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Growth and biomass of *Populus* irrigated with landfill leachate

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Abstract

Resource managers are challenged with waste disposal and leachate produced from its degradation. Poplar (*Populus* spp.) trees offer an opportunity for ecological leachate disposal as an irrigation source for managed tree systems. Our objective was to irrigate *Populus* trees with municipal solid waste landfill leachate or fertilized well water (control) (N, P, K) during the 2005 and 2006 growing seasons and test for differences in tree height, diameter, volume, and biomass of leaves, stems, branches, and roots. The trees were grown at the Oneida County Landfill located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Eight clones belonging to four genomic groups were tested: NC13460, NC14018 [(*Populus trichocarpa* Torr. & Gray × *Populus deltoides* Bartr. ex Marsh) × *P. deltoides* 'BC_1']; NC14104, NC14106, DM115 (*P. deltoides* × *Populus maximowiczii* A. Henry 'DM'); DN5 (*P. deltoides* × *Populus nigra* L. 'DN'); NM2, NM6 (*P. nigra* × *P. maximowiczii* 'NM'). The survival rate for each of the irrigation treatments was 78%. The total aboveground biomass ranged from 0.51 to 2.50 Mg ha^{-1}, with a mean of 1.57 Mg ha^{-1}. The treatment × clone interaction was not significant for tree diameter, total volume, dry mass of the stump or basal roots, or root mass fraction ($P > 0.05$). However, the treatment × clone interaction was significant for height, total tree dry mass, aboveground dry mass, belowground dry mass, and dry mass of the leaves, stems + branches (woody), and lateral roots ($P < 0.05$). There was broad clonal variation within the BC_1 and DM genomic groups, with genotypes performing differently for treatments. In contrast, the performance of the NM and DN genomic groups was relatively stable across treatments, with clonal response to irrigation being similar regardless of treatment. Nevertheless, selection at the clone level also was important. For example, NC14104 consistently performed better when irrigated with leachate compared with water, while NC14018 responded better to water than leachate. Overall, these data will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects.
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Keywords: Hybrid poplar; Phytoremediation; *Populus deltoides*; *Populus maximowiczii*; *Populus nigra*; *Populus trichocarpa*; Root mass fraction

1. Introduction

Poplars (*Populus* spp.) have been extensively studied in short rotation woody biomass production systems for multiple uses such as fiber, fuel and environmental benefits (Dickmann, 2001; Isebrands and Karnosky, 2001; Coleman and Stanturf, 2006). Exemplary traits that have contributed to the success of such uses include: ease of rooting, quick establishment, fast growth, and elevated rates of photosynthesis and water usage (Ceulemans et al., 1992; Pontailler et al., 1999; Zalesny et al., 2006). Broad genetic diversity among poplar genomic groups and selection of specific genotypes within such groups increase the potential enhancement of growth and establishment for various uses across heterogeneous sites (Heilman and Stettler, 1985; Heilman et al., 1994). The combination of appropriate cultural practices and well-suited genotypes helps to maximize poplar performance for improved biomass yields (Buhler et al., 1998; Stanturf et al., 2001).

Environmental benefits have been realized from poplar culture when used as components in riparian buffers along streams (Schultz et al., 2004) and as vegetative filters for phytoremediation applications (Licht and Isebrands, 2005). Several phytoremediation projects utilized wastewater in the form of landfill leachate as an irrigation and fertilization source

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for poplar trees (Shrive et al., 1994; Erdman and Christenson, 2000; Zalesny and Bauer, in press). Proper clonal selection practices must be utilized given the genetic variability within the genus *Populus* (Rajora and Zsuffa, 1990; Eckenwalder, 1996) and the variable concentrations of inorganic and organic components in the leachate (Gettinby et al., 1996). Leachate production occurs through natural degradation processes aided by the movement of water through the landfill profile (Christensen and Kjeldsen, 1989). Due to the variation associated with residential, commercial, and industrial waste material, the leachate is highly variable and compositional changes occur seasonally and annually (Shrive et al., 1994; Kjeldsen et al., 2002).

A great deal of information has been reported using poplars for short rotation forestry (Heilman, 1999; Riemenschneider et al., 2001), but there are relatively fewer reports about using poplars for leachate phytoremediation systems. Thus, researchers and resource managers need information that is currently lacking about tree establishment with leachate irrigation. Such information will help increase the success of using poplars for remedial benefits, especially with ecologically damaging contaminants such as those found in most leachate. Overall, the use of short rotation woody crop management for remediation supports improved environmental quality and secondary benefits such as carbon sequestration, a harvestable product, aesthetic improvements, and erosion control (Isebrands and Karnosky, 2001; Duggan, 2005).

This project expands on our previous work investigating phyto-recurrent selection, which was defined as a method using crop and tree improvement strategies to identify and select superior performing clones for remediation projects (Zalesny et al., in press). Clonal selections were made after three successive cycles of evaluation (i.e. three separate greenhouse studies) testing 23 traits relating to height growth, leaf development, and root initiation at 14 (cycle 1; 25 clones), 45 (cycle 2; 12 clones), and 30 (cycle 3; 12 clones) days after planting. The best eight clones were selected for testing in the current *in situ* study (cycle 4) out of the original 25 genotypes belonging to six distinct genomic groups: (1) (*Populus trichocarpa* Torr. & Gray × *Populus deltoides* Bartr. ex Marsh) × *P. deltoides* 'BC_1'; (2) *P. deltoides* × *P. deltoides* 'DD'; (3) *P. deltoides* 'D'; (4) *P. deltoides* × *Populus maximowiczii* A. Henry 'DM'; (5) *P. deltoides* × *Populus nigra L.* 'DN'; (6) *P. nigra* × *P. maximowiczii* 'NM'.

