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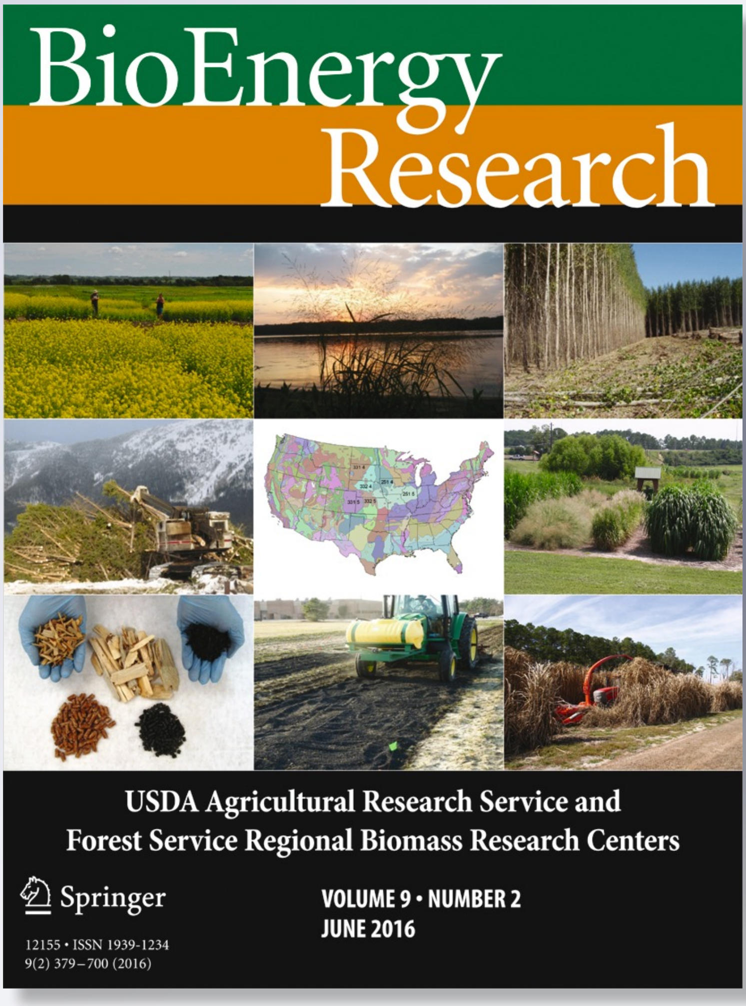

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Environmental Technologies of Woody Crop Production Systems

Ronald S. Zalesny Jr[1] · **John A. Stanturf**[2] · **Emile S. Gardiner**[3] · **Gary S. Bañuelos**[4] · **Richard A. Hallett**[5] · **Amir Hass**[6] · **Craig M. Stange**[7] · **James H. Perdue**[8] · **Timothy M. Young**[9] · **David R. Coyle**[10,11] · **William L. Headlee**[12,13]

Abstract Soil erosion, loss of productivity potential, biodiversity loss, water shortage, and soil and water pollution are ongoing processes that decrease or degrade *provisioning* (e.g., biomass, freshwater) and *regulating* (e.g., carbon sequestration, soil quality) ecosystem services. Therefore, developing environmental technologies that maximize these services is essential for the continued support of rural and urban populations. Genotype selection is a key component of these technologies, and characteristics of the species used in short rotation woody biomass systems, as well as the silvicultural techniques developed for short rotation woody crops are readily adapted to environmental applications. Here, we describe the development of such woody crop production systems for the advancement of environmental technologies including phytoremediation, urban afforestation, forest restoration, and mine reclamation. The primary goal of these collective efforts is to develop systems and tools that can help to mitigate ecological degradation and thereby sustain healthy ecosystems across the rural to urban continuum.

Keywords Forest restoration · Mine reclamation · *Populus* · Phytoremediation · *Salix* · Urban afforestation

Abbreviations

B	Boron
C	Carbon
Cl	Chloride

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DDT	Dichlorodiphenyltrichloroethane
EC	Electrical conductivity
FLR	Forest landscape restoration
IAES	Institute for Applied Ecosystem Studies
Mg ha^{-1} $year^{-1}$	Megagrams per hectare per year
MSW	Municipal solid waste
Na	Sodium
PAL	Phenylalanine ammonia lyase
PCE	Perchloroethylene
PMLU	Post-mining land use
PPO	Polyphenol oxidase
Se	Selenium
SRWC	Short rotation woody crops
TCE	Trichloroethylene
US	United States
USDA ARS	USDA Agricultural Research Service
USDA FS	USDA Forest Service
VOCs	Volatile organic compounds

Introduction

Soil erosion, loss of productivity potential, biodiversity loss, water shortage, and soil pollution are ongoing processes that decrease or degrade ecosystem services [1]. Degradation ranges from severe erosion of surface soil to chemical, biological, or radiological contamination or altered inundation regime. Ecosystem services are generally categorized as *provisioning*, *regulating*, *cultural*, and *supporting services* [2]. Ecosystem services are key drivers in almost all Research and Development from the USDA Forest Service (USDA FS) and USDA Agricultural Research Service (ARS), and the USDA Biomass Research Centers focus specifically on *provisioning services* (e.g., biomass, freshwater) and *regulating services* (e.g., carbon sequestration, soil quality). Developing environmental technologies that maximize these services is essential to meet the needs of rural and urban populations. To accomplish this, USDA scientists are leaders in developing phytoremediation, urban afforestation, forest restoration, and mine reclamation technologies. The characteristics of the species used in short rotation woody biomass applications, and the silvicultural techniques developed for short rotation woody crops (SRWCs), are readily adapted to environmental applications. In particular, poplars (*Populus* species and their hybrids) and willows (*Salix* species and their hybrids) have been used successfully in a variety of situations where the primary aims are environmental quality and protection rather than biomass production [3–5]. Most of the poplar and willow genomic groups in Table 2 of Zalesny et al. [6] have been used for phytoremediation and associated environmental technologies. For *Populus*, the primary species used include the following: eastern cottonwood (*Populus deltoides* Bartr. ex Marsh), European black poplar (*Populus nigra* L.), Japanese poplar (*Populus suaveolens* Fischer subsp. *maximowiczii* A. Henry), and western black poplar (*Populus trichocarpa* Torr. & Gray). For *Salix*, six species are predominantly used for environmental technologies: Missouri willow (*Salix eriocephala* Michx.), sandbar willow (*Salix interior* Rowlee), Japanese willow (*Salix miyabeana* Seemen.), black willow (*Salix nigra* Marshall), basket willow (*Salix purpurea* L.), and dragon willow (*Salix sachalinensis* F. Schmidt).

A common feature of the environmental technologies discussed below is that they are designed for situations where the physical environment, especially the soil resource, has been altered to the point of biological degradation (or exceedance of regulatory thresholds) or presents conditions that are atypical of the 'natural' conditions of the sites. Identifying species or provenances adapted to these conditions is commonly the first requirement for developing appropriate technologies [7, 8]. High biomass productivity per se may not be the top priority; rather, successful establishment and persistence or high uptake of contaminants may be the primary goal. A genetically improved seedling with fast growth, proper form, or desirable wood quality is required for production plantings but plant material for environmental applications may need other qualities such as precocious flowering [9] or an ability to take up and sequester sodium (Na) and chloride (Cl) into leaf, woody, and root tissues [10, 11].

