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Genotypic variability and stability of poplars and willows grown on nitrate-contaminated soils

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ABSTRACT

Phyto-recurrent selection is an established method for selecting tree genotypes for phytoremediation. To identify promising *Populus* (poplar) and *Salix* (willow) genotypes for phytotechnologies, our objectives were to (1) evaluate the genotypic variability in survival, height, and diameter of poplar and willow clones established on soils heavily contaminated with nitrates; and (2) assess the genotypic stability in survival and diameter of selected poplar clones after one and eleven growing seasons. We tested 27 poplar and 10 willow clones planted as unrooted cuttings, along with 15 poplar genotypes planted as rooted cuttings. The trees were tested at an agricultural production facility in the Midwestern, United States. After 11 growing seasons, using phyto-recurrent selection, we surveyed survival and measured the diameter of 27 poplar clones (14 unrooted, 13 rooted) that were selected based on superior survival and growth throughout plantation development. Overall, willow exhibited the greatest survival, while poplar had the greatest height and diameter. At 11 years after planting, superior clones were identified that exhibited above-average diameter growth at the establishment- and rotation-age, most of which had stable genotypic performance over time. Selection of specific clones was favorable to genomic groups, based on the geographic location and soil conditions of the site.

KEYWORDS

Fertilizer residue; phyto-recurrent selection; phytotechnologies; *Populus* L.; *Salix* L

Introduction

Contamination from nitrates (NO_3^-) into groundwater is a critical concern in the Midwestern United States (David *et al.* 1997; Stites and Kraft 2001; Syswerda *et al.* 2012). Coarse soils and shallow groundwater in much of the Midwest, coupled with tile drainage, vegetation management practices, and the use of fertilizers and pesticides, have contributed to the environmental degradation caused by NO_3^- (Jaynes *et al.* 2001; Zhao *et al.* 2001; Calderon and Jackson 2002). Thus, solutions for inexpensive NO_3^- remediation techniques have been developed and tested. However, most systems are not sustainable over extended periods of time without additional soil and water amendments (Woodard *et al.* 2003). Regardless, there is an urgent need to develop and deploy systems for NO_3^- reduction that is efficient and sustainable (Udawatta *et al.* 2002).

Phytoremediation, which is defined as using plants and associated microorganisms to remediate contaminated soils, is a potentially sustainable system (McIntyre and Lewis 1997; Rockwood *et al.* 2013; Zalesny *et al.* 2016b). Agricultural crops such as corn (*Zea mays* L.) and alfalfa (*Medicago sativa* L.) have proven successful at phytoremediation of NO_3^--contaminated groundwater (Russelle *et al.* 2001; Cavero *et al.* 2003). However, phytoremediation with trees can be very efficient and effective because of their perennial growth habit with more root development and biomass accumulation than agricultural crops.

One group of trees currently used for phytoremediation is a limited number of selected *Populus* species and their hybrids, excluding the aspens (*i.e.* poplars) (Isebrands and Karnosky 2001; Zalesny *et al.* 2016b). Poplars have been used to remediate sites with contamination from NO_3^-, petroleum hydrocarbons, salts, landfill leachates, heavy metals, pesticides, solvents, explosives, and radionuclides (O'Neill and Gordon 1994; Rockwood *et al.* 2004; Isebrands *et al.* 2014). In addition, species and hybrids belonging to the genus *Salix* (*i.e.* willows) comprise another group of trees used for phytoremediation (Perttu and Kowalik 1997; Mirck *et al.* 2005). Willows have been studied extensively for phytoremediation purposes in regions of North America (Kopp *et al.* 2001) and Europe (Mirck *et al.* 2005; Isebrands *et al.* 2014). Willows (primarily *S. viminalis* L., *S. dasyclados* Wimm., *S. fragilis* L., *S. kinuyanagi* Kimura, and *S. discolor* Muhl) have been used worldwide for phytoremediation of dairy-farm effluent (Roygard *et al.* 2001), wastewater sludge (Labrecque *et al.* 1998), municipal wastes (Perttu 1993), and cadmium phytoextraction (Klang-Westin and Eriksson 2003).

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Selected genotypes of poplar and willow are ideal for phytoremediation because of their ability to produce extensive root systems and aboveground biomass while growing on contaminated soils. Superior varieties also have elevated hydraulic control potential, leading to the capacity for increased transpiration on water-rich sites and decreased water demands on water-limited areas (Isebrands *et al.* 2014; Zalesny *et al.* 2016a). In addition, the broad genetic diversity of both genera has led to successful interspecific hybridization (Ronald 1982; Eckenwalder 1984; Kopp *et al.* 2001), which supports greater phytoremediation potential than with other tree species (Schreiner 1971; Dickmann 2001). Vegetative propagation of poplars and willows allows for repeated use of favorable clones once they are identified and selected (Stanturf *et al.* 2001; Volk *et al.* 2004).

The aforementioned clonal variation among and within poplar and willow genotypes leads us to assert that successful phytoremediation applications using these trees require genotypic screening using phyto-recurrent selection prior to large-scale deployment (Zalesny *et al.* 2007). Thus, we assume that phytoremediation potential is proportional to survival, height, and diameter, which are our measures of potential benefit because successful tree establishment and subsequent growth are required for all long-term phytoremediation applications. Unfortunately, in the current study, specific experimental location and soil data were not available due to a landowner confidentiality agreement. Therefore, we were not able to test for nitrate uptake from the contaminated soils into the tree tissues. However, there is evidence of nitrate phytoremediation using these trees (O'Neill and Gordon 1994; Pilipović *et al.* 2009). The objectives of this study were to (1) evaluate the genotypic variability in survival, height, and diameter of poplar and willow clones established on soils heavily contaminated with fertilizer residues (*i.e.* NO_3^-); and (2) assess the genotypic stability in survival and diameter of selected poplar clones after one and eleven growing seasons. In the current study, *in situ* phyto-recurrent selection was used to select 27 out of the original 42 poplar clones that were measured at eleven years. Despite not being able to test for uptake of NO_3^-, we assert that our allometric data are important for long-term success of the current application and for future researchers and managers to identify promising genotypes of poplar and willow for potential use in phytotechnologies of this nature.

Materials and methods

Clone selection and plot establishment

Forty-two poplar clones and 10 willow clones were sampled from seven genomic groups per genus. The genomic groups were selected based on their utilization and associated growth potential in the Midwestern United States. Fifteen clones of poplar were represented as rooted planting stock and 27 clones were represented as unrooted planting stock, while all willow clones were unrooted (Table 1). Rooted cuttings of poplar, approximately 30-cm long with up to six dominant lateral roots, were processed from excavated trees grown in a nursery in Ames, IA (42.0°N, 93.6°W) for one growing season. Unrooted cuttings of poplar clones, 20-cm long, were processed from whips grown for one growing season in stool beds established at Hugo Sauer Nursery in Rhinelander, WI (45.6°N, 89.4°W). Cuttings of willow were obtained from the State University of New York, Syracuse, NY (43.0°N, 76.2°W). For all planting stock, at least one primary bud was located within 2.5 cm from the apical end of each cutting.

All cuttings were soaked in water for 5 d before planting at a former agricultural site located adjacent to an agricultural production facility, where the soils exhibited heavy localized concentrations of fertilizer residues (*i.e.* NO_3^-). Corn and soybeans previously had been grown on these soils, which were silt loam, formed in loamy deposits underlain by silty clay loam and friable silt loam, with 2–5% slopes, moderate permeability, and high available water capacity. Unfortunately, due to a landowner confidentiality agreement, data on NO_3^- concentrations and specific site

Table 1. Genomic groups, stock types (i.e. rooted versus unrooted cuttings), and clones.

