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Growth response of cottonwood roots to varied NH_4:NO_3 ratios in enriched patches

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Summary Maximization of short-rotation forest plantation yield requires frequent applications of nutrients, especially nitrogen (N). Whole-plant growth is known to be sensitive to the proportion of ammonium to nitrate (NH_4:NO_3). However, the extent to which N form affects root growth, branching and morphology is poorly understood, and these variables may have substantial impacts on plant nutrient and water acquisition. We used rooted cuttings of cottonwood (*Populus deltoides* Bartr. ex Marsh.) to investigate the effect of various NH_4:NO_3 ratios on root growth in N-enriched patches. A sand culture study with split-root systems was carried out in which 1–3% of the total root system of each cutting was supplied with 2 mM N at NH_4:NO_3 ratios of 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 (molar basis), with the rest of the plant supplied with 0 mM N, resulting in the whole plant becoming N deficient. During the experiment, whole-plant growth was unaffected by the treatments. Of the NH_4:NO_3 ratios tested, greatest total root length, specific root length, and root N concentration of roots in enriched patches occurred in the 20:80 NH_4:NO_3 treatment. The largest response of roots in enriched patches was third- and fourth-order root production. We conclude that N form has a profound effect on root development and morphology in enriched patches.

Keywords: ammonium:nitrate ratio preference, ammonium nutrition, nitrate nutrition, nitrogen-enriched patches, root foraging, root morphology, root proliferation, sand culture.

Introduction

Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) and other *Populus* species are fast growing and are often grown under intensive management to maximize growth potential (Zsuffa et al. 1996). Because of the high growth potential of *Populus*, its physiology and genetics have been studied in detail (Stettler et al. 1996). However, our understanding of the sensitivity of whole-plant growth of this genus to root-related variables is rudimentary. It is known that *Populus* invests a large amount of carbon below ground (Pregitzer and Friend 1996), but the degree to which, and mechanisms by which, this investment can be manipulated to increase shoot growth remain unclear. We focused on root growth in nutrient-enriched patches of soil because drip irrigation in *Populus* plantations commonly supplies both nutrients and water to isolated portions of the root system.

Soil nutrient availability is highly variable. Roots proliferate in patches of soil that are enriched in nutrients (Caldwell 1994), especially nitrogen (N) (Friend et al. 1990, 2000, Pregitzer et al. 1993), and phosphorus (P) (Drew 1975, Drew and Saker 1978). To maintain overall plant growth, roots growing in nutrient-enriched microsites compensate for the roots growing in nutrient-poor soil (Drew 1975, Drew and Saker 1978, Caldwell 1994). Compensatory uptake results from an increase in fine lateral root production and an increase in ion uptake rate from nutrient-enriched patches, thereby allowing adequate amounts of nutrients to be absorbed and translocated to nutrient-deficient areas of the plant. In addition, the sensitivity of root proliferation to enriched patches varies genetically and may confer a competitive advantage to those species or genotypes with a strong proliferation response (Hodge et al. 1999, Friend et al. 2000).

The form of N (ammonium (NH_4^+) or nitrate (NO_3^-)) available to the plant is another important environmental variable affecting plant growth. Root proliferation and overall plant growth are usually greater with a mixture of NH_4^+ and NO_3^- than with either form alone (Wang and Below 1992, Saravitz et al. 1994, Schortemeyer and Feil 1996). The precise NH_4:NO_3 ratio for optimum growth of a given species varies with internal physiological conditions (e.g., root carbohydrate status and nitrate reductase activity) and external environmental conditions (e.g., pH and total N concentration) (Marschner 1995). However, broad NH_4:NO_3 growth optima show substantial variation among species reflecting edaphic adaptation. In general acid-tolerant species such as evergreen conifers grow best in NH_4^+-dominated solutions, whereas species adapted to more basic soils, such as deciduous hardwoods, grow best in NO_3^--dominated solutions (Lavoie et al. 1992, Falkengren-Gerup 1995, Kozlowski and Pallardy 1997; Kronzucker et al. 1997).