Many environmental plantings may have secondary uses to produce wood products. For example, windbreaks or stream buffers protect crops or watercourses but may be managed to produce wood products or bioenergy [4, 9]. In any case, the techniques used to establish biomass plantations, whether for production or environmental purposes, are based on the same technologies [12]. Here, we describe the advancement of environmental technologies including phytoremediation, urban afforestation, forest restoration, and mine reclamation. The primary goal of these collective efforts is to develop systems and tools that can help to sustain healthy ecosystems across the rural to urban continuum despite elevated levels of ecological degradation.

Phytoremediation

Background

The International Phytotechnology Society (http://phytosociety.org/) defines phytotechnologies as *the strategic use of plants to solve environmental problems by remediating the qualities and quantities of our soil, water, and air resources and by restoring ecosystem services in managed landscapes*. Phytoremediation is the most well-known phytotechnology, being the direct use of plants to clean up contaminated soil, sediment, sludge, or groundwater

[13–15]. Much of the biomass research conducted in the Maximum Yield Work Unit of the Lake States Forest Experiment Station's Northern Institute of Forest Genetics in Rhinelander, WI (45.64° N, 89.47° W) (Fig. 1) during the 1970s and 1980s focused on feedstock production for energy and fiber, with an emphasis on developing and refining silvicultural systems for productive and sustainable plantations [6, 16]. Genetics, physiology, and vegetation management were at the forefront of research priorities. During these decades, emerging concerns about waste management created an opportunity to repurpose SRWC intensive forestry techniques to meet additional goals of remediating contaminated soil and water resources [12, 17]. As a result, phytotechnology research in Rhinelander began in the mid-1990s. The primary emphasis of initial phytoremediation research at Rhinelander was to evaluate the use of poplars and willows as biological filters atop or adjacent to closed landfills. The practical implications for resource managers included being able to recycle and reuse municipal solid waste (MSW) landfill leachate on-site to reduce the economic and ecological costs associated with treating the wastewaters, along with maintaining regional environmental integrity of groundwater aquifers and nearby water bodies while simultaneously growing a biomass crop.

Phyto-recurrent Selection

Early phytotechnology research in Rhinelander involved testing the performance and phytoremediation capabilities of SRWCs in greenhouses and growth chambers, then progressed to field tests in tanks with engineered soil layers and ultimately field-scale plantations. Current research involves a combination of greenhouse and field tests. Adopting crop and tree improvement strategies used in forestry, horticulture, and agronomy, *phyto-recurrent selection* was developed to choose superior-performing genotypes for specific remediation efforts [7, 18]. The method involves using multiple testing cycles to evaluate, identify, and select favorable clones based on the response of genotypes to variable leachate chemistries and site conditions. Early cycles are relatively short and data collected are easy to acquire (typically done in the greenhouse or growth chamber), while later cycles require more time and resources to increase knowledge of genotypes advancing (typically done in the field). Fewer clones are tested as the complexity of the data increases, and multiple-trait selection strategies are used to evaluate the combination of complex phenotypic expressions regulated by quantitative traits (e.g., height, diameter, biomass—see below for more details). The ultimate goal is to deploy a combination

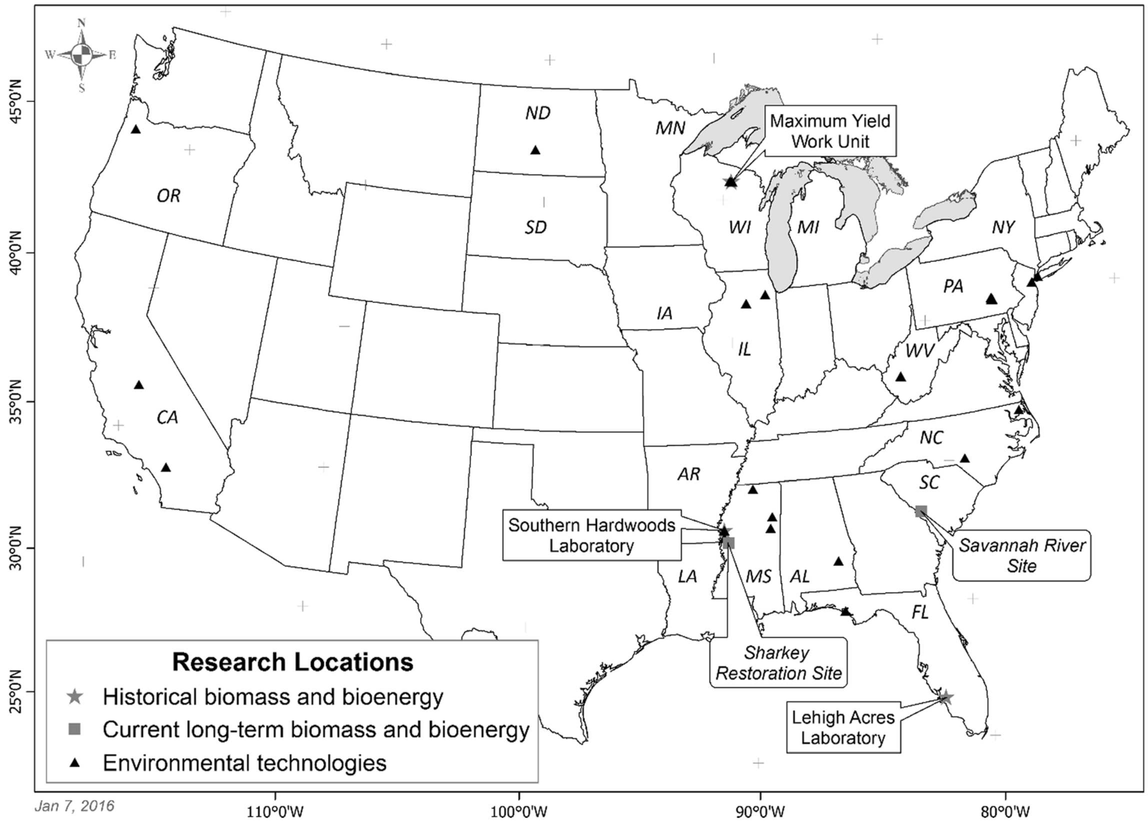

Fig. 1 Map of research sites for environmental technologies of woody crop production systems in the USA. The three sites indicated in *rectangular boxes (with square edges)* are historic locations for USDA Forest Service woody crop biomass research and development, and the two sites indicated in the remaining *two boxes (with round edges)* are current, long-term biomass and bioenergy research locations

of genotypes with improved phytoremediation potential over the original set of clones tested for the particular phytoremediation application, as well as adequate genetic variation to guard against insect/disease outbreaks, changes in soil conditions (especially those induced by the wastewaters), and unfavorable genotype × environment interactions [7, 18]. The first production bioenergy/phytoremediation plantation was established at the Oneida County Landfill (Online Resource 1) using clones selected from early phyto-recurrent selection cycles [19]. Most recently, phyto-recurrent selection has been used for urban afforestation (see below) [20], biochar applications [21], and mine reclamation (see below).

