

Supplementary Materials

Establishment of regional phytoremediation buffer systems for ecological restoration in the Great Lakes Basin, USA. II. New clones show exceptional promise

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Table S1. Probability values from analyses of variance for poplar clone groups grown in sixteen phytoremediation buffer systems (i.e., phyto buffers) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA and the Lake Michigan watershed of eastern Wisconsin, USA. Buffer groups correspond to year of planting. Significant values highlighted in the Results are bolded. See **Table 3** for clone group descriptions.

	Buffer	Clone group	Buffer × Clone group
----- 2017 Buffer group -----			
Health ₂₀₁₇₍₂₀₁₇₎	0.0006	<0.0001	0.1300
Health ₂₀₁₇₍₂₀₁₈₎	<0.0001	<0.0001	0.0233
Health ₂₀₁₇₍₂₀₁₉₎	<0.0001	0.0010	0.0010
MAI ₂₀₁₇₍₂₀₂₀₎	<0.0001	0.0010	0.9533
----- 2018 Buffer group -----			
Health ₂₀₁₈₍₂₀₁₈₎	<0.0001	0.1664	0.5329
Health ₂₀₁₈₍₂₀₁₉₎	<0.0001	<0.0001	0.0023
----- 2019 Buffer group -----			
Health ₂₀₁₉₍₂₀₁₉₎	<0.0001	0.6821	0.6166

MAI: Mean annual increment

Table S2. Probability values from repeated measures analyses of variance for poplar clone groups measured in 2017, 2018, and 2019 in sixteen phytoremediation buffer systems (i.e., phyto buffers) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA and the Lake Michigan watershed of eastern Wisconsin, USA. Buffer groups correspond to year of planting. Significant values highlighted in the Results are bolded. See **Table 3** for clone group definitions.

	Buffer	Clone group	Buffer × Clone group	Year	Buffer × Year	Clone group × Year	Buffer × Clone group × Year
----- 2017 Buffer group -----							
Height	<0.0001	0.2895	0.9852	<0.0001	<0.0001	<0.0001	0.2929
Diameter	<0.0001	0.1673	0.9951	<0.0001	<0.0001	0.0184	0.7866
Volume	<0.0001	0.2292	0.9973	<0.0001	<0.0001	0.0449	0.8963
----- 2018 Buffer group -----							
Height	<0.0001	0.0109	0.2562	<0.0001	<0.0001	0.0776	0.0791
Diameter	<0.0001	0.1525	0.4226	<0.0001	<0.0001	0.0192	0.0036
Volume	<0.0001	0.0325	0.0487	<0.0001	<0.0001	0.0213	<0.0001
----- 2019 Buffer group -----							
Height	<0.0001	0.0602	0.1271	<0.0001	<0.0001	0.2193	0.0542
Diameter	<0.0001	0.0599	0.0272	<0.0001	<0.0001	0.0034	0.0293
Volume	<0.0001	0.0038	0.0001	<0.0001	<0.0001	0.0014	<0.0001

Table S3. Diameter (cm) (\pm one standard error) of three poplar clone groups tested in five phytoremediation buffer systems (i.e., phyto buffers) established in 2018 (i.e., the **2018 Buffer Group**) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA and the Lake Michigan watershed of eastern Wisconsin, USA. Trees were measured following the 2018, 2019, and 2020 growing seasons.