The overall objective of all phyto-recurrent selection cycles was to test the effectiveness of poplars for uptake of inorganic and organic contaminants found in landfill leachate. More specifically, the objective of the current study was to test for differences in growth and biomass distribution of eight *Populus* clones when irrigated with municipal solid waste landfill leachate or fertilized well water (control) (N, P, K) for two growing seasons. In addition to actual phytoremediation success, tree growth and biomass accumulation are important for evaluating the overall effectiveness of the biological attenuation system. These data will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects.

Table 1
Mean temperature, total precipitation, and total number of growing degree days (GDD; base = 10 °C) from May to October during 2005 and 2006 in Rhinelander, Wisconsin, USA (45.6°N, 89.4°W)

Month	Temperature (°C)		Precipitation (cm)		GDD	
	2005	2006	2005	2006	2005	2006
May	10	12	4.44	17.14	133	218
June	16	17	4.51	1.26	374	383
July	20	21	13.04	11.18	597	649
August	16	18	5.43	9.92	380	460
September	12	na[a]	3.90	na	161	na
October	5	na	3.44	na	17	na

[a] Not applicable because trees were harvested 18 August 2006.

2. Materials and methods

2.1. Site and leachate description

The study was conducted at the Oneida County Landfill (municipal solid waste) located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Temperature, precipitation, and growing degree days across the experimental period are listed in Table 1. The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0–6% slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash. Soil pH, along with carbon and nitrogen content, is listed in Table 2.

Leachate was collected from the Oneida County Landfill and its chemistry was analyzed (Northern Lake Service Inc., Crandon, Wisconsin, USA) using approved United States Environmental Protection Agency methods. The leachate was brown in color and had a putrid odor. Concentrations of nitrogen (N), phosphorous (P), and potassium (K) were 610 ± 68 mg N L^{-1} (157 kg N ha^{-1}), 2.3 ± 0.4 mg P L^{-1} (0.6 kg P ha^{-1}), and 450 ± 30 mg K L^{-1} (115 kg K ha^{-1}). The primary toxicity concern was the relatively high chloride (Cl^-) concentration of 1114 ± 140 mg L^{-1} (286 kg Cl^- ha^{-1}). In contrast, the Cl^- concentration in the well water (control) at the time of harvest was 3.5 mg L^{-1} (0.9 kg Cl^- ha^{-1}). Other than Cl^-, the leachate concentrations of inorganics, organics, and metals have declined annually since final closure and capping of the landfill in 2002. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development. Variation of pH, salinity, biological oxygen demand, chemical oxygen demand, and Cl^- concentration since landfill closure is presented in Table 3.

2.2. Clone selection and experimental design

Eight *Populus* clones were selected from 25 original genotypes during three phyto-recurrent selection cycles based on 23 traits relating to height growth, leaf development, and root initiation (Zalesny et al., in press). The clones and their parentages (i.e. genomic groups) were: NC13460, NC14018

Table 2
Soil pH ($n = 3$), along with carbon and nitrogen content ($n = 4$), at a depth of 0–30 cm at nine sampling points for each treatment

Sampling point	pH		C ($g\ kg^{-1}$)		N ($g\ kg^{-1}$)	
	Control	Leachate	Control	Leachate	Control	Leachate
1	5.21 ± 0.03	6.07 ± 0.04	7.03 ± 0.21	22.35 ± 2.44	0.60 ± 0.06	1.73 ± 0.20
2	5.68 ± 0.07	6.15 ± 0.02	7.43 ± 1.36	36.25 ± 2.00	0.67 ± 0.19	3.05 ± 0.17
3	5.54 ± 0.07	5.71 ± 0.02	5.83 ± 0.84	24.40 ± 1.25	0.50 ± 0.07	2.00 ± 0.11
4	5.31 ± 0.07	6.21 ± 0.09	10.23 ± 0.76	45.70 ± 2.23	0.88 ± 0.06	3.80 ± 0.17
5	5.93 ± 0.04	6.32 ± 0.03	16.83 ± 1.80	51.30 ± 5.45	1.55 ± 0.10	4.53 ± 0.52
6	6.35 ± 0.02	6.25 ± 0.02	42.50 ± 3.77	49.55 ± 2.24	3.60 ± 0.32	4.35 ± 0.22
7	5.70 ± 0.03	6.37 ± 0.01	33.63 ± 2.47	50.23 ± 2.57	2.95 ± 0.19	4.38 ± 0.18
8	6.16 ± 0.03	6.11 ± 0.03	5.03 ± 0.39	39.03 ± 1.30	0.53 ± 0.05	3.45 ± 0.10
9	5.86 ± 0.05	6.35 ± 0.00	11.80 ± 0.43	41.85 ± 1.17	1.05 ± 0.05	3.75 ± 0.10
Overall	5.75 ± 0.21	6.17 ± 0.12	15.82 ± 2.25	40.07 ± 1.89	1.39 ± 0.19	3.45 ± 0.18

The control treatment was well water applied at a volume equal to that of the leachate.