Salts and Salinity

Sodium and Cl are constituents of primary concern in many landfills, which have led to saline wastewaters and soil conditions with elevated electrical conductivity (EC) levels. Phyto-recurrent selection has been used to choose clones exhibiting superior growth and development combined with accumulation of Na and Cl into leaf, woody (stem + branch), and root tissues when irrigated and fertilized with high-salinity landfill leachate. To date, USDA FS researchers have conducted 11 studies associated with phytoremediation of MSW landfill leachate in northern Wisconsin. The studies were conducted at the Institute for Applied Ecosystem Studies (IAES) (formerly known as the Northern Institute of Forest Genetics), Oneida County Landfill, and former City of Rhinelander Landfill in Rhinelander, WI, US (45.6° N, 89.4° W). Both landfills have been closed for some time; the Oneida County Landfill currently serves as a MSW transfer station. Two synthesis and analysis projects were completed at the IAES facilities [7, 11], two research studies were conducted in greenhouses at the IAES [17, 18], four field studies took place at the Oneida County Landfill [10, 19, 22, 23], and three projects were conducted at the Rhinelander Landfill [8, 24, 25].

Both allometric and physiological stress indicators were measured in the aforementioned studies. Allometric traits included the following: height, diameter, volume, number of leaves, root morphology traits, and biomass of leaves, stems, and roots. Physiological indicators were concentrations of heavy metals and salts in leaves, stems, and roots (i.e., uptake data), as well as sap flow and other water usage variables. Both Na and Cl supplied via the MSW landfill leachate has been accumulated at high concentrations in poplar tissues [8, 10]. Zalesny et al. [10] reported the least amount of Na and Cl was accumulated in the woody tissue (i.e., harvestable biomass) of poplars, which corroborated common physiological trends that Cl generally being transported out of the root is stored in leaves and branches versus Na that is restricted more to the root [26].

While phenotypic responses have ranged from little observed stress to complete mortality, there was minimal foliar disruption (i.e., leaf wilting or necrosis) and associated decrease of average aboveground productivity across the study area due to high-salinity leachate chemistries [8, 10, 17–19, 22, 23]. The overall mortality rate at the Oneida County Landfill ranged from 6 % (*P. nigra* × *P. suaveolens* subsp. *maximowiczii* 'NM6') to 56 % [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* 'NC13460'], with a mean of 22 % [10]. There were presumed osmotic effects associated with elevated EC and salt concentrations that led to some water stress (i.e., leaf wilting), yet the responses were short-lived and sustained toxicity symptoms (e.g., scorched leaf margins, excessive leaf abscission, yellow mottling of leaves, leaf size reduction) were not evident [17]. Overall, aboveground responses were highly clone specific [8, 17]. For example, the estimated percent of leachate Na accumulated in the total tree biomass (leaf + woody + root) of eight clones tested in phyto-recurrent selection cycle 4 at the Oneida County Landfill following 2 years of establishment ranged from 1 % (*P. deltoides* × *P. nigra* 'DN5') to 6 % ('NM6'), with a mean of 3 %, while that for Cl ranged from 3 % ('NC13460') to 21 % ('NM6'), with a mean of 10 % [11]. Fast growth rates of favorable poplar clones have exceeded loss of abscised foliage and tissue chlorosis that often lead to reduced photosynthetic area, carbon assimilation, and/or biomass accumulation [23, 27]. Certain genotypes at the Oneida County Landfill exhibited lower levels of salts in their leaves and therefore preserved more biomass in photosynthetic tissue. Ultimately, this substantially influenced total tree biomass and helped to choose clones for further testing and deployment.

High-salinity leachate irrigation did not substantially impact root production, which was also genotype specific. Although there is minimal information about mechanisms of salt tolerance in poplars [28], optimal EC levels typically range from 1.0 to 5.0 mS cm^{-1} [29]. The variability in salt tolerance of the genotypes subjected to leachate with 10.2 mS cm^{-1} during initial greenhouse cycles [18] was indicative of broad genetic variation among poplars at the section, species, and clone level [30]. Osmotic stress was detrimental to young tissues during root initiation, but this response diminished as the trees developed and as such environmental pressure eliminated less-tolerant genotypes during subsequent phyto-recurrent selection cycles [18]. Zalesny et al. [19] tested the effects of high-salinity leachate irrigation on poplar root system morphology and reported some impacts of leachate on the presence and abundance of fine roots (0 to 2 mm diameter). One important observation was that 23 % of the trees fertigated with leachate exhibited necrotic fine root tissue dieback that appeared to have been caused by elevated EC levels ($\geq$9.4 mS cm^{-1}) with high Na ($\leq$1200 mg L^{-1}) and Cl ($\leq$1250 mg L^{-1}) concentrations. However, subsequent regrowth from the same nodal locations was prevalent and had balanced out negative impacts of the leachate. For example, given strong genetic control, trees receiving leachate fertigation had only 4 % less fine root biomass than those receiving well-water alone, which was not significantly different. Plant roots often increase growth to utilize areas with more nutrients and water and decrease growth or abscise tissue in response to environmental stressors [31]. Zalesny et al. [19] observed plastic root responses that enabled tissue avoidance to reduce exposure to the high-salinity leachate environment.

A comparison of clones 'NM6' and 'NM2' (*P. nigra* × *P. suaveolens* subsp. *maximowiczii*) illustrates the importance of selecting specific clones rather than genomic groups when fertigating poplar trees with high-salinity wastewaters. These clones have exhibited similar establishment potential and productivity throughout the North Central USA [32, 33], yet have had drastically different above- and below-ground responses to MSW leachate application [24]. For example, Zalesny et al. [17] reported positive growth and biomass responses of 'NM6' to leachate versus well-water control, while differences between treatments were non-existent for 'NM2'. Likewise, Zalesny et al. [10] reported a 10 % difference in total amount of Cl accumulated in leaf tissue, despite the fact that Cl distribution among plant tissues was nearly identical for both clones.

Currently, there are two follow-up studies related to this landfill work. First, at the Oneida County Landfill, 8-year-old trees of 'NM2' were measured in 2013 as part of the meta-analysis of long-term phytoremediation installations described below. The objective of these efforts was to assess biomass productivity and carbon (C) storage potential of the trees as they neared the end of their economic rotation. Individual-tree, annual biomass productivity ranged from 2.6 to 56.4 dry Mg ha^{-1} year^{-1} with a stand-level mean of 21.7 dry Mg ha^{-1} year^{-1}, which was 14.3 % greater than the expected productivity of this clone grown under typical biomass production conditions in northern Wisconsin. Similar results were found for C, which had 10.3 Mg C ha^{-1} year^{-1}. Second, at the former City of Rhinelander Landfill, 15.5-year-old trees belonging to clones 'NM6' and 'DN34' (*P. deltoides* × *P. nigra*) were harvested during July 2015 to assess the same allometric and physiological traits detailed for the biomass ecosystem services network described in Fig. 2 of Zalesny et al. [6]. In addition, long-term phytoremediation effectiveness is being assessed via testing of salts and heavy metals in tree tissues and soils.

