

The interaction between nitrogen source, soil pH, and drought in the growth and physiology of three poplar clones¹

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Abstract: The objective of this study was to understand how nitrogen (N) source, soil pH, and drought interact in the growth and water-use efficiency of poplars. Rooted cuttings from two hybrid poplar clones, 24 (Walker) (*Populus deltoides* × *Populus ×petrowskyana*), and 794 (Brooks 6) (*Populus deltoides* × *Populus ×petrowskyana*), and one native balsam poplar clone 1004 (*Populus balsamifera* L.), were grown in a greenhouse for a period of 8 weeks. Trees received a combination of two N sources (ammonium sulphate [(NH₄)₂SO₄] and calcium nitrate [Ca(NO₃)₂]), two soil pHs (5 and 7), and two drought levels (85% and 40% field capacity, as determined by mass). Trees had reduced growth, net photosynthesis, and stomatal conductance with NO₃⁻ fertilization, and these effects were accentuated at soil pH 7. The drought treatment only slightly reduced growth and rarely interacted with N source or soil pH. Drought also did not affect instantaneous measurements of water-use efficiency, but it increased δ¹³C by an average of 0.55‰. The poplar clones used in this study appear to be better adapted to take up N from NH₄⁺ than from NO₃⁻-N sources.

Key words: drought stress, fertilization, N source, poplar, stable isotope, water-use efficiency.

Résumé : Les auteurs cherchent à comprendre comment la source d'azote, le pH du sol et la sécheresse interagissent dans la croissance et l'utilisation efficace de l'eau, chez les peupliers. Ils ont cultivé, pendant 8 semaines, des boutures enracinées de deux clones de peupliers hybrides, 24 (Walker) [*Populus deltoides* × *Populus ×petrowskyana*], et 794 (Brooks 6) [*Populus deltoides* × *Populus ×petrowskyana*], ainsi que du clone 1004 de peuplier baumier indigène (*Populus balsamifera* L.). On a fertilisé les arbres avec une combinaison de deux sources d'azote (N) (sulfate d'ammonium [(NH₄)₂SO₄] et nitrate de calcium [Ca(NO₃)₂]), deux pH du sol (5 et 7), et deux degrés de sécheresse (85 % et 40 % de la capacité au champ, telle que déterminée par le poids sec). On observe une croissance, une photosynthèse nette et une conductance stomatale réduites avec la fertilisation au NO₃⁻, et ces effets augmentent à pH 7 du sol. Le traitement à la sécheresse ne réduit la croissance que faiblement et interagit rarement avec l'efficacité de l'utilisation de l'eau, mais augmente le δ¹³C en moyenne de 0.55 ‰. Les clones de peupliers utilisés dans l'étude semblent mieux adaptés à utiliser le NH₄⁺ que le NO₃⁻, comme source de N.

Mots-clés : résistance à la sécheresse, fertilisation, source de N, peuplier, isotopes stables, efficacité de l'utilisation de l'eau.

[Traduit par la Rédaction]

Introduction

Interest in establishing hybrid poplar (*Populus* spp.) plantations under intensive management systems is rapidly expanding in Canada (Lester 1995; Gordon 2001). Throughout the country, farmers are encouraged by government incentives to install new plantations on abandoned farmland to increase carbon sequestration to meet Canada's goals towards the Kyoto Protocol agreement (Natural Resources Canada 2005). Just as in modern agriculture, hybrid poplar plantations require a great deal of maintenance to meet growth

rate expectations (Dickmann et al. 2001), especially in boreal regions where the growing season is relatively short. Having historically used wood from native forests, however, silvicultural expertise with fast growing plantations is yet to be developed for these regions.

In an attempt to promote establishment and early growth of plantations in agricultural fields of the boreal mixed-wood region of Alberta, we established a series of field-fertilization trials where multiple combinations of nitrogen (N), phosphorus (P), and potassium (K) were applied at

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Table 1. Means and SEs for height, basal diameter, and dry-mass growth of each clone after 8 weeks of greenhouse growth.

Clone	Height (cm)	Basal diameter (mm)	Dry mass (g)
1004	32.53a (1.17)	3.04a (0.12)	8.12a (0.71)
24	42.73b (1.15)	4.02b (0.12)	10.50b (0.70)
794	62.18c (1.15)	5.84c (0.12)	26.38c (0.70)

Note: Values with the same letter in a column are not significantly different at $P \leq 0.05$.

Table 2. Analysis of variance giving sources of variation, degrees of freedom (df), and F values for height, basal diameter, dry-mass growth, and root-to-leaf ratio at the end of the experimental period.

Source of variation	df	Height growth	Basal diameter growth	Dry-mass growth	Root/leaf ratio
Block	2	0.06	0.82	1.00	0.03
Clone	2	175.91*	144.04*	237.94*	30.27*
Drought	1	2.09	17.25*	6.17*	0.59
pH	1	147.37*	94.52*	72.57*	0.82
N source	1	50.73*	18.61*	44.92*	25.47*
Clone×drought	2	0.81	0.60	0.04	0.54
Clone×pH	2	15.08*	1.34	3.66*	23.66*
Drought×pH	1	0.07	1.57	2.43	1.06
Clone×N source	2	7.45*	7.75*	18.28*	3.97*
Drought×N source	1	2.51	1.26	1.61	2.39
pH×N source	1	42.98*	23.91*	37.14*	15.12*
Clone×drought×pH	2	0.08	0.48	0.10	0.19
Clone×drought×N source	2	1.91	1.05	2.84	2.10
Clone×pH×N source	2	5.81*	4.69*	8.96*	19.29*
Drought×pH×N source	1	0.18	0.09	0.87	1.35
Clone×drought×pH×N source	2	0.29	1.45	0.95	0.77
Error	188				

Note: Asterisks indicate significant probabilities for F tests: *, $P \leq 0.05$.

planting. Repeatedly, N fertilization with ammonium nitrate (NH_4NO_3) failed to increase growth rates (DesRochers et al. 2006) and even decreased survival (van den Driessche et al. 2003, 2005). Under greenhouse conditions, aspen (*Populus tremuloides* Michx.) responded to NH_4NO_3 fertilizer similarly to a nitrate (NO_3^-) only N source, and trees had reduced growth under higher soil pH (DesRochers et al. 2003). Recent work by Lu et al. (2005) also showed that NH_4NO_3 fertilizer had similar effects to NO_3^- -N fertilizer on the physiology of tobacco (*Nicotiana tabacum* L.) plants. Field results (Choi et al. 2005; DesRochers et al. 2006) suggest that poplars may not be well-adapted to NO_3^- -N sources of fertilizers. This is surprising, since it is often found that plants grow better under NO_3^- fertilization (Allen and Raven 1987; Raab and Terry 1994; Guo et al. 2002), and since the nitrate reductase enzyme is active in poplar roots without induction (Min et al. 1998). Evers (1964) also found that *Populus ×euramericana* Dode grown at soil pH 3 to pH 8 had greater dry-matter production with NO_3^- -N fertilization than with NH_4^+ -N, although an interaction between N sources and pH revealed no difference between N sources at pH 8.

