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Relationships among Root–Shoot Ratio, Early Growth, and Health of Hybrid Poplar and Willow Clones Grown in Different Landfill Soils

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Abstract: Root–shoot allocation of biomass is an underrepresented criterion that could be used for tree selection in phytoremediation. We evaluated how root–shoot allocations relate to biomass production and overall health of poplar and willow clones grown in landfill soil treatments. Fifteen poplar clones and nine willows were grown in a greenhouse for 65 days in soils from five Wisconsin landfills and one greenhouse control. We tested for treatment, clone, and interaction differences in root–shoot ratio (RSR), health, and growth index, along with relationships between RSR with diameter, health, height, total biomass, and growth index. Treatments, clones, and their interactions were not significantly different for poplar RSR, but willow clones differed ($p = 0.0049$). Health significantly varied among willow clones ($p < 0.0001$) and among the clone × treatment interaction for poplars ($p = 0.0196$). Analysis of means showed that willow clones 'Allegany' and 'S365' exhibited 28% and 21% significantly greater health scores than the overall mean, respectively. Root–shoot ratio was not significantly correlated with health in either genus but was positively correlated with growth index for poplars, which was corroborated via regression analyses. Selecting clones based on a combination of biomass allocation, health, and growth indices may be useful for using phyto-recurrent selection to satisfy site-specific ecosystem services objectives.

Keywords: phytoremediation; hybrids; health; biomass production; phytotechnologies

1. Introduction

There are numerous phytotechnologies available for remediating contaminated sites. For example, through the use of woody plants like trees, phytoremediation aims to remediate many forms of soil and water pollution [1,2]. Common targets of phytoremediation include metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and industrial byproducts [3]. Remediation occurs through decontamination, the removal of pollutants through plant uptake, or stabilization, which alters the soil chemistry to stabilize the pollutant(s) [4]. Phytoextraction is a mode of decontamination in which plants accumulate contaminants and are later harvested. This removal of pollutants rather than stabilization prevents transfer to nearby groundwater, streams, and lakes. The degree of phytoextraction possible is specific to site conditions, pollutant bioavailability and plant characteristics [5,6]. Soil texture, pH, and pollutant concentration must all be within the limits of the plants used to enhance productivity and growth, which in turn will promote maximum phytoextraction. Because of the range in performance and tolerance among tree species, methods of species selection are vital to maximize potential remediation.

A recent development in phytotechnologies, phyto-recurrent selection is the screening and selection of readily available clones based on performance in experimental trials [7,8]. Superior clones are selected for their survivability, rooting ability, yield, and pest/disease resistance. Multiple testing cycles are employed to eliminate less desirable clones. As the number of clones left in a trial decreases, the complexity of the data increases. The data collected from the last cycle of a trial determines which clones to plant at a specific site. In one study, phyto-recurrent selection was used to select *Populus* clones for phytoremediation of landfill leachate [7]. The clones responded differently to leachate treatments for all traits tested in the first growing cycle. In the third growing cycle rooting differences were evident between leachate treatments, which was corroborated in the field [9]. This highlights the need for testing and selecting clones for site-compatible characteristics, which has been a focus of phytotechnologies research throughout the past decade [8].

Phytoremediation research has focused on ways to simultaneously maximize yield and root growth. *Populus* and *Salix* genera are ideal short rotation woody crops (SRWC) due to their high yield and fast growth [10]. *Populus* is also one of the most popular genera used for phytoremediation because of its rapid juvenile growth, ease of hybridization, and vegetative propagation [11]. Rapid growth and proper establishment at the start of a plant's life, especially when grown in contaminated soils, is essential to successful phytoremediation. Poplars can also regrow after multiple harvests and effectively remove inorganics from contaminated soil [12,13].

Like *Populus*, the genus *Salix* is a main source of clones for phytoremediation [4]. Willows and poplars are similar in that they both have the potential for high biomass productivity, effective nutrient uptake, and clone-specific capacity for taking up heavy metals [14]. Willows also exhibit high evapotranspiration, a key factor determining rapid chemical uptake in plants [15]. Such uptake of contaminants is useful in a variety of situations on different contaminated sites. For example, willows planted on land contaminated with dredged sediment containing mineral oil and polyaromatic hydrocarbons decreased the mineral oil composition almost four times more than decreases on a paired site where all vegetation was removed [16]. Willows can also experience enhanced growth when immersed in contaminated conditions. Higher yield, increased number of shoots, and increased plant dry mass have all occurred in willows treated with wastewater compared to control plants [14,17].

Currently, phytoremediation research on poplars and willows is focusing on understanding the role of biomass distribution, across genera and even among specific hybrid clones in response to contaminated soils [18]. The relationship between below- and above-ground biomass of a plant is known as the root–shoot ratio (RSR). Understanding the RSR provides insight to a plant's ability to perform across different sites or contaminant levels. Biomass ratios can vary among species, between plants of different sizes, and in differing moisture conditions [19,20]. In addition, nutrient availability, oxygen, light, and temperature can impact the biomass ratio in plants [21–23]. These factors emphasize the need to better understand RSRs and the genotype × environment interactions that influence them.

Biomass allocation is important to phytoremediation because it constitutes criteria for selecting superior site-specific clones. Research to date has shown immense variability in above- and below-ground biomass production among clones [24–26]. Not only does variation exist among clones but between clones grown in different soils as well. Willows specifically have been shown to have a near twofold increase in number of leaves and coarse roots when grown in clay versus sand substrates [27]. In particular, cuttings grown in the clay substrate exhibited higher net primary production, dry mass distribution, shoot height, or fine root number than those grown in sand. Marginal sites such as landfills exhibit similar soil heterogeneity that will likely result in variable biomass production and phytoremediation capability among clones. Across this diversity of site types, there is a need to determine which clones can survive, maximize growth and are most effective at remediating the site. For example, species with greater belowground biomass allocations may provide the greatest benefit in sites where the primary objective involves the roots, such as phytostabilization, rhizofiltration, or rhizodegredation. On the other hand, if the objective involves the aboveground processes of the plant, such as phytovolatilization or phytodegredation, species with greater aboveground biomass allocations should be implemented.

In addition to the variation in poplar and willow biomass allocation and health across different soil treatments, differences among clones have been shown to dictate success of these production systems [13]. For example, Wullschleger et al. [26] found that biomass allocation in poplars varies with genotype and age. They also conclude that root–shoot relationships should be investigated because of the importance of root distribution to carbon sequestration and phytoremediation. In a similar study, Barigah et al. [28] tested the differences in aboveground biomass production among five hybrid poplar clones after one year of growth and reported that all clones differed from one another, with the best clone producing over 3.5 times more aboveground biomass and aboveground biomass per area than the poorest-performing clone. Variation was attributed to phenotypic traits of the individual clones such as large individual leaf size, total leaf area, and photosynthetic rates. Results were similar for another small-scale study comparing four poplar clones, though the variation was not as distinct [29]. The range in RSRs was 0.26 to 0.36, with significant shifts in clonal biomass rankings across the two-year study. Thus, not only was there variation among same-aged clones in biomass production and allocation, but clonal variation over time as well. Knowledge of such variation in biomass allocations among clones, especially in regard to temporal variation, is important to the success of a phytoremedation site. Clones that exhibit greater belowground biomass allocations early on have the potential for better initial establishment in harsh field conditions (i.e., contamination, available nutrients, moisture levels) when compared to clones that do not. Successful establishment can lead to better tree health, and increased overall effectiveness of the phytoremediation at a site.

More research must be done to determine which clones are best for each allocation strategy, be it higher mass dedicated to shoots or roots, a balance between the two, or adaptability among all three. Scientists can then use biomass allocation data to choose clones that meet site-specific remediation goals like high root allocations to stabilize riparian buffer zones, high shoot allocations to allow for maximum evapotranspiration, or the ability to change between both at highly dynamic sites. In the current study, we tested the hypothesis that poplar and willow clones will vary in their root–shoot allocations when grown in different landfill soil treatments, both among clones and treatments. Furthermore, we assessed the relationships among RSR and the early growth and health of the clones. Methods and results will provide researchers with another criterion for the phyto-recurrent selection process regardless of specific genotypes or geographic locations.

