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Growth and physiological responses of three poplar clones grown on soils artificially contaminated with heavy metals, diesel fuel, and herbicides

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ABSTRACT

We tested the growth and physiological responses of three poplar clones [*Populus deltoides* Bartr. ex Marsh. 'Bora', 'PE 19/66'; *Populus* × *euramericana* (Dode) Guinier 'Pannonia'] grown for 3 years on soils artificially contaminated with heavy metals, diesel fuel, and herbicides at the Experimental Estate of the Institute of Lowland Forestry and Environment (ILFE), University of Novi Sad, Serbia. Within three field blocks, clonal whole-plots were divided into seven subplots containing a non-contaminated control and six artificially-contaminated soil treatments: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}. Significant clone × treatment interactions governed growth and physiology throughout the study ($p < 0.05$), and the influence of inorganics *versus* organics varied with tree age. Heavy metals had a more substantial influence on growth and physiology as the trees matured, while diesel and herbicide treatments were most pronounced during the first growing season ($p < 0.0001$). Clones 'Bora' and 'PE 19/66' exhibited greater biomass than 'Pannonia', with trees growing in the control soils exhibiting 13.8 and 19.6 times greater biomass than 'Pannonia', respectively.

KEYWORDS
Biomass; photosynthesis; phytotechnologies; *Populus*; transpiration; water use efficiency

Introduction

Woody biomass from short rotation coppice (SRC) plantations play a substantial role in feedstock production for alternative energy sources throughout the world, thus helping to mitigate climate change driven by excessive use of fossil fuels (Zalesny, Berndes, *et al.* 2019). Establishment of such energetic plantations for biomass production presents the basis for more efficient usage of renewable energy sources while avoiding additional emissions of carbon dioxide (CO_2) (Rončević *et al.* 2013). Although high costs are main obstacle to widespread use of biomass for energy from SRC plantations, the use of such biomass is still sought as a viable option in energy portfolios (Lazarus *et al.* 2015). Increasing biomass yields and optimizing fuel quality are among potential options for reducing such costs associated with production, and these alternatives help to promote the use of biomass from SRC plantations as sustainable energy feedstocks (Klašnja *et al.* 2008).

Historically, one of the most effective means of enhancing biomass productivity and energy-related traits has been through intra- and inter-sectional hybridization and subsequent selection (Orlović *et al.* 2003; Mahama *et al.* 2011), often resulting in substantial gains from breeding efforts (Riemenschneider *et al.* 2001; Nelson *et al.* 2018). In eastern Europe, for example, biomass production of superior poplar clonal selections increased ten-fold following decades of breeding and selection at the Institute of Lowland Forestry and Environment in Novi Sad, Serbia (Gardiner et al. 2018), while poplar biomass of superior genotypes was nearly three times greater than commercial clones in the Upper Great Lakes, United States (Zalesny *et al.* 2009). Regardless of the geographic location of deployment, poplar production has focused on optimizing genotype × environment interactions in order to increase potential biomass yield, including both traditional applications on marginal lands (Zalesny, Stanturf, Gardiner, Bañuelos, *et al.* 2016) and newer systems that incorporate poplar production with phytotechnologies (Zalesny, Stanturf, Gardiner, Perdue, *et al.* 2016), such as remediation of soils polluted with heavy metals (Trudić *et al.* 2013; Pilipović *et al.* 2019) and crude oil (Pilipović *et al.* 2012).

Considering the increase of degraded land such as industrial brownfields and municipal landfills, the production of biomass in SRC plantations can be combined with phytoremediation which, according to Salt *et al.* (1995), presents the technology that uses plants and their associated organisms for environmental cleanup. Although many annual and perennial herbaceous plants can be used for phytoremediation, there are fewer tree species exhibiting high potential for this purpose. In particular, poplars are ideal for

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phytoremediation due to their pioneer species biology that is characterized by rapid growth and well-developed roots. As such, poplars are capable for uptake of large amounts of water on water-rich sites and high water use efficiency (WUE) on moisture-limited areas (Licht and Isebrands 2005; Zalesny, Berndes, *et al.* 2019). Phytoremediation potential should be considered in the aforementioned breeding of poplars for SRC biomass production. In these systems, growth and biomass are the most commonly-evaluated parameters (Felix et al. 2008; Fortier *et al.* 2010), wherein extensive genotypic variability has led to differing uptake of heavy metals (Laureysens *et al.* 2004; Polle *et al.* 2013; Baldantoni *et al.* 2014), as well as responses to petroleum hydrocarbons (El-Gendy *et al.* 2009; Pilipović *et al.* 2012; Cook and Hesterberg 2013) and herbicides (Donahue *et al.* 1994; Strauss *et al.* 1997; Meilan *et al.* 2002).

In addition, physiological traits play a substantial role in breeding and selection of poplars for wide-ranging applications, including phytoremediation (Pilipović *et al.* 2019). For example, there were strong, positive correlations among photosynthetic processes, growth, and biomass production traits among multiple poplar genomic groups (Orlović *et al.* 1998; Marron and Ceulemans 2006). However, various effects such as drought, low soil fertility, and soil contaminants significantly disturb growth and physiological processes, especially during phytoremediation. The decreased intensity of photosynthesis of plants impacted by heavy metals is due to changes in chloroplast structure, disturbance of chlorophyll synthesis, disturbance in electron transport, inhibition of Calvin cycle enzymes, and lack of carbon dioxide due to stomatal closure (Seregin and Ivanov 2001). Heavy metal accumulation in chloroplasts caused impacts to photosystem II (PSII) resulting in disturbed chlorophyll fluorescence, which in normal conditions accounts for 3% of total absorbed light (Semane *et al.* 2010). Considering individual effects of heavy metals on physiological processes, higher concentrations of Cd inhibit Fe metabolism and cause chlorosis, leading to a decrease in intensity of photosynthesis (Borišev *et al.* 2016). Similarly, leaf chlorosis, disturbed water balance, and reduced stomatal opening are major stress responses to toxic Ni concentrations (Clemens 2006). In contrast, Ni is one of the essential micronutrients involved in nitrogen metabolism (Stanković *et al.* 2006), and Ni can have positive effects on photosynthesis when accumulated in lower concentrations (Xue *et al.* 2013). Like Ni, excess Cu in soil solution can disturb photosynthetic processes, nitrogen metabolism, and oxidative pathways (Borghi *et al.* 2008; Trudić *et al.* 2013). Similarly, organic pollutants such as petroleum hydrocarbons also impact growth of plants either by affecting soil properties (Zalesny and Bauer 2007b; Pilipović 2012) or plant physiological processes (Chan and Chiu 1985; Pajević *et al.* 2009; Han *et al.* 2016), with higher contamination levels often leading to tree mortality. The same is true for many herbicides. For example, Oxyfluorfen belongs to the group of photobleaching biphenyl ether (DPE) herbicides that inhibit protoporphyrinogen oxidase, which is the last common enzyme in heme and chlorophyll biosynthesis (Yanagida *et al.* 1999), while Pendimethalin affects specific growth rate and chlorophyll A content, while decreasing net photosynthesis and increasing dark respiration (Shabana *et al.* 2001).

Integrating poplar SRC with phytotechnologies such as phytoremediation offers opportunities for the production of woody biomass for energy feedstocks with the clean-up of polluted sites such as brownfields and landfills. However, knowledge of the responses of poplar genotypes to soils with concentrations of heavy metals and organic pollutants are not well understood. Therefore, the objective of this study was to test the growth and physiological responses of three poplar clones grown on field-soils artificially contaminated with heavy metals, diesel fuel, and herbicides. In particular, we assessed the performance of two, newly-selected high-yield clones (‘Bora’ and ‘PE 19/66’) relative to one commonly used clone (‘Pannonia’) for both biomass production and phytoremediation potential. While the soil conditions and clonal material are specific to Serbia, the results and applications of this study are useful for researchers, managers, and academicians growing poplar SRC throughout the world in regions experiencing similar ecological concerns, such as the Great Lakes Basin, USA.

Materials and methods

Site description and preparation

The study was conducted on a 0.24-ha plot that was previously used for poplar nursery production at the Experimental Estate of the Institute of Lowland Forestry and Environment (ILFE), University of Novi Sad, Serbia (former Poplar Research Institute) (45.29444°N, 19.88556°E). Soil properties of the study area are shown in Tables 1 and 2, along with depth to water table (Figure 1). General site preparation for the experiment consisted of plowing and tilling to a depth of 40 cm. After this initial soil preparation, the study area was divided into seven subplots for each of

Table 1. Soil physico-chemical properties in a study testing the growth and physiological responses of three poplar clones grown on soils artificially contaminated with heavy metals, diesel fuel, and herbicides.

Soil type	Fluvisol (loamy form)
pH	7.89
Bulk density (g cm^{-3})	1.42
Porosity (%)	49.47
Humus (%)	1.20
Total N (%)	0.10
$CaCO_3$ (%)	19.82
P_2O_5 (mg 100 g^{-1})	3.63
K_2O (mg 100 g^{-1})	12.40

Table 2. Soil water properties in a study testing the growth and physiological responses of three poplar clones grown on soils artificially contaminated with heavy metals, diesel fuel, and herbicides.