Recent research with eastern cottonwood suggests that

60–80% NO_3^- (balanced with NH_4^+) optimizes whole-plant growth when the entire root system is in a homogeneous nutrient solution (Woolfolk 2000). However the optimal NH_4:NO_3 ratio for root proliferation in N-rich patches may differ from the optimal ratio for whole-plant growth. In addition, root responses to a whole-plant treatment may be confounded by shoot–root feedbacks. For instance, changes in root growth rates may be influenced more by carbon allocation from shoots than by direct responses to the root environment (Friend et al. 1994). Our objective was to evaluate the degree to which root growth and morphology change in response to N form with as few confounding factors as possible. We used a split root approach whereby the plant and most of its roots were treated uniformly and one small portion of the roots was exposed to an N-enriched patch, which varied only in the proportion of NH_4^+ and NO_3^-.

Methods

The experiment was conducted in a greenhouse at Mississippi State University from November to December 1998 in a photoperiod extended to 15.5-h with high-pressure sodium lamps, and a mean daily photosynthetic photon flux density (PPFD) of 334 μmol m^{-2} s^{-1}. Day/night air temperature averaged 21/18 °C.

Greenwood cuttings of an eastern cottonwood (*Populus deltoides*) clone, D–105, from central Wisconsin (92°40′ W, 45°30′ N; Riemenschneider and Isebrands 1996) were rooted in a peat and vermiculite mixture for 4–6 weeks in a misting chamber. After rooting, cuttings were transplanted to 700-ml (25 cm long) leach tubes containing washed silica sand for further growth and acclimation to sand culture. Transplanted cuttings received 100 ml of a 2 mM N complete nutrient solution based on the formulation of Ingestad and Lund (1979), with N supplied as 50:50 NH_4:NO_3 (Table 1) daily until at least five leaves reached a lamina length of 4 cm. The solution was adjusted with 0.1 M NaOH or 0.1 M HCl to pH 5.0 ± 0.5. Thirty-five uniformly sized plants were then transferred to 2-l PVC pots with one primary lateral root from the cutting (secondary roots removed) placed in a smaller 200-ml adjacent pot through a side hole connecting the pots. The smaller pot was held adjacent to the large pot by metal hose clamps. The small side holes in both pots produced a path (surrounded by open-cell PVC foam) through which the isolated root could extend. The larger pot received 250 ml of a 2 mM N complete nutrient solution (50% NO_3^-) and the smaller pot received 50 ml of a 0 mM, N but otherwise complete nutrient solution every day. After 7 days of this pretreatment, five randomly selected plants were harvested and 30 plants were randomly assigned to one of six root treatments (n = 5): 0, 20, 40, 60, 80 and 100% NO_3^- (molar basis, balanced with NH_4^+ in complete nutrient solution, Table 1). Pots were leached with 1 l of distilled H_2O immediately followed by 500 ml of the assigned treatment. Treatments were arranged by row and rotated daily to minimize response to any variation in the greenhouse environment. The experiment was arranged in a completely randomized design and analyzed accordingly. Plants were treated for 14 days with 250 ml of a 0 mM N nutrient solution applied to all large pots every day and with 50 ml of 2 mM N solution at the assigned NH_4:NO_3 ratio applied every 12 h to the small pots. These solutions were adequate to saturate and leach both pot volumes. Actual NH_4^+ and NO_3^- concentrations of solutions applied were analyzed with a DX-500 ion chromatograph (Dionex, Sunnyvale, CA) and were within 5% of the target ratios. Every seventh day, the first 125 ml of leachate from the large pot and all leachate from the small pot were collected by displacement with the treatment solution and analyzed for conductivity, pH, NH_4^+ concentration and NO_3^- concentration.

Table 1. Concentrations and source salts for elements supplied in the complete nutrient solution, based on Ingestad and Lund (1979).

