WIREs Wiley Interdisciplinary Reviews

ADVANCED REVIEW

Ecosystem services of poplar at long-term phytoremediation sites in the Midwest and Southeast, United States

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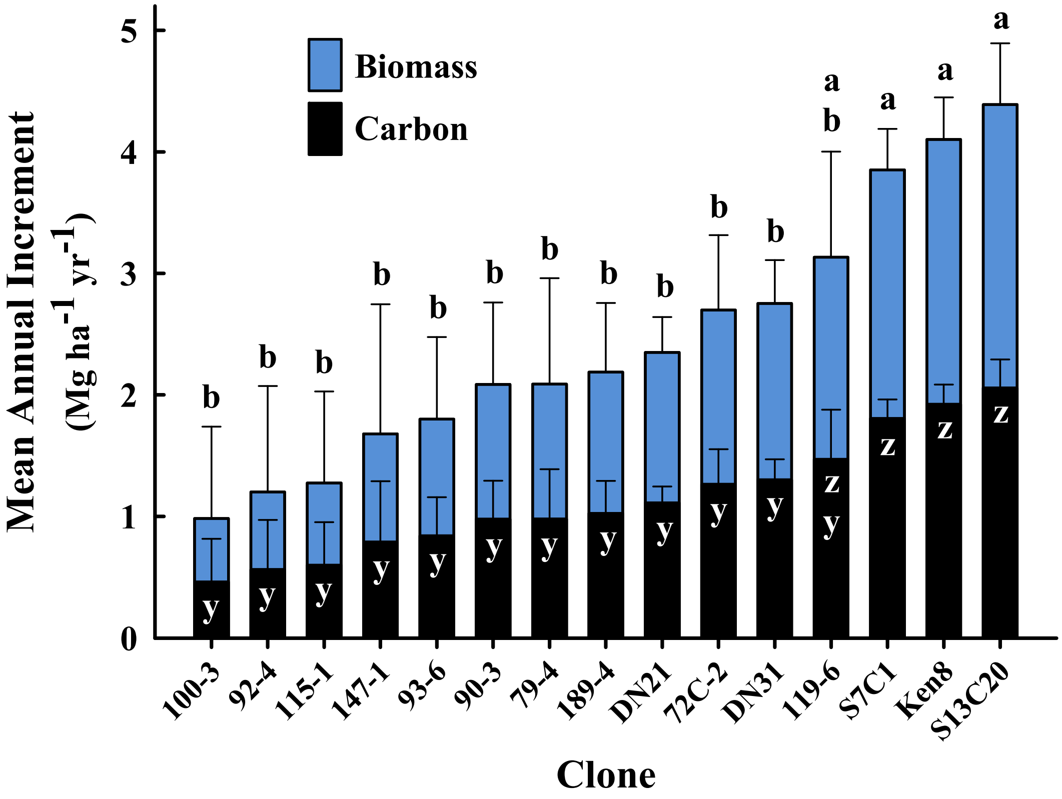
Conflict of interest: The authors have declared no conflicts of interest for this article.

ABSTRACT

Short rotation woody crops (SRWCs) including *Populus* species and their hybrids (i.e., poplars) are ideal for incorporating biomass production with phytotechnologies such as phytoremediation. To integrate these applications, fifteen poplar plantings from nine long-term phytoremediation installations were sampled from 2012 to 2013 in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) United States. In this review, we report summary results of this sampling and how performance at each site compared with comparable phytoremediation systems in the literature. We review significant genotypic differences from each planting within the context of *provisioning* (i.e., biomass production) and *regulating* (i.e., carbon sequestration) ecosystem services and how they relate to the need for a cleaner environment during times of accelerated ecological degradation. Overall, the contaminated poplar sites provided these ecosystem services comparable to non-contaminated poplar sites used for bioenergy and biofuels feedstock production. For example, phytoremediation trees at the Midwestern sites had biomass values ranging from 4.4 to 15.5 Mg ha^{-1} y^{-1}, which was ~20% less relative to bioenergy trees (P = 0.0938). Results were similar for diameter and carbon, with some genotype × environment interactions resulting in phytoremediation trees exhibiting substantially greater growth and productivity (i.e., +131% at one site). As illustrated in the current review, phytoremediation success can be increased with the identification and deployment of genotypes tailored to grow well and tolerate a broad diversity of contaminants (generalists) (i.e., 'DN34', 'NM6', '7300501') versus those that significantly outperform their counterparts under unique site conditions (specialists) (i.e., '220-5', '51-5', 'S13C20').

GRAPHICAL/VISUAL ABSTRACT AND CAPTION

Least-squares means of mean annual increment (MAI) of aboveground total (stem + branch) dry biomass and MAI of aboveground total carbon for fifteen poplar clones evaluated at an industrial brownfield in Panama City, FL (planting H1). With the exception of 'DN21' and 'DN31' that are both *Populus deltoides* × *P. nigra* F_1 hybrids, all clones belong to the *P. deltoides* genomic group. Different letters above bars within a trait represent statistically significant differences ($P < 0.05$). Error bars equal one standard error of the mean.



INTRODUCTION

Worldwide environmental degradation has reached alarming levels over the past decades, causing substantial ecological, economic, and social problems in both rural and urban areas (Donohoe, 2003). Regardless of where communities lie along the urban-to-rural gradient (McDonnell and Pickett, 1990), much of this degradation has resulted from anthropogenic impacts associated with agriculture, industrial manufacturing, and disposal of municipal and industrial waste (UNEP, 2012). Phytotechnologies are sustainable solutions that utilize plants to mitigate such degradation while moving towards restoration of ecosystem services across a diversity of spatial and temporal scales (Gopalakrishnan et al., 2009). Phytoremediation is one of the most common phytotechnologies, directly using plants to clean up contaminated soil, sediment, sludge, or groundwater (Arthur et al., 2005; Burges et al., 2018; Cunningham and Ow, 1996; McIntyre and Lewis, 1997; Schnoor et al., 1995). Mirck et al. (2005) described processes of phytoremediation with purpose-grown trees (e.g., phytostabilization, rhizofiltration, phyto- and rhizosphere-degradation, phytoextraction, phytovolatilization), which also include gaining hydraulic control of sites in order to contain the contaminants in one area or control the migration of the chemicals from the area (Burges et al., 2018; Ferro et al., 2001; Landmeyer, 2001; Vose et al., 2000; Zalesny et al., 2006).

Short rotation woody crops such as *Populus* species and their hybrids (hereafter referred to as poplars) are ideal for phytoremediation given their genetics and physiology (Dickmann and Keathley, 1996), in addition to having well-established silvicultural prescriptions that can be directly applied to phytotechnologies (Licht and Isebrands, 2005; Rockwood et al., 2004; 2013; Zalesny et al., 2016b). As model woody plants, poplars are among the most-studied trees in the world, with their genome (i.e., *P. trichocarpa* Torr. & Gray) being the first of all trees to be sequenced (Tuskan et al., 2006). There has also been extensive breeding and development of poplars for specific end uses (Stanton et al., 2014; Zalesny et al., 2016a), including phytoremediation (Isebrands et al., 2014). Hybridization of poplars is common given the broad amount of genetic diversity in parental populations which often involves transfer of favorable traits of interest (e.g., fast growth, extensive rooting, elevated water usage) leading to heterosis (i.e., hybrid vigor) (Ronald, 1982; Willing and Pryor, 1976). Selection of open-pollinated genotypes has also resulted in substantial gains from such tree improvement efforts (Eckenwalder, 1984). One of the primary objectives of such breeding is to choose generalist genotypes that perform well over a broad geographic range (over a broad range of contaminants in need of remediation) and/or to select specialist genotypes adapted to local site conditions (used for specific contaminants) (Orlovic et al., 1998; Stanturf et al., 2017; Zalesny et al., 2005a; Zalesny and Bauer, 2007c). As a result, current phytoremediation efforts utilize phyto-recurrent selection, a method involving the use of multiple testing cycles to evaluate, identify, and select favorable clones based on the response of genotypes to variable contaminants and site conditions (Zalesny et al., 2007b).

Matching superior genotypes with contaminants and their specific tissues (i.e., roots, wood, leaves) where the aforementioned processes take place helps to enhance the ecosystem services resulting from phytoremediation (Zalesny and Bauer, 2007a; Zalesny et al., 2016b). The Millennium Ecosystem Assessment (2005) defines four categories of ecosystem services: 1) *cultural* (the nonmaterial benefits obtained from ecosystems, e.g. values), 2) *supporting* (the natural processes that maintain other services, e.g. nitrogen cycle), 3) *provisioning* (the goods or products obtained from ecosystems, e.g. freshwater), and 4) *regulating* (the benefits obtained from an ecosystem's control of natural processes, e.g. soil quality). Among the primary objectives of using poplars for phytoremediation in the United States is to enhance aboveground biomass production (*provisioning* services) and carbon sequestration (*regulating* services) while mitigating environmental degradation in urban and rural communities.

Among other constraints (Nixon et al., 2001), one of the most challenging responsibilities for phytoremediation programs is the commitment and ability to continue measurements and monitoring throughout the rotation. Some long-term information exists, however, and has been useful for advancing the use of poplars in phytoremediation (Doucette et al., 2013; Erdman and Christenson, 2000; Madejón et al., 2013; Smesrud et al., 2012). To address this need for long-term results, during 2012 and 2013 we sampled 15 poplar plantings from 9 long-term phytoremediation installations located in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) United States. We determined diameter growth, biomass productivity, and carbon storage at various stages of poplar plantation development under site conditions with inorganic and/or organic contaminants, ranging in complexity from salts to petroleum hydrocarbons. In this review, we report summary results of this sampling and how performance at each site compared with comparable phytoremediation systems in the literature. We review significant genotypic differences from each planting within the context of *provisioning* (i.e., biomass) and *regulating* (i.e., carbon) ecosystem services and how they relate to the need for a cleaner environment during times of accelerated ecological degradation. Our results are useful for future researchers and resource managers developing phytoremediation projects tailored to their specific contaminant(s) and site conditions, especially in the context of integrating ecological restoration with ecosystem services.

MATERIALS AND METHODS

Phytoremediation Installations

Fifteen poplar plantings from nine long-term phytoremediation installations located in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) United States were sampled (Figure 1). A list of published studies from these installations is provided in Table 1. The locations (including their latitude and longitude), the number of plantings at each location, mean summer (i.e., June through August) temperature, and mean annual precipitation are described in Table 2. Specific latitude and longitude coordinates were not available for two locations (i.e., D: Midwest; I: Northeast NC), given landowner confidentiality agreements. Weather data represented 30-year climate normals (1981 to 2010) obtained from the National Climatic Data Center (NCDC) of the National Oceanic and Atmospheric Administration (NOAA) (www.ncdc.noaa.gov). In summary, latitudes ranged from 45.63 to 30.21 °N and longitudes ranged from 89.48 to 76.21 °W, with corresponding ranges for temperature of 18.2 to 27.7 °C and precipitation of 675 to 1,551 mm. Table 3 is a summary of individual plantings at each location. Contaminants of concern were both inorganic and organic, ranging in complexity from salts to petroleum hydrocarbons. Stocking ranged from very open at 434 trees ha^{-1} to very dense at 4,310 trees ha^{-1}. The plantings were established from 1998 to 2008 and were 5 to 15 years old at the time of measurements. Some plantings were comprised of single-clone tests, while the greatest number of genotypes sampled at any site was 34 clones, resulting in the number of experimental units at individual plantings ranging from 99 to 1,637 trees. In total, 55 clones belonging to ten genomic groups were tested (Table 4). These genotypes represented eight species from three taxonomic sections of the genus *Populus*. The clones represented superior selections from three breeding programs in the Midwest, one in the Pacific Northwest, one in the Southeast, and a collection of clones representing experimental and commercial controls (most of Canadian and European origin) – which to our knowledge is the greatest diversity of genotypes ever reported for phytoremediation in North America.

Measurements and Calculations

At each site, trees were measured for stem diameter at 1.37 m aboveground (aka diameter at breast height; DBH), and stand density (number of stems per unit area) was determined from stem spacing. These measurements were then used to estimate biomass mean annual increment ($\text{BIOMASS}_{\text{MAI}}$) and carbon mean annual increment ($\text{CARBON}_{\text{MAI}}$), as described below.

Aboveground woody biomass (stem + branches) was estimated from DBH using existing allometric biomass equations. Traditionally poplar researchers in the Midwest and Southeast have used a limited number of generalized biomass equations that do not allow for genotype-specific biomass estimation (Netzer et al., 2002; Shelton et al., 1982). Most recently, Zalesny et al. (2015) reported differences among genomic groups used in the current study (but not clones within the groups) for total aboveground biomass equations. These group-specific equations (Biomass = $a \times \text{DBH}^{b}$; see Table 5) were therefore used to estimate aboveground biomass from DBH for each tree, and the average biomass per tree was then multiplied by stand density to estimate total aboveground biomass per unit area. Finally, biomass per unit area was divided by stand age to determine biomass mean annual increment ($\text{BIOMASS}_{\text{MAI}}$), which is a commonly-used metric for biomass production and facilitates comparisons among sites by accounting for differences in stand age.

