

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

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**Abstract** 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. Finer roots were scanned and analyzed for architecture. 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 specific root lengths than control or ammonium treated trees. These allocation differences affected the water uptake capacity of the plants as reflected by the higher sapflow rate in the nitrate treatment. The diameter of proximal roots at the tree base predicted well the total root biomass and length. The diameter of smaller lateral roots also predicted the lateral root mass, length, surface area and the number of tips. The effect of nitrogen fertilisation on the fine root structure translated into an effect on the functioning of the fine roots forming a link between form (architecture) and function (water uptake).

**Keywords** Nitrogen fertilisation · Hybrid poplars · Root structure · Fine roots · Proximal roots · Water uptake · Sapflow

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## 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—have been 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 have 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 and Berntson 2001), fine root architecture (Woolfolk and Friend 2003) and fine root vitality (Clemensson-Lindell and Persson 1995). However, the differential effect of nitrate and ammonium in inducing such changes is not known. 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 efficiency. Just as root architecture can be affected by the different nitrogen forms, it follows that the nitrogen form can also potentially affect plant water relations.

The aim of the research was to understand how fertilisation affects the balance between root morphology and root function. 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 roots to coarse roots ratios than trees fertilised with nitrate. The hypothesis is that these changes in plant morphology will result in different water uptake efficiencies between ammonia and nitrate-fertilized trees. We also hypothesize that fertilisation will increase growth aboveground and decrease allocation to belowground parts for both forms of nitrogen (Poorter and Nagel 2000); we expect the unfertilized trees to have a higher root mass, length and link number.

## Materials 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°C in July and -10.4°C in January. The mean annual precipitation is 920 mm, 90.1 mm in July and 70.4 mm in January (Environment Canada, Ste-Anne-De-Belleveu weather station).

In June of 2004, two hybrid poplar clones (*P. maximowiczii* × *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 (40 cm above root collar) and mean stem height of the 311 clone was  $11.17 \pm 1.59$  mm and  $174.93 \pm 24.25$  cm, respectively. For the 313 clone, the mean initial diameter (40 cm above root collar) and mean stem height was  $11.42 \pm 1.52$  mm and

184.31 ± 36.94 cm, respectively. The experiment was set up with four blocks of four trees per clone (311 or 313) per treatment (control, ammonium and nitrate fertilisation). Fertilisation was applied to individual trees three times during each of the two growing seasons (2004: June 26th, July 16th and August 6th; 2005: June 3rd, June 24th, July 15th) totalling 200 kg ha<sup>-1</sup> 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. A systemic herbicide (RoundUp<sup>®</sup>) was applied uniformly throughout the site to remove competing herbaceous vegetation. Application was done manually to avoid contact with the trees. Throughout the 2004 and 2005 growing seasons, we recorded growth in diameter at 40 cm above root collar and height monthly.

Soil nutrient monitoring was conducted using PRS (Plant Root Simulator)<sup>TM</sup>-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<sup>TM</sup> probes revealed the following nitrogen supply (µg/10 cm<sup>-2</sup>): control treatment NO<sub>3</sub><sup>-</sup> = 64, NH<sub>4</sub><sup>+</sup> = 5; nitrate treatment NO<sub>3</sub><sup>-</sup> = 234, NH<sub>4</sub><sup>+</sup> = 5; ammonium treatment NO<sub>3</sub><sup>-</sup> = 147, NH<sub>4</sub><sup>+</sup> = 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 from clone 313 for total root system excavations (6 from each treatment type: control, nitrate and ammonium). Total root system excavations were limited to roots greater than 2 mm. The roots were excavated by hand (using various hand tools and brushes) and great care was taken to maintain the integrity of the root system architecture.

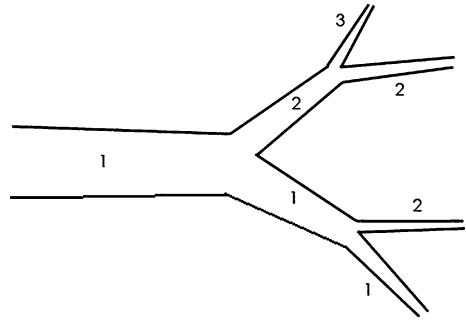
Once excavated, the root systems were severed from the stem at the root collar. The aboveground parts of trees were then separated into the following compartments for biomass analysis: leaves, branches and stem. From each excavated root system, we randomly chose, and severed from the root base, two whole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

We also took 36 random leaf samples from the mid crown for nutrient analysis. The samples were taken systematically across treatment and clones (6 samples per treatment/clone type combination). The analyses were performed using a wet oxidation procedure (Parkinson and Allen 1975). The samples were homogenised in a mortar and then analysed for their contents in N, P, K, Ca and Mg using colorimetric analysis.