2. Materials and Methods

2.1. Soil Collection and Site Description

Soils were collected from five closed solid waste municipal landfills in eastern Wisconsin, USA; Bellevue, Caledonia, Menomonee Falls, Slinger, and Whitelaw (Figure 1). Annual mean temperature across the landfills ranges from 6.8 to 8.8 °C while precipitation ranges from 749 to 876 mm [30]. Soil was collected down to a depth of 0.8 m from between 5 and 16 randomly selected points from each site. The total amount of soil collected from each site was 3.8 m^3. Soil samples were sieved (0.6 cm screen) and homogenized by site. The sieved soils were loaded into Agrimaster poly stock tanks (Behlen Manufacturing Company, Columbus, NE, USA) with a resulting individual tree soil volume of 0.02 m^3.

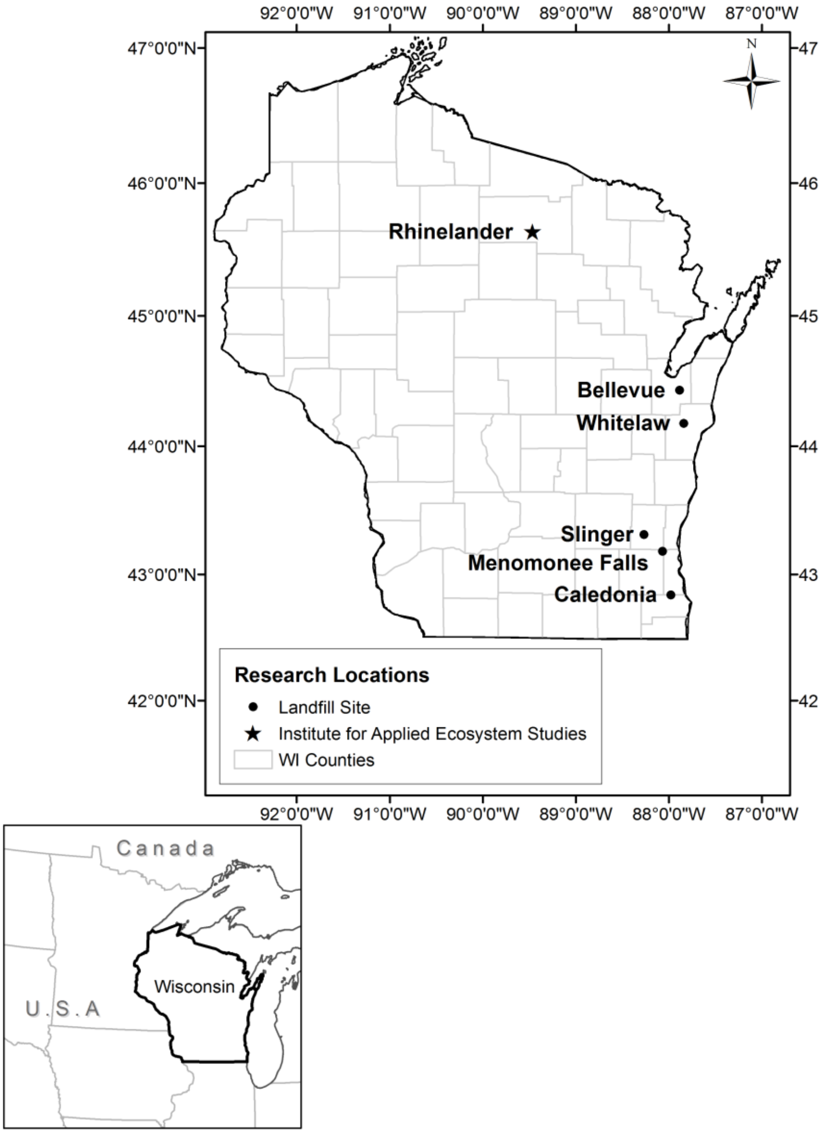

Figure 1. Locations of the five landfill sites where soil was collected and the phytotechnologies greenhouse where phyto-recurrent selection was conducted (Rhinelander, WI, USA) in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in the landfill soils.

2.2. Soil Properties

Six 25-mg subsamples of soil were collected from the sieved and homogenized soil of each site, processed, and subsequently sent to Northern Lake Service, Inc. (Crandon, WI, USA). The subsamples were analyzed for volatile organic compounds, including vinyl chloride, which were later determined to be undetectable at soil harvesting depths. Soil pH, texture, and chemical properties are listed in Table 1. The aim of the current study was to determine if RSR varied among clones, soils, or their interaction, and therefore we did not evaluate the uptake of particular soil components, nor their effect on tree growth.

Table 1. pH, texture, and chemical properties of soils collected from five landfills in eastern Wisconsin, USA in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown in the landfill soils for 65 days.

	Soil Treatment					
	Bellevue	**Caledonia**	**Menomonee Falls**	**Slinger**	**Whitelaw**	**Control**
pH	7.64 ± 0.02	7.20 ± 0.01	7.52 ± 0.02	7.37 ± 0.01	7.74 ± 0.02	4.49 ± 0.01
Texture	Clay Loam	Clay	Clay Loam	Clay Loam	Sandy Loam	-
	Percent					
Clay	38	48	28	28	7	-
Sand	37	21	33	25	55	-
Silt	35	31	39	47	38	-
C	0.024 ± 0.001	0.029 ± 0.004	0.064 ± 0.000	0.024 ± 0.001	0.041 ± 0.003	0.310 ± 0.005
N	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.007 ± 0.000
	g kg^{-1}					
Al	41.82 ± 0.56	52.46 ± 4.21	31.68 ± 0.30	45.71 ± 0.32	35.43 ± 4.62	22.75 ± 0.67
Ca	43.19 ± 1.78	30.51 ± 8.27	97.14 ± 1.12	23.22 ± 0.24	62.74 ± 8.14	11.24 ± 0.20
Fe	22.19 ± 0.30	29.56 ± 2.21	20.34 ± 0.18	26.29 ± 0.27	21.14 ± 2.11	16.61 ± 0.38
K	20.81 ± 0.24	19.57 ± 1.27	11.58 ± 0.36	13.88 ± 0.20	14.78 ± 1.69	4.43 ± 0.40
Na	5.40 ± 0.05	4.45 ± 0.12	4.14 ± 0.03	6.21 ± 0.05	4.95 ± 0.16	2.71 ± 0.15
Ti	2.56 ± 0.02	3.39 ± 0.24	2.03 ± 0.01	3.23 ± 0.01	2.35 ± 0.24	2.48 ± 0.06
Si	255.47 ± 3.59	234.58 ± 6.19	163.75 ± 1.42	263.10 ± 1.00	209.66 ± 3.23	76.98 ± 2.51
	mg kg^{-1}					
Mg	17.68 ± 0.63	21.14 ± 4.06	47.25 ± 0.31	15.08 ± 0.17	37.21 ± 3.85	25.53 ± 0.65
Mn	396.80 ± 8.50	648.96 ± 66.74	512.31 ± 4.55	790.67 ± 5.27	392.88 ± 66.65	257.87 ± 5.76
P	435.47 ± 8.75	426.05 ± 4.79	357.47 ± 5.89	445.51 ± 3.14	381.91 ± 9.96	380.88 ± 12.91
Sr	114.21 ± 0.58	101.06 ± 1.55	100.34 ± 0.27	103.01 ± 0.43	101.50 ± 1.36	56.84 ± 1.60
Zr	16.58 ± 0.15	16.55 ± 0.51	11.92 ± 0.38	23.77 ± 0.26	13.30 ± 0.94	2.00 ± 0.13

2.3. Genotype Selection and Experimental Design

Twenty-four clones were used in this study, fifteen poplar clones and nine willow clones. *Populus* and *Salix* cuttings were obtained from the USFS NRS Hugo Sauer Nursery (Rhinelander, WI, USA), Iowa State University Clonal Orchard at the Iowa Department of Natural Resources State Nursery (*Populus* only) (Ames, IA, USA), Michigan State University Clonal Orchard at the Tree Research Center (*Populus* only) (Lansing, MI, USA), University of Minnesota Natural Resources Research Institute Clonal Orchard at the North Central Research and Outreach Center Nursery (*Populus* only) (Grand Rapids, Minnesota, USA), and Double A Willow (*Salix* only) (Fredonia, NY, USA). These sources grew whips of the clones for one growing season in stool beds. Dormant, unrooted cuttings were processed to a length of 20.32 cm. Cuts were made to position at least one primary bud within 2.54 cm from the top of each cutting. Cuttings were then stored in polyethylene bags at 5 °C and subsequently soaked in water to a height of 10.16 cm for 48 h before planting. The trees were grown in the greenhouse with a 16-h photoperiod. Temperatures were held at 24 °C in the daytime and 20 °C in the nighttime. Trees received irrigation with unfiltered well water based on water demand. The trees of each selection cycle were arranged in a split-plot design with random block effects, fixed soil treatment whole plots, and fixed clone sub-plots. Three blocks and six soil treatments (soil from each of the five landfills plus a control of Jolly Gardner Pro-Line C/G Custom Growing Mix (a mix of Canadian sphagnum peat, medium perlite, and vermiculite) (Atlanta, GA, USA) were tested. For the purposes of this study, "soil treatment" is defined as these six different types of soil; soils were not treated or amended in any way. Clones themselves were arranged in randomized complete blocks in order to account for potential greenhouse environmental gradients. A total of 432 cuttings were planted, with 18 cuttings per clone, and three cuttings per clone per soil treatment. Trees were established in Agrimaster poly stock tanks with individual tank volumes of 0.26 m^3.