[(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* 'BC_1']; NC14104, NC14106, DM115 (*P. deltoides* × *P. maximowiczii* 'DM'); DN5 (*P. deltoides* × *P. nigra* 'DN'); NM2, NM6 (*P. nigra* × *P. maximowiczii* 'NM'). In this paper we use the *Populus* section names as specified by Eckenwalder (1996), but we have retained the species nomenclature for *P. maximowiczii* (Japanese poplar) that has been previously used in the *Populus* literature. *Populus maximowiczii* is currently classified as a subspecies of *Populus suaveolens* Fischer (Eckenwalder, 1996; Dickmann, 2001). Throughout this paper, we have qualitatively compared genomic groups because we were interested in evaluating genotypes at the strategic level of selection. However, given the lack of statistically adequate clonal representation within genomic groups, rigorous testing among genomic groups was not conducted.

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, with cuts made to position at least one primary bud not more than 2.54 cm from the top of each cutting. Cuttings were stored at 5 °C and soaked in water to a height of 15 cm for 3 d before planting on 14 June 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks, two treatments (whole plots), and eight clones (sub plots) at a spacing of 1.2 m × 2.4 m (i.e. 3472 trees ha^{-1}). Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen, 1981; Zavitkovski, 1981).

Water (control) from a well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 d. Following establishment, trees were hand irrigated with either leachate or fertilized water, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects. The 2005 weekly application rate was 3.8 L $tree^{-1}$ (23.1 mm ha^{-1} assuming an irrigated soil surface area of 0.16 m^2 $tree^{-1}$). Given eight applications, a total of 1.9 kL of each treatment was applied across the growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006

Table 3
Oneida County Landfill leachate composition over time of parameters relevant to the current study compared with those in the published literature

Sampling date	pH	Electrical conductivity ($mS\ cm^{-1}$)	Biological oxygen demand ($mg\ L^{-1}$)	Chemical oxygen demand ($mg\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)
19 April 2001	8.0	8.7	1600	2800	1000
9 April 2002	7.9	8.7	270	1300	980
10 October 2002	7.7	10.0	1600	2600	1100
30 April 2003	8.1	6.8	380	1500	1300
28 October 2003	8.6	13.0	690	2300	1600
6 April 2004	8.1	7.0	69	880	790
15 October 2004	8.9	3.4	210	1100	1200
25 January 2005	8.0	10.2	14	1100	1400
23 February 2005	8.8	10.2	48	1000	1400
28 April 2005	8.8	5.7	16	670	820
19 October 2005	8.8	6.6	26	650	750
12 April 2006	8.2	9.6	190	1100	1200
Reported leachate[a]	4.5–9.0	2.5–35.0	20–57,000	140–152,000	150–4500

Table adapted from Zalesny et al. (in press).
[a] Ranges based on 14 studies cited in Kjeldsen et al. (2002).

was increased to 22.7 L tree^{-1} (34.6 mm ha^{-1} assuming an irrigated soil surface area of 0.66 m^2 tree^{-1}) because of root system development. Given twelve applications, a total of 17.4 kL of each treatment was applied across the growing season. To prevent substantial leaching from the experimental plot, application of treatments was adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 d prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.

Mechanical and hand weeding were performed weekly in 2005 and 2006 to ensure maximum tree survival. Electric fencing was used to prevent deer browse and injury to the trees. Polyvinylchloride (PVC) tubing, 15.24 cm in diameter, was installed after leaf senescence in November 2005 on each tree to protect the trunk from girdling by rodents during the winter.

2.3. Data collection and analysis

Height (to the nearest 1.0 cm) and diameter (to the nearest 0.01 mm) were measured on 15 August 2006. Height was measured from ground level to the base of the apical bud on the terminal shoot. To reduce experimental error associated with stump swell, diameter was measured at 10 cm above the soil surface. Volume (cm^3) was estimated using the generalized equation: volume = diameter^2 × height, according to Avery and Burkhart (1994).

On 17 August 2006, each tree was rated for presence or absence of sylleptic branches, which are defined as branches that emerge from buds without a period of dormancy (Wu and Stettler, 1998). In addition, one branch from each of the basal, middle, and apical thirds of each tree was randomly chosen for harvest. Total leaf area of the three branches was determined for each tree (Li Cor Model 3100 Area Meter), and the subsampled woody components (stems + branches) and leaves were placed in a drying oven at 70 °C for dry mass determination.

All trees were destructively harvested in two stages on 18 August 2006. First, the aboveground portion of each tree was cut at 10 cm above the soil surface, and woody and leaf components were separated and dried at 70 °C. Woody, leaf, and aboveground (woody + leaf) biomass was determined when dry mass values reached a constant mass. Total tree leaf area (TTLA) was estimated according to the following equation: TTLA = (area of subsampled leaves/dry mass of subsampled leaves) × total tree leaf dry mass. Second, root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter × depth = 0.28 m^3) for each tree. Root systems were washed and divided into the stump, lateral roots, and basal roots. Lateral and basal root separation was based on organ development from the stump associated with these two primary *Populus* rooting ontogenies. Lateral roots develop from latent root primordia distributed throughout the length of the original cutting, while basal roots develop from callus as a result of wounding at the base of the cutting (Luxova and Lux, 1981; Zalesny et al., 2005). Stump, lateral root, basal root, and belowground (stump + lateral + basal) dry mass was determined identically to shoot components. Root mass fraction was calculated as the ratio between belowground dry mass and total tree dry mass (Coyle and Coleman, 2005).