### Measurements on proximal roots

Prior to oven drying for biomass computation, we analysed the proximal roots in the laboratory. Each link (segment between branching events) was separated by order using

**Fig. 1** Schematic representation of the hybrid poplar root order used for architectural analysis. Number 1s represent first order roots, number 2s represent second order roots and number 3s represent third order roots



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 (Fig. 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 0.001 g.

#### Measurements on sapflow and sampled lateral roots

At the end of the 2005 growing season, we randomly selected 18 trees from each clonal type (36 trees in total), and six from each treatment (control, nitrate and ammonium). For each tree, we randomly selected two 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 (72 in total), measurements had to be staggered over a 6-week period starting July 25th. Approximately 12 roots were monitored simultaneously for seven consecutive days then the sap flow equipment was 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 and Leuscher (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  $\text{g h}^{-1}$  (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. A total of 20 roots were rejected either because they did not have a minimum of 4 days of monitoring or calculated sapflow data revealed technical problems (indicated by extreme values or unusually large fluctuations). When sapflow monitoring was complete, each sampled lateral root was carefully excavated by hand from the point where the gauge was installed to the finest of root tips (including fine roots smaller than 2 mm).

**Table 1** Summary of measurements, data and related clonal type

Measurements	Data	Clone	
		311	313
Growth	Diameter	All trees	All trees
	Height	All trees	All trees
Foliar nutrient analysis	N, P, K, Ca and Mg	18	18
Biomass analysis	Leaves, stems, branches	n/a	18 trees (6/treatment)
Whole root excavations	Total root system biomass	n/a	18 trees (6/treatment)
Proximal roots	Length, mass, link analysis	n/a	36 roots (2/excavation)
Sapflow	Hourly flow	18 trees (6/treatment)	18 trees (6/treatment)
Lateral root architecture	Surface area, length, tips	18 trees (6/treatment)	18 trees (6/treatment)

The 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, the roots were washed then scanned (400 dpi on an LC4800-II scanner with a double lighting 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 (<2 mm) and coarse root (>2 mm) using digital callipers. Coarse and fine roots were then oven dried at 60°C. All measurements and data collected per clonal type are detailed in Table 1.

### Statistical analysis

Analyses were performed using JMP 8 (SAS Institute Inc. Cary, NC, USA). Mixed models (REML) were used to test the effect of fertilisation treatment (nitrate, ammonium, and control), clonal type (311 and 313) and their interaction (fixed effects) on tree growth, sap flow, and sampled lateral root architecture. For biomass and proximal roots, only one clone was used so the only fixed effect was fertilisation treatment. Random blocks were used to reduce sources of variation caused by possible soil heterogeneity. Measurements from sapflow gauges were not averaged per tree but were assessed independently. Where fixed effects were found to be statistically significant, the Tukey–Kramer HSD procedure was used for comparison of means. Regressions were used to establish relationships between proximal root architecture characteristics as well as for establishing linear relationships between sampled lateral root diameters and various root characteristics. The significance level was considered when  $P$  was less than 0.05.

## Results

### Tree growth and biomass

Fertilisation treatment had a significant effect on diameter growth ( $P < 0.05$ ) but not on height growth (Table 2). Both nitrate and ammonium treatments showed significantly greater diameter growth over the control treatment (Fig. 2a, b).

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

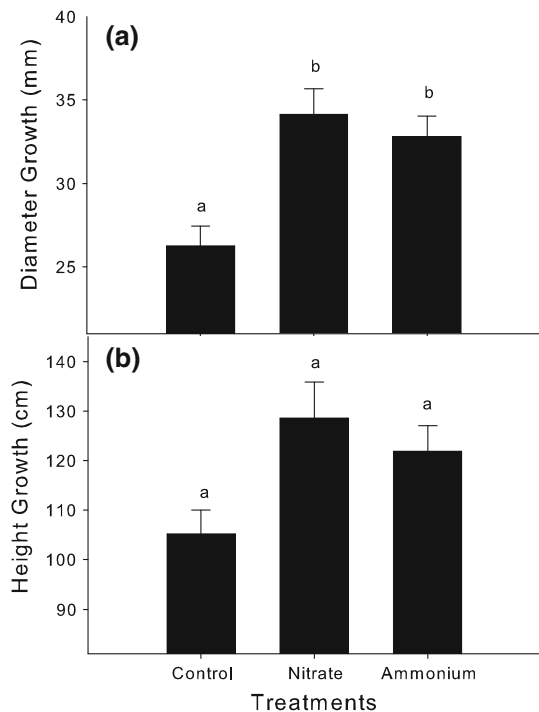
**Table 2** Effect of fertilisation treatment, clone and treatment  $\times$  clone interaction (fixed effects) on diameter and height growth

	<i>df</i>	Diameter growth (mm)		Height growth (cm)	
		<i>n</i> = 81		<i>n</i> = 81	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	2	79.943	0.025*	4.048	0.205
Clone	1	3.532	0.320	4.362	0.322
Treatment $\times$ clone	2	0.322	0.758	0.068	0.937

