Title: Woody biomass development of seven Mid-Atlantic wetland species

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Abstract

The primary goal of forested wetland creation and restoration is to replace or return ecosystem structure and functions to the landscape. Production of plant biomass is an important ecosystem function that will develop in successfully created and restored forested wetlands. Quantifying accumulation of biomass of planted trees is important in understanding this development. Destructive harvests and tissue elemental analysis of saplings (*n*=567) planted across a hydrologic gradient were used to develop biomass estimation models for seven species common to the Mid-Atlantic region of the United States. The model established that stem cross-sectional diameter at groundline was an adequate predictor of total biomass. The model was then applied to all living trees (*n*=1,258) to evaluate species and stocktype performance over 6 years across the hydrologic gradient. Early colonizing species accumulated more biomass than late succession species. Larger stocktypes (1-gallon container) accumulated more biomass than smaller stocktypes (bare root and tubeling) in areas with greater hydrologic stress and accumulated biomass decreased with increasing hydrologic stress regardless of stocktype. Biomass in conjunction with elemental concentrations measured in this study can be used to evaluate the development of restored or created forested wetlands and to determine sapling contributions to these global chemical cycles.

Keywords: Allometric Equations, Forested Wetlands, Stocktype Assessment, Biomass

Introduction

Production and accumulation (storage) of plant biomass is an important ecosystem function that will develop in created and restored forested wetlands. In early successional created/restored forested wetlands the majority of plant biomass is produced by herbaceous vegetation (Atkinson et al. 2005; DeBerry & Perry 2012). However, as created and restored forested wetlands develop, the production and accumulation of biomass shifts to perennial woody vegetation (woody vines, shrubs, and trees) (Noon 1996; Odland 1997; Battaglia et al. 2002; DeBerry & Perry 2012, Mitsch et al. 2012).

In order to accurately determine amount of woody biomass accumulated in early successional created/restored wetlands it is necessary to measure or estimate the amount of biomass accumulated in woody saplings (defined here as trees with a stem diameter at breast height (dbh) less than 3 cm or trees shorter than 1.37 m tall that lack a dbh entirely). Woody biomass is estimated using mathematical (allometric) relationships between biomass and one or more morphological characteristics such as stem diameter and/or height (e.g. Jenkins et al. 2003). These allometric equations are referred to as biomass estimation models (BEM).

There are few BEM’s for saplings compared to numerous ones for trees greater than 3 cm dbh (See Chojnacky et al. 2014). BEMs for saplings often relate a stem diameter measurement from another part of the main stem (at groundline, root collar, or some distance above groundline) to aboveground and/or belowground biomass (Tefler 1969, Roussopoulos and Loomis 1979, Smith and Brand 1983, Williams and McClenahem 1984, Wagner and Ter-Mikaelian 1999, Geudens et al. 2004, Henry et al. 2011).

Studies have reported poor survival and morphological growth (and therefore biomass production) of planted trees in created/restored wetlands (Morgan & Roberts 2003; Sharitz et al.

2006; Matthews & Endress 2008). Factors that influence survival and biomass production of saplings planted in created/restored forested wetlands including selection of species and stocktype and environmental conditions present at the time of planting (soil physical and chemical properties and hydrology in particular) (Denton 1990; Niswander and Mitsch 1995; Stanturf et al. 2004; Bergshneider 2005; Daniels et al. 2005; Bruland and Richardson 2006; McLeod et al. 2006; Pennington and Walters 2006; Dickinson 2007; Roquemore et al. 2014).

The purpose of this study was 1) to construct BEMs relating total biomass (leafless woody above and belowground coarse root dry biomass) to sapling stem cross-sectional diameter at groundline for seven native Mid-Atlantic woody wetland species, 2) to determine how stocktype influenced amount of biomass accumulated following planting (using the developed BEM) for each species, and 3) determine how soil and hydrologic conditions influenced the amount of biomass accumulated following planting. We hypothesized that the larger more expensive stocktype (1-gallon container) would have greater survival and biomass accumulation than smaller stocktypes (bare root and tubeling). Additionally, we hypothesized that individual species survival and biomass accumulation would be dependent upon soil and hydrologic conditions and that increased soil and hydrologic stress would reduce survival and biomass accumulation of all species.