	Water retention (%)				
Soil depth (cm)	0.33 (bar)	6.25 (bar)	15 (bar)	Available water (%)	Capillarity (cm)
0–45	46.46	24.49	24.24	22.22	17.1
45–80	48.25	28.12	18.00	30.25	16.2
80–120	46.53	21.22	20.52	26.01	16.2

three blocks, with one subplot for a non-contaminated control and the remaining subplots for six artificially-contaminated soil treatments: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}. After subplot delineation, treatment plots were sprayed with each of the contaminants using a 200-L tractor tank sprayer, and contaminants were incorporated into the surface soil layer by disking to a depth of 30 cm. In order to obtain sufficient contamination in the upper soil layer associated with the root zone during establishment (*i.e.*, the first 3 years), heavy metal treatment concentrations were based on a doubling of maximum allowable limits of: 3 mg Cd kg^{-1}, 100 mg Cu kg^{-1}, and 50 mg Ni kg^{-1}.

Plant material and experimental design

One-year-old coppice shoots from three poplar clones [*Populus deltoides* Bartr. ex Marsh. 'Bora', 'PE 19/66'; *Populus* × *euramericana* (Dode) Guinier 'Pannonia'] were collected from the gene bank at the ILFE Experimental Estate and processed into 30-cm dormant, hardwood cuttings with at least one bud in the upper 5 cm of each propagule. Prior to planting, cuttings were soaked in water for 48 h to stimulate rooting and treated with a 0.5% copper sulfate solution to reduce potential fungal growth that could negatively impact root initiation and subsequent tree establishment. Immediately after pretreatment, cuttings were planted in a split-plot experiment design with three clone whole plots and seven soil treatment subplots. Clone whole plots were arranged in three random blocks with individual-tree spacing of 2.0 × 0.5 m, resulting in a planting density of 10,000 trees ha^{-1} within every soil treatment subplot. Post-planting, site maintenance consisted of mechanical tilling and hand weeding during the first two growing seasons. Given canopy closure, weed management was not necessary during the third year. Precipitation was the water source each growing season (*i.e.*, no supplemental irrigation was provided).

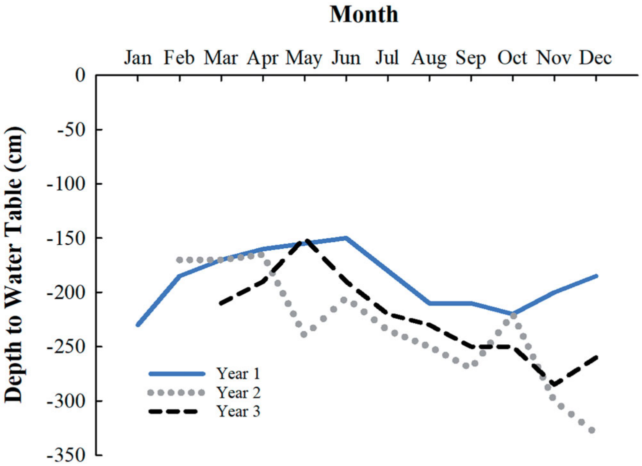

Figure 1. Depth to water table during one, two, and three years after planting at a field site where three poplar clones were tested on soils artificially contaminated with heavy metals, diesel fuel, and herbicides.

Data collection and analysis

Tree height (0.1 cm) and diameter (0.01 mm) were determined following first, second, and third year budset, with 10 trees per clone × treatment interaction per block (*i.e.*, 30 trees were measured for each subplot). Height was measured from the point of attachment of the primary stem to the tip of the terminal bud, and diameter was measured at 1.37 m aboveground [*i.e.*, at diameter at breast height (DBH)]. Third-year diameter data were used to estimate aboveground dry biomass according to the following genomic-group specific allometric equations reported in Zalesny *et al.* (2015):

$$\text{Biomass}_{\text{'Bora', 'PE 19/66'}} = 10^{-0.65} \times \text{DBH}^{2.01} \tag{1}$$

$$\text{Biomass}_{\text{'Pannonia'}} = 10^{-1.02} \times \text{DBH}^{2.36} \tag{2}$$

During August of the first two growing seasons, physiological parameters were measured on fully developed leaves at the seventh Leaf Plastochron Index (*i.e.*, LPI = 7) (Larson and Isebrands 1971) from five trees per clone × treatment interaction per block (*i.e.*, 15 trees were sampled for each subplot). Gas exchange measurements were conducted using an ADC LCPro + Portable Photosynthesis System (ADC Bioscientific, Ltd., Hoddesdon, UK) under controlled, constant light conditions of 1000 μmol m^{-2} s^{-1}, constant ambient air supply of 100 μmol s^{-1}, and ambient levels of air humidity and temperature. Direct gas exchange parameters included photosynthetic rate (A) (μmol m^{-2} s^{-1} CO_2), transpiration rate (E) (mmol m^{-2} s^{-2} H_2O), substomatal CO_2 concentration (C_i) (vpm), and stomatal conductance (g_s) (mol m^{-2} s^{-1}). Instantaneous WUE (μmol O_2 mmol H_2O^{-1}) was computed as the ratio of A/E (Farquhar *et al.* 1989). In addition to these gas exchange parameters, in the first growing season, the leaves were dark-adapted for 30 min and chlorophyll A fluorescence was measured on the middle section of leaf blades using an OS1-FL portable fluorometer (Opti Sciences OS1-FL, Hudson, NH) (Öquist and Wass 1988). During measurement, the maximum quantum efficiency of PSII photochemistry or maximum quantum yield of PSII (F_v/F_m) was recorded.

All growth and physiological 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 the aforementioned split-plot design including the main effects of block (random), clone (fixed whole-plots), and soil treatment (fixed subplots), and their interactions. Block × treatment interactions with $p > 0.25$ were pooled with the error term, which was then used to test treatment main effects. The following two linear additive models were used:

$$Y_{ijk} = \mu + B_i + C_j + BC_{ij} + T_k + BT_{ik} + CT_{jk} + \text{Error (without pooling)}$$

$$Y_{ijk} = \mu + B_i + C_j + BC_{ij} + T_k + CT_{jk} + \text{Pooled Error (with pooling)}$$

where Y_{ijk} = response variable to be analyzed, μ = overall mean, B_i = main effect of ith block, C_j = main effect of jth

Table 3. Probability values from analyses of variance comparing growth and physiological responses of three poplar clones (C = clone) grown on non-contaminated, alluvial control soils and those artificially contaminated with six soil treatments (T = treatment), including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}.

		Source of variation		
Age	Parameter	C	T	C × T
Growth				
Year 1	Diameter	<0.0001	<0.0001	**<0.0001**
	Height	<0.0001	<0.0001	**<0.0001**
Year 2	Diameter	0.0002	<0.0001	**0.0031**
	Height	0.2821	0.0877	0.1723
Year 3	Diameter	**0.0010**	**0.0004**	0.4311
	Height	0.0004	<0.0001	**0.0043**
	Biomass	**0.0007**	**0.0039**	0.6387
Physiology				
Year 1	Net photosynthesis (*A*)	0.0674	<0.0001	**<0.0001**
	Substomatal CO_2 concentration (C_i)	<0.0001	<0.0001	**<0.0001**
	Chlorophyll fluorescence (F_V/F_M)	0.0004	0.2021	**0.0083**
	Transpiration rate (*E*)	0.0014	<0.0001	**0.0001**
	Stomatal conductance (g_s)	0.0494	<0.0001	**<0.0001**
	Water use efficiency (*WUE*)	0.0228	<0.0001	**<0.0001**
Year 2	Net photosynthesis (*A*)	0.0012	0.0031	**<0.0001**
	Substomatal CO_2 concentration (C_i)	<0.0001	<0.0001	**<0.0001**
	Transpiration rate (*E*)	0.0016	<0.0001	**<0.0001**
	Stomatal conductance (g_s)	<0.0001	<0.0001	**<0.0001**
	Water use efficiency (*WUE*)	<0.0001	<0.0001	**<0.0001**

Significant values listed in bold were compared in the results and illustrated in Tables 4–6 and Figures 2–3.

clone, BC_{ij} = effect of interaction between ith block and *j*th clone, T_k = main effect of *k*th treatment, BT_{ik} = effect of interaction between *i*th block and *k*th treatment, CT_{jk} = effect of interaction between *j*th clone and *k*th treatment, and pooled error = error term resulting from pooling of BT_{ik} and BCT_{ijk} terms, defined as: effect of interaction between *i*th block and *k*th treatment, and effect of interaction among *i*th block, *j*th clone, and *k*th treatment, respectively.

Fisher's protected least significant difference (LSD) was used to separate means of main effects at a probability level of $p < 0.05$.

