



Photosynthesis and aboveground carbon allocation of two co-occurring poplar species in an urban brownfield[☆]



Diane Radwanski^a, Frank Gallagher^b, Dirk W. Vanderklein^c, Karina V.R. Schäfer^{a, d, *}

^a Department of Biological Sciences, Rutgers University, Newark, NJ, United States

^b Department of Landscape Architecture, Rutgers University, New Brunswick, NJ, United States

^c Department of Biology, Montclair State University, Montclair, NJ, United States

^d Earth and Environmental Science Department, Rutgers University, Newark, NJ, United States

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ABSTRACT

Phytoremediation, a technique used to reclaim heavy metal-contaminated soils, requires an understanding of plant physiological responses to heavy metals. However, the majority of studies documenting heavy metal impact on plant functioning have been performed in laboratory or greenhouse settings. We predicted that increased soil heavy metal concentrations reduce photosynthesis and biomass production in trees growing in metal contaminated soil in a naturally re-vegetated urban brownfield. Leaf gas exchange, leaf carbon and nitrogen concentration, and tree biomass were recorded and compared for *Populus deltoides* and *Populus tremuloides* growing in an urban brownfield. The CO₂ compensation point (CCP) differed significantly between soil metal concentrations and species, with *P. deltoides* displaying a greater CCP and *P. tremuloides* displaying a lower CCP as soil metal concentration increased, despite no changes in dark respiration for either species. In terms of biomass, only total branch weight (TBW) and leaf area (LA) differed significantly between soil metal concentrations, though the difference was largely attributable to variation in diameter at breast height (DBH). Furthermore, TBW and LA values for *P. deltoides* did not decrease with increasing soil metal concentration. Soil metal concentration, thus, had minimal effect on the relationship between tree age and DBH, and no effect on relationships of tree age and height or LA, respectively. Significant differences between soil metal concentrations and species were found for $\delta^{15}\text{N}$ (isotopic nitrogen ratio) while leaf nitrogen content (% N) also differed significantly between species. Long-term water use efficiency derived from carbon isotope analysis (iWUE_{isotope}) differed significantly between trees grown on different soil metal concentrations and a significant species-metal concentration interaction was detected indicating that the two study species responded differentially to the soil metal concentrations. Specifically, *P. tremuloides* enhanced while *P. deltoides* reduced long-term iWUE_{isotope} as soil metal concentration increased, further emphasizing the importance of species and possible genotype selection for phytoremediation.

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1. Introduction

Numerous areas across the globe are suffering from heavy metal contamination (Lone et al., 2008; McGrath et al., 2001; Meers et al., 2010). Heavy metals pose a serious threat due to their non-biodegradable nature, difficulty of removal, health risks, and buildup in water and soils (Giller et al., 1998; Lone et al., 2008;

Mendola et al., 2002). In the United States, there are over 450,000 brownfields, and nearly 80% of Superfund sites and over 50 million cubic meters of soil are contaminated with heavy metals (Dermont et al., 2008; EPA, 2012). It has been estimated that brownfields in the U.S. alone account for the loss of more than 205,000 ha of valuable land that could be used for agriculture, pasture, or forests (Lone et al., 2008). As such, phytoremediation has become widely recognized as a cost-effective, efficient, eco-friendly, and *in situ* applicable method for remediating metal-contaminated soils (Ali et al., 2013; Saier and Trevors, 2010; Vithanage et al., 2012; Wang and Jia, 2010). Species with traits that include fast growth, extensive root systems, and large biomass production have become a

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* Corresponding author. Department of Biological Sciences, Rutgers University, 195 University Ave, Newark, 07102, NJ, United States.

E-mail address: karinavr@andromeda.rutgers.edu (K.V.R. Schäfer).

focus of many phytoremediation studies (Jabeen et al., 2009; Pulford and Watson, 2003; Seth, 2012). Among these plants, members of the genus, *Populus*, are known to comprise a large number of species that are able to grow in metal-contaminated soils (Wang and Jia, 2010).

Many studies on the effects of heavy metals on plant function have been carried out in laboratory or greenhouse settings (Ali et al., 2013; Dos Santos Utmazian et al., 2007; Giachetti and Sebastiani, 2006; Hermle et al., 2006, 2007; Robinson et al., 2007), which, while being effective in controlling environmental variables and isolating cause and effect, do not reflect field conditions and included one year old seedlings to 6 year old trees, hydroponic pot studies and chambered field studies but not established trees in a naturally assembled brownfield.

Studies that have examined the impact of heavy metals on many plant species have found negative effects on photosynthesis and biomass production. Leaf damage, alterations in plant cell structure, and damage to both photosynthetic and water-conducting tissues have been reported in plants dealing with heavy metal contamination (Barylka et al., 2001; Hermle et al., 2007). In response to treatments with copper (Cu), nickel (Ni), and cadmium (Cd), *Populus deltoides* and *Populus euramericana* undergo oxidative stress, leading to damaged chlorophyll molecules followed by reductions in photosynthetic capacity (Trudić et al., 2012). Young trees of *Populus x euramericana* suffered from reduced foliage, impaired gas exchange, and decreased photosynthesis rates in response to zinc (Zn) treatments (Di Baccio et al., 2003). Young trees of *Populus tremula* experienced decreases in transpiration, diameter growth, foliage area, fine root biomass, aboveground biomass, and leaf area, resulting in lower photosynthetic ability when grown with topsoil treated with Cu, Zn, Cd, and lead (Pb) (Hermle et al., 2006; Menon et al., 2007). When grown in soils treated with Cd, Zn, and Cu, two-year-old seedlings of *Populus canadensis* only reached 75% of their biomass production compared to individuals of the same species and age growing in untreated soil (Wang and Jia, 2010). Mature trees of *Betula populifolia* displayed reduced productivity when growing in soils with higher Zn levels (Gallagher et al., 2008a). Mature trees of *P. deltoides* did not significantly differ in terms of photosynthetic functioning between low and high soil metal concentrations, though it was found that trees of *P. deltoides* that were of similar size, tended to be younger when growing on soils with lesser metal contamination (Renninger et al., 2013). Older trees may simply have greater ability to tolerate heavy metals due to their larger size, which conveys greater number of water- and nutrient-conducting, as well as photosynthetic tissues, which may enhance the ability to offset negative impacts of heavy metals. In addition, the established trees may be able to cope better with heavy metal contamination, because their roots explore deeper soil volumes with less contamination, there may be less plant available heavy metals due to complexation with organic matter, confining heavy metals in foliage that gets discarded by the end of the season, other elements that ameliorate damaging effects or the harmful heavy metals are translocated throughout the soil volume (Nocito et al., 2011; Qian et al., 2012; Shen et al., 2016).