Methods

Study Site

The experimental site (hereto referred to as "the site") was established in New Kent County, Virginia, USA at the Virginia Department of Forestry, New Kent Forestry Center in 2008-2009 (Figure 1). The site is located in the Coastal Plain Region of Virginia and average yearly temperature is 15^{o} C and the average yearly precipitation is 116.2 cm/year (39 year average; WEST POINT 2 NW, Coop ID: 449025). The 1.4-ha experimental site is located 8.8 m above sea level and approximately 1 km north of the Chickahominy River (lat 37° 25' 25.9026" N, long -77° 0' 53.3628" W). The site is located on a terrace adjacent to a mature palustrine forested wetland to the west and north with managed upland fields to the east and south. Soil series on the site include Catpoint fine sand, State very fine sandy loam and Altavista fine sandy loam (Soil Survey Staff NRCS 2015). These are classified as somewhat excessively drained, well drained, and moderately well drained respectively. Based on observations at the site, the depth to the natural water table is estimated to be greater than 1 m prior to cell construction.

The site consisted of three hydrologically distinct cells (ambient (AMB), saturated (SAT) and flooded (FLD)) each 49 m x 95 m in size (Figure 1). Each cell was equipped with an on-site irrigation system capable of producing a minimum of 2.54 cm of irrigation per hour. The three cells were hydrologically manipulated to include an ambient treatment (AMB - received only precipitation), saturated treatment (SAT - kept saturated for a minimum of 90% of the growing season within the root-zone (10 cm) of the plantings and irrigated to supplement precipitation), and a flooded treatment (FLD - inundated above the root crown for a minimum of 90% of each year). Irrigation water was drawn from the non-tidal portion of the Chickahominy River approximately 8 km upriver above the Walkers Dam, Walkers, Virginia. Soils in the AMB and SAT treatments were tilled using a finger plow to a depth of 20 cm in February 2009 prior to planting while the FLD treatment was excavated using a 5-ton backhoe to a depth of 1 m to an existing clay layer. In addition to the hydrologic differences among the cells, there were differences in soil physical and chemical characteristics (Table 1).

<u>Planting Material</u>

The common name and wetland indicator status for each of the seven planted species (Lichvar et al. 2012; Lichvar 2013) was: *Betula nigra* L. (river birch, FACW), *Liquidambar styraciflua* L. (sweetgum, FAC), *Platanus occidentalis* L. (American sycamore, FACW), *Quercus bicolor* Willd. (swamp white oak, FACW), *Quercus palustris* Münchh. (pin oak, FACW), *Quercus phellos* L. (willow oak, FACW) and *Salix nigra* Marshall (black willow, OBL). To facilitate analysis and interpretation of plant material selection, species were assigned to early or late successional categories based on common Mid-Atlantic Coastal Plain regional trends in dominance during stand development as expressed through differences in maturation and growth rates, dispersal mechanisms, and disturbance tolerance. Early successional species consisted of four species (*B. nigra, L. styraciflua, P. occidentalis* and *S. nigra*) that are typically dominant during the early stages of succession, have rapid growth and maturation rates, have wind dispersed seeds and are moderately tolerant of disturbance. The late successional species group consisted of three species (*Q. bicolor, Q. palustris,* and *Q. phellos*) typically dominant in the later stages of succession, have slower growth and maturation rates, have large seeds that are dispersed mainly by animals and are generally less tolerant of disturbance.

Three stocktypes of each of the seven species were used in this study: bare root (BR), tubeling (TB), and 1-gallon containers (GAL) (tubelings of *P. occidentalis*, *Q. phellos*, and *S. nigra* had their soil removed by the nursery prior to shipment). Bare root seedlings range in age from one to three years old and were planted during dormancy without soil surrounding the roots. Tubelings are typically similar in age to bare root seedlings; however, they were planted with potting soil surrounding the roots and were grown in small square containers. One gallon containerized seedlings are larger and older than BR and TB and were planted with potting soil intact around the root system. The GAL was most expensive, followed by TB, with BR being the least expensive.