This project is part of a larger experiment that consisted of three phyto-recurrent selection growing cycles. The 24 clones used in this study were those that were selected for cycle 3 trials using weighted summation indices (described below). There were 15 hybrid poplar clones from four genomic groups and nine willow clones from six genomic groups (Table 2).

Table 2. *Populus* and *Salix* genomic groups and clones tested in phyto-recurrent selection cycle 3 in a study assessing how root–shoot allocations contribute to biomass production and overall health when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Genomic Group	Clone
Populus [a]	
P. deltoides × *P. maximowiczii* 'DM'	313.55, DM111, NC14106
P. deltoides × *P. nigra* 'DN'	9732-32, 9732-36, 21700, 99038022, 99038026, BR 3960, DN5, DN34, DN177
P. nigra × *P. maximowiczii* 'NM'	NM2, NM6
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* 'TDD'	NC13820
Salix [b]	
S. caprea hybrid 'C'	S365
S. miyabeana 'M'	SX67
S. purpurea 'P'	Allegany
S. purpurea × *S. miyabeana* 'PM'	Millbrook
S. sachalinensis × *S. miyabeana* 'SM'	Canastota
S. viminalis × *S. miyabeana* 'VM'	Fabius, Owasco, Tully Champion
S. viminalis × (*S. sachalinensis* × *S. miyabeana)* 'VSM'	Preble

[a] Sections and authorities for *Populus* are: *Aigeiros* Duby—*P. deltoides* Bartr. ex Marsh, *P. nigra* L.; *Tacamahaca* Spach—*P. maximowiczii* A. Henry, *P. trichocarpa* Torr. & Gray. [b] Sections and authorities for *Salix* are: *Cinerella* Seringe—*S. caprea* L.; *Helix* Dumortier—*S. purpurea* L., *S. miyabeana* Seemen; *Vimen* Dumortier—*S. sachalinensis* F. Schmidt; *Viminella* Seringe—*S. viminalis* L.

2.4. Weighted Summation Indices

In all cycles, weighted summation indices were used for phyto-recurrent selection [7]. Allometric traits (root dry mass; combined leaf and stem dry mass; number of roots; height; diameter; total leaf number; stem dry mass; leaf dry mass) were given weights (sum of weight = 1) based on their relative contribution to initial survival and early establishment. The weights were then multiplied by the adjusted or unadjusted means for each trait, and the values were added across all traits. Clones that exhibited greater relative index scores were selected to move on to the next cycle. The present study involves clones that advanced to cycle 3 through superior index scores in the previous two cycles.

2.5. Data Collection

Tree health was measured at 58 days after planting using a four-category qualitative scale ranging from 0 to 3, where 0 = dead, 1 = poor health, 2 = moderate health, and 3 = optimal health (Figure 2). Two researchers measured the health of all the clones to promote consistency in ratings. At 65 days after planting, height, measured from the point of attachment of the primary stem to the original cutting, and diameter, taken 1.5 cm from the point of attachment to avoid stem swell, were measured. Trees were harvested, washed, and dissected into roots, stems, leaves, and cuttings. All components were oven dried at 70 °C until constant mass was obtained. Root–shoot ratio (RSR) was calculated as the ratio of root dry mass to dry mass of stems + leaves. Cutting dry mass was not included in the RSR calculations.

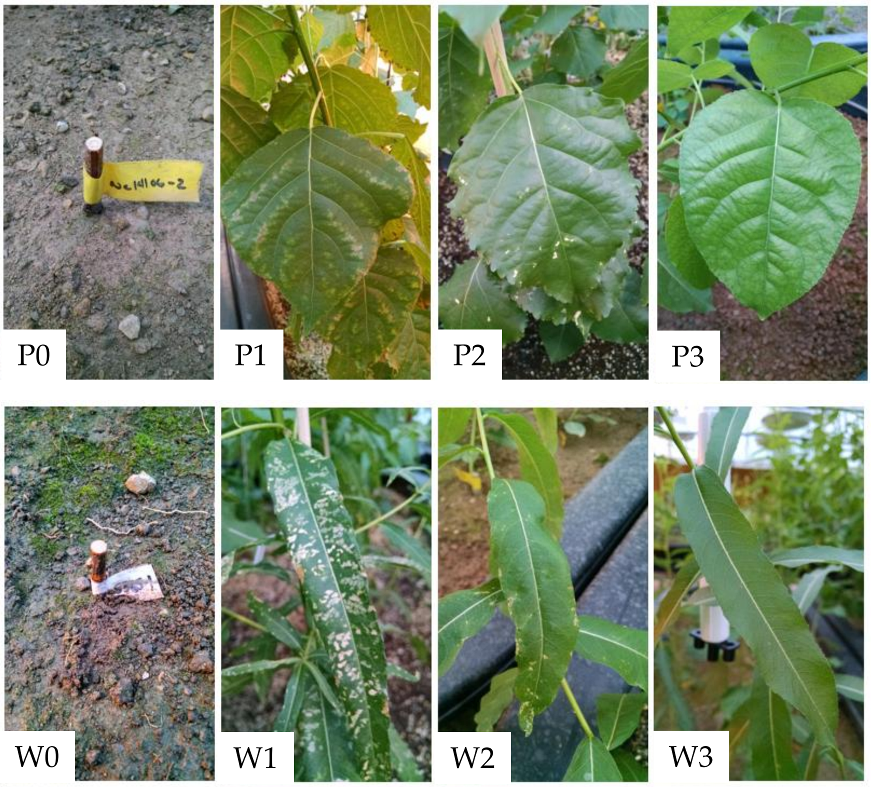

Figure 2. Photographs of tree health at 58 days after planting using a four-category qualitative scale ranging from 0 to 3, where 0 = dead, 1 = poor health, 2 = moderate health, and 3 = optimal health in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar (P) and willow (W) clones grown in soils from five landfills in eastern Wisconsin, USA.

2.6. Growth Index

A simplified growth index was created by calculating z-scores [31] for diameter, height, and number of leaves and then averaging the scores for each tree. This index was created separately for each genus. To make the index more intuitive we made all values positive by adding a constant equal to the absolute value of the lowest score in the data set. Higher values represented more robust trees.

2.7. Data Analysis

Height, diameter, total dry mass (above- plus below-ground), RSR, and growth index data were analyzed using analyses of variance, analyses of means, and correlation analyses according to SAS® (PROC GLM; PROC ANOM; PROC CORR; SAS Institute, Inc., Cary, NC, USA). These analyses assumed the split plot design with a random block effect and fixed main effects for soil treatment and clone. Analyses of covariance (ANCOVA) were conducted to test for the effect of cutting dry mass on all traits because of its broad variation at 65 days after planting (0.79 to 22.97 g). Cutting dry mass was a significant covariate for leaf dry mass, stem dry mass, and aboveground dry mass ($p < 0.05$) but did not have a significant effect on root dry mass ($p > 0.05$). Main effects and their interactions were considered significantly different at $p < 0.05$ according to comparison analyses using Tukey's adjustment. The Kruskal-Wallis test was used to test for differences in tree health scores. Again, significant effects were differentiated using Tukey's adjustment. Clones were classified as generalists or specialists based on their mean health scores. Generalists were clones whose mean health score (Section 2.5) was 2.7 or better in four or more of the six soil treatments. Specialist clones exhibited mean health scores of 2.7 or better in three or less of the treatments. All analyses were performed to assess the relationships among root–shoot allocations, early growth, and health of the trees for soil treatment × clone interactions, within genera.