The purpose of this study is to examine and compare the effects of heavy metals on photosynthesis and aboveground biomass production in *Populus deltoides* W. Bartram ex Marshall (USDA, 2015a) and *Populus tremuloides* Michx. (USDA, 2015b), growing in a naturally re-vegetated brownfield in New Jersey, which contains a persisting soil metal contamination (Evans et al., 2015; Gallagher et al., 2008a, b; Hagemann et al., 2015). Here, it is predicted that *P. deltoides* and *P. tremuloides* will exhibit a higher photosynthetic capacity in sites containing lower soil metal contamination compared to sites with higher soil metal contamination. As a result, productivity for both species is expected to be higher in sites with

lower soil metal contamination. Trees of both species growing in highly metal-contaminated soils are expected to have higher leaf dark respiration compared to trees growing in lesser metal-contaminated soils because more energy is required in order to maintain leaf structural and photosynthetic integrity in a heavy metal soil environment (Hermle et al., 2007). Similarly, transpiration is expected to be lower for trees in sites with higher soil metal contamination in order to tolerate metal-induced water stress (Hermle et al., 2007; Lamoreaux and Chaney, 1978; Schlegel et al., 1987). Photosynthetic and intrinsic water use efficiency should then be higher for trees growing in sites of higher soil metal contamination due to lower transpiration or lower stomatal conductance, respectively (Gallagher et al., 2008a; Renninger et al., 2013).

2. Materials and methods

2.1. Study site and species selection

The study was carried out in Liberty State Park (LSP), which is located in Jersey City, New Jersey, U.S.A. Liberty State Park originally existed as an intertidal mudflat and salt marsh before undergoing a land use change and being filled with waste from New York City in order to be made usable as a rail yard for the Central Railroad of New Jersey (CRRNJ) (Gallagher et al., 2008b). The area was used by the CRRNJ until 1967, leaving the area abandoned (NJ Department of Environmental Protection (2009)). Later, the area was purchased with government funds and upon the completion of cleanup efforts (<http://www.nan.usace.army.mil/Portals/37/docs/civilworks/projects/nj/ecor/HRELiberty/htrw.pdf>), the area was officially opened as a public park in 1976 (NJ Department of Environmental Protection (2009)). The LSP consists of approximately 490 ha, and while the park has been largely restored, a 102 ha area still exists that is fenced off and closed to public access (NJDEP, 2002; Renninger et al., 2013) because of its classification as a brownfield due to soil heavy metal contamination (Gallagher et al., 2008a). Specifically, the area has been found to be contaminated with arsenic (As) from 9 to 31 $\mu\text{g g}^{-1}$, Cr ranging from 18 to 96 $\mu\text{g g}^{-1}$, Cu ranging from 52 to 76 $\mu\text{g g}^{-1}$, Pb ranging from 177 to 414 $\mu\text{g g}^{-1}$, vanadium (V) in the range of 21–137 $\mu\text{g g}^{-1}$, and Zn ranging from 69 to 140 $\mu\text{g g}^{-1}$ (Hagemann et al., 2015), of which soil concentrations of As, Cr, Pb, V, and Zn are considered to be above normal for New Jersey soils (Gallagher et al., 2008a; Sanders, 2003). Previous soil sampling done in 2005 indicated that soil contamination was highly heterogeneous across the area (Gallagher et al., 2008a) and remains so to this day (Hagemann et al., 2015). Despite the presence of metal-contaminated soils, the area has been naturally re-vegetated, with *Betula populifolia* Marsh. (35%), *Populus deltoides* W. Bartram ex Marshall (16%), and *Populus tremuloides* Michx. (14%) representing the dominant species in the hardwood areas (Evans et al., 2015; Gallagher et al., 2008a; Hagemann et al., 2015; Renninger et al., 2013).

Within this 102 ha fenced off area, six sites were chosen for this study, each representing areas of high, medium, or low soil metal contamination based on the composite soil metal concentration present using the composite soil metal concentration map and site designations in Gallagher et al. (2008a). Details on the heterogeneity of metal concentrations and specifically which metals have higher or lower concentrations at the different sites have been previously reported in Gallagher et al. (Gallagher et al., 2008a, b; Renninger et al., 2013), and more recently in Hagemann et al. (2015) and Qian et al. (2012). Sites 41 and 48 were selected as areas of low soil metal concentrations (LM) and sites 14/16 and 25 as areas of high soil metal concentrations (HM) in order to examine heavy metal effects on gas exchange and leaf nutrient

concentration. Sites 11, 41, and 48 were selected to represent LM, site 18 to represent medium soil metal concentrations (MM), and finally, sites 14/16 and 25 to represent HM for analysis of tree biomass. The species of interest for this study were *P. deltooides* and *P. tremuloides*, as previous studies conducted at the same study site have focused on *B. populifolia* (Gallagher et al., 2008a) and *P. deltooides* (Renninger et al., 2013), but have not focused on *P. tremuloides* nor a comparison between *P. deltooides* and *P. tremuloides*.

2.2. Photosynthesis measurements

Photosynthetic measurements were taken during the last week of each month during the period of June 2013 to September 2013, and from the last week of each month during the period of May 2014 to September 2014, using an infra-red gas analyzer (LiCor 6400 XT, LiCor Biosciences, Inc., Lincoln, NE, USA). Measurements were made each day from the morning to the afternoon, during peak photosynthetic activity, while avoiding the presence of early morning dew on leaves and the natural closure of stomata in the late afternoon. Measurements were taken from one leaf for each of 5 trees of *P. deltooides* and 5 trees of *P. tremuloides* at sites 41, 48, and 25. At site 14/16, however, due to a lack of presence of *P. deltooides*, measurements were taken for *P. tremuloides* only. Samples were taken by cutting branches, located in open areas of the canopy, off of the trees and then keeping and cutting them underwater so as to avoid stomatal closure due to water stress. Leaves of the cut branches were measured within 90 min after each branch cutting (Asao and Ryan, 2015; Venturas et al., 2015). Measurement of leaves at approximately the same canopy height, time of day and weather conditions controls for as many variables as possible and being able to compare primarily for the edaphic conditions selected for in this study. Assimilation-carbon dioxide response (A/C_i) curves and assimilation-light response curves were made for each leaf measured. The A/C_i curves were constructed by setting a constant photosynthetically active photon flux density level (PPFD) to $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and altering the CO_2 concentration in the leaf chamber from 50 ppm to 1500 ppm. Light response curves were constructed by setting a constant CO_2 concentration in the leaf chamber to 400 ppm, and then varying PPFD from $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The data from the A/C_i curves were used to calculate the maximum carboxylation rate as limited by Rubisco (V_{cmax} in $\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum electron-transport-limited carboxylation rate (J_{max} in $\mu\text{mol m}^{-2} \text{s}^{-1}$), carboxylation rate as limited by triose-phosphate use (TPU in $\mu\text{mol m}^{-2} \text{s}^{-1}$), mesophyll conductance (g_m in $\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$), carboxylation efficiency (CE in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \text{ppm}^{-1}$), and the CO_2 compensation point (CCP in ppm). Using the A/C_i Curve Fitting Utility Version 2007.1 (Sharkey et al., 2007), a best fit model was used to minimize the difference of the sum of squares between measured and modeled data for the Rubisco-limited, electron-transport-limited, and TPU-limited section of the A/C_i curve to estimate V_{cmax} , J_{max} , and TPU, and g_m . Final values of V_{cmax} , J_{max} , TPU, and g_m were taken as the respective values scaled by the model to a leaf temperature of 25°C to avoid comparison of values at different observed leaf temperatures.

