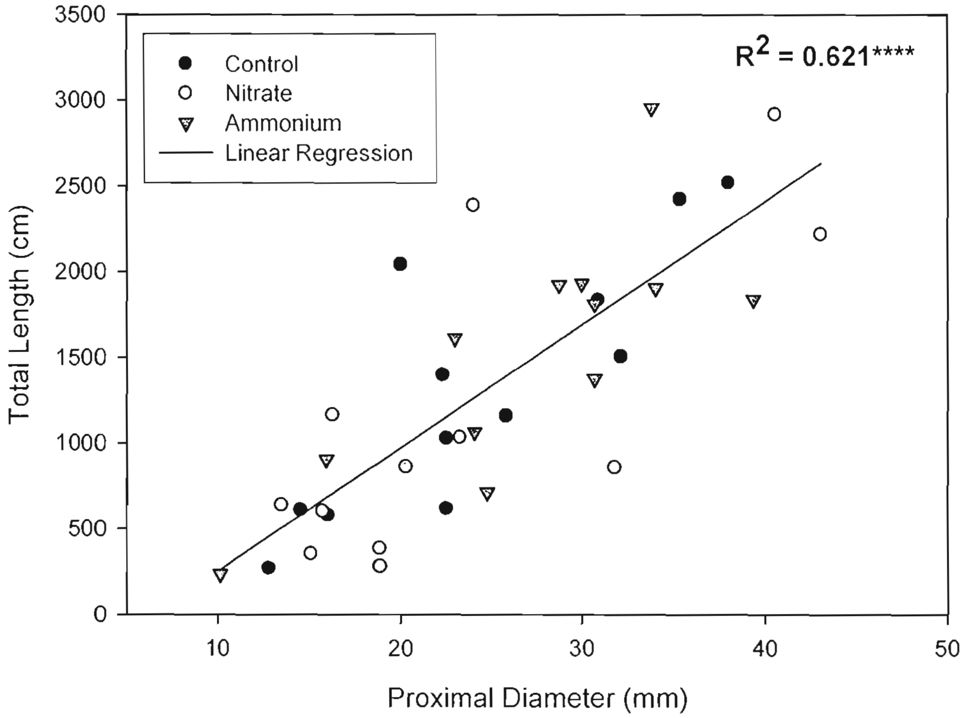

Figure 1.9 Total length (cm) as a function of proximal diameter (mm) for clone 313. Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control and 200 kg N ha^{-1} year^{-1} of nitrate and 200 kg N ha^{-1} year^{-1} of ammonium. Least-squared linear regression ($P < 0.0001$) for combined treatments is shown since no significant difference was found between treatments.

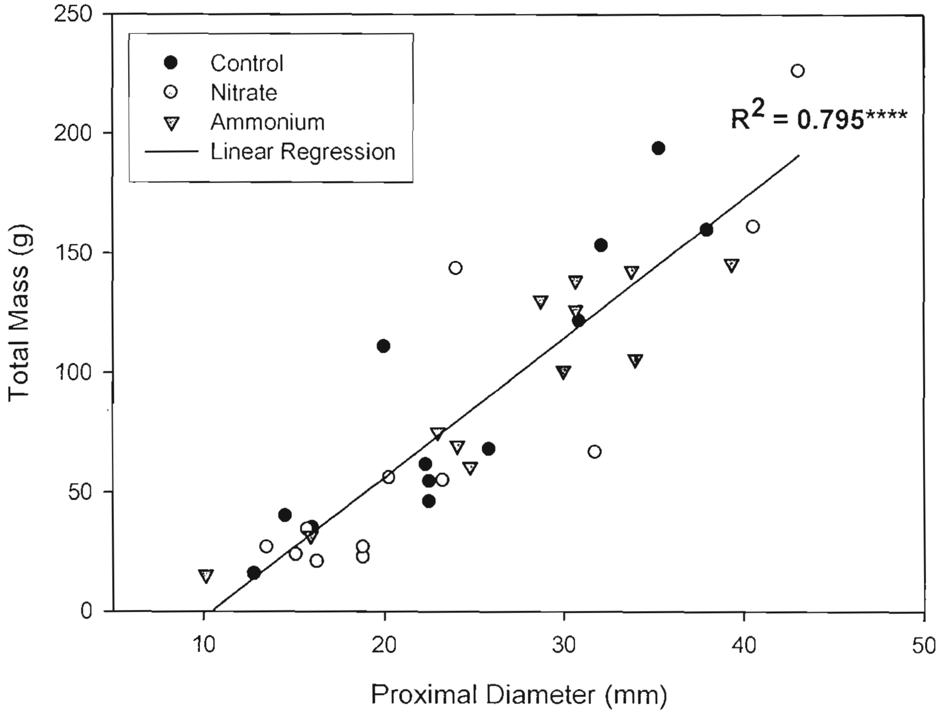

Figure 1.10 Total mass (g) as a function of proximal diameter (mm) for clone 313. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression ($P < 0.0001$) for combined treatments is shown since no significant difference was found between treatments.

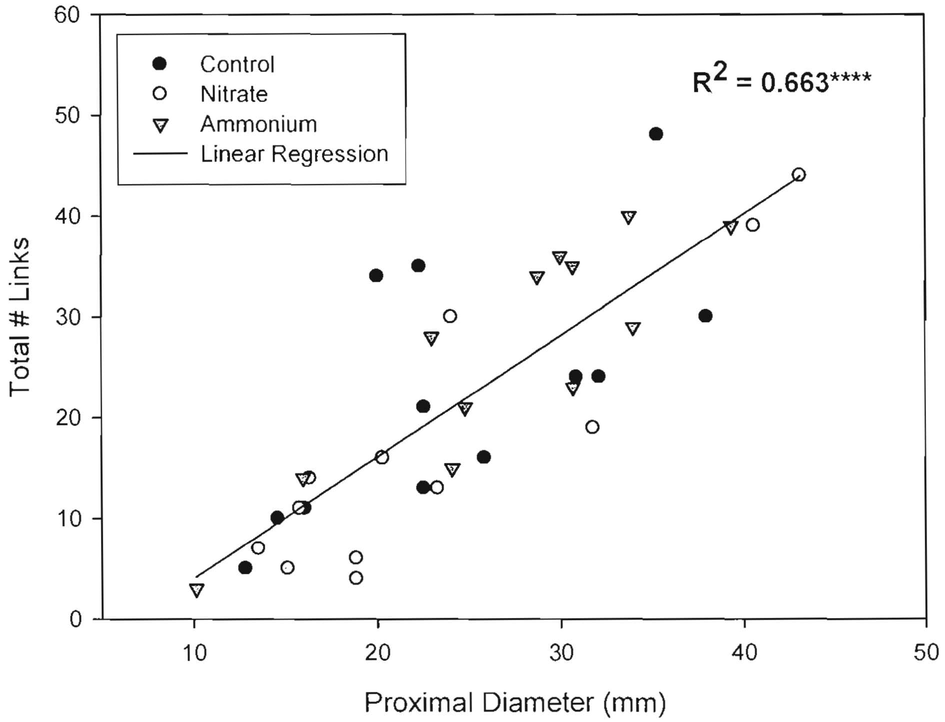

Figure 1.11 Total # links as a function of proximal diameter (mm) for clone 313. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression ($P < 0.0001$) for combined treatments is shown since no significant difference is found between treatments.

1.5 RESULTS

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Overall, the treatment had a significant effect on diameter growth ($P < .0001$) and clonal types had different reactions to fertilisation treatments. The 311 clone reacted positively and significantly to the ammonium and nitrate treatments whereas the 313 clone had no significant reaction to either of the fertilisation treatments. The nitrate treatment for the 311 clone had the greatest growth of any other treatment x clone category (Figure 1.3). However, growth was not significantly greater than in the 311 ammonium-treated trees.

1.5.2 Biomass and allocation

Biomass and allocation measurements were restricted to the 313 clone. The results reflect the previous results on growth where fertilisation had no effect on the biomass allocation of the 313 clone. Mass ratios were similar across treatments (Figure 1.4). On average for all treatments, root biomass accounted for 23% of the total biomass whereas leaves accounted for 26% of the biomass.

Similarly, when total biomass, leaf biomass, and root biomass were plotted as a function of diameter, treatment had no significant effect on the strongly significant positive linear relationships (Figures 1.5, 1.6, 1.7). Likewise, treatment had no significant effect on the root to shoot ratios plotted as a function of diameter. Here the relationship was a negative linear relationship. As diameter increased, less biomass was allocated to roots versus to shoots (Figure 1.8).