Data were analyzed using analyses of variance (PROC MIXED; SAS Institute Inc., 2004) assuming the split plot design described above. Blocks were considered random in the analysis, while treatments were fixed whole plots and clones were fixed sub plots. Therefore, means were evaluated rather than variances. The following linear additive model was used:

$$Y_{ijk} = \mu + B_i + T_j + BT_{ij} + C_k + TC_{jk} + \text{pooled error}$$

where Y_{ijk} is the response variable to be analyzed, μ the overall mean, B_i the main effect of *i*th block, T_j the main effect of *j*th treatment, BT_{ij} the effect of interaction between *i*th block and *j*th treatment, C_k the main effect of *k*th clone, TC_{jk} the effect of interaction between *j*th treatment and *k*th clone, and pooled error is the error term resulting from pooling of BC_{ik} and BTC_{ijk} terms, defined as: effect of interaction among *i*th block and *k*th clone, and effect of interaction among *i*th block, *j*th treatment, and *k*th clone, respectively. Means were considered different at probability values of $P < 0.05$.

3. Results

3.1. Tree growth

The survival rate of the trees at the time of harvest was the same for each treatment at 78% (50/64). Height did not differ between leachate and well water (control) treatments, but there were differences among clones. The treatment × clone interaction was significant (Table 4). *Populus nigra* × *P. maximowiczii* "NM" clones NM2 and NM6 had the greatest height across both irrigation treatments (Fig. 1). Despite substantial clonal variation among genotypes belonging to the

Table 4
Probability values from analyses of variance comparing growth and biomass traits of eight *Populus* clones (see Section 2 for descriptions) irrigated once-weekly with treatments of fertilized well water (control) or landfill leachate during the 2005 and 2006 growing seasons

Trait	Source of variation		
	Treatment	Clone	Treatment × clone
Height (cm)	0.1642	**0.0094**	**0.0494**
Diameter (cm)	0.2552	0.1027	0.1368
Volume (cm^3)	0.1336	0.1504	0.0910
Dry mass (g)			
Total tree	0.4965	0.0620	**0.0397**
Aboveground	0.5987	0.0550	**0.0464**
Belowground	0.0956	0.0921	**0.0146**
Leaf	0.3767	0.1495	**0.0400**
Woody (stem + branch)	0.8124	**0.0180**	**0.0515**
Stump	0.2954	0.0716	0.0971
Basal root	0.2355	0.4944	0.0616
Lateral root	**0.0185**	**0.0102**	**0.0119**
Root mass fraction	0.1031	**<0.0001**	0.9099

Significant values are in bold.

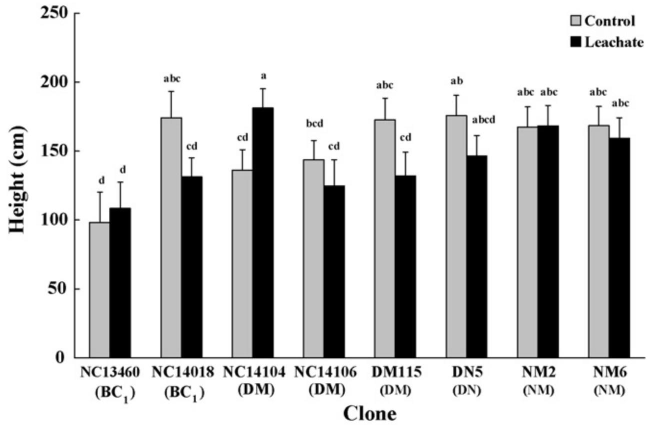

Fig. 1. Height of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree^{-1} week^{-1}) and 2006 (22.7 L tree^{-1} week^{-1}) growing seasons. The control treatment was water applied at a volume equal to that of the leachate. Each bar represents the mean of 3–8 trees with one standard error. Bars labeled with different letters were different at $P < 0.05$.

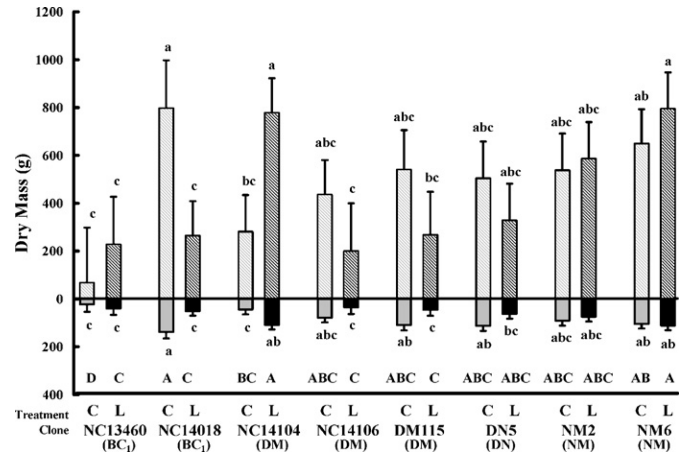

Fig. 2. Above- and below-ground biomass of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree^{-1} week^{-1}) and 2006 (22.7 L tree^{-1} week^{-1}) growing seasons. The control (C) treatment was water applied at a volume equal to that of the leachate (L). Zero on the *y*-axis denotes the groundline. Each bar represents the mean of 3–8 trees with one standard error. Bars labeled with different lowercase [aboveground {above 0} and belowground {below 0}] and uppercase (total tree biomass) letters were different at $P < 0.05$.

P. deltoides × *P. maximowiczii* 'DM' (DM115, NC14106, NC14104) and [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*] 'BC_1' (NC14018, NC13460) genomic groups, all but one clone exhibited similar performance across treatments. Only clone NC14104 had significantly greater height when irrigated with leachate than water, while significantly greater height for water versus leachate did not exist within any genotype. Overall, the mean height was 149.3 ± 16.0 cm. Treatment and clone main effects, along with their interaction, were negligible for diameter and volume (Table 4).