Element	μM	Salt
N	2000	NH_4NO_3 (NH_4Cl, $NaNO_3$)[1]
K	460	KCl
P	120	KH_2PO_4
Mg	90	$MgSO_4$
Ca	60	$CaCl_2$
B	5.30	H_3BO_3
Fe	3.56	$Fe(NO_3)_3$[2]
Mn	2.08	$MnCl_2$
Zn	0.26	$Zn(NO_3)_2$[2]
Cu	0.13	$CuCl_2 \cdot 2H_2O$
Co	0.028	$CoCl_2$
Mo	0.042	Na_2MoO_4

[1] The dominance of NH_4^+ and NO_3^- was varied, according to treatment, by supplementation with NH_4Cl or $NaNO_3$, respectively.

[2] Total NO_3^- concentration was not significantly affected by these salts.

At harvest, the split root was excised from the whole plant (just inside the small pot). Each whole plant was divided into leaves, stems plus cutting, coarse roots (> 2.0 mm diameter), fine roots (< 2.0 mm diameter) and dried at 70 °C to constant mass (about 48 h). Each split root was divided into root orders and scanned with WinRhizo® (Régent Instruments, Québec, Canada) to determine total length and mean diameter. The first-order root was defined as the primary root in the split-root pot. The highest category measured was third-order and greater roots, which included fourth-order roots in several samples. Split roots were then dried and weighed by order. Dried tissues (foliage, stem + roots, and split roots) were ground through a 60-mesh screen and analyzed for N with an NA-1500 NCS analyzer (Fisons Instruments, Beverly, MA).

Analyses of variance (ANOVA) were performed with SAS® software (SAS Institute, Cary, NC). An unequal t-test was performed between pre-treatment and post-treatment tissue N contents. Mean separations among treatments were performed with Tukey's studentized range test at $\alpha = 0.05$.

Results

Soil solution responses

Small-pot leachate pH was between 5 and 6 (Table 2). Al-

though the solution added to small pots was pH 5 for every NH_4:NO_3 ratio, relatively abundant NO_3^- resulted in small but statistically significant increases in pH. Small pot leachate N H_4^+ and NO_3^- concentrations were within 10% of the target values, indicating minimal solution depletion by roots.

Whole-plant responses

Whole-plant biomass increased ($P = 0.02$) from pretreatment (3.1 g) to harvest (3.9 g), but whole-plant N content did not change over this time. The distribution of N within the plant changed over time, with a decrease in foliar N and an increase in stem + root N in post-treatment samples compared with pretreatment samples (Table 3). Split root NH_4:NO_3 treatments had no effects on whole-plant biomass, N content or N distribution.

Split-root responses

Although no plant mortality occurred, two split roots died in the 0 and 100% NO_3^- treatments, and one split root died in the 20 and 60% NO_3^- treatments. These plants were excluded from analysis. All roots survived in the 80 and 40% NO_3^- treatments. Most root growth resulted from second-, third- and higher-order roots arising from the primary root. Split-root biomass differed significantly between treatments and was driven by second-order root biomass, which was nearly twofold higher in the 100% NO_3^- treatment than in other treatments (Table 4). Split-root length was also affected by treatments, with greater total length in the 80% NO_3^- treatment compared with the 0 and 20% NO_3^- treatments (Table 4). This effect was caused by stimulation of second-, third- and higher-order roots with increasing percent NO_3^-, but not pure NO_3^-. Specific root length (SRL) of first- and second-order roots was unaffected by treatment. However, SRL of the entire split root followed the same trend as total length, with a peak in the 80% NO_3^- treatment (Table 4).

Nitrogen concentration of split roots was 13.6 ± 0.2, 12.8 ± 1.6, 17.5 ± 0.5, 19.3 ± 2.5, 19.5 ± 2.0 and 17.7 ± 0.7 mg N g^{-1} in the 0, 20, 40, 60, 80 and 100% NO_3^- treatments, respectively (mean ± SEM), with a broad optimum from 60 to 80% NO_3^- ($P = 0.057$). Nitrogen content of split roots was 0.25 ± 0.07, 0.11 ± 0.04, 0.27 ± 0.03, 0.43 ± 0.08, 0.45 ± 0.11 and 0.50 ± 0.12 mg N per root in the 0, 20, 40, 60, 80 and 100% NO_3^- treatments, respectively (mean ± SEM). As with biomass, N content of split roots increased with increasing percent NO_3^- ($P = 0.055$), but contributed less than 1% to total plant nitrogen (Table 3).