Carbon sequestration in aboveground biomass was estimated from $\text{BIOMASS}_{\text{MAI}}$ and information on carbon concentration of hybrid poplars. For carbon concentration, standard assumptions of wood being 50% carbon (Birdsey, 1992) are most often applied but are not robust considering the genetic variability across poplar genomic groups or clones. Thus, we used refined estimates based on a region-wide study testing the carbon storage potential of hybrid poplar in the North Central United States. In that study, clone-specific carbon estimates were developed for 11 clones grown across 17 sites (Headlee et al., 2013), from which we used mean genomic group values in the current study (Table 5). Specifically, $\text{BIOMASS}_{\text{MAI}}$ was multiplied by the group-specific carbon concentration to determine $\text{CARBON}_{\text{MAI}}$. Estimating belowground carbon sequestration was beyond the scope of the current sampling efforts because it was not possible to excavate root systems during aboveground measurements.

Statistical Analyses

Data for the growth parameters of DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were subjected to analysis of variance (ANOVA) using PROC GLM in SAS® (SAS Institute Inc., Cary, North Carolina, USA). For sites with only 1 genotype and no silvicultural comparisons, only means and standard errors of the growth parameters were computed. For sites with multiple genotypes and/or silvicultural treatments, ANOVA techniques (Littell et al., 2002) were used to test the null hypotheses of no significant differences among genotypes, treatments, and/or genotype × treatment interactions. If significant differences were detected ($P < 0.05$), then the least significant difference (LSD) approach was used to identify which genotype, treatment, and/or genotype × treatment means differed significantly from one another (here also, $P < 0.05$).

Spatial Analysis of Aboveground Total Carbon

Spatial information (i.e., tree locations within each planting, tree spacing) was available for seven of the fifteen plantings (Table 3). Of these seven sites, single genotypes were tested at plantings A3 [Oneida County Landfill (I)] and B1 (Argonne National Laboratory) while the other five contained 6 to 27 clones. Where data was available, the spatial distribution of $\text{CARBON}_{\text{MAI}}$ was developed for each planting using open source software QGIS 2.8 and statistical software R. The distribution of carbon hotspots and variation among clones for the 75th quantile of $\text{CARBON}_{\text{MAI}}$ was assessed for each planting and for all sites combined. For plantings A3 and B1 with single genotypes, only the spatial map of $\text{CARBON}_{\text{MAI}}$ was generated. The 75th quantile was selected because these are the locations where carbon storage was maximized for the sites and hence is of most interest in determining ecosystem services provided.

REVIEW OF ECOSYSTEM SERVICES AT PHYTOREMEDIATION INSTALLATIONS

Diameter, Biomass, and Carbon

Across All Plantings

Overall, diameter at breast height (DBH) ranged from 4.3 ± 0.1 cm for 5-year-old trees growing at a U.S. Coast Guard base in Elizabeth City, NC with petroleum hydrocarbons (planting E3) to 23.3 ± 0.6 cm for 14-year-old trees exposed to volatile organic compounds (VOCs) and tritium at the Argonne National Laboratory in Lemont, IL (planting B1). Mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$) ranged from 1.3 ± 0.1 to 15.5 ± 0.4 Mg ha^{-1} y^{-1} for 5-year-old trees with petroleum hydrocarbons at planting E2 in Elizabeth City, NC and 11-year-old trees subjected to a combination of salts, metals, and nitrates at an anonymous agricultural production facility in the Midwest (planting D1), respectively. Similarly, MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) ranged from 0.6 ± 0.1 to 7.3 ± 0.2 Mg C ha^{-1} y^{-1} for these sites.

A: Rhinelander, WI

Zalesny et al. (2006) described testing of poplar clone 'NM6' (*P. nigra* × *P. maximowiczii*) for phytoremediation of leachate containing nitrates at a former municipal landfill in Rhinelander, WI (plantings A1 and A2), which was also established with clone 'DN34' (*P. deltoides* × *P. nigra*). The primary objective of the phytoremediation efforts was to capture hydraulic control of the site in order to mitigate subsurface infiltration and off-site movement of the contaminants into an adjacent wetland near Slaughterhouse Creek. Hydraulic control consisted of volatization of most of the precipitation before it completely leached through the landfill content as well as uptake and filtering of contaminants through the transpiration stream. At 14.5 years after planting (planting A1), mean stand-level DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were 15.3 ± 0.4 cm, 5.0 ± 0.2 Mg ha^{-1} y^{-1}, and 2.4 ± 0.1

Mg C ha^{-1} y^{-1}, respectively. Clone 'NM6' produced significantly greater DBH (+66%), $BIOMASS_{MAI}$ (+305%), and $CARBON_{MAI}$ (+306%) than 'DN34' ($P < 0.0001$) (Table 6). Clonal means for DBH, $BIOMASS_{MAI}$, and $CARBON_{MAI}$, respectively, were: 18.2 ± 0.3 cm, 6.7 ± 0.2 Mg ha^{-1} y^{-1}, and 3.2 ± 0.1 Mg C ha^{-1} y^{-1} for 'NM6' and 10.9 ± 0.4 cm, 2.2 ± 0.3 Mg ha^{-1} y^{-1}, and 1.0 ± 0.1 Mg C ha^{-1} y^{-1} for 'DN34'. Based on the survival and performance of 'NM6' during establishment, the phytoremediation system was expanded to include a second planting of this genotype one year after the initial study was planted (planting A2). At 13.5 years after planting, DBH ranged from 3.4 to 26.4 cm, with a mean of 14.0 ± 0.3 cm, while mean $BIOMASS_{MAI}$ was 4.4 ± 0.2 Mg ha^{-1} y^{-1} and mean $CARBON_{MAI}$ was 2.1 ± 0.1 Mg C ha^{-1} y^{-1}.

Two additional phytoremediation studies were established at the Oneida County Landfill, located 6 km west of Rhinelander, WI. First, Zalesny et al. (2007b) described the use of phyto-recurrent selection to choose superior poplar clones for a phytoremediation system utilizing landfill leachate as irrigation and fertilization for poplar energy crops. Salts (primarily sodium and chloride) were the primary concern in the leachate. Previous studies had shown broad genetic variability in salt tolerance among poplar genomic groups and genotypes (Chen et al., 2002; Fung et al., 1998). In particular, Smesrud et al. (2012) provided information about the long-term (i.e., 15 years) implications of poplar silviculture (including clonal selection) on the success of high-salinity landfill leachate recycling systems. In the current study, as a result of three cycles of greenhouse testing, 25 clones were reduced to 8 genotypes that were outplanted in an *in situ* trial at the landfill (cycle 4). Zalesny et al. (2007a) described field testing of the 8 clones followed by selection of the most favorable genotype, that being clone 'NM2' (*P. nigra* × *P. maximowiczii*). A total of 136 trees of 'NM2' were left on site to serve as a long-term testing trial (planting A3). At 8 years after planting, 90% of the trees were still alive, which was similar survival to a 27-month-old phytoremediation system in north Florida recycling tertiary treated municipal wastewater with 14 *P. deltoides* clones (Minogue et al., 2012). The trees of the current study exhibited DBH ranging from 5.4 to 21.8 cm, with a mean of 13.7 ± 0.3 cm, while mean $BIOMASS_{MAI}$ was 11.2 ± 0.5 Mg ha^{-1} y^{-1} and mean $CARBON_{MAI}$ was 5.3 ± 0.3 Mg C ha^{-1} y^{-1}.

These results are comparable to those previously reported from similar leachate and effluent irrigation sites (Carlson, 1992; Minogue et al., 2012; Moffat et al., 2001; Shrive et al., 1994). For example, the mean stand-level DBH was 10.6 cm and $BIOMASS_{MAI}$ reached a maximum of 5.1 Mg ha^{-1} y^{-1} at 4 years after planting and declined to 3.8 Mg ha^{-1} y^{-1} at 5 years (which was within the low end of the range reported above) across 22 *P. trichocarpa* × *P. deltoides* F_1 hybrids in Vernon, British Columbia, Canada. These results were comparable given that the trees were at similar stages of plantation development when considering the shorter time period to crown closure at Vernon given its much denser initial spacing (6,419 trees ha^{-1}) versus planting A3 in Rhinelander (1,789 trees ha^{-1}) (Carlson, 1992). In addition, trees of 'NM6' irrigated with high-salinity (580 mg Na L^{-1}; 1,039 mg Cl L^{-1}) municipal landfill leachate had significantly greater diameter (+ 256%) and height (+ 212%) relative to those irrigated with water (control) or not irrigated at all after two growing seasons in Hamilton, Ontario, Canada (Shrive et al., 1994). Similarly, landfill leachate with lower overall salt concentrations (424 mg Na L^{-1}; 429 mg Cl L^{-1}) than those reported above produced significantly greater total biomass (+ 117%) relative to a control treatment with tap water (2 mg Na L^{-1}; 8 mg Cl L^{-1}) for trees of *P. deltoides* clone 'I-69/55' after 11 weeks of growth in Vrhnika, Slovenia (Zupanc and Zupančič-Justin, 2010; Zupančič-Justin et al., 2010). Furthermore, waste water irrigation with sewage sludge effluent during the first three years of plantation establishment significantly increased $BIOMASS_{MAI}$ of clones 'Beaupré' (*P. trichocarpa* × *P. deltoides*) and 'Trichobel' (*P. trichocarpa*) relative to non-irrigation treatments, with 'Beaupré' exhibiting 50% greater $BIOMASS_{MAI}$ than 'Trichobel' over that duration (Moffat et al., 2001). Lastly, $BIOMASS_{MAI}$ at 27 months after planting ranged from 20.9 to 49.8 Mg ha^{-1} y^{-1} for 14 *P. deltoides* clones irrigated with tertiary treated municipal wastewater, albeit with much denser spacing (i.e., 11,960 trees ha^{-1}) than the current study (1,789 trees ha^{-1}) (Minogue et al., 2012).

The second study established at the Oneida County Landfill consisted of 'NM6' and clone 'DN182' (*P. deltoides* × *P. nigra*) that were used for phytoremediation of paper mill fiber cake effluent recycling (planting A4). More specifically, fiber cake from a local paper production facility was distributed onto asphalt pads that sloped into an effluent collection lagoon. The nitrogen-rich effluent was irrigated onto the trees, providing essential fertilization and water requirements. At 12.5 years after planting in the current study, mean stand-level DBH, $BIOMASS_{MAI}$, and $CARBON_{MAI}$ were 19.0 ± 0.3 cm, 9.4 ± 0.3 Mg ha^{-1} y^{-1}, and 4.5 ± 0.1 Mg C ha^{-1} y^{-1}, respectively. Clone 'NM6' produced significantly greater DBH (+26%), $BIOMASS_{MAI}$ (+54%), and $CARBON_{MAI}$ (+58%) than 'DN182' ($P < 0.0001$) (Table 6). Clonal means for DBH, $BIOMASS_{MAI}$, and $CARBON_{MAI}$, respectively, were: 20.5 ± 0.3 cm, 10.8 ± 0.3 Mg ha^{-1} y^{-1}, and 5.2 ± 0.1 Mg C ha^{-1} y^{-1} for 'NM6' and 16.3 ± 0.4 cm, 7.0 ± 0.4 Mg ha^{-1} y^{-1}, and 3.3 ± 0.2 Mg C ha^{-1} y^{-1} for 'DN182'. In a similar study, Carpenter and Fernandez (2000) manufactured 7 topsoil blends consisting of various proportions of pulp sludge (from a Kraft process pulp mill), sand, and/or flume grit (recovered from a pulp mill wood-yard flume) and tested survival and growth of poplars grown in the topsoil at an unreclaimed gravel pit in Howland, Maine, USA. At 15 months after planting, DBH was significantly greater for all blends relative to sandy loam control topsoil (Carpenter and Fernandez, 2000). In contrast, despite non-significant treatment differences, Howe and Wagner (1996) reported 15% increases in stem biomass of six-month-old 'Fraser' cottonwood (*P. deltoides*) grown in controlled environments in soils with and without papermill sludge amendments.

B: Lemont, IL

Quinn at el. (2001) and Gopalakrishnan et al. (2007) described testing of poplar clone 'NE308' (*P. charkowiensis* × *P.* cv *incrassata*) for phytoremediation of volatile organic compounds (VOCs) [i.e., trichloroethylene (TCE), perchloroethylene (PCE), and carbon tetrachloride (CCl_4)] and tritium at the Argonne National Laboratory in Lemont, IL (planting B1). Building on prior laboratory evidence of the ability of 'DN34' to take up, translocate, and transpire TCE and other VOCs (Burken and Schnoor, 1998; 1999) as well as controlled short-term (i.e., 3 years) field trials with clones 'H11-11' and '50-189' (*P. trichocarpa* × *P. deltoides*) resulting in nearly 100% of TCE being removed from subsurface influent water streams (Gordon et al., 1998; Newman et al., 1999), planting B1 was one of the first large-scale, long-term installations for VOCs in the United States. Ma and Burken (2003) described other field sites, while Doucette et al. (2013) described significant TCE volatilization through soil and leaves from 8-year-old poplar trees of clones '184-111' (*P. trichocarpa* × *P. deltoides*), 'OP-367' (*P. deltoides* × *P. nigra*), and 'Eridano' (*P. deltoides* × *P. maximowiczii*).

