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Species Trials of Short Rotation Woody Crops on Two Wastewater Application Sites in North Carolina, USA

Shawn Dayson Shifflett · Dennis W Hazel · Douglas J Frederick · Elizabeth Guthrie Nichols

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Abstract Forty-two *Populus* spp. clones, *Eucalyptus benthamii*, and seven tree species native to North Carolina were evaluated for survival and height growth through the establishment phase at two municipal wastewater application sites. Groundwater was monitored at each site to determine if establishment of the species trials resulted in exceedances of nutrient mitigation requirements. At the Gibson Wastewater Treatment Facility, 26 *Populus* clones had 100 % survival, with mean height growths ranging between 152 to 260 cm, and basal diameters ranging between 11.4 and 28.8 mm. Green ash, planted in 2011 and 2012, had high survivorship (>95 %) with first year mean height growth of 30±28 cm (2012) and second year mean height growth of 101±52 cm (2011). Basal diameter for green ash was 33.3±12.6 mm. *E. benthamii* had moderate survivorship (>77 %) and first year mean height growth of 47±27 cm. At the Jacksonville Wastewater Treatment Facility, green ash and bald cypress had high survivorship (>96 %), first year mean height growths of 14±25 cm and 27±16 cm, and basal diameters of 13.1±3.9 mm and 11.6±4.8 mm, respectively. Survivorship for 12 *Populus* clones ranged from 50 and 94 % with mean first year height growths between 58 to 121 cm, and basal diameters between 6.8 and 12.5 mm. *E. benthamii* had low survivorship (43 %) with mean first year height growths of 17±17 cm and basal diameters of 12.0±7.7 mm. Groundwater concentrations of NO_3+NO_2 and N-NH_4 remained below regulatory requirements at both sites with one exceedance in February 2012 in Jacksonville, NC. The results show that some *Populus* clones are excellent candidates for woody biomass production on municipal wastewater application fields. Native green ash and bald cypress are also good candidates, but these trees may require longer rotations than *Populus* to achieve similar biomass yields.

Keywords *Populus* · Native trees · Municipal wastewater · Bioenergy · Marginal and degraded lands

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S. D. Shifflett · D. W. Hazel · D. J. Frederick · E. G. Nichols (✉)
Department of Forestry and Environmental Resources, North Carolina State University, North Carolina, NC, USA
e-mail: egnichol@ncsu.edu

S. D. Shifflett
e-mail: sdshiffl@ncsu.edu

D. W. Hazel
e-mail: dennis_hazel@ncsu.edu

D. J. Frederick
e-mail: doug_frederick@ncsu.edu

Introduction

Short rotation woody crops (SRWCs) are intensively managed plantations characterized by closely spaced tree plantings and short harvest rotations (1 to 20 years) [1–6]. In the southeastern United States, *Populus* spp., *Eucalyptus* spp., and *Pinus* spp. have gained popularity for use in SRWC systems due to their high growth rates and their provision of conventional forest products for wood manufacturing [7]. Planting density for these systems can range from 5,000 to 20,000 stems ha^{-1}, but can be as low as 1,000 to 2,500 stems ha^{-1} [4]. Research on SRWC systems first began in the 1960s by evaluating "sycamore silage" [4, 5]. In the 1970s and 1980s, SRWCs were part of a national energy research focus to develop renewable energy resources [4, 6]. As the need for energy security recently increased, SRWC systems have simultaneously regained public interest [8–10]. Large-scale production of SRWCs may provide local bioenergy sources and environmental benefits such as the reduction of greenhouse gases, soil conservation, and nutrient sequestration [11–13]. Despite these positive qualities, there are concerns about the negative impacts caused by expanding SRWC plantations. One major controversy is where SRWC plantations should be established. Tenenbaum [14] has argued that promoting energy production from bioenergy sources will lead to the conversion of productive food agriculture lands for energy and fiber production, potentially causing food shortages. High water demand by these plantations is a second concern [15, 16].

Researchers have proposed that degraded or marginal lands can provide significant SRWC biomass production without compromising food production or the conventional management of forested lands [17–20]. Marginal lands represent a small but important source for SRWC production at the global scale [17]. Abandoned and degraded lands could amount to 10 to 52 % of current liquid fuel consumption by converting these lands to SRWCs production [19]. Municipal wastewater application fields are a potential marginal land resource for SRWC production, but these lands not always defined within the framework of marginal or degraded lands [20–24]. Studies in the 1980s considered these lands to be "disturbed lands and marginal lands," [25] representing valuable production acreage to be sourced for biomass and bioenergy generation.

Various studies have evaluated SRWC production, particularly *Populus* spp. and *Eucalyptus* spp., on municipal wastewater application lands in the United States, Europe, Australia, and the Middle East [25–29]. Hopmans et al. [30] observed that species selection played a critical role for biomass production in Australia where biomass production was greatest for *Populus* spp. and *Eucalyptus* spp., followed by *Casuarina cunninghamiana*, (Miq.), and *Pinus radiate* (D. Don). Borjesson and Berndes [26] showed that SRWC production on wastewater application sites had added economic value due to the removal of nutrients and minimization of eutrophication in Swedish surface waters. Dimitriou and Rosenqvist [27] found that fertilizing *Salix* SRWCs with wastewater increased biomass production and reduced fertilization costs. Shah et al. [28] concluded that *Eucalyptus* seedlings grew more when irrigated with wastewater versus tap water in Pakistan. Zalesny et al. [29] successfully established *Populus* spp., *Eucalytpus* spp., *Pinus* spp., *Khaya ivorensi*, African mahogany *(*A. Chev.), *Tectona grandis*, teak (L.), and *Gmelina arborea*, beechwood (Roxb.) plantations using municipal wastewater for irrigation in Egypt. Collectively, these studies note that careful management is required to avoid negative impacts of wastewater irrigation on local water sources. SRWCs on municipal wastewater land application sites must produce biomass, effectively remove nutrients, and protect surface and groundwater quality.

Potential contamination of groundwater and surface water from municipal wastewater irrigation is a concern in North Carolina (USA) with its high incidence of nutrient-sensitive waters [31] and an emerging bioenergy industry for woody biomass, bioenergy, and biofuels [32]. Globally, there is some disagreement about the impact of SRWC production on nutrient groundwater quality at wastewater land application sites. Some SRWC studies have reported that groundwater concentrations may initially exceed the regulatory limits during establishment at these sites [33, 34]. Reviews of the literature have shown that willow SRWC establishment can maintain nitrogen contamination in groundwater below regulatory requirements [35]. Similar to other studies [26, 36], Minogue et al. [37] observed that nitrogen taken up by *Populus deltoides* clones exceeded inputs from irrigation and atmospheric deposition, thus protecting shallow groundwater. One objective of this study was to evaluate the impact of establishment and early growth of SRWCs on nutrient concentrations in groundwater at two municipal wastewater application facilities in North Carolina.

In the USA, most woody biomass production on municipal wastewater sites have not been managed as SRWC plantations, but instead were studies evaluating conventional forest management on municipal waste application fields using a wide range of species [25, 33, 37–40]. The best SRWC candidate for biomass production and survival is most often eastern cottonwood and its improved hybrid poplar clones [25, 37, 38]. Overman [38] evaluated ten tree species under wastewater irrigation in Florida and found significantly high production for *P. deltoides* (Bartr. ex Marsh.) as well as black locust and American sycamore. These three species grew more than 150 cm in height within the first year, with *P. deltoides* reaching approximately 250 cm in the first year. Minogue et al. [37] found that improved *P. deltoides* clones produced above ground biomass yields as high as 112 Mg ha^{-1} after 27 months. In this study, *Eucalyptus* spp. grew more than 300 cm in the first 6 months but died during winter. Collectively, these studies show that irrigation with municipal wastewater consistently increased woody biomass production; however, the establishment phase is critical for long-term performance [41, 42].

In North Carolina (USA), land application of municipal wastewater is a prominent method for reducing contaminant discharges to local surface waters with some economic return by harvesting the land cover. These lands represent 3,540 ha (~0.3 % of land) in North Carolina with sites ranging from 0.2 to 944 ha in size [43]. Only a few of these sites apply municipal wastewater to trees. Most municipal facilities in North Carolina produce hay and other herbaceous crops [43]. Based on our review of the literature, only one study has evaluated the productivity of SRWCs on wastewater application sites in North Carolina. Frederick [44] evaluated the growth of *Liquidambar styraciflua* (L.) and *Platanus occidentalis* (L.) at a moderate density (1,788 stems ha^{-1}) and found trees to have high survival (91 to 93 %) and modest biomass production (7.7–22.3 oven dry metric tons ha^{-1} after 60 months). It is important to note that this plantation was not intentionally established as a SRWC system, but conditions of the study fit within the definition of SRWC provided above.