3.2. Biomass distribution

The main effects of treatment and clone for total tree dry mass were not significant, but the treatment × clone interaction was (Table 4). The NM clones exhibited the greatest overall total tree dry mass, while the BC_1 and DM genotypes had the most clonal variation (i.e. variation between or among clones within a specific genomic group) (Fig. 2). Clones NC13460 and NC14104 had significantly greater total tree dry mass with leachate over water, while clone NC14018 exhibited greater total dry mass with water over leachate. Overall, the mean total tree dry mass was 529.6 ± 189.2 g.

Aboveground and belowground dry mass accumulation was similar among treatments and clones. Treatment and clone main effects for aboveground dry mass were not significant, but the treatment × clone interaction was (Table 4). The NM clones exhibited the greatest overall aboveground dry mass, while the BC_1 and DM genotypes had the most clonal variation (Fig. 2). Clone NC14104 was the only clone that had significantly greater aboveground dry mass when irrigated with leachate than water. In contrast, clone NC14018 exhibited greater aboveground dry mass with water than leachate. Overall, the mean aboveground dry mass was 453.3 ± 167.2 g. Moreover, treatment and clone main effects were not significant for belowground dry mass, but the treatment × clone interaction was (Table 4). A distinct genomic group advantage for overall belowground dry mass was non-existent, but the BC_1 and DM genotypes had the most clonal variation (Fig. 2). Clone NC14104 was the only clone that had significantly greater belowground dry mass when irrigated with leachate than water. In contrast, clones NC14018 and DM115 exhibited greater belowground dry mass with water than leachate. Overall, the mean belowground dry mass was 76.4 ± 22.7 g.

The main effects of treatment and clone did not differ for leaf dry mass. However, there was a significant treatment × clone interaction (Table 4). The NM clones exhibited the greatest overall leaf dry mass, while the DM genotypes had the most clonal variation (Table 5). Clone NC14104 was the only clone that had significantly greater leaf dry mass when receiving leachate irrigation compared with water. In contrast, clone NC14018 exhibited greater leaf dry mass when receiving water irrigation compared with leachate. Overall, the mean leaf dry mass was 217.6 ± 73.0 g. There was a highly significant ($P < 0.0001$) linear relationship between leaf area and stem volume and between leaf area and woody dry mass (Fig. 3). Leachate treatment did not affect woody dry mass, but there were differences among clones. The treatment × clone interaction was significant (Table 4). The NM clones exhibited the greatest overall woody dry mass, while the BC_1 and DM genotypes had the most clonal variation (Table 4). Clone NC14104 was the only genotype that had significantly greater woody dry mass when irrigated with leachate versus water, while clone NC14018 exhibited greater stem dry mass with water versus leachate. Overall, the mean woody dry mass was 235.7 ± 94.9 g. The ranking of our genomic groups for relative sylleptic branching from most to least was BC_1:DM:NM:DN.

Treatment and clone main effects, along with their interaction, were not significant for stump dry mass or basal root dry mass (Table 4). However, the main effects of treatment and clone, along with the treatment × clone interaction, were

Table 5
Dry mass (g) of tree components for each combination of clone and treatment (n = 3–8) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree^{-1} week^{-1}) and 2006 (22.7 L tree^{-1} week^{-1}) growing seasons

Clone	Treatment	Biomass component				
		Leaf	Stem + branch	Stump	Lateral root	Basal root
NC13460	Control	49.4 ± 100.7 d	18.1 ± 130.8 d	7.7 ± 12.3	5.7 ± 11.5 d	10.5 ± 13.4
	Leachate	119.3 ± 87.5 cd	107.3 ± 113.7 cd	18.9 ± 10.7	9.6 ± 9.9 cd	11.6 ± 11.6
NC14018	Control	368.5 ± 87.5 a	428.0 ± 113.7 ab	40.6 ± 10.7	61.0 ± 9.9 a	35.2 ± 11.6
	Leachate	128.3 ± 62.8 cd	135.7 ± 81.8 c	21.6 ± 7.6	12.9 ± 7.0 cd	15.7 ± 8.2
NC14104	Control	153.3 ± 66.9 bcd	127.5 ± 87.1 c	16.1 ± 8.2	19.9 ± 7.5 bcd	6.9 ± 8.8
	Leachate	373.3 ± 62.8 a	404.8 ± 81.8 ab	41.5 ± 7.6	33.5 ± 7.0 b	33.2 ± 8.2
NC14106	Control	246.9 ± 62.8 abcd	189.0 ± 81.8 bc	25.4 ± 7.6	30.3 ± 7.0 bc	22.5 ± 8.2
	Leachate	120.9 ± 87.5 cd	78.6 ± 113.7 cd	14.2 ± 10.7	10.9 ± 9.9 cd	10.7 ± 11.6
DM115	Control	272.6 ± 72.0 abcd	267.6 ± 93.7 abc	38.3 ± 8.8	34.8 ± 8.1 b	36.7 ± 9.5
	Leachate	139.6 ± 78.6 bcd	127.8 ± 102.2 c	17.1 ± 9.6	20.4 ± 8.9 bcd	8.9 ± 10.4
DN5	Control	230.8 ± 66.9 abcd	272.9 ± 87.1 abc	41.6 ± 8.2	26.1 ± 7.5 bcd	44.0 ± 8.8
	Leachate	143.7 ± 66.9 bcd	184.4 ± 87.1 bc	28.7 ± 8.2	9.3 ± 7.5 d	23.6 ± 8.8
NM2	Control	262.1 ± 66.9 abcd	275.4 ± 87.1 abc	38.2 ± 8.2	24.6 ± 7.5 bcd	27.3 ± 8.8
	Leachate	260.9 ± 66.9 abcd	324.5 ± 87.1 abc	30.4 ± 8.2	26.3 ± 7.5 bcd	17.4 ± 8.8
NM6	Control	294.7 ± 62.8 abc	354.2 ± 81.8 ab	43.2 ± 7.6	39.7 ± 7.0 ab	21.6 ± 8.2
	Leachate	317.5 ± 66.9 ab	476.2 ± 87.1 a	41.7 ± 8.2	40.0 ± 7.5 ab	28.4 ± 8.8