These unexpected effects of NH_4NO_3 on growth and survival of hybrid poplars in the field might be caused by an increased susceptibility to drought brought about by the fertilization (Harvey and van den Driessche 1997). This is of particular concern for the prairie provinces where drought periods commonly occur during the summer months (Envi-

ronment Canada 2004). Gas exchange and leaf carbon isotope ratio ($\delta^{13}\text{C}$) analyses suggested that N fertilization induced water stress (DesRochers et al. 2003, 2006; van den Driessche et al. 2005): During the year of planting, different poplar clones fertilized with NH_4NO_3 produced smaller leaves, had reduced stomatal conductance and net photosynthesis rates, and fixed proportionally more ^{13}C than unfertilized trees (DesRochers et al. 2006). As plants become moisture stressed, leaf stomatal apertures are reduced to limit water loss, leading to greater water-use efficiency (WUE) and less discrimination against ^{13}C (Farquhar et al. 1989; Livingston et al. 1999). Different N sources can also affect plant $\delta^{13}\text{C}$ by influencing plant WUE and carbon assimilation (Yin and Raven 1998). It is not clear, however, how N source (NO_3^- vs. NH_4^+) and soil pH interact with soil moisture in the growth of poplars. Uptake of NO_3^- is usually more difficult in alkaline soils because the reduction of NO_3^- produces hydroxide (OH^-) ions, which then have to be neutralized. If the pH of the rooting medium is high, the neutralization process is more difficult and may disrupt the organic synthesis, affecting in turn the overall uptake of NO_3^- (Huffaker and Rains 1978; Miller and Cramer 2004). The influence of N source on water relations is variable according to the plant species and the experimental conditions (see Lu et al. 2005). Høgh-Jensen and Schjoerring (1997) found that WUE of white clover (*Trifolium repens* L.) was affected by an interaction between N source and water conditions, such that plants were more water-efficient when

supplied with NO_3^- -N. Walch-liu et al. (2000) found that tobacco plants had higher root-to-shoot ratios and stomatal conductance under NH_4^+ and higher water uptake under NO_3^- fertilization. Guo et al. (2002) found that bean plants (*Phaseolus vulgaris* L.) supplied with NO_3^- had lower stomatal conductance, assimilation, and evaporation rates.

The objective of this study was to understand how N source, soil pH, and drought interact in the growth and WUE of poplars. We tested two hybrid poplar clones used in fast-growing plantations and one clone of a poplar native to Canada, balsam poplar (*Populus balsamifera* L.). The latter may be more widely used in afforestation programs in the future, owing to its ease of propagation and to the fact that it preserves the composition of the natural landscape.

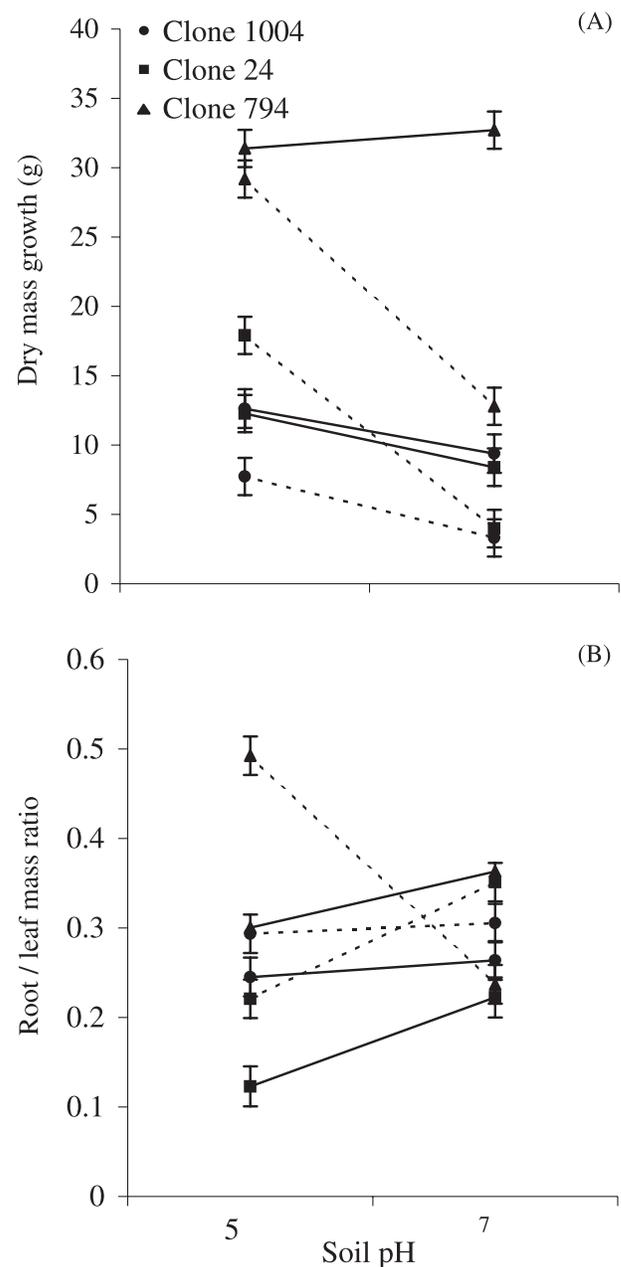
Materials and methods

Rooted cuttings from two hybrid poplar clones, 24 (Walker) (*P. deltoides* × *P. ×petrowskyana*), and 794 (Brooks 6) (*P. deltoides* × *P. ×petrowskyana*), and one native balsam poplar clone 1004 (*P. balsamifera* L.) collected near Slave Lake, Alberta (55°N, 114°W), were chosen for the experiment. The trees were planted in 6.6 L pots containing an artificial soil mixture of 2:1 peat-vermiculite (by volume) and grown in a greenhouse for the duration of the experiment. An 18 h photoperiod (0600–2400 h) was maintained with natural light supplemented with artificial lights (400 W high pressure sodium bulbs, Litmore Distributors Ltd., Edmonton, Alta.), providing a photosynthetic photon flux density (PPFD) of 270 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at pot level. Temperature was set at 25 °C during the light period, and 15 °C during the dark period.

The pH of the soil mixture was adjusted with calcium carbonate (CaCO_3) to obtain the two levels of pH (5 and 7). Nitrogen was supplied as ammonium sulphate [$(\text{NH}_4)_2\text{SO}_4$] (NH_4^+ -N source) and calcium nitrate [$\text{Ca}(\text{NO}_3)_2$] (NO_3^- -N source). The possible confounding effects of S in the $(\text{NH}_4)_2\text{SO}_4$ and the Ca in the $\text{Ca}(\text{NO}_3)_2$ was dealt with by having an adequate supply of S and Ca in all treatments, so that there was unlikely to be a response to the additional S or Ca. Both N sources were applied at a concentration of 200 $\text{mg}\cdot\text{L}^{-1}$ (4.29 $\text{mmol}\cdot\text{L}^{-1}$ of N). A 100 mL aliquot per tree supplied 20 mg N per tree, once a week. Half of the trees (108) received a given N treatment. Each pot also received 3 g of phosphorus (P), which was supplied as monobasic calcium orthophosphate [$\text{Ca}(\text{H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O}$] at the beginning of the experiment. Calcium requirements were satisfied by the CaCO_3 used for pH control, and by the Ca in the calcium orthophosphate. Other macronutrients were supplied once per week. Potassium (K), Mg, and S were supplied in macronutrient solutions of K_2SO_4 and $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, giving 20 mg of K, 18.6 mg of S, and 8 mg of Mg, once per week. Micronutrients were given at the same time as the macronutrients, and included 0.4 mg of chelated Fe, 0.004 mg Mn and Mo, and 0.04 mg Cu, Zn, and B.

