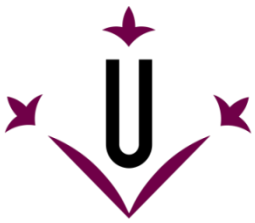


Nitrogen forms affect root structure and water uptake in the hybrid poplar

SUSY DOMENICANO [1, 3], LLUIS COLL [1, 2], CHRISTIAN MESSIER [1] AND FRANK BERNINGER [1]

1) Université du Québec a Montréal

   Département des Sciences Biologiques
   CP 8888 Succ Centre Ville
   Université du Québec à Montréal
   Montréal, Qc H3C 3P8
   Canada

2) Forest Technology Center of Catalonia, Ctra Sant Llorenç, km 2. 25280 Solsona, Spain

3) Corresponding author:
   s.domenicano@sympatico.ca

   Tel.: (514) 987-3000 (ext: 1644)

   Fax: (514) 987-4647

Summary

The study analyses the effects of two different forms of nitrogen fertilisation (nitrate and ammonium) on root structure and water uptake of two hybrid poplar (*Populus maximowiczii x P. balsamifera*) clones in a field experiment. Water uptake was studied using sap flow gauges on individual proximal roots and coarse root structure was examined by excavating 18 whole-root systems. Nitrogen forms did not affect coarse-root system development, but had a significant effect on fine-root development. Nitrate-treated trees presented higher fine:coarse root ratios and higher fine-root surface areas by unit of mass than control or ammonium treated trees for one clone. These structural differences affected the water uptake capacity of the plants as reflected by the higher sapflow rate in the nitrate treatment. The fertilised trees presented higher aboveground growth but no changes in biomass distribution. The diameter of proximal roots at tree base predicted well the total root biomass and length. No explanation was found for the lack of effect on the other clone, however.

Key words: nitrogen fertilisation, hybrid poplars, root structure, fine roots, proximal roots, water uptake, sapflow.

Introduction

Below-ground resource acquisition, be it for nutrients or water, is intimately linked with root soil exploration and exploration efficiency, and therefore with root morphology and architecture, the latter referring 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 (Lynch 1995, Fitter 2002). Furthermore, plants with higher specific root lengths—one of many root morphological 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).

Many studies have investigated the effects of soil fertility on root growth, morphology and architecture (see for example Pregitzer et al. 2002, Hodge 2004), but none has yet investigated the functional linkage between changes in root morphology and architecture induced by fertilisation and root water conductivity in the field. Nitrogen has been shown to be an important determinant of vertical root distribution in the soil (Fujimaki *et al*. 2004), root biomass (Bauer & Berntson 2001), fine root architecture (Woolfolk & Friend 2003) and fine root vitality (Clemensson-Lindell & Persson 1995). However, the differential effect of nitrate and ammonium in inducing such changes is not known. Nonetheless, the physical, chemical and biological processes associated with the different forms of nitrogen are vastly different (Min et al. 1999) and their relative abundances could affect root morphology, architecture and its efficiency. 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.

The aim of the research was to understand how fertilisation affects the balance between root morphology and root function taken size as a possible covariate. Since poplars have a *slight* preference for ammonium (Dickmann et al. 2001) and because ammonium is less mobile in the soil, we hypothesize that trees fertilised with ammonium will have lower fine-root to coarse-root ratios than trees fertilised with nitrate. We hypothesize that these changes in plant morphology will result in different water uptake efficiencies, especially ammonium-fertilised trees. We also predict that fertilisation will increase growth aboveground and decrease allocation to belowground parts (Poorter & Nagel 2000).

Material and methods

Experimental design

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.

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. 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, respectively. 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, respectively. The experiment was set up as a full factorial experiment with four blocks of four trees per clone (311 or 313) per treatment (control, ammonium and nitrate fertilisation). 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^{-1} of nitrogen at the end of each growing season. For the nitrate treatments, 3 equal applications of potassium nitrate were applied manually at the base of the tree (1 m radius) and for the ammonium treatments, 3 equal applications of ammonium sulphate were applied manually at the base of the tree (1 m radius). Fertilisation was applied at equal intervals and trees were given time to establish themselves and show signs of growth prior to the initial treatment. Herbaceous vegetation was removed using a systemic herbicide (RoundUp®). Throughout the 2004 and 2005 growing seasons, we recorded growth in diameter at 40cm above root collar and height.

Soil nutrient monitoring was conducted using PRS probes (Western Ag Innovations, Saskatoon, SK, Canada). During the summer of 2004 we found that the ratio of ammonium to nitrate ions was low (21% ammonium) in the ammonium treatment. Suspecting a large amount of nitrification, we applied a nitrification inhibitor, dicyandiamide (DCD) to the ammonium treatment, to maintain higher levels of available ammonium in the soil the year after (2005). In 2004, following a three-week burial period (21 days), the PRS™ probes revealed the following nitrogen supply (μg/10 cm^{-2}):

control treatment NO_3^- = 64, NH_4^+ = 5; nitrate treatment NO_3^- = 234, NH_4^+ = 5; ammonium treatment NO_3^- = 147, NH_4^+ = 358. As a result of the DCD application in 2005, the nitrate dominated the nitrogen supply in the nitrate and control treatments (less than 10% ammonium), while ammonium supplied 70 % of the nitrogen in the ammonium treatment.

Tree measurements

During August 2005, we randomly selected 18 trees of the 313 clones for total root excavations (6 from each treatment type: control, nitrate and ammonium). Total root excavations were limited to roots greater than 2mm. The roots were excavated by hand and great care was taken to maintain the integrity of the root system architecture.