The control treatment was water applied at a volume equal to that of the leachate. See Section 2 for genotypic descriptions. Means within each component labeled with different letters were different at $P < 0.05$. The treatment × clone interaction was negligible for stump (P = 0.0971) and basal root (P = 0.0616) dry mass. Aboveground, belowground, and total tree biomass are illustrated in Fig. 2.

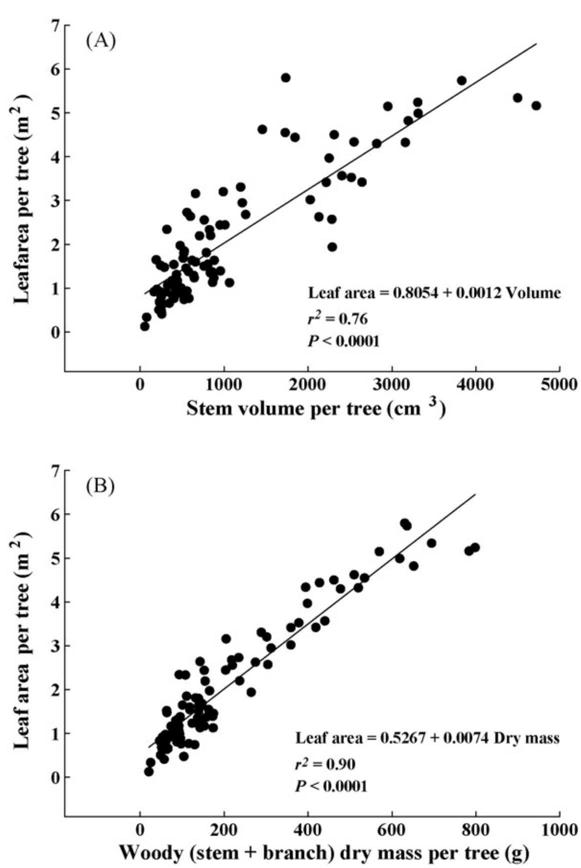

Fig. 3. Leaf area vs. stem volume (A) and woody (stem + branch) dry mass (B), per tree (n = 100 for each).

significant for lateral root dry mass (Table 4). The DM and NM clones exhibited the greatest overall lateral root dry mass, while the BC_1 genotypes had the most clonal variation (Table 4). No clones had significantly greater lateral root dry mass when irrigated with leachate compared with water, but clone NC14018 exhibited greater lateral root dry mass with water compared with leachate. Overall, the mean lateral root dry mass was 25.3 ± 8.2 g.

Treatments did not affect root mass fraction (RMF), but clones were significantly different. There was no treatment × clone interaction for RMF (Table 4). The BC_1 clones and DN5 exhibited the greatest overall RMF, while the DM genotypes had the most clonal variation (Fig. 4). Overall, genotypes within genomic groups performed similarly, showing a lack of clonal differences. The mean RMF was 0.16 ± 0.01.

4. Discussion

Although leachate irrigation did not enhance tree growth and biomass for most genotypes in the current study, significant productivity reductions associated with the leachate also were not observed. Therefore, there is a great potential for remediation of landfill leachate using *Populus*. Selection within the clonal variation that resulted from variable responses to leachate or well water (control) treatments will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects. Further examinations are needed, however, that test similar responses throughout the entire rotation. The objective of this study

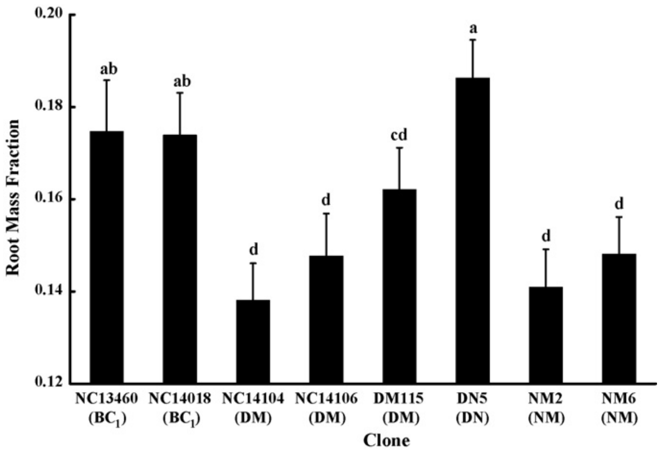

Fig. 4. Root mass fraction across leachate and water (control) irrigation treatments of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting. Each bar represents the mean of 7–15 trees with one standard error. Bars labeled with different letters were different at $P < 0.05$.

was to irrigate *Populus* with landfill leachate or water and to test for differences in height, diameter, and volume, along with biomass of the leaves, stems, branches, and roots. Some of the genomic groups and clones exhibited broad variation for most traits, while the performance of other genotypes was relatively stable. Specifically, there were two trends in the performance of the four genomic groups. First, broad clonal variation existed within the BC_1 and DM genomic groups, with clones performing differently for treatments. Second, the productivity of the NM and DN genomic groups was relatively stable across treatments, with the NM clones having the greatest growth and biomass accumulation for nearly all tissue components. Clone NC14104 was the only genotype to uniformly exhibit greater height and biomass for multiple tissues when irrigated with leachate compared with water, while NC14018 consistently exhibited greater levels of biomass accumulation with water versus leachate.