To evaluate whether the relationships with RSR from the correlation analyses differed significantly by treatment or clone, ANCOVAs were carried out using PROC GLM in SAS®. Linear equations were fit for each response variable (diameter, height, total dry mass, and growth index) with experimental effects of treatment or clone, as well as the covariate RSR and interactions between RSR and the experimental effects. This approach allows testing for differences in the intercepts (via treatment or clone), whether the overall relationship with RSR differs from zero (via RSR), and whether the relationship with RSR differs by treatment or clone (via interactions with RSR). In all cases, statistical significance ($\alpha = 0.05$) was tested using F-ratios based on Type III sums of squares, and the coefficient of determination (r^2) for each model was recorded. When RSR interactions were significant, slope estimates for each treatment or clone were generated and t-tests were conducted to determine which parameter estimate(s) differed significantly from zero. When justified by the presence of significant experimental effects, the slope estimates were generated using treatment- or clone-specific intercepts.

3. Results

3.1. Analyses of Variance

Soil treatment, clone, and the treatment × clone interaction were not significant for RSR in poplars (Table 3). However, there were differences among clones for willows ($p = 0.0049$), with RSR ranging from 0.006 ± 0.011 (clone 'SX67') to 0.061 ± 0.009 ('Owasco') and an overall mean of 0.035 ± 0.009 (Figure 3). Root–shoot ratios among willow genomic groups differed without any noticeable patterns. In general, RSR was not significantly different among the top six clones. Although mean RSR for willow clones varied, they were not significantly different from the overall mean RSR (Figure 3). Despite a significant clone main effect ($p < 0.0001$), the treatment × clone interaction governed tree health for poplar ($p = 0.0196$) (Table 3). Of the poplars, 48% had optimal health (score = 3), 34% had moderate health (score = 2), 2% had poor health (score = 1), and 16% were dead (score = 0). Across clones, mean health scores were stable in individual soil treatments, with values varying by 0.2 health points (Table 4). Similarly, nine of the 15 poplar clones tested in cycle 3 performed as generalists, with mean health scores not significantly differing across soil treatments. The remaining six clones were specialists according to health scores. Clone 'DN34' had significantly greater health in soils from Menomonee Falls, Bellevue, and Slinger than soils from the other three sites, which were not different from one another. The remaining five specialist clones had significantly better health in one or two of the soil treatments.

Table 3. Probability values from analyses of variance in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Source of Variation	Root–Shoot Ratio	Tree Health	Growth Index
		Populus	
Soil treatment	0.1427	0.2877	**<0.0001**
Clone	0.0726	**<0.0001**	**0.0003**
Soil treatment × clone	0.9778	**0.0196**	0.1750
		Salix	
Soil treatment	0.0697	0.1842	**<0.0001**
Clone	**0.0049**	**<0.0001**	**0.0029**
Soil treatment × clone	0.3145	0.9550	0.0755

Means for root–shoot ratio of both genera were adjusted for the variation in cutting dry mass, which was a significant covariate (Populus, $p = 0.0027$; Salix, $p = 0.0048$). Significant effects are shown in bold.

Table 4. Mean tree health scores (n = 3) for each combination of soil treatment and hybrid poplar clone in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Performance Group	Genomic Group	Clone	Soil Treatment											
			Bellevue		Caledonia		Menomonee Falls		Slinger		Whitelaw		Control	
Generalist	DM	313.55	2.7 ± 0.3	a	3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a	2.3 ± 0.3	ab	2.7 ± 0.3	a
Generalist	DM	DM111	3.0 ± 0.0	a	3.0 ± 0.0	a	ne		2.3 ± 0.3	ab	2.0 ± 0.0	bc	2.3 ± 0.7	ab
Generalist	DM	NC14106	2.7 ± 0.3	a	3.0 ± 0.0	a	ne		3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a
Specialist	DN	9732-32	2.5 ± 0.4	ab	2.0 ± 0.0	bc	2.0 ± 0.0	bc	**2.7 ± 0.3**	**a**	2.3 ± 0.3	ab	2.0 ± 0.0	bc
Specialist	DN	9732-36	**3.0 ± 0.0**	**a**	2.0 ± 0.0	bc	2.0 ± 0.0	bc	2.5 ± 0.4	ab	**3.0 ± 0.0**	**a**	1.7 ± 0.3	c
Specialist	DN	21700	2.0 ± 0.0	bc	2.0 ± 0.0	bc	**3.0 ± 0.0**	**a**	2.3 ± 0.3	ab	**3.0 ± 0.0**	**a**	2.0 ± 0.6	bc
Specialist	DN	99038022	2.0 ± 0.0	bc	**2.7 ± 0.3**	**a**	2.0 ± 0.0	bc	**2.7 ± 0.3**	**a**	2.0 ± 0.0	bc	2.0 ± 0.0	bc
Generalist	DN	99038026	2.0 ± 0.0	bc	2.5 ± 0.4	ab	2.0 ± 0.6	bc	1.7 ± 0.3	c	2.0 ± 0.0	bc	2.0 ± 0.0	bc
Specialist	DN	BR 3960	**2.7 ± 0.3**	**a**	2.3 ± 0.3	ab	2.0 ± 0.0	bc	2.3 ± 0.3	ab	2.0 ± 0.0	bc	2.0 ± 0.6	bc
Generalist	DN	DN5	3.0 ± 0.0	a	2.7 ± 0.3	a	3.0 ± 0.0	a	3.0 ± 0.0	a	2.5 ± 0.4	ab	3.0 ± 0.0	a
Specialist	DN	DN34	**3.0 ± 0.0**	**a**	2.0 ± 0.0	bc	**3.0 ± 0.0**	**a**	**2.7 ± 0.3**	**a**	2.0 ± 0.0	bc	2.0 ± 0.0	bc
Generalist	DN	DN177	2.7 ± 0.3	a	2.5 ± 0.4	ab	3.0 ± 0.0	a	3.0 ± 0.0	a	2.3 ± 0.3	ab	2.7 ± 0.3	a
Generalist	NM	NM2	3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a	2.5 ± 0.4	ab	3.0 ± 0.0	a
Generalist	NM	NM6	3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a	3.0 ± 0.0	a	2.7 ± 0.3	a	3.0 ± 0.0	a
Generalist	TDD	NC13820	2.5 ± 0.4	ab	3.0 ± 0.0	a	3.0 ± 0.0	a	2.7 ± 0.3	a	3.0 ± 0.0	a	2.7 ± 0.3	a
		Across	2.7 ± 0.1		2.6 ± 0.1		2.6 ± 0.1		2.7 ± 0.1		2.4 ± 0.1		2.4 ± 0.1	

The control treatment was a standard greenhouse potting mix. Clones were categorized into performance groups based on their stability for tree health across sites. Means with the same letter were not significantly different at p = 0.05. ne = not estimable. Bold values indicate soil treatments wherein specialist clones performed better than with other landfill soils. Means labelled as ‘across’ represent the overall soil treatment means averaged across clones within a particular soil treatment. See Table 2 for genomic group descriptions.

Willows, on the other hand, only exhibited a significant clone main effect ($p < 0.0001$), with mean health scores ranging from 1.9 ± 0.2 ('Canastota') to 2.8 ± 0.1 ('Allegany') and an overall mean of 2.20 ± 0.05 (Figure 3). Of the willows, 30% had optimal health, 56% had moderate health, 10% had poor health, and 4% were dead. All genomic groups containing *Salix viminalis* showed below average health, in addition to those with *S. sachalinensis* × *S. miyabeana* parentage (i.e., 'Canastota' and 'Preble'). In contrast, analysis of means showed that clones 'Allegany' and 'S365' exhibited 28% and 21% significantly greater tree health than the overall mean, respectively (Figure 3).