We took among all treatments a sample of 38 leaf samples from the mid crown for nutrient analysis. The samples were taken systematically across treatment and clones. The samples were homogenised in a mortar and then analysed for their contents in N, P, K, Ca and Mg using colorimetric analysis.

Once excavated, the root systems were severed from the stem at the root collar. The aboveground parts of trees were then separated into the following compartments for biomass analysis: leaves, branches and stem. From each excavated root system, we randomly chose and severed two main roots for root architecture analysis. These will be referred to as proximal roots. Plant parts were brought to the lab where the root systems were carefully washed and dried at 60°C until constant mass.

Measurements on proximal roots

Prior to oven drying for biomass computation, 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). All links 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.

Measurements on sapflow and sampled whole lateral root systems

At the end of the 2005 growing season, we randomly selected 18 trees from each clonal type (311 and 313), and six from each treatment (control, nitrate and ammonium). For each tree, we randomly selected two whole lateral roots for sapflow and root measurements. Because the trees selected from the 313 clone were the same as those for the whole-root excavation and that sapflow 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 sapflow, 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.

Sapflow was measured using the stem heat balance methods (Sakuratani 1981). We used Dynagage SGA5 microsensors (Dynamax Inc. Houston, TX, USA) and adapted Coners & Leuschner's (2002) field protocol to our experimental design. We exposed suberized roots of approximately 4-5 mm in diameter at a distance of 1-2 m from the stem for sapflow measurements. A small pit was dug around the root to allow easy installation of the gauges. After installation, the hole was filled with insulating packing material to reduce temperature fluctuations. A foam board covered the pit opening to further protect the gauges and diminish temperature fluctuations. We calculated sapflow in g h^{-1} (grams per hour) from 6 am to 9 pm for seven consecutive days. Because of technical problems, 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 sapflow data revealed technical problems (indicated by extreme values or unusually large fluctuations). Problems in the functioning of sapflow systems and rejection of portions of the data are normal procedures in research using these sapflow sensors.

When sapflow monitoring was complete, each sampled whole lateral root was carefully excavated by hand from the point where the gauge was installed to the finest of root tips. The whole lateral roots were kept intact and fresh in a sealed bag in a cooler for further analysis using image analysis software (WinRHIZO Pro v. 2005B, Regent Instrument Inc. Montréal, QC, Canada). Once at the lab, roots were washed then scanned (400 dpi on an LC4800-II scanner with a double lightning system). Average diameter, total length, length by diameter class and the total surface area, were analysed for each sample. After

scanning, 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.

Statistical analysis

Tree growth was tested using a repeated measures analysis using the PROC MIXED 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 sapflow. In the case of sapflow, two gauges were installed per tree hence the ANOVAs were nested (gauge within treatment) to take this into account. Regressions were used to establish relationships between root architecture parameters and tree size diameters. For every analysis, the significance level was considered when P was less than 0.05.

Results

Tree growth

The 311 clone reacted positively and significantly to the ammonium and nitrate treatments in diameter growth ($P < 0.0001$), whereas the 313 clone had no significant reaction to either of the fertilisation treatments.

Because of the time and cost constraints for below-ground excavation, only the 313 clone was selected randomly for biomass and allocation measurements. Fertilisation had no effect on the biomass allocation of the 313 clone. Mass ratios were similar across

treatments. On average, root biomass accounted for 23% of the total biomass and leaves accounted for 26% (data not shown). Poplars of both clones varied in diameter at 40 cm between 38 and 58 cm and in total biomass between 1900 g and 4800 g.

Similarly, when total biomass, leaf biomass, and root biomass were plotted as a function of diameter, treatments had no significant effects on the strongly significant positive linear relationships (Figure 2). Likewise, treatments had no significant effects on the root to shoot ratios plotted as a function of tree basal diameter. As diameter increased, less biomass was allocated to roots versus to shoots (Figure 3).

Foliar nutrient concentrations

The average content of N in the samples was 3.2 % (with a range from 0.9 to 4.2 %). Ca contents varied from 0.09??? to 0.98 % with a mean of 0.19. For Mg, the concentrations varied between 0.07 and l.49 % with a mean 0.3, while Potassium had contents between 0.68 and 1.84 % with a mean of 1.42. The gravimetric potassium to nitrogen ratio was 0.45. This ratio decreases with increasing N content ($p<0.001$). The ratios of the concentrations of the other nutrients to the concentration of N were not correlated with the N –concentration nor vary with treatments (data not shown)

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 ($R^2 = 0.663$, $P < 0.0001$) (Figure 4). ANOVAs revealed that the nitrogen fertilisation treatments had no significant effects on total root

length, mass or link number when the parameters were used as covariates. However, even though not statistically different, nitrate-treated trees had consistently lower values of these variables 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 (data not shown). For link number per proximal diameter, the mean nitrate values were 25% lower than the control and 27% lower than the ammonium. 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.

Treatments also had no effects 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 (Figure 5). These relations 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 orders 1, 2 and 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).

The relative biomass allocation to the different root orders was not significantly different across treatments. 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%).

Sampled lateral roots

For clone 311, the nested ANOVA on log-transformed data demonstrated that treatments had no significant effects on fine to coarse root ratio (F:C ratio), surface area/mass, or SRL (Table 1). The same tests on clone 313 showed that treatments had a significant effect on F:C ratio ($P < 0.01$) and surface area/mass ($P < 0.05$). We also noted a marginal effect in SRL ($P = 0.063$).

For clone 313, the results of a GLM (Table 2) indicated that lateral root mass (g), treatment, and lateral root mass x treatment had significant effects on total root surface area (cm), surface area <2mm, total length (cm), length <2mm (cm), and tips. All these parameters increased in this clone when increasing total mass and were higher in the nitrate treatments. The trend is similar with the 311 clone but the effects were not statistically significant (see Table 2).