Irrigation and fertilization effects on *Populus* productivity have been previously tested (Coleman et al., 2004; Brown and van den Driessche, 2005; Coyle and Coleman, 2005). This information is useful for increasing yield when applying an alternative irrigation and fertilizer source such as landfill leachate. Shrive et al. (1994) irrigated NM6 for two seasons with 3.5 mm d^{-1} of leachate, a volume similar to the current study, and found height to be significantly greater than with the water treatment. In contrast, fertilization effects from the leachate were not present in the current study. We standardized the nutrient content of our water irrigation treatments for N, P, and K (i.e. we added fertilizer at concentrations equal to the leachate for each element) in order to identify impacts resulting from the negative and potentially toxic chemical constituents of the leachate, without giving the leachate treatment a fertilization advantage. This standardization was important because N is the most limiting factor in short rotation woody crop systems, and N addition is a proven method for increasing overall productivity of the trees (Hansen et al., 1988; Brown and van den Driessche, 2002; Coyle and Coleman, 2005). In contrast, it has been reported that N-fertilization did not increase growth during establishment. DesRochers et al. (2006) tested growth responses to fertilization of one *Populus balsamifera* L. (B) × *Populus simonii* Carr. (S) hybrid '33 cv. P38P38' and two *P. deltoides* (D) × *Populus petrowskyana* (P) hybrids '24 cv. Walker', '794 cv. Brooks6' and reported negligible fertilization responses after the second growing season. In addition, variation in fertilization growth responses of *Populus tremuloides* Michx. seedlings as a result of different soil pH levels were reported (DesRochers et al., 2003).

The elevated Cl^- concentration (1100 mg L^{-1}) and electrical conductivity (EC) (8.3 mS cm^{-1}) was a concern in the current study, considering poplars have been reported to be sensitive to salt and have optimal growth at an EC ranging from 1 to 5 mS cm^{-1} (Neuman et al., 1996). However, there were no treatment differences for aboveground dry mass. Therefore, the leachate did not negatively impact this trait, which may have been partially due to dilution of the leachate by the soil and/or precipitation. Nevertheless, there was some genetic variation in sensitivity to Cl^- and EC among the *Populus* genotypes studied, with minimal productivity losses or increased plant stresses that are common responses related to excessive Cl^- and elevated EC (Neuman et al., 1996; Shannon et al., 1999). Aside from NC14018, all clones showed similar or better aboveground biomass with the leachate compared with the water treatment. The elevated Cl^- content of the leachate was likely a factor in the clonal sensitivity of NC14018 to leachate irrigation, which was illustrated by the greater biomass of NC14018 when irrigated with water. Thus, proper clonal selection for elevated Cl^- and EC is essential for deployment in future systems.

Biological productivity of short rotation woody crops is measured by the combination of aboveground and belowground growth, economic yield, and associated environmental benefits (Dickmann, 2001). Though difficult to quantify, ecological benefits such as carbon sequestration, erosion control, reduced pollution, and improved landscape processes are compelling reasons for the deployment of phytoremediation systems. Economic benefits are relatively easier to quantify and can be obtained from phytoremediation projects by harvesting the aboveground biomass of the trees (i.e. harvestable yield). The total aboveground biomass in the current study ranged from 0.51 to 2.50 Mg ha^{-1}, with a mean of 1.57 Mg ha^{-1}. The NM clones had a clear genomic group advantage, with the greatest overall biomass of 2.50 Mg ha^{-1} for NM6 and 1.95 Mg ha^{-1} for NM2. These results were similar to Baker and Blackmon (1977), who reported 2.42 Mg ha^{-1} of biomass for D after one growing season in Stoneville, Mississippi, USA (33.4°N, 90.9°W). This growth from one season in Stoneville (216 frost-free days) is greater than two seasons of growth of our clonal material in northern Wisconsin (103 frost-free days). Therefore, the longer growing season, extending into November, is largely responsible for the greater biomass accumulation in the southern United States versus the North Central region. The 2006 growing season in the current study was shortened given the mid-August harvest. This time frame was used to harvest the trees during their vigorous growth at the end of the leachate applications. Other reports of *Populus* biomass were similar to those in the current study. Pontailler et al. (1999) reported 1.15–4.22 Mg ha^{-1} of aboveground dry mass after one growing season in Orsay, France (49.0°N, 2.5°E) for one *P. trichocarpa* (T), one DN, and two *P. trichocarpa* × *P. deltoides* (TD) genotypes. Likewise, our leaf dry mass (217.6 g tree^{-1}) was within the range (169–235 g tree^{-1}) reported by Ceulemans et al. (1996) for second year growth of TD and DN clones. In contrast, our stem dry mass (235.7 g tree^{-1}) was less than the range (504–717 g tree^{-1}) reported by Tschaplinski and Blake (1989) for second year growth of three DN clones.