Results

Growth

The clone × treatment interaction was significant for diameter during the first ($p < 0.0001$) and second ($p = 0.0031$) growing seasons (Table 3). Diameter of one-year-old trees ranged from 0.77 ± 0.09 ('Pannonia' control) to 3.25 ± 0.13 cm ('PE 19/66' Pendimethalin), with an overall mean of 1.85 ± 0.09 cm (Table 4). All three clones differed from one another, with 'PE 19/66' exhibiting the largest diameter that was 19% and 43% greater than 'Bora' and 'Pannonia', respectively. Also, 'Pannonia' and 'PE 19/66' differed from the overall mean. In general, trees subjected to organic contaminants exhibited 44% greater first-year diameter than heavy metals, and Cu, Ni, Oxyfluorfen, and Pendimethalin treatments differed from the overall mean. Within specific clone × treatment interactions, organic contaminants had 36%, 41%, and 49% larger diameter than their heavy metal counterparts for 'Bora', 'Pannonia', and 'PE 19/66', respectively (Table 4). Similar results were shown for year 2, wherein diameter ranged from 1.2 ± 0.1 ('Pannonia' control) to 4.9 ± 0.2 cm ('PE 19/66' Oxyfluorfen), with an overall mean of 3.1 ± 0.1 cm (Table 4). Once again, 'PE 19/66' was significantly greater than the other two genotypes, exhibiting 13% and 46% larger diameter than 'Bora' and 'Pannonia', respectively. Trees of the organic contaminant treatments had 29% greater diameter than trees grown on heavy metal-contaminated soils, and specific diameter-advantages for each clone equaled 26%, 33%, and 33% for 'Bora', 'Pannonia', and 'PE 19/66', respectively (Table 4). Moreover, the clone × treatment interaction became negligible during year 3 ($p = 0.4311$), at which time clone ($p = 0.0010$) and treatment ($p = 0.0004$) main effects governed this trait (Table 3). In particular, 'PE 19/66' exhibited significantly greater diameter than 'Bora' (19%) and 'Pannonia' (56%). Treatment differences were less pronounced than those for clones, with trees subjected to heavy metals exhibiting 21% smaller diameter than that for organics, yet none of the treatments differed from the overall mean (Figure 2).

Clone × treatment interactions were significant for height at one ($p < 0.0001$) and three ($p = 0.0043$) years after planting yet negligible during year 2 ($p = 0.1723$) (Table 3). After the first growing season, height ranged from 1.1 ± 0.0 ('Pannonia' control) to 2.7 ± 0.1 m ('PE 19/66' Pendimethalin), with an overall mean of 1.9 ± 0.1 m (Table 4). Similar to diameter, all three clones differed from each other for height, with 'PE 19/66' exhibiting the tallest trees that were 5% and 24% greater than those for 'Bora' and 'Pannonia', respectively. In general, trees of the organic contaminant treatments exhibited 35% greater first-year height than heavy metals, and all contaminant treatments differed from the overall mean. Clonal responses to specific

Table 4. Mean value (± standard error) for diameter (cm) and height (m) at one, two, or three years after planting for three poplar clones [*Populus deltoides* Bartr. ex Marsh 'Bora', 'PE 19/66'; *P.* × *euramericana* 'Pannonia' (Dode) Guinier] grown on non-contaminated, alluvial control soils and those artificially contaminated with six soil treatments, including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}.

Soil treatment	Diameter$_{year1}$	Diameter$_{year2}$	Height$_{year1}$	Height$_{year3}$
'Bora'				
Cadmium (Cd)	1.43 ± 0.07 gh*	3.2 ± 0.3 defg	1.6 ± 0.1 e*	8.1 ± 0.1 bcde
Copper (Cu)	1.42 ± 0.08 gh*	2.6 ± 0.2 ghi	1.6 ± 0.1 e*	7.2 ± 0.7 e
Nickel (Ni)	1.59 ± 0.05 fg	2.9 ± 0.1 efg	1.8 ± 0.0 e	7.7 ± 0.6 de
Diesel (DIE)	2.14 ± 0.08 de	3.9 ± 0.3 cd*	2.3 ± 0.1 bcd*	9.1 ± 0.3 bc
Oxyfluorfen (OXY)	2.29 ± 0.12 cd*	3.9 ± 0.2 cd*	2.3 ± 0.0 bcd*	9.0 ± 0.5 bc
Pendimethalin (PEN)	2.51 ± 0.08 bc*	3.9 ± 0.1 cd*	2.4 ± 0.1 bc*	9.0 ± 0.1 bc
Control (CON)	1.84 ± 0.10 ef	3.5 ± 0.2 de	1.8 ± 0.1 e	9.1 ± 0.3 bc
'Pannonia'				
Cadmium (Cd)	1.08 ± 0.09 ij*	1.6 ± 0.1 jk*	1.3 ± 0.0 f*	4.7 ± 0.2 g*
Copper (Cu)	1.00 ± 0.06 ij*	1.7 ± 0.0 jk*	1.2 ± 0.1 f*	5.8 ± 0.1 fg*
Nickel (Ni)	1.04 ± 0.08 ij*	2.0 ± 0.2 ij*	1.3 ± 0.1 f*	6.0 ± 0.6 f*
Diesel (DIE)	1.57 ± 0.09 fg	2.5 ± 0.1 ghi	1.8 ± 0.1 e	7.7 ± 0.5 de
Oxyfluorfen (OXY)	1.82 ± 0.10 ef	2.8 ± 0.2 fgh	2.1 ± 0.1 d	8.6 ± 0.0 bcd
Pendimethalin (PEN)	2.10 ± 0.10 de	2.7 ± 0.1 gh	2.2 ± 0.0 bcd*	7.8 ± 0.6 de
Control (CON)	0.77 ± 0.09 j*	1.2 ± 0.1 k*	1.1 ± 0.0 f*	5.8 ± 0.2 fg*
'PE 19/66'				
Cadmium (Cd)	1.72 ± 0.19 fg	3.5 ± 0.5 de	1.7 ± 0.2 e	9.3 ± 0.5 b
Copper (Cu)	1.23 ± 0.06 hi*	2.5 ± 0.1 ghi	1.3 ± 0.1 f*	8.1 ± 0.4 bcde
Nickel (Ni)	1.57 ± 0.03 fg	3.4 ± 0.1 def	1.7 ± 0.0 e	9.3 ± 0.4 b
Diesel (DIE)	2.51 ± 0.22 bc*	4.3 ± 0.3 abc*	2.5 ± 0.0 ab*	9.6 ± 0.5 b*
Oxyfluorfen (OXY)	3.22 ± 0.06 a*	4.9 ± 0.2 a*	2.7 ± 0.1 a*	11.1 ± 0.2 a*
Pendimethalin (PEN)	3.25 ± 0.13 a*	4.7 ± 0.2 ab*	2.7 ± 0.1 a*	11.0 ± 0.2 a*
Control (CON)	2.77 ± 0.17 b*	4.2 ± 0.2 bc*	2.5 ± 0.1 ab*	9.7 ± 0.1 b*
Overall mean	1.85 ± 0.09	3.1 ± 0.1	1.9 ± 0.1	8.3 ± 0.2

Means with different letters within a column for each parameter were different at $p < 0.05$, and those indicated with an asterisk (*) were different than the overall mean.

treatments also showed similar trends as diameter, with organic contaminants producing trees that were 26%, 40%, and 38% taller than those grown in heavy metal-amended soils for 'Bora', 'Pannonia', and 'PE 19/66', respectively (Table 4). In the third growing season, height ranged from 4.7 ± 0.2 ('Pannonia' Cd) to 11.1 ± 0.2 m ('PE 19/66' Oxyfluorfen), with an overall mean of 8.3 ± 0.2 m (Table 4). The ranking of individual clones remained consistent with first-year results, though the magnitude of differences increased, with 'PE 19/66' exhibiting 13% and 32% taller trees than those for 'Bora' and 'Pannonia', respectively. Responses of genotypes to individual treatments varied by genotype, with 'Bora' and 'Pannonia' corroborating growth advantages of the organic contaminant group relative to the heavy metals (Table 4). Specifically, height was 14% and 31% taller for organics versus heavy metals of 'Bora' and 'Pannonia', respectively. In contrast, the site management benefits of diesel application (*e.g.*, weed control) decreased over time, with height of trees grown on diesel-contaminated soils similar to the heavy metals, which were 19% shorter than for herbicide treatments.

The clone × treatment interaction was negligible for third-year biomass ($p = 0.6387$), yet the clone ($p = 0.0007$) and treatment ($p = 0.0039$) main effects were both significant for this trait (Table 3). 'PE 19/66' had the greatest biomass that was 33% and 87% heavier than that for 'Bora' and 'Pannonia', respectively. Both 'PE 19/66' and 'Pannonia' exhibited biomass that was different from the overall mean. Treatment differences corroborated the general trends shown for diameter and height (Figure 2). Specifically, trees of the organic contaminant group had 35% greater biomass than that of the heavy metal group, although not all within-treatment comparisons were significant.