In North Carolina, native tree species such as *Fraxinus pennsylvanica* (L.), *Taxodium distchum* (L.), *Pinus taeda* (*L.*), and *L. styraciflua* have potential as SRWC biomass resources and may be better received by land managers [45]. *Populus* spp. clones and *Eucalyptus* spp. are more likely to produce more biomass, but their growth and survival is not well documented in North Carolina. Thus, a second objective of this study was to evaluate the establishment, survival, and growth of North Carolina native trees to a variety of *Populus* spp. clones and *Eucalyptus benthamii* (Maiden & Cambage) on two wastewater land application sites.

Materials and Methods

Site Description, Groundwater Monitoring, and Site Establishment

SRWC plantations were established on two municipal wastewater application facilities in eastern North Carolina. Each location was selected based on the availability of an established irrigation system, active permits for land application of municipal wastewater, and cooperation of facility administration. Both facilities receive similar rainfall precipitation and wastewater irrigation but differ by land application size due to the volume of municipal wastewater treated (see Table 1). The study site at the Gibson Wastewater Treatment Facility (Gibson, NC) has well-drained soils and receives little shading from surrounding land cover. This site has loamy sands (Table 1) and moderate soil slopes ranging from 0–6 % to 12–15 % [46]. Wastewater application rates and nutrient loading for Gibson are provided in Table 1. The study site at the Jacksonville Wastewater Treatment Facility (Jacksonville, NC) has poorly drained, loamy fine sand soils, and substantial shading due to an adjacent 35-year-old stand of loblolly pine. The site is moderately flat with 2 to 6 % slopes [46] and is prone to pooling after either rain or wastewater applications. Wastewater application rates and nutrient loading for Jacksonville are provided in Table 1.

Shallow groundwater monitoring wells were installed at Gibson (n=9) and Jacksonville (n=12) across the planting sites (locations not shown but available in Supplementary Material). These wells were used to collect groundwater samples to evaluate leaching of nitrate and ammonia to groundwater before, during, and after site establishment. Two-inch (50-mm) boreholes were hand-augured to 1 m below groundwater depth (2 to 7 m below ground surface) in accordance with the North Carolina Department of Environment and Natural Resources, Division of Water Quality Well Construction Standards [47]. PVC-screens (50 mm diameter by 1.5 m height) were installed in the boreholes, supplemented with 2-inch PVC piped as needed to reach 1.5 m above the land surface. Capped and concreted galvanized shrouds (1.6 m) were installed over the PVC wells. A 1-L Teflon dedicated bailer (Forestry Supplies Inc., Jackson, MS, USA) was provided for each well and used for required purging and sample collection. Wells were conditioned by repeated purging for 1 month prior to the first sample collection.

Two establishment trials were conducted at the Gibson Waste Water Application Facility. The first trial, referred to as "Gibson 2011," was initiated in October 2010 by removing a diseased stand of American sycamore [48]. Subsequently, the terrain was disced to 18 cm and treated with a

Table 1 Climate, site, and experiment characteristics for Gibson and Jacksonville, North Carolina, USA

	Gibson	Jacksonville
Location	34°45′ N, 79°36′ W	34°45′ N, 77°25′ W
Facility size	7.3 ha	293.7 ha
Size of experimental layout	2.0 ha	0.4 ha
Average annual precipitation[a]	1,300 mm	1,400 mm
Average annual irrigation	1,102 mm ha^{-1}[b]	1,109 mm ha^{-1}[c]
Total applied nitrogen	223 kg[d]	93.0 kg[d]
Total applied phosphorous	31.1 kg[d]	14.2 kg[d]
Total nitrogen per tree	0.11 kg N $tree^{-1}$	0.17 kg N $tree^{-1}$
Total phosphorous per tree	0.015 kg P $tree^{-1}$	0.025 kg P $tree^{-1}$
Daily wastewater outflow	275 m^3[b]	19,700 m^3[d]
Soil series[e]	Ailey loamy sand and Pelion loamy sand	Norfolk loamy fine sand
Soil pH	5.8–6.4	5.3–7.0
Mean depth to groundwater	1.9±0.24 m	0.83±0.26 m
Mean depth of monitoring wells	3.2±1.3 m	2.8±0.26 m
Previous use	Managed American sycamore plantation	Fallow ground
Planting date(s)	03/2011, 03/2012, 05/2012[g]	03/2012, 05/2012[g]
Experimental design	Randomized block design	Randomized block design
Tree spacing	1.8×1.8 m	1.8×1.8 m

[a] Climate data provided by the State Climate Office of North Carolina

[b] Personal communication—Greg Leonard

[c] Personal communication—Jill Puff

[d] Calculated from monthly non-discharge monitoring reports supplied to Division of Water Quality North Carolina Department of Environment and Natural Resources

[e] Data provided by City of Jacksonville website

[f] Data provided by USDA Web Soil Survey [46]

[g] *E. benthamii* planting date

41 % glyphosate solution for weed control. The site was planted in two sections. One section contained seven native tree species provided by Claridge State Nursery (Goldsboro, NC, USA) and one *P. deltoides* clone provided by ArborGen, LLC (Ridgeville, SC). The native tree species included Atlantic white cedar (*Chamaecyparis thyoides* (L.)), green ash (*F. pennsylvanica* (Marshall)), loblolly pine (*P. taeda* (L.)), cherrybark oak (*Quercus pagoda* (Raf.)), water oak (*Quercus nigra* (L.)), willow oak (*Quercus phellos* (L.)), and bald cypress (*Taxodium distichum* (Rich.)). Willow oak and water oak were concurrently planted in the same blocks. The remaining native tree species were planted as an incomplete block design with each species replicated in two monoculture blocks. All native species were planted as 1-year-old bare rooted seedlings; thus all native trees were planted with an initial height and basal diameter that varied from seedling to seedling. The other section in Gibson 2011 was planted with six *P. deltoides* clones provided by ArborGen, LLC (Ridgeville, SC). Clones were planted in a completely randomized block design with three blocks. Each block contained two rows representing one clone. Clones were planted as 20–60 cm dormant cuttings, with no initial growth. Table 2 shows the total number of plantings for each tree species in Gibson 2011. A detailed planting layout is not provided but available in Supplementary Material.

A second trial was initiated at Gibson after first season survival was low with 62 % mortality in the Gibson 2011 trial. An early summer drought and lack of rainfall did not require land application of wastewater to manage lagoon water levels. Most trees died due to the drought. The second trial, referred to as Gibson 2012, was initiated by removing the *Populus* clones section as well as a new portion of the diseased American sycamore. The native tree section from Gibson 2011 was left intact, but dead trees were replaced by green ash, loblolly pine, or bald cypress. The newly available terrain was disced to 18 cm and treated for weeds using Karmex® (Dupont™, Wilmington, DE, USA). In December 2011, wheat was planted to meet facility permit requirements for a cover crop until trees were planted in March 2012. Tree locations were spot-sprayed with herbicide to maintain a surrounding wheat cover crop and reduce weed competition. In March 2012, trees were planted in two new sections. The first section was planted with *Populus* clones in a randomized block design with five blocks. Each block contained 40 *Populus* clones with *P. deltoides* × *Populus trichocarpa*, *P. deltoides*, *P. deltoides* × *Populus maximowiczii* or unknown parentage (ArborGen, LLC, Ridgeville, SC, USA). See Table 2 to identify clone parentage by ID. In the same section, eight additional monoculture blocks were planted with clones 187, 221, 302, 303, 304, 339, 380, and 444. The second section planted at Gibson 2012 was planted as a randomized block design with monoculture blocks of native trees (sweetgum, bald cypress, green ash, loblolly pine) and *E. benthamii. E. benthamii* seedlings were provided and planted by the North Carolina State University Forest Productivity Cooperative. Each species was replicated in two blocks. Table 2 shows the total number of plantings for each tree species in Gibson 2012. Clone ID and parentage can be found in Table 2, and planting layouts for 2012 are provided in Supplementary Material.

An establishment trial was conducted at the Jacksonville Waste Water Application Facility in late 2011. This trial was referred to as "Jacksonville 2012." The trial was initiated in October 2011 by discing the soil to 18 cm and applying a 41 % glyphosate solution to reduce weeds. In March 2012, part of the site was planted with six blocks in a randomized complete block design with nine replicates of bald cypress, green ash, sweetgum, and *E. benthamii* as well as six replicates of loblolly pine. In a separate section, four blocks were planted in a randomized complete block design with four replicates of 12 *Populus* clones. A detailed planting layout for Jacksonville is provided in Supplementary Material (Figure S3).