The primary objective of the current phytoremediation efforts was to capture hydraulic control of the site in order to mitigate subsurface infiltration and off-site movement of the contaminants. Additional objectives included: 1) extraction and transpiration of contaminants, 2) sequestration of pollutants in tree biomass, and 3) co-metabolization of the VOCs in the root zone (Quinn et al., 2001). All trees were planted so that root development targeted the areas of soil and groundwater contamination (down to depths of 9 m), using methods that included the patented TreeWell® and TreeMediation® systems (Applied Natural Sciences, Inc., Hamilton, Ohio, USA). At 14 years after planting in the current study, DBH ranged from 3.0 to 41.7 cm, with a mean of 23.3 ± 0.6 cm, while mean $\mathrm{BIOMASS_{MAI}}$ was 5.4 ± 0.3 Mg $\mathrm{ha^{-1}\ y^{-1}}$ and mean $\mathrm{CARBON_{MAI}}$ was 2.5 ± 0.1 Mg C $\mathrm{ha^{-1}\ y^{-1}}$. Since the site was established, there have been very few reports of field-scale phytoremediation systems testing the response of poplar trees to CCl_4; those described have been laboratory-based (Ferrieri et al., 2006; Ma and Burken, 2002) or field-based with test beds, allowing for minimal numbers of experimental units to be tested (Wang et al., 2004). Relatively more field work has been done with TCE and PCE, albeit for short durations and under controlled conditions (James et al., 2009; Stanhope et al., 2008). For example, 4-year-old trees of 'OP-367' were associated with a 99% reduction of chlorinated ethenes in PCE-contaminated soils (James et al., 2009). In contrast, Legault et al. (2017) reported a reduced level of TCE removal from field-soils relative to the quantities exhibited in the greenhouse, though transgenic poplars were associated with greater levels of TCE removal than their wild-type counterparts. To increase TCE removal in the field, Doty et al. (2017) inoculated poplar trees with a natural bacterial endophyte, *Enterobacter* sp. strain PDN3, and reported 32% greater biomass and better health of the treated trees relative to non-inoculated controls. They concluded that combining the TCE-degrading bacteria with the poplar trees supported a field-based method for TCE phytoremediation (Doty et al., 2017).

C: LaSalle, IL

Isebrands et al. (2004) described testing of 19 poplar clones for phytoremediation of a PCE contaminated plume of soil and groundwater at the former LaSalle Electric Utilities site in LaSalle, IL (plantings C1 and C2) (Table 7). Rockwood et al. (2004; 2013) also described the phytoremediation, including that of TCE at the site. Two phytoremediation plantings were deployed: 1) growing trees in the open (i.e., without restrictions on rooting) atop the contaminated plume (planting C1), and 2) growing trees in groundwater treatment units (GTUs) to control the subsurface water flow in the rhizosphere (i.e., the area surrounding the tree roots) (planting C2). At 11 years after planting, clones differed for DBH ($P < 0.0001$), $\mathrm{BIOMASS_{MAI}}$ ($P = 0.0001$), and $\mathrm{CARBON_{MAI}}$ ($P = 0.0001$) at planting C1 (Table 6), where these dependent variables ranged from 4.7 to 22.9 cm (DBH), 0.7 to 16.2 Mg $\mathrm{ha^{-1}\ y^{-1}}$ ($\mathrm{BIOMASS_{MAI}}$), and 0.3 to 7.6 Mg C $\mathrm{ha^{-1}\ y^{-1}}$ ($\mathrm{CARBON_{MAI}}$). In contrast to Shifflett et al. (2014), who reported a lack of significant differences in establishment-year basal diameter among 42 poplar clones [belonging to three genomic groups (*P. deltoides*; *P. trichocarpa* × *P. deltoides*; *P. deltoides* × *P. maximowiczii*)] at a wastewater application site in Gibson, North Carolina, the broad genetic variability among clones was similar to that for other field-based phytoremediation studies (Bañuelos et al., 2010; Laureysens et al., 2004; Shannon et al., 1999).

More specifically, despite close genetic relationships within genomic groups reported in the literature, clones had differential responses to the contaminants and site conditions (Zalesny et al., 2005b). For example, Shannon et al. (1999) irrigated seven genotypes belonging to two genomic groups [1) '49-177', '50-194', '15-29', '50-197' (*P. trichocarpa* × *P. deltoides*); 2) 'DN34', 'OP-367', 'PC1' (*P. deltoides* × *P. nigra*)] with seven salinity treatments ranging from 1.5 to 15 dS $\mathrm{m^{-1}}$ and reported significant inter-family variability for total shoot mass (per tree). At the lowest salinity level, the four *P. trichocarpa* × *P. deltoides* hybrids comprised the top clones, while their *P. deltoides* × *P. nigra* counterparts ranked 5 to 7. However, at 15 dS m^{-1} clone ‘DN34’ exhibited the greatest total shoot mass that was significantly heavier than the second-ranked clone (‘49-177’); all remaining clones were equal to one another yet significantly less than ‘DN34’ and ’49-177’. From a phytoremediation perspective, the rise of ‘DN34’ to the top position in the highest salinity level corroborates the need for genotypic selection as gains from such selection are proportional to variation. Similarly, Bañuelos et al. (2010) conducted five micro-field plot screening trials testing dozens of poplar clones belonging to eleven genomic groups and concluded that variability within genomic groups was substantial enough to require clonal selection for salinity and boron tolerance. In Europe, Laureysens et al. (2004) concluded similar results for phytoextraction of heavy metals from polluted soils. At 6 years after planting, they reported a range of nearly 14 Mg ha^{-1} y^{-1} for $\text{BIOMASS}_{\text{MAI}}$ across 13 poplar clones belonging to five genomic groups (Laureysens et al., 2004). The effectiveness of phytoextraction and phytostabilization has been shown to be highly clone-specific. For example, Baldantoni et al. (2014) showed a nearly tenfold increase in cadmium phytoextraction for clone ‘N12’ (*P. nigra*) relative to ‘AL22’ (*P. alba*), while the latter was superior for phytostabilization of copper. Likewise, genotypes of *P. euphratica* and *P.* × *canescens* (i.e., *P. tremula* × *P. alba*) significantly differed for tolerance to cadmium exposure in a short-term hydroponic system, with *P.* × *canescens* exhibiting greater cadmium tolerance levels (Polle et al., 2013). To aid in selection of genotypes for field-based phytoremediation applications, quantitative trait loci (QTLs) and candidate genes for cadmium tolerance in a pseudo-backcross genomic group [(*P. trichocarpa* ‘93-968’ × *P. deltoides* ‘ILL-101’) × *P. deltoides* ‘D124’] have been identified (Induri et al., 2012) and are indicative of the need for combining traditional tree improvement with the ever-growing field of molecular genetics (Dickmann and Keathley, 1996).

In the current study, there were three notable trends in clonal ranks for all traits (Table 8). First, with the exceptions of clones ‘220-5’ and ‘51-5’ that consistently ranked in the top four genotypes, intraspecies *P. deltoides* × *P. deltoides* F_1 crosses generally exhibited greater diameter growth than their pure open-pollinated *P. deltoides* counterparts. Second, the two hybrids involving *P. maximowiczii* (‘Belgian25’, *P. deltoides* × *P. maximowiczii*; ‘NM2’) were ranked last and second-to-last for all traits, which corroborated expected genecological results (Farmer, 1996). That is, *P. maximowiczii* belongs to the taxonomic section *Tacamahaca*, which is better adapted to colder climates and shorter growing seasons (Fortier et al., 2010). These two clones, in particular, have exhibited above-average biomass productivity in the northern parts of the region (i.e., above 45 °N latitude) but have been outperformed by species and hybrids with parentage exclusively from the section *Aigeiros* in more southern latitudes (Riemenschneider et al., 2001; Zalesny et al., 2009c). Third, the only hybrid aspen genotype tested (‘Crandon’; *P. alba* × *P. grandidentata*) ranked second for all traits, which was very promising from a clonal selection standpoint as relatively little is known about the performance of these hybrids for phytoremediation in the region.

Furthermore, comparisons of both plantings (i.e., open-grown versus GTUs) at LaSalle, IL revealed significant clone and system main effects ($P < 0.05$) along with negligible interactions for all traits ($P > 0.05$) (Table 6). Eight clones were tested in both plantings (Table 7) and relative ranks were similar to those of planting C1 described above, with one exception (Table 9). Clone ‘7300501’ outranked two genotypes that performed better in planting C1, indicating that this genotype may be less impacted by the GTU-imposed rooting restriction than other clones. Future research potential includes further testing of this clone in non-GTU versus GTU treatments, as well as conducting root harvests to elucidate potential differences among clones in their belowground biomass production – which has been shown to differ in other phytoremediation applications (Zalesny et al., 2009a). Lastly, this restriction on the lateral and vertical extent of rooting likely led to significant differences between management systems, with open-grown trees having 79% greater diameter growth and 43 to 44% greater annual biomass and carbon accumulation per unit area (Table 9), despite being planted at roughly half as many trees per unit area compared to the GTUs. These results are in contrast to others using systems similar to the GTUs in the current study. For example, Ferro et al. (2013) reported a lack of differences in diameter growth rate for 'NM6' and 'DN34' grown in 20-cm polyvinylchloride (PVC) pipe extending to 7.5 m below the soil surface into a groundwater plume contaminated with total petroleum hydrocarbons versus nearby control trees growing in similarly sized boreholes that were not directly accessing the plume. In addition, Abichou et al. (2012) reported 32-month-old trees of 20 *P. deltoides* clones and two *P. deltoides* × *P. nigra* F_1 hybrids exhibited similar height and DBH when grown in lysimeters versus unlined test sections at a landfill in Tallahassee, Florida. The primary difference between the GTU- and lysimeter-based systems, however, was that the lysimeters imposed far less rooting restrictions, on a soil volume basis.

D: Midwest

A total of 27 clones representing six genomic groups were grown at an anonymous agricultural production facility in the Midwest that had salts, metals, and nitrates in the soils (Table 7) (planting D1) (Zalesny and Bauer, 2019). At 11 years after planting, clones differed for DBH ($P < 0.0001$), $\text{BIOMASS}_{\text{MAI}}$ ($P < 0.0001$), and $\text{CARBON}_{\text{MAI}}$ ($P < 0.0001$) (Table 6), where these dependent variables ranged from 9.4 to 26.5 cm (DBH), 3.1 to 26.4 Mg $\text{ha}^{-1}\ \text{y}^{-1}$ ($\text{BIOMASS}_{\text{MAI}}$), and 1.5 to 12.4 Mg C $\text{ha}^{-1}\ \text{y}^{-1}$ ($\text{CARBON}_{\text{MAI}}$) (Table 10). In general, open-pollinated *P. deltoides* clones and *P. deltoides* × *P. deltoides* F_1 hybrids outperformed interspecies genomic groups, with the best of such hybrids ('DN182') being ranked 10th for carbon, 11th for biomass, and 16th for diameter. Furthermore, similar to the clonal rankings at LaSalle, IL, the genotypic positions for DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ at planting D1 did not perfectly match each other. This was most noticeable for the middle rankings wherein the clones shifted most when moving from DBH to the other traits (Table 8, Table 10). This trend highlights the importance of having biomass equations and carbon concentrations that account for genetic differences, as such differences dictate that higher DBH does not necessarily equate to higher biomass and carbon accumulation in the wood.

Three clones at the Midwest agricultural production facility were planted as both unrooted and rooted cuttings. For these trees, clone and stock type main effects were significant for DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ ($P < 0.0001$), while their interactions were negligible ($P > 0.05$) (Table 6). Clone '80X00601' (*P. deltoides* × *P. deltoides*) had significantly greater DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ than '119.16' (*P. deltoides* × *P. deltoides*) and 'DN34', which did not differ from one another (Table 9). For planting stock, trees established as unrooted cuttings had 22% greater diameter growth and 49 to 50% greater biomass and carbon accumulation (Table 9). While unrooted cuttings performed better at this site, it is worth noting that, in certain circumstances, rooted cuttings may be preferred given contaminated or dry soils where cuttings fail to root and substantial replanting is necessary. In addition, poor establishment survival may cause a delay in the phytoremediation system if trees need to be re-established the following year.