Phyto buffer	Clone group ^a					
	NRRI				Experimental	Common
	9732-11	9732-24	9732-31	9732-36		
----- 2018 Measurement year -----						
BC: Bellevue (Central)	0.79 \pm 0.07	0.74 \pm 0.07	0.88 \pm 0.07	0.64 \pm 0.07	0.70 \pm 0.07	0.68 \pm 0.07
BE: Bellevue (East)	0.84 \pm 0.07	0.78 \pm 0.07	0.82 \pm 0.08	0.73 \pm 0.07	0.68 \pm 0.07	0.71 \pm 0.07
CW: Caledonia (West)	1.05 \pm 0.07	1.00 \pm 0.07	1.13 \pm 0.07	1.05 \pm 0.07	1.02 \pm 0.07	1.03 \pm 0.07
MA: Maniwoc	0.96 \pm 0.07	0.88 \pm 0.07	0.95 \pm 0.07	0.92 \pm 0.08	0.83 \pm 0.07	0.98 \pm 0.07
MQ: Marquette	0.50 \pm 0.08	0.63 \pm 0.09	0.67 \pm 0.08	0.64 \pm 0.09	0.57 \pm 0.07	0.56 \pm 0.07
----- 2019 Measurement year -----						
BC: Bellevue (Central)	3.04 \pm 0.28	3.05 \pm 0.28	3.07 \pm 0.28	2.66 \pm 0.28	2.78 \pm 0.28	2.97 \pm 0.28
BE: Bellevue (East)	2.92 \pm 0.28	2.80 \pm 0.28	2.79 \pm 0.30	2.78 \pm 0.28	2.78 \pm 0.28	2.84 \pm 0.28
CW: Caledonia (West)	3.86 \pm 0.28	3.38 \pm 0.28	4.28 \pm 0.28	3.33 \pm 0.28	3.85 \pm 0.28	3.97 \pm 0.28
MA: Maniwoc	4.31 \pm 0.28	3.78 \pm 0.28	3.95 \pm 0.28	3.57 \pm 0.30	3.91 \pm 0.28	4.39 \pm 0.28
MQ: Marquette	1.63 \pm 0.30	1.86 \pm 0.35	2.15 \pm 0.30	2.26 \pm 0.35	1.82 \pm 0.28	1.63 \pm 0.28
----- 2020 Measurement year -----						
BC: Bellevue (Central)	3.87 \pm 0.39	3.96 \pm 0.39	4.07 \pm 0.39	3.29 \pm 0.39	3.41 \pm 0.39	3.41 \pm 0.39
BE: Bellevue (East)	3.74 \pm 0.39	3.64 \pm 0.39	3.58 \pm 0.41	3.26 \pm 0.39	3.13 \pm 0.39	3.01 \pm 0.39
CW: Caledonia (West)	4.20 \pm 0.39	3.69 \pm 0.39	6.03 \pm 0.39	3.43 \pm 0.39	5.00 \pm 0.39	4.88 \pm 0.39
MA: Maniwoc	5.53 \pm 0.39	4.83 \pm 0.39	4.96 \pm 0.39	4.54 \pm 0.41	4.88 \pm 0.39	5.32 \pm 0.39
MQ: Marquette	1.36 \pm 0.41	1.52 \pm 0.49	2.04 \pm 0.41	2.10 \pm 0.49	1.89 \pm 0.39	1.61 \pm 0.39

^a 'NRRI' = promising genotypes bred, tested, and selected at the University of Minnesota Duluth, Natural Resources Research Institute (NRRI) for broad-ranging applications [36,38].

'Experimental' = genotypes with a rich history of testing but that are still at the experimental stage.

'Common' = genotypes commonly used for commercial and/or research purposes in the region.

Table S4. Diameter (cm) (\pm one standard error) of three poplar clone groups tested in five phytoremediation buffer systems (i.e., phyto buffers) established in 2019 (i.e., the **2019 Buffer Group**) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA. Trees were measured following the 2019 and 2020 growing seasons.