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 and ammonium

treatments were significantly different from each others, 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.

Sapflow

For the combined clones and treatments, mean hourly flow during the daytime (6 am to 9 pm) ranged from 1.686 g h^{-1} to 51.986 g h^{-1} and averaged 16.120 (± 1.54 SE) g h^{-1}. 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 treatments had significant effects ($P < 0.05$) on the log-transformed mean hourly flow per surface area data (Table 3). The Tukey-Kramer HSD test showed that the nitrate treatment had a significantly higher hourly flow per surface area rate than the control (Figure 6a). 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 6b).

Discussion

We hypothesized that under favourable belowground conditions, the production of belowground plant parts would be reduced compared to aboveground growth (in agreement with Pregitzer et al. 1990, Ericsson 1995, Glynn et al. 2003, Cooke et al. 2005,

Karacic & Weih 2006). But contrary to our expectations, the results indicate 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 between aboveground and belowground fractions. Our results agree with those of Rippulone et al. (2004) and Coleman et al. (2004) where biomass ratios were unaffected by nitrogen treatments. Changes in allocation did not occur due to fertilisation, but rather due to ontogenic development caused by the fertilisation. Root shoot ratio decline with tree size, but there was no treatment effect as in Albaugh et al. (2006). This further indicates that ontogenic control of allocation seems to be more important than resource-based control (Delagrange et al. 2004). However, the nitrogen contents of the unfertilised trees were quite high and it is possible that trees were already saturated with nitrogen.

A problem of our experiment was that the high nitrogen concentrations in our trees were not matched by equally high concentrations of other nutrients. While absolute concentration of potassium were quite high, the ratios of potassium to nitrogen concentrations were below normal and indicated that our trees might have become limited by potassium (Van den Driessche and Rieche 1974). In particular the lack of correlation between growth and nitrogen concentration could be caused by other nutrients becoming more and more limiting. We do not know to what extend our results on root structure and function could be caused by low potassium concentrations. However, the trees did not show any signs of potassium deficiency such as chlorotic spots on leaves or necroses close to the leaf corners. Altogether, this indicates that potassium levels were in the deficient range, but we estimate that the effects on growth and structure of our trees

were limited. It has, however, been reported that potassium deficiency reduces elongation of lateral roots (Armengaud *et al.*, 2004).

Our prediction of higher root mass, length and link number in roots of unfertilised trees (when compared with fertilised trees) was not supported by our results although the roots for the nitrate-treated trees tended to present less mass and links per proximal diameter than either control or ammonium-treated trees. In any case, the pattern was rather variable and warrants further investigation. Our methods did however reveal that by measuring the diameters of sampled lateral roots at the root base, root length, link number and mass for that sampled root could easily be estimated. The method requires the measurements of only one parameter (lateral root diameter) and the process itself is non-destructive. This approach seems to be a stable, less laborious, albeit less informative, alternative to more sophisticated geometry based models (Van Noordwijk et al. 1994, Ozier-Lafontaine et al. 1999, West et al. 1999, Salas et al. 2004, Coll et al. 2008). The ease by which root mass, total length, and link number can be predicted using only the lateral root diameter merits more attention.

Unlike our predictions, fertilisation also had no effect on biomass allocation among different 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. Our study did, however, reveal how the architecture and morphology of different root orders change with root size. These results offer potential insights into the development of the root orders as a function of the whole root. For the first order, the change in architecture reflects the increasing functional importance of support and transport since an increase in

diameter and mass accompanied by little growth in length translates in more tissue for water and nutrient transport. The functional role of soil exploration would appear to be taken up by the second root order, accounting for over half the root system's length and number of links. Unlike the first and second order, the length and link number associated to the third root order increases exponentially. The architectural differences of the third root order reflect an increasing functional importance for root exploration with increasing root size. The fact that allocation to different root orders did not change as a function of nitrogen form indicates that allocation was rather altered by ontogenic development and less by fertilisation.

Where fine roots are concerned, we predicted that investment in fine roots structures would be greater in non-fertilised trees. Fine roots reacted significantly to the fertilisation treatments for clone 313 but not for 311. Comparing our findings 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 among environmental and soil conditions that can alter fine root characteristics within the same taxa (Pregitzer et al. 2000, Block et al. 2006). 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 & Bernston 2001, Pregitzer et al. 2002, Guo et al. 2004), 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 & Friend 2003).

The GLM analysis revealed that fine root architecture characteristics, such as surface area, total length and number of tips, were all strongly related to root mass for both clones suggesting that the effects of the fertilisation treatments are limited to fine roots for the clone 313. One trend worth noting is that for both clones, fine roots was always most responsive to root mass in the nitrate fertilisation treatment, 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. Likewise, research focusing on the varying effects of nitrogen forms on plant growth is confusing at best. Of the 70 studies cited in Martinez-Loucao & 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. Bauer & Bernston (2001) showed in a hydroponics experiment that both *Betula* and *Pinus* species 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. In a hydroponic experiment using *Populus deltoides* Bartr. ex Marsh (eastern cottonwood), nitrogen forms were shown to alter root architecture such that an increase in nitrate resulted in higher-order root development or branching (Woolfolk & Friend 2003). However, at 100% nitrate, higher-order root development was hindered. The authors concluded that when nitrogen is provided in a nitrate-dominating form, high root length density is produced

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), 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 sapflow in the nitrate-treated trees compared to the control trees. We hypothesized that sapflow would increase with increasing surface area since the surface of fine roots is the location of water uptake. Nitrate fertilisation led to the development of more fine roots compared to the ammonium and control treatments. Although not significant, the nitrate treatment demonstrates higher hourly sap flow than the control and ammonium treatment confirming that surface area is an important parameter in understanding sapflow. When normalized, flow per surface area is significantly higher in the nitrate treatment (vs. control) which may suggest higher hydraulic conductivity (axial, radial or both) in the nitrate treatment. We cannot make any assumptions concerning the effect of nitrogen availability on the physiological qualities of the fine root structures, but it 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 & Van den Driessche 1997, 1999). Perhaps the nitrate-treated trees developed larger vessels enabling higher rates of sapflow.