Genomic group	Stock type	Clone[a]
Populus		
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*	Unrooted	NC13466, NC13548, NC13552, NC13863, **NC13992**, NC13999
P. deltoides × *P. deltoides* (F_1 hybrid)	Rooted	**80X00601**, **80X01107**, **ISU25-4**, **ISU25-12**, **ISU25-21**, **ISU25-35**, **ISU25-R2**, **ISU25-R4**, **ISU25-R5**
P. deltoides × *P. deltoides* (F_2 hybrid)	Unrooted	**119.16**
P. deltoides × *P. maximowiczii*	Rooted	**Belgian25**
"	Unrooted	**313.23**, 313.55, DM101, DM109, DM113, DM114, **DM115**, DM121, NC14104, NC14107
P. nigra × *P. maximowiczii*	Rooted	NM2
"	Unrooted	**NM6**
P. deltoides × *P. nigra*	Rooted	Eugenei (a.k.a. DN34), **I45-51**
"	Unrooted	**DN5**, **DN34** (a.k.a. Eugenei), **DN182**
P. deltoides	Rooted	**252-4**, **42-7**
"	Unrooted	**7300501**, **8000105**, **91.05.02**, **D121**, **D123**, **D124**
Salix		
S. purpurea	Unrooted	FC185, FC189, FC190, PUR12
S. eriocephala	"	S25
S. interior × *S. eriocephala*	"	S301
S. discolor	"	S365
S. ×*dasyclados*	"	SV1
S. sachalinensis	"	SX61
S. miyabeana	"	SX67

[a]Poplar clones listed in **bold** were measured at 1 and 11 growing seasons after planting.
Note: Authorities for the aforementioned species of *Populus* and *Salix* are as follows: *P. deltoides* Bartr. ex Marsh; *P. trichocarpa*; Torr. & Gray; *P. nigra* L.; *P. maximowiczii* A. Henry; *S. purpurea* L.; *S. eriocephala* Michx.; *S. interior* Rowlee.; *S. discolor* Muhl.; *S. sachalinensis* F. Schmidt; *S. miyabeana* Seemen.

Table 2. Percent survival by replication (rep), genus, and planting stock after the first growing season.

	Genus – planting stock			
Rep	Poplar rooted	Poplar unrooted	Willow unrooted	Across genera and planting stock[a]
1	100	96	97	98 z
2	20	13	27	20 x
3	77	64	79	73 y
4	97	94	96	96 z
Across Reps[b]	74ab	67b	75a	72

[a]Means followed by the same letter within the column are not different; $LSD_{0.05} = 8$.
[b]Means followed by the same letter within the row are not different; $LSD_{0.05} = 7$.

and soil physicochemical information was not available. Nevertheless, the soils exhibited good potential for the cultivation of trees.

Site preparation consisted of tilling the soil with a chisel tooth plow and wing blades to a maximum depth of 30 cm. The experimental design was randomized complete blocks with four replications (hereafter referred to as reps) and variable numbers of ramets per clone per rep, which was due to availability of the cuttings, orientation of the reps relative to the perceived direction of contaminant movement, and aspect (rep 1 = west; reps 2 and 3 = South; rep 4 = East). Spacing was 2.4 × 2.4 m and 0.9 × 2.4 m between cuttings for poplars and willows, respectively. An extruded plastic fence (Hoover Fence Co., Newton Falls, OH) with a height of 2 m successfully protected the plots from deer browse.

Data collection and analysis

Tree height to the nearest 0.1 cm and tree diameter to the nearest 0.01 mm were determined following first-year budset. The diameter was measured consistently at 10 cm above the soil surface and the same person measured all trees to reduce experimental error. In addition, diameter at breast height (DBH) was measured at 1.37 m aboveground following eleventh-year budset. Diameter data were used to estimate total aboveground dry biomass according to biomass equations developed for specific genomic groups (Zalesny *et al.* 2015). Mortality, damage by fauna, competition from weeds, and other noticeable damage also were recorded for both measuring cycles.

Height (year 1) and diameter (years 1 and 11) data were subjected to analyses of variance (ANOVA) and analyses of means (ANOM) according to SAS® (PROC GLM; PROC ANOM; SAS INSTITUTE, INC., Cary, NC) assuming a simple all random effects model including the main effects of rep and clone, the interaction between rep and clone, and experimental error. Additionally, Pearson (*i.e.* phenotypic) correlation coefficients were generated using PROC CORR of SAS® in order to test for relationships among survival and growth-related traits between the first and eleventh growing seasons. There was one situation where the same clone was planted as rooted and unrooted cuttings. In this case, the two entries were coded differently for the analysis. Specifically, the rooted stock was identified as 'Eugenei' while the unrooted stock was designated as 'DN34.' Although the genotype has numerous clonal designations, both of these sources were the same genotype. Genera and planting stock were compared separately using a paired *t*-test. Fisher's protected least significant difference (LSD) was used to test for differences among means of main effects at a probability level of $p < 0.05$.

Results and discussion

Genotypic variability in survival, height, and diameter of poplars and willows during establishment

The percent survival of trees across reps, genera, and planting stock ranged from 13% (rep 2, unrooted poplar) to 100% (rep 1, rooted poplar), with an experiment-wide survival rate of 72% (Table 2). Rep 1 had the greatest survival rate (98%), while rep 2 had the lowest survival rate (20%). Willow (75%) had a greater survival rate than poplar (71%), and rooted poplar (74%) had a greater survival rate than unrooted poplar (67%). Overall survival rates were best for reps 1 and 4, moderate for rep 3, and worst for rep 2. Expected survival rates for rooted planting stock are about 95% and for unrooted planting stock are about 80%. Schnoor (2000) reported survival rates of 2.5% and >50% in South Dakota, USA, and Kansas, USA, respectively, for poplars grown under conditions of heavy zinc and cadmium concentrations. Reporting values between Schnoor (2000) and the current study, Zalesny *et al.* (2005b) reported an experiment-wide survival rate of 67% for poplars and willows grown on soils heavily-contaminated with petroleum hydrocarbons.

Poplar and willow differed for height ($p < 0.0001$) and diameter ($p < 0.0001$) (Table 3). Confidence intervals ($\alpha = 0.05$) for the difference between the means for height and diameter ranged from 6.8 to 16.4 cm and 3.47 to 4.54 mm, respectively. Year one mean height of poplar and willow across reps was 101.5 ± 1.7 cm and 89.9 ± 1.5 cm, respectively. Likewise, the mean diameter of poplar and willow was 10.83 ± 0.20 mm and 6.82 ± 0.12 mm, respectively. These differences for height and diameter were clone-specific. In addition, given these and other dependent variables of the current study (*i.e.* survival and biomass), we were not able to test whether such differences were due to the biology of the trees, the soil, or the interaction between the trees and soil. Nevertheless, an important morphological difference to note is that willow usually produces multiple stems that initially are smaller in diameter and shorter in height than poplar, which often produces one or two larger and taller stems (Hall *et al.* 1998). Subsequent production following establishment substantially increases when the growth of willow stems and leaves increases potential evapotranspiration (Christersson 1986; Labrecque *et al.* 1993; Mirck and

Table 3. Estimates from *t*-tests testing for differences between genera and between planting stock for height and diameter after the first growing season.

Class variable	Dependent variable	n_1	n_2	$\text{Mean}_{(\bar{a}1-\bar{a}2)}$	$\text{SE}_{(\bar{a}1-\bar{a}2)}$	95% CI $_{(\bar{a}1-\bar{a}2)}$	*t*-Statistic	*p* Value
Genera[a]	Height	983	660	11.6	2.5	(6.8, 16.4)	4.73	**<0.0001**
	Diameter	983	660	4.00	0.27	(3.47, 4.54)	14.69	**<0.0001**
Planting stock[b]	Height	259	1384	18.6	3.3	(12.2, 25.1)	5.66	**<0.0001**
	Diameter	259	1384	1.95	0.39	(1.19, 2.71)	5.03	**<0.0001**

Significant values are listed in bold.

[a]Genera: 1=*Populus*; 2=*Salix*; n_1 and n_2 = # of poplar and willow experimental units, respectively; $\text{mean}_{(\bar{a}1-\bar{a}2)}$, $\text{SE}_{(\bar{a}1-\bar{a}2)}$, and 95% CI $_{(\bar{a}1-\bar{a}2)}$ = mean, standard error, and confidence interval of the difference between the means, respectively ($\alpha = 0.05$).

[b]Planting stock: 1 = rooted, 2 = unrooted, n_1 and n_2 = # of rooted and unrooted experimental units, respectively; $\text{mean}_{(\bar{a}1-\bar{a}2)}$, $\text{SE}_{(\bar{a}1-\bar{a}2)}$, and 95% CI $_{(\bar{a}1-\bar{a}2)}$ = mean, standard error, and confidence interval of the difference between the means, respectively ($\alpha = 0.05$).

Note: Despite different sample sizes for planting stock, the *t*-test can be used because sample standard deviations are similar (height: rooted = 60.2, unrooted = 46.1; diameter: rooted = 6.7, unrooted = 5.5). The above *t*-test for planting stock compares rooted poplar versus unrooted poplar and willow combined. A t-test for rooted poplar versus unrooted poplar (excluding willow) expressed differences for height [$p < 0.0001$, 95% CI $_{(\bar{a}1-\bar{a}2)}$ (7.4, 22.6)] and no differences for diameter [$p = 0.9281$, 95% CI $_{(\bar{a}1-\bar{a}2)}$ (−0.88, 0.97)].