The two levels of moisture supply were created by bringing the pots to field capacity and letting them dry to reduce weight by 15% (high moisture level) or 60% (low moisture level) before rewatering. Soil water potentials were not measured; however, for trees subjected to low soil moisture

Fig. 1. Mean (A) dry-mass growth and (B) root-to-leaf mass ratio for each N source, soil pH, and clone combinations. Broken lines are for NO_3^- -N and solid lines are for NH_4^+ -N. Error bars are SEs.

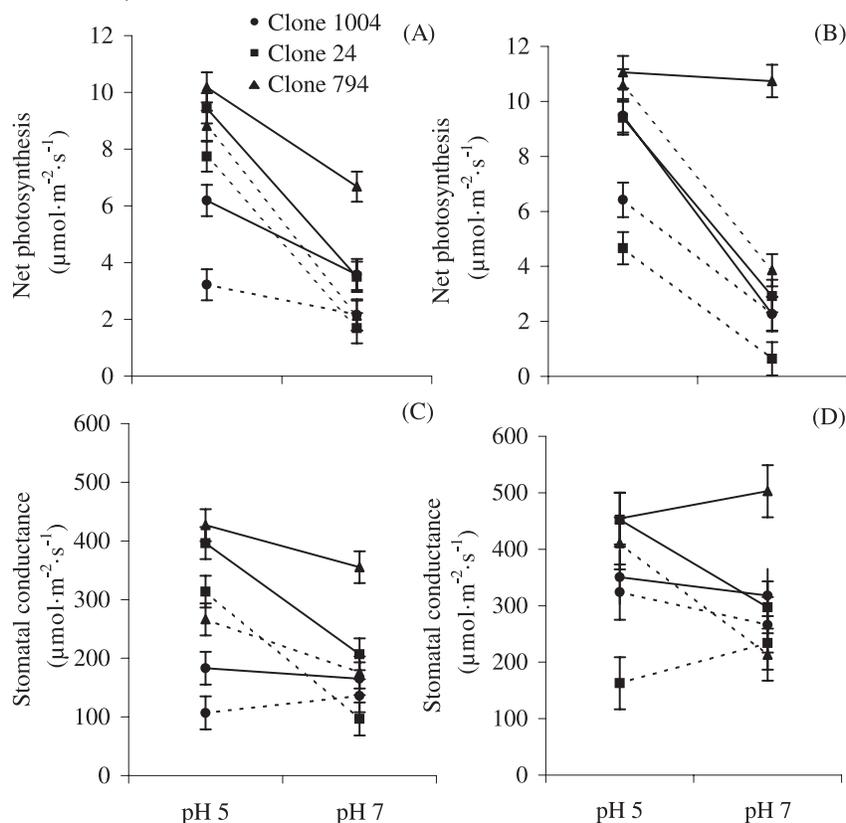


level, leaves would visibly begin to loose turgor if they were not rewatered shortly after having reached this low moisture level. Each pot was wrapped in a heavy plastic bag closed around the root collar to avoid evaporation from the soil surface and to calculate the amount of water used by the trees. Gravimetric WUE (WUE_g) was calculated as the ratio of total plant dry mass gain during the experiment to water used, as measured by weighing the pots daily.

Measurements

Height and basal diameter of each tree were measured immediately after planting, and prior to destructive harvesting after 8 weeks of growth, while the trees were still actively

Fig. 2. Mean net photosynthesis and stomatal conductance during (A, C) the first and (B, D) second measurement dates. Broken lines are for NO_3^- -N and solid lines are for NH_4^+ -N. Error bars are SEs.



growing. After 5 and 8 weeks of growth (first and second measurement periods), measurements of net photosynthesis (A), transpiration (E), and stomatal conductance to CO_2 (G_s) were made between 1000 and 2000 h using an infrared gas analyzer (IRGA, CIRAS I, PP Systems, Haverhill, Mass.) equipped with an automatic broadleaf cuvette (PLC(B), PP Systems). Measurement PPFD was $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while CO_2 concentration and humidity were set at $350 \mu\text{L}\cdot\text{L}^{-1}$ and 40%, respectively. Leaf temperature was set to ambient and averaged $31.9 \pm 3.6^\circ\text{C}$ during the first measurement period and $28.2 \pm 1.5^\circ\text{C}$ during the second measurement period. Instantaneous WUE (WUE_i) was calculated as A/E .

At harvest, the rooted cuttings were carefully washed clean of all rooting medium, separated into leaves, stems, and roots, dried at 80°C for 2 d, then weighed. A sample of 24 trees per clone was also destructively sampled at the beginning of the experiment to calculate average dry mass for each clone. All tissue samples were ground with a Cyclone Sample Mill with a 1 mm mesh size (UDY Corp., Fort Collins, Colo.) and chemical analysis of total N, P, K, Ca, Na, Mg, S, Fe, Mn, Cu, Zn, Mo, and B were carried out (Norwest Labs, Lethbridge, Alta.) for all plant parts. All leaf samples were individually analyzed, whereas stem and root samples were pooled into two replicate samples per treatment combination. Subsamples of the leaves were ground more finely with a Brinkmann MM2 ball grinder and sent to the lab (Soil Biochemistry Laboratory, University of Alberta, Edmonton, Alta.) for $\delta^{13}\text{C}$ determination using a Carlo-Erba 1500 NCS directly interfaced to a V.G. Sira mass spectrometer (Middlewich, Cheshire, UK) tuned for

continuous flow ^{13}C IRMS measurements. The isotopic composition of the samples was calculated from:

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1]1000$$

where R_{sample} and R_{standard} are the ratios of $^{13}\text{C}/^{12}\text{C}$ in the sample and standard, respectively. Spring-wheat grain was used as the working standard, with a carbon isotope composition of -23.47‰ relative to Pee Dee Belemnite (PDB).

Experimental design

The experiment was designed as a completely randomized block design with two N sources ($\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{SO}_4$) \times two drought treatments (85% and 40% field capacity) \times three clones (24, 794, and 1004) \times two pH levels (5 and 7). Each treatment combination was applied to three trees and replicated in three blocks to compensate for possible environmental gradients in the greenhouse ($N = 216$). The growth and nutrient data were statistically analyzed with the General Linear Model procedure of SAS' statistical package (version 8.0, SAS Institute Inc. Cary, N.C.). Gas-exchange measurements were analyzed in a repeated measures analysis of variance (PROC MIXED, SAS). Measurements were blocked according to the experimental design and time of day was used as a covariate. A significance level of $P \leq 0.05$ was chosen.

Results

Growth

There were large growth differences between clones; on

Table 3. Repeated measures analysis of variance giving source of variation, degrees of freedom (df), and *F* values for net photosynthesis, stomatal conductance, and instantaneous WUE.