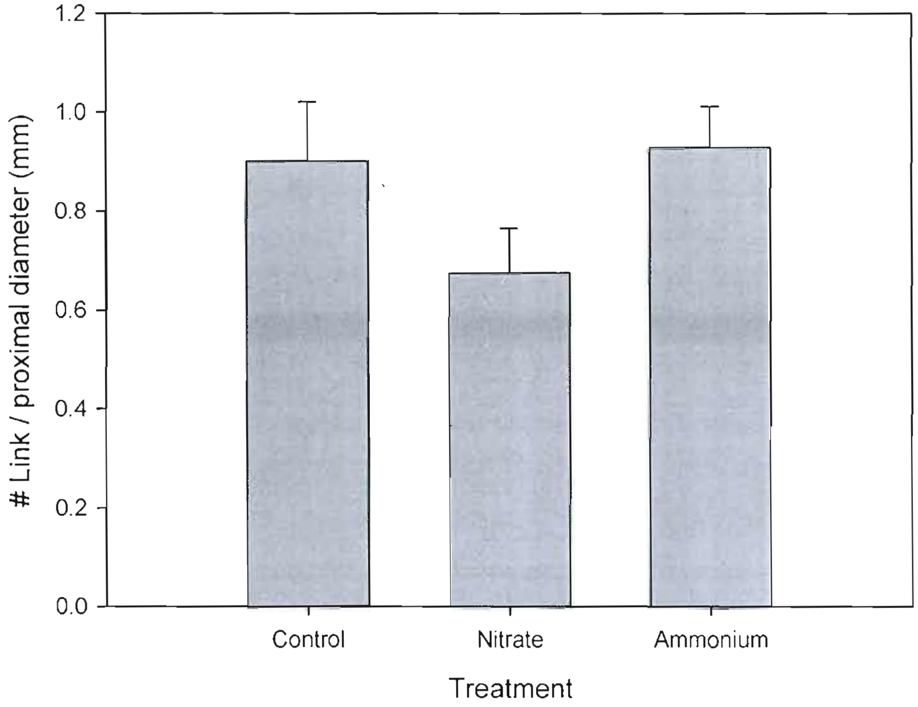

Figure 1.13 Number of links/proximal diameter (mm) of 313 trees excavated in August 2005. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Each bar represents the mean of 6 trees. No significant difference was found across treatments

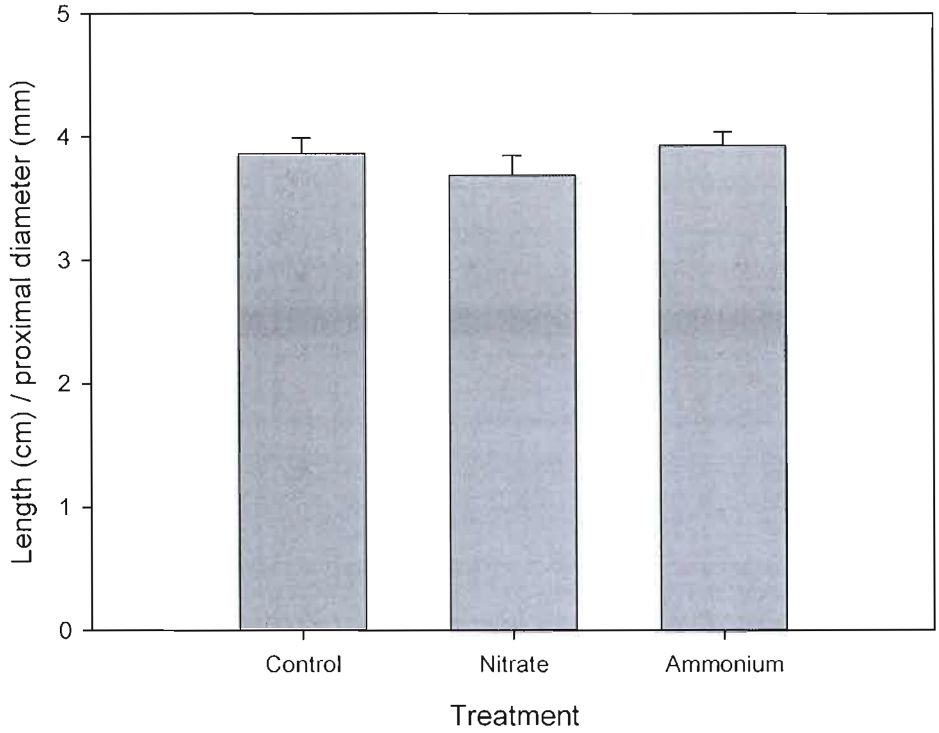

Figure 1.14 Length (cm)/proximal diameter (mm) of 313 trees excavated in August 2005. Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control and 200 kg N ha^{-1} year^{-1} of nitrate and 200 kg N ha^{-1} year^{-1} of ammonium. Each bar represents the mean of 6 trees. No significant difference was found across treatments.

Treatment also has no effect on root order length, mass or link number. However, when mean order length, mass and link number are plotted as a function of total root length, total mass and total link number respectively, we discover highly significant regressions (Figures 1.15, 1.16, 1.17). The relation types varied with root order. For mean order length, order 1 maintained a significant log regression with total root length (R^2 = 0.344, $P < 0.0001$), order 2 a highly significant linear relationship (R^2 = 0.872, $P < 0.0001$) and order 3 a significant exponential relationship (R^2 = 0.691, $P < 0.0001$). Similar results were found for the mean order link number: mean link number for order 1 was best correlated to total link number with a log regression (R^2 = 0.659, P < 0.0001), order 2 with a linear regression (R^2 = 0.981, P < 0.0001) and order 3 with an exponential relationship (R^2 = 0.510, P < 0.0001). For mean order mass however, the relationships between order 1, order 2 and order 3 mean mass and the total mean mass were all linear (R^2 = 0.838, P < 0.0001; R^2 = 0.736, P < 0.0001; R^2 = 0.354, P < 0.001, respectively).

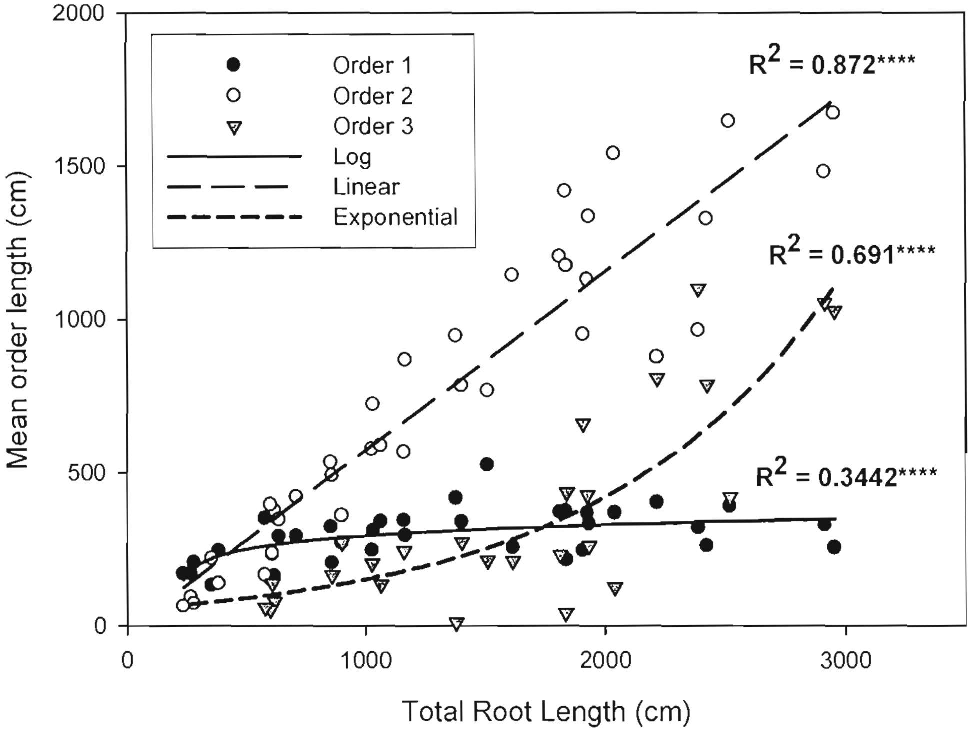

Figure 1.15 Mean order length as a function of total root length (cm) for clone 313. Best fit regressions for combined treatments are as follows: Log regression (*P* < 0.0001) is shown for order 1, least-squared linear regression (*P* < 0.0001) is shown for order 2 and exponential regression (*P* < 0.0001) is shown for order 3. No significant differences were found between treatments.

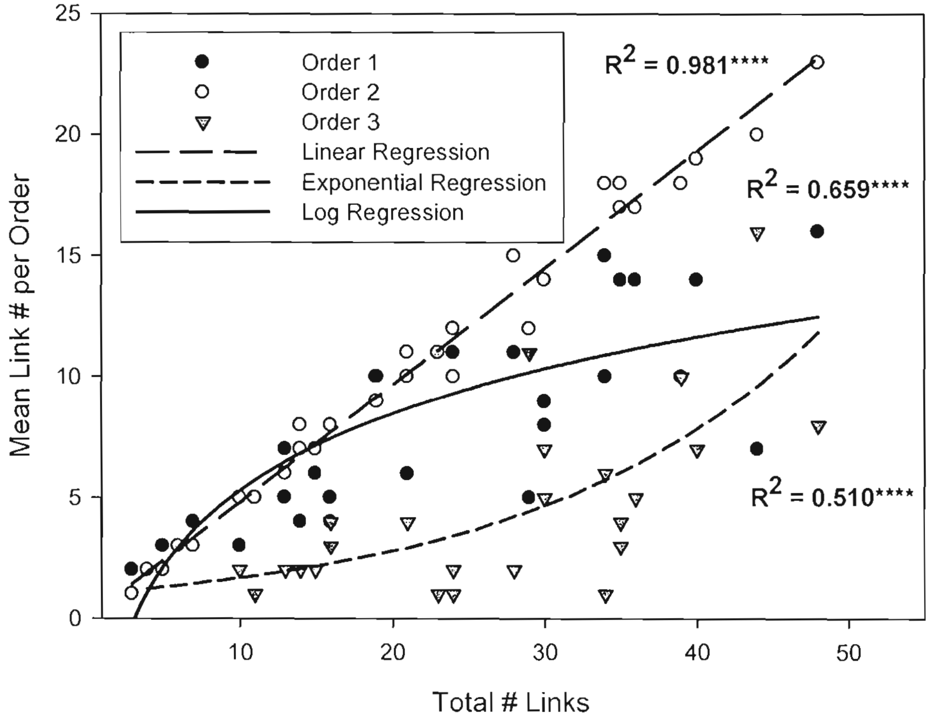

Figure 1.16 Mean number of links per order as a function of total number of links for clone 313. Best fit regressions for combined treatments are as follows: Log regression ($P < 0.0001$) is shown for order 1, least-squared linear regression ($P < 0.0001$) is shown for order 2 and exponential regression ($P < 0.0001$) is shown for order 3. No significant differences were found between treatments.