Table shows results of mixed model analysis using restricted maximum likelihood estimation for each variable tested

\*  $P < 0.05$

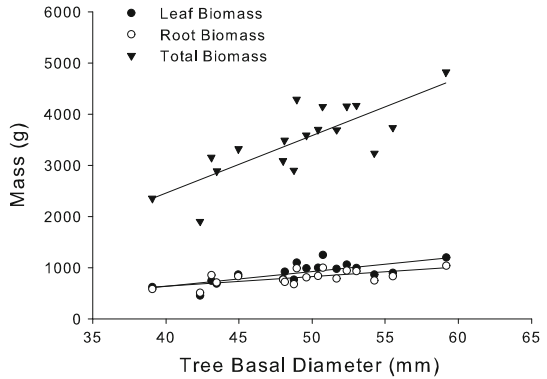
**Fig. 2** Overall tree growth for combined clones (a) height growth (cm) (b) and diameter growth (mm) per treatment type. Fertilisation treatments were 0 kg N ha<sup>-1</sup> year<sup>-1</sup> for the control and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of nitrate and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of ammonium. Treatment bars with the same letter are not significantly different (Tukey's HSD,  $\alpha = 0.05$ )



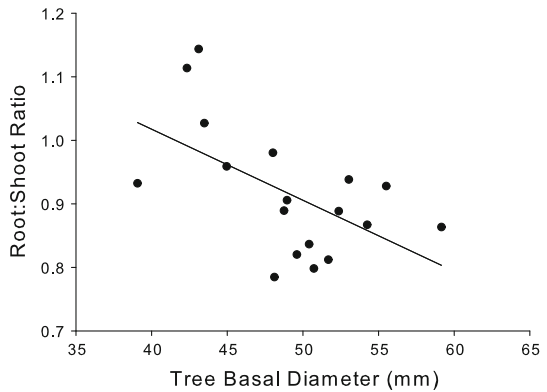
biomass allocation. On average, root biomass accounted for 23% of the total biomass and leaves accounted for 26% (data not shown). Poplars varied between 38 and 58 cm in diameter and between 1,900 and 4,800 g in total biomass.

Significant positive linear relationships resulted when total biomass (total biomass =  $111.775(\text{diameter}) - 2008.981$ ;  $R^2 = 0.627$ ;  $P < 0.0001$ ,  $n = 18$ ), leaf biomass (leaf biomass =  $29.137(\text{diameter}) - 532.173$ ;  $R^2 = 0.544$ ;  $P < 0.0005$ ,  $n = 18$ ), and root biomass (root biomass =  $18.302(\text{diameter}) - 89.284$ ;  $R^2 = 0.432$ ;  $P < 0.003$ ,  $n = 18$ ) were plotted as a function of tree basal diameter (Fig. 3). Likewise, there was a negative linear relationship between root:shoot ratio and tree basal diameter (root:shoot ratio =  $-0.011(\text{diameter}) + 1.464$ ;  $R^2 = 0.315$ ;  $P < 0.0153$ ,  $n = 18$ ) (Fig. 4).

**Fig. 3** Total biomass (g) (a), leaf biomass (g) (b) and root biomass (g) (c) of clone 313 trees as a function of diameter (40 cm above root collar). Fertilisation treatments were 0 kg N ha<sup>-1</sup> year<sup>-1</sup> for the control and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of nitrate and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of ammonium. Least-squared linear regression ( $P < 0.0001$ ) for combined treatments is shown since no significant difference is found between treatments



**Fig. 4** Root:shoot ratio of clone 313 trees as a function of diameter (40 cm above root collar). Fertilisation treatments were 0 kg N ha<sup>-1</sup> year<sup>-1</sup> for the control and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of nitrate and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of ammonium. Least-squared linear regression ( $P < 0.001$ ) for combined treatments is shown since no significant difference was found between treatments