Table 4. Analyses of variance of height and diameter after the first growing season.

Source of variation	df	Mean square	*F*-variance ratio	*p*-Value	Expected mean squares
Height					
Replication	3	250963.00	77.92	**<0.0001**	$\sigma^2 + 4.0489\sigma^2\text{RC} + 178.880\sigma^2\text{R}$
Clone	51	11269.00	2.23	**0.0002**	$\sigma^2 + 7.2028\sigma^2\text{RC} + 24.124\sigma^2\text{C}$
Replication × clone	116	6038.51	6.95	**<0.0001**	$\sigma^2 + 8.9012\sigma^2\text{RC}$
Within plot	1472	869.38			σ^2
Total	1642				
Diameter					
Replication	3	3725.27	85.36	**<0.0001**	$\sigma^2 + 4.0489\sigma^2\text{RC} + 178.880\sigma^2\text{R}$
Clone	51	149.93	2.17	**0.0003**	$\sigma^2 + 7.2028\sigma^2\text{RC} + 24.124\sigma^2\text{C}$
Replication × clone	116	82.85	7.59	**<0.0001**	$\sigma^2 + 8.9012\sigma^2\text{RC}$
Within plot	1472	10.92			σ^2
Total	1642				

Significant values are listed in bold.

Volk 2010). At that point in time, willow may experience growth greater than or equal to poplar. In addition, willow may expend more energy and resources into developing an extensive root system during the establishment growing season. However, the growth of aboveground transpiring surface and belowground root spread and depth are not directly measured by simple stem length and diameter metrics (Mirck and Zalesny 2015). Therefore, more extensive studies testing for differences between poplar and willow and among genotypes within genera are needed.

Rooted and unrooted planting stock differed also in height ($p < 0.0001$) and diameter ($p < 0.0001$) (Table 3). Confidence intervals ($\alpha = 0.05$) for the difference between the means for height and diameter ranged from 12.2 to 25.1 cm and 1.19 to 2.71 mm, respectively. The mean height of rooted and unrooted planting stock across reps was 112.6 ± 3.7 cm and 93.9 ± 1.2 cm, respectively. Likewise, the mean diameter of rooted and unrooted planting stock was 10.86 ± 0.42 mm and 8.91 ± 0.15 mm, respectively. Although rooted cuttings exhibited greater survival rates and significantly better growth, they required an extra year and care in the nursery, and it was more difficult to plant the rooted cuttings relative to the unrooted cuttings. In contrast, unrooted cuttings required less units of resources to work with, but traditionally had lower survival rates when certain biological requirements such as rooting were not fulfilled. As illustrated by Zalesny *et al.* (2005b), choice of propagule is often more of a financial decision rather than being based on the biology of the silvicultural prescription.

A similar situation could be found for phytotechnologies involving fertilizer residues. For example, the spacing used for poplar in the current study resulted in 1680 trees per hectare planted. At the survival rates shown in Table 2 (i.e. 74% for rooted cuttings and 67% for unrooted cuttings), a total of 2270 and 2508 trees per hectare would be needed to achieve the desired stocking for rooted and unrooted cuttings, respectively. Assuming a cost of $0.29 per unrooted cutting (Lazarus *et al.* 2015), the total cost of unrooted cuttings equals $727.32 per hectare, resulting in a break-even cost of $0.32 per rooted cutting. The typical cost range for rooted cuttings is $2.00–$4.00, which is well above the $0.32 break-even cost. Thus, a land manager would likely choose a lower survival rate followed by replanting rather than the upfront investment in rooted cuttings. Nevertheless, we recommend continued testing of both types of propagules in order to identify favorable characteristics of variation present across all genotypes, regardless of planting stock.

Reps differed for height ($p < 0.0001$) and diameter ($p < 0.0001$) (Table 4). Despite an interaction between rep and clone for height and diameter, Fisher's protected least significant difference (LSD) was used to separate the means of the main effect of rep for each dependent variable. We chose to analyze main effects in addition to the interactions because the variation among reps, irrespective of genotypes, expressed the importance of selecting promising genotypes that can survive under soil conditions of heavy localized concentrations of NO_3^-. Rep 4 exhibited the greatest height (122.8 ± 2.1 cm), while rep 2 expressed the lowest height

Table 5. Rank (R) and mean height (cm) by replication (rep) for each clone after the first growing season.

Clone	R	Rep 1		R	Rep 2		R	Rep 3		R	Rep 4		R	Overall	
ISU25-35	1	178.1	(5.1)		x	x	1	134.9	(25.4)	2	221.9	(6.1)	1	186.0	(12.0)
ISU25-R5	5	164.4	(17.2)		x	x	4	87.5	(4.3)	6	197.8	(14.6)	2	172.5	(13.9)
313.55	2	171.7	(13.6)		x	x	28	52.2	(11.5)	1	246.9	(16.1)	3	166.4	(25.4)
NC13863	16	129.2	(8.1)	11	48.2	(0.0)		x	x	8	183.8	(6.7)	4	144.4	(15.7)
NC13552	20	116.8	(5.6)		x	x		x	x	15	162.0	(5.4)	5	139.4	(9.3)
ISU25-R4	3	168.9	(14.1)	8	52.1	(0.0)	14	68.8	(7.1)	3	212.3	(4.8)	6	135.4	(14.8)
SX67	4	166.5	(6.3)		x	x	8	77.5	(7.4)	20	146.8	(7.4)	7	132.8	(6.4)
ISU25-21	6	163.2	(6.6)		x	x	2	95.2	(48.7)	30	129.6	(11.7)	8	132.0	(10.9)
DM101	14	130.7	(17.7)		x	x	15	65.0	(2.8)	12	166.2	(20.4)	9	125.7	(15.5)
NC13466	11	136.2	(11.8)		x	x	38	42.3	(11.5)	17	154.3	(18.0)	10	124.6	(16.1)
DM121	47	43.2	(8.5)		x	x	42	40.5	(9.9)	5	202.9	(5.5)	11	122.4	(30.6)
ISU25-R2	9	154.8	(15.6)	2	88.1	(0.0)	5	87.3	(8.2)	31	126.1	(6.6)	12	121.5	(7.8)
DM115	7	162.4	(11.5)		x	x	30	51.0	(10.8)	19	146.9	(21.3)	13	120.1	(16.9)
ISU25-4	8	161.4	(8.1)	19	34.9	(0.0)	47	22.2	(0.0)	33	120.9	(18.0)	14	119.3	(15.3)
Eugenei	35	83.0	(2.7)		x	x	3	92.0	(7.1)	24	142.2	(10.0)	15	114.8	(8.8)
DM113	12	134.1	(10.4)		x	x	41	40.7	(13.6)	29	131.4	(14.7)	16	114.3	(14.1)
SX61	10	136.9	(9.8)	4	62.8	(8.2)	19	61.6	(3.6)	16	160.9	(6.8)	17	113.9	(6.6)
80X00601	41	67.8	(16.3)		x	x	7	80.5	(15.1)	14	162.8	(6.9)	18	113.3	(11.0)
313.23	40	72.7	(11.4)		x	x		x	x	13	166.1	(18.2)	19	112.7	(21.0)
ISU25-12	46	48.6	(0.9)		x	x	18	62.9	(6.0)	9	180.9	(14.6)	20	112.2	(16.3)
NM6	23	114.6	(5.8)	14	42.8	(6.5)	24	59.1	(3.3)	11	170.0	(10.2)	21	111.4	(5.2)
FC190	15	130.3	(4.8)		x	x	21	60.9	(3.5)	35	116.2	(5.4)	22	111.2	(4.3)
Belgian25	38	75.3	(4.1)		x	x	27	52.7	(4.5)	4	208.4	(10.6)	23	110.6	(19.0)
NC14107	49	30.4	(6.7)		x	x		x	x	7	190.1	(7.6)	24	110.2	(30.5)
NC13999	28	101.7	(5.1)	7	57.2	(0.0)	25	55.8	(4.2)	10	174.2	(5.7)	25	106.5	(14.3)
119.16	25	105.0	(9.2)	17	36.7	(6.8)	34	45.9	(7.5)	27	135.8	(8.9)	26	103.4	(7.9)
NM2	44	54.7	(8.9)	1	93.0	(0.0)	26	54.9	(10.6)	18	152.7	(12.2)	27	100.0	(13.6)
8000105	17	125.5	(5.9)		x	x	40	41.2	(6.2)	23	142.6	(53.6)	28	99.5	(19.1)
NC13992	42	63.0	(13.8)		x	x		x	x	25	141.0	(5.7)	29	96.4	(17.6)
NC14104	13	132.5	(21.1)	15	41.1	(19.5)	32	49.6	(5.8)	21	145.1	(32.0)	30	95.2	(16.4)
D121	34	83.3	(14.3)	5	62.1	(0.0)	13	70.0	(15.9)	22	142.8	(1.5)	31	94.0	(11.4)
I45-51	30	91.3	(3.5)		x	x	6	81.5	(6.7)	38	103.3	(7.7)	32	93.3	(4.6)
DN182	31	90.5	(5.7)		x	x	20	61.1	(4.3)	28	132.8	(6.9)	33	91.5	(4.1)
FC185	22	115.0	(5.5)	3	71.3	(6.7)	10	72.6	(3.3)	42	92.2	(4.4)	34	90.3	(3.1)
DN5	32	89.5	(6.1)	16	40.9	(4.3)	16	64.7	(5.4)	34	119.5	(5.4)	35	88.3	(3.9)
DN34	27	102.8	(3.5)	9	51.0	(5.1)	29	52.0	(4.6)	40	100.3	(11.4)	36	86.1	(3.7)
S301	21	115.5	(4.1)	6	60.3	(3.7)	22	60.3	(3.5)	39	100.8	(6.0)	37	85.4	(3.6)
FC189	24	105.4	(5.8)		x	x	17	63.8	(5.0)	48	77.7	(4.1)	38	81.0	(3.3)
NC13548	50	29.2	(14.4)		x	x	39	42.2	(13.9)	26	138.6	(3.0)	39	80.7	(19.0)
7300501	37	76.6	(18.4)		x	x	45	27.6	(5.5)	32	122.7	(30.9)	40	80.3	(18.0)
80X01107	18	120.5	(6.8)		x	x	11	72.0	(8.8)	50	61.9	(17.7)	41	80.2	(10.6)
S365	26	103.0	(3.2)	18	35.3	(11.6)	31	50.9	(2.3)	44	88.8	(3.8)	42	79.3	(3.5)
DM114	19	119.4	(5.4)	20	32.2	(15.0)	43	38.6	(10.3)	36	105.5	(12.9)	43	77.9	(12.0)
PUR12	33	88.9	(4.4)		x	x	46	27.3	(4.5)	46	80.0	(3.3)	44	77.9	(3.7)
D123	39	73.2	(19.1)		x	x	33	46.5	(10.5)	41	92.4	(10.1)	45	72.9	(9.5)
D124	29	92.9	(20.0)		x	x	23	59.6	(15.9)	49	66.0	(6.0)	46	72.9	(9.0)
S25	36	80.7	(4.9)		x	x	35	45.5	(2.5)	45	81.2	(5.6)	47	69.6	(3.4)
91.05.02	45	51.7	(8.1)		x	x	9	77.0	(4.2)	47	78.3	(9.8)	48	67.3	(5.9)
SV1	43	58.8	(3.4)	10	48.9	(3.1)	44	37.1	(2.9)	43	90.7	(3.8)	49	60.0	(2.9)
DM109	52	20.4	(5.9)	12	47.3	(3.2)	37	44.0	(3.8)	37	103.8	(31.9)	50	56.6	(12.2)
252-4	51	24.0	(2.9)		x	x	12	70.4	(15.4)	51	60.0	(6.3)	51	52.5	(6.3)
42-7	48	35.5	(2.9)	13	43.5	(10.7)	36	45.5	(14.1)	52	57.0	(13.9)	52	49.1	(7.5)
Overall		104.1	(1.8)		54.5	(2.1)		59.8	(1.3)		122.8	(2.1)		96.9	(0.9)