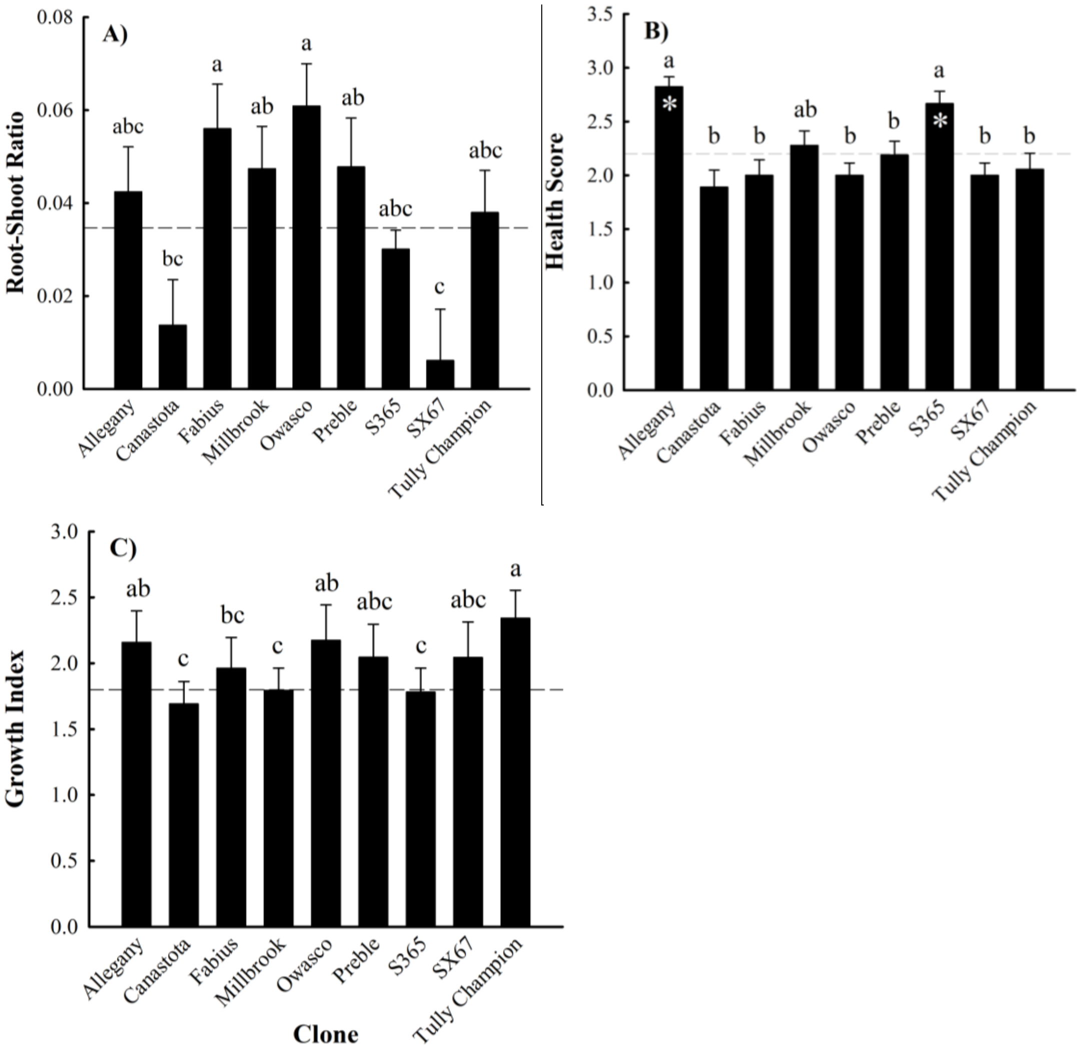

Figure 3. Root–shoot ratio (**A**), health score (**B**), and growth index (**C**) for the *Salix* clone main effect in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA. 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 Tukey's adjustment at $p > 0.05$.

Soil treatment and clone main effects were significant for growth indices in poplars and willows ($p < 0.05$), while their interaction was not significant for both genera ($p > 0.05$) (Table 3). Trends in growth indices for soil treatments were similar for poplars and willows, with the control exhibiting nearly twice the magnitude of any other treatment and 72% to 75% percent higher indices than the overall mean, respectively (Figure 4). For both genera, the growth index for Bellevue, Caledonia, and Slinger was the same, and Whitelaw and Menomonee Falls had the lowest indices of all soil treatments. Furthermore, the variability in growth index for willow clone main effects was intermediate of that for RSR and health score, and no clone exhibited a distinct advantage nor were any clones significantly different than the overall mean (Figure 3). In contrast, for poplars, clone 'BR 3960' exhibited 30% to 117% higher growth indices than the second best ('NM6') and worst ('313.55') clones, respectively (Figure 5). Also, the growth index of clone 'BR 3960' was 53% greater than the overall mean. Overall, trends for individual poplar genomic groups were non-existent for growth indices.

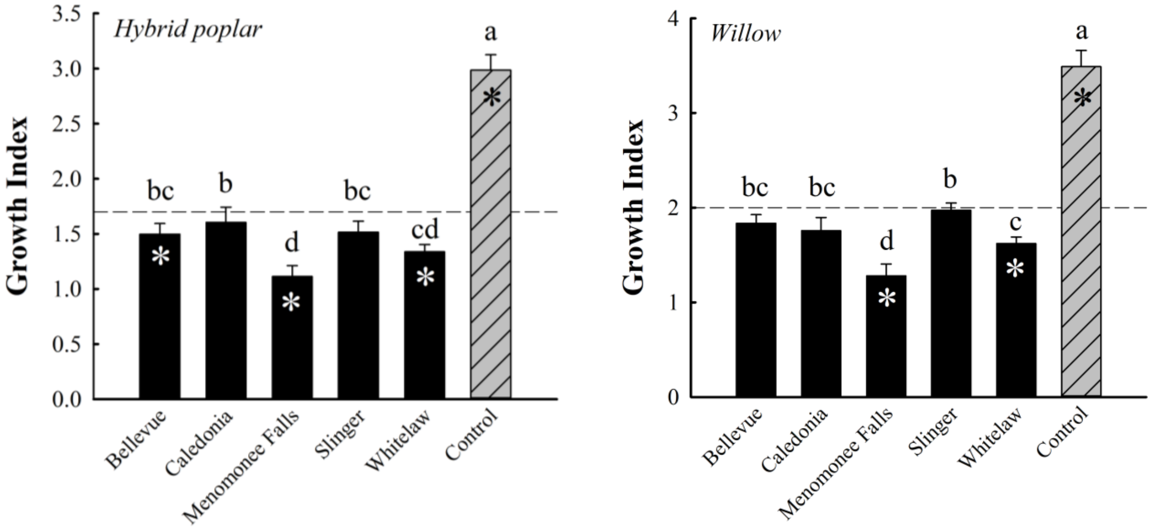

Figure 4. Growth index for the soil treatment main effect in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA. The dashed line represents the overall 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 Tukey's adjustment at $p > 0.05$.

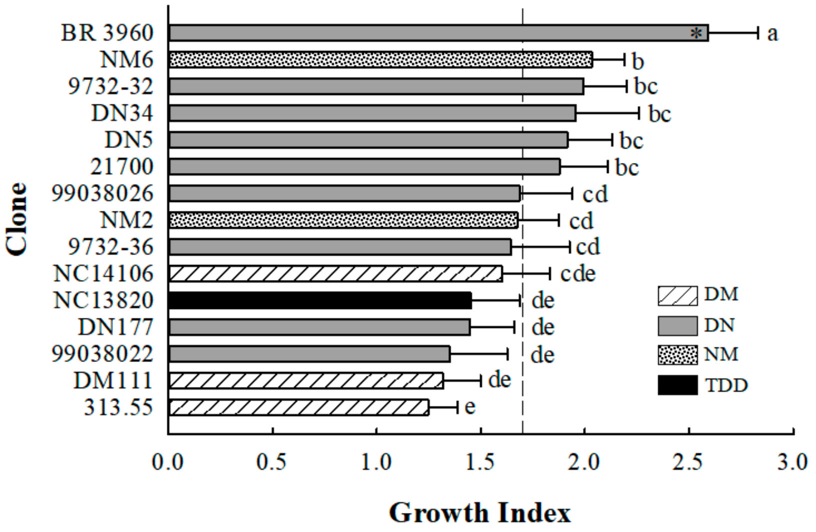

Figure 5. Growth index for the hybrid poplar clone main effect in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA. The dashed line represents the 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 Tukey's adjustment at $p > 0.05$. Shading of bars indicates different genomic groups; see Table 2 for their descriptions.