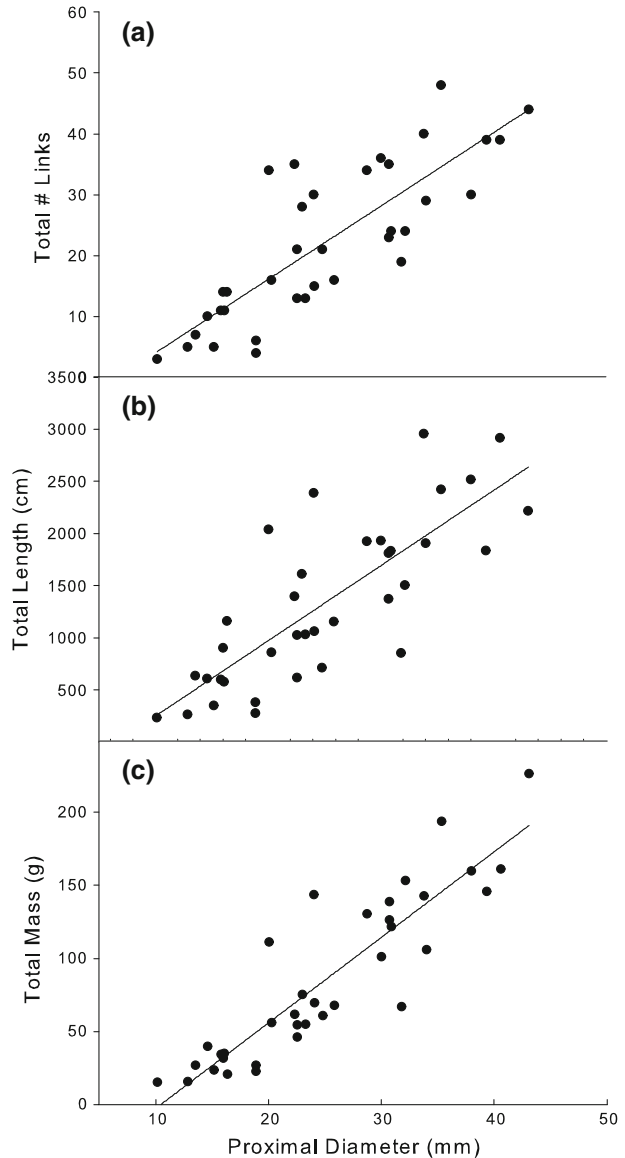
Foliar nutrient concentrations

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 1.49% with a mean 0.3, while K 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 Pearson correlation,  $P < 0.001$ ). The K/N ratio was, however, not significantly different between treatments. The ratios of the concentrations of the other nutrients to the concentration of N were not significantly correlated with the N -concentration nor did they 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 (root length = 72.031 (proximal diameter) - 469.945;  $R^2 = 0.639$ ,  $P < 0.0001$ ), mass (root mass = 5.854 (proximal diameter) - 61.231;  $R^2 = 0.801$ ,  $P < 0.0001$ ) and link number (link # = 1.205 (proximal diameter) - 8.036;  $R^2 = 0.673$ ,  $P < 0.0001$ ) (Fig. 5a-c). The fertilisation treatments had no significant effects on these correlations. However, even though not statistically significant, nitrate-treated trees had consistently lower values (around 25%) of root mass and link number per proximal diameter in comparison to the control and ammonium treatments. The

**Fig. 5** Total # links (a), Total length (cm) (b) and Total mass (g) (c) as a function of proximal diameter (mm) for clone 313. Fertilisation treatments were  $0 \text{ kg N ha}^{-1} \text{ year}^{-1}$  for the control and  $200 \text{ kg N ha}^{-1} \text{ year}^{-1}$  of nitrate and  $200 \text{ kg N ha}^{-1} \text{ year}^{-1}$  of ammonium. Least-squared linear regression ( $P < 0.0001$ ) for combined treatments is shown since no significant difference was found between treatments