Physiology

The clone × treatment interaction governed all physiological traits after the first and second growing seasons (Table 3). During the first growing period, net photosynthesis (A) ranged from 5.10 ± 1.40 ('PE 19/66' Diesel) to 15.63 ± 0.99 μmol CO$_2$ m^{-2} s^{-1} ('Pannonia' Ni), with an overall mean of 11.76 ± 0.34 μmol CO$_2$ m^{-2} s^{-1} ($p < 0.0001$) (Table 5). 'Pannonia' had 10% and 13% greater A than 'Bora' and 'PE 19/66', respectively, despite that none of the genotypes differed from the overall mean. Across all clone × treatment combinations, trees growing on soils amended with heavy metals were not different than the control but had 1.5 times higher A than those with diesel and herbicide treatments. In particular, A of trees grown in heavy metal plots was 32% and 55% higher than those with organic amendments for 'Pannonia' and 'PE 19/66', respectively (Table 5). The magnitude of results was similar for the second growing season, where A ranged from 8.26 ± 0.40 ('PE 19/66' Cd) to 16.47 ± 0.88 μmol CO$_2$ m^{-2} s^{-1} ('Pannonia' Cd), with an overall mean of 11.93 ± 0.25 μmol CO$_2$ m^{-2} s^{-1} ($p < 0.0001$) (Table 6). 'Bora' had 15% and 10% greater A than 'Pannonia' and 'PE 19/66', respectively, with the latter being the only genotype that did not differ from the overall mean. In contrast to the first year, there was no difference between heavy metals and organics, except that A was 15% less for trees on Cu soils relative to the control. The interaction between 'Bora' and Pendimethalin was

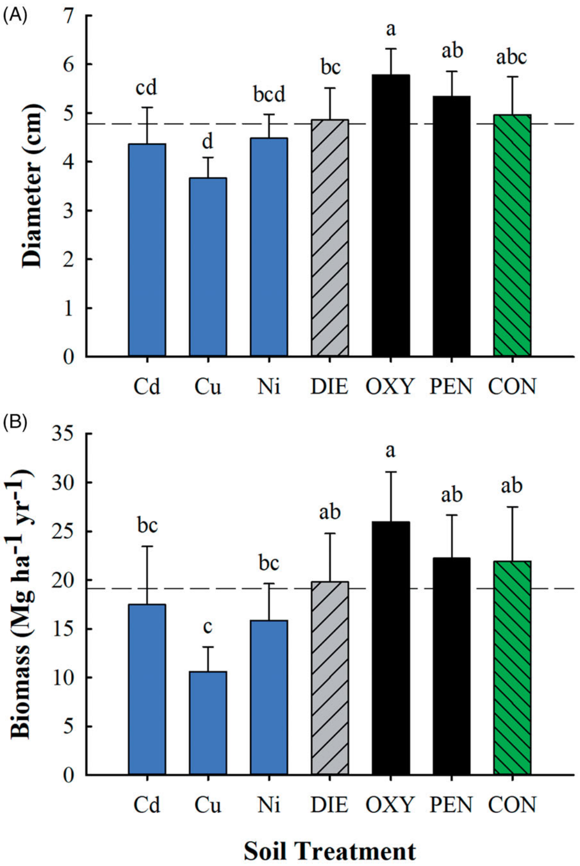

Figure 2. Diameter and aboveground biomass for the soil treatment main effect of three-year-old poplar trees grown on non-contaminated, alluvial control soils (CON) and those artificially contaminated with six soil treatments, including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1} (DIE), (5) 236 g Oxyfluorfen ha^{-1} (OXY), and (6) 1,320 g Pendimethalin ha^{-1} (PEN). Values are averages across three poplar clones [*Populus deltoides* Bartr. ex Marsh 'Bora', 'PE 19/66'; *P.* × *euramericana* 'Pannonia' (Dode) Guinier]. The dashed line represents the overall mean, while bars with the same letters were not different according to Fisher's protected LSD at $p < 0.05$.

the only combination that was significantly higher than the overall mean for the clone, despite only having significantly greater *A* than Cd and Oxyfluorfen treatments (Table 6). While *A* was consistent for 'Pannonia' subjected to diesel and herbicides (which did not differ from the control), responses of the genotype to heavy metals were highly variable and all different from one another. The Cd treatment produced the greatest *A*, which was 45% and 29% greater than Cu and Ni soils, respectively. Similar trends within contaminant groups were not apparent for 'PE 19/66', which exhibited contaminant-specific responses (Table 6).

Clone × treatment interactions were significant for substomatal CO_2 concentration (C_i) during both years ($p < 0.0001$) (Table 3). For year 1, C_i ranged from 201 ± 6 ('Pannonia' Oxyfluorfen) to 270 ± 12 vpm ('PE 19/66' diesel), with an overall mean of 227 ± 2 vpm (Table 5). All three clones differed from the overall mean and each other, with 'PE 19/66' exhibiting the highest C_i that was 9%, 12%, and 16% greater than the overall mean, 'Bora', and 'Pannonia', respectively. Trees of the diesel and control soils had the highest and lowest C_i, respectively, and they were the only treatments to differ from the overall mean. Overall, clone × treatment interactions produced relatively stable C_i values, with one exception. Soils contaminated with diesel produced the highest C_i that was 1.1 to 1.2 times significantly greater than all other treatments for 'Bora' (Table 5). The overall mean for year 2 (203 ± 4 vpm) was 11% less than year 1 for C_i (Table 6), with values ranging from 145 ± 7 ('Bora' Oxyfluorfen) to 278 ± 7 vpm ('PE 19/66' diesel). All three clones differed from one another, while 'Bora' and 'PE 19/66' were the only clones differing from the overall mean. Clone 'PE 19/66' had the greatest C_i, which was 24% and 8% higher than 'Bora' and 'Pannonia', respectively. Despite that trees of the diesel-contaminated soils had 6 to 19% higher C_i than all other treatments, trends within contaminant groups were not evident. However, the responses of 'Pannonia' and 'PE 19/66' across groups were significant yet opposite. For 'Pannonia', the heavy metals produced 19% higher C_i than soils amended with organics, while for 'PE 19/66' diesel- and herbicide-contaminated soils exhibited 27% higher C_i than Cd-, Cu-, and Ni-contaminated soils (Table 6).

Chlorophyll fluorescence (F_v/F_m) was measured during the first growing season, and the clone × treatment interaction was significant for the one-year-old trees ($p = 0.0083$) (Table 3). During that time, F_v/F_m ranged from 0.653 ± 0.032 ('PE 19/66' Pendimethalin) to 0.757 ± 0.008 ('Pannonia' Cd), with an overall mean of 0.704 ± 0.005 (Figure 3). All three clones differed from each other, with 'Pannonia' exhibiting the highest F_v/F_m that was 7% and 10% greater than 'Bora' and 'PE 19/66', respectively. The latter genotypes differed from the overall mean, while 'Pannonia' exhibited similar F_v/F_m. None of the treatments differed from the overall mean, and clone × treatment interactions exhibited minimal changes in both rank and magnitude. In general, with the exception of Cu, 'Pannonia' had the highest F_v/F_m values for all treatments, though most interactions were not significantly different from one another (Figure 3). Though it ranked third for all other treatments, 'PE 19/66' had the highest F_v/F_m for Cu-contaminated soils, that being 9% and 7% greater than 'Bora' and 'Pannonia', respectively.

During the first two growing seasons, clones differed in their responses to soil treatments for transpiration rate (*E*) ($p < 0.0001$) (Table 3). In year 1, *E* ranged from 2.93 ± 0.07 ('PE 19/66' Ni) to 4.50 ± 0.15 mmol H_2O m^{-2} s^{-2} ('Bora' control), with an overall mean of 3.63 ± 0.06 mmol H_2O m^{-2} s^{-2} (Table 5). 'Bora' had 9% and 10% greater E than 'Pannonia' and 'PE 19/66', respectively, and was the only genotype to significantly differ from the overall mean (*i.e.*, being 6% greater). Both inorganic and organic soil treatments reduced *E* across clones, with control treatment trees having 27% and 14% higher *E* than both contaminant groups, respectively. More specifically, *E* for Cd, Cu, and Ni treatments was significantly less than the overall mean, while that of diesel, Oxyfluorfen, and Pendimethalin did not differ from the mean. The variation in clonal responses to soil treatments was similar to the other physiological parameters, with contaminant groups differing within clones, performing similarly within clones, or failing to exhibit discernible trends. For example, 'Bora' grown in the

Table 5. Mean value (± standard error) for net photosynthesis (A; μmol CO_2 m^{-2} s^{-1}), substomatal CO_2 concentration (C_i; vpm), transpiration rate (E; mmol H_2O m^{-2} s^{-2}), and stomatal conductance (g_s; mol m^{-2} s^{-1}) after the **first growing season** for three poplar clones [*Populus deltoides* Bartr. ex Marsh 'Bora', 'PE 19/66'; *P.* × *euramericana* 'Pannonia' (Dode) Guinier] grown on non-contaminated, alluvial control soils and those artificially contaminated with six soil treatments, including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}.