Standard errors are in parentheses.

(54.5 ± 2.1 cm), and rep 1 (104.1 ± 1.8 cm) and rep 3 (59.8 ± 1.3 cm) ranked second and third, respectively (Table 5). All reps differed according to the LSD ($\alpha = 0.05$, LSD critical value = 5.2, $n = 569$, 97, 402, and 575 for reps 1, 2, 3, and 4, respectively). Similar results were shown for diameter. Rep 4 exhibited the greatest diameter (12.05 ± 0.27 mm), while rep 2 expressed the lowest diameter (3.95 ± 0.15 mm), and rep 1 (10.35 ± 0.19 mm) and rep 3 (4.83 ± 0.11 mm) ranked second and third, respectively. All reps differed according to the LSD ($\alpha = 0.05$, LSD critical value = 0.59, $n = 569$, 97, 402, and 575 for reps 1, 2, 3, and 4, respectively). Thus, height and diameter were affected similarly by heavy NO_3^- concentrations in the soil, which was corroborated by a phenotypic correlation of $r = 0.84$ ($p < 0.0001$). The practical importance of these results is that reps may be considered pseudo-treatments. Selection of better-performing genotypes was important because of the broad amount of variation in soil conditions among reps. Unfortunately, the aforementioned landowner confidentiality agreement prohibited the reporting of specific soil contaminant levels. However, given observable contamination indicators such as the lack of broadleaf and grass competition, reps 2 and 3 (that were located the closest to the residue source) appeared to have much worse contamination than reps 1 and 4. These apparent levels of contamination were consistent with tree height and diameter data reported above. Overall, these high levels of soil heterogeneity warrant the need to conduct phyto-recurrent selection in future

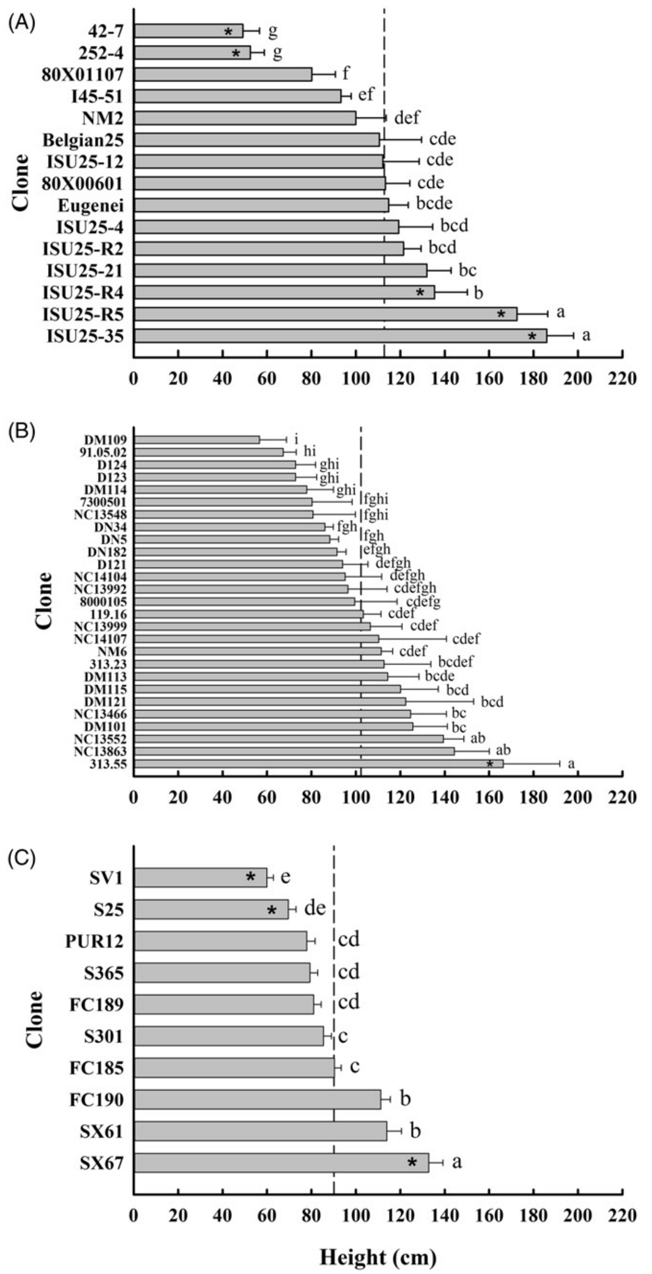

Figure 1. Height of rooted poplar cuttings (A.), unrooted poplar cuttings (B.), and unrooted willow cuttings (C.). Error bars represent one standard error of the mean. The dashed line represents the overall mean, while asterisks within bars indicate individual means that were significantly different from the overall mean ($p < 0.05$). Means followed by the same letter within each planting stock were not different ($p > 0.05$).

studies with similar variability in site conditions (Zalesny *et al.* 2007).