E: Elizabeth City, NC

Cook et al. (2010) and Nichols et al. (2014) described testing of poplar clones for phytoremediation of petroleum hydrocarbons at a U.S. Coast Guard Base in Elizabeth City, NC (plantings E1, E2, E3). Two distinct phytoremediation objectives were tested: 1) using poplar trees for hydraulic control to retard water movement toward the Pasquotank River while decreasing on-site recharge and preventing further migration of the contaminated water into the river, and 2) using poplar trees to enhance biodegradation of the residual petroleum via rhizodegradation (i.e., degradation of chemical contaminants into less harmful compounds in the rhizosphere) (Cook et al., 2010; Nichols et al., 2014). To accomplish these objectives, they utilized three silvicultural prescriptions differing in diameter and depth of the planting holes, use of contaminated or clean soil for backfilling (or no backfill at all), and long whips versus unrooted cuttings as planting propagules. Regardless of these treatments, all three plantings (i.e., E1, E2, E3) included four interspecific hybrid poplar clones belonging to two genomic groups (‘15-29’ and ‘49-177’, *P. trichocarpa* × *P. deltoides*; ‘DN34’ and ‘OP367’, *P. deltoides* × *P. nigra*) (Cook et al., 2010). Clone effects were non-significant for all traits at planting E1 yet highly significant for plantings E2 and E3 ($P < 0.05$) (Table 6). At planting E1, mean stand-level DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were 6.8 ± 0.4 cm, 2.4 ± 0.2 Mg ha^{-1} y^{-1}, and 1.2 ± 0.1 Mg C ha^{-1} y^{-1}, respectively. At planting E2 (where 1-m unrooted whips were planted into 23-cm diameter holes that were 1.2 m deep and backfilled with clean topsoil), mean stand-level DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were 4.5 ± 0.2 cm, 1.3 ± 0.1 Mg ha^{-1} y^{-1}, and 0.6 ± 0.1 Mg C ha^{-1} y^{-1}, respectively. Clones within genomic groups exhibited similar diameter growth, with *P. trichocarpa* × *P. deltoides* hybrids generally (albeit not statistically) being larger than the *P. deltoides* × *P. nigra* genotypes (Figure 2). At 5 years after planting, the DBH of ‘49-177’ was not different from ‘15-29’, ‘DN34’, or ‘OP367’ yet ‘15-29’ exhibited significantly greater diameter than both of the *P. deltoides* × *P. nigra* clones. These trends were identical for $\text{BIOMASS}_{\text{MAI}}$ and $\text{CARBON}_{\text{MAI}}$ at planting E2. At planting E3, mean stand-level DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were 4.3 ± 0.1 cm, 2.6 ± 0.1 Mg ha^{-1} y^{-1}, and 1.3 ± 0.0 Mg C ha^{-1} y^{-1}, respectively. In contrast to planting E2, the genomic group trends broke down at planting E3 (where 30-cm unrooted cuttings were planted into 8-cm diameter holes that were 30 cm deep and lacked backfilling), with all four clones differing for DBH (clone rank = ‘OP367’ > ‘49-177’ > ‘DN34’ > ‘15-29’) at 5 years after planting. Clones ‘OP367’ and ‘49-177’ exhibited similar $\text{BIOMASS}_{\text{MAI}}$ and $\text{CARBON}_{\text{MAI}}$ that was greater than for ‘DN34’ and ‘15-29’, which were not different from one another (Figure 2).

This clonal instability within genomic groups corroborated previous results from numerous studies testing the ability of trees for rhizoremediation of petroleum hydrocarbons (Cook and Hesterberg, 2013). In particular, clonal instability was reported in a study testing 20 poplar clones in their ability to survive and grow in soils contaminated with a mean of 25% total petroleum hydrocarbons, by mass (Zalesny et al., 2005b). Trees established with 20-cm unrooted cuttings in sand-filled, augered holes achieved successful rooting and survival, resulting in mean height ranging from 14 ± 2 to 51 ± 15 cm after one growing season. The greatest height differential occurred within the [(*P. deltoides* × *P. trichocarpa*) × *P. deltoides*] backcross hybrids, wherein the best clone (‘NC13377’) exhibited 3.6 times greater height than the worst clone (‘NC13570’) (Zalesny et al., 2005b). In addition to the aforementioned results illustrating the efficacy of growing poplar in soils heavily-contaminated with petroleum hydrocarbons, Jordahl et al. (1997) illustrated the potential of rhizosphere degradation in soils of 7-year-old ‘DN34’ that exhibited significantly greater numbers of microbes involved in phytoremediation relative to adjacent soils without trees. Gunderson et al. (2007) showed better tolerance of hydrocarbon-contaminated soils for poplar clone 'Walker' [*P. deltoides* × (*P. laurifolia* × *P. nigra*)] with the addition of ectomychorrhizal colonization with the fungus *Pisolithus tinctorious* (Pers.) Coker and Couch. Similarly, Ferro et al. (1999) reported a lack of phytotoxic effects on tree growth and water use of clone 'DN34' established as 1.2-m long whips and grown in barrels in a range of VOC mixtures for up to 88 days. Overall, however, there have been limited reports of steady removal of these pollutants at long-term phytoremediation installations. For example, El-Gendy et al. (2009) reported consistent reductions of benzene, toluene, ethylbenzene, and xylene (BTEX) throughout the first 8 years of plantation development for 'DN34' at a reclaimed oil tank farm site in Cabin Creek, West Virginia, USA.

Felix et al. (2008) reported mean stand DBH of 9.5 ± 2.5 cm and associated $\text{BIOMASS}_{\text{MAI}}$ of 3.2 Mg ha^{-1} yr^{-1} at 5 years after planting in a production system where poplar trees of clone 'OP-367' were grown on deep trench rows treated with municipal biosolids near Washington, DC, USA. The mean DBH of trees at plantings E2 and E3 was approximately 50% of those reported by Felix et al. (2008), while $\text{BIOMASS}_{\text{MAI}}$ was approximately 60%. These results were not surprising, however, given the benefits of growing trees in high-nutrient, high-organic matter biosolids versus harsh growing conditions imposed by petroleum hydrocarbons. Nevertheless, there have been reports of elevated tree growth in the presence of hydrocarbon-contaminated soils. For example, Gunderson et al. (2008) reported increased fine root production of three-year-old poplar trees of clone 'Griffin' (*P. deltoides* × *P. petrowskyana*) growing at a decommissioned diesel and gasoline fuel tank storage site in eastern Saskatchewan, Canada. The practical implication of the extensive root production for phytoremediation was that greater root biomass stimulated enhanced microbial activity that led to significant petroleum degradation in the rhizosphere, which is directly related to the second objective in the current study.

F: Aberdeen, NC

Poplar clones were tested for phytoremediation of dichlorodiphenyltrichloroethane (DDT) and lindane at an industrial brownfield in Aberdeen, NC (planting F1). At 15 years after planting, mean stand-level DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were 12.3 ± 0.2 cm, 5.5 ± 0.2 Mg ha^{-1} y^{-1}, and 2.6 ± 0.1 Mg C ha^{-1} y^{-1}, respectively. Clone 'NE308' produced significantly greater DBH (+14%), $\text{BIOMASS}_{\text{MAI}}$ (+32%), and $\text{CARBON}_{\text{MAI}}$ (+32%) than clone 'NE41' (*P. maximowiczii* × *P. trichocarpa*) ($P < 0.05$) (Table 6). Clonal means for DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$, respectively, were: 13.4 ± 0.4 cm, 6.5 ± 0.4 Mg ha^{-1} y^{-1}, and 3.1 ± 0.2 Mg C ha^{-1} y^{-1} for 'NE308' and 11.8 ± 0.3 cm, 4.9 ± 0.3 Mg ha^{-1} y^{-1}, and 2.3 ± 0.1 Mg C ha^{-1} y^{-1} for 'NE41'.

G: Union Springs, AL

Poplar clones were tested for phytoremediation of miscellaneous organic contaminants from an industrial brownfield in Union Springs, AL (planting G1). At 5 years after planting, differences in DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ were negligible ($P > 0.05$) for the six open-pollinated *P. deltoides* clones tested at the site ('189-4', '3-1', '94-4', 'Ken8', 'S13C20', 'S7C1') (Table 6). Nevertheless, across clones DBH ranged from 1.1 to 20.7 cm, with a mean of 6.5 ± 0.4 cm, while mean $\text{BIOMASS}_{\text{MAI}}$ was 11.2 ± 0.3 Mg ha^{-1} y^{-1} and mean $\text{CARBON}_{\text{MAI}}$ was 5.3 ± 0.6 Mg C ha^{-1} y^{-1}.

H: Panama City, FL

Poplar clones were tested for phytoremediation of arsenic at an industrial brownfield in Panama City, FL (planting H1). Previous poplar studies reporting arsenic tolerance and phytoremediation are very limited (Merkle, 2006). While LeBlanc et al. (2011) reported increased arsenic resistance of tissue-cultured plants of clone 'C-175' (*P. deltoides*), we are unaware of field reports in the United States highlighting the productivity of poplar trees grown on arsenic-contaminated soils. In the current study a total of 15 poplar clones were tested, with 13 being open-pollinated *P. deltoides* selections and two being *P. deltoides* × *P. nigra* F_1 hybrids (Table 7). Clone effects were highly significant for DBH, $\text{BIOMASS}_{\text{MAI}}$, and $\text{CARBON}_{\text{MAI}}$ ($P < 0.0001$) at 5.4 years after planting (Table 6). Diameter ranged from 4.1 ± 0.9 to 8.3 ± 0.6 cm, with a mean of 7.0 ± 0.2 cm (Figure 3). The top three clones ('S13C20', 'Ken8', 'S7C1') exhibited nearly 3.5 times more $\text{BIOMASS}_{\text{MAI}}$ and $\text{CARBON}_{\text{MAI}}$ than the bottom three clones ('100-3', '92-4', '115-1'), with ranges in these traits from 1.0 ± 0.8 to 4.4 ± 0.5 Mg ha^{-1} y^{-1} ($\text{BIOMASS}_{\text{MAI}}$; mean = 2.9 ± 0.1 Mg ha^{-1} y^{-1}) and 0.5 ± 0.4 to 2.1 ± 0.2 Mg C ha^{-1} y^{-1} ($\text{CARBON}_{\text{MAI}}$; mean = 1.4 ± 0.1 Mg C ha^{-1} y^{-1}) (Figure 3). The F_1 hybrids were in the top half of clones for all three dependent variables.

The impact of arsenic contamination on poplar biomass productivity was evident at this site. For example, despite differences in planting density (i.e., 11,960 trees ha^{-1} versus 1,346 trees ha^{-1} at planting H1), Minogue et al. (2012) reported that $\text{BIOMASS}_{\text{MAI}}$ ranged from 20.9 to 49.8 Mg ha^{-1} y^{-1} for 27-month-old *P. deltoides* trees growing at a municipal waste sprayfield in Tallahassee, Florida, located 160 km from planting H1. Given the relatively close proximity of both plantings, this example shows that the trees grown without arsenic-contaminated soils exhibited nearly twelve times greater biomass than their arsenic-grown counterparts. Nevertheless, in addition to stocking, the primary difference in the phytoremediation systems was recycling of high-nitrogen and high-phosphorus wastewater at the sprayfield site, with both nutrients known to increase biomass of poplars (Minogue et al., 2012).

I: Northeast NC

An undefined *P. deltoides* × *P. nigra* F_1 hybrid was tested for phytoremediation of nitrates in a hog lagoon in northeast North Carolina (planting I1). The success of using poplars for nitrate management has been reported previously. For example, O'Neill and Gordon (1994) reported significant increases in total root biomass after testing 'Carolina' poplar (*P. deltoides* × *P. nigra*) in an artificial riparian zone engineered to mimic subsurface water (i.e., nitrate-nitrogen) flow through the rhizosphere. More specifically, poplars have exhibited significantly greater growth and productivity than sycamore (*Platanus occidentalis* L.) short rotation woody crops grown for nutrient uptake and biomass feedstock production at a decommissioned swine lagoon in Stillwater, Oklahoma (Dipesh et al., 2015). Among 25 pure *P. deltoides* genotypes, clonal selections based on provenance resulted in substantial gains from selection (height = +10%; DBH = +144%; aboveground woody biomass = +483%) (Dipesh et al., 2015). At 10 years after planting in the current study, DBH ranged from 1.8 to 25.7 cm, with a mean of 12.5 ± 0.4 cm, while mean $\text{BIOMASS}_{\text{MAI}}$ ranged from 0.1 to 35.8 Mg ha^{-1} y^{-1} (mean $\text{BIOMASS}_{\text{MAI}}$ was 8.3 ± 0.6 Mg ha^{-1} y^{-1} and mean $\text{CARBON}_{\text{MAI}}$ was 3.9 ± 0.3 Mg C ha^{-1} y^{-1}.

Spatial Distribution of Aboveground Total Carbon

Figure 4 illustrates the spatial distribution of $\text{CARBON}_{\text{MAI}}$ at the seven plantings listed in Table 3. All of the plantings analyzed showed the presence of several "hotspots" or locations where $\text{CARBON}_{\text{MAI}}$ was substantially greater than the average within each site. This pattern was present in both single- and multiple-genotype plantings, which suggests that spatial variability in soil heterogeneity from contamination dominated genotype × environment interactions. Several studies, including Gopalakrishnan et al. (2007) and Limmer et al. (2011), have mapped spatial variability in contaminant concentrations at specific sites, while others have evaluated the behavior of clones (i.e., growth, biomass) as a function of contaminant levels (Zalesny et al., 2016b). However, the combined impact of both spatial variability and clonal selection has not been evaluated at long-term phytoremediation sites. Our current results suggest that such a study could prove valuable in further elucidating the dominant variables influencing biomass production, carbon sequestration and ecosystem services of phytotechnologies.

The distribution of clones present in the hotspots within individual plantings is presented in Table 11. Of the seven plantings evaluated, sites with greater than 15 clones showed an effect from clonal selection on the presence of $\text{CARBON}_{\text{MAI}}$ hotspots. Approximately 45 to 60% of the clones present at these three plantings displayed increased carbon accumulation when compared to the other clones present within each site. While this response likely resulted from differences in treatments and reductions of growth from higher contaminant levels, some of the clones appeared to perform better than others at specific sites. This is similar to results shown by Baldantoni et al. (2014) in the laboratory who tested poplar heavy metal phytoextraction and by Zalesny and Bauer (2007a) in the field who tested poplar petroleum phytoremediation, and suggests that phyto-recurrent selection of clones at field sites is advisable when carbon sequestration is a consideration of phytoremediation systems.