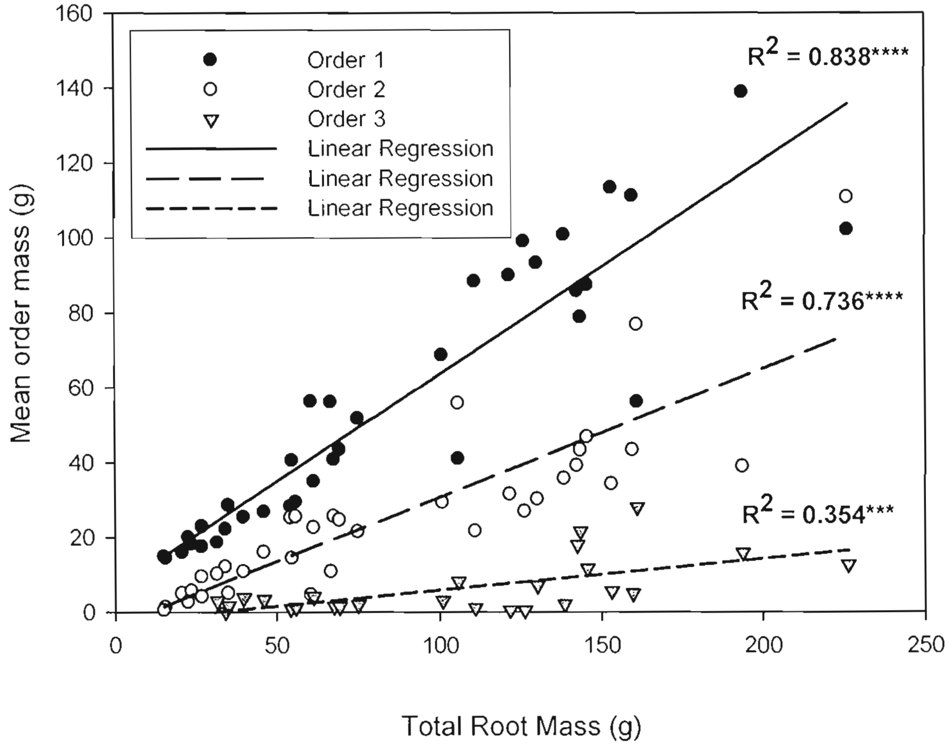

Figure 1.17 Mean order mass (g) as a function of total root mass (g) for clone 313. Best fit regressions for combined treatments are as follows: least-squared linear regression ($P < 0.0001$) is shown for order 1, least-squared linear regression ($P < 0.0001$) is shown for order 2 and least-squared linear regression ($P < 0.001$) is shown for order 3. No significant differences were found between treatments.

The relative biomass allocation to the different root orders was not significantly different across treatments (Figure 1.18). For the combined treatments, order 1 accounted for 68.42 ± 2.4% of the biomass allocated to roots, order 2 for 27.68 ± 2%, and order 3 for 3.90 ± 0.8%. Unlike the allocation of biomass, where the first order accounts for the largest proportion of mass, the 2nd order accounted for the most root length (55.07 ± 2.3%), followed by order 1 (30.24 ± 2.9%) and then order 3 (14.7 ± 2.3%).

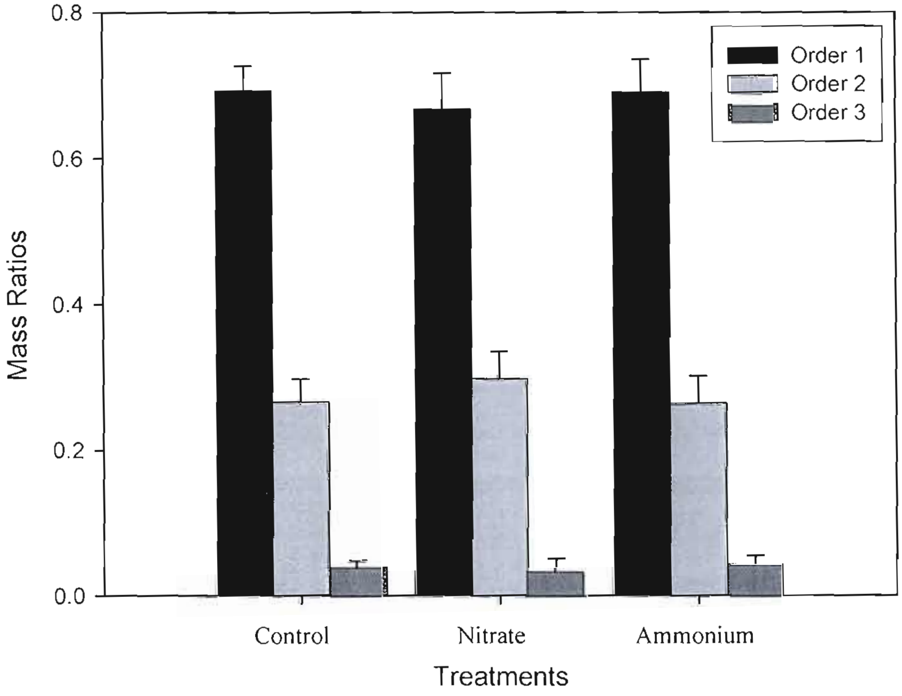

Figure 1.18 Mass ratios (root order biomass/total root biomass) of 313 trees excavated in August 2005. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. No significant differences were found across treatments.

1.5.4 Fine roots

Fine root measurements obtained using WinRHIZO are summarized in Appendix A.1. For clone 311, a nested ANOVA on log-transformed data demonstrates that treatment has no significant effect on fine to coarse root ratio (F:C ratio), surface area/mass, or SRL (Table 1.3). The same tests on clone 313 shows that treatment has a significant effect on F:C ratio ($P<0.01$), surface area/mass ($P<0.05$). We also note a marginal effect in SRL ($P = 0.063$).

For clone 313, the results of a GLM (Table 1.4) indicate that total root mass (g), treatment, and total root mass x treatment have significant effects on total root surface area (cm), surface area <2mm, total length (cm), length <2mm (cm), and tips. For surface area and length >2mm, total mass has a significant effect (P<0.0001), but treatment and mass x treatment do not. The overall models for each of the dependent variables are highly significant for both the 313 and the 311, however the effect of treatment and mass x treatment for clone 311 are not similar to those of 313—they do not have a significant effect on any of the models. Mass x treatment was significant for length > 2mm (*p*<0.05) and for number of tips (*p*<0.05).

Table 1.3 Analysis of variance (Nested ANOVA) summaries for log-transformed F:C ratio, Surface area/Mass, and Specific Root Length (SRL) for clone 313 and clone 311.

Source of variation	df	F:C ratio		Surface area/mass		Specific Root Length	
		MS	P	MS	P	MS	P
Clone 311							
Treatment	2	0.360	0.394	0.102	0.424	0.184	0.495
Gauge[Treatment]	3	0.459	0.318	0.131	0.350	0.360	0.260
Error	30	0.375		0.115		0.255	
Clone 313							
Treatment	2	1.944	0.008	0.317	0.015	0.523	0.063
Gauge[Treatment]	3	0.443	0.294	0.011	0.919	0.041	0.868
Error	29	0.342		0.064		0.172	

Table 1.4 Summary table of GLM testing the effects of total root mass, treatment and total root mass x treatment on fine root architecture parameters. *P* values less than 0.05 were considered significant. R^2 values refer to the overall general linear model. (*SA = surface area; L = length).

Dependent*	Independent	Clone 313				Clone 311			
		F	df	P	R^2	F	df	P	R^2
Total SA	Overall	93.182	34	<.0001	0.931	16.200	35	<.0001	0.685
	Mass	228.484	1	<.0001		72.612	1	<.0001	
	Treatment	6.605	2	0.0043		1.571	2	0.2245	
	Mass x Treatment	10.524	2	0.0004		0.883	2	0.4242	
SA >2mm	Overall	118.518	34	<.0001	0.945	35.779	35	<.0001	0.832
	Mass	213.225	1	<.0001		169.570	1	<.0001	
	Treatment	0.287	2	0.7526		1.062	2	0.3583	
	Mass x Treatment	2.574	2	0.0935		1.943	2	0.1609	
SA <2mm	Overall	17.598	34	<.0001	0.709	6.388	35	0.0004	0.435
	Mass	59.908	1	<.0001		25.984	1	<.0001	
	Treatment	6.336	2	0.0052		1.540	2	0.2308	
	Mass x Treatment	6.789	2	0.0038		0.365	2	0.6973	
Total L	Overall	18.664	34	<.0001	0.722	4.358	35	0.0042	0.324
	Mass	61.543	1	<.0001		17.138	1	0.0003	
	Treatment	3.768	2	0.0351		1.220	2	0.3094	
	Mass x Treatment	7.254	2	0.0028		0.661	2	0.5236	
L >2mm	Overall	54.789	34	<.0001	0.888	21.324	35	<.0001	0.744
	Mass	115.248	1	<.0001		98.573	1	<.0001	
	Treatment	0.388	2	0.6819		1.375	2	0.2684	
	Mass x Treatment	3.548	2	0.0418		3.374	2	0.0477	
L <2mm	Overall	14.516	34	<.0001	0.665	3.808	35	0.0087	0.286
	Mass	50.176	1	<.0001		14.669	1	0.0006	
	Treatment	3.661	2	0.0382		1.169	2	0.3243	
	Mass x Treatment	6.632	2	0.0042		0.589	2	0.5614	
# of Tips	Overall	9.175	34	<.0001	0.546	8.084	35	<.0001	0.503
	Mass	33.073	1	<.0001		24.763	1	<.0001	
	Treatment	5.157	2	0.0121		3.129	2	0.0583	
	Mass x Treatment	7.797	2	0.002		3.917	2	0.0308	

For the 313 clone, trees subjected to the nitrate treatment had more surface area (total and <2mm), length (total and <2mm), and number of tips with increasing total mass than for the ammonium and control treatment. The trend is similar with the 311 clone but the treatment did not have a statistically significant effect (see Table 1.4). For every dependent variable and for both clones, the trend is the same with nitrate having the greatest slope, followed by ammonium and control (ex.: Figure 1.19). The Tukey-Kramer HSD test showed that for the 313 clone, the nitrate treatment is significantly different from the ammonium and control treatments except for total length (>2mm) and total surface area (>2mm). For number of tips, the nitrate treatment and ammonium treatment were significantly different from each other, but not significantly different from the control treatment. For the 311 clone, the Tukey-Kramer HSD test showed no significant differences between treatments for any dependent variable.