The two hybrid poplar clones 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—sapflow 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.

REFERENCES

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

Armengaud P., R. Breitlingand A. Amtmann. 2004. The potassium-dependent transcriptome of Arabidopsis reveals a prominent role of jasmonic acid in nutrient signaling. Plant Physiol. 136: 2556–2576.

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

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

Clemensson-Lindell A. and H. Persson. 1995. Fine-root vitality in a Norway spruce stand subjected to various nutrient supplies. Plant Soil 1: 167-172.

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

Coll L., C. Potvin, C. Messier and S. Delagrange. 2008. Root architecture and allocation patterns of eight native tropical species with different successional status used in open-grown mixed plantations in Panama. Trees, 22: 585-596.

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

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

Delagrange S., C. Messier, M.J. Lechowicz and P. Dizengremel . 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., J.G. Isebrands, T.J. Blake, K. Kosola and J. Kort. 2001. Physiological ecology of poplars. *In*: Dickmann DI, ed. Poplar Culture in North America. Ottawa, Canada: NRC Research Press, 77-118.

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

Eissenstat D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15: 763-782.

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

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

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

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

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

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

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

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

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 162[1]: 9-24.

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

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

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

Martins-Louçao M.A. and C. Cruz. 1999. Role of Nitrogen Source in Carbon Balance . *In*: Srivastava HS, Singh RP, eds. Nitrogen Nutrition and Plant Growth. Enfield, USA: Science Publishers, Inc. 205-230.

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

Ozier-Lafontaine H., F. Lecompte and J.F. Sillon. 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 Soil 209: 167-180.

Poorter H. and O. Nagel. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Aust. J. Plant Physiol. 27: 1191–1191.

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

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

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

Pregitzer K.S. 2002. Fine roots of trees - a new perspective. New Phytol. 154: 267-270.

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

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

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

Sakuratani T. 1981. A heat balance method for measuring water flux in the stem of intact plants. J. Agric. Meteorol.37: 9-17.

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

Van den Driesche R. and K. Rieche. 1974. Prediction of Mineral Nutrient Status of Trees by Foliar Analysis. Bot. Rev. 40: 347-394.

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

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

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

TABLES

Table 1. Analysis of variance (Nested ANOVA) summaries for log-transformed Fine:Coarse root ratio (F:C), Surface area:Mass, and Specific Root Length (SRL) for sampled whole lateral root systems from clone 313 and clone 311.

Source of variation	df	F:C ratio MS	F:C ratio *P*	Surface area/mass MS	Surface area/mass *P*	Specific Root Length MS	Specific Root Length *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	3	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 2. Summary table of GLM testing the effects of root mass, treatment and root mass x treatment on fine root architecture parameters of sampled lateral root systems. *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	

Table 3. 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	

FIGURES

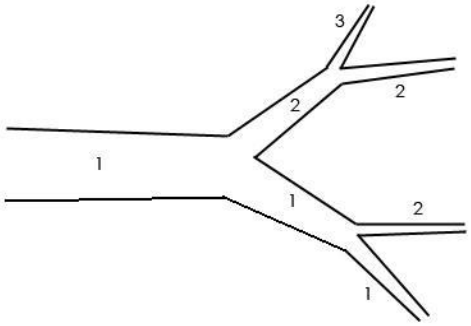


Figure 1. Schematic representation of the hybrid poplar root order used for architectural analysis.