Sampling and Analyses

All tree locations and monitoring wells were recorded using a handheld unit GPS (Tremble GeoXT Handheld, Sunnyvale, CA, USA). Recorded locations were plotted using ArcGIS 10 (ESRI ArcGIS, Redlands, CA, USA) for site maps. All height (cm) measurements were collected using a Crain CMR series Measuring Rod (Crain Enterprises, Inc., Memphis, TN, USA). All basal diameter measurements were collected using MyCal-Lite Series 700 Digital Calipers (Mitutoyo America, Chicago, IL, USA). Because *E. benthamii* and native tree species were planted as 1-year-old seedlings with an initial height at planting, two measurements were required to determine height. Height was determined at the time of planting and then again several months afterward. Because *E. benthamii* was planted in April 2012 to avoid late frost, initial height measurements were not taken until July 2012. Initial heights for all other trees were determined in July 2012 at Gibson and in April 2012 at Jacksonville. Initial height growth measurements were not required for *Populus* as they were planted as dormant sticks with no pre-existing growth. No initial diameter measurements were collected for any trees. Final basal diameter was collected in October 2012 for all living trees. Mortality measurements were scored as binary traits (0=dead, 1=alive). Computing the total number of dead trees and dividing by the total number of plantings determined percent mortality. Tree height and mortality were quantified in September 2011, July 2012, and October 2012 for Gibson and in March 2012, July 2012, and October 2012 for Jacksonville. Quality control and quality assurance measures are reported in Supplementary Material.

Groundwater samples were collected monthly from November 2011 until September 2012 and were analyzed for nitrate and nitrite (NO_3+NO_2) and ammonium (N-NH_4).

Table 2 Species and number of trees planted in Gibson and Jacksonville, North Carolina, USA

Species		Jacksonville 2012	Gibson 2012 Number planted	Gibson 2011
Chamaecyparis thyoides	Atlantic white cedar	–	–	94
Eucalyptus benthamii	Camden White Gum	84	247	–
Fraxinus pennsylvanica	Green Ash	82	399	78
Liquidambar styraciflua	Sweetgum	82	270	–
Pinus taeda	Loblolly Pine	50	355	102
Quercus pagoda	Cherrybark Oak	–	–	93
Q. phellos, *Q. nigra*	Willow/Water Oak	–	370	–
Taxodium distichum	Bald Cypress	70	–	–
Populus spp.	Clone ID (Parentage)			
140 (*P. deltoides*)		–	5	–
176 (*P. deltoides*)		–	5	–
185 (*P. deltoides*)		–	5	97
198 (*P. deltoides*)		–	5	–
200 (*P. deltoides*)		–	5	–
221 (*P. deltoides*)		–	25	–
224 (*P. deltoides*)		–	5	–
345 (*P. deltoides*)		–	5	–
379 (*P. deltoides*)		–	–	5
380 (*P. deltoides*)		16	30	–
381 (*P. deltoides*)		–	5	–
406 (*P. deltoides*)		–	5	–
409 (*P. deltoides*)		–	5	–
410 (*P. deltoides*)		–	5	–
411 (*P. deltoides*)		–	5	–
412 (*P. deltoides*)		–	5	–
413 (*P. deltoides*)		–	5	–
414 (*P. deltoides*)		–	5	–
423 (*P. deltoides*)		–	5	–
427 (*P. deltoides*)		–	5	–
429 (*P. deltoides*)		–	5	–
432 (*P. deltoides*)		–	5	–
434 (*P. deltoides*)		–	5	–
439 (*P. deltoides*)		–	–	97
443 (*P. deltoides*)		–	–	96
444 (*P. deltoides*)		–	25	93
445 (*P. deltoides*)		–	–	82
448 (P. deltoides)		16	5	–
449 (*P. deltoides*)		16	5	–
450 (*P. deltoides*)		16	5	–
451 (*P. deltoides*)		16	5	–
187 (*P. trichocarpa x P. deltoides*)		16	30	–
188 (*P. trichocarpa x P. deltoides*)		16	5	–
229 (*P. trichocarpa x P. deltoides*)		16	5	–
302 (*P. trichocarpa x P. deltoides*)		16	30	–
303 (*P. trichocarpa x P. deltoides*)		16	30	–
304 (*P. trichocarpa x P. deltoides*)		16	30	–
337 (*P. trichocarpa x P. deltoides*)		–	5	–
339 (*P. trichocarpa x P. deltoides*)		16	30	–
341 (*P. trichocarpa x P. deltoides*)		–	5	–
342 (*P. trichocarpa x P. deltoides*)		–	5	–
6 (Unknown)		–	–	297

Table 2 (continued)

Species	Jacksonville 2012	Gibson 2012 Number planted	Gibson 2011
138 (Unknown)	–	5	–
147 (Unknown)	–	5	–
148 (Unknown)	–	5	–
230 (*P. deltoides x P. maximowiczii*)	–	5	–

Before collecting samples, wells were purged using dedicated Teflon® bailers for three well volumes or until wells were empty to assure removal of any stagnant water [49]. Samples were stored in pre-cleaned high density 125-mL polyethylene Nalgene® bottles (Rochester, NY, USA), preserved with sulfuric acid to a pH less than 2.0, kept cool at 4 °C or below, and analyzed at the Center for Applied Aquatic Ecology (CAAE, Raleigh, NC, USA) within 28 days of collection. Analysis for NO_3+NO_2 was performed on a Bran and Luebbe QuAAtro Segmented Flow Analyzer (Bran+Luebbe Inc., Delavan, WI, USA) following Standard Method 4500NO_3F and United States Environmental Protection Agency (USEPA) Method 353.2 by means of Automated Cadmium Reduction with a reportable detection limit of 5.6 μg L^{-1}. Analysis of N-NH_4^+ followed the Standard Method 4500 NH_3H, USEPA Method 350.1, by means of automated phenate, with a reportable detection limit of 7.0 μg L^{-1}. Quality control and quality assurance measures are reported in Supplementary Material.

Statistical Analyses

Mortality data was not subjected to statistical analyses because binary data with a mean incidence outside certain boundaries (e.g., 30 to 70 %) are not advised for analysis using common statistical practices [50]. Data on height and basal diameter was subjected to either one-way or two-way analyses of variance according to the randomized complete block design with an α=0.05 (SAS, PROC GLM, or PROC MIXED, Cary, NC, USA). Height, basal diameter, block, and interaction effects (height by block and basal diameter by block) were evaluated for significance where appropriate. Trees that died during the experiment were not analyzed for height growth and were not included in statistical models.

Results

Mortality and Tree Species Establishment

Gibson 2011

Figure 1 shows percent mortality data for trees planted in 2011 and 2012; actual inventory data are provided in Supplementary Material (Tables S1–S3). In 2011, the percent mortality for all tree plantings was 62 % due to a late spring and early summer drought. Native tree species survived better and had lower percent mortalities than *Populus* (67 %; data not shown but provided in Supplementary Material). Green ash had the lowest percent mortality (3 %) and best survival of all species planted at Gibson, followed by bald cypress (42 %), cherrybark oak (44 %), water/white oak (57 %), Atlantic white cedar (65 %), and loblolly pine (66 %). *Populus* clones (n=6) had higher percent mortalities between 50 % and 90 %. *E. benthamii* was not planted at Gibson in 2011. Most of the *Populus* clones planted in 2011 were removed except for *Populus* clone 6 due to its survival and high growth.

Gibson 2012

In 2012, rainfall was normal throughout the growing season, and all trees, including surviving trees from 2011, were inventoried for mortality in October 2012 (Table S2). Overall, tree survivorship improved with declines in mortality from 62 % in 2011 to 26 % in 2012. For trees planted in 2012, native trees had a lower percent mortality (14 %) than *Populus* and *E. benthamii* (18 %). For native trees planted from 2011, green ash had the lowest mortality (5 %) and best survival followed by bald cypress (47 %), cherry bark oak (52 %), water/white oak (52 %), loblolly pine (69 %), and Atlantic white cedar (71 %; Fig. 1). A similar mortality trend was observed for native trees planted in 2012, with higher mortalities observed for loblolly pine (34 %) and lower mortalities observed for bald cypress (16 %), sweetgum (5 %), and green ash (3 %) (Fig. 3, Table S2). *Populus* clone 6, that was planted in 2011 and not removed in 2012, had a percent mortality of 20 % in 2012.

Populus clones planted in 2012 had lower mortalities (15 %) compared to clones planted in 2011 (60 %). Twenty-six of the 42 clones planted in 2012 had no mortality (Fig. 1 or Table S2). The remaining 16 clones had mortalities ranging from 3 to 53 %. Evaluating clone parentage for mortality revealed that clones with unknown parentage (n=3 clones, 0 % mortality) and *P. deltoides*×*P. maximowiczii* parentage (n=1 clones, 0 % mortality) survived superior to *P. deltoides* parentage (n=28 clones, 13 %) and *P. trichocarpa*×*P. deltoides* parentage (n=10 clones, 18 %). See Table 2 for parentage identification.