Phyto buffer	Clone group ^a						
	NRRI					Experimental	Common
	99038022	9732-11	9732-24	9732-31	9732-36		
----- 2019 Measurement year -----							
EE: Escanaba (East)	0.93 \pm 0.07	0.75 \pm 0.07	0.90 \pm 0.07	0.89 \pm 0.07	0.73 \pm 0.07	0.80 \pm 0.07	0.77 \pm 0.07
EW: Escanaba (West)	1.54 \pm 0.07	1.30 \pm 0.07	1.13 \pm 0.07	1.26 \pm 0.07	1.46 \pm 0.07	1.40 \pm 0.07	1.43 \pm 0.07
MU: Munising	0.98 \pm 0.07	0.64 \pm 0.07	0.78 \pm 0.07	0.62 \pm 0.07	0.84 \pm 0.07	0.70 \pm 0.07	0.81 \pm 0.07
ON: Ontonagon (North)	0.81 \pm 0.07	0.62 \pm 0.07	0.60 \pm 0.07	0.67 \pm 0.07	0.56 \pm 0.07	0.56 \pm 0.07	0.59 \pm 0.07
OS: Ontonagon (South)	0.83 \pm 0.07	0.77 \pm 0.07	0.79 \pm 0.07	0.67 \pm 0.07	0.65 \pm 0.07	0.66 \pm 0.07	0.66 \pm 0.07
----- 2020 Measurement year -----							
EE: Escanaba (East)	1.71 \pm 0.23	1.46 \pm 0.23	2.21 \pm 0.23	1.90 \pm 0.25	1.49 \pm 0.23	1.83 \pm 0.23	1.77 \pm 0.23
EW: Escanaba (West)	3.06 \pm 0.23	2.83 \pm 0.23	2.94 \pm 0.23	2.87 \pm 0.23	3.40 \pm 0.23	3.57 \pm 0.23	4.02 \pm 0.23
MU: Munising	2.70 \pm 0.23	2.03 \pm 0.23	2.70 \pm 0.23	2.07 \pm 0.23	2.69 \pm 0.23	2.70 \pm 0.23	3.07 \pm 0.23
ON: Ontonagon (North)	1.93 \pm 0.23	1.26 \pm 0.23	1.27 \pm 0.23	1.48 \pm 0.23	1.44 \pm 0.23	1.27 \pm 0.23	1.42 \pm 0.23
OS: Ontonagon (South)	1.58 \pm 0.23	2.07 \pm 0.23	1.94 \pm 0.23	1.69 \pm 0.25	1.72 \pm 0.23	1.67 \pm 0.23	1.73 \pm 0.23

^a ‘NRRI’ = promising genotypes bred, tested, and selected at the University of Minnesota Duluth, Natural Resources Research Institute (NRRI) for broad-ranging applications [36,38].

‘Experimental’ = genotypes with a rich history of testing but that are still at the experimental stage.

‘Common’ = genotypes commonly used for commercial and/or research purposes in the region.