Table 12 illustrates intra-site variability among clones for the $\text{CARBON}_{\text{MAI}}$ hotspots, including the: 1) distribution of clones present in the hotspots, 2) distribution of hotspot locations by clone, and 3) distribution of clones over the entire site. As shown in Table 12, each planting had at least two clones dominating the hotspots. For example, at planting H1 (industrial brownfield at Panama City, FL), clones 'Ken8' and 'S7C1' were present in hotspots in significantly higher numbers than all other clones. Additionally, 'Ken8' and 'S7C1' outperformed the other genotypes in the hotspots, as indicated by greater percentages of these clones with increased $\text{CARBON}_{\text{MAI}}$ relative to their presence across the site. For example, 'Ken8', 'S7C1', and 'S13C20' were present in the hotspots at 1.7 to 2.3 times the rate over the entire site. By comparison, 'DN21', 'DN31', '189-4', and '72C-2' were present at the same or lower rate in hotspots compared to their distribution across the site (Table 12). These results suggested that specific clones can be selected at a given site in order to maximize carbon accumulation and ecosystem services. Similar trends were shown for the other four plantings (Table 12).

CONCLUSIONS

Phytoremediation and associated phytotechnologies have been used successfully throughout the world to bridge the gap between ecological degradation and ecosystem restoration along urban-to-rural gradients. The extensive variability in aboveground biomass production and carbon sequestration in the current review illustrated the importance of long-term monitoring and data collection at phytoremediation installations. Despite being exposed to harsh site conditions, these ecosystem services were comparable to those at non-contaminated sites used for bioenergy and biofuels feedstock production. In general at the Midwestern sites, phytoremediation trees exhibited ~20% reduction in diameter and biomass relative to their non-contaminated counterparts. More specifically, there were no differences in diameter (P = 0.0614) nor biomass (P = 0.0938) between trees grown on liability lands versus typical production systems in the Midwest, where the percent difference in diameter (DBH_{Δ}) ranged from -53.6 to +22.6% and that for biomass ($\mathrm{BIOMASS}_{\Delta}$) ranged from -78.6 to +131.3% (Table 13).

Furthermore, results of the current review also showed that multiple silvicultural prescriptions should also be tested at individual sites in order to maximize the provision of ecosystem services while optimizing the mitigation of contaminants. For example, open-grown trees at LaSalle, IL exhibited significantly greater biomass and carbon benefits relative to those in groundwater treatment units. The overall key to the success of such systems is the balance between the potential break down of pollutants in the rhizosphere and/or uptake into tree tissues with the need for control of subsurface water movement. The choice of planting propagule is another silvicultural decision that impacts the biological and economic success of phytoremediation. For example, trees established as unrooted cuttings at a Midwestern agricultural production facility significantly outperformed those that were nursery-grown for a year, excavated, and root pruned before being planted as rooted cuttings (Zalesny and Bauer, 2019).

During project planning, propagule cost and ease of planting should be balanced with expected tree survival and potential for long-term phytoremediation benefits. Based on survival rates during establishment, Zalesny and Bauer (2019) reported a break-even cost of $0.32 per rooted cutting to accomplish the same desired rotation-age stocking as trees planted from unrooted, hardwood cuttings. Given that rooted cuttings typically cost $2.00 to $4.00 per tree, and despite potential phytoremediation advantages from the rooted cuttings, their costs may preclude their use. Genotype selection was the third, and arguably most important, silvicultural component directly compared in the current review. Only well-adapted clones should be grown at the sites. Diameter, biomass, and carbon varied greatly among and within genomic groups, which corroborated the need for methodologies such as phyto-recurrent selection that are used for matching specialized genotypes with individual pollutants and the need for breakdown and uptake in the soil and/or specific tree tissues (i.e., roots, wood, leaves). As illustrated in the current study, phytoremediation success can be increased with the identification and deployment of genotypes tailored to grow well and tolerate a broad diversity of contaminants (generalists) (i.e., ‘DN34’, ‘NM6’, ‘7300501’) versus those that significantly outperform their counterparts under unique site conditions (specialists) (i.e., ‘220-5’, ‘51-5’, ‘S13C20’).

ACKNOWLEDGMENTS

RSZ presented this information during the Environmental Applications of Poplar and Willow session of the Sixth International Poplar Symposium (IPS-VI: Domestication of *Populus* and *Salix*: How far have we come, and how far do we still have to go?) held July 20 to 23, 2014, in Vancouver, British Columbia, Canada. We thank our numerous phytotechnologies, short rotation woody crops, and forestry colleagues for many worthwhile discussions on the importance of integrating these lines of science to enhance ecological restoration. In addition, we thank all of the resource managers who collaborated on these projects and offered us access to their unique sites. We are also grateful to Dr. David Coyle (University of Georgia) for reviewing earlier versions of this paper, Sue Lietz for developing Figure 1, as well as those who spent countless hours conducting fieldwork: Bruce Birr, Erin Clements, Samantha Hoppe, Christina Ives, Ben Klosiewski, Kricket Koehn, Matt Kramer, Hailey Laska, Brendan McGuire, Larry Moos, John Quinn, Taylor Preul, Sophie Schmidt, John Schutts, Nathan Visser, and Molly Willging.

DISCLAIMER

Commercial products mentioned in this review were used because they met specific research needs. Selection of these products is left to the discretion of the users. Endorsement is not intended by the Forest Service, United States Department of Agriculture.

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TABLES

TABLE 1 | Published studies from fifteen poplar plantings across nine long-term phytoremediation installations in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) United States that were evaluated in the current review for tree diameter, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon.

TABLE 2 | Descriptions of study locations used in a network of poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States. Due to landowner confidentiality agreements, latitude and longitude cannot be listed for locations D and I. Weather data represent 30-year climate normals (1981 to 2010), with summer temperatures defined as June through August (data obtained from www.ncdc.noaa.gov).

TABLE 3 | Names and descriptions of the individual poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States.

TABLE 4 | Genomic groups and clones used in a network of poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States.

TABLE 5 | Aboveground total (stem + branch) dry biomass equations (biomass = $a \times \text{DBH}^{b}$), fit statistics, and mean carbon percentages by genomic group (from Headlee et al., submitted).

TABLE 6 | Probability values from analyses of variance comparing diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) of poplar (*Populus* spp.) clones grown for phytoremediation applications in the Midwest and Southeast, United States. In addition to clone, sources of variation for plantings C2 and D2 included engineering system and planting stock type, respectively. Significant values are in bold.

TABLE 7 | Poplar plantings with at least eight clones being tested for phytoremediation in the Midwest and Southeast, United States. See Table 4 for genomic group definitions.

TABLE 8 | Least-squares means and clonal ranks of diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for 19 poplar clones evaluated at an industrial brownfield in LaSalle, IL (planting C1). Different letters within a column represent statistically significant differences ($P < 0.05$). Standard errors of stand-level means are indicated in parentheses.

TABLE 9 | Clone and treatment effects for diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for poplars evaluated at LaSalle, IL (plantings C1 and C2) and Midwest agriculture production facility (planting D1). For each location, different letters within a column represent statistically significant differences ($P < 0.05$); clonal differences are denoted in lower-case while treatment differences are denoted in upper-case.

TABLE 10 | Least-squares means and clonal ranks of diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for 27 clones evaluated at Midwest agriculture production facility (planting D1). Different letters within a column represent statistically significant differences ($P < 0.05$). Standard errors are indicated in parentheses.

TABLE 11 | Percentage of clones present in $\text{CARBON}_{\text{MAI}}$ hotspots ($\text{HOTSPOT}_{\text{CLONE}}$) within seven individual plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. $\text{CARBON}_{\text{MAI}}$ = mean annual increment (MAI) of aboveground total carbon.

TABLE 12 | Percentage of: 1) clones present in $\text{CARBON}_{\text{MAI}}$ hotspots ($\text{HOTSPOT}_{\text{CLONE}}$), 2) $\text{CARBON}_{\text{MAI}}$ hotspots present by clone ($\text{CLONE}_{\text{HOTSPOT}}$), and 3) clones distributed across the entire site ($\text{SITE}_{\text{CLONE}}$) within five individual plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. $\text{CARBON}_{\text{MAI}}$ = mean annual increment (MAI) of aboveground total carbon.

TABLE 13 | Observed stand-level diameter at breast height (DBH_{OBS}) and mean annual increment of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{OBS}}$) at poplar phytoremediation plantings in the Midwest, United States, along with expected diameter (DBH_{EXP}) and biomass ($\text{BIOMASS}_{\text{EXP}}$) of equally-aged poplar grown for bioenergy and biofuels near the phytoremediation plantings. Overall, there were no differences in diameter ($P = 0.0614$) or biomass ($P = 0.0938$) between phytoremediation and biomass trees. The percent difference in diameter (DBH_{Δ}) and biomass (BIOMASS_{Δ}) of trees grown on liability lands versus typical production systems is also shown. See Headlee et al. (2013) for a description of the biomass plantings.

FIGURES

FIGURE 1 | Map of long-term phytoremediation sites in the Midwest and Southeast, United States.

FIGURE 2 | Least-squares means of diameter at breast height, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon for four poplar clones (‘DN34’ and ‘OP367’, *P. deltoides* × *P. nigra*; ‘15-29’ and ‘49-177’, *P. trichocarpa* × *P. deltoides*) evaluated at a U.S. Coast Guard Base in Elizabeth City, NC (plantings E2 and E3). Different letters above bars within a planting for each trait represent statistically significant differences ($P < 0.05$). Error bars equal one standard error of the mean.

FIGURE 3 | Least-squares means of diameter at breast height, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon for fifteen poplar clones evaluated at an industrial brownfield in Panama City, FL (planting H1). With the exception of 'DN21' and 'DN31' that are both *Populus deltoides* × *P. nigra* F_1 hybrids, all clones belong to the *P. deltoides* genomic group. Different letters above bars within a trait represent statistically significant differences ($P < 0.05$). Error bars equal one standard error of the mean.

FIGURE 4 | Spatial distribution of mean annual increment (MAI) of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) at seven poplar plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. Descriptions of the seven plantings are provided in Table 3.

TABLE 1 | Published studies from fifteen poplar plantings across nine long-term phytoremediation installations in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) United States that were evaluated in the current review for tree diameter, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon.

Location	Planting(s)	Soil Type	Contaminant Concentration(s)[1]	Reference(s)[2]
A: Rhinelander, WI	A1: Rhinelander Landfill (I)	Padus Loam	S: NH_3^+, 13,639 mg Fe kg^{-1}; L: 420 mg N L^{-1}; 1,100 mg Na L^{-1}; 1,200 mg Cl L^{-1}	i,j,p,v
	A2: Rhinelander Landfill (II)	"	"	---
	A3: Oneida County Landfill (I)	Padus-Pence Sandy Loam	S: 203 mg Na kg^{-1}; 91 mg Cl kg^{-1}; L: 598 mg N L^{-1}; 690 mg Na L^{-1}; 1,093 mg Cl L^{-1}	b,k,m,n,o,q,r,s,t,u
	A4: Oneida County Landfill (II)	Padus Loam	NA[3]	---
B: Lemont, IL	B1: Argonne National Lab	Ozaukee Silt Loam	S (for adjacent willow plots): 300-40,000 µg TCE kg^{-1}; 114-71,000 µg PCE kg^{-1}; 66-770,000 µg CCl_4 kg^{-1}; W (for poplar plots): 100-36,000 µg TCE L^{-1}	c,f
C: LaSalle, IL	C1: Industrial Brownfield (I)	Drummer Silty Clay Loam	NA	d,g,h
	C2: Industrial Brownfield (II)	Elburn Silt Loam	NA	d,g,h
D: Midwest	D1: Ag Production Facility	NP[4]	NP	i
E: Elizabeth City, NC	E1: US Coast Guard Base (I)	Udorthent, loamy	W: 2,100 µg benzene L^{-1}; 2,500 µg MTBE L^{-1}; G: 18,710 µg TPH (mass), 459 µg BTEX (mass)	a,e
	E2: US Coast Guard Base (II)	"	"	a,e
	E3: US Coast Guard Base (III)	"	"	a,e
F: Aberdeen, NC	F1: Industrial Brownfield	Vaucluse Loamy Sand	NA	---
G: Union Springs, AL	G1: Industrial Brownfield	Blanton-Bonifay Loamy Sand	NA	---
H: Panama City, FL	H1: Industrial Brownfield	Chipley Sand	NA	---
I: Northeast NC	I1: Hog Lagoon	NP	NP	---

[1] S = Soil; L = Leachate; W = Ground Water; G = Soil Gas

[2] References: a) Cook et al. (2010); b) Coyle et al. (2011); c) Gopalakrishnan et al. (2007); d) Isebrands et al. (2004); e) Nichols et al. (2014); f) Quinn et al. (2001); g,h) Rockwood et al. (2004, 2013); i,j,k,l) Zalesny and Bauer (2007a, 2007b, 2007c, 2019); m,n,o) Zalesny and Zalesny (2009a, 2009b, 2011); p,q,r,s,t,u,v) Zalesny et al. (2006, 2007a, 2007b, 2008a, 2008b, 2009a, 2009b)

[3] Not available.