Source of variation	df	Net photosynthesis	Stomatal conductance	Instantaneous WUE
Block	2	6.82	8.21	2.51
Clone	2	69.53*	18.26*	18.74*
Drought	1	1.17	1.95	0.12
pH	1	299.96*	22.68*	227.77*
N source	1	139.03*	57.48*	51.55*
Clone×drought	2	2.29	0.82	0.30
Clone×pH	2	9.91*	2.86	7.06*
Drought×pH	1	1.41	0.43	0.02
Clone×N source	2	0.72	4.53*	2.20
Drought×N source	1	2.15	0.12	1.51
pH×N source	1	3.86*	0.09	11.33*
Clone×drought×pH	2	1.81	1.49	1.35
Clone×drought×N source	2	1.20	3.08*	0.02
Clone×pH×N source	2	14.95*	4.14*	4.80*
Drought×pH×N source	1	1.98	0.47	0.72
Clone×drought×pH×N source	2	3.27*	0.52	2.02
Date	1	34.87*	49.96*	2.18
Date×clone	2	16.46*	8.16*	14.28*
Date×drought	1	0.18	1.91	1.88
Date×pH	1	0.09	1.89	1.14
Date×N source	1	7.88*	0.36	3.71
Date×clone×drought	2	3.36*	3.77*	0.16
Date×clone×pH	2	7.22*	5.46*	3.55*
Date×drought×pH	1	2.30	0.37	0.86
Date×clone×N source	2	0.35	0.70	0.49
Date×drought×N source	1	0.57	1.98	0.10
Date×pH×N source	1	1.42	0.23	2.37
Date×clone×drought×pH	2	0.34	0.32	1.44
Date×clone×drought×N source	2	1.47	0.41	1.66
Date×clone×pH×N source	2	4.44*	5.10*	0.06
Date×drought×pH×N source	1	0.04	0.37	0.93
Date×clone×drought×pH×N source	2	0.33	0.64	0.55
Time of day covariate	1	9.42*	13.34*	0.19

Note: Asterisks indicate significant probabilities for *F* tests: *, $P \leq 0.05$.

average, clone 1004 produced 8.1 g of dry matter, while the hybrid clones 24 and 794, respectively, produced 1.3- and 3.2-fold the biomass of clone 1004 (Table 1). Both pH and N source had major effects on biomass growth and interacted with clones (Table 2). Dry-mass growth was greater at pH 5 than 7 for clones 1004 and 24, and was especially low for all clones at pH 7 when NO_3^- -N was supplied (Fig. 1A). Clone 794 also showed decreased growth between pH 5 and pH 7 when supplied NO_3^- -N, but was not affected by pH when supplied NH_4^+ -N (Fig. 1A). The drought treatment reduced biomass growth by only 12%, by reducing basal diameter but not height (Table 2). Moisture did not interact with any of the other treatments for biomass growth (clone, N source, or pH; Table 2). Root/leaf mass ratios were unaffected by the drought treatment. Clones 1004 and 24 had greater root/leaf mass ratios when given NO_3^- -N under both soil pHs, whereas clone 794 growing under a soil pH of 7 had greater root/leaf ratios when supplied NH_4^+ -N (Fig. 1B).

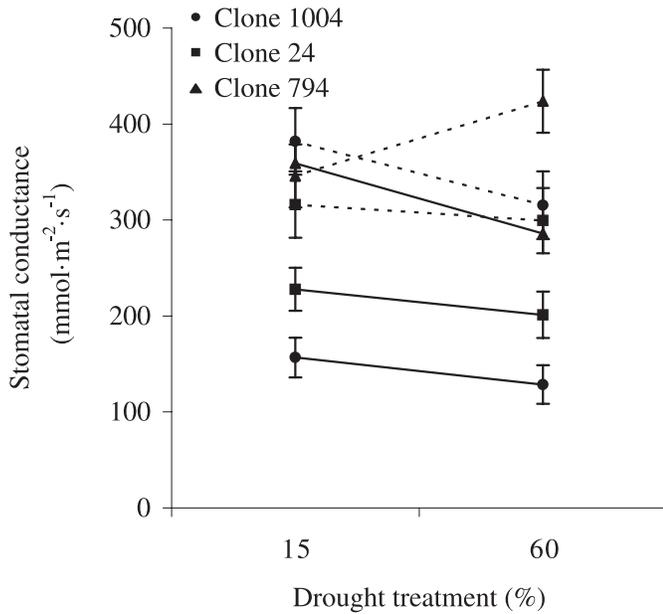
Gas exchange

Like the biomass growth of trees, net photosynthesis rates

(A) were higher at pH 5 and under NH_4^+ -N fertilization (Figs. 2A and 2B). Rates of A were similar for both measurement dates, however, A of clone 794 was no longer reduced by pH 7 when supplied with NH_4^+ -N or by NO_3^- -N at pH 5 (Fig. 2B), leading to significant interactions with date (Table 3). In addition, A of clone 24 fertilized with NO_3^- -N dropped below that of the native clone 1004 at the second measurement date (Fig. 2B). The drought treatment generally did not affect A, except for clone 1004 during the second measurement date where it was significantly reduced by 23% under the low moisture treatment (date × clone × drought interaction, Table 3).

During the first measurement date, the hybrid clones showed reduced stomatal conductance (G_s) at soil pH 7, while G_s of the native clone (1004) was unaffected by soil pH (Fig. 2C). NO_3^- -N fertilization reduced G_s by 31.0%, 31.3%, and 42.8% for clones 1004, 24, and 794, respectively, compared with NH_4^+ -N fertilization (Fig. 2C). G_s was also reduced with NO_3^- -N fertilization during the second measurement date, and was generally lower at pH 7 except for clone 794 under NH_4^+ -N fertilization or for clone 24 under NO_3^- -N fertilization (Fig. 2D). The main effect of

Fig. 3. Mean stomatal conductance for each clone and drought treatment. Solid lines are for the first measurement date and broken lines are for the second measurement date. Error bars are SEs.