22 = 99038022; 16 = 99059016; 36 = 9732-36; CO = Common; EX = Experimental

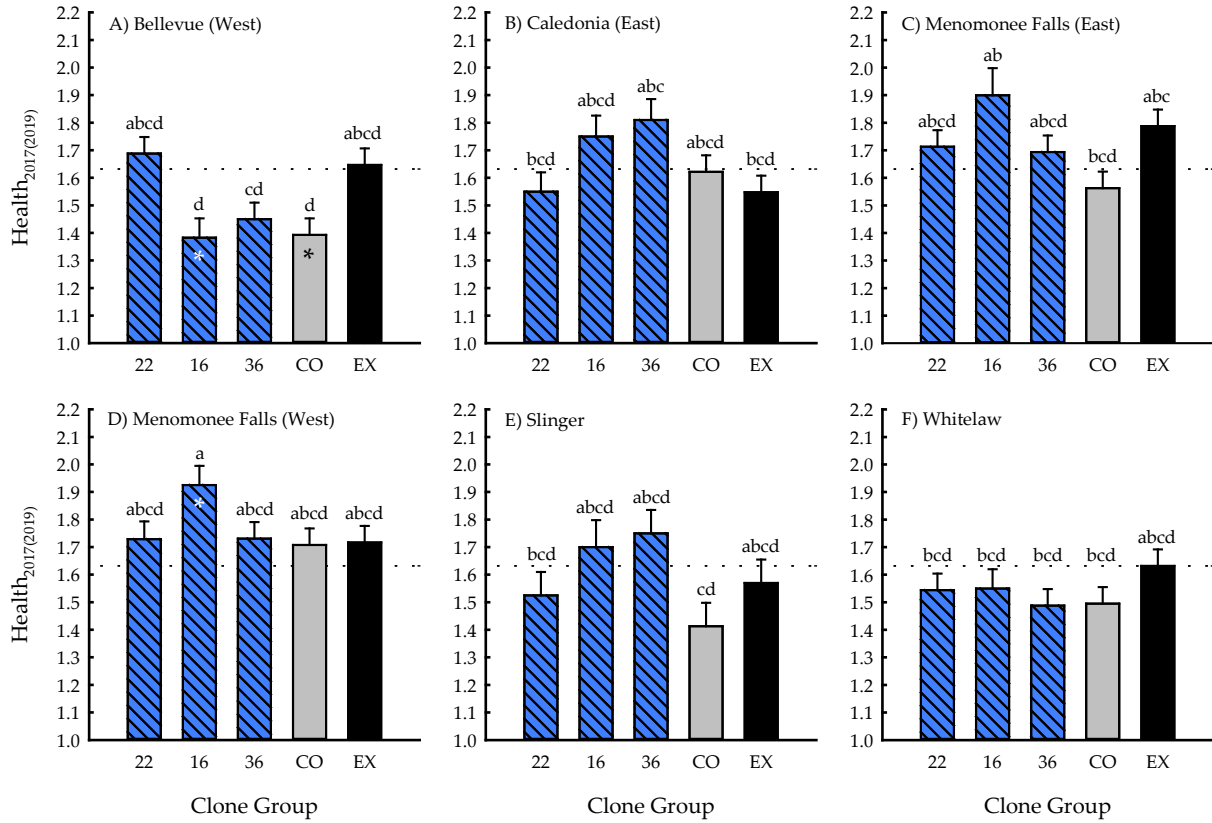


Figure S1. Tree health (\pm one standard error) determined after the 2019 growing season of three clone groups (i.e., NRRI = 22, 12, 36; Common; Experimental; see Table 3 for definitions) tested in six phyto remediation buffer systems (i.e., phyto buffers) established in 2017 (i.e., the 2017 Buffer Group) in the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times clone group combinations are different at $P < 0.05$. See Materials and Methods for complete tree health definitions (1 = optimal health, 2 = good health, 3 = moderate health, 4 = poor health, and 5 = dead).

11 = 9732-11; 24 = 9732-24; 31 = 9732-31; 36 = 9732-36; CO = Common; EX = Experimental