Clones differed in height ($p = 0.0002$) and diameter ($p = 0.0003$) (Table 4). Height across reps for all genera and planting stock ranged from 49.1 ± 7.5 to 186.0 ± 12.0 cm. Height of rooted poplar clones ranged from 49.1 ± 7.5 to 186.0 ± 12.0 cm, while the height of unrooted poplar clones ranged from 56.6 ± 12.2 to 166.4 ± 25.4 cm, and height of unrooted willow clones ranged from 60.0 ± 2.9 to 132.8 ± 6.4 cm (Figure 1). The results for diameter showed similar trends. Diameter across genera and planting stock ranged from 5.26 ± 0.31 to 18.64 ± 1.88 mm. Diameter of rooted poplar clones ranged from 5.94 ± 0.80 to 18.64 ± 1.88 mm, while the diameter of unrooted poplar clones ranged from 5.89 ± 1.30 to 16.75 ± 2.78 mm, and diameter of unrooted willow clones ranged from 5.26 ± 0.31 to 8.64 ± 0.46 mm.

Rooted poplar clones exhibited the greatest height and diameter of all combinations of genera and planting stock. The two best clones overall were rooted stock of poplar from the breeding program of Dr. Richard B. Hall at Iowa State University. Overall, the 'ISU25-X' series of clones from Iowa State University performed the best, with each clone expressing heights well over 1 m by the end of year one. A set of clones from a first-generation backcross population from the USDA Forest Service, Northern Research Station also grew very well (clone series 'NC13XXX'). Selected genotypes from this backcross population have exhibited exceptional rooting ability from unrooted cuttings under varying soil textures and soil temperatures in the North Central United States (Zalesny *et al.* 2004, 2005a, 2005c). Other clones not included in the aforementioned genomic groups that performed well were poplar clone '313.55' and willow clone 'SX67' (heights of 166.4 ± 25.4 cm and 132.8 ± 6.4 cm, respectively).

The broad range of variability among our genotypes provides greater potential for identification and selection of good-rooting clones that also can be established successfully under conditions of heavy NO_3^- concentrations (Pilipović *et al.* 2009; Borišev *et al.* 2010). O'Neill and Gordon (1994) reported Carolina poplar (*Populus* ×*canadensis* Moench; also known as 'Eugenei' and 'DN34') exhibited great ability to remediate NO_3^- from soils of an artificial riparian zone. Nitrate concentration in the soils decreased as uptake by the trees increased, with the tree roots serving as primary sinks for NO_3^-. Such genotypes will be very beneficial for future projects. For example, working with similar *Populus* and *Salix* genotypes as in the current study, Zalesny *et al.* (2005b) reported genotype-dependent phytoremediation potential when trees were established on soils contaminated with petroleum hydrocarbons. In addition, a selected poplar genotype ('H11-11') resulting from a cross between *P. trichocarpa* and *P. deltoides* efficiently remediated contamination from trichloroethylene (TCE) (Gordon *et al.* 1997), and recent results have shown a 32% increase in diameter of poplar using endophyte-assisted phytoremediation for TCE (Doty *et al.* 2017). Greger and Landberg (2001) and Landberg and Greger (1994) tested the ability of *S. viminalis* for phytoextraction of cadmium from soil and reported broad genetic variation among clones. In addition, researchers using agricultural crops reported similar results of successful genotype-dependent remediation (Yang and Blanchar 1993; Wiltse *et al.* 1998; Glenn *et al.* 2001), while others showed a lack of varietal differences resulting in less opportunity for selection of superior genotypes (Russelle *et al.* 2001). Nevertheless, breeding and selection of any plant genotypes for specific phytoremediation uses are important for the success of plant-enhanced bioremediation (Zalesny *et al.* 2016b).

Table 6. Percent survival by replication (rep) and planting stock (i.e. rooted versus unrooted cuttings) across 27 poplar clones measured after one and eleven growing seasons.

	Year 1			Year 11		
Rep	Rooted	Unrooted	Across stock[a]	Rooted	Unrooted	Across stock[a]
1	100	97	98 z	80	32	40 Z
2[b]	–	–	–	–	–	–
3	76	66	69 y	52	25	35 Z
4	97	94	96 z	72	36	52 Z
Across Reps[c]	91 a	86 a	87	67 A	31 B	42

[a]Means followed by the same letter within the columns are not different: (Year 1 $LSD_{0.05} = 12$; Year 11 $LSD_{0.05} = 32$).
[b]Rep 2 was not measured given extensive mortality at 11 years.
[c]Means followed by the same letter within the row are not different: (year 1 $LSD_{0.05} = 10$; year 11 $LSD_{0.05} = 26$).

Table 7. Phenotypic correlations among traits and their rankings for 27 poplar clones following the first and eleventh growing seasons.

Year 1	Year 11				
	Diameter	$Diameter_{RANK}$	Biomass	$Biomass_{RANK}$	Survival
Diameter	0.19	−0.19	0.19	−0.17	0.12
	0.3412	0.3314	0.3399	0.3950	0.5671
$Diameter_{RANK}$	−0.07	0.08	−0.06	0.06	0.01
	0.7204	0.6828	0.7481	0.7831	0.9616
Height	0.15	−0.18	0.15	−0.13	0.24
	0.4593	0.3590	0.4509	0.5317	0.2188
$Height_{RANK}$	−0.07	0.14	−0.06	0.05	−0.20
	0.7180	0.4945	0.7778	0.8206	0.3193
Survival	0.38	−0.38	0.40	−0.41	0.38
	0.0529	**0.0489**	**0.0412**	**0.0331**	**0.0474**

Significant correlations are shown in bold ($p < 0.05$).

There was an interaction between rep and clone for height ($p < 0.0001$) and diameter ($p < 0.0001$) (Table 4). Given the similar nature of the interactions for each dependent variable, the means for height are given as a representative expression of clonal performance across reps (Table 5). There were few clones that performed consistently across reps (see below), regardless of whether height and diameter were above average or below average. The extreme variation in genotypic performance within any given rep most likely was due to the variability in soil $NO3^-$ levels. Thus, for phytoremediation purposes, we recommend extensive physiological tests of $NO3^-$ levels within the roots, shoots, and cuttings, as well as in the soil.

Genotypic stability in survival and diameter of poplars after one and 11 growing seasons

Using *in situ* phyto-recurrent selection (Zalesny *et al.* 2007), 27 clones that were measured after the first growing season were re-measured after eleven growing seasons, with 13 genotypes planted as rooted cuttings and fourteen clones as unrooted cuttings (Table 1). For year 1, the percent survival of trees across reps, clones, and planting stock ranged from 66% (rep 3, unrooted) to 100% (rep 1, rooted), with an overall survival rate of 87% (Table 6). After eleven years, the percent survival ranged from 25% (rep 3, unrooted) to 80% (rep 1, rooted), with an overall survival rate of 42%. The survival rates across planting stock and reps exhibited inverse relationships during both points in time. Specifically, there were no differences in the overall survival rate of rooted versus unrooted cuttings at year 1, yet trees planted as rooted cuttings had more than a two-fold greater survival relative to their unrooted counterparts at year 11. Across planting stock, the survival rates of reps 1 and 4 were roughly 40% better than for rep 3 after the first growing season, while reps did not differ for survival rate across planting stock after 11 years (Table 6). These results corroborated the general trends of trees established from rooted poplar cuttings having greater survival rates than unrooted cuttings as trees reach rotation-age (Stanturf *et al.* 2001).

Survival is an important early indicator of phytoremediation potential (Zalesny *et al.* 2005b), and in this study, establishment survival was significantly correlated with the rank in diameter ($r = -0.38$, $p = 0.0489$), biomass ($r = 0.40$, $p = 0.0412$), rank in biomass ($r = -0.41$, $p = 0.0331$), and survival ($r = 0.38$, $p = 0.0474$) at 11 years after planting (Table 7). As expected, clones with greater survival had better ranks and thus greater diameter and biomass. Although the correlation between first-year survival and 11-year diameter was not significant, a previous study found increased survival at establishment led to greater rotation-age biomass across clones (Zalesny *et al.* 2015). In particular, genotype-specific responses were observed for differences in survival, diameter, and rank of diameter measured after one and 11 growing seasons (Table 8).