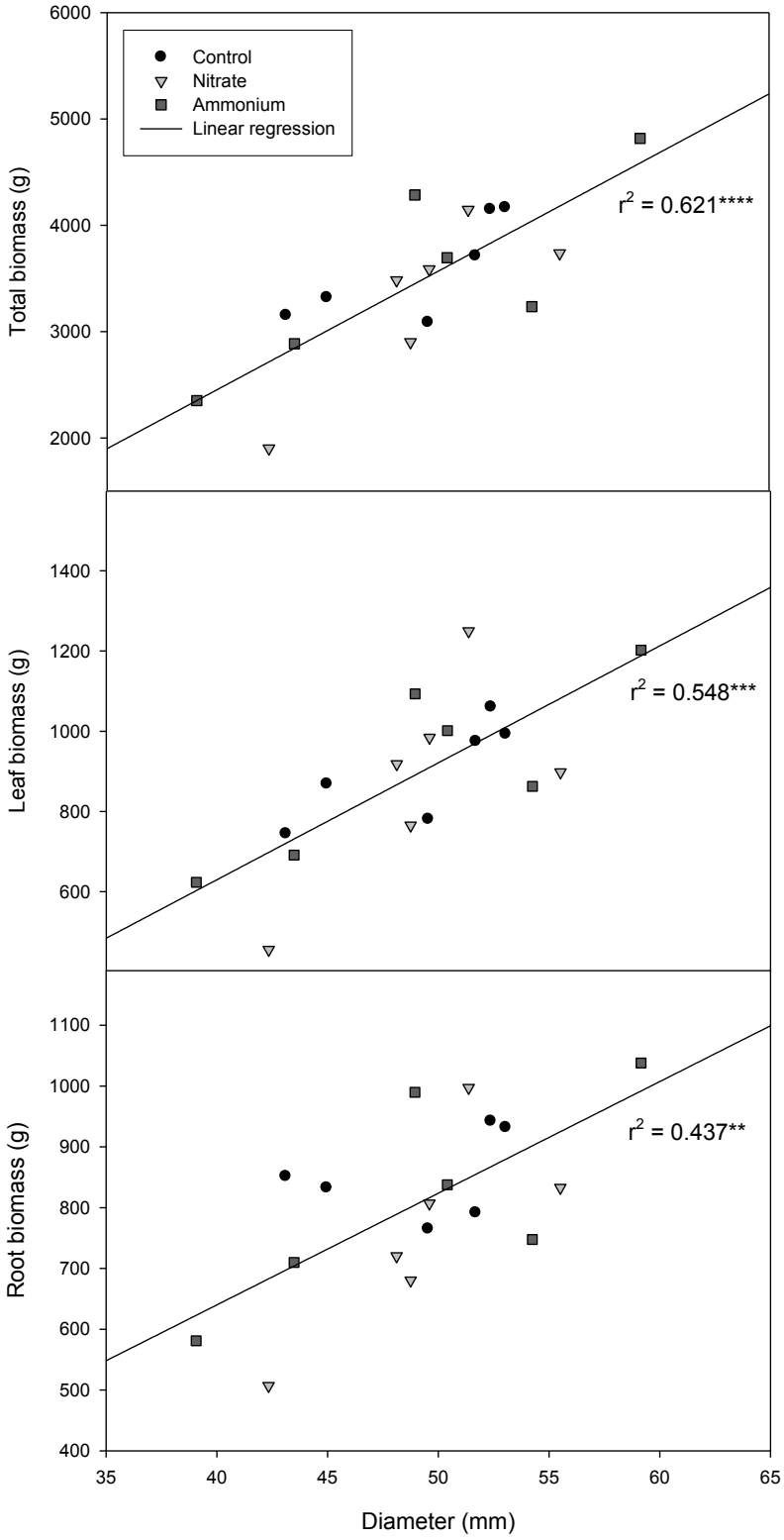


Figure 2. Total biomass (a), leaf biomass (b) and root biomass (c) of clone 313 trees as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression ($P < 0.0001$) for combined treatments is shown since no significant difference is found between treatments.

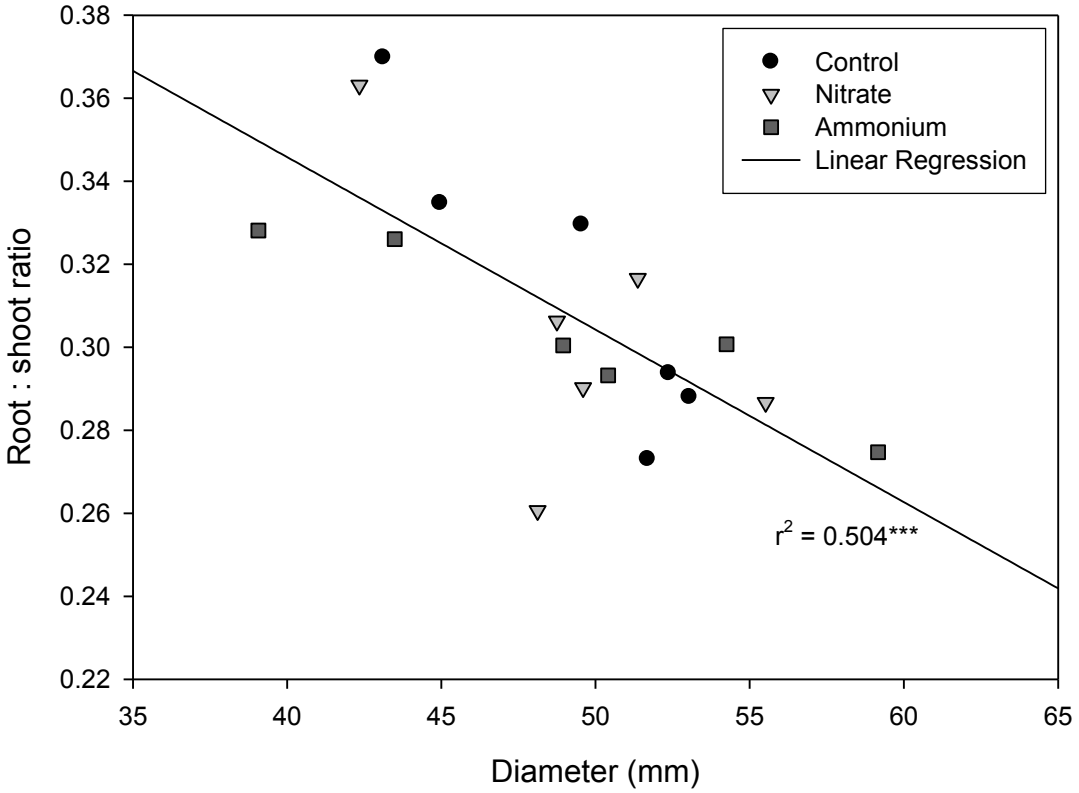


Figure 3. Root:shoot ratio of clone 313 as a function of diameter (40 cm above root collar). Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression ($P < 0.001$) for combined treatments is shown since no significant difference was found between treatments.