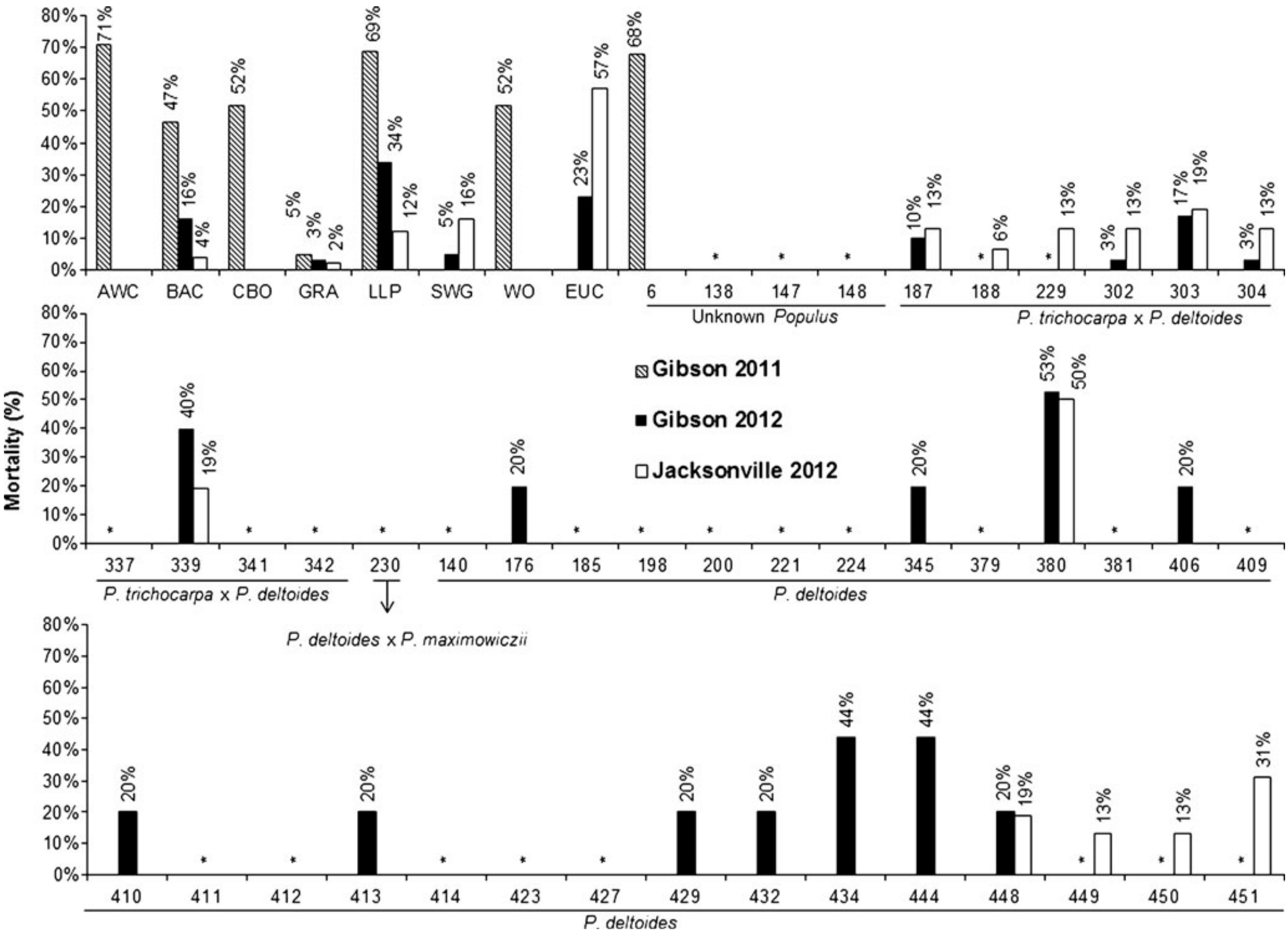

Fig. 1 Mortality among tree species and clones planted in 2011 and 2012. *Asterisks* indicate 0 % mortality for the corresponding tree planting in 2012 at Gibson. Clones sharing similar parentage are *underlined*. *AWC* Atlantic White Cedar, *BAC* Bald Cypress, *CBO* Cherry Bark Oak, *GRA* Green Ash, *LLP* Loblolly Pine, *SWG* Sweetgum, *WO* White Oak, *EUC Eucalyptus benthamii*

Jacksonville

In 2012, site conditions at Jacksonville were very wet with frequent, if not constant, soil saturation and pooling from rainfall and wastewater application. Evidence of herbivory was present on a variety of tree species, but had no clear effect on first year survival (see Section 3.2.3 for damage description). The overall site percent mortality for all trees was 18 %. For native trees, green ash had the lowest percent mortality (2 %) and best survival, followed by bald cypress (4 %), loblolly pine (12 %), and sweetgum (16 %). *Populus* clones had a cumulative percent mortality of 19 % and *E. benthamii* had a percent mortality of 57 % (Fig. 3). Only one *Populus* clone, HP 188, had a percent mortality less than 10 % (HP 188, 6 %; Fig. 1, Table S3). The remaining *Populus* clones had mortalities between 13 and 50 % (Fig. 1). Aggregating *Populus* mortality data by clone parentage showed similar trends to Gibson in that *P. trichocarpa* × *P. deltoides* parentage (n=7 clones) had a lower percent mortality (14 %) than *P. deltoides* parentage (28 %; n=5 clones).

Tree Height Growth and Basal Diameter

Height growth for all trees was determined by measuring the change in height from the initial height measurement for each tree at the start of the growing season and at the end of the growing season. In cases where height measurements were not collected at the time of planting, the earliest available measurement was substituted as the initial height growth. Basal diameter was only collected at the end of the growing season in October 2012. Figure 2 shows box plots of *Populus* tree height growth for 2012 plantings. Figure 3 shows box plots of *Populus* basal diameter for 2012 plantings. Figure 5 shows height growth and basal diameter for native trees planted at Gibson in 2011, and height growth and basal diameter for native trees and *E. benthamii* planted at Jacksonville and Gibson in 2012. Results of analysis of variance for height growth between clones, parentage types, species, blocks, and interactions can be found in Table 3. Results of analysis of variance for basal diameter between clones, parentage types, species, blocks, and interactions can be found in Table 4.

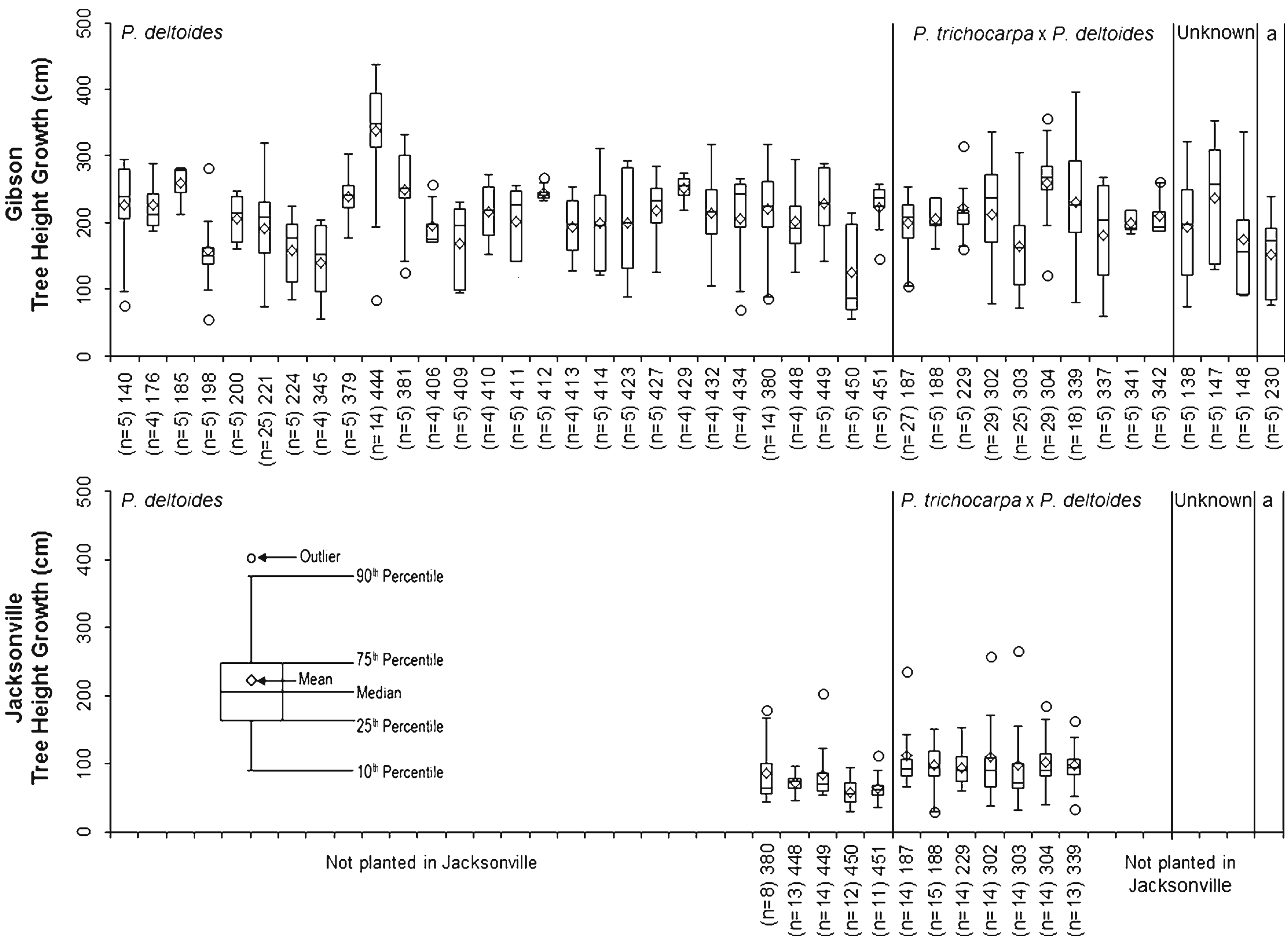

Fig. 2 Tree height growth (cm) for *Populus* clones. *Populus* clones are grouped by parentage; *a—P. deltoides* × *P. maximowiczii*

Gibson 2011

Inventoried growth for all 2011 plantings at Gibson are not shown but can be found in Table S1 of Supplementary Material. *Populus* clones were planted in March 2011 and final measurements were collected in September 2011. Four out of six clones grew to a mean height of 100 cm or more. However, statistical analysis of *Populus* clones found no significant differences in their mean height growth (p=0.55). Basal diameter was not collected at the end of the Gibson 2011 growing season.