Table 2. Mean weekly small-pot leachate and solution pH ± SE, with probabilities ($P > F$). Similar letters or no letters indicate no significant differences among treatment means for individual variables ($\alpha = 0.05$, Tukey's studentized range test).

Treatment	Split root pH	
% NO_3^-	Week 1	Week 2
0	4.8 ± 0.2 d	4.8 ± 0.1 d
20	5.5 ± 0.1 bc	5.3 ± 0.1 bc
40	5.3 ± 0.1 c	4.9 ± 0.1 d
60	5.4 ± 0.1 bc	5.0 ± 0.1 cd
80	5.7 ± 0.1 ab	5.6 ± 0.1 b
100	6.1 ± 0.1 a	6.1 ± 0.1 a
$P > F$	< 0.01	< 0.01
Stock solution	5.0 ± 0.1	4.6 ± 0.1

Table 3. Changes in tissue N content (mg $plant^{-1}$) ± SE over the course of treatment. Values are for pretreatment ($n = 5$) and treatment plants ($n = 23$) with results from an unequal t-test. Similar letters indicate no significant differences between pretreatment and treatment plants within tissue type ($\alpha = 0.05$, Tukey's studentized range test).

Tissue type	N content (mg $plant^{-1}$)		$P > \|t\|$
	Pretreatment	Post treatment	
Foliage	27.2 ± 2.3 a	22.9 ± 0.8 a	0.13
Stem + roots	16.9 ± 1.1 b	20.9 ± 0.6 a	0.01
Split root	0.1 ± 0.1 b	0.3 ± 0.1 a	0.01
Total	44.3 ± 3.3 a	44.2 ± 1.3 a	0.99

Discussion

Cottonwood roots grew most in the presence of abundant NO_3^-, but not when pure NO_3^- was the sole N source. Our findings for isolated roots with varied N form in patches were remarkably similar to previous studies of whole plants with varied N form in the entire soil volume. Specifically, whole-plant biomass increased from 2 to 3 g in 0–30% NO_3^- to 4 to 5 g in 70–100% NO_3^-. Whole-plant N content increased from 44 to 49 mg N in 0–30% NO_3^- to 111 to 127 mg N in 70–100% NO_3^- (Woolfolk 2000). Overall, growth and N accumulation nearly doubled from NH_4^+-dominated to NO_3^--dominated solutions for both whole plants and for isolated roots. Although root structural measurements were less intensive in the first study, specific root length responses of the data sets were similar. Both data sets reflected an inhibition of high-order root development in solutions of pure NO_3^- compared with solutions dominated by NO_3^-, but containing some NH_4^+. This consistency of response to N form, between patch and uniform applications, suggests that root:shoot feedbacks were of minor importance, and that root responses were driving whole-plant responses to N form.

Unbalanced application of NH_4^+ or NO_3^- is widely acknowledged to decrease or increase, respectively, soil pH (Marschner 1995, p. 248), as was noted in our treatments (Table 2). Although changes in soil pH are reported to alter root growth and morphology (Stoffela et al. 1991, Hirano and Hijii 1998, 2000), we ascribe treatment effects to variations in N form and not to pH for two reasons. First, root growth is generally unresponsive to pH in the range of 5.0 to 7.5 (Marschner 1995). Our solutions were adjusted to pH 5 at the start of the experiment and changed, over the course of 12 h, to pH values no lower than 4.8 and no higher than 6.1 (Table 2). Frequent solution applications prevented the pH from drifting outside of this favorable range. Second, treatments changed root growth

Table 4. Mean biomass (mg), length (cm), specific root length (m g^{-1}) ± SE and probabilities ($P > F$) of eastern cottonwood split roots (live roots only) to varied NH_4:NO_3 ratios at 2 mM N applied to isolated patches. Similar letters or no letters indicate no significant differences among treatment means for individual variables ($\alpha = 0.05$, Tukey's Studentized Range Test). Number of samples, *n*, is given in parentheses. Pretreatment is shown for reference only and was not included in the analysis.