Overall, planting stock influenced the genotypic stability in diameter of the 27 poplar clones, resulting in four general response groups when comparing the difference between observed diameter and mean diameter at the establishment- and rotation-age (Figure 2). In general, clones established from unrooted cuttings were more stable than those from rooted cuttings, as indicated by tighter genotype clustering. The segregation of genotypes into response groups corroborated previous results from landfill leachate applications using a subset of these clones (Zalesny *et al.* 2008). In the current study, the first group consisted of clones that were planted from rooted cuttings, had high survival rates, and exhibited above-average diameter at years 1 and 11 ('ISU25-35'; 'ISU25-R5'; 'ISU25-R4'; '80X00601'; 'ISU25-12'; 'ISU25-21') (upper right quadrant of Figure 2). These clones represented the most optimal genotype mix for phytoremediation of fertilizer residues. The second group was comprised of seven genotypes that exhibited above-average diameter during the 11th growing season despite poor growth during establishment ('ISU25-4'; 'DN182'; 'NM6'; 'DN34'; 'NC13992'; 'DM115'; '313.23') (lower right quadrant of Figure 2). With the exception of 'ISU25-4,' all of these genotypes were planted as unrooted cuttings. Similar to group 1, these clones were considered favorable due to their long-term performance. The third group consisted of a mixture of four rooted and three unrooted clones that had above-average diameter during the establishment year yet below-average growth at rotation-age (rooted: '42-7'; '252-4'; '80X01107'; 'ISU25-R2'; unrooted: '7300501'; 'D121'; 'D124') (upper left quadrant of Figure 2). Lastly, the fourth response group was a mixture of two rooted and five unrooted clones that had below-average diameter at both measuring periods (rooted: 'Belgian25'; 'I45-51'; unrooted: '8000105'; '119.16'; 'DN5'; 'D123'; '91.05.02') (lower left quadrant of Figure 2).

Table 8. Survival, diameter, and rank of diameter for 27 poplar clones measured after one and eleven growing seasons.

	Survival		Diameter		$Diameter_{RANK}$	
Clone	Year 1 (%)	Year 11 (%)	Year 1 (mm)	Year 11 (cm)	Year 1 (position)	Year 11 (position)
ISU25-35	94.4	100.0	8.6	26.5	1	1
ISU25-R5	100.0	78.6	17.8	21.1	2	8
ISU25-R4	100.0	72.7	14.4	24.0	3	3
NC13992	58.3	25.0	13.2	15.1	4	25
ISU25-21	76.5	70.6	13.0	19.9	5	10
313.23	58.3	33.3	12.7	12.4	6	26
ISU25-12	100.0	83.3	12.5	21.9	7	6
ISU25-4	65.0	60.0	12.4	18.3	8	17
80X00601	96.4	71.4	12.0	22.6	9	4
DM115	100.0	50.0	11.9	15.1	10	24
NM6	86.2	2.5	11.3	17.7	11	18
DN34	87.6	22.9	10.8	17.1	12	21
DN182	89.5	34.5	10.7	18.5	13	16
D121	83.3	75.0	10.5	21.0	14	9
119.16	85.0	65.0	10.0	18.7	15	15
DN5	84.3	27.5	9.9	17.7	16	19
Belgian25	83.3	33.3	9.5	9.4	17	27
8000105	91.7	66.7	9.4	18.8	18	14
ISU25-R2	100.0	100.0	9.4	19.9	19	11
7300501	83.3	75.0	8.2	21.5	20	7
I45-51	100.0	16.7	7.8	15.5	21	23
80X01107	88.2	64.7	7.7	19.8	22	13
D123	91.7	91.7	6.9	17.5	23	20
D124	100.0	83.3	6.7	19.9	24	12
252-4	83.3	72.2	6.5	22.4	25	5
91.05.02	83.3	58.3	6.1	16.4	26	22
42-7	82.4	47.1	5.9	25.0	27	2

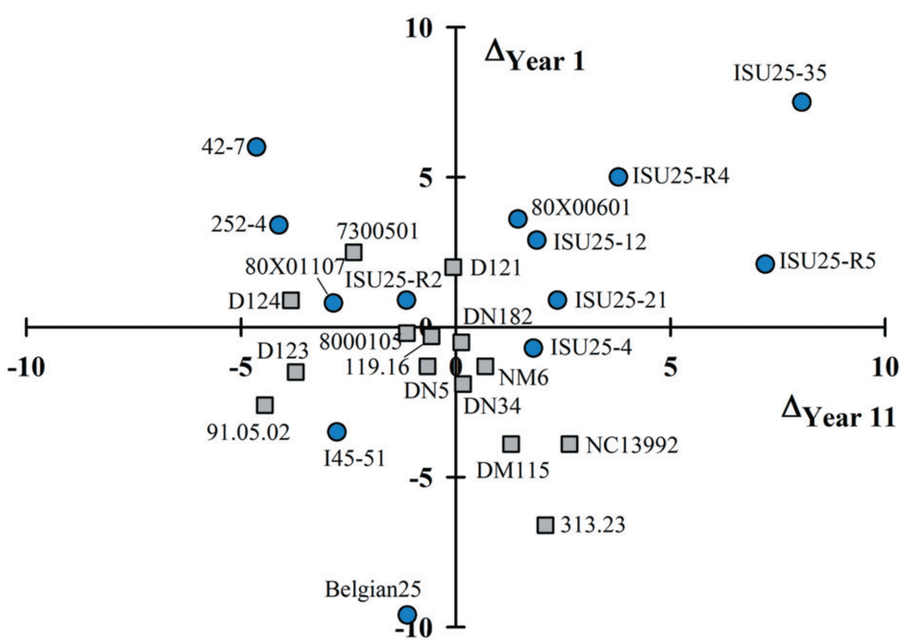

Figure 2. Difference between observed diameter and mean diameter for 27 poplar clones measured after one ($^{\Delta}$Year 1; mm) and eleven ($^{\Delta}$Year 11; cm) growing seasons. Clones planted as rooted cuttings are shown with blue circles, while those planted as unrooted cuttings are gray squares.

While these genotypes were the least favorable for the current study, many have been successful during other phytoremediation applications (Zalesny *et al.* 2016b). Therefore, these clones warrant further investigation in future studies with similar site conditions.

Genotypic stability in diameter is illustrated in Figure 3, which depicts changes in the rank of the 27 poplar clones measured after one and 11 growing seasons. Contrary to the trends in the difference in magnitude of diameter growth shown in Figure 2, a relationship between planting stock and diameter rank changes was non-evident. Overall, 12 genotypes had positive rank changes, 12 genotypes had negative rank changes, and three genotypes had the same rank at the establishment- and rotation-age. The latter three clones are of particular interest, given their stability in growth despite a decade of climatic, edaphic, and other external influences. The four genotypes at the opposite ends of the changes in rank are also important (*i.e.* '252-4' and '42-7' that improved rank versus '313.23' and 'NC13992' that lost substantial rank). Clones '252-4' and '42-7' ranked

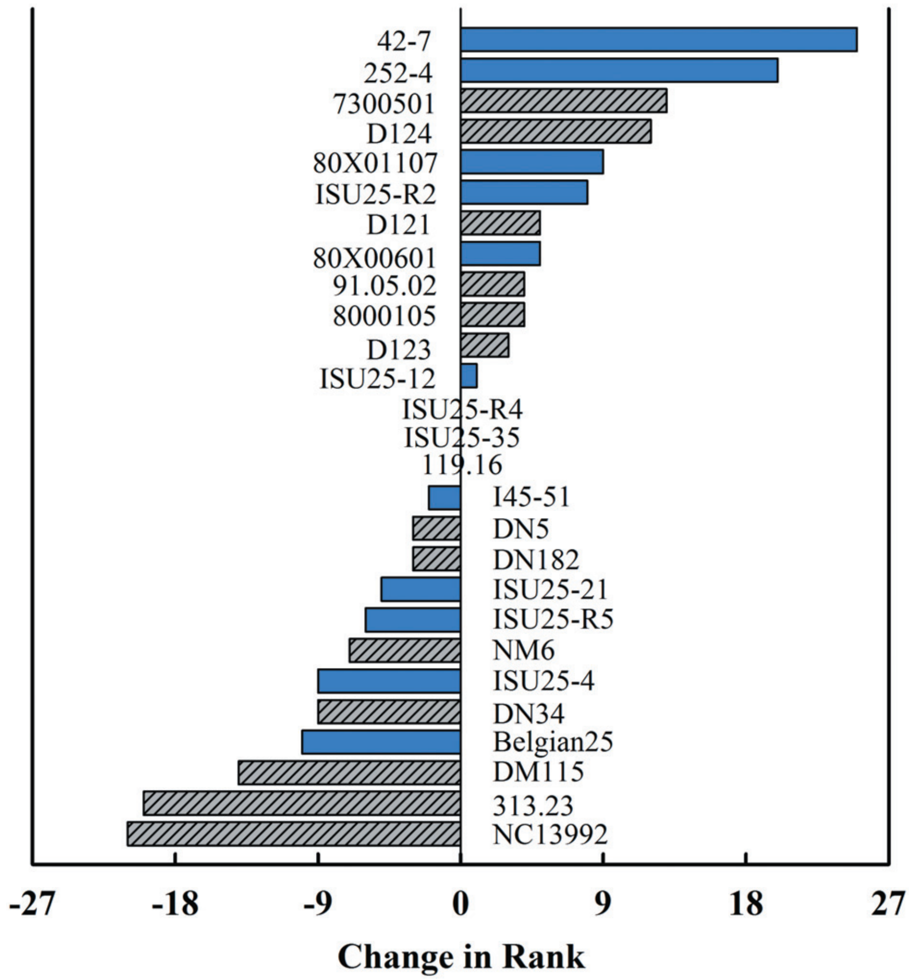

Figure 3. Changes in rank of diameter for 27 poplar clones measured after one and 11 growing seasons. Clones planted as rooted cuttings are shown with blue bars (including 'ISU25-R4' and 'ISU25-35'), while those planted as unrooted cuttings are gray-hashed bars (including '119.16').