1.5.5 Sap flow

For the combined clones and treatments, mean hourly flow during the daytime (6am to 9pm) ranged from 1.686 g/h to 51.986 g/h and averaged at 16.120 (± 1.54 SE) g/h. Mean hourly flow per fine root surface area was 275.510 (±25.68 SE) with a minimum flow of 31.466 g h-1 m-2 and a maximum flow of 792.462 g h-1 m-2. A nested ANOVA showed that treatment had a significant effect ($P<0.05$) on the log-transformed mean hourly flow per surface area data (Table 1.5). The Tukey-Kramer HSD test showed that the nitrate treatment had a significantly higher hourly flow per surface area rate than the control (Figure 1.20). As for mean hourly flow, although treatments did not have a significant effect, nitrate also had the highest mean flow of all three treatments (Figure 1.20).

Table 1.5 Analysis of variance (Nested ANOVA) summaries for log-transformed mean hourly flow per surface area (g h-1 m-2) and mean hourly flow (g h-1) for combined clones.

Source of variation		Hourly flow per surface area		Hourly flow	
	df	MS	P	MS	P
Treatment	2	1.736	0.039	1.086	0.181
Gauge[Treatment]	3	1.058	0.109	0.684	0.352
Clone	1	1.289	0.113	0.666	0.302
Error	41	0.492		0.610	

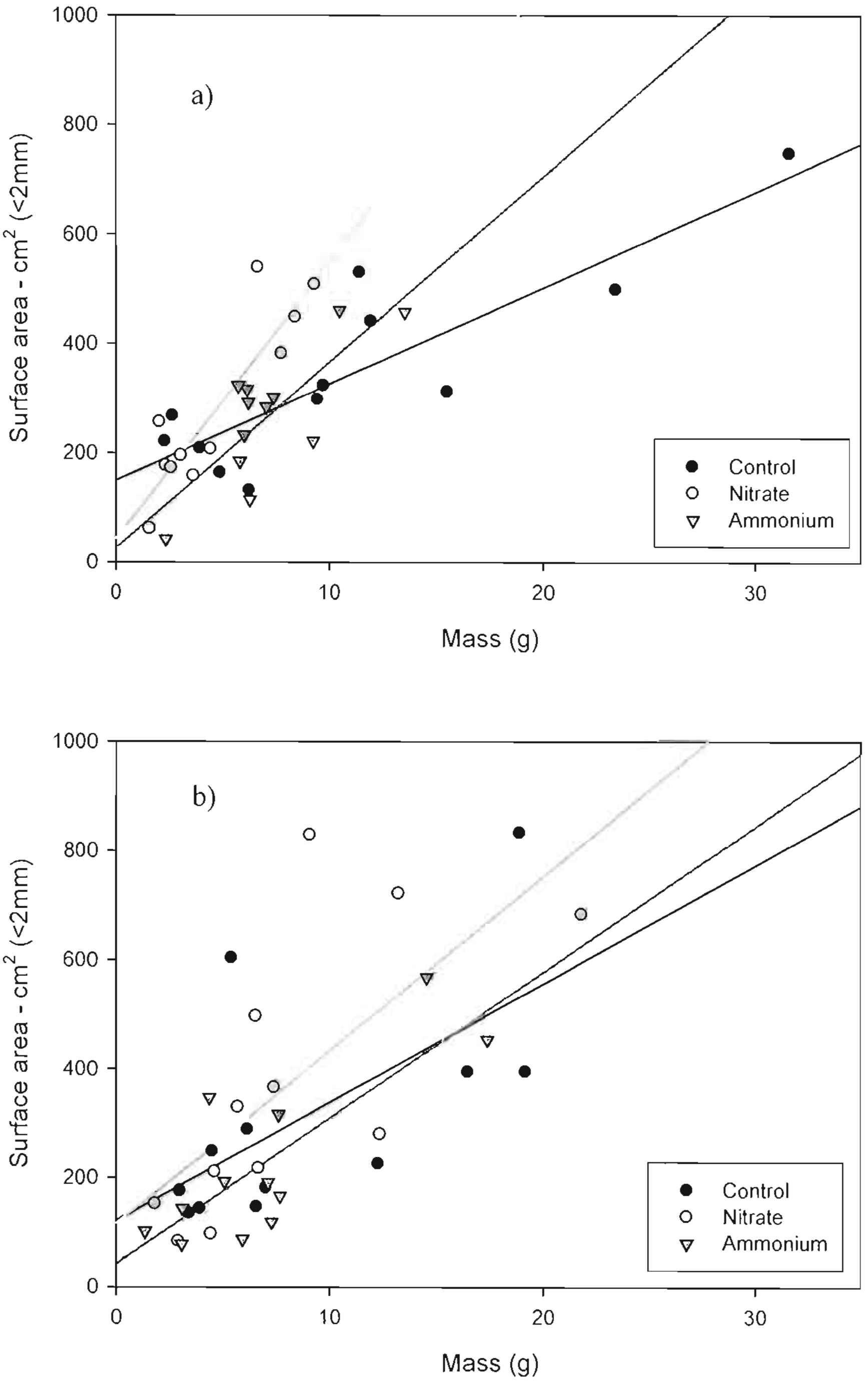

Figure 1.19 Least-square linear regressions for total surface area-cm^2 (<2mm) as a function of mass (g). for each treatment type. a) clone 313, b) clone 311.

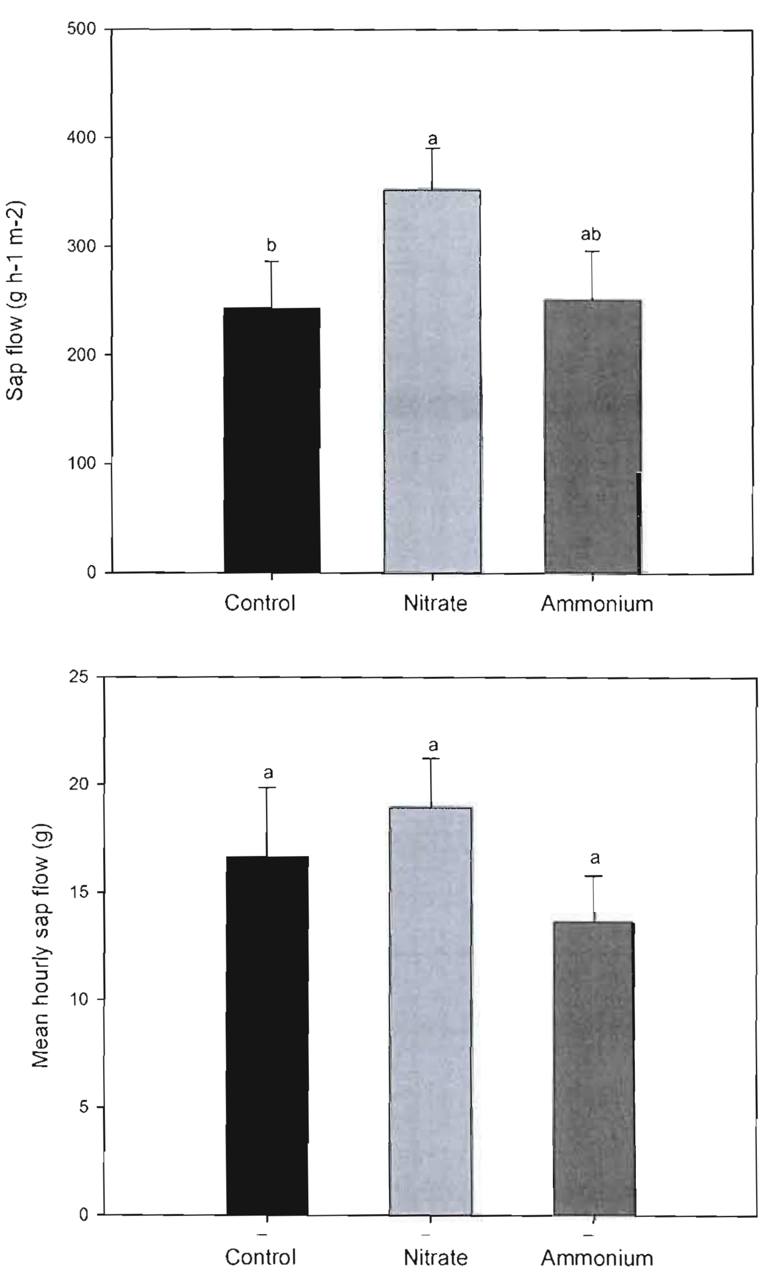

Figure 1.20 a) Sap flow (g h-1 m-2) and b) mean hourly sap flow. Fertiliser treatments were 0 kg N ha^{-1} year^{-1} for the control (T0) and 200 kg N ha^{-1} year^{-1} of nitrate (T1) and 200 kg N ha^{-1} year^{-1} of ammonium (T2). Each bar represents the mean ± SE for each treatment type for combined clones. Treatment bars with the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$).

1.6 DISCUSSION

1.6.1 Treatment effect on overall growth and biomass allocation

Results indicate that clonal types did not react similarly to the fertilisation treatments. Although the nitrate treatments showed most growth in both the 313 and the 311 clonal types, growth for the nitrate-treated trees was only significantly greater than the control treatment in the 311 clone. These findings are coherent with other studies that demonstrated significant differences in overall DBH and height or stem volume growth not only for varying hybrid combinations (Brown and van den Dreissche, 2002; Fang *et al.,* 1999; Michael *et al.*, 1988) but also in hybrids sharing the same genotype (Dickson *et al.*, 1998; Swamy *et al.,* 2006).