In order to calculate carboxylation efficiency (CE) and CO_2 compensation points (CCP), the A/C_i data were plotted in Sigmaplot 11.0 (Sysstat Software Inc., San Jose, CA, USA) for each measured leaf. A linear regression was fit through the initial, linear section of the A/C_i curve, roughly from 50 to 200 ppm CO_2 . The linear equation of the regression was then used to calculate the slope (CE), and x-intercept (CCP).

The data from light response curves were used to calculate the maximum rate of net photosynthesis (A_{max} in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$),

quantum yield (Φ in $\mu\text{mol CO}_2 \mu\text{mol}^{-1}$ photons), dark respiration rate (R_D in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), and light compensation point (LCP in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for each measured leaf. As per Lobo et al. (2013), multiple models were used to best fit the original, observed light response data and the model with the lowest sum of the squares of the errors (SSE) was used to estimate A_{max} , Φ , R_D , and LCP for each measured leaf. Three light response curves were calculated for each leaf, using 3 different models as outlined in Lobo et al. (2013) and based on work by Webb et al. (1974), Prioul and Chartier (1977), and Ye (2007). The 3 models included an exponential curve-fitting model (Lobo et al., 2013), a non-rectangular hyperbola-fitting model, and a modified non-rectangular hyperbola model which utilized equations (6), (8) and (11) in Lobo et al. (2013), respectively.

Transpiration (Transp in $\text{mmol H}_2\text{O}$), the ratio of intercellular CO_2 to ambient CO_2 (C_i/C_{airinst}), photosynthetic water use-efficiency (WUE in $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{H}_2\text{O}$), defined as photosynthetic carbon assimilation/transpiration, and intrinsic water use efficiency (iWUE in $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{H}_2\text{O}$), defined as photosynthetic assimilation/stomatal conductance, were estimated from A/C_i and light response curves from points where PPFD was $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and CO_2 concentrations were 400 ppm for each leaf.

2.3. Leaf carbon and nitrogen concentration

Leaves that were used for photosynthesis measurement were collected from five trees of *P. deltooides* and *P. tremuloides* in each of the four study sites, with the exception of site 14/16, which did not contain *P. deltooides*, in order to calculate leaf carbon (C) and nitrogen (N) concentrations, as well as C and N isotopic ratios. The leaves were first dried at 60°C for at least 48 h in a convection oven (Thermo Scientific Precision 3050 Series premium oven, Thermo Fisher Scientific, USA) then ground into a fine powder, placed in tin capsules, and sent out to the University of California's Davis Stable Isotope center located in Davis, CA, USA, for analysis of leaf C and N concentrations (C and N in %), C:N ratios, isotopic C ratios ($\delta^{13}\text{C}$), and isotopic N ratios ($\delta^{15}\text{N}$). Nitrogen concentrations (%) were scaled to a per unit leaf area by multiplying N concentration by the leaf mass per area measured from the respective collected leaves in order to calculate nitrogen use efficiency (NUE). Total leaf N (TLN) was calculated by multiplying leaf N concentration by total leaf weight. Average N concentration was determined from leaf composition data categorized by species and site, and then multiplied by the total leaf weight of trees harvested for biomass analysis that were classified as the same species and site. In the case of *P. tremuloides* growing in site 11, which was not represented in leaf nutrient concentration data, N concentrations were averaged from *P. tremuloides* from sites 41 and 48, since sites 41, 48, and 11 are all classified as LM sites, and have similar amounts of heavy metals that do not exceed a certain composite soil metal concentration (Gallagher et al., 2008a), and then multiplied by the total leaf weight of *P. tremuloides* from site 11. Trees from site 18 were excluded, as leaf N concentration was not determined for trees at the MM sites. Additionally, nitrogen use efficiency (NUE in $\mu\text{mol CO}_2 \text{ g}^{-1} \text{N s}^{-1}$) was calculated by dividing the net photosynthesis rate, which was estimated from A/C_i and light response curves from points where PPFD was $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and CO_2 concentrations were 400 ppm for each leaf, by the concentration of N, scaled per unit leaf area, in each of the measured leaves. Carbon isotopic discrimination (Δ in ‰) was also calculated from the C isotope data using equation (4) in Renninger et al. (2014) based on work by Farquhar et al. (1989). Isotopic intrinsic water use efficiency (iWUE_{isotope}) was calculated using equation (5) in Renninger et al. (2014) based on work by Farquhar et al. (1989). The isotopic ratio of intercellular CO_2 to ambient CO_2 ($C_i/C_{\text{a, isotope}}$) was calculated

using the following equation based on Farquhar et al. (1989):

$$C_i / C_a, \text{ isotope} = \frac{(\Delta - 4.4)}{(27 - 4.4)} \quad (1)$$

where Δ is in ‰, 4.4 is the diffusive discrimination of ^{13}C in air through the stomata, and 27 is the discrimination of Rubisco to ^{13}C .

2.4. Biomass harvest and aboveground growth

The harvest protocol for *P. deltoides* was previously reported in Renninger et al. (2013). In addition, twenty-two trees of *P. tremuloides* were harvested in the spring of 2010 and summer of 2011 to comprise a sufficiently broad diameter at breast height range from 0.85 cm to 14.7 cm and a sample size of 5–7 in different soil metal concentration sites, with total sample size of 22 per species being typical for felled tree studies (Arias et al., 2011; Karlik and Chojnacky, 2014; Suchomel et al., 2012). Additionally, the branch diameter was recorded from every branch taken from each harvested tree in 2011. *P. deltoides* was harvested in sites 41, 18, and 25, while *P. tremuloides* was harvested in sites 11, 18, 48, 14/16, and 25. Trees were cut at 10 cm from the ground after recording the diameter at breast height. The main axis of the fallen tree was cut into one-meter segments and fresh weight of each of the one-meter segments was recorded using scales in the field. From the base of each one-meter segment, one slice of the bottom of the stem (cookie) was collected and fresh weight of the cookie was recorded. For each of the one-meter segments, branch diameters of all the branches were recorded. All the branches and leaves were labeled and were collected in separate bags. All the bags with branches, leaves, and cookies for individual trees were dried in a convection oven (at 60 °C, 6 days) in the laboratory (Thermo Scientific Precision 3050 Series premium oven, Thermo Fisher Scientific, USA). The ratio of the dry to fresh weight of the cookies was used to estimate the dry weight of the one-meter segments of tree stem previously recorded in the field. Ring widths of each tree cookie were measured along two perpendicular radii to the nearest 0.1 mm. This allowed for determination of the age of each tree, as well as overall growth pattern. Tree height was divided into three segments (bottom, middle, and top) for selecting the number of leaves to estimate leaf area (LA). The numbers of leaves selected for scanning were proportional to the number of branches on each segment. Total leaf weight (TLW) was estimated by adding the dry weight of all the leaves of all the branches from an individual tree. Total branch weight (TBW) was determined by adding the dry weight of all the branches. To calculate the LA for individual trees, leaves collected from a tree in the field were scanned on the same day in the laboratory using a commercial scanner and analyzing the images using Image J (<http://rsbweb.nih.gov/ij/>). Specific leaf area (SLA) was calculated as the ratio of LA per unit of leaf dry mass. Canopy level SLA was then used to determine total leaf area by multiplying it with total leaf mass of each canopy level. Total aboveground tree mass (TTM) was estimated by adding the dry weight of leaves, branches, and total stem mass.