In addition to landfills, our salt phytoremediation research has expanded to other sites across the rural to urban continuum. Most recently, phyto-recurrent selection has been used to identify hybrid poplar genotypes that are tolerant of highly sodic soils in central North Dakota, where recorded salinity levels have exceeded 10.0 mS cm^{-1}, a threshold not typically suitable for trees [28]. The overall objective of the work is to identify, select, and deploy genotypes that survive and develop on slightly saline soils (i.e., 4.0 to 8.0 mS cm^{-1}) given that Russian olive (*Elaeagnus angustifolia* L.) is the only current option available to the USDA Natural Resources Conservation Service across similar sites in the upper Great Plains. Despite its tolerance to the sodic conditions, Russian olive is not desirable due to its invasive characteristics. Hybrid poplar clones tolerant of salinity conditions ranging from 4.0 to 6.0 mS cm^{-1} (i.e., slightly saline) could be added to the Field Office Technical Guide for conservation plantings (i.e., windbreaks), in addition to producing a biomass crop for landowners. From a practical standpoint, successful plantings could provide protection to roads and building sites and slow the rate of saline soil expansion through reduced soil surface evaporation and greater transpiration, along with capturing more snow which may further dilute surface salinity. In the current North Dakota project, seven hybrid poplar genotypes belonging to three genomic groups (*P. nigra* × *P. suaveolens* subsp. *maximowiczii* 'NM2', 'NM6'; *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* 'NC14104', 'NC14106'; *P. deltoides* × *P. nigra* 'DN5', 'DN17', 'DN182') from the USDA FS in Rhinelander are being tested and compared with Russian olive. At 2 years after planting, survival across hybrid poplar clones ranged from 62 to 95 % (mean = 82 %) compared with 86 % survival for Russian olive. The two *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* hybrids were the only poplar clones to exhibit lower survival than Russian olive while all other genotypes had similar or better success. Similarly, only 'NM2' had shorter average height (84.7 cm) than Russian olive (96.1 cm) while height for the remaining genotypes ranged from 97.3 to 157.1 cm (hybrid poplar mean = 113.5 cm). Therefore, phyto-recurrent selection has worked through two growing seasons, although continued monitoring of genotypic performance is needed throughout the rotation before final selections can be made.

Additional work on salts has been conducted by USDA ARS scientists in California (Fig. 1), which has recently experienced one of its worst droughts in history. For the past 4 years, irrigated crop production has been drastically reduced. Water reuse strategies are an imperative technology to withstand the severe drought conditions, especially considering increasing shortages of high-quality water and attempts to maintain agronomic production coupled with the need to protect water quality in rivers, lakes, streams, and groundwater. In this regard, trees have been viewed as potential candidates for receiving poor-quality water because of the ability of selected genera to accumulate salts and specific ions while producing economically valued biomass that is not biologically hazardous. Rapid-growing tree species, such as poplars and their hybrids, may be ideal for water irrigation strategies involving poor-quality waters (i.e., those with high levels of salinity and boron (B)) in central California, because the trees transpire large quantities of water, produce large quantity of renewable resources, have long life spans relative to agronomic crops, possess deep root systems, and coppice after they have been cut [22]. As described above, poplar species are especially promising because of their genetic diversity, and the genus can be easily manipulated through hybridization and selective breeding. Nevertheless, quantitative information for both identifying and describing the mechanisms associated with salt and B tolerance in hybrid poplars is still lacking. Due to their broad genetic diversity, there is a high potential to achieve multiple ecosystem services using methods such as phyto-recurrent selection to identify physiological mechanisms in potential salt- and B-tolerant hybrid poplar clones that can be grown under adverse water and soil conditions. Inter-clonal differences may also be identified and exploited to help identify specific hybrid poplar clones that possess cellular mechanisms that effectively culminate in a tolerance for high concentrations of toxic ions such as Na, Cl, and B in poor-quality waters of Central California. For this reason, evaluating the initial ability of specific poplar genotypes to absorb, accumulate, exclude, or manage the transport (i.e., upward and downward mobility) of ions may all be contingent upon finding cellular adaption mechanisms that prevent toxic concentrations of ions from disrupting cellular function and structure.

Zalesny et al. [10] and Zalesny and Bauer [8] have reported impacts of excessive Cl and other elements on the growth of *Populus* clones. Additionally, Shannon et al. [34] and Bañuelos et al. [35] evaluated the impacts of saline and B irrigation on eight hybrid poplar clones belonging to three genomic groups (*P. deltoides* × *P. nigra* 'DN34', 'OP367', 'PC1'; *P. trichocarpa* × *P. deltoides* '50194', '50197', '1529', '49177'; *P. trichocarpa* × *P. nigra* 'D01') that were tested for salt tolerance and uptake of B and Cl in sand culture studies. After hardwood cuttings were planted and established under non-saline conditions, young saplings were treated with artificial wastewaters containing different levels of salts, B, and selenium (Se). High salt concentrations affected B- and Se-accumulation in leaves, reduced growth, and led to leaf damage and shedding; however, B and Se had no detrimental impact on growth. There was broad genetic variation in salt tolerance among the eight genotypes. The salinity at which dry mass was reduced ranged from 3.3 to 7.6 mS cm^{-1} depending on clone, and the relative decrease in dry mass with increasing salinity varied among clones and ranged from 10 to 15 % per salinity unit (i.e., mS cm^{-1}). Importantly, leaf Cl values increased in relation to the Cl concentrations in the irrigation waters, but the concentrations also were subject to clonal variation. Salt tolerance in the poplar clones was generally related to Cl content in the leaves and stems but was also influenced by growth and vigor characteristics, as well as the allometric relationships between leaves and stems that influenced the sinks in which ions could accumulate before reaching toxic levels. Defoliation occurred as a function of salinity concentration and characteristically began at the base of the main stem and continued toward the apex over time. Leaf analyses indicated that this damage coincided with Cl accumulation and some clones had a greater ability to restrict Cl accumulation in the shoot when compared with most other genotypes. Salt tolerance of hybrid poplars is related to their ability to (1) avoid the accumulation of Cl in shoot tissue or exclude salts from the shoot, (2) maintain low leaf Cl content by restricting uptake at the root and transport from root to shoot, or (3) avoid high leaf Cl by maintaining higher proportions of sink tissues. Clearly, mechanisms associated with Cl restriction in poplars need to be examined.

To address this need, Bañuelos et al. [36] studied the joint impact of salinity and B on hybrid poplar growth and reported significant variation in leaf B concentration across tolerant clones. Differences were associated with clones, treatments (irrigation with non-saline versus irrigation with high salinity and high B water), and leaf positions. Specifically, B concentration was significantly greater in leaves of tolerant *P. trichocarpa* × *P. nigra* hybrids treated with poor-quality water than those grown under control conditions. A notable exception was observed with clone '345-1' [(*P. trichocarpa* × *P. deltoides*) × *P. nigra*], whose lower leaves accumulated the same average B concentration under both control and high salinity/high B conditions. Lower leaves tended to accumulate significantly greater B concentrations than upper leaves in both control and salt/B treatment conditions, except in control-grown '13-366' (*P. deltoides* × *P. nigra*). Notable exceptions were also observed under salt/B treatment in 'Simp-Alk' (*P. deltoides* × *P. nigra*) and in '302-4' (*P. trichocarpa* × *P. nigra*) and '309-72' (*P. deltoides* × *P. nigra*) under control growing conditions. In these cases, the concentration of B was higher in upper leaves than in lower leaves. Boron concentrations in lower and upper leaves of less tolerant clones were significantly higher than in leaves from similar positions collected from the most salt- and B-tolerant clones (e.g., '13-366'; '302-4'; '309-72'). In soils with high salinity (5.0 to 7.0 mS cm^{-1}) and B (4 to 6 mg L^{-1}) that are associated with the presence of poor-quality groundwater (i.e., 8.0 to 10.0 mS cm^{-1}; 5 to 8 mg B L^{-1}; 0.12 mg Se L^{-1}) in Central California, seasonal accumulation of Cl and B in the most salt- and B-tolerant clones [(*P. trichocarpa* × *P. deltoides*) × *P. nigra* '13-366'; '345-1'; '347-14'] fluctuated over time (Figs. 2 and 3). Specifically, leaves collected at the end of summer each year from up to 56 trees per clone in an ongoing multi-year field study had significantly greater concentrations of both Cl and B compared to early spring collected leaves, irrespective of the year. Throughout six years of growth, leaf Cl concentrations were sometimes as high as 10,000 mg Cl kg^{-1} leaf dry mass, while leaf B concentrations were as high as 1000 mg B kg^{-1} leaf dry mass. Robinson et al. [37] also reported leaf concentrations as high as 845 and 1200 mg B kg^{-1} leaf dry mass in poplars grown in non-saline soils containing between 30 and 40 mg B kg^{-1} soil, respectively. These high leaf B concentrations at both the saline and non-saline