Furthermore, the leaves and woody biomass of the current study each comprised 50% of the total aboveground dry mass, which was relatively similar to the leaves (37%) and stems + branches (63%) for 1-year-old D genotypes (Baker and Blackmon, 1977). In addition, leaf and woody biomass components of our study were within the range reported by Friend et al. (1991) for two TD clones after two growing seasons in the Pacific Northwest (PNW) region of the United States (131 frost-free days). In their study, 35–81% of aboveground biomass was comprised of stems, while 19–65% was in the leaves. Aboveground biomass of T, D, and two TD clones also was evaluated in the PNW (Scarascia-Mugnozza et al., 1997). After 2 years of growth, stems + branches comprised 59–74% of the aboveground biomass over all clones.

The relationships between leaf area and volume, and between leaf area and aboveground dry mass, are important for phytoremediation given the need for early prediction of potential remedial effectiveness. There was a positive linear relationship for these traits in the current study (Fig. 3). Although similar correlations among numerous allometric traits often have been reported for *Populus* (Isebrands and Nelson, 1982; Ridge et al., 1986; Rogers et al., 1989; van den Driessche, 1999), this information remains relevant. Evaluation of specific correlations in any study is necessary, because such correlations may not hold true across studies. For example, the development of sylleptic branching is an important morphometric trait associated with enhanced early productivity and increased photosynthetic carbon for tree development (Scarascia-Mugnozza et al., 1999; Dickmann, 2001). Well-developed correlations between sylleptic branching and tree yield have been reported (Wu and Stettler, 1998; Scarascia-Mugnozza et al., 1999). However, based on a survey of sylleptic branching in the current study, a positive relationship between sylleptic branching and biomass was not observed. Likewise, Ceulemans et al. (1992) reported a weak correlation between sylleptic branching and stem volume after one and four growing seasons for T female parents, D male parents, and their TD F_1 hybrids. Interestingly, they reported the greatest number of sylleptic branches occurred in the T genotypes, with the F_1 hybrids exhibiting intermediary scores and the D genotypes the fewest number of sylleptic branches (Ceulemans et al., 1992). The ranking of our genomic groups for relative sylleptic branching from most to least was BC_1:DM:NM:DN. Our BC_1 clones were the only genomic group with T parentage, but the *P. maximowiczii* (M) males of the DM and NM F_1 hybrids also belong to the section *Tacamahaca*. Sylleptic branching was nearly non-existent for DN5, whose parentage is limited to the section *Aigeiros*. Similar intersectional differences have been reported for rooting among these genomic groups (Zalesny and Wiese, 2006).

Indirect selection for a desirable characteristic based on direct selection of an easily measurable trait can be useful in identification of favorable clones if the intertrait correlation is strong enough. Leaf area is an important trait for many remediation processes, especially given its relationship to photosynthetic productivity (Larson and Isebrands, 1972). Contaminants may either be sequestered and/or degraded in the leaves and other tissues (Burken and Schnoor, 1997; Newman et al., 1997) or be volatilized through leaf stomata and transpired into the atmosphere (Newman et al., 1997; Thompson et al., 1998; Mirck et al., 2005). However, it is difficult for researchers and resource managers to determine whole-tree leaf area on trees beyond the first growing season. At the time of harvest in the current study, some trees that were sampled for total leaf counts had nearly 2000 leaves. Therefore, there is an ongoing need to identify easily measurable traits that can be used as predictors of the correlative variables (Larson and Isebrands, 1972; Isebrands and Nelson, 1982; Harrington et al., 1997). If the desired phytoremediation processes involve the direct need for increased leaf area, then simple, non-destructive volume calculations can be used to estimate leaf area. Aboveground dry mass, albeit a destructive method, also would be easier than whole-tree leaf area determinations. Isebrands and Nelson (1982) used similar methods to test whether leaf characteristics could be estimated from less complex variables, with the overall goal of using such information for improving biomass productivity of *Populus* in short rotation intensive forestry systems. Likewise, Harrington et al. (1997) reported that leaf production (area or mass) was a useful predictor of potential productivity of a TD (11-11) and T (7-75) *Populus* clone. Given the results of the current study, we believe this type of information can be adapted for similar assessment needs during the establishment phase in almost all phytoremediation settings.

5. Conclusion

Overall, given that every leachate source should be regarded as unique, there is an essential need for initial genotype screening followed by the establishment and evaluation of test plots to ascertain clonal performance prior to large-scale deployment. The lack of overall differences in response to treatments in the current study was a result of extensive genotypic screening during phyto-recurrent selection cycles 1–3 that reduced the variability among the clones deployed, relative to the original 25 genotypes (Zalesny et al., in press). However, from a practical standpoint, the variation that was observed was useful for further selection of clones that could be used in a large-scale system. For example, clone NC14018 would not be suitable for further deployment if irrigated with the leachate used in the current study, but NC14104 would be an ideal candidate relative to the other clones. Thus, similar tree-based bioremediation technologies can be beneficial for the reduction of environmental damage resulting from such pollution (Mirck et al., 2005). Phytoremediation merges the science of plantation forestry with environmental clean-up methodologies to achieve the following important ecological benefits: (1) phytoremediation utilizes natural plant processes whereby the leachate can be biologically cleansed to remove many of the excessive nutrients and chemicals; (2) depending on the contaminants, phytoremediation plantations may be harvested in 8–10 years for fiber or energy, utilizing short rotation forestry to offset demand and conserve natural forest stands (Gladstone and Ledig, 1990); (3) when plants remove and sequester excess nutrients and chemicals found in the leachate, it prevents the unwanted leaching of potentially harmful contaminants into nearby watersheds.

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