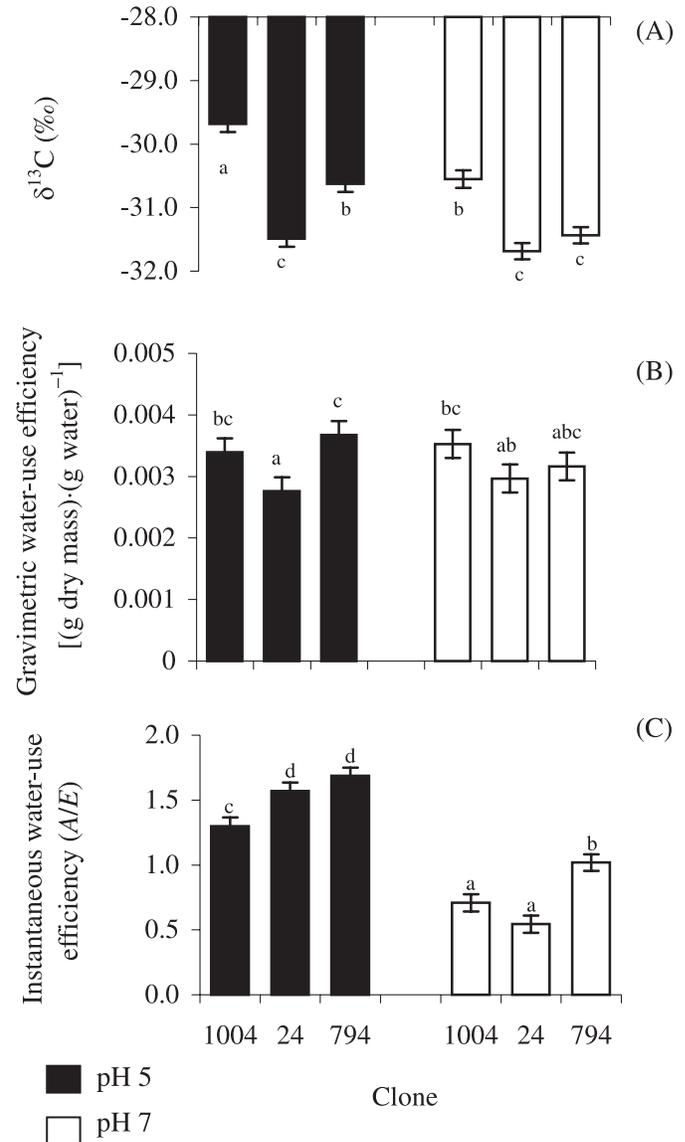
drought on G_s was not significant (Table 3), however the significant date \times clone \times drought interaction shows that clone 794 maintained high G_s under the low moisture treatment at the second measurement date (Fig. 3).

Water use and leaf $\delta^{13}\text{C}$

The native clone (1004) had higher (less negative) mean values of $\delta^{13}\text{C}$ than the hybrids (Fig. 4A). Increasing soil pH from 5 to 7 decreased $\delta^{13}\text{C}$ of clones 1004 and 794 by 0.87‰ and 0.81‰, respectively, while the decrease in $\delta^{13}\text{C}$ of clone 24 (0.19‰) was not significant (Fig. 4A). Clone 24, which had the lowest $\delta^{13}\text{C}$ values, also had the lowest WUE_g (Fig. 4B). Clone 1004 had lower WUE_i than the hybrid clones at pH 5, and WUE_i declined for all clones at pH 7 (Fig. 4C).

The drought treatment did not affect measurements of WUE_i (Table 3), while it increased $\delta^{13}\text{C}$ by an average of 0.55‰ (Table 4). WUE_g also increased by 36% and 32% under drought conditions for clones 1004 and 24, respectively, while clone 794 had similar WUE_g under both moisture treatments (Table 5). The drought treatment further interacted with soil pH for WUE_g (Table 4), such that trees had higher WUE_g at pH 5 under well watered conditions and at pH 7 under water-limited conditions (Table 5). Dry matter production and water use over the experimental period were well-correlated for each clone (Fig. 5). The greater slope of the linear regression for clone 794 shows its greater water use efficiency (Fig. 5C). Although high WUE_g could also be achieved in clones 1004 and 24 (Table 5), it was mainly at the expense of dry matter production (Fig. 6). The behaviour of clone 794 contrasted with that of 1004 and 24 in that dry matter production increased almost linearly with WUE_g . At a WUE_g of 0.006 g·g⁻¹, clone 24 was making minimal growth, whereas clone 794 was making maximum growth (approximately 10-fold the dry matter production).

Fig. 4. Mean (A) leaf carbon isotope ratios, (B) gravimetric WUE and (C) instantaneous WUE for each clone and soil pH combination. Error bars are SEs. Bars with the same letters are not significantly different at $P \leq 0.05$.



$\text{NH}_4^+\text{-N}$ fertilization decreased WUE_g of clones 1004 and 24, while it increased it for clone 794 (Table 5). There was a lot of variation, however, and the effect was only significant for clone 1004. The effect of N source on WUE_g also interacted with soil pH, such that an increase in pH from 5 to 7 increased WUE_g with $\text{NH}_4^+\text{-N}$ fertilization, but reduced it with $\text{NO}_3^-\text{-N}$ fertilization (Table 5). This interaction was also significant for WUE_i (Table 3); however, it showed that an increase in pH from 5 to 7 always reduced WUE_i and that the decrease was greater under $\text{NO}_3^-\text{-N}$ fertilization (Fig. 7).

Mineral nutrition

Clone 794 utilized leaf N 3.7-fold and leaf P 2-fold more productively than clone 1004 (Table 6). Clone 794 showed the greatest utilization efficiency for all macronutrients, but

Table 4. Analysis of variance giving source of variation, degrees of freedom (df), and *F* values for leaf carbon isotope ratio ($\delta^{13}\text{C}$) and WUE_g .

Source of variation	df	$\delta^{13}\text{C}$ (‰)	WUE_g
Block	2	2.14	0.04
Clone	2	64.89*	4.38*
Drought	1	27.35*	18.79*
pH	1	35.25*	0.10
N source	1	1.10	1.91
Clone×drought	2	0.19	7.57*
Clone×pH	2	4.25*	1.57
Drought×pH	1	0.41	9.64*
Clone×N source	2	2.95*	6.43*
Drought×N source	1	0.33	2.92
pH×N source	1	1.40	7.26*
Clone×drought×pH	2	1.76	1.84
Clone×drought×N source	2	0.33	1.26
Clone×pH×N source	2	1.26	0.42
Drought×pH×N source	1	0.02	0.82
Clone×drought×pH×N source	2	0.77	0.14
Error	188		

Note: Asterisks indicate significance probabilities for *F* tests: *, $P \leq 0.05$.

differences between the other two clones varied according to nutrient (Table 6). Biomass growth increased linearly with plant N ($R^2 = 0.81$, $P < 0.001$), P ($R^2 = 0.88$, $P < 0.001$), and K ($R^2 = 0.95$, $P < 0.001$) uptake. N uptake by plants was 12% greater under well watered conditions (0.34 vs. 0.30 $\text{g}\cdot\text{plant}^{-1}$; $P = 0.01$), and was generally greater with $\text{NH}_4^+\text{-N}$ fertilization and at pH 5 (Fig. 8A). However, clone 794 was able to absorb similar amounts of N under both soil pH when given $\text{NH}_4^+\text{-N}$, and N uptake by clone 24 was unaffected by N source at pH 5 (Fig. 8A). The drought treatment decreased K uptake by 13% ($P = 0.03$), but it did not affect P ($P = 0.87$) or Ca uptake ($P = 0.12$). P uptake was greater at pH 5 and was reduced by $\text{NO}_3^-\text{-N}$ fertilization under soil pH of 7 (Fig. 8B). At soil pH of 5, however, $\text{NO}_3^-\text{-N}$ fertilization increased P uptake for clone 24, reduced it for clone 1004, while N source did not affect P uptake by clone 794 (Fig. 8B). K uptake was similar to P uptake showing interactions between soil pH, N source, and clone (Fig. 8C). Trees from clone 1004 and 24 fertilized with $\text{NH}_4^+\text{-N}$ had similar Ca uptake under both soil pHs, while an increase from pH 5 to 7 increased Ca uptake for clone 794. When fertilized with $\text{NO}_3^-\text{-N}$, an increase in soil pH reduced Ca uptake (Fig. 8D).