As the large *P. tremuloides* trees (diameter at breast height > 7 cm) were harvested during the leafless stage in the spring of 2010, ten branches from all sites where the trees were harvested, were collected in the summer of 2013 and a branch diameter to leaf mass and branch diameter to leaf area relationship was generated for each site separately (with an R^2 of 0.62 and $P < 0.0001$, respectively), given that there is a clear, allometric relationship between branch diameter, LA, and leaf mass (Hunt et al., 1999; McDowell et al., 2002; Zhang and Cao, 2009) based largely on pipe model theory (Shinozaki et al., 1964). These relationships, separated for each soil metal concentration and

generated for each site (high or low), were used to calculate each individual tree leaf biomass and LA for the trees harvested previously.

2.5. Statistical analysis

For all statistical evaluations of gas exchange, leaf carbon and nitrogen concentration, and biomass data, data were combined from 2013 to 2014, and 2010 and 2011. Means and standard errors were calculated for gas exchange variables and leaf C and N concentrations for *P. deltoides* and *P. tremuloides* growing in LM and HM. A linear mixed effects model was created in Matlab R2014b (The Mathworks Inc, Natick, MA) to determine if means differed between soil metal and/or species. Model parameters included two independent, fixed effects, which were soil metal (designated as either high or low) and species, with both soil metal and species nested within sites. An interaction effect between species and soil metal was also included. The month and year of measurement for each tree was included as a single random effect as the primary focus was on comparing species and sites and not seasonal variation. Analysis of variance (ANOVA) used within the model was set to indicate significant difference at $\alpha = 0.05$. Adjusted R^2 values of the model varied from 0.07 to 0.32 and from 0.25 to 0.50 for the gas exchange and leaf carbon and nitrogen data, respectively (see Tables 1 and 2).

Similar procedure was used to analyze biomass and TLN means, with the following differences. In order to improve fit to the model, all values were log transformed (log, base 10). Model parameters additionally included diameter at breast height (DBH) and age as independent, fixed effects, and another interaction term between species and age. Adjusted R^2 values of the model varied from 0.77 to 0.98 (see Table 3).

In order to investigate the effects of heavy metals on allometric growth patterns, the relationships between tree age and DBH, height (H), or LA, as well as between DBH and total tree mass (TTM), were plotted for *P. deltoides* and *P. tremuloides* growing in LM, MM, and HM. Using aocool in Matlab R2014b, linear regressions were performed to fit separate lines to the data of tree age versus DBH, height, or LA, and DBH versus TTM. Due to the nonlinear relationships between tree age and the aforementioned variables, data were log transformed (log, base 10) before regression analysis. Regressions were followed by an analysis of covariance (ANCOVA) to determine if slopes differed at $\alpha = 0.05$. For the ANCOVA, tree age was designated as the explanatory variable, and DBH, height, or LA as the response variable. For DBH versus TTM, DBH and TTM were the explanatory and response variables, respectively. All variables were categorized by species and soil metal concentration differences (high or low). Tukey's HSD test was employed if the ANCOVAs signified differences in the slopes and/or y-intercepts at $\alpha = 0.05$.

3. Results

3.1. Gas exchange parameters

The V_{Cmax} , TPU, g_m , and CE did not differ significantly between soil metal concentration or species (Table 1). Also, J_{max} did not differ significantly between soil metal concentration, but did differ between species. In both LM and HM sites, J_{max} was greater for *P. deltoides* compared to *P. tremuloides* (by approximately 24% and 17%, respectively, Table 1). The CCP differed significantly between trees due solely to the effects of soil metal concentration, but not between species (Table 1). Additionally, a significant interaction effect between soil metal and species was found. *P. deltoides* had a greater CCP compared to *P. tremuloides* in both LM and HM, but was 63% greater in HM and only 11% greater in LM (Table 1). In HM,

Table 1

Comparison of gas exchange performance of *Populus deltoides* (PD) and *Populus tremuloides* (PT) between high and low soil metal contamination sites at Liberty State Park.

	LM ^a		HM ^a		P-values*			Adj R ²
	PD ^a	PT ^a	PD	PT	S	ML	S:ML	
V _{cmax}	65.2 (5.7)	61.9 (4.8)	83.0 (16.0)	77.4 (6.9)	0.43	0.14	0.91	0.12
J _{max}	101.1 (6.9)	81.5 (3.8)	102.5 (19.1)	87.7 (3.2)	0.04	0.88	0.74	0.12
TPU	7.7 (0.52)	5.9 (0.32)	7.3 (1.4)	6.1 (0.18)	0.051	0.75	0.70	0.09
g _m	11.1 (2.4)	14.5 (2.1)	9.3 (2.5)	8.9 (1.7)	0.25	0.85	0.39	0.17
CE	0.056 (0.004)	0.079 (0.015)	0.092 (0.034)	0.069 (0.007)	0.49	0.15	0.23	0.13
CCP	68.3 (4.9)	61.2 (5.5)	83.7 (13.7)	51.4 (3.1)	0.69	0.05	0.008	0.25
A _{max}	10.7 (0.59)	10.6 (0.59)	10.1 (1.00)	9.4 (0.54)	0.72	0.53	0.74	0.30
Φ	0.069 (0.001)	0.069 (0.003)	0.063 (0.002)	0.068 (0.003)	0.94	0.14	0.26	0.07
R _D	1.7 (0.09)	1.46 (0.16)	1.8 (0.18)	1.43 (0.11)	0.08	0.78	0.75	0.08
LCP	27.2 (1.7)	21.7 (2.3)	33.0 (3.9)	22.0 (1.4)	0.02	0.13	0.21	0.18
Transp	3.1 (0.17)	2.4 (0.17)	2.6 (0.19)	2.5 (0.14)	<0.001	0.13	0.04	0.30
C _i /C _a inst	0.68 (0.02)	0.61 (0.01)	0.69 (0.03)	0.62 (0.01)	0.15	0.62	0.79	0.33
WUE	3.9 (0.25)	4.4 (0.14)	3.5 (0.32)	4.3 (0.14)	0.55	0.48	0.81	0.25
iWUE	64.5 (5.5)	83.3 (3.4)	63.8 (8.6)	81.3 (3.2)	0.09	0.75	0.82	0.31

Values are means and standard errors, and are defined as follows: Maximum carboxylation rate as limited by Rubisco (V_{cmax}; μmol m⁻² s⁻¹); maximum electron-transport-limited carboxylation rate (J_{max}; μmol m⁻² s⁻¹); carboxylation rate as limited by triose-phosphate use (TPU; μmol m⁻² s⁻¹); mesophyll conductance (g_m; μmol m⁻² s⁻¹ Pa⁻¹); carboxylation efficiency (CE; μmol CO₂ m⁻² s⁻¹ ppm⁻¹); CO₂ compensation point (CCP; ppm); maximum rate of net photosynthesis (A_{max}; μmol CO₂ m⁻² s⁻¹); quantum yield (Φ; μmol CO₂ μmol⁻¹ photons); dark respiration rate (R_D; μmol CO₂ m⁻² s⁻¹); light compensation point (LCP; μmol photons m⁻² s⁻¹); transpiration (Transp; mmol H₂O); C_i/C_ainst; ratio of intercellular CO₂ to ambient CO₂; photosynthetic water use efficiency (WUE; μmol CO₂ mmol⁻¹ H₂O); intrinsic water use efficiency (iWUE; μmol CO₂ mmol⁻¹ H₂O).