3.2. Correlations

Poplar phenotypic correlations ranged from −0.17 (health with diameter) to 0.95 (height with growth index) (Table 5). Root–shoot ratio was not significantly correlated with health ($r = -0.08$, $p = 0.2215$) but was correlated with height ($r = 0.36$, $p < 0.0001$), diameter ($r = 0.45$, $p < 0.0001$), total biomass ($r = 0.56$, $p < 0.0001$), and growth index ($r = 0.50$, $p < 0.0001$). All correlations not including RSR were significant except health with height ($r = -0.12$, $p = 0.0687$). For willows, phenotypic correlations ranged from −0.18 (health with diameter) to 0.96 (diameter with growth index) (Table 5). Root–shoot ratio was not correlated with health ($r = -0.09$, $p = 0.2826$), height ($r = 0.12$, $p = 0.1381$), nor growth index ($r = 0.14$, $p = 0.0865$), but was significantly correlated with diameter ($r = 0.20$, $p = 0.0143$) and total biomass ($r = 0.22$, $p = 0.0068$). All correlations not including RSR were significant ($p < 0.05$), except the correlation between health and growth index ($r = -0.13$, $p = 0.1155$).

Table 5. Phenotypic correlations among six traits in a study assessing the relationships among root–shoot ratio (RSR), early growth, and health of hybrid poplar (above diagonal) and willow (below diagonal) clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

	Diameter	Health	Height	RSR	Total Biomass	Growth Index
Diameter		−0.17 **0.0120**	0.91 **<0.0001**	0.45 **<0.0001**	0.89 **<0.0001**	0.94 **<0.0001**
Health	−0.18 **0.0245**		−0.12 0.0687	−0.08 0.2215	−0.15 **0.0213**	−0.18 **0.0066**
Height	0.91 **<0.0001**	−0.16 **0.0439**		0.36 **<0.0001**	0.85 **<0.0001**	0.95 **<0.0001**
RSR	0.20 **0.0143**	−0.09 0.2826	0.12 0.1381		0.56 **<0.0001**	0.50 **<0.0001**
Total biomass	0.80 **<0.0001**	−0.17 **0.0390**	0.75 **<0.0001**	0.22 **0.0068**		0.88 **<0.0001**
Growth index	0.96 **<0.0001**	−0.13 0.1155	0.94 **<0.0001**	0.14 0.0865	0.91 **<0.0001**	

Probability values are shown below the correlations. Significant correlations are shown in bold.

3.3. Analyses of Covariance

The ANCOVA results indicated that the relationship with RSR differed significantly by treatment for diameter, height, total dry mass, and growth index, but not for health (Table 6). This was true both for poplars and for willows. In general, the fit of treatment models was weak for health ($r^2 = 0.07$ to 0.10) but relatively strong for the other variables ($r^2 = 0.57$ to 0.81). Clones differed significantly in their relationship with RSR only for diameter and height, and even then only for willows and not for poplars. The fit of clone models was relatively weak in all cases ($r^2 = 0.16$ to 0.48), even for the willow models in which the relationship with RSR was found to differ significantly among clones ($r^2 = 0.19$ to 0.21) (Table 6).

For the models in which the relationship with RSR differed significantly by treatment or clone, the estimates of the slopes for each treatment or clone are shown in Tables 7 and 8, respectively. For poplars, the Whitelaw soils only showed a significant positive relationship of RSR with total dry mass, whereas all other soil treatments showed significant positive relationships with diameter, height, and growth index. All but one treatment (i.e., Menomonee Falls) also showed a significant positive relationship between RSR and total dry mass (Table 7). For willows, three treatments (Caledonia, Control, and Menomonee Falls) showed significant positive relationships between RSR and diameter, total dry mass, and growth index; two of those treatments (Caledonia and Menomonee Falls) also showed significant positive relationships between RSR and height (Table 7). In addition, four willow clones (‘Allegany’, ‘Owasco’, ‘SX67’, and ‘Tully Champion’) showed significant positive relationships between RSR and height; three of those clones ('Owasco', 'SX67', and 'Tully Champion') also showed significant positive relationships for diameter (Table 8).

Table 6. Results of analyses of covariance (ANCOVA) (i.e., *p*-values from *F*-tests of model effects and model r^2 values) for diameter, height, total dry biomass, and growth index in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Genus	Model	Diameter	Height	Total Biomass	Growth Index
Populus	T	**<0.0001**	**<0.0001**	**0.0451**	**<0.0001**
	RSR	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	T × RSR	**<0.0001**	**0.0009**	**<0.0001**	**0.0003**
	r^2	0.76	0.64	0.81	0.66
	C	0.6784	0.0233	0.5592	**0.0473**
	RSR	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	C × RSR	0.0597	0.0770	0.0553	0.0630
	r^2	0.43	0.40	0.48	0.42
Salix	T	**<0.0001**	**<0.0001**	**0.0008**	**<0.0001**
	RSR	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	T × RSR	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	r^2	0.69	0.57	0.76	0.72
	C	0.1344	0.0613	0.5170	0.2447
	RSR	**0.0016**	**0.0103**	**0.0004**	**0.0159**
	C × RSR	**0.0341**	**0.0202**	0.0572	0.0505
	r^2	0.19	0.21	0.20	0.16

Significant interactions with root–shoot ratio (RSR) (bold) indicate that soil treatments (T) or clones (C) differed in their relationship with RSR.

Table 7. Slope parameter estimates and associated *p*-values for the relationship between root–shoot ratio (RSR) and diameter, height, total dry biomass, and growth index in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Genus	Slope (Treatment)	Diameter		Height		Total Biomass		Growth Index	
		Est	*p*-Value	Est	*p*-Value	Est	*p*-Value	Est	*p*-Value
Populus	Menomonee Falls	45.4	**0.0006**	709.9	**0.0035**	106.9	0.0696	19.9	**0.0057**
	Control	57.1	**<0.0001**	628.7	**<0.0001**	419.8	**<0.0001**	19.2	**<0.0001**
	Bellevue	44.7	**<0.0001**	631.3	**0.0001**	161.2	**<0.0001**	23.0	**<0.0001**
	Slinger	32.2	**0.0056**	497.2	**0.0202**	128.1	**0.0143**	16.7	**0.0087**
	Caledonia	73.6	**<0.0001**	1011.7	**0.0003**	312.3	**<0.0001**	33.9	**<0.0001**
	Whitelaw	7.8	0.1527	105.9	0.2957	61.0	**0.0141**	4.0	0.1809
Salix	Menomonee Falls	39.9	**<0.0001**	992.3	**<0.0001**	112.9	**0.0029**	19.1	**<0.0001**
	Control	30.3	**<0.0001**	360.9	0.0573	353.6	**<0.0001**	19.2	**<0.0001**
	Bellevue	−1.1	0.6915	−115.4	0.1426	14.3	0.2771	−1.7	0.2933
	Slinger	4.6	0.4018	86.7	0.5639	43.8	0.0828	1.5	0.6220
	Caledonia	25.3	**0.0014**	698.1	**0.0013**	98.7	**0.0062**	12.9	**0.0034**
	Whitelaw	11.0	0.0584	112.3	0.4797	48.9	0.0675	3.1	0.3404

Significant slopes (bold) indicate that the relationship with RSR for a given soil treatment differs from zero.

Table 8. Slope parameter estimates and associated *p*-values for the relationship between root–shoot ratio (RSR) with diameter and height in a study assessing the relationships among root–shoot allocations, early growth, and health of hybrid poplar and willow clones when grown for 65 days in soils from five landfills in eastern Wisconsin, USA.

Genus	**Slope (Clone)**	**Diameter**		**Height**	
		Est	***p*-Value**	**Est**	***p*-Value**
Salix	Allegany	17.4	0.0597	497.6	**0.0199**
	Canastota	12.8	0.3139	267.5	0.3615
	Fabius	0.5	0.9155	−41.4	0.6776
	Millbrook	12.4	0.0833	212.3	0.1955
	Owasco	17.3	**0.0045**	307.8	**0.0273**
	Preble	12.9	0.1058	126.3	0.4906
	S365	10.4	0.4382	−382.9	0.2166
	SX67	29.4	**0.0212**	663.7	**0.0237**
	Tully Champion	23.2	**0.0079**	376.9	0.0590

Significant slopes (bold) indicate that the relationship with RSR for a given clone differs from zero.