[4] Not possible due to landowner confidentiality agreements.

TABLE 2 | Descriptions of study locations used in a network of poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States. Due to landowner confidentiality agreements, latitude and longitude cannot be listed for locations D and I. Weather data represent 30-year climate normals (1981 to 2010), with summer temperatures defined as June through August (data obtained from www.ncdc.noaa.gov).

Location	**Number of Plantings**	**Latitude (°N)**	**Longitude (°W)**	**Mean Summer Temperature (°C)**	**Mean Annual Precipitation (mm)**
A: Rhinelander, WI	4	45.63	89.48	18.2	675
B: Lemont, IL	1	41.60	88.08	22.3	1,018
C: LaSalle, IL	2	41.35	89.11	22.3	964
D: Midwest	1	--	--	22.7	946
E: Elizabeth City, NC	3	36.31	76.21	25.2	1,183
F: Aberdeen, NC	1	34.99	79.22	25.3	1,182
G: Union Springs, AL	1	32.01	85.75	25.9	1,408
H: Panama City, FL	1	30.21	85.68	27.7	1,551
I: Northeast NC	1	--	--	25.3	1,125

TABLE 3 | Names and descriptions of the individual poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States.

Planting	**Issue**	**Stocking (trees ha^{-1})**	**Year Planted**	**Age (y)**	**Number of Clones**	**Number of Trees**
A1: Rhinelander Landfill (I)	Nitrates, hydraulic control	1,076	1999	14.5	2	165
A2: Rhinelander Landfill (II)	Nitrates, hydraulic control	1,076	2000	13.5	1	200
A3: Oneida Co. Landfill (I)[1]	Salts in leachate	1,789	2005	8.0	1	123
A4: Oneida Co. Landfill (II)	Fiber cake recycling	1,076	2001	12.5	2	531
B1: Argonne National Lab[1]	VOCs, tritium	434	1999	14.0	1	179
C1: Industrial Brownfield (I)[1]	TCE, PCE	1,328	2002	11.0	19	144
C2: Industrial Brownfield (II)[1]	TCE, PCE	2,691	2002	11.0	8	68
D1: Ag Production Facility[1]	Salts, metals, nitrates	1,681	2002	11.0	27	359
E1: US Coast Guard Base (I)	Petroleum hydrocarbons	1,111	2006	6.0	4	99
E2: US Coast Guard Base (II)	Petroleum hydrocarbons	1,111	2007	5.0	4	263
E3: US Coast Guard Base (III)	Petroleum hydrocarbons	2,500	2007	5.0	4	1,637
F1: Industrial Brownfield	DDT, lindane	2,315	1998	15.0	2	178
G1: Industrial Brownfield[1]	Misc. organics	4,310	2008	5.0	6	101
H1: Industrial Brownfield[1]	Arsenic	1,346	2008	5.4	15	135
I1: Hog Lagoon	Nitrates	1,795	2003	10.0	1	180

[1] Planting where the spatial distribution of MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) was evaluated.

TABLE 4 | Genomic groups and clones used in a network of poplar (*Populus* spp.) plantings grown for phytoremediation applications in the Midwest and Southeast, United States.

Genomic group[1]	**Clone(s)**
P. deltoides 'D'	7300501; 8000105; 91.05.02; 220-5; 252-4; 42-7; 51-5; 3-1; Ohio Red; D121; D123; D124; 79-4; 90-3; 92-4; 93-6; 94-4; 100-3; 115-1; 119-6; 147-1; 189-4; 72C-2; Ken8; S13C20; S7C1
P. deltoides × *P. deltoides* 'DD'	80X01107; 80X00601; 80X01015; ISU.25-4; ISU.25-12; ISU.25-21; ISU.25-35; ISU.25-R2; ISU.25-R4; ISU.25-R5; 119.16
P. deltoides × *P. nigra* 'DN'	DN5; DN21; DN31; DN34; DN182; OP-367; I4551
P. nigra × *P. maximowiczii* 'NM'	NM2; NM6
P. trichocarpa × *P. deltoides* 'TD'	15-29; 49-177
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* 'TDD'	NC13992
P. maximowiczii × *P. trichocarpa* 'MT'	NE41
P. charkowiensis × *P.* cv *incrassata* 'CI'	NE308
P. deltoides × *P. maximowiczii* 'DM'	DM115; Belgian25; 313.23
P. alba × *P. grandidentata* 'AG'	Crandon

[1] Authorities for the aforementioned species are: *P. alba* L.; *P. charkowiensis* R.I. Schrod.; *P. deltoides* Bartr. ex Marsh; *P. grandidentata* Michx.; *P. incrassata* Dode; *P. maximowiczii* A. Henry; *P. nigra* L.; *P. trichocarpa* Torr. & Gray.

TABLE 5 | Aboveground total (stem + branch) dry biomass equations (biomass = $a \times \text{DBH}^{b}$), fit statistics, and mean carbon percentages by genomic group (from Zalesny et al., 2015).

Genomic Group(s)	***a***	***b***	**R^2**	**Carbon (%)**
D, DD	0.224	2.01	0.87	46.85
DN	0.095	2.36	0.91	47.31
NM	0.316	1.94	0.73	47.71
TD, TDD	0.380	1.78	0.69	47.48
AG, CI, DM, MT	0.093	2.33	0.86	47.28

TABLE 6 | Probability values from analyses of variance comparing diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) of poplar (*Populus* spp.) clones grown for phytoremediation applications in the Midwest and Southeast, United States. In addition to clone, sources of variation for plantings C2 and D2 included engineering system and planting stock type, respectively. Significant values are in bold.

Planting			DBH	$\text{BIOMASS}_{\text{MAI}}$	$\text{CARBON}_{\text{MAI}}$
A1	Rhinelander Landfill (1999)		**<0.0001**	**<0.0001**	**<0.0001**
A2	Rhinelander Landfill (2000)		na[1]	na	na
A3	Oneida County Landfill (Leachate)		na	na	na
A4	Oneida County Landfill (Fibercake)		**<0.0001**	**<0.0001**	**<0.0001**
B1	Argonne National Laboratory		na	na	na
C1	Industrial Brownfield (I)		**<0.0001**	**0.0001**	**0.0001**
D1	Midwest Ag Production Facility		**<0.0001**	**<0.0001**	**<0.0001**
E1	U.S. Coast Guard Base (I)		0.9721	0.8215	0.8187
E2	U.S. Coast Guard Base (II)		**0.0260**	**0.0048**	**0.0045**
E3	U.S. Coast Guard Base (III)		**<0.0001**	**<0.0001**	**<0.0001**
F1	Industrial Brownfield		**0.0008**	**0.0004**	**0.0004**
G1	Industrial Brownfield		0.4374	0.4294	0.4294
H1	Industrial Brownfield		**<0.0001**	**<0.0001**	**<0.0001**
I1	Hog Lagoon		na	na	na
C2	Industrial Brownfield (II)				
		Clone	**0.0092**	**0.0132**	**0.0129**
		System[2]	**<0.0001**	**0.0057**	**0.0057**
		Clone × System	0.4812	0.6061	0.6010
D2	Midwest Ag Production Facility				
		Clone	**<0.0001**	**<0.0001**	**<0.0001**
		Stock Type[3]	**<0.0001**	**<0.0001**	**<0.0001**
		Clone × Stock Type	0.3174	0.3620	0.3548

[1] na = not applicable because only one clone was tested.

[2] Groundwater treatment units where trees grown in wells were compared to open-grown trees.

[3] Trees established as unrooted cuttings were compared to rooted cuttings with 5 to 7 lateral roots.

TABLE 7 | Poplar plantings with at least eight clones being tested for phytoremediation in the Midwest and Southeast, United States. See Table 4 for genomic group definitions.

Planting	Genomic Group / Clone	
C1: Industrial Brownfield (I)	AG	Crandon
(LaSalle, IL)	D	7300501, 220-5, 252-4, 42-7, 51-5, OhioRed
	DD	119.16, 80X00601, 80X01015, 80X01107, ISU.25-21, ISU.25-35, ISU.25-R4, ISU.25-R5
	DM	Belgian25
	DN	DN34, I4551
	NM	NM2
C2: Industrial Brownfield (II)	AG	Crandon
(LaSalle, IL)	D	7300501, 220-5, 51-5
	DD	80X01107, ISU.25-21, ISU.25-R4
	DN	I4551
D1: Ag Production Facility	D	252-4, 7300501, 8000105, 91.05.02, D121, D123, D124
(Midwest)	DD	119.16, 42-7, 80X00601, 80X01107, ISU.25-12, ISU.25-21, ISU.25-35, ISU.25-4, ISU.25-R2, ISU.25-R4, ISU.25-R5
	DM	313.23, Belgian25, DM115
	DN	DN182, DN34, DN5, I4551
	NM	NM6
	TDD	NC13992
H1: Industrial Brownfield	D	79-4, 90-3, 92-4, 93-6, 100-3, 115-1, 119-6, 147-1, 189-4, 72C-2, Ken8, S13C20, S7C1
(Panama City, FL)	DN	DN21, DN31

TABLE 8 | Least-squares means and clonal ranks of diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for 19 poplar clones evaluated at an industrial brownfield in LaSalle, IL (planting C1). Different letters within a column represent statistically significant differences ($P < 0.05$). Standard errors of stand-level means are indicated in parentheses.

Clone	DBH (cm)		Rank	$\text{BIOMASS}_{\text{MAI}}$ (Mg ha^{-1} y^{-1})		Rank	$\text{CARBON}_{\text{MAI}}$ (Mg C ha^{-1} y^{-1})		Rank
220-5	22.9	a	1	16.2	a	1	7.6	a	1
Crandon	20.9	abc	2	13.6	abc	2	6.4	abc	2
51-5	20.4	ab	3	12.4	abc	4	5.8	abc	4
ISU.25-R5	19.1	abc	4	10.6	abcdef	5	5.0	abcdef	5
ISU.25-35	18.7	abc	5	10.4	abcdef	7	4.9	abcdef	7
I4551	18.6	abc	6	12.8	ab	3	6.0	ab	3
80X00601	18.0	abc	7	10.5	abcd	6	4.9	abcd	6
ISU.25-R4	17.3	abcd	8	8.6	bcdefgh	11	4.0	bcdefgh	11
DN34	16.7	abcde	9	9.8	abcdefgh	8	4.7	abcdefgh	8
80X01015	16.5	bcd	10	8.7	bcdefg	10	4.1	bcdefg	10
80X01107	15.0	bcde	11	6.7	cdefgh	12	3.1	cdefgh	12
7300501	14.7	cde	12	9.0	bcdefg	9	4.2	bcdefg	9
ISU.25-21	13.9	bcdef	13	6.6	cdefgh	13	3.1	cdefgh	13
Ohio Red	11.9	def	14	5.0	defgh	14	2.3	defgh	14
252-4	11.0	def	15	4.5	efgh	15	2.1	efgh	15
119.16	10.7	def	16	3.9	efgh	16	1.8	efgh	16
42-7	7.8	ef	17	2.1	gh	17	1.0	gh	17
Belgian25	6.9	f	18	1.8	h	18	0.8	h	18
NM2	4.7	f	19	0.7	fgh	19	0.3	fgh	19
Overall Mean	16.1	(0.6)		9.3	(0.6)		4.4	(0.3)	

TABLE 9 | Clone and treatment effects for diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for poplars evaluated at LaSalle, IL (plantings C1 and C2) and Midwest agriculture production facility (planting D1). For each location, different letters within a column represent statistically significant differences ($P < 0.05$); clonal differences are denoted in lower-case while treatment differences are denoted in upper-case.

Location	Planting(s)	Clone	Treatment[1]	DBH (cm)		$\text{BIOMASS}_{\text{MAI}}$ (Mg ha^{-1} y^{-1})		$\text{CARBON}_{\text{MAI}}$ (Mg C ha^{-1} y^{-1})	
C: LaSalle, IL	C1, C2	220-5	--	18.1	a	13.3	a	6.2	a
	C1, C2	Crandon	--	17.5	ab	12.7	ab	6.0	ab
	C1, C2	51-5	--	15.6	abc	9.7	abc	4.5	abcd
	C1, C2	I4551	--	14.2	bc	9.5	bc	4.5	abc
	C1, C2	7300501	--	13.1	bc	8.5	bc	4.0	bcd
	C1, C2	ISU.25-R4	--	12.8	bc	6.5	c	3.1	cd
	C1, C2	80X01107	--	12.3	c	6.1	c	2.8	d
	C1, C2	ISU.25-21	--	11.8	c	6.4	c	3.0	cd
	C1	--	Open	17.9	A	10.7	A	5.0	A
	C2	--	GTU	10.9	B	7.4	B	3.5	B
D: Midwest	D1	80X00601	--	22.8	a	19.5	a	9.1	a
	D1	119.16	--	18.7	b	13.2	b	6.2	b
	D1	DN34	--	16.1	b	11.0	b	5.2	b
	D1	--	Unrooted	21.1	A	17.5	A	8.2	A
	D1	--	Rooted	17.3	B	11.7	B	5.5	B

[1] LaSalle, IL: groundwater treatment units where trees grown in wells were compared to open-grown trees. Midwest: trees established as unrooted cuttings were compared to rooted cuttings with 5 to 7 lateral roots.