*P-values indicate significant difference at α = 0.05 (in bold) between gas exchange performance of species (S), soil metal concentration (ML), and for an interaction between S and ML (S:ML) based on a linear mixed effects model; n = 65, 70, 33, and 72 for PD LM, PT LM, PD HM, and PT HM, respectively.

^a LM, low soil metal concentration; HM, high soil metal concentration; PD, *Populus deltoides*; PT, *Populus tremuloides*.

Table 2

Comparison of leaf carbon and nitrogen concentration between *Populus deltoides* and *Populus tremuloides* between high and low soil metal contamination sites at Liberty State Park.

	LM ^a		HM ^a		P-values*			Adj R ²
	PD ^a	PT ^a	PD	PT	S	ML	S:ML	
C	45.6 (0.17)	48.3 (0.24)	45.6 (0.53)	47.0 (0.31)	<0.0001	0.93	0.051	0.26
N	2.2 (0.06)	2.5 (0.06)	2.2 (0.09)	2.5 (0.06)	0.001	0.45	0.75	0.50
TLN	0.05 (0.03)	0.01 (0.008)	0.04 (0.02)	0.005 (0.002)	0.29	0.86	0.61	0.07
C:N	21.0 (0.74)	19.8 (0.46)	21.3 (1.0)	19.4 (0.43)	0.03	0.43	0.27	0.44
δ ¹³ C	-29.9 (0.14)	-30.0 (0.10)	-30.9 (0.23)	-29.7 (0.15)	0.83	<0.001	<0.0001	0.26
δ ¹⁵ N	-0.17 (0.14)	-2.3 (0.07)	-1.5 (0.12)	-1.8 (0.12)	<0.0001	<0.0001	<0.0001	0.47
Δ (‰)	17.9 (0.15)	18.0 (0.10)	19.0 (0.24)	17.7 (0.16)	0.83	<0.001	<0.0001	0.26
C _i /C _a isotope	0.60 (0.006)	0.60 (0.004)	0.64 (0.01)	0.59 (0.007)	0.83	<0.001	<0.0001	0.26
iWUE _{isotope}	99.5 (1.6)	98.9 (1.2)	88.3 (2.7)	102.3 (1.8)	0.83	<0.001	<0.0001	0.26
SLA	124.1 (34.4)	149.2 (45.5)	132.6 (25.3)	141.4 (42.6)	0.03	0.83	0.79	0.45
NUE	6.2 (0.39)	5.9 (0.40)	6.1 (0.82)	6.1 (0.37)	0.52	0.98	0.72	0.28

Values are means and standard errors, and are defined as follows: Leaf carbon concentration (C, %); leaf nitrogen concentration (N, %); total leaf nitrogen content (TLN, kg); C:N, leaf carbon to nitrogen ratio; δ¹³C, isotopic carbon ratio; δ¹⁵N, isotopic nitrogen ratio, carbon isotopic discrimination (Δ, ‰); C_i/C_aisotope, isotopic ratio of intercellular CO₂ to ambient CO₂; iWUE_{isotope}, isotopic intrinsic water use efficiency; SLA specific leaf area in cm² g⁻¹; nitrogen use efficiency (NUE, μmol CO₂ g⁻¹ N s⁻¹).

*P-values indicate significant difference at α = 0.05 (in bold) in leaf composition between species (S), soil metal concentration (ML), and for an interaction between species and soil metal concentration (S:ML), respectively, based on a linear mixed effects model; n = 65, 70, 33, and 72 for PD LM, PT LM, PD HM, and PT HM, respectively, except for TLN, where n = 6, 4, 7, and 11 for PD LM, PT LM, PD HM, and PT HM, respectively.

^a LM, low soil metal concentration; HM, high soil metal concentration; PD, *Populus deltoides*; PT, *Populus tremuloides*.

Table 3

Comparison of biomass parameters between *Populus deltoides* and *Populus tremuloides* between high and low soil metal contamination sites at Liberty State Park.

	LM ^a		MM ^a		HM ^a		P-values*					Adj R ²	
	PD ^a	PT ^a	PD	PT	PD	PT	DBH	S	ML	Age	S:M		S:A
DBH	8.8 (3.3)	7.0 (1.9)	4.0 (1.0)	4.9 (1.8)	8.9 (2.8)	3.6 (0.9)	n/a	0.57	0.059	<0.001	0.72	0.55	0.77
H	7.2 (1.6)	7.7 (1.2)	4.8 (0.6)	6.4 (1.8)	8.0 (1.4)	5.1 (1.0)	<0.0001	0.58	0.50	0.89	0.46	0.07	0.94
TBW	6.8 (5.2)	3.4 (2.1)	2.5 (1.9)	3.2 (2.1)	11.1 (7.9)	1.2 (0.42)	<0.0001	0.04	0.04	0.54	0.06	0.06	0.91
TLW	2.6 (1.6)	0.69 (0.33)	0.78 (0.52)	0.49 (0.26)	1.9 (0.99)	0.21 (0.09)	<0.0001	0.059	0.11	0.94	0.55	0.93	0.96
LA	34.1 (21.6)	8.6 (3.9)	10.8 (7.0)	6.0 (3.2)	25.0 (12.5)	2.6 (1.0)	<0.0001	0.98	0.03	0.52	0.26	0.79	0.91
TTM	30.2 (21.3)	13.5 (8.8)	9.4 (7.5)	13.1 (8.6)	35.6 (22.6)	4.4 (2.2)	<0.0001	0.74	0.07	0.09	0.69	0.94	0.98

Values are means and standard errors, and are defined as follows: Diameter at breast height (DBH, cm); tree height (H, m); total branch weight (TBW, kg); total leaf weight (TLW, kg); leaf area (LA, m²); total tree mass (TTM, kg).

*P-values under 'DBH', 'S', 'ML', and 'Age', 'S:M', and 'S:A' indicate significant difference at α = 0.05 (in bold) between means due to DBH, species (S), soil metal concentrations (ML), age, and for interactions between species and metal load (S:M) and species and age (S:A), respectively, based on a linear mixed effects model; n = 6, 4, 7, 7, and 11 for PD LM, PT LM, PD MM, PT MM, PD HM, and PT HM, respectively.