Soil treatment	A	C_i	E	g_s
'Bora'				
Cadmium (Cd)	12.26 ± 0.65 bcdef	216 ± 5 ef	3.33 ± 0.06 cde	0.20 ± 0.01 efg
Copper (Cu)	11.53 ± 0.41 defgh	211 ± 3 ef	3.20 ± 0.10 cde	0.17 ± 0.01 gh
Nickel (Ni)	11.53 ± 0.43 defgh	210 ± 6 ef	3.24 ± 0.08 cde	0.17 ± 0.01 gh
Diesel (DIE)	9.61 ± 1.19 fgh	254 ± 10 ab*	4.37 ± 0.17 a*	0.30 ± 0.02 abc*
Oxyfluorfen (OXY)	10.28 ± 1.04 fgh	221 ± 5 de	4.08 ± 0.21 ab	0.21 ± 0.02 efg
Pendimethalin (PEN)	11.64 ± 0.66 defg	219 ± 4 de	4.41 ± 0.16 a*	0.25 ± 0.01 cde
Control (CON)	13.65 ± 0.65 abcde	209 ± 4 ef	4.50 ± 0.15 a*	0.27 ± 0.02 bcd
'Pannonia'				
Cadmium (Cd)	14.57 ± 0.94 abc	217 ± 5 ef	3.54 ± 0.18 c	0.25 ± 0.02 cde
Copper (Cu)	14.16 ± 1.60 abcd	235 ± 4 cd	2.96 ± 0.14 de*	0.27 ± 0.04 bcd
Nickel (Ni)	15.63 ± 0.99 a*	220 ± 5 de	3.53 ± 0.19 c	0.28 ± 0.03 abcd
Diesel (DIE)	11.32 ± 0.86 efgh	211 ± 7 ef	3.65 ± 0.16 bc	0.18 ± 0.01 fgh
Oxyfluorfen (OXY)	9.25 ± 0.84 gh	201 ± 6 f*	3.11 ± 0.24 cde	0.14 ± 0.02 h*
Pendimethalin (PEN)	9.85 ± 0.88 fgh	216 ± 7 ef	3.54 ± 0.15 c	0.17 ± 0.01 gh*
Control (CON)	14.45 ± 0.81 abc	179 ± 3 g*	4.35 ± 0.15 a*	0.21 ± 0.02 efg
'PE 19/66'				
Cadmium (Cd)	14.92 ± 2.12 ab*	256 ± 4 ab*	2.98 ± 0.26 de*	0.31 ± 0.03 ab*
Copper (Cu)	14.86 ± 1.55 ab	251 ± 6 bc*	3.17 ± 0.23 cde	0.33 ± 0.03 a*
Nickel (Ni)	15.29 ± 0.54 a*	249 ± 4 bc*	2.93 ± 0.07 e*	0.30 ± 0.01 abc*
Diesel (DIE)	5.10 ± 1.40 j*	270 ± 12 a*	3.31 ± 0.27 cde	0.16 ± 0.02 gh*
Oxyfluorfen (OXY)	8.86 ± 0.95 hi	245 ± 7 bc	4.21 ± 0.22 a*	0.23 ± 0.02 def
Pendimethalin (PEN)	6.26 ± 0.52 ij*	253 ± 2 ab*	3.48 ± 0.18 cd	0.17 ± 0.01 gh
Control (CON)	12.02 ± 0.47 cdef	222 ± 5 de	4.34 ± 0.13 a*	0.25 ± 0.02 cde
Overall mean	11.76 ± 0.34	227 ± 2	3.63 ± 0.06	0.23 ± 0.01

Means with different letters within a column for each parameter were different at $p < 0.05$, and those indicated with an asterisk (*) were different than the overall mean.

treatments with organic contaminants had 24% greater E than inorganic amendments, there was a general lack of soil treatment differences for 'Pannonia' (despite the control exhibiting significantly higher E than any of the additions), and E for 'PE 19/66' was not linked to combined, contaminant-group responses. The magnitude of E decreased during the second growing season, where E values ranged 2.10 ± 0.09 ('PE 19/66' diesel) to 3.92 ± 0.27 mmol H_2O m^{-2} s^{-2} ('Pannonia' control), with an overall mean of 2.95 ± 0.06 mmol H_2O m^{-2} s^{-2} ($p < 0.0001$) (Table 6). Clones 'Pannonia' and 'PE 19/66' did not differ from one another but had 10% and 7% higher E than 'Bora', respectively. In contrast to the first growing season, none of the clones or treatments differed from the overall mean during year 2. While treatment responses were relatively consistent for 'Bora', trends in E for 'Pannonia' and 'PE 19/66' across groups were significant yet opposite. For 'Pannonia', the diesel- and herbicide-contaminated soils exhibited 22% higher E than Cd-, Cu-, and Ni-contaminated soils, while for 'PE 19/66' heavy metals produced 37% higher E than soils amended with organics (Table 6).

Clone × treatment interactions were significant for stomatal conductance (g_s) during both years ($p < 0.0001$) (Table 3). For year 1, g_s ranged from 0.14 ± 0.02 ('Pannonia' Oxyfluorfen) to 0.33 ± 0.03 mol m^{-2} s^{-1} ('PE 19/66' Cu), with an overall mean of 0.23 ± 0.01 mol m^{-2} s^{-1} (Table 5). 'PE 19/66' had 12% and 15% greater g_s than 'Bora' and 'Pannonia', respectively, and was the only genotype to significantly differ from the overall mean (*i.e.*, being 8% greater). In general, trees subjected to heavy metals exhibited 21% greater g_s than the organic contaminant group, yet none of the individual treatments differed from the overall mean. Contaminant groups segregated within clones such that for 'Bora' organic contaminants had 28% greater g_s than for heavy metals, while g_s for 'Pannonia' and 'PE 19/66' was 41% and 39% greater for heavy metals relative to organics, respectively (Table 5). For year 2, g_s ranged from 0.15 ± 0.02 ('Pannonia' Oxyfluorfen) to 0.83 ± 0.02 mol m^{-2} s^{-1} ('Pannonia' Cd), with an overall mean of 0.31 ± 0.02 mol m^{-2} s^{-1} (Table 6). All three clones differed from one another, while 'Bora' and 'PE 19/66' were the only clones differing from the overall mean. Clone 'PE 19/66' had the greatest g_s, which was 47% and 19% better than 'Bora' and 'Pannonia', respectively. In general, treatments did not segregate according to contaminant group. The Cd and Pendimethalin treatments, which were similar to one another, had trees exhibiting the highest g_s that was 22% (Ni) to 50% (Cu) greater than all other treatments. The response of 'Bora' was consistent across soil treatments, with g_s being marginally (yet not significantly) less for Oxyfluorfen than the other treatments. The g_s trend was similar for 'Pannonia', with two exceptions: 1) trees grown on Cd-contaminated soils had 80% higher g_s than the control treatment and 2) those subjected to Ni had a 61% increase relative to the control. For 'Pannonia', the diesel- and herbicide-contaminated soils exhibited 66% higher g_s than Cd-, Cu-, and Ni-contaminated soils (Table 6).

During the first two growing seasons, clones differed in their responses to soil treatments for WUE ($p < 0.0001$) (Table 3). In the first growing period, WUE ranged from 1.454 ± 0.318 ('PE 19/66' diesel) to 5.312 ± 0.948 μmol CO_2

Table 6. Mean value (± standard error) for net photosynthesis (*A*; μmol CO_2 m^{-2} s^{-1}), substomatal CO_2 concentration (C_i; vpm), transpiration rate (*E*; mmol H_2O m^{-2} s^{-2}), and stomatal conductance (g_s; mol m^{-2} s^{-1}) after the **second growing season** for three poplar clones [*Populus deltoides* Bartr. ex Marsh 'Bora', 'PE 19/66'; *P.* × *euramericana* 'Pannonia' (Dode) Guinier] grown on non-contaminated, alluvial control soils and those artificially contaminated with six soil treatments, including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1}, (5) 236 g Oxyfluorfen ha^{-1}, and (6) 1,320 g Pendimethalin ha^{-1}.