Height growth and basal diameter for native tree species planted in 2011 at Gibson is shown in Fig. 5. This figure shows height growth from the first documented height measurement in September 2011 to the final documented measurement in October 2012, representing 14 months of growth. Basal diameter shown in the figure represents final basal diameter after 19 months of growth. As dead trees from 2011 were replanted in March 2012, native tree species from 2011 were not subjected to statistical analysis. Five of the six species had plantings with little to no growth from September 2011 to October 2012. Green ash grew the most with a mean height growth±one standard deviation of 99±52 cm, followed by Atlantic white cedar (75±28 cm), loblolly pine (69±34 cm), cherrybark oak (48±30 cm), white/willow oak (42±33 cm), and bald cypress (35±22 cm). Basal diameter differed slightly; green ash had the largest final basal diameter ± one standard deviation (33.3±12.6 mm), followed by bald cypress (24.3±10.8 mm), loblolly pine (22.5±8.9 mm), cherrybark oak (13.7±5.8 mm), and white/willow oak (12.1±4.1 mm). These results suggest that green ash was the only native species at Gibson that achieved height growth comparable to the most successful *Populus* clones. Otherwise, *Populus* appear to outperform natives in height growth. This finding is reinforced by the longer growth period permitted for native tree species (19 months) versus *Populus* (6 months).

Gibson 2012

Figure 2 shows box plots of *Populus* tree height for 2012 at Gibson. Figure 3 shows box plots of *Populus* basal diameter for 2012. Figure 5 shows height and basal diameter of native tree species. Damage to *Populus* and native species was evident in October 2012. Evidence of herbivory was present on 75 % of green ash and 25 % of *Populus* clones. Three

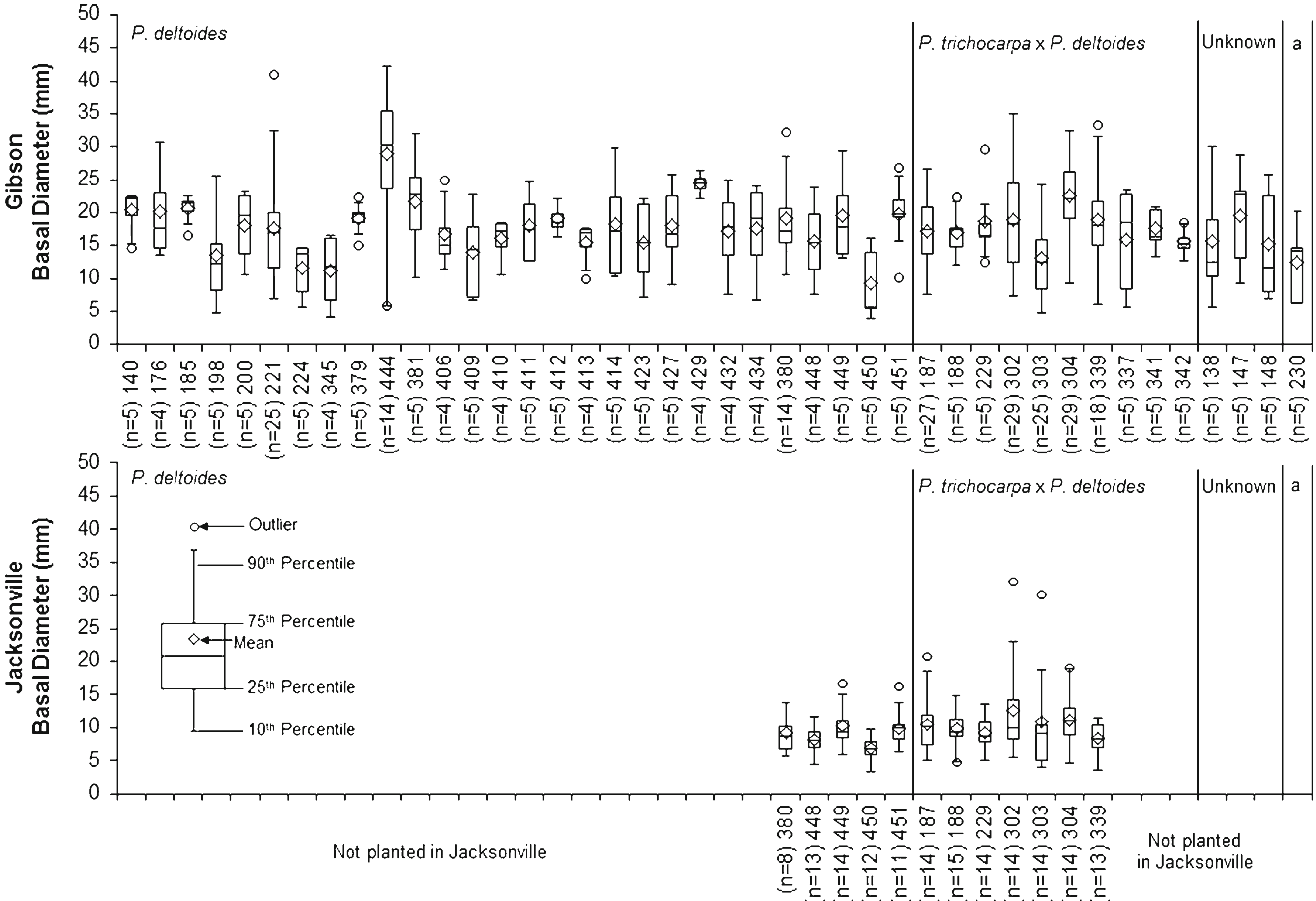

Fig. 3 Basal diameter (mm) for *Populus* clones and *E. benthamii*. *Populus* clones are grouped by parentage; *a*—*P. deltoides* × *P. maximowiczii*

percent of *Populus* clones endured physical damage, most likely due to rutting from *Odocoileus virginianus*, white tail deer. Ten percent of bald cypress appeared to endure some damage to branches and primary shoots as many appeared to have been broken. In cases where damage to trees resulted in negative height growth (n=156), growth was modified to zero growth; this number accounted for 11 % of all trees planted.

Despite damage to trees, *Populus* clones grew much better in 2012 than in 2011. Final height measurements were collected in October of 2012 and evaluated for differences in mean height. Mean height was 207±68 cm for all *Populus* compared to 121±48 cm in 2011. Height for individual clones can be found in Table S2. The 2012 *Populus* clones did not differ significantly in height or basal diameter (p=0.067, Table 3; p=0.148, Table 4). Clones were evaluated by parentage type and segregated into three groups: *P. deltoides*, *P. deltoides* x *P. trichocarpa*, and unknown. The unknown group included *Populus* 138, 147, 148, and 230. Clone 230 was included as an unknown because no other clones with *P. deltoides*×*P. maximowiczii* parentage were planted. Grouping clones by parentage did not show significant differences between parentage types for height or basal diameter (p=0.52, Table 3; p=0.56, Table 4).

Native tree species and *E. benthamii* were separately evaluated for height and basal diameter. Evaluation of these tree species was based on growth between July 2012 and October 2012. Tree heights and basal diameter are shown in Fig. 5. Growth differed significantly (p<0.0001 for both height and basal diameter) between tree species with *E. benthamii* achieving the most height at 47±27 cm. Deer damage to native trees resulted in a wide distribution of height within each tree species. Green ash and bald cypress grew to 30±28 cm and 26±17 cm during the 3-month period, respectively. Despite having low mortality, sweetgum grew very little with 18±15 cm of growth. Loblolly pine grew the least with 13±14 cm of growth from July to October. Green ash achieved the greatest basal diameter at 16.5±5.4 mm. All other tree plantings had a mean basal diameter less than 10.5 mm (see Figs. 3 and 4).