4. Discussion

Understanding biomass allocation among hybrid clones is important for phytoremediation because it can be used as a selection criterion in phyto-recurrent selection to determine which clones best match site-specific goals. The high variation in previous studies in biomass allocation among treatments, moisture conditions and even individual clones of poplars and willows highlights the need for incorporating biomass allocation into the phyto-recurrent selection process. Results of the present study were consistent with those of Rytter [27] regarding biomass allocations and health across different soil treatments (Table 3). As with Rytter [27], biomass production was significantly different across soil treatments, but not biomass allocations. The variation in RSR across treatments was marginally significant for willow clones ($p = 0.0697$), but the variation was less apparent among poplars ($p = 0.1427$). Similarly, soil treatments did not influence health of either genus, which may have resulted from the smaller sample sizes of cycle 3, short growing cycle (i.e., 65 days) relative to an entire field growing season (i.e., 110 days), and uniform climatic conditions in the greenhouse. Field-simulating moisture conditions in greenhouse trials have been linked with considerable variation of RSR in black willow cuttings [32]. Our consistent, adequate watering of trees prevented this heterogeneity from becoming evident.

Results of this study corroborated previous findings of variation among clones. In poplars, tree health varied significantly among clones, while in willows, both tree health and RSR varied significantly among clones (Table 3). Nevertheless, despite these univariate results, health was not significantly related to RSR in general, and the relationship did not differ significantly among treatments or clones. Thus, tree health in the current study does not appear to be strongly related to any sort of imbalance in above- versus below-ground partitioning of resources. In contrast, most metrics of poplar size (i.e., diameter, height, total dry mass, and growth index) showed significant positive relationships with RSR under most treatments. The two exceptions were Menomonee Falls and Whitelaw; the former showed significant positive relationships for all metrics except total dry mass, and the latter showed a significant positive relationship only for total dry mass (Table 6). For willows, the metrics of plant size showed significant positive relationships with RSR for only half the treatments. Thus, it appears the different soil conditions were mediating the extent to which RSR influenced enhanced growth, particularly for willow (Table 6). Furthermore, when accounting for treatment differences, total dry mass had the strongest relationship with RSR of all the variables considered in this study, as demonstrated by the associated models having the best fit for both poplar ($r^2 = 0.81$) and willow ($r^2 = 0.76$). Notably, the highest slope for total dry mass in both poplars and willows was observed for the control soil, which suggests that the levels and/or types of contaminants in the other soils may serve to “dull” the relationship between RSR and total biomass production to varying degrees.

The weaker fit of the clone models (Table 8) compared to the treatment models (Table 7) suggested that genotype differences were less important than soil differences in explaining the relationship between RSR and plant size metrics. However, significant differences among clones were observed for some size metrics in willows, with about a third of the willow clones having slopes that differ significantly from zero. This suggested that genotype can also play a role at times in determining the extent to which higher RSR is associated with improved performance. Overall, treatment and clone main effects were examined independently for the ANCOVA in this study, without regard for their potential interactions. While the evaluation of such interactions may provide further insights (e.g., whether treatment-specific slopes differ among clones), the replication within each treatment × clone combination ($n = 3$) in the current study was insufficient to justify fitting unique slopes for each of these combinations. Future studies with larger sample sizes are therefore recommended, so that these potential interactions can be explored.

Moreover, the soil treatment × clone interaction for health scores in poplars exhibited enough variation to classify specialist and generalist clones (Table 4). Average health scores for individual clones were compared across treatments, with nine clones being classified as generalists and six as specialists. Variation among clones was to be expected, due to genotypic and phenotypic differences. However, one rather surprising finding was that 'DN34' was classified as a specialist not a generalist, as anticipated. Clone 'DN34' is a classic generalist; it was bred over 100 years ago and has been used since then by countless researchers across many continents [33,34]. As such, 'DN34' is known across the poplar community to not only thrive but also outperform other clones in a multitude of conditions. In the present study, 'DN34' defied expectations and did not perform equally well in all of the six soil treatments. Instead, 'DN34' only had significantly superior health in three out of the six landfill soils. Similarly, clear underperforming and superior performing willows were also made apparent through the analyses of variance and analyses of means. Clones 'Canastota' and 'Preble' along with all genomic groups containing *Salix viminalis* had below average health, while clones 'Allegany' and 'S365' had greater than average health. These results may lead researchers to select 'Allegany' and 'S365' if clone health is a priority. Further research needs to be done to quantify the differences among clones so that best-performing clones may be selected based on RSR, health score, and growth index.

Despite promising results from the analyses of variance and analyses of means, correlation analyses did not reveal anticipated results. Although root biomass was significantly related to growth indices such that the indices explained 52% of its variability in poplars and 39% in willows (data not reported), RSR itself was not significantly correlated with health in either poplars or willows (Table 5). Two correlations were of particular interest: (1) RSR with total biomass and (2) health with total biomass, as they were both significant in poplars and willows. This study may not directly prove that balanced root–shoot ratios lead to increased health in poplar and willow hybrids, but the "health" component should be interpreted with caution. The health scores used in this study were not all-inclusive by any means; they did not take into account biomass production among other variables. Often in weighted summation indices of phyto-recurrent selection, total biomass production is heavily weighted. Researchers use total biomass production as an indicator of health. Clones with high production are viewed as strong, healthy, and superior, whereas those that produce relatively less biomass are considered weak or unfit. Keeping that in mind, results of this study related the biomass component of health to RSR. Since it is shown that a higher RSR is correlated to higher total biomass production, it can be concluded that RSR indirectly affects health.

5. Conclusions

Root–shoot ratio (RSR) is a valuable selection criterion that can be incorporated into phyto-recurrent selection of poplars and willows used for phytoremediation and associated phytotechnologies, particularly when used in weighted summation indices. In willows, RSR has been shown to vary among individual clones. This suggests that researchers should implement RSR as a selection criterion in weighted summation indices for the phyto-recurrent selection of willows.

The weight given to RSR will depend on site objectives, i.e., if soil stabilization is the main objective, clones with higher RSR may result in the greatest benefit. On the other hand, poplars did not show any variation in RSR among treatment, clone, or their interaction. RSR in poplars should not be discounted for use as a selection criterion, however. Only 15 poplar clones, out of the hundreds that exist, were used in this study, so there should be continued research on a larger array of poplar clones. Furthermore, these 15 clones were already selected for performance in these soils in the first two selection cycles; a relationship may exist among the clones that had been rejected. Both poplars and willows showed correlations between RSR and total biomass. These results indicate that to some degree, aboveground biomass production can be used to predict belowground biomass production but should be tested further in order to develop models that relate the two. Results of this study can serve as a baseline for similar analyses relating health, growth index, and RSR of these short rotation woody crops. It is worth noting that RSR is not intended to be a stand-alone metric for evaluating clone performance. Root–shoot ratio is most useful when weighed with and against other factors, as in the weighted summation indices mentioned above. Further research is needed to address the shortcomings depicted here, namely: cycle length and simplicity of clone health assessments. Furthermore, analyses should be advanced to the field where there will likely be a marked difference in biomass allocation, especially given varying climatic conditions and soil moisture regimes. Whether or not a correlation exists between the RSR of final-cycle greenhouse trees and biomass production of trees established in the field also warrants additional study. Scientists and site managers can use the methodologies and results of this study as a means for selecting the best clones to fulfill their individual objectives.