It is generally thought that under favourable belowground conditions, the production of belowground plant parts are not favoured compared to aboveground parts. But contrary to the results of several studies (Cooke *et al.*, 2005; Ericsson, 1995; Glynn *et al.,* 2003; Karacic and Weih, 2006; Pregitzer *et al.,* 1990), our results show that biomass allocation to roots, stems, branches or leaves was not significantly different across treatments demonstrating that nitrogen availability or form did not affect biomass allocation in aboveground or belowground fractions. Our results are similar to those of Rippulone *et al.* (2004) and Coleman *et al.* (2004). The latter's results demonstrated that mass ratios did not differ from the control when nitrogen fertilisation was applied at a rate of 200kg N ha-1 yr-1, the same rate applied in this study. Furthermore, the study suggests that changes in allocation are not directly due to fertilisation per se, but rather to the accelerated development caused by the fertilisation. Our results concur with their results in that the mass ratios of the fertilised trees (200kg N ha-1 yr-1 rate) were similar to those of the control treatment. Furthermore, total biomass, leaf biomass and root biomass had a strongly significant positive linear relationship with diameter but had no treatment effect. This further indicates that allocation is largely ontogenetically controlled rather than controlled by resource availability (Delagrange *et al.* 2004).

This study also demonstrated that root: shoot ratios declined as a function of diameter, a phenomenon also demonstrated by Albaugh *et al.* (2006). This relationship was not affected by fertilisation. Other studies on resource availability have focused on water availability rather than soil fertility. These studies have found that allocation to roots is affected by water supply, regardless of the size of the tree (Barton and Montagu, 2006). A study on loblolly pine also showed that resource availability had a small effect on biomass partitioning and that the observed shifts in allocation were greatly affected by the ontogenetic development of the trees (King, 1999). The aim of the present study was not to resolve the issue surrounding biomass allocation theories, but nevertheless, our results demonstrates that ammonium and nitrogen fertilisation did not affect biomass allocation in one of our poplar hybrids. This might not have been the case if irrigation was supplied and perhaps the results would have varied if the analysis was undertaken on the 311 clone. Another point to make is that soil fertility was relatively high in the control treatment since the trees were planted on agricultural grounds versus a more infertile forest environment. Had the experiment been undertaken in a natural forest environment, the results might have been different. Furthermore, future studies might benefit from comparing different plant organ ratios. Root to shoot ratios will decline with tree size since trees accumulate "non-living" biomass in the stem with age, whereas comparing fine root mass to leaf root mass (or area) compares the organ that takes up water and nutrients to the organ that uses the nutrients. The use of such ratios would better reflect the relationship between aboveground ground and belowground dynamics. Studies using advanced genetic testing, such as the one conducted by Wullschleger *et al.* (2005) using quantitative trait loci analysis showed that biomass distribution to aboveground and belowground parts in poplar hybrids is under genetic control. Such studies could help resolve issues related to allocation plasticity.

1.6.2 Treatment effect on proximal roots

Our study demonstrates a positive linear relationship for proximal diameters and total root length, mass and link number independently of fertilisation. By measuring the diameters of proximal roots at the root base, total root length, total link number and mass could easily be estimated. The method requires parameters that are easy to measure and the process itself is non-destructive. More sophisticated and complex models using allometric, fractal, and pipe-model principles have been developed and refined to predict root structure, especially in the field of agroforestry where understanding root structure, and hence root competition for resources, is essential (Ozier-Lafontaine *et al.* 1999; Salas *et al.* 2004; Van Noordwijk *et al.* 1994; West *et al.* 1999). This approach offers a simpler, albeit perhaps less precise, research tool alternative. The proximal root diameter relationships established in this study cannot be generalized to other hybrids because they were established using only the 313 clone, however the ease by which root mass, total length, and link number can be predicted using only the proximal diameter merits more attention. Precision could be enhanced by testing whether water availability or other nutrient resources affect this relationship.

Although this study demonstrates that nitrate and ammonium fertilisation does not significantly affect this relationship, it revealed a trend of increased growth for nitrate-treated trees was noticed. The roots for the nitrate-treated trees had less mass and links per proximal diameter than either control or ammonium-treated trees, but this did not hold true for length per proximal diameter. This trend warrants further investigation.

1.6.3 Treatment effect on root order

Similar to previous findings, fertilisation had no effect on root order biomass allocation, and there were no significant differences between the nitrate and ammonium-treated trees (Figure 1.18). Nutrient availability did not alter the allocation of carbon to the root orders. Even when taking a closer look at root architecture by comparing order root length, order link number, and order root mass to the entire root, there was no treatment effect. Few studies have attempted to understand the effects of fertilisation on root order dynamics of larger roots. Studies by Pretziger *et al* (2002) and Guo *et al* (2004) have investigated the dynamics related to soil fertility and root order dynamics but only in fine roots. Studies looking at the effect of fertilisation on larger roots (roots severed from the root base), as is the case with this study, were not found.

One interesting finding in this study is the different relations between mean order length and total root length for the different orders (Figure 1.15). As total root length increases, the mean length of the first order does not increase very much, order 2 mean root length increases linearly and order 3 increases dramatically when the total root length reaches over 2000 cm. For root order links (Figure 1.16), the same pattern emerges. Order 1 appears to plateau as demonstrated by a log regression between mean link # as a function of total link #, order 2 increases linearly, and order 3 increases exponentially when the total number of links is between 20 and 30. When investigating root order mass however (Figure 1.17), the pattern is strictly linear for all orders—as total root mass increases, individual mean order masses increase linearly. Naturally, mean order root length, link # and mass are all related architecturally. Roots with long mean order lengths, few links and low masses are vastly different from roots with short mean order lengths, many links and high masses. In this case, the 1st order roots do not necessarily increase in length or link number with increasing total root length or increasing link # (or with increasing root size), but their mass constantly accounts for the majority of the total root mass. Architecturally and functionally, we can deduct that these roots are vastly different from 2nd order roots. The 2nd order roots have a linear progression where mean order root length and mean order link # are concerned; the order accounts for the largest proportion of total root length and total link #. The mean order mass also progresses linearly with increasing total root mass but is second in proportion compared to the 1st root order. The 3rd order roots demonstrate a starkly different pattern. Mean order length and link number increase exponentially, but mean order mass remains a very low proportion of the total mass.

These results must be interpreted with caution because the link between form (architecture) and function has not been firmly established and remains a subject of debate in root studies. Nonetheless, one might suggest that these results offer potential insights into the development of the root orders as a function of the whole root. The architectural differences reflect an increasing functional importance for root exploration in the 3rd root order with increasing total root size. For the 1st order, the change in architecture perhaps reflects the increasing functional importance of support and transport since an increase in diameter translates in more tissue for water and nutrient transport.

1.6.4 Treatment effect on fine roots and sap flow

The different clones did not react similarly where fine roots were concerned. Fine roots reacted significantly to the fertilisation treatments for clone 313 but not for 311. Treatments had no effect on F:C root ratio, surface area/mass, or SRL for the 311 clone. For the 313 clone however, the nitrate treatment was found to be significantly different from the ammonium and control treatment for F:C root ratio, surface area/mass and almost significant for SRL (*P* = 0.063). Nitrate-treated trees had greater F:C root ratios than the control and ammonium treatments (approximately 43% and 56% respectively), had greater surface area/mass rates (approximately 19% and 24% respectively), and had greater SRLs (approximately 20% and 27% respectively). Comparing the actual numbers with data from other studies has serious limitations since there is noteworthy variation in fine root characteristics not only among taxon (Pregitzer *et al.*, 2002), but also, environmental and soil conditions can alter fine root characteristics within the same taxa (Block *et al.* 2006; Pregitzer *et al* 2000). Results from other studies are indicative of the variability in fine root response to nutrients. Nitrogen availability has been shown to have no significant effect on fine root biomass, SRL, mean diameter, or root length (Bauer and Bernston 2001; Guo *et al* 2004; Pregitzer *et al* 2002), yet in other studies, we see evidence that nitrogen availability has an effect on fine root biomass, fine root production and mortality (Kern *et al* 2004), lateral root elongation (Lopez-Bucio *et al* 2003), and higher root-order development and branching (Woolfolk and Friend 2003). Our study confirms the notion that individual root response is highly variable and that it is not only species-specific, but in our case, clone-specific.

The GLM analysis revealed that fine root architecture characteristics, such as surface area, total length and number of tips, were all strongly related to mass for both clones. Treatment had a significant effect on these linear relationships for total surface area, surface area<2mm, total length, length <2mm, and number of tips for clone 313. This did not hold true for length >2mm or surface area >2mm, suggesting that the effect of the fertilisation treatments is limited to the smallest of root structures for clone 313. Mass x treatment effect was significant for all fine root characteristics, with the exception of surface area >2mm and was weakly significant for length >2mm. For both the clones, all the graphs in Figure 18 show a similar trend: the nitrate fertilisation treatment always maintains the greatest positive slope, followed by ammonium and then control. Interestingly, the fertilisation treatment had significant effects on overall growth (large scale) and fine roots (small scale), but little effect on the coarse root structure or the allocation of carbon to plant organs.

Few studies have attempted to compare the effects of ammonium versus nitrate nutrition on fine root dynamics or root architecture for that matter. Likewise, research focusing on the varying effects of nitrogen forms on plant growth is confusing at best. Of the 70 studies sited in Martinez-Loucao and Cruz (1999) where nitrate and ammonium fertilisation were compared in various plants, 21 plants supplemented with ammonium showed growth stimulation effects, 39 showed inhibition effects and the rest showed equal or slight improvement of growth. More specific to trees, Bauer and Bernston (2001) have shown in a hydroponic experiment that for both the *Betula* and *Pinus* species, plants grown with nitrate as their sole source of N were smaller than those grown with ammonium—this is in contrast with our growth results where the nitrate-treated trees showed significantly more growth—and nitrate-grown plants allocated a significantly more biomass to roots than ammonium-grown plants, which also contrasts our results for biomass allocation. Although specific root length (SRL) did not vary according to nitrogen form, nitrate-grown plants had a significantly greater root density and fine root diameter (confined to *Pinus*) than ammonium-grown plants. In a hydroponic experiment using *Populus deltoides* Bartr. ex Marsh (eastern cottonwood), nitrogen form was shown to alter root architecture such that an increase in nitrate resulted in higher-order root development or branching (Woolfolk and Friend 2003). However, at 100% nitrate, higher-order root development was hindered. The authors conclude that when nitrogen is provided in a nitrate-dominating form, high root length density results and soil exploitation is facilitated. This would appear to be the case with our results. The nitrate-treated poplars had significantly more surface area (<2mm) and length (<2mm) and tips than the ammonium-treated trees.