45th and 48th out of 52 (including willow) for diameter after the first growing season and 5th and 2nd out of 27 after 11 years, respectively. Although not tested in the current study, this change in rank was likely due to the investment of the genotypes in developing extensive root systems during the establishment year, along with favorable genotype × environment interactions that developed over time.

Conclusions

Our results verified the broad clonal variation in survival and subsequent growth among and within poplar and willow genotypes established on soils heavily contaminated with NO_3^-. Such variation was an important positive indicator for the potential of long-term phytoremediation of NO_3^--contaminated soils by poplar and willow because gains from genotype selection are proportional to variation and establishment is the first requirement for all poplar and willow production systems. While poplar exhibited greater overall height and diameter growth, willow exhibited above-average survival and potential for excellent growth and development in subsequent years following establishment. Thus, we recommend that both genera continue to be considered for phytoremediation of contaminated soils. Given the broad range in height and diameter among genotypes and the ability to select better clones within this variation, our findings suggest that selection of specific clones was favorable to selection at the genomic group level, based on the geographic location and soil conditions of the site.

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Disclosure statement

Both authors declare no conflict of interest.

References

Borišev M, Pajević S, Nikolić N, Krstić B, Pilipović A. 2010. Influence of Cd and Ni on content of N, P, K, nitrates and activity of nitrate reductase in clones of *Salix* Spp. Biotechnol Biotechnol Equip. 24(Supp 1):228–235. doi:10.1080/13102818.2010.10817841.

Calderon FJ, Jackson LE. 2002. Rototillage, disking, and subsequent irrigation: effects on soil nitrogen dynamics, microbial biomass, and carbon dioxide efflux. J Environ Qual. 31(3):752–758. doi:10.2134/jeq2002.7520.

Cavero J, Beltran A, Aragues R. 2003. Nitrate exported in drainage waters of two sprinkler-irrigated watersheds. J Environ Qual. 32: 916–926. doi:10.2134/jeq2003.9160.

Christersson L. 1986. High technology biomass production by *Salix* clones on a sandy soil in southern Sweden. Tree Physiol. 2(1_2_3): 261–272. doi:10.1093/treephys/2.1-2-3.261.

David MB, Gentry LE, Kovacic DA, Smith KM. 1997. Nitrogen balance in and export from an agricultural watershed. J Environ Qual. 26(4): 1038–1048. doi:10.2134/jeq1997.00472425002600040015x.

Dickmann DI. 2001. An overview of the genus *Populus*. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar culture in North America. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada. p. 1–42, ISBN 978-0-660-18145-5.

Doty SL, Freeman JL, Cohu CM, Burken JG, Firrincieli A, Simon A, Khan Z, Isebrands JG, Lukas J, Blaylock MJ. 2017. Enhanced degradation of TCE on a Superfund site using endophyte-assisted poplar tree phytoremediation. Environ Sci Technol. 51(17):10050–10058. doi:10.1021/acs.est.7b01504.

Eckenwalder JE. 1984. Natural intersectional hybridization between North American species of *Populus* (*Salicaceae*) in sections *Aigeiros* and *Tacamahaca*. II. Taxonomy. Can J Bot. 62(2):325–335. doi: 10.1139/b84-051.

Glenn EP, Waugh WJ, Moore D, McKeon C, Nelson SG. 2001. Revegetation of an abandoned uranium millsite on the Colorado Plateau, Arizona. J Environ Qual. 30(4):1154–1162. doi:10.2134/jeq2001.3041154x.

Gordon M, Choe N, Duffy J, Ekuan G, Heilman P, Muiznieks I, Newman L, Ruszaj M, Shurtleff BB, Strand S, *et al.* 1997. Phytoremediation of trichloroethylene with hybrid poplars. In: Kruger EL, Anderson TA, Coats JR, editors. Phytoremediation of soil and water contaminants. Washington (DC): American Chemical Society. p. 177–185, ISBN 978-0-841-23503-8.

Greger M, Landberg T. 2001. Investigation on uptake of Cd, Cu, and Zn in *Salix viminalis* of different ages and rotations. In: Greger M, Landberg T, Berg B, editors. *Salix* clones with different properties to accumulate heavy metals for production of biomass. Stockholm, Sweden: University of Stockholm. p. 6–18.

Hall RL, Allen SJ, Rosier PTW, Hopkins R. 1998. Transpiration from coppiced poplar and willow measured using sap-flow methods. Agric For Meteorol. 90(4):275–290. doi:10.1016/S0168-1923(98)00059-8.

Isebrands JG, Aronsson P, Carlson M, Ceulemans R, Coleman M, Dickinson N, Dimitriou J, Doty S, Gardiner E, Heinsoo K, *et al.* 2014. Environmental applications of poplars and willows. In: Isebrands JG, Richardson J, editors. Poplars and Willows: Trees for Society and the Environment. Rome, Italy: Food and Agriculture Organization (FAO) of the United Nations; Boston (MA): CAB International, Inc. Chapter 6, p. 258–336, ISBN 978-1-78064-108-9.

Isebrands JG, Karnosky DF. 2001. Environmental benefits of poplar culture. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar culture in North America. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada. p. 207–218. ISBN 978-0-660-18145-5.

Jaynes DB, Colvin TS, Karlen DL, Cambardella CA, Meek DW. 2001. Nitrate loss in subsurface drainage as affected by nitrogen fertilizer rate. J Environ Qual. 30(4):1305–1314. doi:10.2134/jeq2001.3041305x.

Klang-Westin E, Eriksson J. 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. Plant Soil. 249(1): 127–137. doi:10.1023/A:1022585404481.

Kopp RF, Smart LB, Maynard CA, Isebrands JG, Tuskan GA, Abrahamson LP. 2001. The development of improved willow clones for eastern North America. For Chron. 77(2):287–292. doi:10.5558/tfc77287-2.

Labrecque M, Teodorescu TI, Cogliastro A, Daigle S. 1993. Growth patterns and biomass productivity of two *Salix* species grown under short-rotation intensive culture in southern Quebec. Biomass Bioenerg. 4(6):419–425. doi:10.1016/0961-9534(93)90063-A.

Labrecque M, Teodorescu TI, Daigle S. 1998. Early performance and nutrition of two willow species in short-rotation intensive culture fertilized with wastewater sludge and impact on the soil characteristics. Can J Res. 28(11):1621–1635. doi:10.1139/x98-142.

Landberg T, Greger M. 1994. Can heavy metal tolerant clones of *Salix* be used as vegetation filters on heavy metal contaminated land? In: Aronsson P, Perttu K, editors. Willow vegetation filters for municipal wastewaters and sludges. Uppsala, Sweden: Report 50 of the Swedish University of Agricultural Sciences. p. 133–144.

Lazarus W, Headlee WL, Zalesny RS Jr. 2015. Impacts of supplyshed-level differences in productivity and land costs on the economics of hybrid poplar production in Minnesota, USA. Bioenerg Res. 8(1): 231–248. doi:10.1007/s12155-014-9520-y.

McIntyre T, Lewis GM. 1997. The advancement of phytoremediation as an innovative environmental technology for stabilization, remediation, or restoration of contaminated sites in Canada: a discussion paper. J Soil Contam. 6:227–242. doi:10.1080/15320389709383562.