Discussion

Growth responses of hybrid poplars to mineral fertilization have been widely reported in Europe and North America (Blackmon 1976). N fertilization studies have usually resulted in tree volume increases for various hybrid poplar clones (Curlin 1967; McLaughlin et al. 1987; Heilman and Fu-Guang 1993; Brown and van den Driessche 2002, 2005). However, few studies have dealt with the preference of hybrid poplars for $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$, which can be affected by soil pH (Evers 1964; van den Driessche 1978; Garnett and Smethurst 1999). This work showed that growth of the

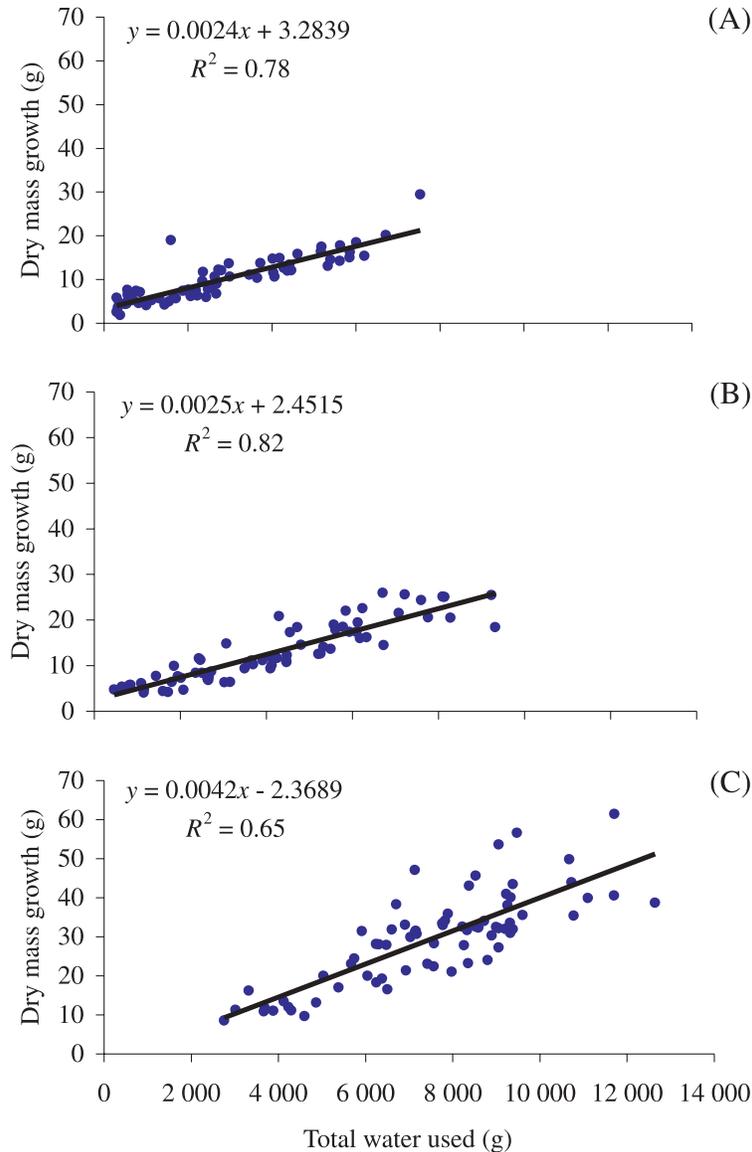
Table 5. Mean WUE_g for each clone and N source, clone and drought, soil pH and drought, and soil pH and N source combination.

Clone	N source	WUE_g	Drought	WUE_g
1004	$\text{NO}_3^-\text{-N}$	3.95c	15%	2.71ab
	$\text{NH}_4^+\text{-N}$	2.97a	60%	4.21d
24	$\text{NO}_3^-\text{-N}$	3.06a	15%	2.32a
	$\text{NH}_4^+\text{-N}$	2.66a	60%	3.41c
794	$\text{NO}_3^-\text{-N}$	3.11ab	15%	3.51c
	$\text{NH}_4^+\text{-N}$	3.73bc	60%	3.32bc
Soil pH				
5	$\text{NO}_3^-\text{-N}$	3.65b	15%	3.16b
	$\text{NH}_4^+\text{-N}$	2.90a	60%	2.53a
7	$\text{NO}_3^-\text{-N}$	3.10a	15%	3.39b
	$\text{NH}_4^+\text{-N}$	3.34ab	60%	3.90c

Note: Values with the same letter in a column are not significantly different at $P \leq 0.05$.

poplar clones used in this study was generally reduced under $\text{NO}_3^-\text{-N}$ fertilization compared with $\text{NH}_4^+\text{-N}$ (Fig. 1A), although others have suggested that *P. tremuloides* is better adapted to $\text{NO}_3^-\text{-N}$ (Kronzucker et al. 1997; Min et al. 1998). The adverse effect of $\text{NO}_3^-\text{-N}$ source was also more pronounced at pH 7 than pH 5. These results are in agreement with previous trials in the field (DesRochers et al. 2006) and with work by Choi et al. (2005) on trembling and hybrid aspens. Reduced growth of plants with $\text{NO}_3^-\text{-N}$ at pH 7 or higher is ascribed to the difficulty of maintaining an internal electrical charge balance (Raven and Smith 1976). Growth of clones 1004 and 794 was nonetheless also reduced by $\text{NO}_3^-\text{-N}$ fertilization under a soil of pH 5 (Fig. 1A), and even if the dry-mass growth of clone 24 was greater under these conditions, net photosynthesis (*A*) and stomatal conductance (G_s) of this clone were greatly reduced towards the end of the experiment (second measurement date, Fig. 2); this suggests that the growth-reducing effect of $\text{NO}_3^-\text{-N}$ was more gradual for this clone and that its dry mass would also have been reduced with $\text{NO}_3^-\text{-N}$ fertilization at pH 5 if the experiment had continued over a longer period of time.

In tobacco plants, Lu et al. (2005) found that $\text{NH}_4^+\text{-N}$, compared with $\text{NO}_3^-\text{-N}$ or $\text{NH}_4\text{NO}_3\text{-N}$ sources, reduced G_s and increased $\delta^{13}\text{C}$. Our results were opposite, since $\text{NO}_3^-\text{-N}$ reduced G_s , by an average of 34% (Fig. 2). When G_s is small in relation to the capacity for CO_2 fixation, it tends to limit CO_2 supply inside the leaf and reduce ^{13}C discrimination (Farquhar et al. 1988). However, since *A* decreased proportionally to G_s in the $\text{NO}_3^-\text{-N}$ treatment in our study (38% reduction), $\delta^{13}\text{C}$ did not significantly change (Table 4; Livingston et al. 1998; Siegwolf et al. 2001; DesRochers et al. 2003). Others also found a decrease in G_s under $\text{NO}_3^-\text{-N}$ in French beans (*Phaseolus vulgaris* L.; Guo et al. 2002) and white clover (*Trifolium repens* L.; Høgh-Jensen and Schjoerring 1997). Additionally, Guo et al. (2002) found that the plants had greater water uptake leading to decreased WUE under $\text{NO}_3^-\text{-N}$, which is in accordance with our instantaneous measurements of WUE . Common wheat (*Triticum aestivum* L.) plants grown in hydroponics were also more water efficient when fertilized with $\text{NH}_4^+\text{-N}$ compared with $\text{NO}_3^-\text{-N}$ and NH_4NO_3 (Yin and Raven

Fig. 5. Dry-mass growth as a function of total water used over the experimental period for clones (A) 1004, (B) 24, and (C) 794.