^a LM, low soil metal concentration; MM, medium soil metal concentration; HM, high soil metal concentration; PD, *Populus deltoides*; PT, *Populus tremuloides*.

P. deltooides displayed a greater CCP when compared to LM (by roughly 23%), while *P. tremuloides* displayed a greater CCP in LM than HM (by approximately 19%, Table 1).

The A_{max} , Φ , and R_D did not differ significantly between soil metal concentration or species. The LCP differed significantly between species, but not soil metal concentration (Table 1). In both LM and HM, the LCP was greater for *P. deltooides* compared to *P. tremuloides* (by roughly 25% and 50%, respectively, Table 1).

The $C_i/C_{a,inst}$, WUE, and $iWUE$ did not differ between soil metal concentration or species. However, transpiration differed significantly between species, but not soil metal concentration. Moreover, a significant interaction effect between soil metal concentration and species was found (Table 1). *P. tremuloides* preferred to restrict transpiration more so than *P. deltooides* in both LM and HM, with lower transpiration by roughly 23% and 4%, respectively (Table 1).

3.2. Leaf carbon and nitrogen concentration

Species differed significantly in leaf carbon content [C (%)], though soil metal concentration had no effect (Table 2). In both LM and HM, *P. tremuloides* contained greater leaf C content (by roughly 6% and 3%, respectively) compared to *P. deltooides* (Table 2). Isotopic C ratios ($\delta^{13}C$), carbon isotopic discrimination ratios ($\Delta\%$), isotopic ratios of intercellular CO_2 to ambient CO_2 ($C_i/C_{a, isotope}$), and isotopic intrinsic water use efficiency ($iWUE_{isotope}$) all differed significantly between soil metal concentration, but not between species, and a significant interaction effect between species and soil metal concentration was found. *P. deltooides* displayed a reduced $\delta^{13}C$ and $iWUE_{isotope}$, while $\Delta\%$ and $C_i/C_{a, isotope}$ were greater when growing in HM compared to LM (Table 2). However, *P. tremuloides* displayed a greater $\delta^{13}C$ and $iWUE_{isotope}$ and reduced $\Delta\%$ and $C_i/C_{a, isotope}$ when growing in HM compared to LM (Table 2).

Leaf nitrogen content [N (%)] differed significantly between species, but not between soil metal concentrations. In both LM and HM, *P. tremuloides* contained greater N content in the leaves compared to *P. deltooides* (Table 2). In the case of isotopic N ratios ($\delta^{15}N$), trees differed significantly between species and soil metal sites (Table 2). A significant interaction effect between species and soil metal sites was also found in the comparison of $\delta^{15}N$. In both LM and HM, *P. deltooides* contained a greater $\delta^{15}N$ compared to *P. tremuloides* (Table 2). *P. deltooides* displayed a greater $\delta^{15}N$ (by roughly 89%) when growing in LM compared to HM (Table 2). *P. tremuloides* showed the opposite response, with a greater $\delta^{15}N$ (by approximately 23%) when growing in HM, compared to LM, sites (Table 2). Specific Leaf Area (SLA) for *P. tremuloides* was significantly greater than for *P. deltooides*, signifying more leaf area per leaf mass (Table 2). Despite differences in leaf N concentration, NUE and TLN did not differ significantly between species or soil metal sites. However, leaf C:N ratios differed significantly between species, with *P. deltooides* having greater leaf C:N compared to *P. tremuloides* in both LM and HM, by roughly 6% and 10%, respectively (Table 2).

3.3. Biomass and allometric relationships

The DBH of trees did not differ significantly between species or soil metal sites (Table 3). The mean age (in years) of *P. deltooides* in LM, MM, and HM was 8.6 ± 0.95 , 6.5 ± 1.4 , and 12.1 ± 1.5 , respectively (data not shown). The mean age (in years) of *P. tremuloides* in LM, MM, and HM was 11.2 ± 1.6 , 7.8 ± 2.1 , and 5.5 ± 0.62 , respectively (data not shown). In terms of tree height, TBW, TLW, LA, and TTM, significant differences were discovered to be due largely to the effect of DBH (Table 3).

Some significant differences between trees, in terms of biomass, were found to be due to effects other than DBH. Total branch weight

did differ significantly between species and soil metal concentration (Table 3). In LM and HM, *P. deltooides* displayed a greater TBW compared to *P. tremuloides*, where the TBW was double and nine-fold that of *P. tremuloides* in LM and HM, respectively (Table 3). Only in the MM sites, was TBW of *P. tremuloides* greater than that of *P. deltooides* (by roughly 28%, Table 3). Furthermore, *P. deltooides* displayed the largest TBW in HM, being roughly 63% and fourfold greater compared to growing in LM and MM, respectively (Table 3). *P. tremuloides* displayed the greatest TBW in LM, being approximately 6% and threefold greater compared to when growing in MM and HM, respectively (Table 3). Leaf area of trees did not differ significantly between species, but did differ significantly between soil metal sites (Table 3). For both *P. deltooides* and *P. tremuloides*, LA was greatest in LM, compared to MM and HM. For *P. deltooides*, LA was greater in LM compared to MM and HM by roughly threefold and 36%, respectively (Table 3). For *P. tremuloides*, LA was greater in LM compared to MM and HM by roughly 43% and threefold, respectively (Table 3).

Trees displayed a significant difference in the relationship between tree age and DBH, with statistically different slopes between soil metal concentration categories ($df = 5$, $P = 0.02$, $\alpha = 0.05$), however, the two study species did not differ in the relationship between tree age and DBH within HM, MM, or LM (Fig. 1). However, *P. deltooides* from LM differed significantly from *P. deltooides* in MM sites with statistically different slopes ($P = 0.03$, $\alpha = 0.05$). In particular, *P. deltooides* generally displayed a greater DBH at a given age when growing in LM, compared to MM, after roughly age 10 (Fig. 1). Trees of different species and with different soil metal concentrations did not differ in the relationships between tree age and height with statistically similar slopes ($P = 0.16$), tree age and LA with statistically similar slopes ($P = 0.10$) or DBH and TTM (Fig. 2), with statistically similar slopes ($P = 0.28$) and y-intercepts