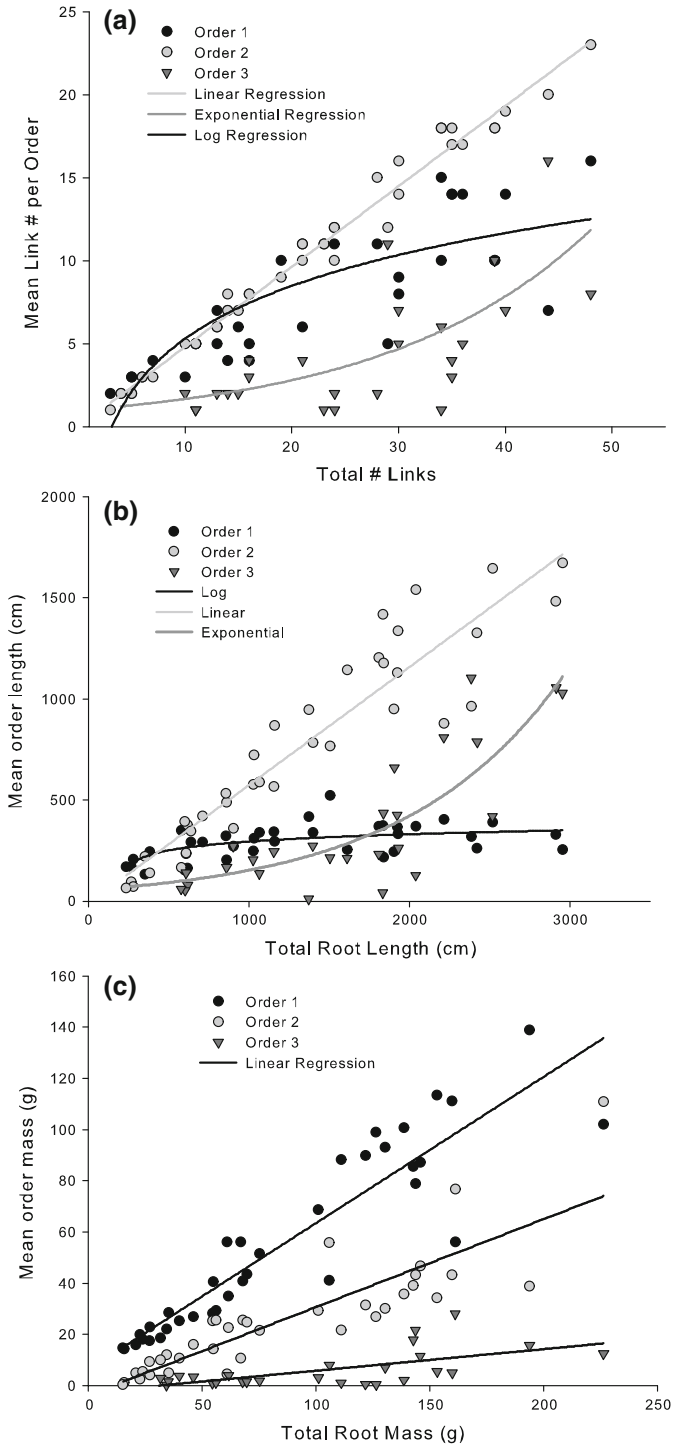


mean values for total length per proximal diameter were only 5 and 6% lower for the control and ammonium treatments, respectively.

Fertilisation treatments also had no effects on root order length, mass or link number. However, when mean order length, mass and link number were plotted as a function of total root length, total mass and total link number respectively, highly significant regressions were obtained (Fig. 6a–c). These relations varied with root order. For mean order

**Fig. 6** Mean link # per order (a), Mean order length (cm) (b), and Mean order mass (g) (c) as a function of total root length (cm) for clone 313. Best fit regressions for combined treatments are shown since no significant differences were found between treatments. \*\*\* = ( $P < 0.001$ ) and \*\*\*\* = ( $P < 0.0001$ )





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  $68.42 \pm 2.4\%$  of the biomass allocated to roots, order 2 for  $27.68 \pm 2\%$ , and order 3 for  $3.90 \pm 0.8\%$ . Unlike the allocation of biomass, where the first order accounts for the largest proportion of mass, the 2nd order accounted for most root length ( $55.07 \pm 2.3\%$ ), followed by order 1 ( $30.24 \pm 2.9\%$ ) and then order 3 ( $14.7 \pm 2.3\%$ ).

### Sapflow

For the combined clones and treatments, mean hourly flow during the daytime (6 am to 9 pm) ranged from  $1.69 \text{ g h}^{-1}$  to  $51.99 \text{ g h}^{-1}$  and averaged  $16.12 (\pm 1.54 \text{ SE}) \text{ g h}^{-1}$ . Mean hourly flow per total root mass was  $2.41 \text{ g h}^{-1} \text{ g}^{-1}$  ( $\pm 0.24 \text{ SE}$ ) with a minimum flow of  $0.20 \text{ g h}^{-1} \text{ g}^{-1}$  and a maximum flow of  $8.00 \text{ g h}^{-1} \text{ g}^{-1}$ . The model showed that treatments had a significant effect ( $P < 0.05$ ) on mean hourly flow per total root mass (Table 3). The Tukey–Kramer HSD test showed that the nitrate treatment had a higher flow ( $\text{g h}^{-1} \text{ g}^{-1}$ ) than the ammonium treatment ( $P < 0.088$ ) and a significantly higher flow ( $\text{g h}^{-1} \text{ g}^{-1}$ ) than the control ( $P < 0.05$ ) (Fig. 7).

### Sampled lateral roots

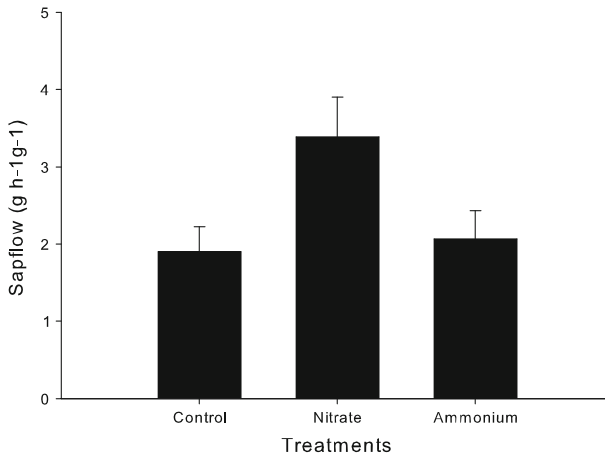
The mixed model (Table 3) showed that fertilisation treatment had an effect on the fine roots to coarse roots ratio with the nitrate treatment showing the highest fine roots to coarse roots ratio compared to ammonium treatment ( $P < 0.053$ ). Treatment was also found to have a significant affect on the SRL of roots greater than 2 mm in diameter ( $P < 0.028$ ) with the nitrate treatment having the highest mean SRL compared to the ammonium