Jacksonville 2012

Figure 2 shows height growth for *Populus* and Fig. 3 shows basal diameter. Figure 4 shows *E. benthamii* and native trees height growth and basal diameter. Severe damage from herbivory, weeds, and other unknown sources appeared throughout

Table 3 Results of analysis of variance for height growth between *Populus* clones, *E. benthamii*, and native tree species at Gibson and Jacksonville, North Carolina, USA

	Source[b]	DF	Mean square	*Z*/*F*	*p* value
2011 Gibson poplar clones	Clone	5	5,786	0.85[c]	0.55
	Block	2	1,221	0.18[c]	0.84
	Clone x block	9	6,845	5.11	<0.0001
	Residual	166	1,339		
2012 Gibson poplar clones–clone[a]	Clone	39	280	1.50[d]	0.067
	Block	4		7.94[d]	<0.0001
	Residual	149	428	8.60[d]	<0.0001
2012 Gibson poplar clones–parentage	Parent	2	3,668.5	0.72[c]	0.52
	Block	4	32,842	6.44[c]	0.013
	Parent×block	8	5,101.4	1.25	0.27
	Residual	176	4,068.9		
2012 Gibson native tree species and *E. benthamii*	Species	4	33,080	73.02	<0.0001
	Residual	1,065	453.04		
2012 Jacksonville poplar clones–clone	Clone	11	4,432	1.99[c]	0.063
	Block	3	3,361	1.61[c]	0.23
	Clone×block	32	2,222	1.07	0.39
	Residual	108	2,077		
2012 Jacksonville poplar clones–parentage	Parent	1	37590	48.84[c]	0.0060
	Block	3	2,862.4	1.37[c]	0.25
	Parent×block	3	769.70	0.37	0.78
	Residual	147	2,087.7		
2012 Jacksonville native tree species and *E. benthamii*	Species	4	778.9	2.29[c]	0.097
	Block	5	603.6	1.78[c]	0.17
	Species×block	14	372.8	2.68	0.0011
	Residual	247	141.0		

Results are reported based on the use of PROC GLM unless otherwise noted

[a] Results reported from PROC MIXED procedure

[b] Computed in SAS v. 9.3 (Cary, NC, USA)

[c] Interaction term used to calculate *F* value

[d] *Z* values

the growing season to *Populus* clones, *E. benthamii*, and native trees. Green ash (99 %) and *Populus* clones (100 %) were damaged with most foliage and some of the terminal buds pruned by the time of final height measurement. In addition to damage from herbivory, weed competition appeared to damage and limit growth of *Populus* plantings. *Cardiospermum grandiflorum* (L.), balloon vine, was found overgrowing plantings on many occasions, and required manual removal. Rapidly growing *Cyperus esculentus* (L.), Johnson grass, and *Sorghum halepense* (L.), nutsedge, were also observed across the site and were managed by mowing. Bald cypress had several breakages to both primary and secondary shoots. The only species that did not have significant external damage were loblolly pine and *E. benthamii*. In cases where damage to trees resulted in negative height (n=156), growth was corrected to be zero growth, accounting for 34 % of all trees planted.

Populus clones grew 93±48 cm in height over 7 months at Jacksonville compared to 207±68 cm in Gibson. *Populus* had final basal diameters of 9.7±4.1 mm at Jacksonville compared to 18.0±7.5 mm at Gibson. Neither height nor basal diameter differed significantly between clones (p=0.063, Table 3; p=0.06, Table 4). Clones were segregated into two groups to evaluate if height growth and basal diameter showed significant differences between parentage types (*P. deltoides* and *P. trichocarpa* × *P. deltoides*). Clones with parentage type *P. trichocarpa* × *P. deltoides* grew significantly more in height than clones with *P. deltoides* parentage (p=0.0060, Table 3). *P. trichocarpa* × *P. deltoides* clones grew a mean height of 106±52 cm and *P. deltoides* clones grew a mean height of 73±32 cm. Four out of six *P. trichocarpa* × *P. deltoides* clones grew more than 100 cm on average during the experimental period whereas no *P. deltoides* clones grew more than 100 cm on average in the same period. This difference may be partly due to herbivory. Basal diameter did not differ significantly between parentage types (p=0.12, Table 4). Native tree species and *E. benthamii* were evaluated for height separately from *Populus* at Jacksonville. Comparisons between trees were made based on height between July 2012 and October 2012. Mean height for all trees was found to be 9.0±13 cm. A two-way ANOVA showed no significant differences between tree species (p=0.097, Table 3). Basal diameter was significantly different between native tree plantings and *E. benthamii* (p=0.005, Table 4). Green ash had the largest basal diameter (13.1±3.9 mm), followed by *E. benthamii* (12.0±7.7 mm), bald cypress (11.6±4.8 mm), sweetgum (8.9±3.8 mm), and loblolly pine (5.8±4.8 mm).

Table 4 Results of analysis of variance for basal diameter between *Populus* clones, *E. benthamii*, and native tree species at Gibson and Jacksonville, North Carolina, USA

	Source[b]	DF	Mean square	*Z*/*F*	*p* value
2012 Gibson poplar clones–clone[a]	Clone	39	2.3	1.05[c]	0.148
	Block	4		5.50[c]	0.0004
	Residual	145	4.0	8.58[c]	<0.0001
2012 Gibson poplar clones–parentage	Parent	2	26.2	0.62[d]	0.56
	Block	4	245.1	5.82[d]	0.017
	Parent×block	8	42.2	1.14	0.3370
	Residual	176	36.9		
2012 Gibson native tree species and *E. benthamii*	Species	4	2,469	99.17	<0.0001
	Residual	1,065	24.90		
2012 Jacksonville poplar clones–clone	Clone	11	27.4	2.01[d]	0.06
	Block	3	42.1	3.09[d]	0.04
	Clone×block	32	2,222	1.07	0.39
	Residual	108	2,608		
2012 Jacksonville poplar clones–parentage	Parent	1	79.6	4.67[d]	0.12
	Block	3	33.9	1.99[d]	0.29
	Parent×block	3	17.1	1.08	0.36
	Residual	147	15.8		
2012 Jacksonville native tree species and *E. benthamii*	Species	4	417.0	8.31[d]	0.005
	Block	5	98.5	1.96[d]	0.13
	Species×block	19	50.2	3.09	<0.0001
	Residual	247	16.2		

Results are reported based on the use of PROC GLM unless otherwise noted

[a] Results reported from PROC MIXED procedure

[b] Computed in SAS v. 9.3 (Cary, NC, USA)

[c] *Z* values

[d] Interaction term used to calculate *F* value

Groundwater Nutrient Concentrations

Results from monitoring groundwater were critical for both wastewater application facilities as both facilities are required to meet permit requirements to protect surface water and groundwater quality on and off site; hence, concentrations of nitrate (NO_3+NO_2) and ammonia (N-NH_4^+) were monitored in groundwater across both sites. The USEPA has established the maximum NO_3+NO_2 concentration at 10 mg L^{-1} in groundwater. North Carolina's wastewater treatment facilities are required to meet criteria set by the USEPA, but states can have stricter guidelines than EPA federal standards. The USEPA does not have a maximum for N-NH_4^+ in groundwater, but the North Carolina Department of Environment and Natural Resources [NCDENR] has a provisional N-NH_4^+ limit of 1.5 mg L^{-1} in groundwater (NCDENR DWQ Groundwater Standards, 2010). Therefore, leaching from wastewater application is permitted but cannot exceed these standards. Monthly concentrations of ammonia and nitrate at both sites are provided in Fig. 5. NO_3+NO_2 ranged from 0.2 to 9.5 mg L^{-1} for Gibson and <0.0056 to 10.95 mg L^{-1} for Jacksonville. N-NH_4^+ ranged from 0.01 to 0.90 mg L^{-1} for Gibson and 0.02 to 1.42 mg L^{-1} for Jacksonville. One sample exceeded the maximum limit for NO_3+NO_2 at Jacksonville in February 2012; otherwise, concentrations of nitrate and ammonia in groundwater were below MCLs for both sites.