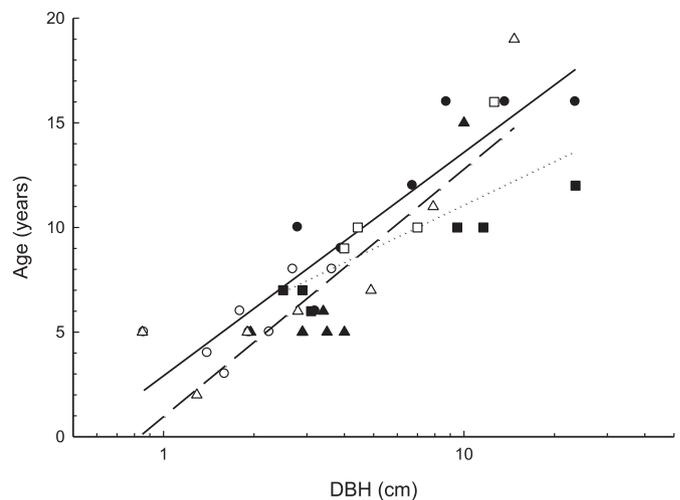


Fig. 1. Relationship between diameter at breast height (DBH) and tree age for *P. deltooides* (PD) and *P. tremuloides* (PT) in high soil metal concentrations (HM), medium soil metal concentrations (MM), and low soil metal concentrations (LM). Closed symbols represent *P. deltooides* and open symbols represent *P. tremuloides*. HM is indicated by circles and solid lines, MM is indicated by triangles and dashed lines, and LM is indicated by squares and dotted lines. Separate linear regressions were fitted to each soil metal concentrations category with both species combined as species did not differ within the same soil metal concentration category: $y = 10.6x + 2.9$ ($r^2 = 0.85$, $P < 0.0001$) for PD and PT in HM ($n = 7$ and 8 , respectively), $y = 11.8x + 0.95$ ($r^2 = 0.73$, $P < 0.0001$) for PD and PT in MM ($n = 7$ and 7 , respectively), and $y = 6.9x + 4.2$ ($r^2 = 0.61$, $P = 0.007$) for PD and PT in LM ($n = 6$ and 4 , respectively). Separate linear regressions (not shown) were calculated for PD in MM and PD in LM as PD differed significantly between MM and LM, where $y = 15.3x - 1.9$ ($r^2 = 0.80$, $P = 0.005$) for PD in MM ($n = 7$) and $y = 5.6x + 4.1$ ($r^2 = 0.94$, $P = 0.001$) for PD in LM ($n = 6$). Note logarithmic scale used on x-axis.

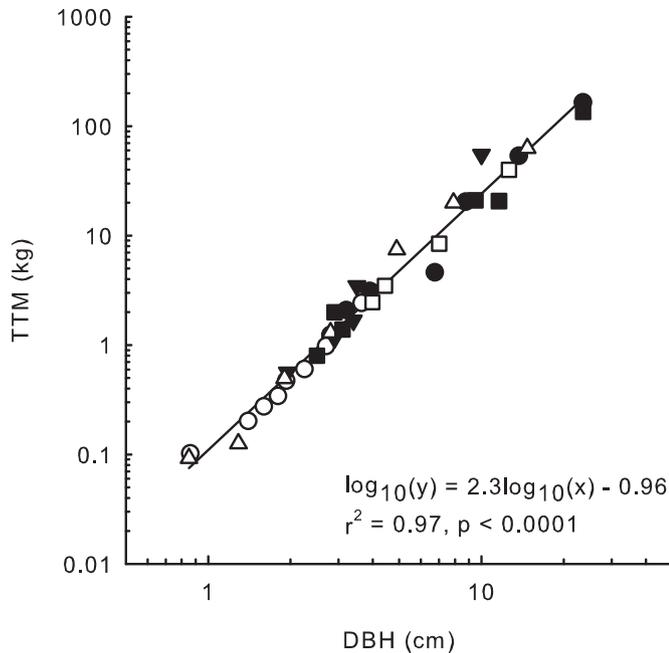


Fig. 2. Relationship between diameter at breast height (DBH) and total tree mass (TTM) for *P. deltooides* and *P. tremulooides* in high soil metal concentrations (HM), medium soil metal concentrations (MM), and low soil metal concentrations (LM). Squares represent LM, triangles represent MM, and circles represent HM, while closed shapes indicate *P. deltooides* and open shapes indicate *P. tremulooides*. A single linear regression was applied to all data points in the plot ($y = 2.3x - 0.96$, $R^2 = 0.97$, Table 3, see text).

($P = 0.28$, Fig. 2).

4. Discussion

Soil metal conditions were not found to have a significant effect, with the exception of the CCP and a species-soil metal concentration interaction for transpiration, on the gas exchange performance of either of the two study species (Table 1). Such findings are not entirely unusual as others have reported finding no apparent effects of heavy metals on photosynthesis from trees growing in the same brownfield (Renninger et al., 2013), though non-field studies have reported reduced rates of photosynthesis in plants exposed to heavy metals (Di Baccio et al., 2003; Hermle et al., 2006; Menon et al., 2007; Romanowska et al., 2002). However, our findings could be expected to differ since natural processes and interactions occurring in the field could offset heavy metal effects (Ahonen-Jonnarth and Finlay, 2001; Gorzelak et al., 2015; Shen et al., 2016; Van Der Heijden and Horton, 2009).

In the case of the CCP, *P. deltooides* experienced a greater CCP when growing in HM, though interestingly, not because CO_2 assimilation was lower or R_D was higher, as neither differed between soil metal sites (Table 1). Therefore, the higher CCP of *P. deltooides* in HM is not indicative of negative, metal-induced effects on CO_2 uptake. While soil metal concentration did not significantly affect transpiration for both species when averaged together, the significant species-metal sites interaction implies that one species was more strongly affected by soil metal conditions. In this case, it appears that *P. deltooides* reduced transpiration more strongly than *P. tremulooides*, which increased transpiration from LM to HM sites (Table 2). Therefore, soil metal concentration may impact water use efficiency in *P. deltooides* more so than in *P. tremulooides*. Isotopic intrinsic WUE was indeed different between soil metal sites, as well as displaying a significant species-soil metal sites interaction. Interestingly, $iWUE_{isotope}$ was actually lower for

P. deltooides in HM rather than LM, though transpiration was reduced in HM (Tables 1 and 2), suggesting that heavy metals may not induce water stress in *P. deltooides*, since if water availability was greater in HM than LM, *P. tremulooides* should have displayed a similar response regarding $iWUE_{isotope}$, rather than increasing $iWUE_{isotope}$ in HM. *P. deltooides* may be able to reduce metal uptake more strongly than *P. tremulooides* (Qian et al., 2012), and this would reflect in different water use efficiency, as uptake by roots is limited and greater uptake of metals reduces uptake of other resources such as water (Menon et al., 2005). Moreover, the two species do differ in leaf N concentrations as well as N source, indicated by $\delta^{15}N$, which also differed between soil metal sites and had a significant species-soil metal site interaction (Table 2), further implying an effect on water use efficiency (Field et al., 1983; van den Boogaard et al., 1995). However, NUE did not differ significantly between species or soil metal sites (Table 2). Considering that LSP is a public greenspace, it is not unlikely that N fertilization of the soil has occurred and that water uptake, rather than N availability, may be a limiting factor regarding differences in water use efficiency between the two species.

Differences in C allocation, which differed between species but not soil metal concentration, as indicated by leaf C:N while TLN did not differ (Table 2), could reflect differences in maintenance demand. Indeed, *P. deltooides* had greater biomass, as indicated by TBW, in HM while *P. tremulooides* had a smaller TBW in HM (Tables 1 and 3), which would reflect a lower maintenance requirement. Additionally, *P. tremulooides* averaged half the age in HM, compared to LM, while *P. deltooides* was roughly 3 years older on average, in HM, and it is reasonable that maintenance respiration increases with increasing age or size. Higher root maintenance and growth could also be a factor influencing respiration in response to nutrient acquisition and mycorrhizal colonization (Birhane et al., 2012).