**Table 3** Effect of treatment, clone and treatment  $\times$  clone interaction (fixed effects) on sapflow, fine roots to coarse roots ratio (F:C), and specific root length (SRL)

	df	Sapflow ( $\text{g h}^{-1} \text{ g}^{-2}$ )		F:C		SRL < 2 mm		SRL > 2 mm	
		$n = 52$		$n = 72$		$n = 72$		$n = 72$	
		F	P	F	P	F	P	F	P
Treatment	2	5.450	0.049*	4.551	0.060**	0.836	0.477	6.562	0.028*
Clone	1	0.028	0.878	0.949	0.399	4.120	0.134	0.326	0.607
Treatment $\times$ clone	2	0.737	0.517	1.581	0.279	1.802	0.241	0.392	0.692

Table shows results of mixed model analysis using restricted maximum likelihood estimation for each variable tested

\*  $P < 0.05$ ; \*\*  $P < 0.06$



**Fig. 7** Mean sap flow ( $\pm$ SE) for each treatment type for combined clones (no significant differences were found between clones). Fertilisation treatments were  $0 \text{ kg N ha}^{-1} \text{ year}^{-1}$  for the control and  $200 \text{ kg N ha}^{-1} \text{ year}^{-1}$  of nitrate and  $200 \text{ kg N ha}^{-1} \text{ year}^{-1}$  of ammonium. Treatment bars with the same letter are not significantly different (Tukey's HSD,  $\alpha = 0.05$ )

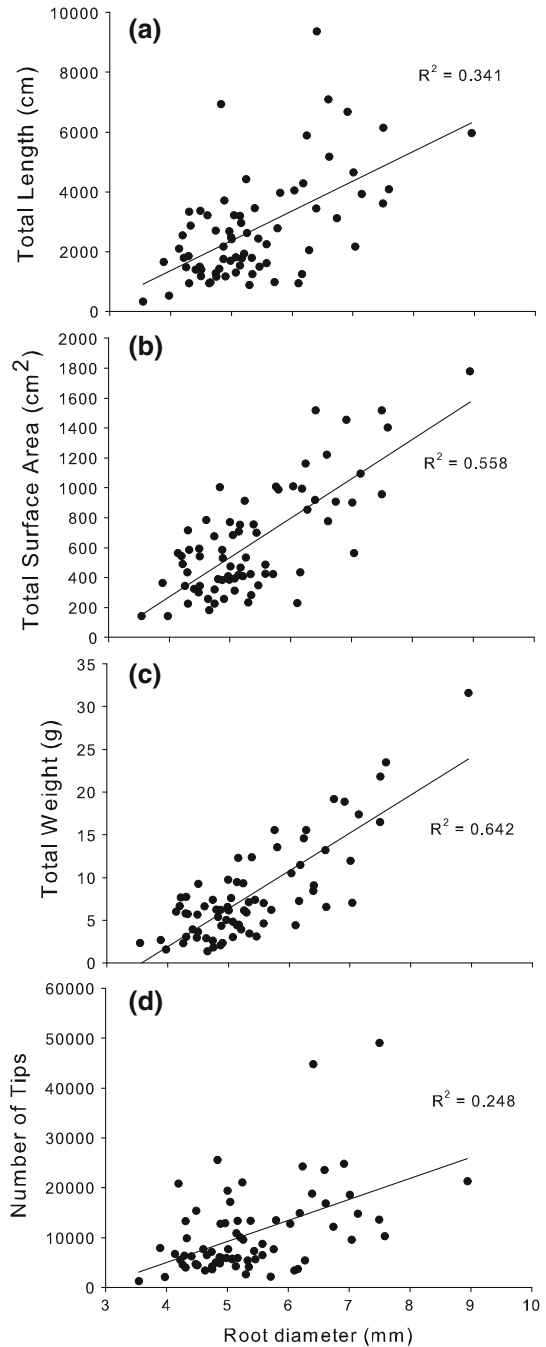
treatment ( $P < 0.026$ ). The diameter of the lateral roots showed significant linear relationships with total length (total length =  $998.653(\text{diameter}) - 2636.759$ ;  $R^2 = 0.341$ ,  $P < 0.0001$ ), total surface area (total surface area =  $263.297(\text{diameter}) - 781.1622$ ;  $R^2 = 0.558$ ,  $P < 0.0001$ ), total lateral root mass (root mass =  $4.441(\text{diameter}) - 15.888$ ;  $R^2 = 0.642$ ,  $P < 0.0001$ ), and total number of tips (number of tips =  $4204.728(\text{diameter}) - 11792.67$ ;  $R^2 = 0.248$ ,  $P < 0.0001$ ) (Fig. 8a–d).