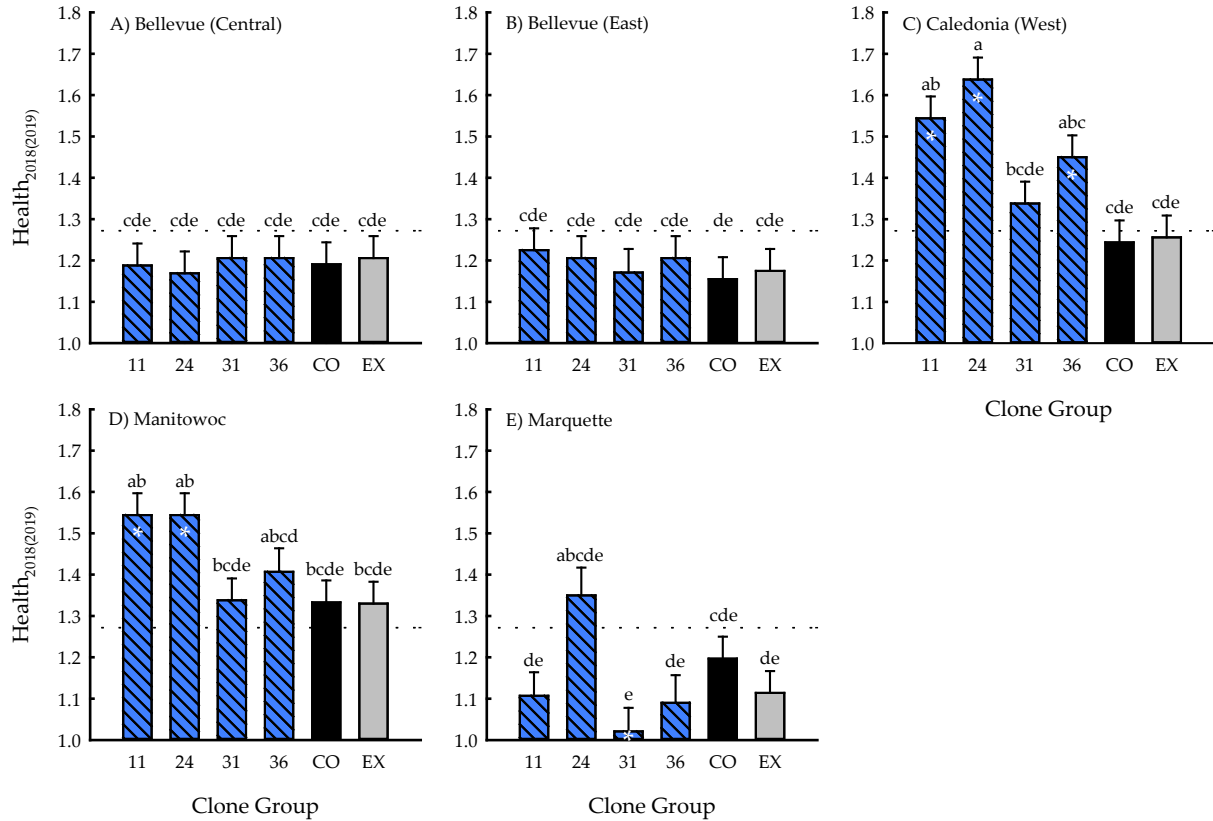


Figure S2. Tree health (\pm one standard error) determined after the 2019 growing season of three clone groups (i.e., NRRI = 11, 24, 31, 36; Common; Experimental; see **Table 3** for definitions) tested in five phyto remediation buffer systems (i.e., phyto buffers) established in 2018 (i.e., the **2018 Buffer Group**) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times clone group combinations are different at $P < 0.05$. See Materials and Methods for complete tree health definitions (1 = optimal health, 2 = good health, 3 = moderate health, 4 = poor health, and 5 = dead).

BW: Bellevue (West); CE: Caledonia (East); ME: Menomonee Falls (East); MW: Menomonee Falls (West); SL: Slinger; WH: Whitelaw