Treatment	Root order			Total
% NO_3^-	First	Second	Third[1]	
Biomass (mg)				
Pretreatment	8 ± 1 (5)	1 ± 0.3 (5)	0 (5)	9 ± 1 (5)
0	12 ± 3 (3)	6 ± 2 b (3)	0 (3)	18 ± 5 ab (3)
20	7 ± 1 (4)	3 ± 2 b (4)	0 (4)	11 ± 3 b (4)
40	7 ± 1 (5)	8 ± 1 ab (5)	0.1 ± 0.1 (1)	15 ± 2 ab (5)
60	11 ± 1 (4)	10 ± 2 ab (4)	0.3 ± 0.2 (2)	21 ± 2 ab (4)
80	9 ± 1 (5)	11 ± 2 ab (5)	1.2 ± 0.7 (5)	22 ± 3 ab (5)
100	11 ± 2 (3)	17 ± 4 a (3)	0 (3)	28 ± 6 a (3)
$P > F$	0.22	< 0.01	0.20	0.05
Length (cm)				
Pretreatment	12 ± 1 (5)	10 ± 2 (5)	0 b (5)	21 ± 1 (5)
0	11 ± < 1 (3)	31 ± 5 ab (3)	0 b (3)	43 ± 6 b (3)
20	9 ± 1 (4)	18 ± 7 b (4)	0 b (4)	28 ± 7 b (4)
40	11 ± < 1 (5)	48 ± 4 ab (5)	1 ± 1 ab (1)	59 ± 4 ab (5)
60	13 ± 3 (4)	60 ± 9 ab (4)	6 ± 4 ab (2)	79 ± 15 ab (4)
80	12 ± 1 (5)	64 ± 12 a (5)	35 ± 15 a (5)	110 ± 22 a (5)
100	12 ± < 1 (3)	60 ± 15 a (3)	0 b (3)	72 ± 14 ab (3)
$P > F$	0.62	0.02	0.03	< 0.01
Specific root length (m g^{-1})				
Pretreatment	16 ± 3 (5)	81 ± 16 (5)	–	24 ± 3 (5)
0	10 ± 2 (3)	71 ± 27 (3)	–	26 ± 5 b (3)
20	15 ± 4 (4)	60 ± 8 (3)	–	27 ± 2 b (4)
40	15 ± 2 (5)	66 ± 11 (5)	152 (1)	41 ± 5 ab (5)
60	12 ± 2 (4)	62 ± 9 (4)	222 ± 56 (2)	37 ± 5 ab (4)
80	13 ± 1 (5)	59 ± 8 (5)	449 ± 101 (5)	49 ± 4 a (5)
100	11 ± 2 (3)	38 ± 11 (3)	–	27 ± 6 b (3)
$P > F$	0.69	0.68	0.32	0.01

[1] Third- and higher-order roots.

independently of treatment effects on pH. For instance, biomass, length and specific root length all nearly doubled from 20% NO_3^- to 60% NO_3^-, with essentially no change in pH (Table 2 and Table 4). Our finding that NH_4^+ generally acidifies soil and that NO_3^- generally makes it more alkaline, is widely reported. Such N-induced changes in soil pH are an important consideration in some soils because they may ameliorate or exacerbate other nutrient deficiencies or elemental toxicities (Marschner 1995).