## 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 and 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 Ripullone 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. King et al. (1999) also demonstrated that in trembling aspen (*Populus tremuloides* Michx), increased N-availability was not a factor controlling biomass allocation. Root to shoot ratio declined with tree size, but there was no fertilisation 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).

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 K were quite high, the ratios of K to N concentrations were below normal and indicated that our trees might have become limited by K (Van den Driesche and Rieche

**Fig. 8** Total length (cm) (a), Total surface area (cm<sup>2</sup>) (b) Total mass (g) (c), and Total number of tips (d) as a function of lateral root diameter (mm) for clone 313. Fertilisation treatments were 0 kg N ha<sup>-1</sup> year<sup>-1</sup> for the control and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of nitrate and 200 kg N ha<sup>-1</sup> year<sup>-1</sup> of ammonium. Least-squared linear regression ( $P < 0.0001$ ) for combined treatments is shown since no significant difference was found between treatments



1974). The lack of correlation between growth and N concentration could then be caused by other nutrients becoming more and more limiting. We do not know to what extent our results on root structure and function could be caused by low K 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 therefore, we estimate that the effects of limited K concentrations on the growth and structure of our trees were limited. It has, however, been reported that K deficiency reduces elongation of lateral roots (Armengaud et al. 2004). It is noteworthy that the N/K ratios did not differ between treatments and the possible limited effects of K deficiency should be, hence, similar for all our treatments.

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. The pattern was rather variable and warrants further investigation. Our results did however reveal that by measuring the diameters of proximal lateral roots at the root base, root length, link number and mass for that sampled root could easily be estimated. This approach seems to be a stable, less laborious, 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.

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, however, did 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 reflected the increasing functional importance of support and transport since an increase in diameter and mass accompanied by little growth in length could mean 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 increased 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 not by nitrogen availability (Delagrangé et al. 2004).

Our results confirmed the prediction that investment in fine roots structures would be less in ammonium-treated trees (than in nitrate-treated trees) since poplars have been shown to have a slight preference for this form of less-mobile nitrogen (Dickmann et al. 2001). This preference would explain how in our experiment, nitrate fertilisation increased the ratio of fine roots to coarse roots and also altered the SRL of roots greater than 2 mm in diameter. Comparing these findings with data from other studies has serious limitations since environmental and soil conditions are found to alter fine root characteristics (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 and Bernston 2001; Pregitzer et al. 2002; Guo et al. 2004), yet in other studies, we see evidence that nitrogen availability had an effect on fine root biomass, fine root production and mortality (King et al. 2002; 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 not only confirms that nitrogen fertilisation affects fine roots, but that the form of nitrogen applied has significant importance on fine root architecture.

We hypothesized that sapflow would increase with increasing fine root mass since the surface of fine roots is the location of water uptake. Our results demonstrated that the

nitrate-treated trees had a significantly higher mean hourly flow per root mass than the control trees (and a nearly-significant difference with the ammonium-treated trees). This might suggest a higher hydraulic conductivity (axial, radial or both) in the roots of the nitrate-treated trees or an easier water uptake in the NO<sub>3</sub> treatment due to a higher fine root to coarse root mass ratio and a lower soil to root resistance. Most importantly, the effect on the fine root structure translated into an effect on the functioning of the fine roots.

The fine root changes induced by nitrate fertilisation, such as a higher F:C ratio as well as a higher SRL (in roots with a diameter > 2 mm) most likely explain the greater rate of water extraction from the soil. Similar results have been reported where higher SRL was associated with rapid root proliferation and higher radial hydraulic conductivity (Eissenstat 1991; Huang and Eissenstat 2000). We cannot make any assumptions regarding the physiological structure of the fine roots in this study, such as a variability in tissue density or xylem vessel diameter which would help us understand the finer details of how nitrate fertilisation affected the roots. For example, N-fertilisation has been shown to increase xylem vessel diameters potentially increasing water uptake capacity, but also making poplars more susceptible to xylem cavitations on dry sites (Harvey and Van den Driessche 1997, 1999).

This study has clearly shown that root form and function are intimately linked and vary depending on the nitrogen form. Further information is needed to increase our knowledge regarding both how the different forms of nitrogen affect the physiological structures of roots and how root structure affects root function, especially in a complex field environment where knowledge can be applied to improve environmental management of poplar plantations.