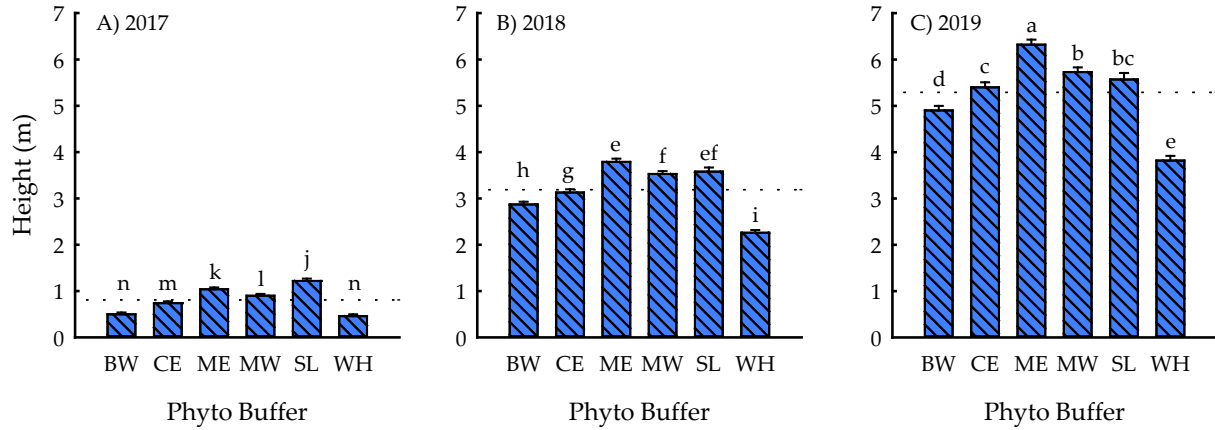


Figure S3. First- (A), second- (B), and third-year (C) height (\pm one standard error) of six phyto remediation buffers (i.e., phyto buffers) established in 2017 (i.e., the **2017 Buffer Group**) in the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times year combinations are different at $P < 0.05$.

BW: Bellevue (West); CE: Caledonia (East); ME: Menomonee Falls (East); MW: Menomonee Falls (West); SL: Slinger; WH: Whitelaw

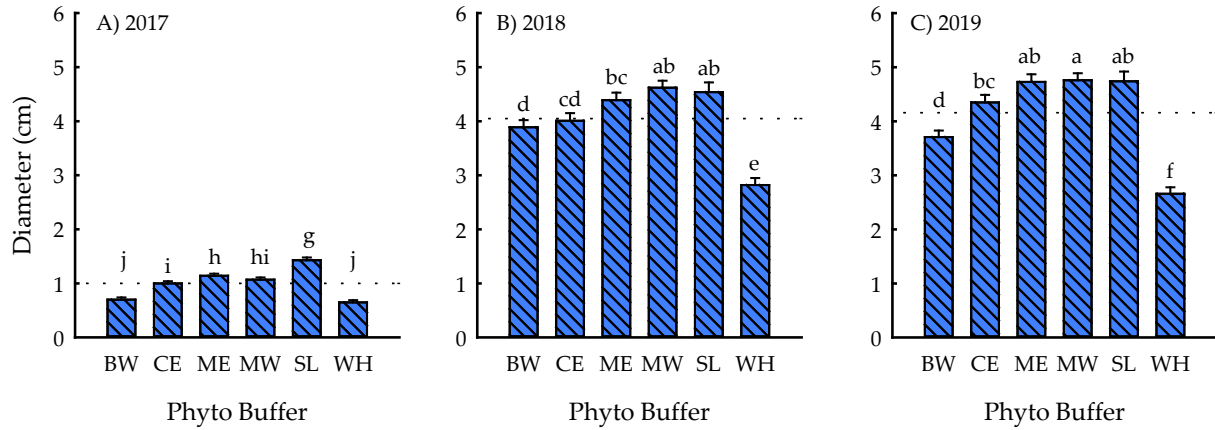


Figure S4. First- (A), second- (B), and third-year (C) diameter (\pm one standard error) of six phyto remediation buffers (i.e., phyto buffers) established in 2017 (i.e., the **2017 Buffer Group**) in the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times year combinations are different at $P < 0.05$.

BC: Bellevue (Central); BE: Bellevue (East); CW: Caledonia (West); MA: Manitowoc; MQ: Marquette