TABLE 10 | Least-squares means and clonal ranks of diameter at breast height (DBH), mean annual increment (MAI) of aboveground total (stem + branch) dry biomass ($\text{BIOMASS}_{\text{MAI}}$), and MAI of aboveground total carbon ($\text{CARBON}_{\text{MAI}}$) for 27 clones evaluated at Midwest agriculture production facility (planting D1). Different letters within a column represent statistically significant differences (*P* < 0.05). Standard errors are indicated in parentheses.

Clone	DBH (cm)		Rank	$\text{BIOMASS}_{\text{MAI}}$ ($\text{Mg ha}^{-1}\text{ y}^{-1}$)		Rank	$\text{CARBON}_{\text{MAI}}$ ($\text{Mg C ha}^{-1}\text{ y}^{-1}$)		Rank
ISU.25-35	26.5	a	1	26.4	a	1	12.4	a	1
42-7	25.0	ab	2	24.2	ab	2	11.3	ab	2
ISU.25-R4	24.0	ab	3	22.5	abc	3	10.5	abc	3
80X00601	22.6	bc	4	19.2	bcd	4	9.0	bcd	4
252-4	22.4	bc	5	18.3	bcde	6	8.6	bcde	6
ISU.25-12	21.9	bcd	6	17.9	bcdef	7	8.4	bcdef	7
7300501	21.5	bcde	7	18.8	bcdef	5	8.8	bcdef	5
ISU.25-R5	21.1	bcdef	8	16.7	cdefg	8	7.8	cdefg	8
D121	21.0	bcdefg	9	16.7	cdefg	9	7.8	cdefg	9
ISU.25-21	19.9	cdefgh	10	15.1	defgh	13	7.1	defgh	13
ISU.25-R2	19.9	cdefgh	11	14.7	defgh	14	6.9	defgh	14
D124	19.9	cdefghi	12	15.6	defgh	10	7.3	defgh	11
80X01107	19.8	cdefghi	13	15.2	defgh	12	7.1	defgh	12
8000105	18.8	cdefghi	14	13.9	defghi	15	6.5	defghi	15
119.16	18.7	efghi	15	13.2	fgh	18	6.2	fgh	18
DN182	18.5	efghi	16	15.5	defg	11	7.4	defg	10
ISU.25-4	18.3	defghi	17	12.4	fghi	20	5.8	fghi	20
NM6	17.7	cdefghij	18	13.3	cdefghij	17	6.4	cdefghij	17
DN5	17.7	ghi	19	13.8	efgh	16	6.5	efgh	16
D123	17.5	efghij	20	11.5	ghi	21	5.4	ghi	21
DN34	17.1	hij	21	12.6	ghi	19	5.9	ghi	19
91.05.02	16.4	ghij	22	10.2	ghij	22	4.8	ghij	22
I4551	15.5	efghijk	23	9.8	efghij	23	4.6	efghij	23
DM115	15.1	ij	24	8.2	hij	24	3.9	hij	24
NC13992	15.1	fghijk	25	7.4	ghij	25	3.5	ghij	25
313.23	12.4	jk	26	5.2	ij	26	2.4	ij	26
Belgian25	9.4	k	27	3.1	j	27	1.5	j	27
Overall Mean	19.6	(0.3)		15.5	(0.4)		7.3	(0.2)	

TABLE 11 | Percentage of clones present in $\mathrm{CARBON_{MAI}}$ hotspots ($\mathrm{HOTSPOT_{CLONE}}$) within seven individual plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. $\mathrm{CARBON_{MAI}}$ = mean annual increment (MAI) of aboveground total carbon.

Planting	Total Number of Clones	$\mathrm{HOTSPOT_{CLONE}}$ (%)[1]
A3: Oneida Co. Landfill (I)	1	100.0
B1: Argonne National Lab	1	100.0
C1: Industrial Brownfield (I)	19	57.9
C2: Industrial Brownfield (II)	8	87.5
D1: Ag Production Facility	27	60.0
G1: Industrial Brownfield	6	100.0
H1: Industrial Brownfield	15	46.7

[1] Number of clones in the 75% $\mathrm{CARBON_{MAI}}$ quantile / total number of clones at each planting.

TABLE 12 | Percentage of: 1) clones present in CARBON$_{MAI}$ hotspots (HOTSPOT$_{CLONE}$), 2) CARBON$_{MAI}$ hotspots present by clone (CLONE$_{HOTSPOT}$), and 3) clones distributed across the entire site (SITE$_{CLONE}$) within five individual plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. CARBON$_{MAI}$ = mean annual increment (MAI) of aboveground total carbon.

Planting	**Clone**	**HOTSPOT$_{CLONE}$ (%)**	**CLONE$_{HOTSPOT}$ (%)**	**SITE$_{CLONE}$ (%)**
C1: Industrial Brownfield (I)	I4551	29.7	39.3	19.4
	80x00601	18.9	63.6	7.6
	220-5	13.5	62.5	5.6
	51-5	10.8	50.0	5.6
	7300501	8.1	27.3	7.6
	Crandon	5.4	40.0	2.8
	ISU.25-35	5.4	28.6	4.9
	Eugenei	2.7	33.3	2.1
	ISU.25-21	2.7	16.7	4.2
	ISU.25-R5	2.7	16.7	3.5
	Ohio.Red	2.7	11.1	6.3
C2: Industrial Brownfield (II)	220-5	26.3	55.6	6.9
	I4551	21.1	17.4	5.9
	Crandon	15.8	50.0	15.8
	7300501	10.5	40.0	5.0
	51-5	10.5	22.2	5.9
	80x01107	5.3	14.3	10.4
	ISU.25-21	5.3	20.0	5.9
D1: Ag Production Facility[4]	ISU.25-35*	14.4	72.2	5.0
	DN182	13.3	31.6	10.6
	ISU.25-R4*	11.1	62.5	4.5
	80X00601	6.7	66.7	2.5
	80X00601*	6.7	54.6	3.1
	DN5	6.7	20.0	8.4
	ISU.25-12*	5.6	41.7	4.2
	7300501	4.4	44.4	2.5

	252-4*	4.4	30.8	3.6
	42-7*	4.4	50.0	2.2
	DN34	4.4	16.7	6.7
	119.16	3.3	23.1	3.6
	D121	3.3	33.3	2.5
	D124	3.3	27.3	2.8
	ISU.25-R5*	3.3	27.3	3.1
	ISU.25-R2*	2.2	12.5	4.5
	8000105	1.1	12.5	2.2
	ISU.25-21*	1.1	8.3	3.3
G1: Industrial Brownfield	S13C20	36.7	34.4	15.8
	Ken8	20.0	28.6	10.4
	S7C1	20.0	50.0	5.9
	3-1	10.0	30.0	5.0
	189-4	10.0	21.4	6.9
	94-4	3.3	8.3	5.9
H1: Industrial Brownfield	Ken8	32.4	57.9	14.1
	S7C1	29.4	50.0	14.8
	S13C20	11.8	44.4	6.7
	DN21	8.8	11.1	20.0
	DN31	8.8	16.7	13.3
	189-4	5.9	28.6	5.2
	72C-2	2.9	16.7	4.4

[1] Number of trees per clone in 25% CARBON$_{MAI}$ quantile / number of trees in 25% quantile across the site.

[2] Number of trees per clone in 25% CARBON$_{MAI}$ quantile / number of trees per clone at the site.

[3] Number of trees per clone / total number of trees at the site.

[4] Clones of planting D1 denoted with an asterisk (*) were established as rooting cuttings.

TABLE 13 | Observed stand-level diameter at breast height (DBH_{OBS}) and mean annual increment of aboveground total (stem + branch) dry biomass ($BIOMASS_{OBS}$) at poplar phytoremediation plantings in the Midwest, United States, along with expected diameter (DBH_{EXP}) and biomass ($BIOMASS_{EXP}$) of equally-aged poplar grown for bioenergy and biofuels near the phytoremediation plantings. Overall, there were no differences in diameter ($P = 0.0614$) or biomass ($P = 0.0938$) between phytoremediation and biomass trees. The percent difference in diameter (DBH_{Δ}) and biomass ($BIOMASS_{\Delta}$) of trees grown on liability lands versus typical production systems is also shown. See Headlee et al. (2013) for a description of the biomass plantings.

Phytoremediation Planting	**Biomass Planting**	**Age (y)**	**DBH_{OBS} (cm)**	**DBH_{EXP} (cm)**	**DBH_{Δ} (%)**	**$BIOMASS_{OBS}$ (Mg ha^{-1} y^{-1})**	**$BIOMASS_{EXP}$ (Mg ha^{-1} y^{-1})**	**$BIOMASS_{\Delta}$ (%)**
A1: Rhinelander Landfill (I) (Rhinelander, WI)	Rhinelander, WI	14.5	15.3	24.3	- 37.0	5.0	20.9	- 76.1
A2: Rhinelander Landfill (II) (Rhinelander, WI)	Rhinelander, WI	13.5	14.0	23.4	- 40.2	4.4	20.6	- 78.6
A3: Oneida County Landfill (I) (Rhinelander, WI)	Escanaba, MI	8.0	13.7	16.6	- 17.5	11.2	9.8	+ 14.3
A4: Oneida County Landfill (II) (Rhinelander, WI)	Rhinelander, WI	12.5	19.0	22.3	- 14.8	9.4	19.9	- 52.8
B1: Argonne National Lab (Lemont, IL)	Lancaster, WI	14.0	23.3	19.0	+ 22.6	5.4	12.1	- 55.4
C1: Industrial Brownfield (I) (LaSalle, IL)	Arlington, WI	11.0	16.1	23.3	- 30.9	9.3	12.1	- 23.1
C2: Industrial Brownfield (II) (LaSalle, IL)	Arlington, WI	11.0	10.8	23.3	- 53.6	7.3	12.1	- 39.7
D1: Ag Production Facility (Midwest)	Ames, IA	11.0	19.6	17.0	+ 15.3	15.5	6.7	+ 131.3

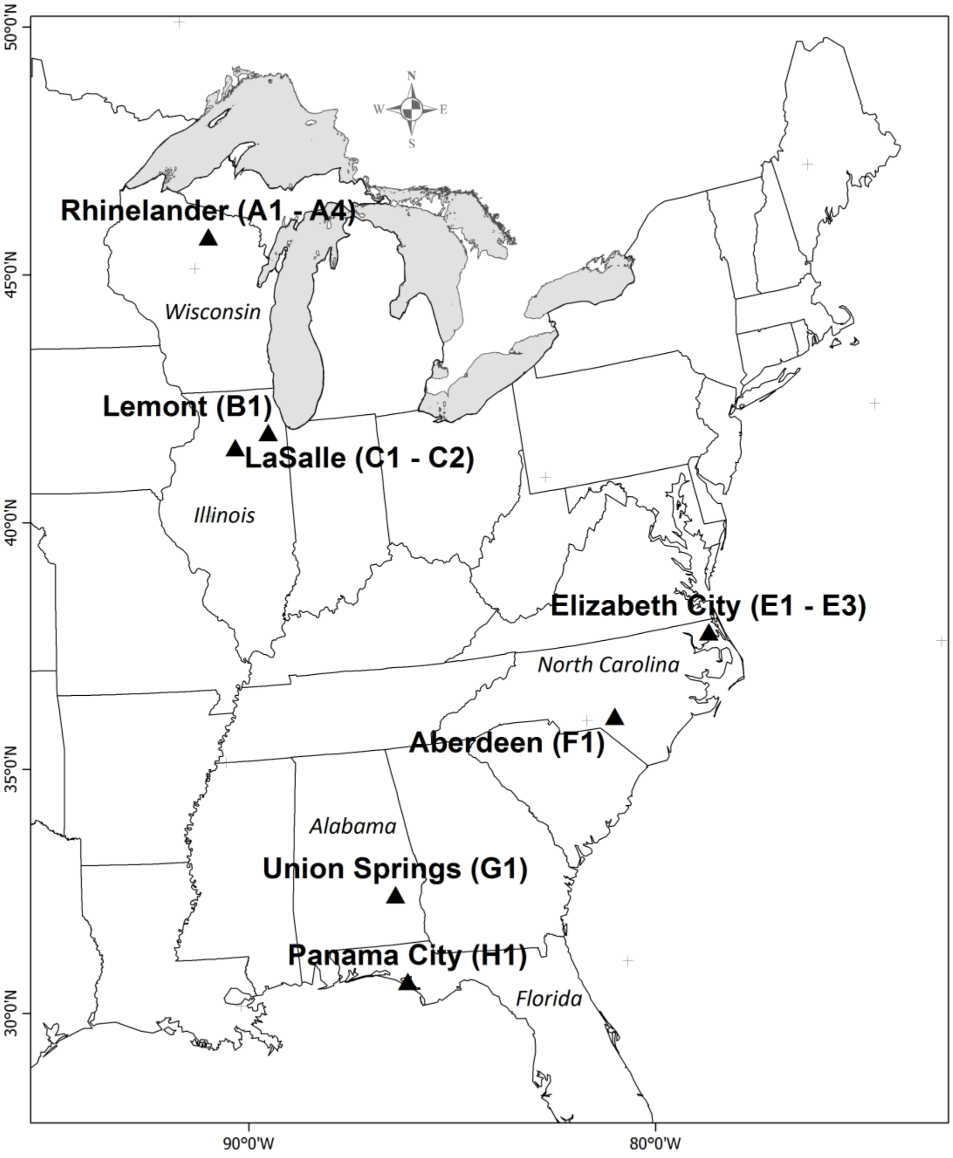

FIGURE 1 | Map of long-term phytoremediation sites in the Midwest and Southeast, United States.