Soil treatment	*A*	C_i	*E*	g_s
'Bora'				
Cadmium (Cd)	12.69 ± 1.21 cde	176 ± 14 ghi*	2.38 ± 0.10 ij*	0.20 ± 0.02 def*
Copper (Cu)	12.96 ± 0.87 bcde	175 ± 10 ghi*	2.76 ± 0.11 fgh	0.22 ± 0.01 def
Nickel (Ni)	12.72 ± 0.67 bcde	178 ± 5 gh*	2.57 ± 0.11 ghi	0.21 ± 0.01 def
Diesel (DIE)	12.99 ± 0.47 bcde	186 ± 6 fg	2.85 ± 0.10 efg	0.26 ± 0.01 de
Oxyfluorfen (OXY)	11.90 ± 0.60 def	145 ± 7 j*	3.07 ± 0.05 def	0.15 ± 0.01 f*
Pendimethalin (PEN)	14.78 ± 0.49 ab*	195 ± 19 fg	2.79 ± 0.12 efgh	0.26 ± 0.04 de
Control (CON)	13.07 ± 0.63 bcd	149 ± 3 j*	3.26 ± 0.10 cd	0.19 ± 0.01 def*
'Pannonia'				
Cadmium (Cd)	16.47 ± 0.88 a*	257 ± 7 abc*	2.67 ± 0.04 ghi	0.83 ± 0.02 a*
Copper (Cu)	9.09 ± 0.50 hi*	219 ± 7 de	2.71 ± 0.22 fghi	0.21 ± 0.02 def
Nickel (Ni)	11.63 ± 1.12 defg	248 ± 7 bc*	2.35 ± 0.06 ij*	0.44 ± 0.08 c*
Diesel (DIE)	9.67 ± 1.04 ghi*	208 ± 8 ef	3.05 ± 0.19 def	0.22 ± 0.03 def
Oxyfluorfen (OXY)	9.26 ± 0.84 hi*	182 ± 5 g	3.13 ± 0.14 cde	0.15 ± 0.02 f*
Pendimethalin (PEN)	10.30 ± 0.61 fghi	194 ± 6 fg	3.71 ± 0.12 ab*	0.22 ± 0.01 def
Control (CON)	10.98 ± 0.46 efgh	159 ± 4 ij*	3.92 ± 0.27 a*	0.17 ± 0.01 ef*
'PE 19/66'				
Cadmium (Cd)	8.26 ± 0.40 i*	194 ± 5 fg	3.81 ± 0.14 ab*	0.16 ± 0.00 ef*
Copper (Cu)	9.16 ± 0.60 hi*	184 ± 6 g	3.91 ± 0.15 a*	0.16 ± 0.01 ef*
Nickel (Ni)	12.42 ± 0.31 de	192 ± 4 fg	3.46 ± 0.13 abc*	0.28 ± 0.01 d
Diesel (DIE)	11.46 ± 0.95 defg	278 ± 7 a*	2.10 ± 0.09 j*	0.41 ± 0.06 c
Oxyfluorfen (OXY)	14.74 ± 0.47 abc*	261 ± 7 ab*	2.55 ± 0.06 ghi*	0.62 ± 0.08 b*
Pendimethalin (PEN)	13.39 ± 0.47 bcd	245 ± 7 bc*	2.44 ± 0.06 hij*	0.70 ± 0.08 b*
Control (CON)	12.59 ± 0.31 de	235 ± 5 cd*	2.43 ± 0.10 hij*	0.45 ± 0.03 c*
Overall mean	11.93 ± 0.25	203 ± 4	2.95 ± 0.06	0.31 ± 0.02

Means with different letters within a column for each parameter were different at $p < 0.05$, and those indicated with an asterisk (*) were different than the overall mean.

mmol H_2O^{-1} ('PE 19/66' Cd), with an overall mean of 3.332 ± 0.118 μmol CO_2 mmol H_2O^{-1} (Figure 3). 'Pannonia' had 16% and 8% greater *WUE* than 'Bora' and 'PE 19/66', respectively. None of the genotypes were significantly different from the overall mean. In contrast, with the exception of the control, all contamination treatments differed from the overall mean. Specifically, trees of the heavy metal treatments had 24% greater *WUE* while those of the organic treatments had 28% less *WUE*, and the control was intermediate between both of these contaminant groups. Clone × treatment interactions for 'Bora', 'Pannonia', and 'PE 19/66' resulted in Cd-, Cu-, and Ni-contaminated soil treatments having 32%, 33%, and 65% greater *WUE* than diesel, Oxyfluorfen, and Pendimethalin treatments, respectively (Figure 3). In the second growing period, *WUE* ranged from 2.171 ± 0.087 ('PE 19/66' Cd) to 6.167 ± 0.314 μmol CO_2 mmol H_2O^{-1} ('Pannonia' Cd), with an overall mean of 4.245 ± 0.128 μmol CO_2 mmol H_2O^{-1} (Figure 3). Clonal ranks for the best and worst genotype shifted from year 1 (*i.e.*, 'Pannonia' > 'PE 19/66' > 'Bora'), with 'Bora' in year 2 having 20% and 9% greater *WUE* than 'Pannonia' and 'PE 19/66', respectively. In addition, *WUE* of 'Bora' and 'Pannonia' was 9% greater and 10% less than the overall mean, respectively. General trends for treatments were nonexistent, wherein no individual soil treatments were different than the overall mean. In contrast, clone × treatment interactions were both generalist and specialist in nature. For example, 'Bora' exhibited relatively stable *WUE* irrespective of soil treatments (*i.e.*, generalist performance), while heavy metal-contaminated soils had 51% lower *WUE* than those amended with diesel and herbicides (*i.e.*, specialist) for 'PE 19/66', and 'Pannonia' was intermediate. In general, 'Pannonia' performed as a generalist; however, trees of its Cd and Ni treatments were 39% and 24% greater than the clone mean, respectively (Figure 3).

Discussion

Given that poplars and their hybrids have proven effective for phytoremediation of a broad spectrum of contaminants, the objective of this study was to test the growth and physiological responses of three poplar clones [*P. deltoides* Bartr. ex Marsh. 'Bora', 'PE 19/66'; *P.* × *euramericana* (Dode) Guinier 'Pannonia'] grown for 3 years on field soils artificially contaminated with heavy metals, diesel fuel, and herbicides. Overall, significant clone × treatment interactions governed growth and physiology throughout the study, and the influence of inorganics versus organics varied with tree age. Specifically, heavy metals had a more substantial influence on growth and physiology as the trees matured, while diesel and herbicide treatments were most pronounced during the first growing season, with diminishing effects over time. Clones 'Bora' and 'PE 19/66' had 5.3 and 7.9 times greater biomass than 'Pannonia', respectively, across soil treatments. Despite non-significant genotype × treatment interactions at the end of the study, 'Bora' and 'PE 19/66' exhibited greater biomass than 'Pannonia', with trees growing in the control soils exhibiting 13.8 and 19.6 times greater biomass than 'Pannonia', respectively. Thus, based on three years of growth, 'Bora' and 'PE 19/66' have greater potential than 'Pannonia' for larger-scale systems, regardless of contaminant source. Although it was beyond the scope of

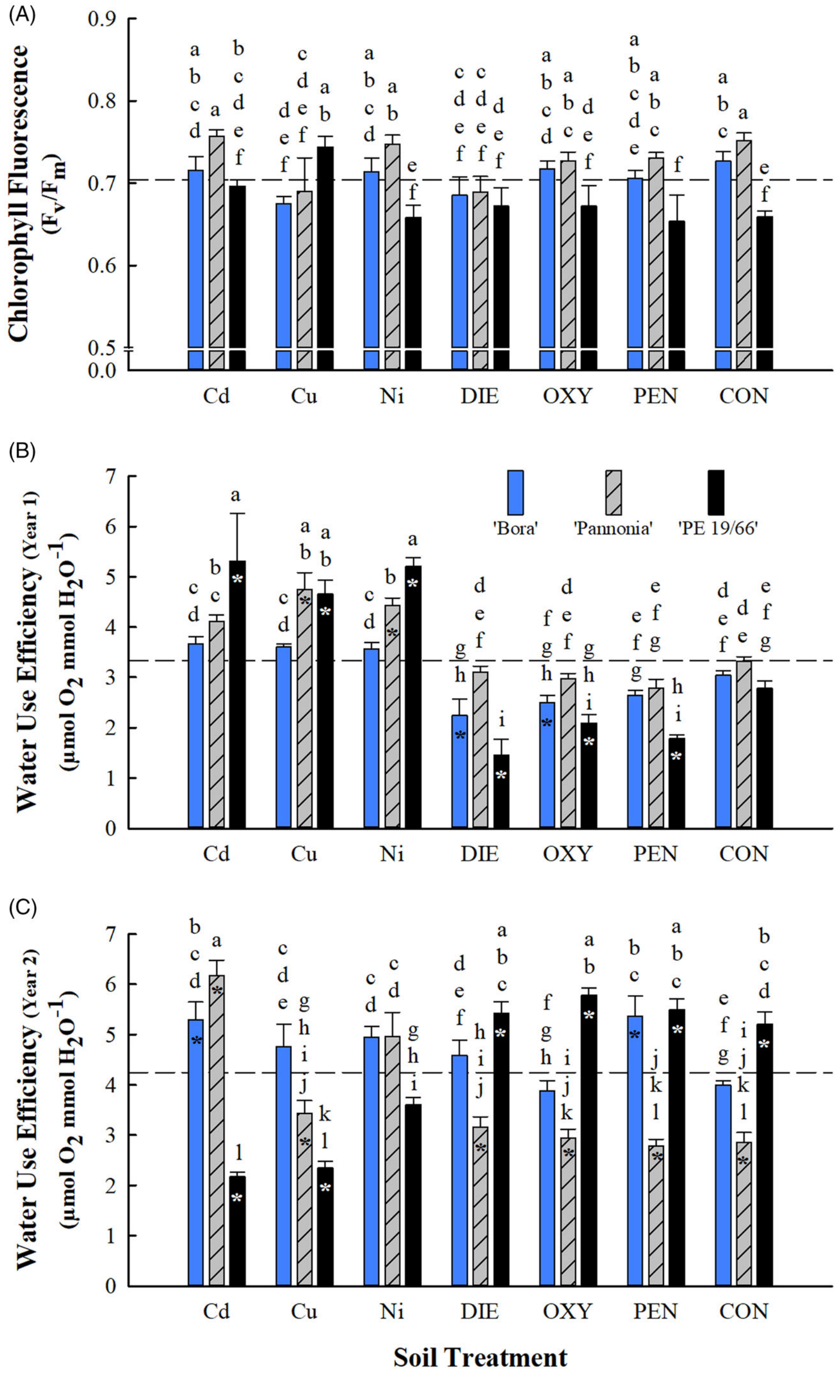

Figure 3. Chlorophyll fluorescence and water use efficiency of three poplar clones [*Populus deltoides* Bartr. ex Marsh 'Bora', 'PE 19/66'; *P.* × *euramericana* 'Pannonia' (Dode) Guinier] grown on non-contaminated, alluvial control soils (CON) and those artificially contaminated with six soil treatments, including: (1) 10.6 kg Cd ha^{-1}, (2) 247 kg Cu ha^{-1}, (3) 183.3 kg Ni ha^{-1}, (4) 6,667 L diesel fuel ha^{-1} (DIE), (5) 236 g Oxyfluorfen ha^{-1} (OXY), and (6) 1,320 g Pendimethalin ha^{-1} (PEN). The dashed line represents the overall mean, while bars with asterisks indicate means that differ from the overall mean at $p < 0.05$. Bars with the same letters were not different according to Fisher's protected LSD at $p < 0.05$.

this study, additional experimentation testing the additive growth and physiological responses to field sites with multiple contamination sources is warranted.