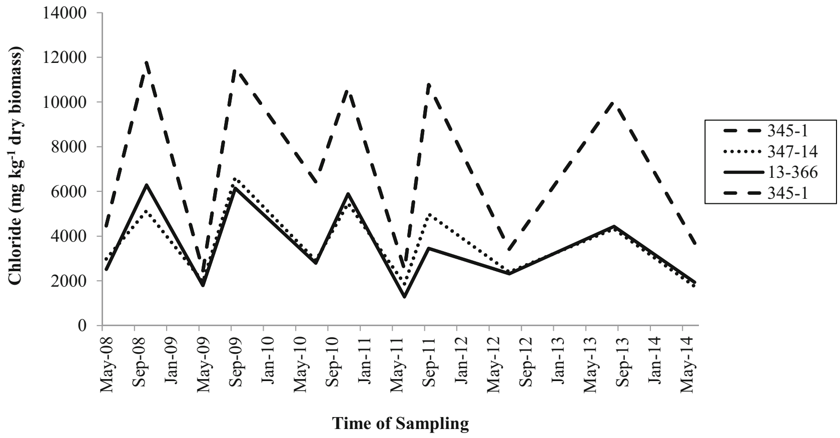

Fig. 2 Chloride concentrations in leaves of salt- and boron-tolerant poplar hybrids field-grown in spring and fall under high saline and boron conditions for 6 years. Values represent means from a minimum of 10 replicates

sites indicate that there is no common B exclusion mechanism among these poplar genotypes. At high B tissue levels, Rees et al. [38] demonstrated with neutron radiography that the ability of a *P. deltoides* × *P. nigra* (i.e., *P.* × *euramericana*) hybrid to accumulate B was associated with B hypertolerance in the living tissue and storage of B in dead leaf tissue.

In an attempt to further understand the salt and B tolerance of these selected clones, Bañuelos et al. [36] tested their root to shoot metal translocation mechanisms. However, uncertainty exists about the detoxification strategies of salt and B tolerant poplar genotypes with high tissue B concentrations. Depending on the clone, these strategies included the following: (1) compartmentation, (2) element localization, (3) tolerance/detoxification mechanisms in leaf tissues, or (4) sub-cellular responses resulting in cellular structure isolating the excessive accumulation of toxic ions. Current studies at the Swiss Federal Institute for Forest, Snow, and Landscape in Birmensdorf, Switzerland, involve using histochemical analyses and x-ray micro analytical methods to identify cellular structure responses to excessive salinity and B in salt- and B-tolerant poplar clones (Arriaga, Bañuelos, and Vollenweider, unpublished data). Relatedly, other physiological responses exhibited by poplars when exposed to excessive salt and B may also be linked to differences in antioxidant responses (i.e., changes in total phenolic content and activity of enzymes such as phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO)) [39–41]. The presence and activities of PAL and PPO are currently being investigated by Sommerhalter and Nguyen (unpublished data) in leaves of salt and B tolerant poplar clones, as these enzymes and their activity can be affected by changes in phenolic metabolism as a result of stress [40]. Generally, plants exposed to harsh growing conditions (e.g., high salinity and B), increase their antioxidant levels, including phenolic compounds, which can help suppress the subsequent free radical activity [39, 42]. At the molecular level, others have examined association linkages between DNA markers and the polygenic traits of salt and B tolerance compared to salt and B sensitive genotypes of poplar trees (Follen, Prince and Bañuelos, unpublished data). In this regard, a map of microsatellite markers linked to salt and B tolerance in poplars is being established. These efforts will be useful for developing new screening methods to identify additional tolerant poplar accessions, as well as for refining current methods such as phyto-recurrent selection. Subsequent efforts have identified some genes that were linked to salt and B tolerance in poplar trees and, thus, may be candidate genes for inferring this tolerance (unpublished data from Gell, Prince, and Bañuelos that is based upon

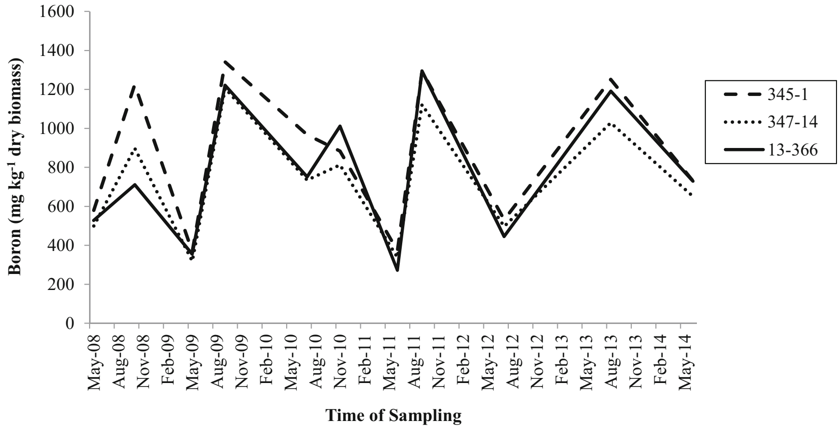

Fig. 3 Boron concentrations in leaves of salt- and boron-tolerant poplar hybrids field-grown in spring and fall under high saline and boron conditions for 6 years. Values represent means from a minimum of 10 replicates

current proteomic research of LeDuc at California State University—East Bay).

Clearly, the physiology and genetics of salt and B tolerance is not completely understood in poplars; however, combining information from current field studies with cellular and molecular investigations may help to elucidate the primary physiological drivers of such tolerance. Following the identification and selection of tolerant poplar genotypes, practical management strategies must be developed and employed to sustain long-term use of poor-quality waters using poplars and their hybrids [43]. With new salt and B tolerance information, the broad variation that is the hallmark of the genus *Populus* may offer opportunities for introducing salt and B tolerance into breeding programs, even though there is much to be learned about physiological response mechanisms to excessive salt and B.