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## References

- Albaugh TJ, Allen HL, Kress LW (2006) Root and stem partitioning of *Pinus taeda*. *Trees-Struct Funct* 20:176–185
- Armengaud P, Breiiting R, Amtmann A (2004) The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signalling. *Plant Physiol* 136:2556–2576
- Bauer GA, Bertson GM (2001) Ammonium and nitrate acquisition by plants in response to elevated CO<sub>2</sub> concentration: the roles of root physiology and architecture. *Tree Physiol* 21:137–144
- Block RMA, Rees KCJ, Knight JD (2006) A review of fine root dynamics in *Populus* plantations. *Agrofor Syst* 67:73–84
- Clemensson-Lindell A, Persson H (1995) Fine-root vitality in a Norway spruce stand subjected to various nutrient supplies. *Plant Soil* 1:167–172
- Coleman MD, Friend AL, Kern CC (2004) Carbon allocation and nitrogen acquisition in a developing *Populus deltoides* plantation. *Tree Physiol* 24:1347–1357
- Coll L, Potvin C, Messier C, Delagrange S (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, Leuscher C (2002) In situ water absorption by tree fine roots measured in real time using miniature sap-flow gauges. *Funct Ecol* 16:696–703

- Cooke JEK, Martin TA, Davis JM (2005) Short-term physiological and developmental responses to nitrogen availability in hybrid poplar. *New Phytol* 1:41–52
- Delagrange S, Messier C, Lechowicz MJ, 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 DI, Isebrands JG, Blake TJ, Kosola K, Kort J (2001) Physiological ecology of poplars. In: Dickmann DI (ed) *Poplar culture in North America*. NRC Research Press, Ottawa, pp 77–118
- Eissenstat DM (1991) On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytol* 118:63–68
- Eissenstat DM (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 AH (1986) The topology and geometry of plant-root systems—influence of watering rate on root-system topology in *Trifolium pratense*. *Ann Bot-Lond* 58:91–101
- Fitter AH (2002) Characteristics and functions of root systems. In: Waisel Y, Eshel A, Uzi Kafkafi U (eds) *Plant roots: the hidden half*, 3rd edn. Marcel Dekker, Inc., New York, pp 15–32
- Fujimaki R, Tateno R, Hirobe M, Tokuchi N, Takeda H (2004) Fine root mass in relation to soil N supply in a cool temperate forest. *Eco Res* 19:559–562
- Glynn C, Herms DA, Egawa M, Hansen R, Mattson WJ (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, Mitchell R, Hendricks J (2004) Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* 140:450–457
- Harvey HP, van den Driessche R (1997) Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiol* 17:647–654
- Harvey HP, van den Driessche R (1999) Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars. *Tree Physiol* 19:943–950
- Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol* 162(1):9–24
- Huang B, Eissenstat DM (2000) Linking hydraulic conductivity to anatomy in plants that vary in specific root length. *J Am Soc Hortic Sci* 125:260–264
- Karacic A, 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 CC, Friend AL, Johnson JMF, Coleman MD (2004) Fine root dynamics in a developing *Populus deltoides* plantation. *Tree Physiol* 24:651–660
- King JS, Pregitzer KS, Zak DR (1999) Clonal variation in above- and below-ground growth responses of *Populus tremuloides* Michaux: influence of soil warming and nutrient availability. *Plant Soil* 217:119–130
- King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytol* 154:389–398
- Lopez-Bucio J, Cruz-Ramirez A, Herrera-Estrella L (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
- Min X, Siddiqi MY, Guy RD, Glass ADM, Kronzucker HJ (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, Lecompte F, Sillon JF (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
- Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Comm Soil Sci Plant Anal* 6:1–11
- Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO<sub>2</sub>, nutrients and water: a quantitative review. *Aust J Plant Physiol* 27:1191
- Pregitzer KS, Dickmann DI, Hendrick R, Nguyen PV (1990) Whole-tree carbon and nitrogen partitioning in young hybrid poplars. *Tree Physiol* 7:79–93
- Pregitzer KS, Zak DR, Maziasz J, Deforest J, Curtis PS, Lussenhop J (2000) Interactive effects of atmospheric CO<sub>2</sub> and soil-N availability on fine roots of *Populus tremuloides*. *Ecol Appl* 10:18–33
- Pregitzer KS, Deforest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. *Ecol Monogr* 72:293–309

- Ripullone F, Lauteri M, Grassi G, Amato M, Borghetti M (2004) Variation in nitrogen supply changes water-use efficiency of *Pseudotsuga menziesii* and *Populus × euroamericana*; a comparison of three approaches to determine water-use efficiency. *Tree Physiol* 24:671–679
- Rose DA (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 Agr Met* 37:9–17
- Salas E, Ozier-Lafontaine H, Nygren P (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, Rieche K (1974) Prediction of mineral nutrient status of trees by foliar analysis. *Bot Rev* 40:347–394
- van Noordwijk M, Spek LY, Dewilligen P (1994) Proximal root diameter as predictor of total root size for fractal branching models. 1. Theory. *Plant Soil* 164:107–117
- West GB, Brown JH, Enquist BJ (1999) A general model for the structure and allometry of plant vascular systems. *Nature* 400:664–667
- Woolfolk WTM, Friend AL (2003) Growth response of cottonwood roots to varied  $\text{NH}_4\text{:NO}_3$  ratios in enriched patches. *Tree Physiol* 23:427–432