We believe that the effect of nitrate on the fine root structures was a factor in the increase of sap flow in the nitrate-treated trees compared to the control trees. We hypothesized that sap flow would increase with increasing surface area since the surface of fine roots is the location of water uptake. Our results confirm that surface area is an important parameter in understanding sap flow. Morphologically, the nitrate-treated trees had significantly more surface area coming from roots with a smaller diameter (<2mm) than in the ammonium and control treatments. The increased surface area would be an important factor in increasing water uptake since one of the functions of smaller roots is water uptake. We cannot make any assumptions concerning the effect of nitrogen availability on the physiological qualities of the fine root structures, but it is has been shown that N fertilisation increases xylem vessel diameters potentially increasing water uptake capacity, but also makes poplars more susceptible to xylem cavitations on dry sites (Harvey and Van den Driessche 1997, 1999). Perhaps the nitrate-treated trees developed larger vessels enabling higher rates of sap flow.

The scope of this study was large; we investigated the effects of different forms of nitrogen from a large scale (i.e. tree growth and biomass allocation) to a fine scale (fine roots and sap flow). This study did not allow us to draw strong conclusions on the effect of fertilisation at all the scales, but the holistic approach does enable us to draw connections between the different plant organs at different scales. For one, nitrate fertilisation led to the development of more fine roots compared to the ammonium and control treatments which potentially led to more efficient absorption of water and nutrients from the soil which was effectively reflected in the higher sap flow rate. Furthermore, the nitrate grown trees had significantly greater overall growth than the control treatment, without altering the biomass allocation to plant organ parts. Several questions remain however, such as how the ammonium-treated trees also manage significantly greater overall growth compared to the control treatment but without the extra fine roots or absorption potential as the nitrate-treated trees? Were the ammonium-treated trees more susceptible to drought than the nitrate-treated trees since they had nearly the same size but not the same amount of fine roots? Had the trees been planted on non-agricultural soil, would the effects of fertilisation been more drastic and significant? Over time, could we expect the trends to maintain, increase or diminish? Longer term and more focused research is necessary to answer these questions.

1.7 CONCLUSION

The two hybrid poplar clones (*P.maximowiczii* x *balsamifera*) used in this study showed different reactions to the nitrogen fertilisation treatments demonstrating that the effect of nutrients on poplar growth and root architecture is not only species-specific but also clone-specific. Both clones exhibited greater growth with increased nitrogen availability, but the effect was only significant for the 311 clone. Nitrogen fertilisation, more specifically the nitrate treatment, also appeared to have an affect on the fine root architecture, and although trends emerged for the larger root structures and architecture, none of these differences were significant. Most importantly, the effect on the fine root structure translated into an effect on the functioning of the fine roots—sap flow was significantly greater in the nitrate treated-trees effectively forming a link between form (architecture) and function (water uptake). The linkages between form and function are not clearly understood where roots are concerned and this study highlights the need for more field studies aimed at understanding how root form affects root function, especially in a complex field environment where knowledge can be more rapidly applied to better environmental management of poplar plantations. From an environmental perspective, this study demonstrates that enriching plantations with ammonium rather than nitrate would potentially lead to the same productivity (as was the case with clone 311) and would decrease the negative effects associated with nitrate leaching in the soil. Nitrate-treated trees did exhibit better sap flow, but information is lacking on the drought sensitivity of ammonium or nitrate-fertilised trees. Additional studies are needed to enhance our knowledge on how nitrate versus ammonium fertilisation would affect poplars in different environmental conditions.

CONCLUSION

The main objective of this research was to address some of the uncertainties surrounding the use of nitrogen fertilisation on hybrid poplar plantations. More specifically, we wanted to better our understanding of how the different forms of nitrogen (NH_4+ versus NO_3) affect poplar growth and root architecture. We suspected that if root architecture was affected by nitrogen fertilisation, then so would hydraulic relations. Thus, a secondary objective was to link form (architecture) to function (sap flow).

There are several approaches to quantifying root architecture, many of which remain highly debated in root science literature. For the sake of simplicity and to encourage knowledge transferability, we opted for methods that were simple yet effective at describing root architecture. At a larger scale (coarse root and proximal roots), we adopted a simple version of the pipe-stem/fractal approach. It allowed us to relate a simple measurement (proximal diameter) to other "hidden" root characteristics. We also adopted a topological approach when attempting to reveal patterns in the different root orders. Deconstructing the roots into links proved useful and revealed interesting results. Finally, for the fine roots, we opted for measurements and ratios that were well established in the literature such as fine root to coarse root ratio, specific root length, total length, etc. Adopting these measurements allowed us to compare our results to other works.

Field work was an important characteristic of this study. Although not performed in a totally natural environment (forest), our plantation in an agricultural setting reflected the reality of future intensive plantations. Maximum yield for poplar plantations is most likely going to occur in a setting where fertility can be controlled and irrigation supplied. If this is the direction of intensive poplar culture, then studies should also be undertaken under the same potential conditions rather than in hyper-controlled environments such as in hydroponic experiments, or in a forest environment where fertilisation is less likely to be applied and hence results are least applicable. Adopting methods which allow for *in situ* measurements are just as equally important. The use of sap flow gauges to quantify the hydraulic effect of fertilisation on poplar roots was not easy to undertake in a field setting, but the results reflect the actual sap flow rather than the "potential" flow if the measurements were taken in a laboratory setting. The sap flow system was designed to work on stems and branches, and perhaps this explains the greater difficulty in using the system on roots. Issues concerning low flow rates and flow reversal at night require attention if the system is to be used to monitor flow for 24 hours rather than just during the day, as was the case with this study.

Excavating whole root systems is extremely time consuming and laborious. Care must be taken to ensure the root system remains intact and methods for excavating the roots must be altered depending on the conditions of the soil, the root diameter size and the degree of precision required. In this study, we only excavated trees from one clone because of the amount of time and labour required to achieve a relatively high degree of precision in root architecture. Studies would benefit from the development of better excavation methods.

Beyond contributing to adapting and developing methods/techniques associated to root studies, this study has allowed us to discover the following:

1) Anecdotal information has led some to believe that a tree's root system will only expand horizontally as far as the tree's vertical height. Although this was not something specifically tested for in this study, it is interesting to point out that poplar roots were found to expand away from the tree a much larger distance than the height of the canopy.

2) Fertilisation, be it in the form of nitrogen or ammonium, positively affects tree growth for clone 311. In our case, allocation to plant organs did not vary amongst treatments but this could be a result of having a relatively fertile control treatment since the experiment took place in an agricultural setting.

3) The diameter of the poplar stem (40 cm above root collar) proved to be a good indicator of total tree biomass, leaf biomass and to a lesser degree root biomass. This method, though not highly precise, does allow a quick estimate of plant biomass. Furthermore, the treatment did not appear to affect this relationship and hence it is perhaps possible to use this method regardless of the soil and environmental conditions.

4) The proximal diameter, a measurement that is easily obtained by non-destructive means, is an excellent indicator of root length, root mass and total link number. The method is not only non-destructive, but requires little time compared to a total root excavation. If this measurement was integrated as a parameter in a model, it could easily be calibrated and validated. Furthermore, the treatments had no affect on the relations therefore, as is the case with the relation between stem diameter and total biomass, it would be possible to use this parameter regardless of the soil and environmental conditions.

5) An attempt was made early on in the project to incorporate fractal principles in our analysis of root architecture. We realized with time that this was perhaps not the most appropriate approach for hybrid poplars. The poplar root cannot be described as a fractal object, that is, as an object that is self-similar resulting from the replicated iteration of its elementary units (links). Our study demonstrated that each root order has a different architecture and that this architecture not only varies between the different root orders, but changes with the size of the root system. A truly fractal object would be similar at all scales. Using fractal geometry to describe the entire poplar root system would be misleading in that it does not fulfill the primary requirement of being a fractal object.

6) The fertilisation treatments have no affect on the root order architecture or on the allocation of carbon to the different root orders. This was to expected considering that the treatments had no effect on the relation between proximal diameter and root length, mass and link number.

7) Fine roots were affected by the fertilisation treatments. Nitrate appeared to have the greatest impact on several fine root characteristics for both clones (but only significantly for one) in that it had a higher fine root to coarse root ratio, significantly more surface area per mass, as well as greater length and number of tips. The impact on the fine root structure also had an impact on the functioning of the roots—the nitrate treated trees also had

the highest sap flow per surface area compared with the control. This forms a crucial link between form and function.

8) A final important, yet perhaps more speculative, point is that the established link between form and function also has a broader environmental application—it can help reduce the negative impacts of fertilisation. It is well known that nitrate leaches easily from the soil and is often found contaminating groundwater and waterways where its use is extensive (Dinnes *et al* 2002). Ammonium, on the other hand, adheres to soil particles and is not easily leached leading to less contamination and hence better plant use of the nitrogen source. There are issues regarding ammonium and nitrification (when ammonium is transformed in the soil to nitrate), however the use of dicyandiamide reduces the effects of nitrification, as was the case in this study. According to our study, there was no significant difference between the two types of fertilisation treatments. Therefore, using ammonium rather than nitrate in plantations could lead to similar yield without the negative impacts associated with nitrate fertilisation. More studies would be required however to ensure that trees treated with ammonium do not become less drought tolerant since they have less fine roots and sap flow than those trees fertilised with nitrate.