Additional Inorganic and Organic Contaminants

In addition to salts, past and current phytoremediation research out of Rhinelander includes heavy metals [7, 8, 23], nitrates [25], and other inorganics as well as organic contaminants [44] (Table 1). One notable current project is an assessment of ecosystem services (i.e., biomass and carbon) of poplar at long-term phytoremediation sites in the eastern USA [3]. The meta-analysis is a collaboration among federal, state,

Table 1 Recent phytoremediation projects of USDA Forest Service Northern Research Station researchers and their collaborators

Site	System	Issue	Lead institution(s)
Landfills			
Rural landfill (Rhinelander, Wisconsin)	Leachate fertigation	Salts, heavy metals	USDA Forest Service Northern Research Station
Rural landfill (Rhinelander, Wisconsin)	Leachate fertigation	Soil fauna diversity	USDA Forest Service Northern Research Station
Rural landfill (Rhinelander, Wisconsin)	Fiber cake effluent fertigation	Fertilization (NPK) plus compost organic matter	USDA Forest Service Northern Research Station
Municipal landfill (Rhinelander, Wisconsin)	Fertigation with hydraulic barrier	Ammonia, nitrates	USDA Forest Service Northern Research Station
Municipal landfill (Rhinelander, Wisconsin)	Fertigation	Inorganics, organics	USDA Forest Service Northern Research Station
Urban landfill (New York City, New York)	Afforestation and soil improvement	Inorganics, organics, ecosystem diversity	USDA Forest Service Northern Research Station
Brownfields			
Industrial brownfield (LaSalle, Illinois)	Soil remediation	TCE, PCE	University of Florida
Industrial brownfield (Aberdeen, North Carolina)	Soil remediation	DDT, lindane	North Carolina State University
Industrial brownfield (Union Springs, Alabama)	Soil remediation	Organics	University of Florida
Industrial brownfield (Panama City, Florida)	Soil remediation	Arsenic	University of Florida
Industrial brownfield (Gary, Indiana)	Riparian buffer	Petroleum hydrocarbons	US Environmental Protection Agency
Urban brownfield (Gary, Indiana)	Soil remediation, urban development	Petroleum hydrocarbons	Fresh Coast Capital and Delta Institute
Urban brownfields (Midwest)[a]	Hydraulic control and overland flow	Inorganics, organics	USDA Forest Service Northern Research Station
Research brownfield (Lemont, Illinois)	Soil remediation	VOCs, tritium	Argonne National Laboratory
Military brownfield (Elizabeth City, North Carolina)	Soil remediation	Petroleum hydrocarbons	North Carolina State University
Agricultural production facility (Iowa)[a]	Riparian buffer and overland flow	Salts, heavy metals, nitrates	USDA Forest Service Northern Research Station
Other			
Agricultural production farm (Boone, Iowa)	Biochar for propagation	Inorganics	Iowa State University
Ethanol plant (South Dakota)[a]	Fly ash for foliar fertilizer	Inorganics	Iowa State University
Rivers and streams (Midwest)[a]	Riparian stabilization	Erosion, nutrient runoff	Iowa State University
Production tree farms (Nile River, Egypt)	Municipal wastewater	Inorganics, organics	USDA Forest Service
Hog lagoon (North Carolina)[a]	Hydraulic control and subsurface flow	Nitrates	North Carolina State University

[a] Due to a landowner confidentiality agreement, it is not possible to list the specific site location

and industrial partners to address the knowledge gap associated with long-term monitoring of woody crop phytoremediation systems throughout their rotations. In summary, biomass productivity and carbon storage potential were evaluated at 15 poplar plantings belonging to nine long-term phytoremediation installations located in the Midwest (Illinois, Iowa, Wisconsin) and Southeast (Alabama, Florida, North Carolina) regions. Sites included landfills, brownfields, agricultural production facilities, and military installations. Inorganic contaminants were as follows: nitrates, salts, and heavy metals, while their organic counterparts were as follows: volatile organic compounds (VOCs), industrial solvents (trichloroethylene (TCE), perchloroethylene (PCE)), petroleum hydrocarbons, insecticides (dichlorodiphenyltrichloroethane (DDT)), and pharmaceuticals (e.g., lindane). In total, 55 clones belonging to ten genomic groups were tested. 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. For example, phytoremediation trees at the Midwestern sites generally exhibited ~20 % non-significant reduction in diameter ($P = 0.0614$) and biomass ($P = 0.0938$) relative to the bioenergy trees, with some genotype × environment interactions resulting in phytoremediation trees exhibiting substantially greater growth and productivity. Results also showed that multiple silvicultural prescriptions (e.g., propagule type, genotype selection) should be tested at individual sites in order to maximize the provision of ecosystem services while optimizing the mitigation of contaminants.

Urban Afforestation

During the five decades that SRWC research has been occurring there have been dramatic changes in where people are living. In 1950, 30 % of the world's population lived in urban settings. The United Nations estimates that by 2050, 66 % of the world's population will live in urban areas [45]. Today, over 80 % of the US population is urban [46]. These population trends are starting to inform new areas of ecosystem research as we begin to recognize that our cities are more than non-forested areas. Municipalities around the world are investing in urban forests with the goal of providing essential ecosystem services. Many of these investments are allocated toward increasing urban tree canopy cover by restoring degraded or destroyed ecosystems. For example, the majority of the trees in New York City's MillionTreesNYC initiative (completed October, 2015) were planted in large restoration sites in urban natural areas.

The urban environment is very different from the environment in which our forested ecosystems successfully evolved. Our ability to create an urban forest is much more challenging than in less populated natural areas. We have a large body of knowledge for making sure trees are growing productively in our rural working forests. For example, questions about which species to plant on which soil types and seedling spacing to maximize growth and productivity have been answered to the extent that we can create prescriptions for maintaining our rural forests. Far less is known about creating forests in urban environments because of the profound influence of human activities. These influences include high levels of atmospheric pollutants and chemical contamination in the soil [47, 48], soils that are made up of construction debris (e.g., asphalt, rebar, concrete, etc.) or covered by gravel fill, higher temperatures, and exotic invasive plant species.

Currently, there is little research on the growth, survival, and recruitment of new native urban forests and most of the existing studies are less than 5 years in duration [49]. Existing research on urban afforestation includes a body of work which examines recruitment, growth, and survival of woody vegetation on urban landfill sites [50, 51], research on impacts of metals on woody vegetation in naturally vegetated industrial sites [48, 52], and designed experiments examining the effect of species diversity and soil amendments on the success of urban forests planted on soils modified by anthropogenic influences [53, 54].

Phytoremediation and urban afforestation projects are both faced with soils modified by anthropogenic influences and environmental conditions that were not part of the evolutionary history of the vegetation that is planted and expected to thrive in the project areas. However, urban afforestation projects must meet a different set of objectives that do not focus on biomass production or remediating environmental contamination. Instead, such projects are installed to provide ecosystem services like carbon sequestration, soil improvement, or storm water runoff mitigation [55, 56]. In addition, urban forests are created and maintained to provide *cultural* and *supporting* ecosystem services. Municipal policies and laws will also play a major role in the design of urban afforestation projects. For example, in NYC, the Parks Department requires that only native tree species can be planted and that the genetic stock must originate from within a 200 mile radius of NYC. In addition, long-term management strategies cannot include removal of trees (Local Law 3; http://legistar.council.nyc.gov).