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References

1. Schnoor, J.L.; Licht, L.A.; McCutcheon, S.C.; Wolfe, N.L.; Carreira, L.H. Phytoremediation of organic and nutrient contaminants. *Environ. Sci. Technol.* **1995**, *29*, 318–323. [CrossRef] [PubMed]
2. Suresh, B.; Ravishanker, G.A. Phytoremediation: A novel and promising approach for environmental clean-up. *Crit. Rev. Biotechnol.* **2004**, *24*, 97–124. [CrossRef]
3. Robinson, B.H.; Green, S.R.; Mills, T.M.; Clothier, B.E.; van der Velde, M.; Laplane, R.; Fung, L.; Deurer, M.; Hurst, S.; Thayalakumaran, T.; et al. Phytoremediation: Using plants as biopumps to improve degraded environments. *Aust. J. Soil Res.* **2003**, *41*, 599–611. [CrossRef]
4. Mirck, J.; Isebrands, J.G.; Verwijst, T.; Ledin, S. Development of short-rotation willow coppice systems for environmental purposes in Sweden. *Biomass Bioenergy* **2005**, *28*, 219–228. [CrossRef]
5. Cunningham, S.D.; Shann, J.R.; Crowley, D.E.; Anderson, T.A. Phytoremediation of contaminated water and soil. In *Phytoremediation of Soil and Water Contaminants*; Kruger, E.L., Anderson, T.A., Coats, J.R., Eds.; American Chemical Society: Washington, DC, USA, 1997; pp. 2–17, ISBN 9780841235038.
6. Cunningham, S.D.; Ow, D.W. Promises and prospects of phytoremediation. *Plant Physiol.* **1996**, *110*, 715–719. [CrossRef] [PubMed]
7. Zalesny, J.A.; Zalesny, R.S., Jr.; Wiese, A.H.; Hall, R.B. Choosing tree genotypes for phytoremediation of landfill leachate using phyto-recurrent selection. *Int. J. Phytorem.* **2007**, *9*, 513–530. [CrossRef] [PubMed]
8. Zalesny, R.S., Jr.; Stanturf, J.A.; Gardiner, E.S.; Bañuelos, G.S.; Hallett, R.A.; Hass, A.; Stange, C.M.; Perdue, J.H.; Young, T.M.; Coyle, D.R.; et al. Environmental technologies of woody crop production systems. *BioEnergy Res.* **2016**, *9*, 492–506. [CrossRef]

9. Zalesny, J.A.; Zalesny, R.S., Jr.; Coyle, D.R.; Hall, R.B.; Bauer, E.O. Clonal variation in morphology of *Populus* root systems following irrigation with landfill leachate or water during two years of establishment. *BioEnergy Res.* **2009**, *2*, 134–143. [CrossRef]
10. Netzer, D.; Tolsted, D.; Ostry, M.E.; Isebrands, J.G.; Riemenschneider, D.; Ward, K. *Growth, Yield, and Disease Resistance of 7- to 12-Year-Old Poplar Clones in the North Central United States*; General Technical Report NC 229; U.S. Department of Agriculture, Forest Service, North Central Research Station: St. Paul, MN, USA, 2002; pp. 1–40.
11. Zalesny, R.S., Jr.; Stanturf, J.A.; Gardiner, E.S.; Perdue, J.H.; Young, T.M.; Coyle, D.R.; Headlee, W.L.; Bañuelos, G.S.; Hass, A. Ecosystem services of woody crop production systems. *BioEnergy Res.* **2016**, *9*, 465–491. [CrossRef]
12. Baldantoni, D.; Cicatelli, A.; Bellion, A.; Castiglione, S. Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements. *J. Environ. Manag.* **2014**, *146*, 94–99. [CrossRef]
13. Laureysens, I.; Blust, R.; De Temmerman, L.; Lemmens, C.; Ceulemans, R. Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. *Environ. Pollut.* **2004**, *131*, 485–494. [CrossRef] [PubMed]
14. Perttu, K.; Kowalik, P. *Salix* vegetation filters for purification of waters and soils. *Biomass Bioenergy* **1997**, *12*, 9–19. [CrossRef]
15. Dietz, A.C.; Schnoor, J.L. Advances in phytoremediation. *Environ. Health Perspect.* **2001**, *109*, 163–168. [CrossRef]
16. Vervaeke, P.; Luyssaert, S.; Mertens, J.; Meers, E.; Tack, F.; Lust, N. Phytoremediation prospects of willow stands on contaminated sediment: A field trial. *Environ. Pollut.* **2003**, *126*, 275–282. [CrossRef]
17. Holm, B.; Heinsoo, K. Municipal wastewater application to short rotation coppice of willows—Treatment efficiency and clone response in an Estonian case study. *Biomass Bioenergy* **2013**, *57*, 126–135. [CrossRef]
18. Isebrands, J.G.; Aronsson, P.; Carlson, M.; Ceulemans, R.; Coleman, M.; Dickinson, N.; Dimitriou, J.; Doty, S.; Gardiner, E.; Heinsoo, K.; et al. Environmental applications of poplars and willows. In *Poplars and Willows: Trees for Society and the Environment*; Isebrands, J.G., Richardson, J., Eds.; Food and Agriculture Organization (FAO) of the United Nations: Rome, Italy; CAB International, Inc.: Boston, MA, USA, 2014; Chapter 6; pp. 258–336, ISBN 978-1-78064-108-9.
19. Monk, C. Ecological importance of root/shoot Ratios. *Bull. Torrey Bot. Club* **1966**, *93*, 402–406. [CrossRef]
20. Lloret, F.; Casanovas, C.; Penuelas, J. Seedling survival of Mediterranean shrubland species in relation to root: Shoot ratio, seed size and water and nitrogen use. *Funct. Ecol.* **1999**, *13*, 210–216. [CrossRef]
21. Van der Werf, A.; Visser, A.J.; Schieving, F.; Lambers, H. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and slow-growing species. *Funct. Ecol.* **1993**, *7*, 63–74. [CrossRef]
22. Gedroc, J.J.; Mcconnaughay, K.D.; Coleman, J.S. Plasticity in root/shoot partitioning: Optimal, ontogenetic, or both? *Funct. Ecol.* **1996**, *10*, 44–50. [CrossRef]
23. Friend, A.L.; Coleman, M.D.; Isebrands, J.G. Carbon allocation to root and shoot systems of woody plants. In *Biology of Adventitious Root Formation*; Davis, T.D., Haissig, B.E., Eds.; Plenum Press: New York, NY, USA, 1994; pp. 245–273.
24. Adler, A.; Karacic, A.; Weih, M. Biomass allocation and nutrient use in fast growing woody and herbaceous perennials used for phytoremediation. *Plant Soil* **2008**, *305*, 189–206. [CrossRef]
25. Weih, M.; Nordh, N. Characterising willows for biomass and phytoremediation: Growth, nitrogen and water use of 14 willow clones under different irrigation and fertilisation regimes. *Biomass Bioenergy* **2002**, *23*, 397–413. [CrossRef]
26. Wullschleger, S.D.; Yin, T.M.; Difazio, S.P.; Tschaplinski, T.J.; Gunter, L.E.; Davis, M.F.; Tuskan, G.A. Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. *Can. J. For. Res.* **2005**, *35*, 1779–1789. [CrossRef]
27. Rytter, R. Biomass production and allocation, including fine-root turnover, and annual N uptake in lysimeter-grown basket willows. *For. Ecol. Manag.* **2001**, *140*, 177–192. [CrossRef]
28. Barigah, T.; Saugier, B.; Mousseau, M.; Guittet, J.; Ceulemans, R. Photosynthesis, leaf area and productivity of 5 poplar clones during their establishment year. *Ann. Sci. For.* **1994**, *51*, 613–625. [CrossRef]

29. Pallardy, S.; Gibbins, D.; Rhoads, J. Biomass production by two-year-old poplar clones on floodplain sites in the Lower Midwest, USA. *Agroforest. Syst.* **2003**, *59*, 21–26. [CrossRef]
30. U.S. Climate Data. Available online: http://www.usclimatedata.com/ (accessed on 23 April 2017).
31. Green, R. *Sampling Design and Statistical Methods for Environmental Biologists*; Wiley: New York, NY, USA, 1979; ISBN 9780471039013.
32. Pezeshki, S.R.; Anderson, P.H.; Shields, F.D. Effects of soil moisture regimes on growth and survival of black willow (*Salix nigra*) posts (cuttings). *Wetlands* **1998**, *18*, 460–470. [CrossRef]
33. Zalesny, R.S., Jr.; Hall, R.B.; Zalesny, J.A.; McMahon, B.G.; Berguson, W.E.; Stanosz, G.R. Biomass and genotype × environment interactions of *Populus* energy crops in the Midwestern United States. *BioEnergy Res.* **2009**, *2*, 106–122. [CrossRef]
34. Marmiroli, M.; Pietrini, F.; Maestri, E.; Zacchini, M.; Marmiroli, N.; Massacci, A. Growth, physiological and molecular traits in *Salicacae* trees investigated for phytoremediation of heavy metals and organics. *Tree Physiol.* **2011**, *31*, 1319–1334. [CrossRef]