1998). Nitrate uptake into guard cells of the stomates would render plants more sensitive to drought stress (Ines Minguez and Sau 1989; Guo et al. 2003). This is an important finding since previous studies showed that NH_4NO_3 , which has been found to behave like an NO_3^- -N source even though half of its N is in the NH_4^+ form (DesRochers et al. 2003; Lu et al. 2005), reduced first year growth (DesRochers et al. 2006) and survival (van den Driessche et al. 2003, 2005) of field-planted hybrid poplars. These negative effects of NO_3^- -N fertilization on WUE_i , however, were not accentuated by drought (Table 3). The drought treatment we used did not cause marked reductions in growth and rarely interacted with the N source or pH treatments. Reducing water content of the soil medium to 60% may not have been a severe treatment for these clones (measurements of xylem water potential (ψ_x) were not made), although it was enough to significantly increase $\delta^{13}\text{C}$ and WUE_g (Table 4). Interestingly, the reduction in G_s under NO_3^- -N fertilization was greater for clone

794 under wet conditions (Fig. 3), perhaps because N uptake was greater under these conditions, so that it had a greater effect on stomatal conductance.

There was generally more agreement between WUE_g and leaf $\delta^{13}\text{C}$ than between WUE_g and WUE_i (Tables 3 and 4). The gravimetric and $\delta^{13}\text{C}$ methods represent integrated effects over time and are more representative of the plant over the entire experimental period. They can thus be expected to show better mutual agreement than with instantaneous measurements, which are more prone to vary between leaves and environmental conditions at the time of measurement. It is not clear why NO_3^- -N would decrease WUE_i and at the same time increase WUE_g . Perhaps since most of the trees fertilized with NO_3^- -N from clones 1004 and 24 were small, they were rarely limited by water supply (because of the size of the pots) and they could grow for much longer time periods without rewatering (Fig. 6). The fact that clone 794 could continue increasing dry matter production even at high WUE_g (Fig. 6) indicates that it is better

Fig. 6. Relationship between dry-mass growth and WUE_g for clones (A) 1004, (B) 24, and (C) 794.

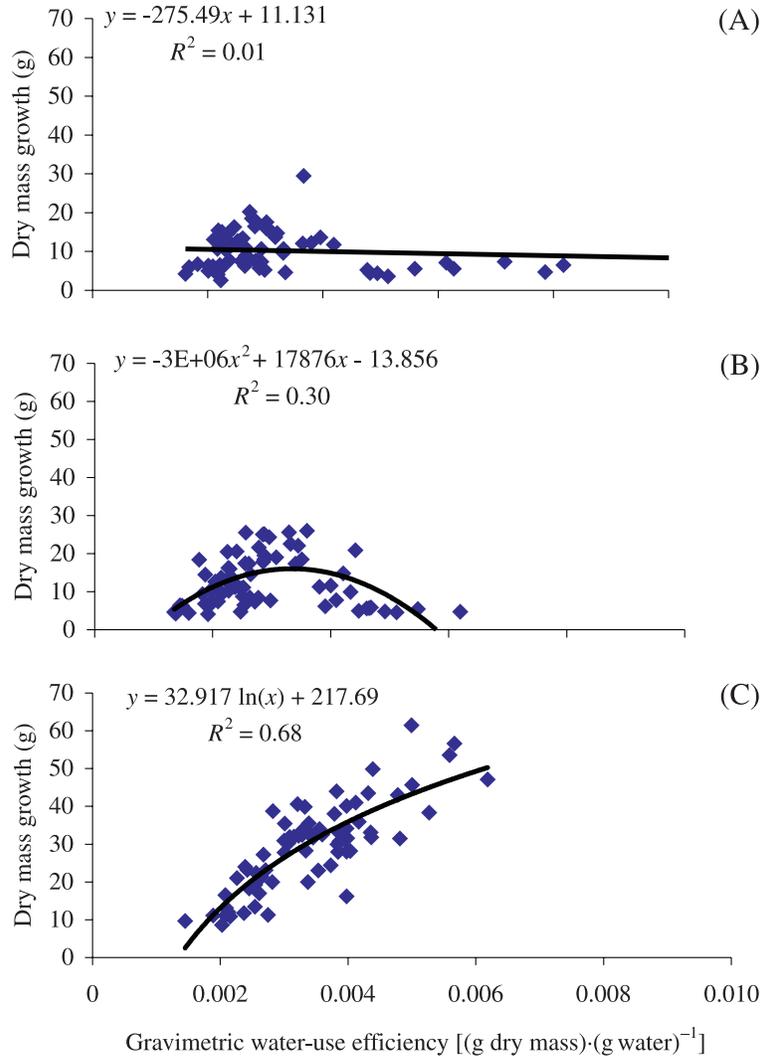
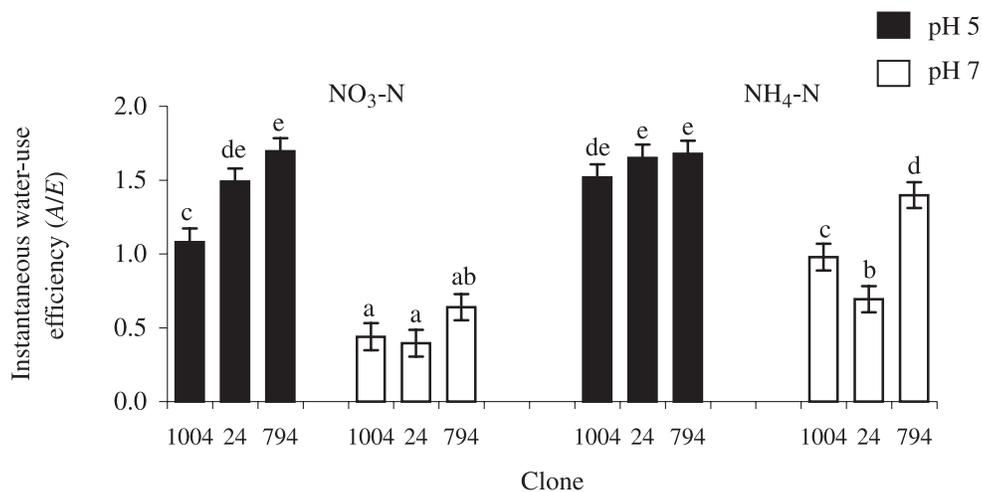


Fig. 7. Mean WUE_i for each N source, soil pH, and clone combination. Error bars are SEs. Bars with the same letters are not significantly different at $P \leq 0.05$.



adapted to tolerate moisture stress of the level applied in this experiment than the other clones. Clones 1004 and 24 achieved high WUE_g in trees that produced very little dry

matter, and it is possible that on experiencing moisture stress both clones avoid it by reducing stomatal aperture to a minimum resulting in high WUE_g , but little growth. Pro-

Fig. 8. Mean leaf (A) N, (B) P, (C) K, and (D) Ca uptake for each N source, soil pH, and clone combination. Broken lines are for NO_3^- -N and solid lines are for NH_4^+ -N. Error bars are SEs.