The current conclusions for growth and biomass corroborate results from field studies throughout the world (Dhillon *et al.* 2010; Sixto *et al.* 2011, 2016), wherein poplar growth and development are typically optimized by matching genotypes to environments where they are grown (Zalesny *et al.* 2009; Lazarus *et al.* 2015; Stanturf *et al.* 2017). Growth and biomass are among the most common parameters assessed for traditional uses such as energy production (Klašnja *et al.* 2008), and are equally relevant for environmental purposes (Felix et al. 2008; Fortier *et al.* 2010). In recent years, consistent with the increased use of phytotechnologies in both rural and urban areas, poplars have become instrumental for heavy metal phytoremediation, especially when additional ecosystem services (*e.g.*, carbon sequestration, water quality, and quantity) are desired outcomes of the remediation system (Burges *et al.* 2018). The relationship between growth/biomass production and genotypic variability among poplars for uptake of heavy metals has been reported for numerous genomic groups (Laureysens *et al.* 2004; Polle *et al.* 2013; Baldantoni *et al.* 2014), including *P. deltoides* and *P.* × *euramericana* that were tested in this study (Zalesny and Bauer 2007a; Zalesny *et al.* 2008; Pilipović *et al.* 2019). While uptake of Cd, Cu, and Ni was beyond the scope of our current objectives, growth and biomass differences among 'Bora', 'PE 19/66', and 'Pannonia' were indicative of variable levels of heavy metal tolerance. This agrees with the results of Laureysens *et al.* (2004) who reported a range of $\sim$14 Mg ha^{-1} y^{-1} for annual biomass production at 6 years after planting for 13 poplar clones belonging to five genomic groups. Despite different genetic backgrounds than those tested in this study, Baldantoni *et al.* (2014) reported that clone 'N12' (*Populus nigra*) had nearly 10 times greater Cd phytoextraction potential than 'AL22' (*Populus alba*), which was better for phytostabilization of Cu.

In addition to heavy metals, growth and biomass responses of poplars established in soils contaminated with petroleum hydrocarbons have been well documented (El-Gendy *et al.* 2009; Pilipović *et al.* 2012; Cook and Hesterberg 2013), including soil-plant interactions and their effects on groundwater (Landmeyer 2001; Ferro *et al.* 2013). In this study, both *P. deltoides* clones ('Bora'and 'PE 19/66') had greater diameter, height, and biomass than 'Pannonia', the *P.* × *euramericana* genotype, when grown in the diesel-contaminated soils. Similarly, Cook *et al.* (2010) and Nichols *et al.* (2014) described phytoremediation of petroleum hydrocarbons using four interspecific poplar clones (*Populus trichocarpa* Torr. & Gray × *P. deltoides* '15-29', '49-177'; *P.* × *euramericana* 'DN34', 'OP367') at a U.S. Coast Guard Base in Elizabeth City, North Carolina (Zalesny, Headlee, *et al.* 2019). While genotypic responses within groups were similar, the *P. trichocarpa* × *P. deltoides* hybrids were larger than the *P. deltoides* × *P. nigra* genotypes. Zalesny *et al.* (2005) reported even greater clonal variability for growth of 20 poplar clones tested in soils contaminated with petroleum hydrocarbons in Gary, Indiana. After the first growing season, height ranged from 14 ± 2 to 51 ± 15 cm across clones, and there was a 3.6-fold increase in height of the best-adapted backcross hybrid [(*P. deltoides* × *P. trichocarpa*) × *P. deltoides* 'NC13377'] relative to the least-adapted clone ('NC13570') within that genomic group. In addition, it is known that soil micro-organisms inhabiting the tree rhizosphere are responsible for remediation of petroleum-based contamination (Jordahl *et al.* 1997), and results of this study corroborated the need to learn more about interactions of favorable genotypes such as 'Bora' and 'PE 19/66' and their associated rhizospheric microorganisms. For example, poplar clone 'Walker' [*P. deltoides* × (*P. laurifolia* × *P. nigra*)] exhibited greater tolerance of diesel-contaminated soils following the incorporation of ectomychorrhizal colonization with the fungus *Pisolithus tinctorious* (Pers.) Coker and Couch (Gunderson *et al.* 2007). Thus, future testing of such clone × micro-organism interactions is warranted, especially as they relate to growth and biomass throughout plantation development.

While the benefits of herbicides as they relate to reducing weed and grass competition from herbaceous and woody crops are well known (Borders and Shiver 1989; Buhler *et al.* 1998), and vegetative buffer systems reducing herbicide runoff from agricultural fields are well-developed (Krutz *et al.* 2005; Lin *et al.* 2011), there are few reports on the impacts of chemicals such as Oxyfluorfen and Pendimethalin on non-target species (*i.e.*, the poplars in this study) during phytoremediation. As reported above, the magnitude of differences in growth and biomass of our trees subjected to herbicide treatments decreased with each growing season. This was expected as the function of these herbicides was to kill targeted species that competed with the poplars for water and nutrients. Nevertheless, there were distinct physiological responses of the poplars to the herbicides (see below), which were directly responsible for tree growth and development. Researchers have developed genetically modified poplars with specific herbicide tolerances (Donahue *et al.* 1994; Strauss *et al.* 1997; Meilan *et al.* 2002), but such genotypes were not tested in this study. Regardless of the types of genotypes used, future phytoremediation research is needed to elucidate how stability of herbicide resistance affects tree development over time (Li *et al.* 2008).

Furthermore, the interdependence and complexity of the investigated physiological processes and parameters were evident in this study. For example, correlations between photosynthesis and water availability are well known in plants, including trees such as poplars. In particular, disturbance of individual processes often reflects disruption of other physiological mechanisms (Bojović *et al.* 2017; Naidoo and Naidoo 2018). Alteration in such physiological processes and enzymatic activity of poplar upon exposure to pollutants often results in environmental inductive effects on phytoremediation that are tightly linked to gene regulation and expression (Yadav *et al.* 2010; Induri *et al.* 2012). Therefore, the effect of contaminants on complementary physiological processes is more appropriate for gauging phytoremediation success than investigating physiological parameters separately.

Heavy metal uptake and transport from roots to stems and leaves are governed by transpiration; therefore, plant tissue water status and soil moisture have crucial importance in photosynthesis and organic assimilation (Marchiol *et al.* 2004). The effect of heavy metals on transpiration differs based on the concentration and species of heavy metal, plant species/genotype, and their interactions, among other factors such as site-specific soil conditions (Tables 1 and 2) (Pietrini *et al.* 2005). In this study, with the exception of 'PE 19/66' in the second growing season, the presence of heavy metals significantly decreased transpiration. These results can be explained by the fact that heavy metals, especially Cd and Ni, affect internal water regimes in plants, thereby decreasing water potential and transpiration, which leads to diffuse resistance and water deficit (Pandey and Sharma 2002). In addition, the presence of heavy metals in a growing medium has significantly affected both root and leaf morphology and anatomical structure, which ultimately resulted in the disturbance of water regimes and assimilation of poplars (Nikolić 2009; Nikolić *et al.* 2017). Despite decreased productivity of poplars due to heavy metals, there was no decrease in net photosynthesis of the trees treated with Cd, Ni, and Cu. In contrast, decreases in biomass with parallel maintenance of high assimilation rates were attributed to intensive respiration and photorespiration (Nikolić 2009). While decreases in photosynthesis were negligible in this study, *WUE* of the tested clones generally increased with decreased transpiration, indicating that the trees economized resources by increasing *WUE* in the presence of heavy metals. Nevertheless, there were some inconsistencies with this overall trend for transpiration. In spite of decreases in stomatal conductance, assimilation, and *WUE*, transpiration of 'PE 19/66' increased during the second growing season. These results corroborated those of Boriŝev *et al.* (2012), who reported increases in transpiration and decreases in *WUE* of willows (*Salix* spp.) affected by Cd, Ni, and other heavy metals. These increases in transpiration are partially explained by the higher number of both adaxial and abaxial stomata on the leaf surface of 'PE 19/66', which was previously observed by Orlović *et al.* (1998). Furthermore, the concentration gradient between ambient air and mesophyll cells is more favorable for water versus carbon dioxide diffusion (Schulze *et al.* 2005).