As with photosynthetic performance, biomass was predicted to be greater for both species in LM. However, biomass, in terms of tree height, TBW, TLW, LA, and TTM, were largely explained by differences in tree DBH (Table 3). Soil metal concentration only significantly affected the TBW and LA, yet both study species did not display trends in TBW and LA that decreased with increasing soil metal concentration (Table 3). Therefore, the claim that biomass should be greater as soil metal contamination declines is not entirely substantiated.

Allometric relationships did not differ significantly, with the exception of DBH versus age (Fig. 1). Interestingly, *P. deltooides* in LM displayed a greater DBH at a given age than when growing in MM, suggesting that growth rate declines could be due to heavy metals, possibly requiring greater resource allocation, i.e. higher respiration to maintenance than growth. However, this difference was seen at around age 10, and *P. deltooides* growing in MM actually obtained greater DBH at a given age than those growing in LM before age 10 (Fig. 1). Additionally, no differences were found in the relationship between DBH and age for *P. deltooides* growing in LM and HM, while *P. tremulooides* did not differ across soil metal sites at all. It should be stressed that having the allometric relationships be conserved over the range of soil metal contamination and over different species is remarkable and hence the relationships are applicable to many situations (Fig. 2). This makes for simplifying scaling and estimating biomass (aboveground net primary production) and more consistency across species and edaphic conditions.

Nutrient availability can strongly influence heavy metal effects on plants (Shen et al., 2016; Silva et al., 2010; Zhang et al., 2011) with nutrient availability and uptake being closely tied to mycorrhizal associations, which also affect C allocation, as there is a C cost to the host plant (Allen et al., 2003). Furthermore, mycorrhizal associations can increase trees' tolerance to heavy metals (Ahonen-

Jonnarth and Finlay, 2001; Krumins et al., 2015; Lingua et al., 2008; Zhang et al., 2010). Members of the genus, *Populus*, are able to establish relationships with arbuscular, ectomycorrhizal, and endomycorrhizal fungi (Gehring et al., 2006; Lukac et al., 2003; Vozzo and Hacskaylo, 1974). *P. tremuloides* and *P. deltoides* associate with *L. bicolor* (Desai et al., 2014; Labbé et al., 2014) though extent of colonization could differ (Tschaplinski et al., 2014) while only *P. deltoides* has been reported to form endomycorrhizal associations (Behie et al., 2012; Vozzo and Hacskaylo, 1974). Considering that $\delta^{15}\text{N}$ differed between species and soil metal sites (Table 2), the two study species may be getting N from different sources due to varying mycorrhizal associations (Krumins et al., 2015). Indeed, recent examination of ectomycorrhizae at the same sites at LSP revealed that mycorrhizal fungal species diversity is similar across soils of varying soil metal sites, yet species composition changes (Evans et al., 2015), suggesting that in LSP, mycorrhizal fungal species do have differing levels of metal tolerance. Naturally, *P. deltoides* and *P. tremuloides* in LSP will experience differences in productivity associated with changes in available and compatible fungal symbionts across varying soil metal concentrations, which could explain differing biomass patterns. Furthermore, the species differences found regarding water use efficiency may be explained by differing mycorrhizal associations, as fungi can aid in excluding heavy metals from entering host plant tissues but also aid in water uptake (Ahonen-Jonnarth and Finlay, 2001; Khalvati et al., 2005; Khan et al., 2000). The mycorrhizal associations and interactions with other nutrients such as magnesium may also explain why these species succeeded in colonizing the abandoned brownfield site in the first place (Bennett et al., 2017; Shen et al., 2016). However, the situation is more complex as information emerges on the occurrence of common mycorrhizal networks (CMNs), which can interconnect different plant individuals, including those of different life stages (Beiler et al., 2010) and species (He et al., 2003) while also transferring C, nutrients, and water between connected individuals (Gorzela et al., 2015; Van Der Heijden and Horton, 2009; Walder et al., 2012).

Many other factors resulting from the nature of conducting field studies may be quite important as well and likely influence our results, though differences in environmental conditions such as precipitation are assumed to be similar for all trees studied given the small area of study. Considering that we examined trees that have already established themselves at the site and that seed germination and sapling growth are severely inhibited by heavy metals (Çurguz et al., 2012; Hermle et al., 2006; Menon et al., 2007; Tanentzap and Ryser, 2015; Wang and Jia, 2010), the existing self-selected trees in LSP may have some genetic advantage allowing them to cope with heavy metal stress. Furthermore, the clonal nature of both study species may exacerbate any such genetic advantages. The heterogeneous nature of metal concentrations in LSP (Gallagher et al., 2008a) is also an important factor, as different sites differ in the specific metal concentrations that are highest and different heavy metals differ in effect (Hermle et al., 2006; Menon et al., 2007; Trudić et al., 2012; Wang and Jia, 2010). These metal concentrations are also likely altered over time through varying environmental interactions, such as translocation, chelation (Khan et al., 2000; Mattina et al., 2003; Mehes-Smith and Nkongolo, 2015; Nocito et al., 2011; Rocciotello et al., 2016; Shen et al., 2016), and including species in LSP that are known to accumulate certain heavy metals (Gallagher et al., 2008a; Qian et al., 2012), however, thus far, not much overall change in soil metal concentration has occurred (Hagmann et al., 2015; Qian et al., 2012).

Contrary to our predictions, photosynthesis and growth performance did not necessarily decline as soil metal concentration increased, and when this pattern emerged, heavy metal concentration did not appear to be the determining factor. Nutrient status,

mycorrhizal associations, and genetic factors are suspected to be ameliorating factors in LSP that may be responsible for this masking effect. *P. deltoides* and *P. tremuloides* did display clear differences in responses to soil metal concentration regarding transpiration, CCP, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ with its derivatives (e.g long term water use efficiency, Table 2) as seen by its significant interaction between species and soil metal concentration, however, emphasizing that even if mitigating factors may be present under field conditions to offset heavy metal impacts, the strength of these factors will vary strongly by species. Therefore, caution should be taken regarding laboratory studies that isolate the impact of these factors on plant-heavy metal interactions as the species selected for study will strongly influence the effect of these mitigating factors in offsetting heavy metal effects and leading to successful phytoremediation projects. Improving the use of plants to remediate the world's contaminated soils will require an ecosystem approach that considers interactions between plants, soil biota, resources, and heavy metals over time.

5. Author contribution statement

Karina V. R. Schäfer designed the experiment. Karina V. R. Schäfer and Frank Gallagher contributed funds. Karina V. R. Schäfer and Dirk W. Vanderklein supervised and conducted the fieldwork. Diane Radwanski performed the data and statistical analysis. Diane Radwanski wrote the manuscript, and Karina V. R. Schäfer, Dirk W. Vanderklein, and Frank Gallagher edited the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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