Discussion

Forty-two *Populus* spp. clones, one *Eucalyptus* species, and seven native North Carolina tree species were evaluated for survival, height, basal diameter, and nutrient mitigation at two municipal wastewater application sites throughout the first year establishment phase. Superior performance for some species was indicated by relatively high tree growth, broad basal diameters, and low percent mortalities that will contribute toward eventual selection criteria. At Gibson, *Populus* clones 185, 140, 412, 379, 381, 147, 449, 451, 229, 427, 342, 200, 188, 411, 414, and 341 had low percent mortalities (<10 %), greater than 200 cm of height, and greater than 17 mm of basal diameter. Differences in height at Gibson from 2011 to 2012 were largely attributed to the regular application of wastewater and precipitation. Growth was generally lower at Jacksonville compared to Gibson. In addition, damage from deer herbivory and rutting to trees at Jacksonville decreased growth for several species. As a result, selection of superior candidates required a lower threshold for height. Bald cypress, green ash, *P. deltoides*×*P. trichocarpa* clones 187, 188, 229, 302, 304, and *P. deltoides* clone 449 had greater than 75 cm of height, greater than 9 mm of basal diameter, and low percent mortality (<13 %) at Jacksonville. The difference in selection criteria between these two sites shows that site differences can be sufficient to vary species and clone selection, despite similar waste treatment, application rates, and general climate. These results emphasize

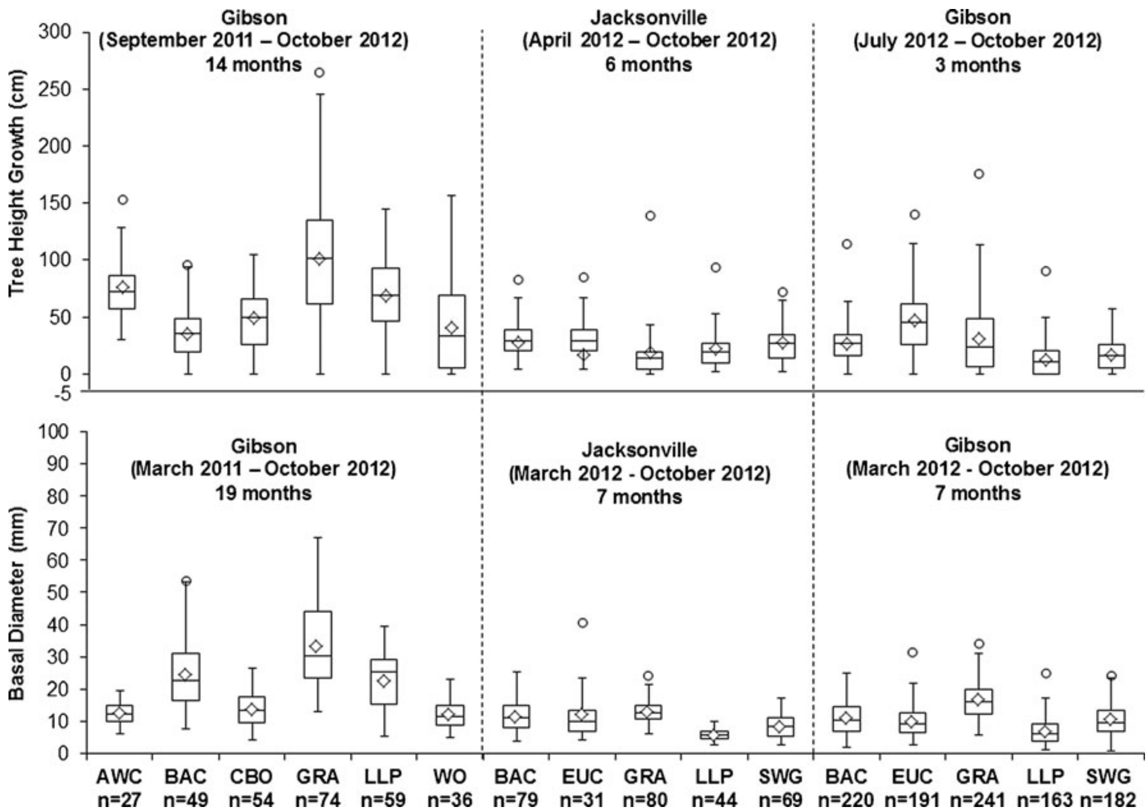

Fig. 4 Tree height growth (cm) and basal diameter (mm) for native trees and *E. benthamii* in Gibson and Jacksonville, North Carolina, USA. Location and months of growth are noted at the heading of each section. Species noted along the *horizontal axis* are: *AWC* Atlantic White Cedar, *BAC* Bald Cypress, *CBO* Cherrybark Oak, *GRA* Green Ash, *LLP* Loblolly Pine, *WO* White oak/Water oak, *SWG* Sweetgum

the need for site-specific screening of tree species and clonal varieties as noted in prior studies [36, 50, 51].

This study compared differences between first year establishment, initial growth (height), and basal diameter for trees native to North Carolina, *Populus* spp. clones, and *E. benthamii* at two study sites irrigated with municipal wastewater. Identical protocols were followed for site preparation and planting spacing at each site, but the two sites differed in the number of trees planted and some specific site attributes. The Jacksonville site had a 35-year-old loblolly pine stand that shaded part of the study site. In addition, soils at Jacksonville tended to be saturated, leading to pooling of water on the surface after irrigation. Also, weed competition at Jacksonville was highly problematic. *C. esculentus*, yellow nutsedge, and *S. halepense*, Johnsongrass, dominated the field site and required multiple herbicide treatments paired with mowing to control through the growing season. Weed competition did not require as much chemical treatment or mowing at Gibson, and surface pooling was not observed after wastewater irrigation. Shading was not an issue at the Gibson experimental site. The Gibson site was planted with a greater variety of species and number of plantings for 2011 and 2012 (see Table 2). Despite these differences, neither tree height growth nor basal diameter was a sensitive parameter to differentiate between *Populus* clones during the establishment period in this study. However, *Populus* parentage was significant in height for conditions at Jacksonville and may be useful when selecting clones for sites with poor conditions.

Populus and *Eucalyptus* are good candidates for wastewater land application because of rapid tree growth and high water use efficiency [12, 41, 52]. In this study, *Eucalyptus* had poor survival in saturated soil conditions, particularly at Jacksonville; however, a few surviving trees did demonstrate rapid height growth superior to *Populus* clones. *Eucalyptus* at Gibson survived better, and some surviving trees grew as well as *Populus* and green ash. *Populus* clones, in general, had good survival and growth; however, select clones (380, 444, 439, and 345) did not perform well. Minogue et al. [37] compared *Eucalyptus* and *Populus* survival and growth at a municipal wastewater application facility in Florida, USA and concluded that *P. deltoides* clones outperformed both *Eucalyptus amplifolia* and *Eucalyptus grandis* clones after 2 years. Both *Eucalyptus* spp. had high mortality due to low temperatures. *Populus* was also found to be the superior species for biomass production in Florida by Overman [38]. These findings and results from our study suggest that *Populus* is a good candidate for biomass production in the southeastern USA, particularly for wastewater land application

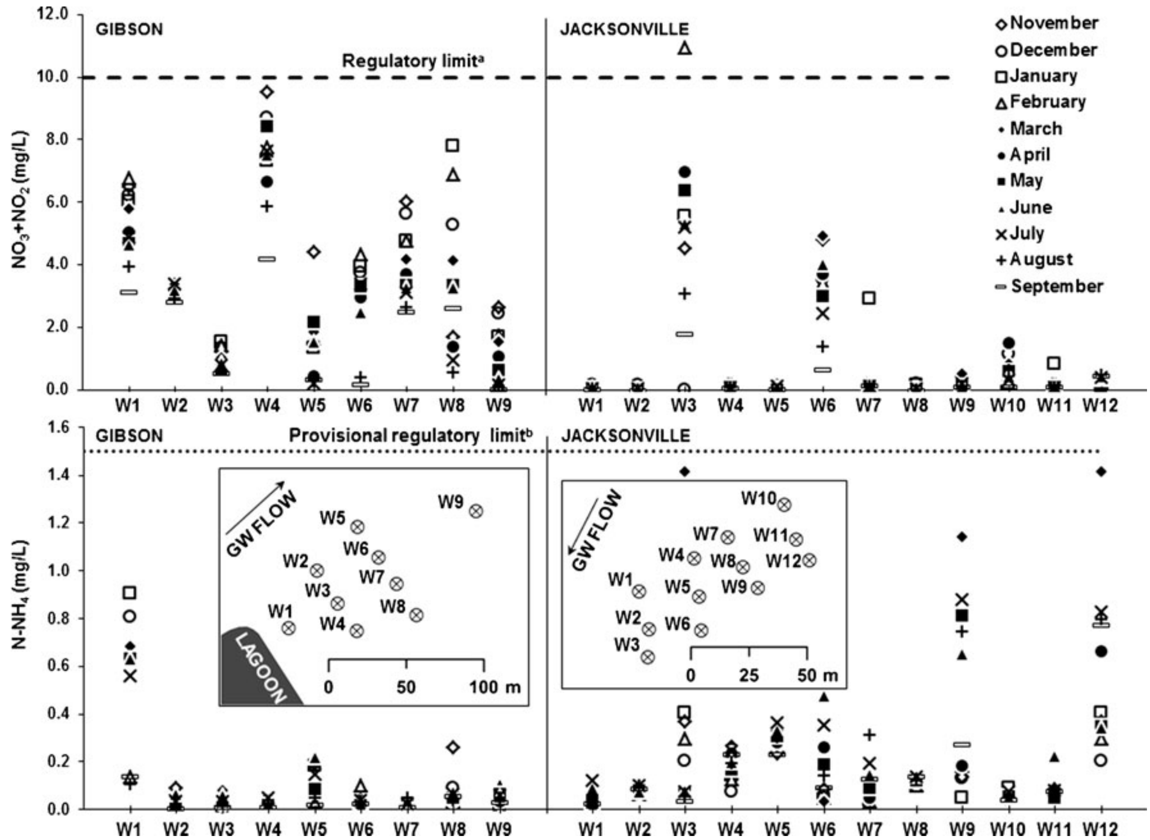

Fig. 5 Nitrate/nitrite (NO_3+NO_2) and ammonia (N-NH4) concentrations in groundwater monitoring wells from Gibson and Jacksonville, North Carolina, USA. [a]NCDENR State Standards, 15A NCAC 02 L.0202 Groundwater Quality Standards; [b]NCDENR State Standards, IMAC 15A NCAC 02OL.202 Groundwater Quality Standards [41]

sites. Native tree species like green ash are productive, but further evaluation of growth over longer periods is needed to determine whether *Populus*, *E. benthamii*, or native tree species are best for meeting wastewater application requirements and biomass production at wastewater land application sites.