We expected that the isolated roots would partially compensate for the N-deficiency in the remainder of the plant (Drew et al. 1978). Despite the severe N deficiency of the main part of the plant and the continuous N supply to the isolated roots, plant N content did not increase over the 2-week period. However, total plant biomass increased 26% and length of roots in the enriched patches increased up to 5-fold. The lack of a quantifiable compensatory N uptake is attributed to the small fraction of isolated root length exposed to treatment (about 1–3%), the low N concentration of the treatment solution and the short time-frame of the experiment. Restricted N accumulation and continued growth resulted in a redistribution of N from the foliage pool to the stem + root pool (Table 3). The shift of N away from foliage and to the stem + root pool is consistent with increases in root fraction associated with decreasing N availability (Mooney and Winner 1991). Although stems were included in our stem + root pool, we speculate that the shift of N out of the foliar pool was driven more by root accumulation than by stem accumulation, based on previous studies of *Populus* under N stress (Ibrihim et al. 1997, 1998).

The most striking treatment response was an alteration in root architecture (Figure 1). Small changes in the NH_4:NO_3 ratio had large effects on higher-order root development. When percent NO_3^- increased from 80 to 100%, total biomass was unaffected but root length decreased because third- and higher-order roots were absent at 100% NO_3^- (Table 4), indicating that excess NO_3^- hinders the development of higher-order roots. In support of this, a positive effect of NH_4^+ on lateral root development has been reported for corn (Anderson et al. 1991). Even in NO_3^--demanding crop species, 100% NO_3^- does not stimulate root growth as much as when about 25% of the N is supplied as NH_4^+ (Wang and Below 1992, Schortemeyer and Feil 1996). The benefits of supplying both forms of N, rather

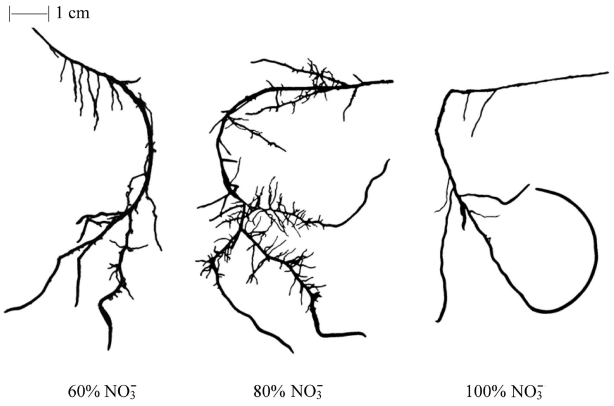

Figure 1. Scanned images of representative split roots from the 60, 80 and 100% NO_3^- treatments. The image illustrates the stimulating effect of 80% NO_3^- on root branching compared with the other treatments.

than NO_3^- alone, are well known (Marschner 1995); however, the sensitivity of root architecture to N form is less well recognized. We note that N form did not alter SRL of individual root orders (Table 4). Higher overall SRL is explained by more branching and the presence of fourth-order roots in the 80% NO_3^- treatment compared with the other treatments (Figure 1). Genetic and environmental effects on total SRL have been reported (Eissenstat 1991, 1992). High SRL values are associated with decreased root diameter (Eissenstat 1992). This presents an image of similar root structure with either thicker or thinner roots. Our findings illustrate how a change in root architecture, caused by the stimulation of higher-order root development, can result in greater overall root system SRL without changing root diameter. This is an important consideration when studying the mechanisms by which genetics and environment control root deployment and the effectiveness of roots at nutrient acquisition under varied environmental conditions (see also Fitter 1994).

It is widely known that tree root growth is plastic and that roots will be deployed preferentially in nutrient-rich soil (Caldwell 1994). We have shown that, by holding N supply constant, root system size and form are sensitive to the balance of NH_4:NO_3. When N was supplied as 80% NO_3^-, cottonwood roots grew in a manner that facilitated soil exploitation (high root length density) more than when N was supplied with more or less NO_3^-. These findings are relevant to understanding the belowground determinants of plant success in ecological communities, and to optimizing the intensive management of woody crops. These data are particularly relevant to situations where trees are used as biofilters (O'Neill and Gordon 1994). We conclude that, to effect greatest absorption of nutrients, NH_4:NO_3 should be balanced to optimize root length development.

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