New research on urban afforestation is setting the stage for including research techniques that have been developed in the environmental technologies discipline (Fig. 4). For example, recent work by Pregitzer et al. [57] examined the performance of four native tree species growing in four categories of NYC urban soil types based on land use history (native glacial till, coal ash, clean fill, and urban fill) in a controlled greenhouse study. The study demonstrated that different species had varying performance depending on soil category illustrating the need for matching species (and perhaps genotypes) to the project site. In another study, Zalesny et al. [20] developed propagation techniques for native poplar and willow

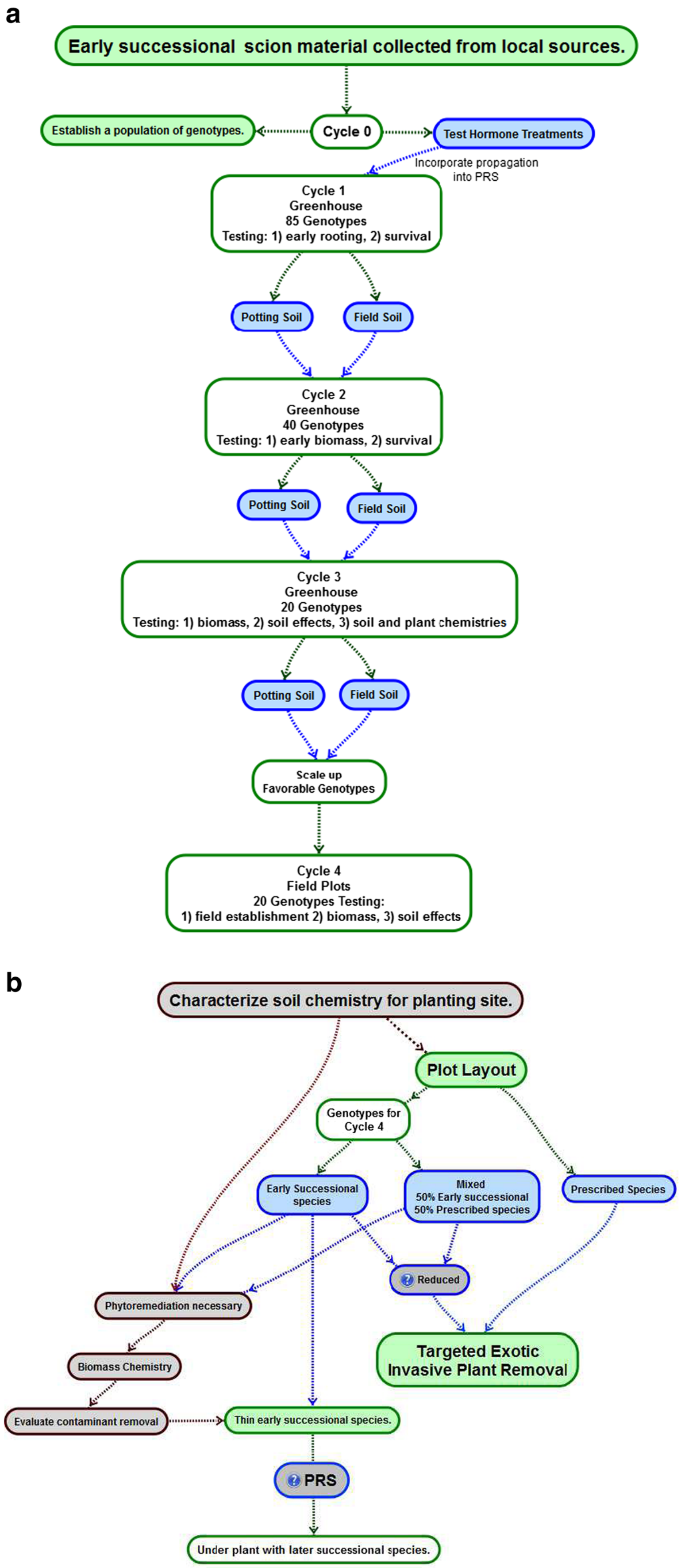

Fig. 4 (**a**) Anthropogenic succession strategy; Phase I: Phyto-recurrent Selection [20]. Urban afforestation using phytorecurrent selection techniques to match genotypes of early successional species to the site. Selected genotypes are advanced to Phase II (Fig. 4b). (**b**) anthropogenic succession strategy; Phase II: Field Trials. Genotypes from Phase I (Fig. 4a) advanced to field trials. Phytoremediation is included depending on site quality. Field trials comparing selected genotypes with standard planting palettes will provide information on whether early successional species are effective at reducing exotic invasive plant invasion

genotypes from Staten Island, NY, in order to provide multiple genotypes for use in phyto-recurrent selection for an afforestation project at Freshkills Park on a legacy landfill site (study installed October, 2015).

Although the long-term goals of creating urban forests might include increased canopy cover, carbon sequestration, or urban heat island mitigation, these goals will not be realized immediately. The near-term goals that managers focus on are as simple as initial tree survival and growth. The NYC Parks and Recreation Department invests in 2 years of mechanical and chemical site preparation to control exotic invasive plant species prior to planting the young trees. Planting stock comes in 7.6-L pots and trees are planted at a spacing of 1.2 × 1.2 m. The ultimate goal is to capture the site and reach canopy closure as quickly as possible to shade out, outcompete, or prevent regeneration of exotic invasive plant species. Post planting, the site is maintained with mechanical and chemical spot treatments aimed at controlling exotic plant species. Costs are high to establish and maintain an urban forest. Continued human intervention is necessary in order to maintain a forest with native tree and plant species.

Forest Restoration

The use of fast-growing tree plantations for forest restoration purposes appears to be gaining greater acceptance with increased emphasis on functional restoration, that is, restoration which emphasizes functional processes rather than structure or composition [9, 58]. On sites degraded by agriculture, plantations of fast-growing, early successional species provide a relatively quick pathway to restoration of ecological functions associated with forests and can produce a range of co-benefits typically not provided by conventional afforestation practices [59–61]. The quick development of forest cover can immediately initiate amelioration of soil degradation, reassembly of nutrient and water cycling processes, development of understory environments conducive to natural regeneration of native species, creation of habitat for native fauna, and generation of revenue for recouping the initial restoration investment. Thus, an increasing base of research supports the premise that judicious use of fast-growing tree plantations can play a role in initiating reconstruction of native plant communities while shifting degradation of some sites toward greater productivity, production of services, and sustainability [9, 58, 62].

Work conducted by the USDA FS Southern Research Station's Center for Bottomland Hardwoods Research at the Sharkey Restoration Research and Demonstration Site (Fig. 1) addresses restoration of bottomland hardwood forests on agricultural land proven to be economically marginal for row crop production [63]. Primary aims of the research are to develop an afforestation system that is (1) capable of initiating recovery toward native bottomland hardwood forests, (2) adaptive enough to address a range of possible landowner objectives beyond forest restoration, and (3) economically sustainable with opportunity for early financial returns [63, 64]. The potential to provide multiple benefits and return income to the landowner quicker than conventional afforestation methods are key drivers of forest restoration in the Mississippi Alluvial Valley where most land is privately owned [65]. Accordingly, research on the site was designed to encompass four afforestation methods that represent a range of restoration intensities from passive (natural recolonization) to intensive (eastern cottonwood-broadleaf interplanting) restoration [59]. This intensive practice builds upon the well-developed silviculture and management of eastern cottonwood plantations advancing this species as a nurse crop for slower growing broadleaf species. Stanturf et al. [59] documented a positive relationship between the rate of development of forested conditions and intensity of restoration, indicating that the environmental benefits of forested conditions can be obtained more readily with the more intensive cottonwood-broadleaf interplanting method. DeSteven et al. [66], who studied herbaceous plant communities relative to intensity of restoration practices, found that ground-layer biomass decreased with restoration intensity and canopy development, but understory species richness and presence of non-native plants were not influenced by restoration intensity 12 years after treatment establishment. Research on the study site continues to examine the trajectory of plant community development and other indices of forest restoration, and future research will likely explore the use of other fast-growing tree species, such as black willow (*S. nigra*), for forest restoration applications.