Finally, although this study does not allow us to make firm conclusions on the interactions of the different fertilisation effects, it is interesting to note that an effect on the form of smallest, most dynamic structure (fine roots) had an impact on the functioning of the root system, which led to an impact in the overall growth of the tree at a large scale. The tree did not appear to be affected by the fertilisation treatment at an intermediate scale. This highlights the importance of undertaking multi-scaled studies that are not as narrow in scope hence allowing for more holistic conclusions and a broader understanding of the interactions between form and function, the fundamental objective in biological inquiry.

APPENDIX A

		311	
	Control	**Nitrate**	**Ammonium**
Mean root diameter (mm)	0.91 ± 0.04	0.75 ± 0.05	0.92 ± 0.08
Mean diameter origin (mm)	5.51 ± 0.29	5.6 ± 0.29	5.31 ± 0.25
Fine root mass (g)	2.13 ± 0.36	2.2 ± 0.42	1.68 ± 0.46
Coarse root mass (g)	6.75 ± 1.48	5.8 ± 1.24	5.3 ± 0.96
Total mass (g)	8.88 ± 1.77	8.03 ± 1.6	7.02 ± 1.35
F : C ratio	0.44 ± 0.08	0.47 ± 0.09	0.32 ± 0.05
Length <2mm (cm)	2723 ± 537	3415 ± 748	2176 ± 419
Length >2mm (cm)	238 ± 32	267 ± 47	201 ± 37
Total Length (cm)	2961 ± 560	3682 ± 782	2377 ± 447
SRL<2mm (m g-1)	13.36 ± 1.69	17.08 ± 3.61	15.98 ± 1.87
SRL >2mm (m g-1)	0.48 ± 0.08	0.52 ± 0.06	0.41 ± 0.04
SRL (m g-1)	4.05 ± 0.84	4.79 ± 0.71	3.96 ± 0.64
Surface area <2mm (cm2)	314 ± 62	372 ± 73	231 ± 45
Surface area >2mm (cm2)	334 ± 52	357 ± 66	287 ± 51
Total surface area (cm2)	649 ± 105	730 ± 134	517 ± 92
SA <2mm / mass (cm2 g-1)	147.4 ± 12.2	183.5 ± 31.1	168.6 ± 20.0
SA >2mm / mass (cm2 g-1)	61.6 ± 8.3	66.6 ± 5.1	57.8 ± 4.4
Total SA / mass (cm2 g-1)	83.8 ± 10.5	95.6 ± 9.0	81.5 ± 8.2
		313	
Mean root diameter (mm)	1.05 ± 0.10	0.89 ± 0.06	1.05 ± 0.08
Mean diameter origin (mm)	5.82 ± 0.41	5.00 ± 0.21	4.95 ± 0.28
Fine root mass (g)	2.59 ± 0.51	1.87 ± 0.30	1.77 ± 0.27
Coarse root mass (g)	8.51 ± 2.14	3.73 ± 1.01	5.40 ± 0.61
Total mass (g)	11.09 ± 2.56	5.60 ± 1.19	7.17 ± 0.82
F : C ratio	0.43 ± 0.09	0.75 ± 0.18	0.33 ± 0.04
Length <2mm (cm)	2605 ± 391	1907 ± 323	2055 ± 279
Length >2mm (cm)	337 ± 60	194 ± 37	247 ± 30
Total Length (cm)	2942 ± 438	2101 ± 348	2303 ± 304
SRL<2mm (m g-1)	12.31 ± 1.76	10.66 ± 0.78	12.45 ± 1.49
SRL >2mm (m g-1)	0.48 ± 0.04	0.66 ± 0.10	0.47 ± 0.04
SRL (m g-1)	3.49 ± 0.49	4.35 ± 0.49	3.19 ± 0.30
Surface area <2mm (cm2)	345 ± 51	281 ± 44	269 ± 35
Surface area >2mm (cm2)	446 ± 86	249 ± 48	314 ± 36
Total surface area (cm2)	790 ± 131	530 ± 82	583 ± 68
SA <2mm / mass (cm2 g-1)	158.8 ± 17.4	157.1 ± 8.9	159.2 ± 12.0
SA >2mm / mass (cm2 g-1	61.8 ± 5.4	82.7 ± 12.4	58.8 ± 3.5
Total SA / mass (cm2 g-1)	86.3 ± 8.5	106.3 ± 8.9	81.2 ± 4.4

BIBLIOGRAPHY

Albaugh, T.J., Allen, H.L., and Kress, L.W. 2006. Root and stem partitioning of *Pinus taeda*. Trees-Structure and Function 20: 176-185.

Arredondo, J.T. and Johnson, D.A. 1999. Root architecture and biomass allocation of three range grasses in response to nonuniform supply of nutrients and shoot defoliation. New Phytologist 143: 373-385.

Atkinson, D. 2000. Root characteristics: why and what to measure. *In* Root methods : a handbook. Springer-Verlag, Berlin, Germany. pp. 1-32.

Barton, C.V.M. and Montagu, K.D. 2006. Effect of spacing and water availability on root : shoot ratio in *Eucalyptus camaldulensis*. Forest Ecology and Management 221: 52-62.

Bauer, G.A. and Berntson, G.M. 2001. Ammonium and nitrate acquisition by plants in response to elevated CO_2 concentration: the roles of root physiology and architecture. Tree Physiology 21: 137-144.

Bauhus, J. and Messier, C. 1999. Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 29: 260-273.

Ben Brahim, M., Gavaland, A., and Cabanettes, A. 2000. Generalized allometric regression to estimate biomass of *Populus* in short-rotation coppice. Scandinavian Journal of Forest Research 15[2]: 171-176.

Bengough, A.G., Castrignano, A., Pages, L., and van Noordwijk, M. 2000. Sampling strategies, scaling and statistics. *In* Root methods : a handbook. Springer-Verlag, Berlin, Germany. pp. 175-210.

Berntson, G.M. 1994. Root systems and fractals - How reliable are calculations of fractal dimensions. Annals of Botany 73: 281-284.

Berntson, G.M. 1997. Topological scaling and plant root system architecture: Developmental and functional hierarchies. New Phytologist 135: 621-634.

Block, R.M.A., Rees, K.C.J., and Knight, J.D. 2006. A review of fine root dynamics in *Populus* plantations. Agroforestry Systems 67: 73-84.

Bouma, T.J., Nielsen, K.L., Van Hal, J., and Koutstaal, B. 2001. Root system topology and diameter distribution of species from habitats differing in inundation frequency. Functional Ecology 15: 360-369.

Brown, K.R. and Van Den Driessche, R. 2002. Growth and nutrition of hybrid poplars over 3 years after fertilization at planting. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 32: 226-232.

Burgess, S.S.O., Adams, M.A., and Bleby, T.M. 2000. Measurement of sap flow in roots of woody plants: a Commentary. Tree Physiology 20: 909-913.

Clemensson-Lindell, A. and Persson, H. 1995. Fine-root vitality in a Norway spruce stand subjected to various nutrient supplies. 168-169[1]:167-172.

Coleman, M.D., Friend, A.L., and Kern, C.C. 2004. Carbon allocation and nitrogen acquisition in a developing *Populus deltoides* plantation. Tree Physiology 24: 1347-1357.

Coners, H. and Leuscher, C. 2002. In situ water absorption by tree fine roots measured in real time using miniature sap-flow gauges. Functional Ecology 16: 696-703.

Cooke, J.E.K., Martin, T.A., and Davis, J.M. 2005. Short-term physiological and developmental responses to nitrogen availability in hybrid poplar. New Phytologist 167[1]: 41-52.

Cruiziat, P., Cochard, H., and Ameglio, T. 2002. Hydraulic architecture of trees: Main concepts and results. Annals of Forest Science 59: 723-752.

Delagrange S., Messier C., Lechowicz M. J. and Dizengremel P. 2004. Physiological, morphological and allocational plasticity in understory deciduous juvenile trees: the additional importance of individual size. Tree Physiol. 24: 775-784.

Dickmann, D.I., Isebrands, J.G., Blake, T.J., Kosola, K., and Kort, J. 2001. Physiological ecology of poplars. *In* Poplar Culture in North America. NRC Research Press, Ottawa, Ontario, Canada. pp. 77-118.

Dickson, R.E., Coleman, M.D., Riemenschneider, D.E., Isebrands, J.G., Hogan, G.D., and Karnosky, D.F. 1998. Growth of five hybrid poplar genotypes exposed to interacting elevated CO_2 and O_3. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 28: 1706-1716.

Dinnes, D.L., Karlen, D.L., Jaynes, D.B., Kaspar, T.C., Hatfield, J.L., Colvin, T.S., and Cambardella C.A. 2002. Nitrogen management strategies to reduce nitrate leaching in tile-drained midwestern soils. Agronomy Journal 94: 153-171.

Drexhage, M., Chauviere, M., Colin, F., and Nielsen, C.N.N. 1999. Development of structural root architecture and allometry of *Quercus petraea*. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 29: 600-608.

Dynamax Inc. Flow 32 Manual. 238. 2005. Dynamax Inc.

Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15: 763-782.

Enquist, B. 2002. Universal scaling in tree and vascular plant allometry: toward a general quantitative theory linking plant form and function from cells to ecosystem. Tree physiology 22: 1045-1064.

Ericsson, T. 1995. Growth and shoot - Root ratio of seedlings in relation to nutrient availability. Plant and Soil 169: 205-214.

Fang, S., Xu, X., Lu, S., and Tang, L. 1999. Growth dynamics and biomass production in short-rotation poplar plantations: 6-year results for three clones at four spacings. Biomass and Bioenergy 17[5]: 415-425.

Fitter, A.H. 2002. Characteristics and functions of root systems. *In* Plant Roots: the Hidden Half. 3rd. ed. Marcel Dekker, Inc., New York, New York, USA. pp. 15-32.

Fitter, A.H. 1987. An architectural approach to the comparative ecology of plant-root systems. New Phytologist 106: 61-77.