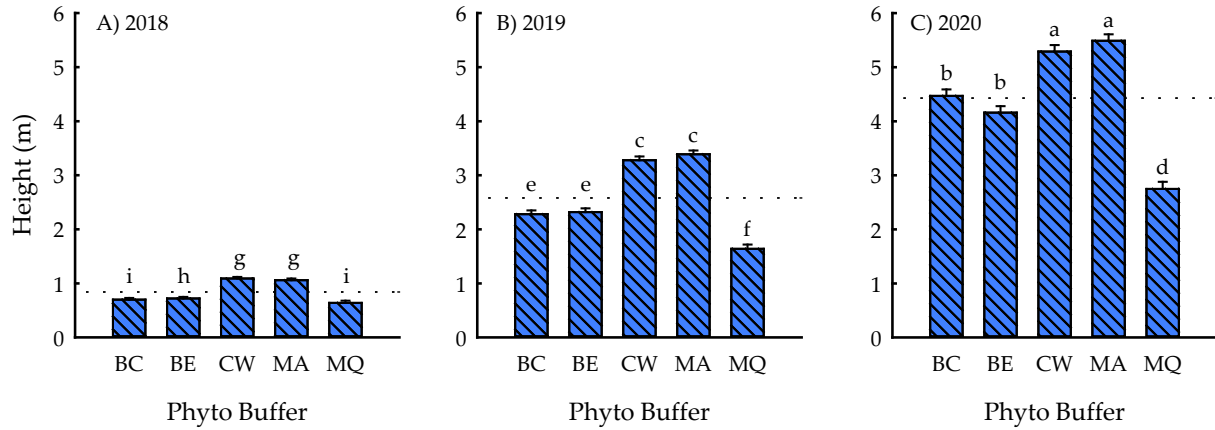


Figure S5. First- (A), second- (B), and third-year (C) height (\pm one standard error) of five phyto remediation buffers (i.e., phyto buffers) established in 2018 (i.e., the **2018 Buffer Group**) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA and the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times year combinations are different at $P < 0.05$.

EE: Escanaba (East); EW: Escanaba (West); MU: Munising
ON: Ontonagon (North); OS: Ontonagon (South)

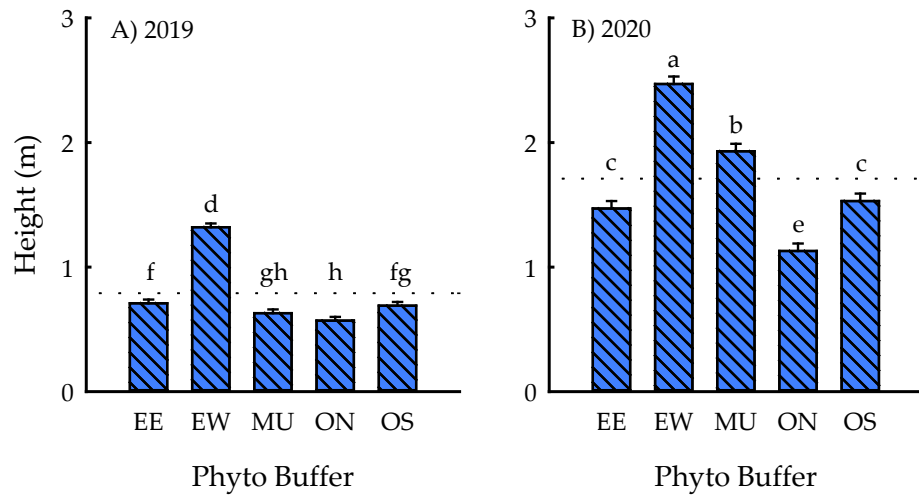


Figure S6. First- (A), second- (B), and third-year (C) height (\pm one standard error) of five phytoremediation buffers (i.e., phyto buffers) established in 2019 (i.e., the **2019 Buffer Group**) in the Lake Superior watershed of the Upper Peninsula of Michigan, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all buffer \times year combinations are different at $P < 0.05$.