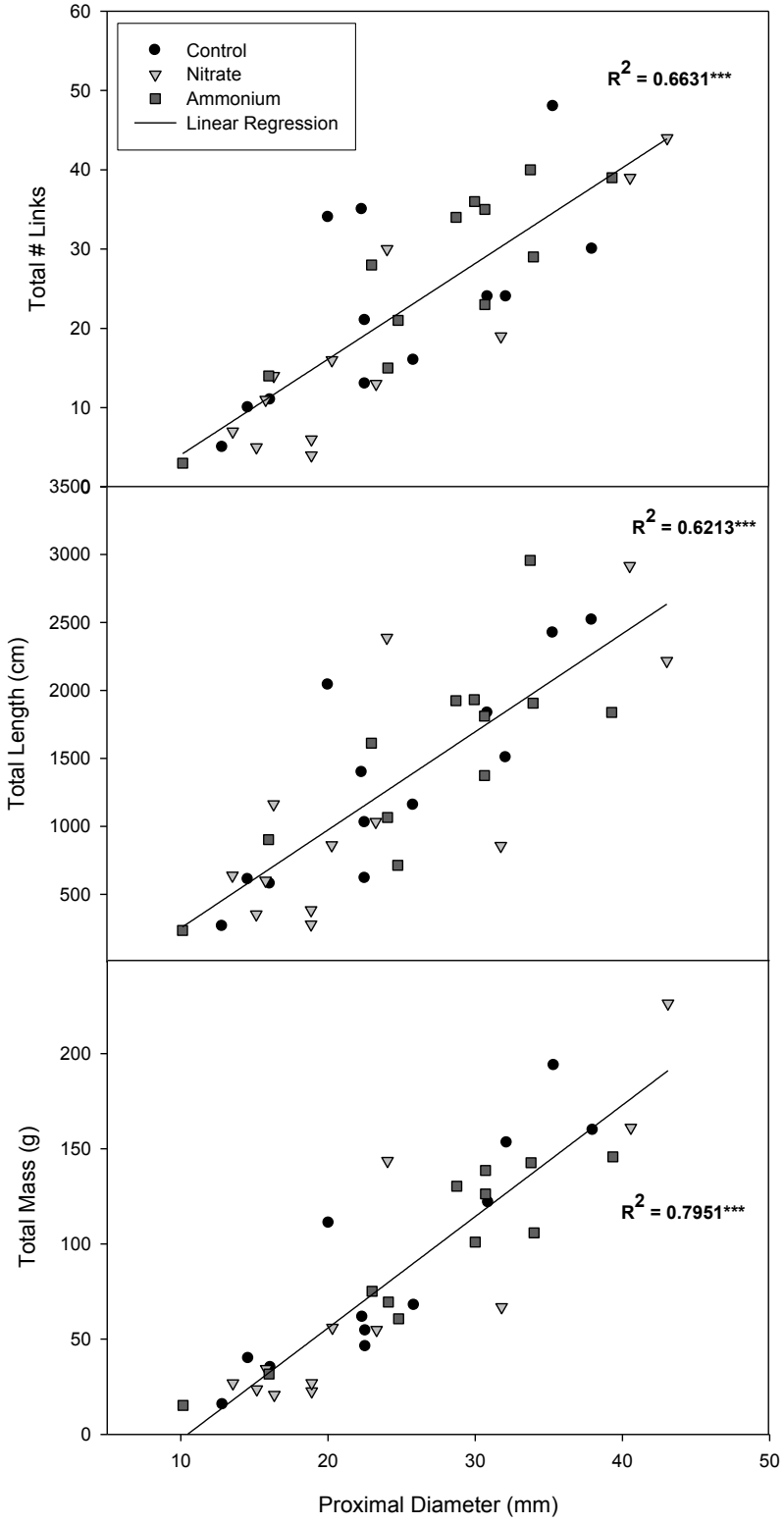
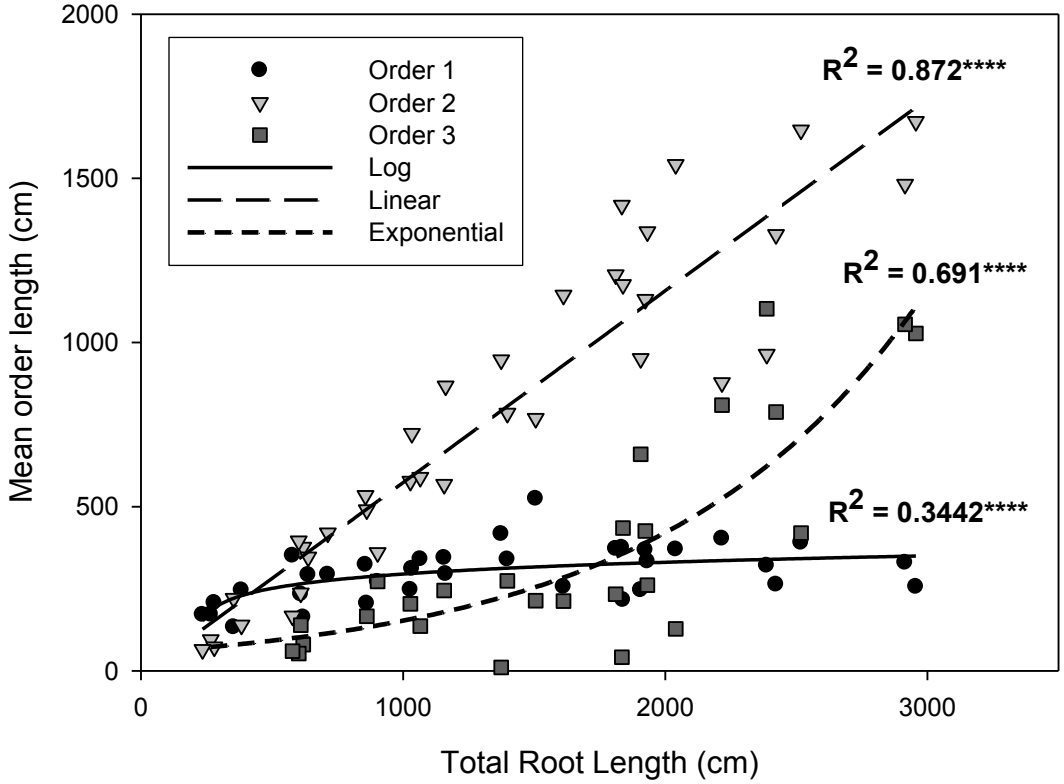