Mirck J, Volk TA. 2010. Seasonal sap flow of four *Salix* varieties growing on the Solvay Wastebeds in Syracuse, NY, USA. Int J Phytoremediat. 12(1):1–23. doi:10.1080/15226510902767098.

Mirck J, Zalesny RS Jr. 2015. Mini-review of knowledge gaps in salt tolerance of plants applied to willows and poplars. Int J Phytoremediat. 17(7):640–650. doi:10.1080/15226514.2014.950414.

Mirck J, Isebrands JG, Verwijst T, Ledin S. 2005. Development of short-rotation willow coppice systems for environmental purposes in Sweden. Biomass Bioenerg. 28(2):219–228. doi:10.1016/j.biombioe.2004.08.012.

O'Neill GJ, Gordon AM. 1994. The nitrogen filtering capability of Carolina poplar in an artificial riparian zone. J Environ Qual. 23: 1218–1223. doi:10.2134/jeq1994.00472425002300060014x.

Perttu KL, 1993. Biomass production and nutrient removal from municipal wastes using willow vegetation filters. J Sustain For. 1(3): 57–70. doi:10.1300/J091v01n03_05.

Perttu KL, Kowalik PJ. 1997. *Salix* vegetation filters for purification of water and soils. Biomass Bioenerg. 12(1):9–19. doi:10.1016/S0961-9534(96)00063-3.

Pilipović A, Orlović S, Katanić M. 2009. The effect of nitrate concentrations on physiology of different leaf age groups. In: Mihailović D, Miloradov MV, editors. Environmental, health and humanity issues in the Down Danubian region, multidisciplinary approaches: Proceedings of the 9th International Symposium on Interdisciplinary Regional Research. Hackensack (NJ): World Scientific Publishing Co. Chapter 13, p. 127–135. ISBN-13 978-981-283-439-3.

Rockwood DL, Isebrands JG, Minogue PJ. 2013. Phytoremediation trees for biofuel. In: Singh BP, editor. Biofuel crops: production, physiology, and genetics. Boston (MA): CAB International, Inc. Chapter 22, p. 474–490. ISBN 978-1-84593-885-7.

Rockwood DL, Naidu CV, Carter DR, Rahmani M, Spriggs TA, Lin C, Alker GR, Isebrands JG, Segrest SA. 2004. Short-rotation woody crops and phytoremediation: opportunities for agroforestry? Agroforestry Syst. 61:51–63. doi:10.1023/B:AGFO.0000028989.72186.e6.

Ronald WG. 1982. Intersectional hybridization of *Populus* sections, *Leuce-aigeiros* and *Leuce-tacamahaca*. Silvae Genet. 31:94–99.

Roygard JKF, Clothier BE, Green SR, Bolan NS. 2001. Tree species for recovering nitrogen from dairy-farm effluent in New Zealand. J Environ Qual. 30(3):1064–1070. doi:10.2134/jeq2001.3031064x.

Russelle MP, Lamb JFS, Montgomery BR, Elsenheimer DW, Miller BS, Vance CP. 2001. Alfalfa rapidly remediates excess inorganic nitrogen at a fertilizer spill site. J Environ Qual. 30(1):30–36. doi:10.2134/jeq2001.30130x.

Schnoor JL. 2000. Phytostabilization of metals using hybrid poplar trees. In: Raskin I, Ensley BD, editors. Phytoremediation of toxic metals: using plants to clean up the environment. New York: John Wiley & Sons, Inc. p. 133–150. ISBN 978-0-471-19254-1.

Schreiner EJ. 1971. Genetics of eastern cottonwood. USDA for. Serv. Res. Pap. WO-RP-11.:19.

Stanturf JA, van Oosten C, Netzer DA, Coleman MD, Portwood CJ. 2001. Ecology and silviculture of poplar plantations. In: Dickmann

DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar culture in North America. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada. p. 153–206. ISBN 978-0-660-18145-5.

Stites W, Kraft GJ. 2001. Nitrate and chloride loading to groundwater from an irrigated north-central U.S. sand-plain vegetable field. J Environ Qual. 30(4):1176–1184. doi:10.2134/jeq2001.3041176x.

Syswerda SP, Basso B, Hamilton SK, Tausig JB, Robertson GP. 2012. Long-term nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. Agric Ecosyst Environ. 149:10–19. doi:10.1016/j.agee.2011.12.007.

Udawatta RP, Krstansky JJ, Henderson GS, Garrett HE. 2002. Agroforestry practices, runoff, and nutrient loss: a paired watershed comparison. J Environ Qual. 31(4):1214–1225. doi:10.2134/jeq2002.1214.

Volk TJ, Ballard B, Robison DJ, Abrahamson LP. 2004. Effect of cutting storage conditions during planting operations on the survival and biomass production of four willow (*Salix* L.) clones. New For. 28(1):63–78. doi:10.1023/B:NEFO.0000031334.86593.4e.

Wiltse CC, Rooney WL, Chen Z, Schwab AP, Banks MK. 1998. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. J Environ Qual. 27(1):169–173. doi:10.2134/jeq1998.00472425002700010024x.

Woodard KR, French EC, Sweat LA, Graetz DA, Sollenberger LE, Macoon B, Portier KM, Rymph SJ, Wade BL, Prine GM, *et al.* 2003. Nitrogen removal and nitrate leaching for two perennial, sod-based forage systems receiving dairy effluent. J Environ Qual. 32(3):996–1007. doi:10.2134/jeq2003.9960.

Yang J, Blanchar RW. 1993. Differentiating chloride susceptibility in soybean cultivars. Agron J. 85(4):880–885. doi:10.2134/agronj1993.00021962008500040019x.

Zalesny JA, Zalesny RS Jr, Wiese AH, Hall RB. 2007. Choosing tree genotypes for phytoremediation of landfill leachate using phyto-recurrent selection. Int J Phytoremediat. 9(6):513–530. doi:10.1080/15226510701709754.

Zalesny JA, Zalesny RS Jr, Wiese AH, Sexton B, Hall RB. 2008. Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. Environ Pollut. 155(1):72–80. doi:10.1016/j.envpol.2007.10.032.

Zalesny RS Jr, Bauer EO, Riemenschneider DE. 2004. Use of belowground growing degree days to predict rooting of dormant hardwood cuttings of *Populus*. Silvae Genet. 53(1–6):154–160. doi:10.1515/sg-2004-0028.

Zalesny RS Jr., Riemenschneider DE, Hall RB. 2005a. Early rooting of dormant hardwood cuttings of *Populus*: analysis of quantitative genetics and genotype × environment interactions. Can. J. For. Res. 35:918–929. doi:10.1139/x05-018.

Zalesny RS Jr., Bauer EO, Hall RB, Zalesny JA, Kunzman J, Rog CJ, Riemenschneider DR. 2005b. Clonal variation in survival and growth of hybrid poplar and willow in an *in situ* trial on soils heavily contaminated with petroleum hydrocarbons. Intl. J. Phytoremediat. 7(3):177–197. doi:10.1080/16226510500214632.

Zalesny RS Jr., Hall RB, Bauer EO, Riemenschneider DE. 2005c. Soil temperature and precipitation affect the rooting ability of dormant hardwood cuttings of *Populus*. Silvae Genet. 54(1-–6):47–58. doi:10.1515/sg-2005-0009.

Zalesny RS Jr., Headlee WL, Gopalakrishnan G, Bauer EO, Hall RB, Hazel DW, Isebrands JG, Licht LA, Negri MC, Guthrie-Nichols E, *et al.* 2015. Ecosystem services of poplar at long-term phytoremediation sites in the Midwest and Southeast, United States. In: Phytotechnologies for Sustainable Development, Proceedings of the 12th International Conference of the International Phytotechnology Society, Manhattan, KN, USA; 27–30 September 2015.

Zalesny RS Jr, Stanturf JA, Gardiner ES, Perdue JH, Young TM, Coyle DR, Headlee WL, Bañuelos GS, Hass A. 2016a. Ecosystem services of woody crop production systems. Bioenerg Res. 9(2):465–491. doi:10.1007/s12155-016-9737-z.

Zalesny RS Jr, Stanturf JA, Gardiner ES, Bañuelos GS, Hallett RA, Hass A, Stange CM, Perdue JH, Young TM, Coyle DR, *et al.* 2016b. Environmental technologies of woody crop production systems. BioEnerg Res. 9(2):492–506. doi:10.1007/s12155-016-9738-y.

Zhao SL, Gupta SC, Huggins DR, Moncrief JF. 2001. Tillage and nutrient source effects on surface and subsurface water quality at corn planting. J Environ Qual. 30(3):998–1008. doi:10.2134/jeq2001.303998x.