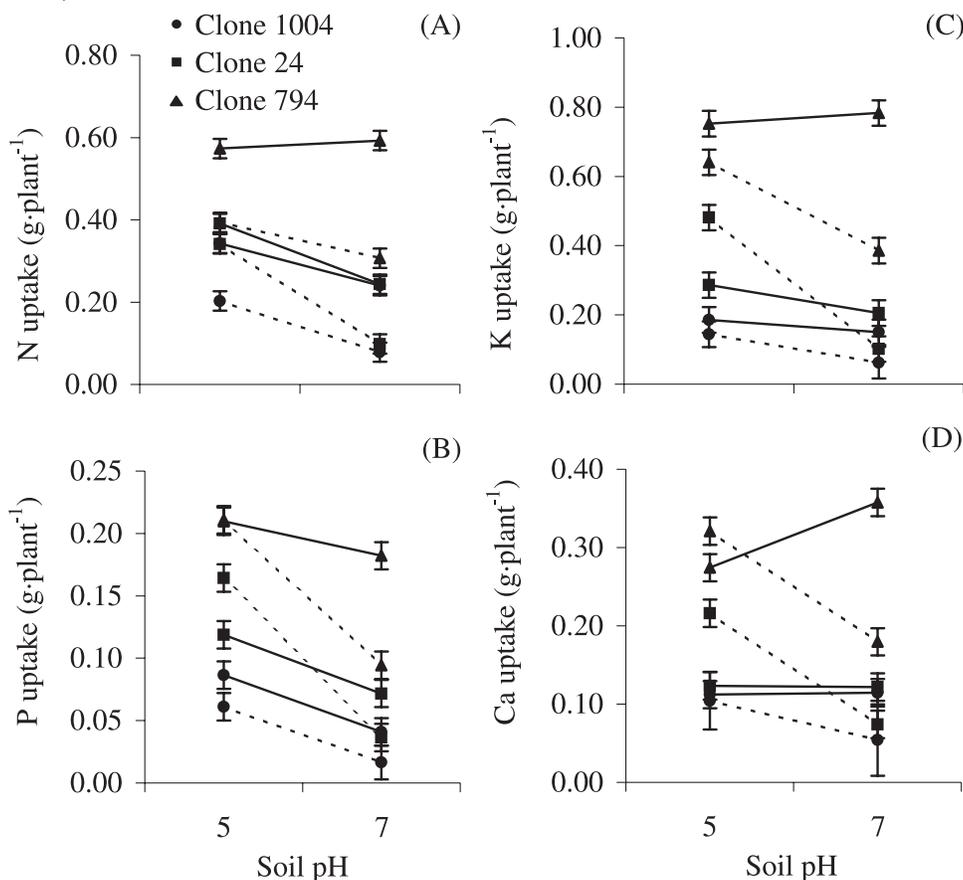


Table 6. Mean nutrient utilization efficiencies [gram of dry-mass growth per leaf nutrient concentration (g·kg⁻¹)] for individual nutrients and clones after 8 weeks of growth.

Nutrient	Clone 1004	Clone 24	Clone 794
N	0.24 (0.04)	0.37 (0.04)	1.11 (0.04)
P	1.16 (0.14)	0.99 (0.14)	3.35 (0.13)
K	0.40 (0.03)	0.34 (0.03)	0.79 (0.03)
Ca	0.60 (0.06)	0.70 (0.06)	1.93 (0.06)
Mg	1.84 (0.19)	2.17 (0.18)	5.63 (0.17)
S	1.39 (0.11)	0.87 (0.11)	3.56 (0.10)

Note: SEs are given in parentheses.

ductive clones can often be more drought-sensitive (Monclus et al. 2005), but in our case, clone 794 was developed from and for the Canadian prairies; hence, it was probably selected for its high productivity despite the relatively dry climate. The low $\delta^{13}\text{C}$ and WUE_g values obtained for clone 24 indicate that it is more drought sensitive (Fig. 4). This clone was somewhat better adapted to use NO_3^- -N (Fig. 1A), which may be why it was less water efficient on average (Ines Miguez and Sau 1989; Guo et al. 2003).

Trees generally responded to the decreased N uptake under NO_3^- -N fertilization (Fig. 8A) by increasing their root-to-leaf ratios (Fig. 1B). Reduced N supply or water stress can lead to increased root-to-shoot ratios (Larcher 2003), however, it is not always the case (Hubick 1990;

Sun et al. 1996; Ripullone et al. 2004; Table 2). For eastern cottonwood (*Populus deltoides* Bartr.), Coyle and Coleman (2005) found that allometric relationships between woody perennial tissues rarely differed in fertilized or irrigated treatments, and that changes in belowground allocation were due to developmental effects.

The concentration of nutrients in foliage is considered to be an indication of its physiological competence, and it was suggested that considering plant growth in relation to nutrient concentration might better explain differences in plant growth performances (Siddiqi and Glass 1981). Nutrient utilization efficiencies (Table 6) also show the superiority of clone 794 in utilizing N and other macronutrients. Leaf P uptake under pH 7 and NO_3^- -N fertilization was greatly reduced (Fig. 8B), which has also been observed in NO_3^- -grown beets (*Beta vulgaris* L.; Raab and Terry 1995) and aspen seedlings (DesRochers et al. 2003). N sources causing an increase in soil pH such as NO_3^- -N sources (Tisdale et al. 1999) will usually decrease P uptake by changing its molecular form, making it less available to plants (Soon and Miller 1977). Reduced P uptake can certainly reduce growth of plants, however it seems that it could also reduce drought resistance of poplars (Harvey and van den Driessche 1999). In the field we have shown that the reduction in P uptake was related to increased $\delta^{13}\text{C}$ of clones 24 and 794 (DesRochers et al. 2006), while Brück et al. (2000) also found this to be true for pearl millet (*Pennisetum glaucum* L.). In this study, P uptake was

linearly related to our WUE_i for the first ($R^2 = 0.53$; $P < 0.001$) and second ($R^2 = 0.60$; $P < 0.001$) measurement dates, while it was not related to $\delta^{13}C$ ($P = 0.37$) or WUE_g ($P = 0.93$). Again, the reason why WUE_i and WUE_g or $\delta^{13}C$ differ is not clear and should be further investigated in poplars by better controlling water stress using xylem water potentials.

In conclusion, we showed that these poplar clones are better adapted to take up N from NH_4^+ than from NO_3^- , and that the negative physiological effects of the NO_3^- -N source were accentuated by higher soil pH. The time-integrated methods of calculating WUE (WUE_g and $\delta^{13}C$) were in better agreement, and sometimes differed with instantaneous measurements (WUE_i). Clone 794 produced the greatest amount of dry matter under high WUE_g and appeared to be better adapted to productive growth in dry conditions, while clone 24 was the least water efficient.

Acknowledgements

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