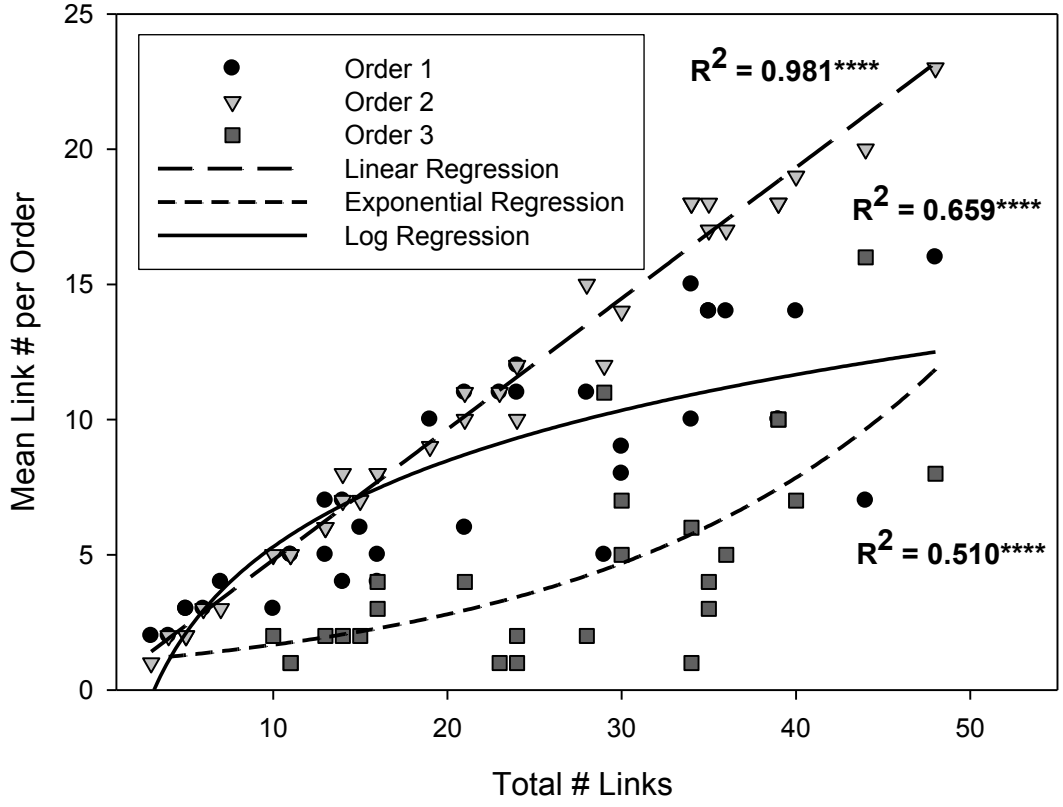