22 = 99038022; 16 = 99059016; 36 = 9732-36; CO = Common; EX = Experimental

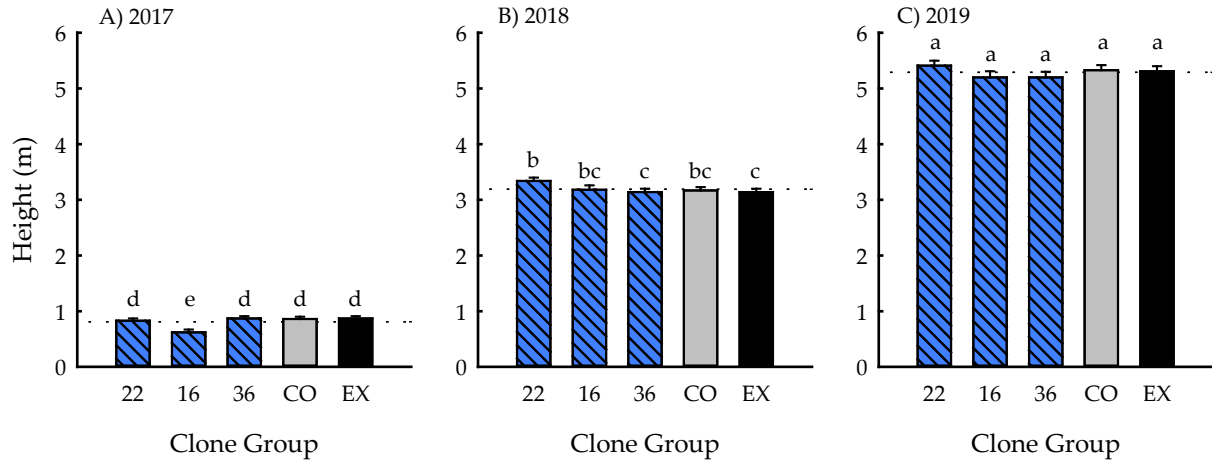


Figure S7. First- (A), second- (B), and third-year (C) height (\pm one standard error) of three clone groups (i.e., NRRI = 22, 16, 36; Common; Experimental; see **Table 3** for definitions) established in 2017 (i.e., the **2017 Buffer Group**) in the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all clone group \times year combinations are different at $P < 0.05$.

22 = 99038022; 16 = 99059016; 36 = 9732-36; CO = Common; EX = Experimental

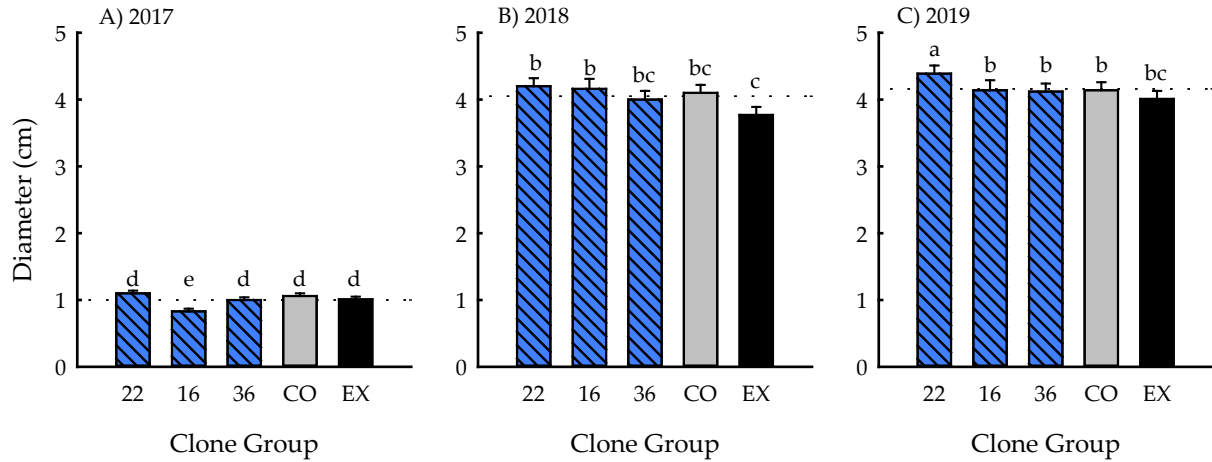


Figure S8. First- (A), second- (B), and third-year (C) diameter (\pm one standard error) of three clone groups (i.e., NRRI = 22, 16, 36; Common; Experimental; see **Table 3** for definitions) established in 2017 (i.e., the **2017 Buffer Group**) in the Lake Michigan watershed of eastern Wisconsin, USA. The dashed line represents the overall mean, and asterisks indicate means different than the overall mean at $P < 0.05$. Bars with different letters across all clone group \times year combinations are different at $P < 0.05$.