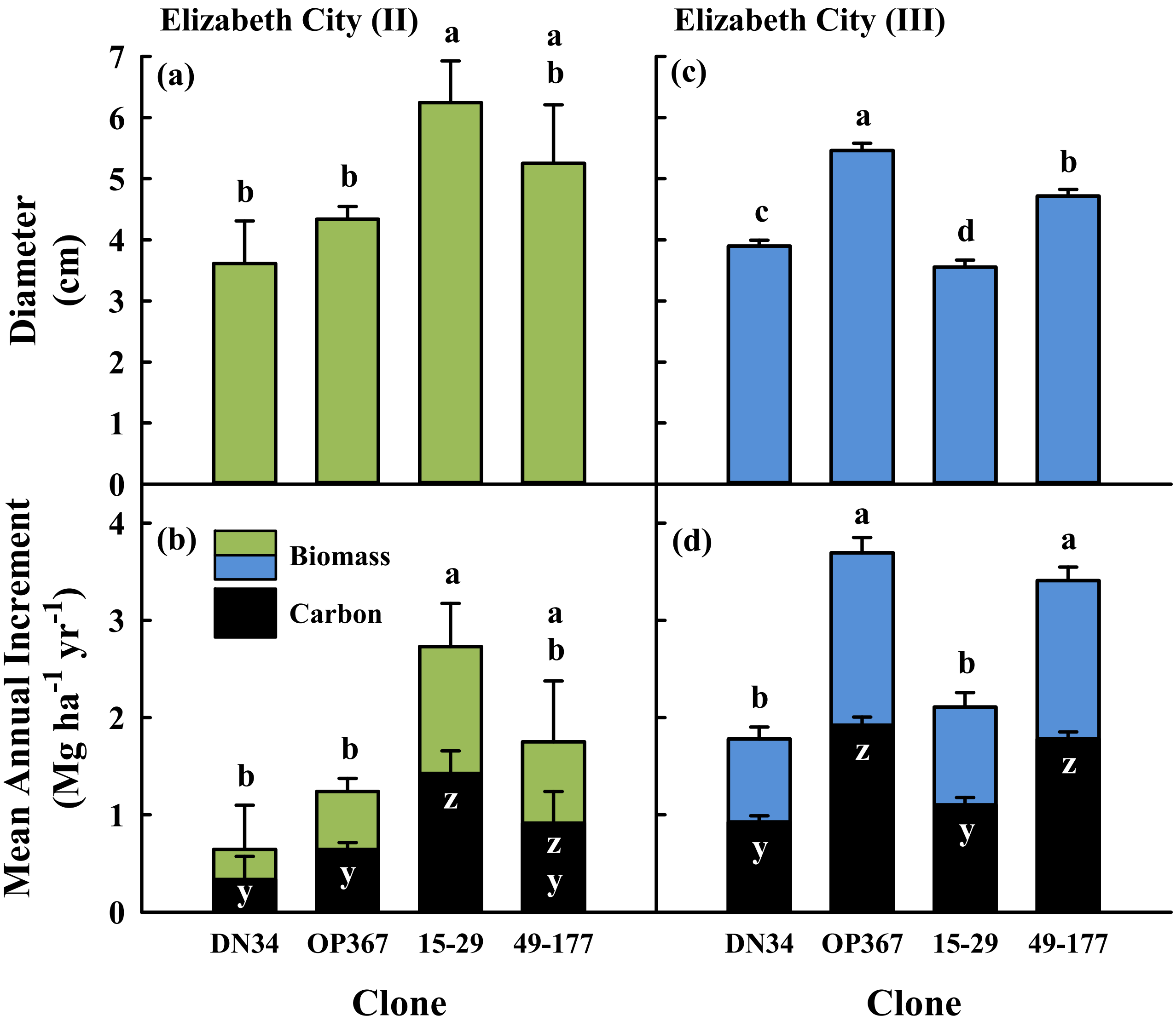

FIGURE 2 | Least-squares means of diameter at breast height, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon for four poplar clones (‘DN34’ and ‘OP367’, *P. deltoides* × *P. nigra*; ‘15-29’ and ‘49-177’, *P. trichocarpa* × *P. deltoides*) evaluated at a U.S. Coast Guard Base in Elizabeth City, NC (plantings E2 and E3). Different letters above bars within a planting for each trait represent statistically significant differences ($P < 0.05$). Error bars equal one standard error of the mean.

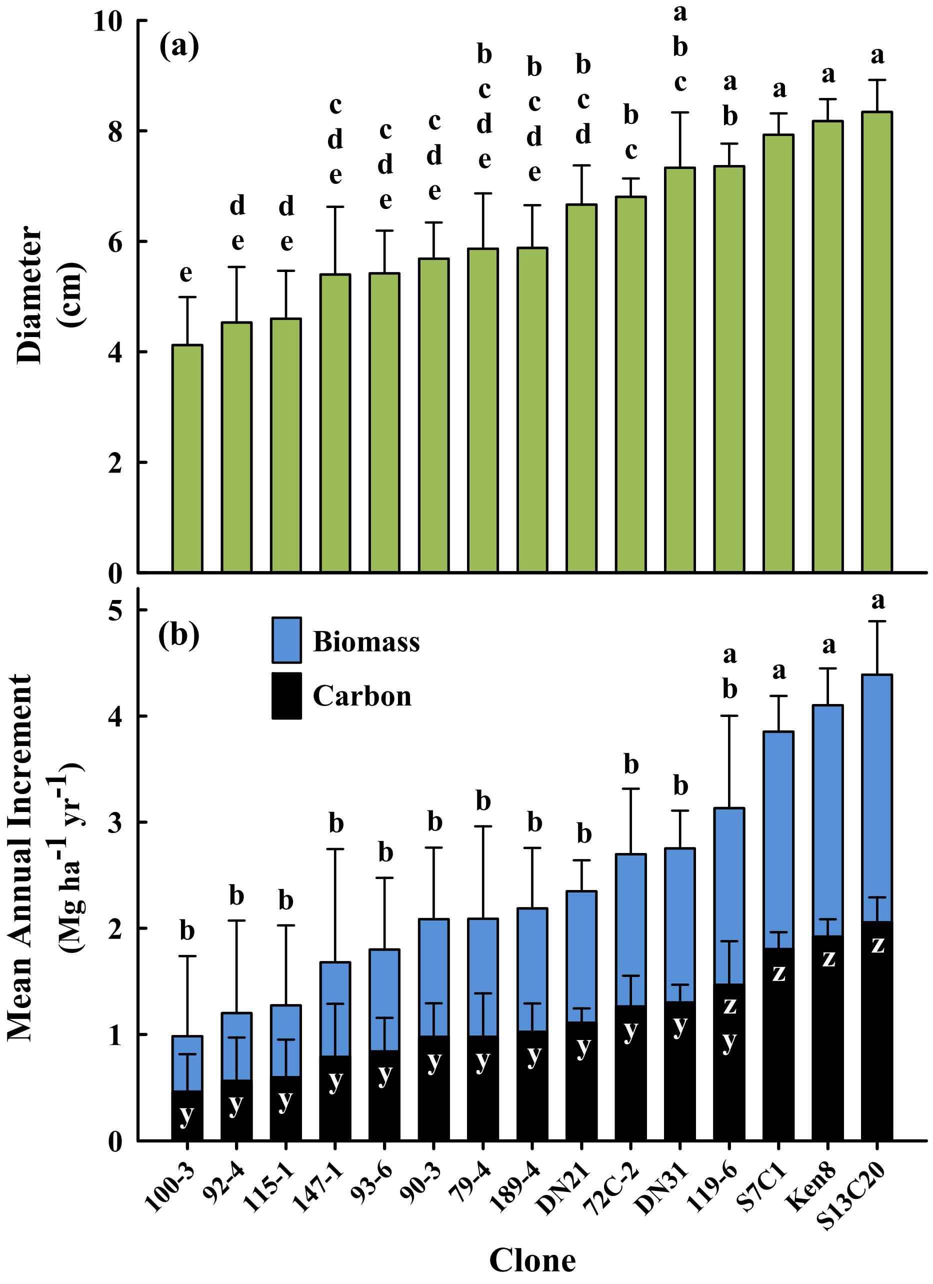

FIGURE 3 | Least-squares means of diameter at breast height, mean annual increment (MAI) of aboveground total (stem + branch) dry biomass, and MAI of aboveground total carbon for fifteen poplar clones evaluated at an industrial brownfield in Panama City, FL (planting H1). With the exception of 'DN21' and 'DN31' that are both *Populus deltoides* × *P. nigra* F_1 hybrids, all clones belong to the *P. deltoides* genomic group. Different letters above bars within a trait represent statistically significant differences ($P < 0.05$). Error bars equal one standard error of the mean.

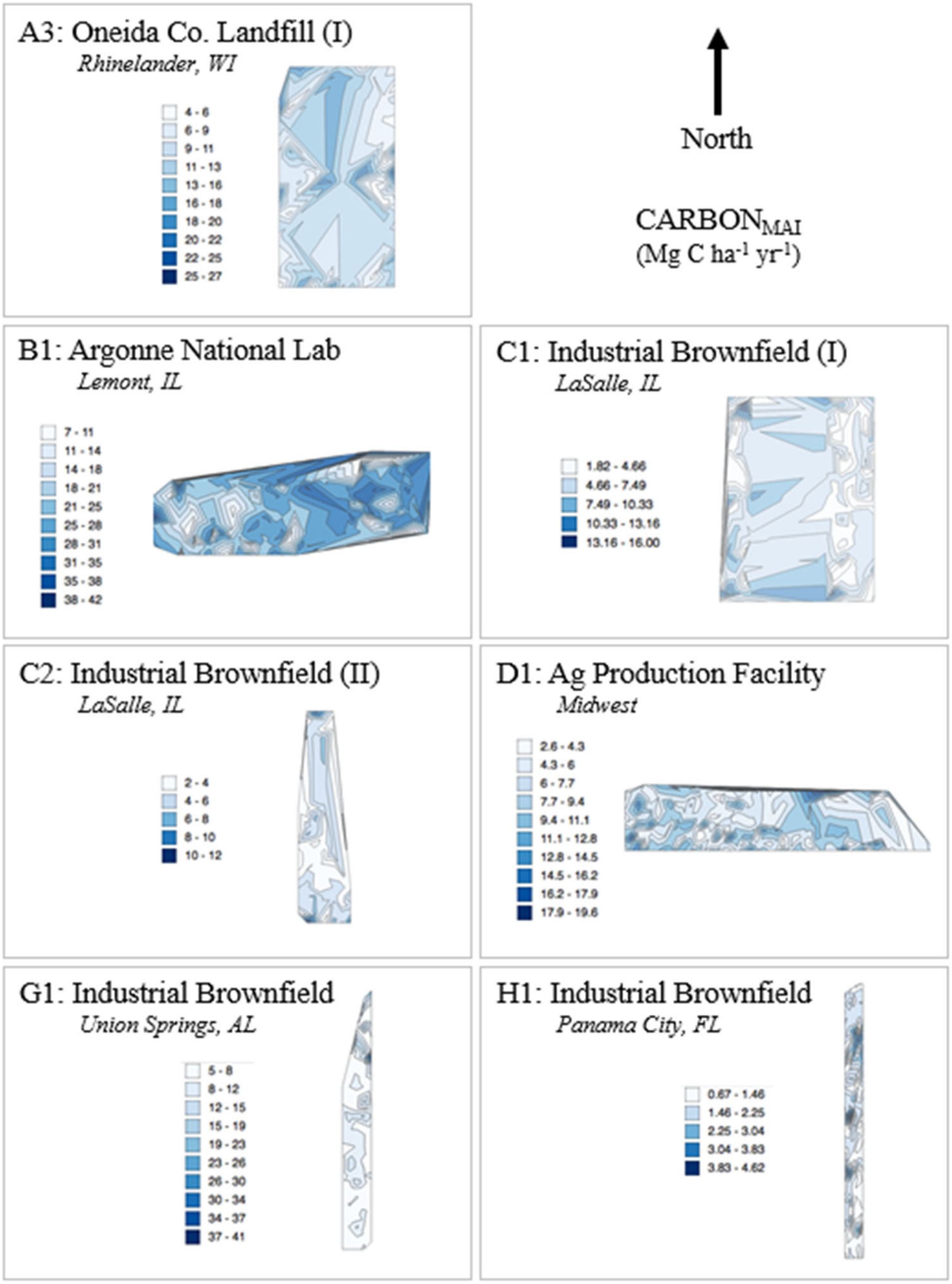

FIGURE 4 | Spatial distribution of mean annual increment (MAI) of aboveground total carbon (CARBON$_{MAI}$) at seven poplar plantings in a review evaluating ecosystem services of phytoremediation applications in the Midwest and Southeast, United States. Descriptions of the seven plantings are provided in Table 3.