Fitter, A.H. 1986. The topology and geometry of plant-root systems - Influence of watering rate on root-system topology in *Trifolium pratense*. Annals of Botany 58: 91-101.

Fitter, A.H. and Stickland, T.R. 1992. Fractal characterization of root-system architecture. Functional Ecology 6: 632-635.

Fujimaki, R., Tateno, R., Hirobe, M., Tokuchi, N., and Takeda, H. 2004. Fine root mass in relation to soil N supply in a cool temperate forest. 19[5]: 559-562.

Glynn, C., Herms, D.A., Egawa, M., Hansen, R., and Mattson, W.J. 2003. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos 101: 385-397.

Godin, C. 2000. Representing and encoding plant architecture: a Review. Annals of Forest Science 57: 413-438.

Guo, D., Mitchell, R., and Hendricks, J. 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. 140[3]: 450-457. 2004.

Hallé, F., Oldeman, R. A. A., and Tomlinson, P. B. Tropical Trees and Forests: An Architectural Analysis. 441. 1978. Berlin, Germany, Springer-Verlag.

Halley, J.M., Hartley, S., Kallimanis, A.S., Kunin, W.E., Lennon, J.J., and Sgardelis, S.P. 2004. Uses and Abuses of Fractal Methodology in Ecology. Ecology Letters 7: 254-271.

Harvey, H.P. and van den Driessche, R. 1999. Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars. Tree Physiology 19: 943-950.

Harvey, H.P. and van den Driessche, R. 1997. Nutrition, xylem cavitation and drought resistance in hybrid poplar. Tree Physiology 17: 647-654.

Isebrands, J.G. and Karnosky, D.F. 2001. Environmenal benefits of poplar culture. *In* Poplar Culture in North America. NRC Research Press, Ottawa, Ontario, Canada. pp. 207-218.

Jourdan, C. and Rey, H. 1997. Architecture and development of the Oil palm (Elaeis Guineensis Jacq) Root System. Plant and Soil 189: 33-48.

Karacic, A. and Weih, M. 2006. Variation in growth and resource utilisation among eight poplar clones grown under different irrigation and fertilisation regimes in Sweden. Biomass & Bioenergy 30: 115-124.

Kern, C.C., Friend, A.L., Johnson, J.M.F., and Coleman, M.D. 2004. Fine root dynamics in a developing *Populus deltoides* plantation. Tree Physiology 24: 651-660.

King, J.S., Albaugh, T.J., Allen, H.L., and Kress, L.W. 1999. Stand-level allometry in *Pinus taeda* as affected by irrigation and fertilization. Tree Physiology 19: 769-778.

Lopez-Bucio, J., Cruz-Ramirez, A., and Herrera-Estrella, L. 2003. The role of nutrient availability in regulating root architecture. Current Opinion in Plant Biology 6: 280-287.

Lynch, J. 1995. Root architecture and plant productivity. Plant Physiology 109: 7-13.

Martins-Louçao, M.A. and Cruz, C. 1999. Role of Nitrogen Source in Carbon Balance . *In* Nitrogen Nutrition and Plant Growth. Science Publishers, Inc., Enfield, New Hampshire. pp. 205-230.

Michael, D.A., Isebrands, J.G., Dickmann, D.I., and Nelson, N.D. 1988. Growth and development during the establishment year of two *Populus* clones With contrasting morphology and phenology. Tree Physiology 4: 139-152.

Min, X., Siddiqi, M.Y., Guy, R.D., Glass, A.D.M., and Kronzucker, H.J. 1999. A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species. Plant Cell and Environment 22: 821-830.

Moravek, Z. and Fiala, J. 2004. Fractal dynamics in the growth of root. Chaos Solitons & Fractals 19: 31-34.

Nielsen, K.L., Miller, C.R., Beck, D., and Lynch, J.P. 1998. Fractal geometry of root systems: Field observations of contrasting genotypes of common bean (*Phaseolus vulgaris L.*) grown under different phosphorus regimes. Plant and Soil 206: 181-190.

Ong, C.K., Deans, J.D., Wilson, J., Mutua, J., Khan, A.A.H., and Lawson, E.M. 1998. Exploring below ground complementarity in agroforestry using sap flow and root fractal techniques. Agroforestry Systems 44: 87-103.

Oppelt, A.L., Kurth, W., Dzierzon, H., Jentschke, G., and Godbold, D.L. 2000. Structure and fractal dimensions of root systems of four co-occurring fruit tree species from Botswana. Annals of Forest Science 57: 463-475.

Oppelt, A.L., Kurth, W., and Godbold, D.L. 2001. Topology, scaling relations and Leonardo's rule in root systems from African tree species. Tree Physiology 21: 117-128.

Ozier-Lafontaine, H., Lecompte, F., and Sillon, J.F. 1999. Fractal analysis of the root architecture of *Gliricidia sepium* for the spatial prediction of root branching, size and mass: model development and evaluation in agroforestry. Plant and Soil 209: 167-180.

Persson, H.A. 1983. The distribution and productivity of fine roots in boreal forests. Plant and Soil 71: 87-101.

Pregitzer, K.S., Deforest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., and Hendrick, R.L. 2002. Fine root architecture of nine north American trees. Ecological Monographs 72: 293-309.

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

Pregitzer, K.S., Zak, D.R., Maziasz, J., Deforest, J., Curtis, P.S., and Lussenhop, J. 2000. Interactive effects ofaAtmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. Ecological Applications 10: 18-33.

Pregitzer, K.S. 2002. Fine roots of trees - a new perspective. New Phytologist 154[2]: 267-270.

Prusinkiewicz, P. 1998. Modeling of spatial structure and development of plants: a review. Scientia Horticulturae 74: 113-149.

Regent Instruments Inc. For root morphology and architecture measurement. 2005. June 2006.

Riemenschneider, D.E., Stanton, B.J., Vallée, G., and Périnet, P. 2001. Poplar breeding strategies. *In* Poplar Culture in North America. NRC Research Press, Ottawa, Ontario, Canada. pp. 43-76.

Ripullone, F., Lauteri, M., Grassi, G., Amato, M., and Borghetti, M. 2004. Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus x euroamericana*; a comparison of three aproaches to determine water-use efficiency. Tree Physiology 24: 671-679.

Rose, D.A. 1983. The description of the growth of root systems. Plant and Soil 75: 405-415.

Sakuratani, T. 1981. A heat balance method for measuring water flux in the stem of intact plants. Journal of Agricultural Meteorology 37: 9-17.

Salas, E., Ozier-Lafontaine, H., and Nygren, P. 2004. A fractal root model applied for estimating the root biomass and architecture in two tropical legume tree species. Annals of Forest Science 61: 337-345.

Sievänen, R., Nikinmaa, E., Nygren, P., Ozier-Lafontaine, H., Perttunen, J., and Hakula, H. 2000. Components of functional-structural tree models. Annals of Forest Science 57: 399-412.

Smith, D.M. 2001. Estimation of tree root lengths using fractal branching rules: a comparison with soil coring for *Grevillea robusta*. Plant and Soil 229: 295-304.

Smith, D.M. and Roberts, J.M. 2003. Hydraulic conductivities of competing root systems of *Grevillea robusta* and maize in agroforestry. Plant and Soil 251: 343-349.

Spek, L.Y. and van Noordwijk, M. 1994. Proximal root diameter as predictor of total root size for fractal branching models 2. Numerical-model. Plant and Soil 164: 119-127.

Stanturf, J.A., Oosten, V.C., Netzer, D.A., and Coleman, M.D.P.C.J. 2001. Ecology and silviculture of poplar plantations. *In* Poplar Culture in North America. NRC Research Press, Ottawa, Ontario, Canada. pp. 153-206.

Stokes, A. 2002. Biomechanics of tree root anchorage. *In* Plant Roots: the Hidden Half. 3rd. ed. Marcel Dekker, Inc., New York, New York, USA. pp. 175-186.

Swamy, S.L., Mishra, A., and Puri, S. 2006. Comparison of growth, biomass and nutrient distribution in five promising clones of *Populus deltoides* under an agrisilviculture system. Bioresource Technology 97[1]: 57-68.

Tatsumi, J., Yamauchi, A., and Kono, Y. 1989. Fractal analysis of plant-root systems. Annals of Botany 64: 499-503.

van den Driessche, R., Rude, W., and Martens, L. 2003. Effect of fertilization and irrigation on growth of Aspen (*Populus tremuloides* Michx.) seedlings over three seasons. Forest Ecology and Management 186: 381-389.

van Noordwijk, M. and Mulia, R. 2002. Functional branch analysis as tool for fractal scaling above- and belowground trees for their additive and non-additive properties. Ecological Modelling 149: 41-51.

van Noordwijk, M. and Purnomosidhi, P. 1995. Root architecture in relation to tree-soil-crop interactions and shoot pruning in agroforestry. Agroforestry Systems 30: 161-173.

van Noordwijk, M., Spek, L.Y., and Dewilligen, P. 1994. Proximal root diameter as predictor of total root size for fractal branching models .1. Theory. Plant and Soil 164: 107-117.

Walk, T.C., Van Erp, E., and Lynch, J.P. 2004. Modelling applicability of fractal analysis to efficiency of soil exploration by roots. Annals of Botany 94: 119-128.

Weih, M. 2004. Intensive short rotation forestry in boreal climates: present and future perspectives. Canadian Journal of Forest Research 34: 369-378.

West, G.B., Brown, J.H., and Enquist, B.J. 1999. A general model for the structure and allometry of plant vascular systems. Nature 400: 664-667.

Woolfolk, W.T.M. and Friend, A.L. 2003. Growth response of cottonwood roots to varied NH_4 : NO_3 ratios in enriched patches. Tree Physiology 23: 427-432.

Wullschleger, S., Yin, T.M., Difazio, S.P., Tschaplinski, T.J., Gunter, L.E., Davis, M.F., and Tuskan, G.A. 2005. Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. Canadian Journal of Forest Research 35: 1779-1789.