Similar to other organic pollutants, as described above for growth and biomass, crude oil and its derivatives such as diesel fuel affect non-woody and woody plants in many different ways. For example, changes in soil properties have caused disturbance of internal water regimes in plants due to hydrophobicity of petroleum derivatives (Pilipović 2012; Han *et al.* 2016). The presence of petroleum in soils has significantly decreased photosynthesis and other physiological parameters in poplars (Pajević *et al.* 2009; Pilipović 2012; Pilipović *et al.* 2012), which corroborated our first-year results. The effect of these organic contaminants on photosynthetic processes have been the result of stomatal limitations caused by decreases in stomatal conductance or non-stomatal effects related to decreases in photosynthetic activities of mesophyll cells (Farquhar and Sharkey 1982). In this study, decreases in stomatal conductance that led to significant reductions of transpiration (as confirmed *via* decreased *WUE)* were only observed for 'PE 19/66'. On the other hand, 'Bora' exhibited significantly decreased *WUE*, which likely resulted from decreases in transpiration and photosynthesis caused by non-stomatal limitations (Naidoo *et al.* 2010). In particular, mangrove trees growing in petroleum-polluted soils had decreased photosynthesis and increased substomatal CO_2 concentration without changes in transpiration (Naidoo *et al.* 2010). In addition, significant decreases of both photosynthesis and transpiration of 'Pannonia' resulted in stable *WUE* in this study, which can be explained by this genotype's adaptation to stress conditions through decreased water uptake (Pajević *et al.* 2009). Furthermore, *WUE* and organic assimilation of the clones tested in this study were not impacted during the second growing season, which can be explained by penetration of the tree roots into deeper, non-contaminated soils or bioremediation of the diesel in the rhizosphere of the trees (due to its lower molecular mass and higher biodegradability by micro-organisms) (Maletić *et al.* 2011).

Although herbicides such as Oxyfluorfen and Pendimethalin have not shown impacts on juvenile growth and performance of some ornamental trees and shrubs (Derr and Salihu 1996; Woeste *et al.* 2005) nor 'Pannonia' grown for nursery production (Vasic *et al.* 2015), the herbicide treatments in this study decreased net photosynthesis in all three clones ('Bora', 'PE 19/66', and 'Pannonia'), with the greatest impact occurring during the first growing season. These early impacts were not unexpected, given that the primary action of more than half of currently-used herbicides is to block photosynthetic functions (Pfister and Antzen 1979). Depending on dose, herbicides can act on crucial physiological pathways or processes in both inhibitory and stimulatory ways. This can have detrimental impacts to such mechanisms in non-target plants (*e.g.*, the poplars in this study), with affected processes ranging from photosynthesis, pigment content, *WUE* parameters (*e.g.*, turgor pressure, stomatal conductance, relative water content, water potential, etc.), and some enzymes such as nitrate reductases and amylases (Wasfi and Samia 2016). For instance, photobleaching diphenyl ether (DPE) herbicides such as Oxyfluorfen used in this study are known to inhibit protoporphyrinogen oxidase, which is the last common enzyme in heme and chlorophyll biosynthesis (Yanagida *et al.* 1999). While less is known about the physiological effects of herbicides on poplars (Pilipović *et al.* 2016), their phytoremediation-relevant impacts in other plants include disturbance of: (1) photosystem II and quantum efficiency (Iftikhar Hussain *et al.* 2010), (2) photosynthetic yield (Haynes *et al.* 2000), (3) chlorophyll content and fluorescence (Shabana *et al.* 2001; Wasfi and Samia 2016), and (4) antioxidant content (Yanagida *et al.* 1999).

As previously mentioned, it is essential to consider the interdependence of physiological parameters and associated assimilation processes when assessing the performance of poplar clones for phytoremediation of both inorganic and organic contaminants. Limitations in assimilation of plants can have stomatal or non-stomatal origin. Stomatal limitation of photosynthesis is reflected in closure of stomata under impacts from contaminants like heavy metals which, when in contact with closing cells of the stomata, cause stomatal closure and decreases of stomatal conductance (Lamoreaux and Chaney 1978). This phenomenon was apparent in this study, with herbicides impacting 'Bora', 'PE 19/66', and 'Pannonia', as well as diesel fuel, Cd, and Cu impacting 'PE 19/66'. In particular, decreases in photosynthesis were generally followed by increases in intercellular carbon dioxide, although stomatal conductance did not always decrease, which indicated non-stomatal limitations of photosynthesis. Nikolić *et al.* (2017) recorded a similar pattern when testing the effect of Cd on photosynthetic parameters of *P. deltoides* and *P.* × *euramericana* clones (*i.e.*, genotypes belonging to the identical genomic groups tested in this study). Mesophyll limitations have also affected photosynthesis of other woody species in the presence of contaminants (Vassilev *et al.* 2005; Han *et al.* 2016).

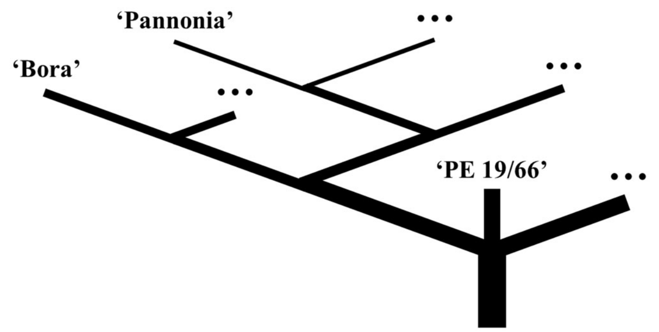

Figure 4. Genetic relatedness of poplar clones 'Bora', 'Pannonia', and 'PE 19/66' based on Figure 2 of Orlović *et al.* (2009), which was originally developed from AFLP markers. Clones 'B-229' and 'PD100' in Orlović *et al.* (2009) are 'Bora' and 'PE 19/66' in this study, respectively.

Non-stomatal limitations often reflect the disturbance of the efficiency of Photosystem II (PS II), which is expressed through changes in chlorophyll fluorescence (F_v/F_m). The F_v/F_m coefficient of chlorophyll fluorescence is typically not a very sensitive stress parameter, although unfavorable conditions that lead to oxidative stress can decrease its value (Linger *et al.* 2005). The overall chlorophyll fluorescence value of 0.704 in this study was within the normal range for poplars and willows (0.7 to 0.8) (Pajević *et al.* 2009; Pilipović *et al.* 2012; Nikolić *et al.* 2015). Although the treatment main effect was negligible, the clone × treatment interaction was significant, especially for 'PE 19/66' where responses for chlorophyll fluorescence were most pronounced. In particular, although there is often a decrease in photosynthesis of poplars affected by organic contaminants, the chlorophyll fluorescence measurements in this study failed to show this trend. Such effects can be attributed to the inactivation and degradation of chlorophyll (Pietrini *et al.* 2003). Other researchers have reported that decreases in photosynthesis resulting from impacts from contaminants are due to decreases in enzymatic activity of the Calvin cycle rather than changes in PS II (Chaffei *et al.* 2004; Burzynski and Zurek 2007; Pajević *et al.* 2009). And yet others have speculated that the lack of the effect on chlorophyll fluorescence could be due to the short recovery period after initial exposure to herbicides (Haynes *et al.* 2000).

Finally, external factors that are not directly related to the contaminants themselves, such as soil water content, nutrient status, and climatic conditions, play a major role in gauging phytoremediation success. The results of this study likely were not only defined by the effect of contaminants on tree growth and physiology, but also by these external factors and the genetic background of the clones. Soil moisture conditions and nutrient status are common limiting factors for plant growth. For example, sufficient available nitrogen increases stomatal conductance in conditions of favorable soil humidity (Shangguan *et al.* 2000). Therefore, in order to supply the photosynthetic apparatus with nitrogen, plants become inefficient and 'waste' water (Donovan *et al.* 2007). Given that soil nitrogen content in this study was in acceptable amounts (Table 1) and that no supplemental irrigation was provided, potential water limitations may have contributed to these results. In particular, groundwater table values substantially decreased as the second growing season progressed (Figure 1), which most likely affected the growth and physiology of all three clones. Significant decreases in transpiration were recorded for 'Pannonia' and 'Bora' in almost all treatments. Such similarity in the clonal responses can be attributed to a greater genetic relatedness between these clones compared with 'PE 19/66' (Figure 4) (Orlović *et al.* 2009). These genetic relationships likely contributed to the different adaptation strategies observed. Nevertheless, based on the results of this study, and in corroboration with Pajević *et al.* (2009), assessing the overall phytoremediation potential of poplar genotypes requires a combination of growth and physiological assessments, wherein adaptive values of specific genotypes are expressed in high photosynthetic potential, relatively high and stable *WUE*, high biomass production, and accumulation of and resistance to high levels of inorganic and/or organic pollutants of interest.

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