This study suggests that establishment is one of the most important stages of producing a successful and highly productive SRWC plantation. Many studies have shown that early growth and mortality affect stand productivity later in rotation [53–55]. Successful plantation establishment depends on many factors such as correct seedling selection [56], effective weed control [54], and site preparation [55, 57]. As shown by the percent mortalities, diameter, and height of each species at Jacksonville and Gibson, this study strongly supports the importance of species selection for biomass production. Challenges in the study, however, show that some additional measures may be needed to support establishment and continued growth. Romagosa and Robison [54] reported that pesticide and weed treatment increased growth 1.6–4.5 times by the end of the second growing season in a natural hardwood plantation in the lower Piedmont. Nilsson and Allen [55] reported that fertilization at the time of planting with high-intensive site preparation could improve volume growth and potentially decrease variability in stand productivity. If a combination of these efforts were applied, increased growth and decreased percent mortality would be expected at both sites.

A unique aspect of this study is the comparison of *Populus* and *Eucalyptus* to trees indigenous to North Carolina. Native trees may offer pest resistance, drought tolerance, and climate adaption traits that non-native species do not possess. Other studies have reported initial height growth of 200 cm or more in a single year for *Populus* spp., *Eucalyptus* spp., and *Salix* spp. [30, 34, 58]. Native trees did not achieve this height at either site throughout the establishment period. These findings support *Populus* clones as a potential source of woody biomass at these municipal wastewater land application sites [37]. The low performance of all tree species at Jacksonville is largely ascribed to the shading by surrounding trees, herbivory from deer, saturated soils, and weed competition, which are significant factors that can impede biomass production [4, 55, 57, 59]. However, the low percent mortality among bald cypress and green ash suggest that native trees might be better candidates than improved *Populus* clones at this particular site.

Native species, like green ash and bald cypress, have been used at other wastewater application sites in North Carolina.

Frederick [60] documented high survival and growth for green ash, bald cypress, and sweetgum on portions of a municipal wastewater irrigation facility that had been degraded due to poor species selection, high hydraulic loading from wastewater application, and soil compaction due to hay production. Frederick [44] also documented survival and biomass production of American sycamore and sweetgum at 2.4×2.4 m spacing on a 145-ha wastewater application facility located in Edenton, NC. By year 5, biomass from American sycamore had 7 % mortality with approximately 4.92 dry Mg ha^{-1} $year^{-1}$ accumulated biomass (total biomass=24.6 dry Mg ha^{-1}). Sweetgum, grown on the same site, had no mortality and accumulated approximately 1.7-Mg ha^{-1} $year^{-1}$ for a total of 8.5 dry Mg ha^{-1} at year 5 [44]. These findings suggest that native species like sweetgum and bald cypress may grow too slowly to meet woody biomass production objectives. On the other hand, the moderate performance of green ash suggests that further evaluation of 10 to 20 years biomass production from these species is needed.

Poor survival and low growth of many native tree species in this study may result from ecosystem preferences of the species. Native trees such as Atlantic white cedar, cherrybark oak, white oak, and water oak displayed high mortality and low growth throughout the first growing season. Initially, this performance was attributed to drought conditions in Gibson. However, slow growth persisted for many native trees throughout 2012 when rainfall and irrigation were adequate. Cherrybark oak, loblolly pine, white oak are facultative or facultative-upland species [see 61] and prefer upland sites with well-drained soils. These trees are not naturally found in saturated, poorly drained soils. *Populus* spp., bald cypress, and green ash are known to be a facultative wetland species that indigenously grow on mesic sites and may be readily adapted for wastewater application lands where soil saturation is frequent [37, 62]. One exception to this finding is Atlantic white cedar, which exhibited high mortality during 2011. This finding should be considered in future studies when evaluating new and less traditional species for wastewater application.

An unexpected finding in this study was the lack of significance in statistical tests for height and basal diameter among *Populus* clones at Gibson. Many studies have found significant differences in biomass production between *Populus* clones with and without wastewater irrigation [41, 51, 63, 64]. One potential reason for the non-significant finding is the early evaluation of all plantings. Many studies evaluate growth 2 to 3 years after planting and as much as 7 to 12 years after [37, 40, 51, 65, 66] to capture responses for pest and disease or cold tolerance. First year growth is less common, but has been used to find differences in clone responses to fertilizer [67] and carbon dioxide concentration [42]. Though this study did not find significant differences between clones in year 1, differences among *Populus* clones are likely to emerge in subsequent years. Coyle et al. [51] documented this effect over three growth seasons with 31 *Populus* clones in South Carolina, USA. At year 10, many of the clones that were documented as preferred clones at year 3 continued to exhibit superior growth [53]. Future evaluations of growth at these two establishment sites will clarify if similar findings are true for municipal wastewater application sites.

Although biomass production potential is frequently considered one of the most important traits when growing SRWC species, this study has shown that mortality is equally as important when selecting species for plantation establishment [41, 51, 68]. A wide range of percent mortality was documented in this study (see Fig. 3). At Gibson, two of the species with greatest height growth demonstrated the highest percent mortalities. *Populus* clones 444 and 380 both grew more than 2 m throughout the experimental period but had percent mortalities of 44 and 53 %, respectively. In contrast, many of the other improved clones and native tree species demonstrated lower height growth as well as lower percent mortalities or higher survival (e.g., green ash, clone 230, 450). Some unlikely candidate species showed moderate percent mortality and relatively little growth (sweetgum and loblolly pine). These species are clearly undesirable for biomass production at these two wastewater application sites. The differences in mortality stress the importance of field trials when selecting for biomass production potential at the stand level.

One consideration in the success of one species over another in this study is the provision of nutrients for establishment and growth. There is some disagreement in the literature about the nutrient demands of SRWCs during establishment. Increased productivity has been reported in *Populus* spp. when applying as little as 50 kg N ha^{-1} $year^{-1}$[69] to as much as 224–336 kg-N ha^{-1} $year^{-1}$ [70]. In contrast, several studies have suggested that high nitrogen application does not contribute to rapid biomass production in the first 2 years of growth [54, 71] and can lead to environmental contamination [69]. In this study, 93 kg N were applied to the Jacksonville site and 223 kg N were applied at Gibson. Though the total amount of N applied at Gibson exceeds the total amount applied at Jacksonville, less nitrogen was provided per tree at Gibson than at Jacksonville (0.11 kg N $tree^{-1}$ versus 0.17 kg N $tree^{-1}$). For our establishment studies, nitrogen application has not lead to increased growth or survivorship during establishment.

In this study, nitrate+nitrite (NO_3+NO_2) and ammonia (N-NH_4) in groundwater at both sites were below regulatory limits throughout the establishment period. These results were different from prior studies on nitrate and ammonia leaching from SRWC systems on wastewater application sites [33, 64, 66]. Generally, nutrient concentrations are expected to increase during the establishment phase due to disturbed organic matter and undeveloped root systems [3, 66]. The ammonification of organic nitrogen and nitrification of ammonia increases water-soluble nitrogen. Thus, high concentrations of NO_3+NO_2 and N-NH_4 would be expected during the establishment phase. However, tree species are known to have different uptake capacities of N-NO_3 and N-NH_4 [72, 73]. Other wastewater application sites may experience exceedances of regulatory limits during the establishment phase based on tree species, but reductions would be expected shortly thereafter either through denitrification or through root uptake. The USEPA reports that denitrification is expected to account for 10 to 25 % of nitrogen loss as nitrous oxide emissions from forested land treatment systems [74]. Lysimeter studies have shown that nitrogen uptake can vary through time and by soil type for *Salix* spp., but soluble nitrogen loss generally decreases with time [64, 75]. Combined, these findings suggest that nitrogen will be absorbed by tree uptake, lost to atmospheric release as nitrous oxide, or lost by ammonia volatilization. Nutrient budgets are needed for other SRWC growth on wastewater application sites to determine how much nitrogen gas and other greenhouse gases are released to the atmosphere. If some species show signs of decreased gas emissions due to high uptake, the presence of greenhouse gases would be expected to decrease. More research is needed in this arena to understand what long-term impacts and environmental benefits can be expected from generating biomass on other marginal and low-productivity lands in the United States.

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