Forest landscape restoration (FLR) is an active area of research for several of the authors, both in the USA and internationally. Primary collaborations have been through the International Union of Forest Research Organizations (IUFRO) that have resulted in several compendia of forest restoration activities globally [67–70]. The current focus is on the key contributions of FLR to climate change mitigation and adaptation [71], consisting of a wide array of policy, governance, and operational aspects that need to be addressed before a landscape can be improved to better meet desired social, environmental, and economic objectives including those related to climate change. Forest landscape restoration and climate-related policy are closely inter-linked and reciprocal. On the one hand, FLR can support achievement of climate-related commitments; on the other, climate policies, tools and funds can accelerate implementation of FLR.

Mine Reclamation

As described above, one of the advantages of woody crop production systems is that they can be grown on marginal and disturbed lands, otherwise not suitable for agriculture and other liability lands where species selections are limited (e.g., in a phytoremediation setting). In both scenarios, competition for the land base and other resources (e.g., water) associated with food production is drastically reduced. In such cases, productive land inventory is increased and excess surface runoff associated with reclaimed mine lands is mitigated. Additionally, overall acreage of land associated with food production is maintained. The southern coalfield of West Virginia, USA, is a primary example of such lands, where post-mining land use (PMLU) often leads to degradation of essential ecosystem services (e.g., water quantity and quality). Incorporating SRWC systems as a viable PMLU alternative increases the potential for biomass production and restoration of natural resources. Similarly, SRWCs may provide an economic stimulus to revitalize local economies, counterbalancing the financial impact of the declining coal industry. To address these needs, USDA FS scientists and collaborators from West Virginia State University have recently established hybrid poplar biomass production farms on surface-mined lands in the southern coalfield of West Virginia. The restoration activities included phyto-recurrent selection to identify favorable hybrid poplar genotypes for broad-scale deployment on these liability lands. In particular, a mine reclamation study was established in 2014 at the Four-Mile Surface Mine near Charleston, West Virginia (38.2° N, 81.7° W) using 60 different hybrid poplar genotypes belonging to seven genomic groups (phyto-recurrent selection cycle 1). After the first growing season (126 days after planting), clonal survival percentages ranged from 19 to 100 %, genomic groups ranged from 56 to 100 %, and the stand-level mean survival was 75 %. Height was measured from the soil line to the apex of the terminal bud, while diameter was measured 2 cm above the point of attachment between the terminal shoot and the original cutting. On average, height and diameter were nearly six times greater for the six most-productive genotypes relative to their least-productive counterparts (Fig. 5). Height ranged from 6.6 cm [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* 'NC13470'] to 38.3 cm ('NM2') and the stand-level mean was 23.1 cm, while diameter went from 0.6 mm ('NC13470') to 3.6 mm ('NM2') with a mean of 2.3 mm. In May 2015, cycle 2 was established, consisting of 32 of the original 60 genotypes. Those data are currently being summarized.

In addition to these current efforts, the USDA FS conducted surface-mine reclamation research from 1976 to 1993 at the laboratory in Berea, Kentucky [72, 73]. Species examined included the following: alders (*Alnus* spp.), birches (*Betula* spp.), white ash (*Fraxinus americana* L.), green ash (*Fraxinus pennsylvanica* Marshall), eastern black walnut (*Juglans nigra* L.), sweetgum (*Liquidambar styraciflua* L.), tulip poplar (*Liriodendron tulipifera* L.), empress tree [*Paulownia tomentosa* (Thunb.) Steud.], pines (*Pinus* spp.), spruces (*Picea* spp.), *Populus* hybrids, oaks (*Quercus* spp.), and black locust (*Robinia pseudoacacia* L.). They found that with proper choice of species, even acidic surface-mined lands have a long-term potential for forest production [74]. Some economically important species can survive and have respectable growth. This research started before extensive re-grading, soil amendments, and herbaceous cover cropping of surface-mined lands were required and seldom done. As requirements for herbaceous cover were instituted for erosion control even where forests were to be established, it became apparent that herbaceous species usually cause an increase in tree seedling mortality and retard tree growth due to resource competition, especially in the first few years after planting [75]. Although results of current reclamation practices may differ from those achieved by earlier USDA FS work at Berea, the foundation for mined land reclamation with trees was established in the eastern coalfields [73].

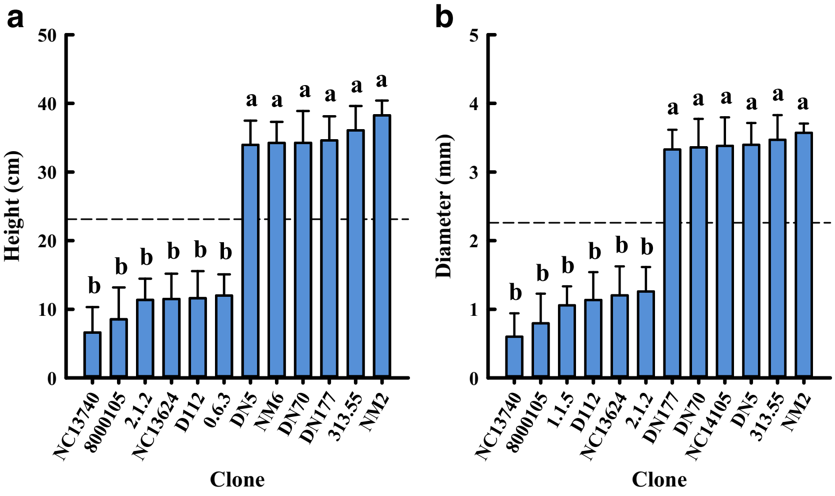

Fig. 5 Height (**a**) and diameter (**b**) of the six least- and most-productive *Populus* clones (out of 60 genotypes that were planted for *phyto-recurrent selection* cycle 1) grown for restoration of degraded mine lands in West Virginia, USA. *Different letters above bars* within a trait represent statistically significant differences ($P < 0.05$). *Error bars* equal one standard error of the mean. The *dashed line* is the mean across all 60 clones

Conclusions

Future Research Directions

Seven areas of continuing research are in progress to further advance phytoremediation, urban afforestation, and mine reclamation:

1. Enhance phyto-recurrent selection methodologies to (1) select favorable tree genotypes for specific remediation, reforestation, and afforestation efforts, (2) deploy a combination of genotypes with improved phytoremediation potential than the original set of genotypes tested for the particular phytoremediation application, and (3) provide adequate genetic variation to guard against insect and disease outbreaks, changes in soil conditions, and unfavorable genotype × environment interactions.
2. Develop phytomatrices to predict the fate of soil and water contaminants into tree tissues (root, shoot, leaf) of particular *Populus* and *Salix* genotypes during phytoremediation and associated phytotechnologies. Include in the matrices a description of genotypes that perform well across a broad range of contaminants (i.e., generalists) versus those that are tolerant of specific concerns (i.e., specialists). Expand genus list to *Eucalyptus*.
3. Identify salt tolerant genotypes and salinity thresholds of woody energy crops irrigated with high-salinity wastewaters or grown on highly-saline/-sodic soils.
4. Identify superior tree species and varieties for afforestation and reforestation activities in urban and rural areas to increase the provision of ecosystem services for long-term ecological sustainability.
5. Demonstrate the capabilities and value of phytoremediation in an urban setting while still meeting all other goals of urban afforestation projects.
6. Utilize phyto-recurrent selection techniques to identify genotypes and species that are likely to perform well at a given site impacted by anthropogenic influences.
7. Use information and techniques gained in items 5 and 6 to develop a replicable and scalable anthropogenic successional strategy for creating forests in cities regardless of geography and climatic zone (Fig. 4).

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