Figure 4. Total # links (a), Total length (b) and Total mass (c) as a function of proximal diameter (mm) for clone 313. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Least-squared linear regression (P < 0.0001) for combined treatments is shown since no significant difference was found between treatments.


Order 1
Order 2
Order 3
Log
Linear
Exponential
R^2 = 0.872****
R^2 = 0.691****
R^2 = 0.3442****
Mean order length (cm)
Total Root Length (cm)
0
500
1000
1500
2000
0
1000
2000
3000


Order 1
Order 2
Order 3
Linear Regression
Exponential Regression
Log Regression
R^2 = 0.981****
R^2 = 0.659****
R^2 = 0.510****
Mean Link # per Order
Total # Links
0
5
10
15
20
25
10
20
30
40
50

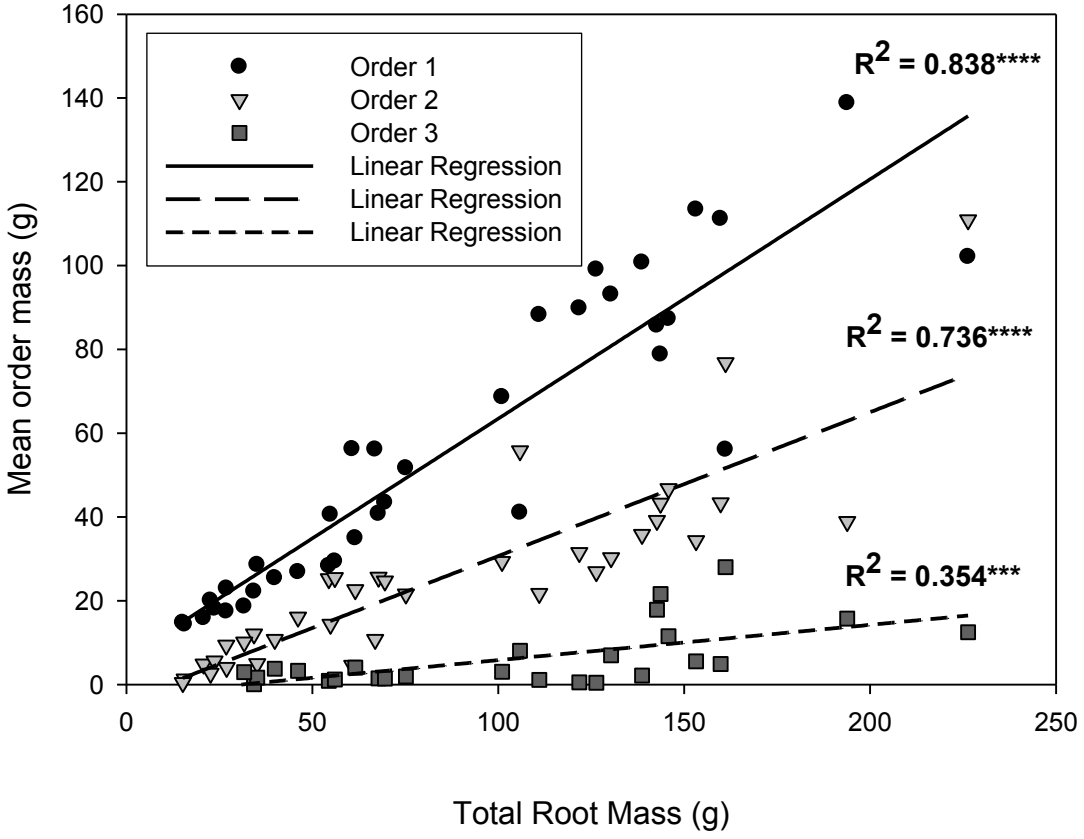


Figure 5. Mean order length (a). Mean link # per order (b), and Mean order mass (c) as a function of total root length (cm) for clone 313. Best fit regressions for combined treatments are shown. *** = ($P < 0.001$) and **** = ($P < 0.0001$). No significant differences were found between treatments.

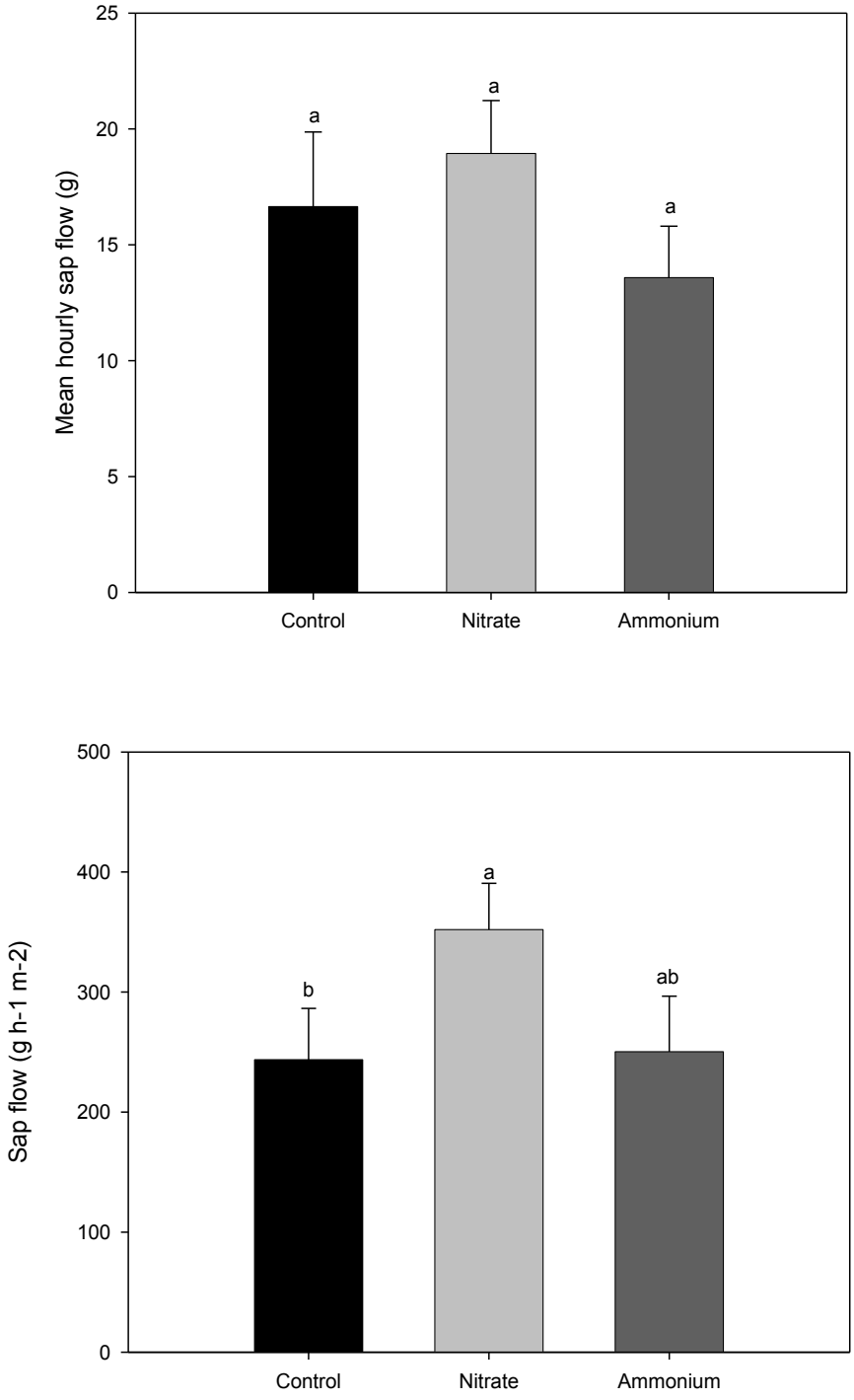


Figure 6. a) Sap flow (g h^{-1} m^{-2}) and b) mean hourly sap flow. Fertiliser treatments were 0 kg N ha^{-1} $year^{-1}$ for the control and 200 kg N ha^{-1} $year^{-1}$ of nitrate and 200 kg N ha^{-1} $year^{-1}$ of ammonium. Each bar represents the mean ± SE for each treatment type for combined clones. Treatment bars with the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$).