In Spring 2009, all combinations of species and stocktypes were planted randomly along rows within each cell. A total of 2,772 trees were planted; approximately 44 of each species/stocktype combination, for a total of 924 trees per cell. Trees were spaced 2.3 m from trees within the row and 2.6 m from trees in adjacent rows resulting in a density of 1,969 stems/ha. In the spring of 2010, a total of 482 additional trees were planted to ensure adequate survival for biomass analyses.

Saplings were obtained from four nurseries located in Virginia, North Carolina, New Jersey, and Tennessee. Replacement stock did not necessarily come from the same nursery as the original stock. No fertilizers were applied and herbaceous competition was controlled around plantings in the AMB and SAT through bimonthly grass cutting and application of glyphosate at the beginning and middle of growing seasons using commercial backpack sprayers.

Morphometric Measurements

Stem cross-sectional diameter at groundline (used to calculate stem cross-sectional area at groundline) of each live sapling were measured in mid-April, mid-August, and mid-October from 2009 to 2014. Individual survival was determined by the presence or absence of green leaves during the growing season and, when green leaves were lacking, tree survival was finally determined by the presence of live cambium obtained via small scratches beginning at the highest point on the stem, then at the vertical midpoint, and finally at the base. Small digital calipers (6-inch stainless steel digital caliper, General, Secaucus, New Jersey) or large calipers (127-cm Mantax Precision Blue Calipers, Haglöf, Inc., Långsele Sweden) were used to measure the diameter of five largest stems above root collar swelling. For trees with multiple stems originating from below ground, the stem cross-sectional area at groundline for each of the five largest stems were summed and then converted to a single stem cross-sectional diameter at groundline (referred to as equivalent stem cross-sectional diameter at groundline (ESD). The sum of the area of the measured stems equals an area equivalent to the corresponding diameter of a single stemmed sapling as described in Paul et al. (2013).

Biomass Sampling

Since the species specific relationships between morphology and biomass change during ontogeny and often in response to environmental conditions, aboveground biomass (AGB) and belowground biomass (BGB) samples were taken in the winter of 2010-2011 and then AGB was sampled from additional trees in late winter 2014. The samples included trees from all planting times, cells and stocktypes to incorporate the maximum variation in morphologies and biomass. A random subsample of saplings (n=346) were removed in the winter of 2010-2011 to measure leafless AGB and coarse root BGB, and a random subsample of trees (n=221) were removed in late winter of 2014 to measure leafless woody AGB. In order to extract roots from the soil matrix a variety of methods were used based on the size of the tree and planting location. Trees removed from the FLD cell were removed by hand or with trowels and pitchforks. Soil remaining on the roots was washed onsite prior to drying. Small trees (<0.5 m tall) were removed using similar methods in the AMB and SAT. For trees taller than 0.5 m, approximately 0.1 m^3 of soil was excavated around the main stem using a tree spade (Dutchman Model 240o, Ontario, Canada) mounted on a skid steer loader (Bobcat S160, Seoul, South Korea). Following excavation the soil was removed from the roots by hand and with trowels and pitchforks. Any roots that were not excavated using the tree spade were subsequently removed from the soil matrix by hand and with shovels, trowels and pitchforks. All spreading and deep roots were followed to their terminus.

Difficult to remove soil around the roots was washed onsite prior to drying. While attempts were made to capture all roots, this method excluded most fine roots smaller than 2 mm diameter.

The complete above and belowground portions of the trees were separated and placed in individual paper bags. Sampling occurred after leaf senescence and leaf biomass was not measured; therefore BGB refers only to coarse roots and AGB refers to stems and branches. All trees were solar dried on-site at approximately 50°C in repurposed greenhouses until constant weight was obtained. The trees were weighed at the end of the summer in 2011 and 2014 following complete drying. The root-to-shoot ratio (r:s) was calculated for trees harvested in winter 2010-2011 where AGB and BGB was harvested.

Subsamples of trunk, stem and twig AGB were collected from trees that were harvested in early spring 2014 (n=103). Subsamples were manually reduced in size and then mechanically ground using a Thomas Wiley Mill Model 4 and Mini-Mill (Thomas Scientific, Swedesboro, NJ). Using a PE2400 CHNS/O Elemental Analyzer (Perkin Elmer, Massachusetts, USA) duplicate dry ground samples were analyzed for percentage carbon and nitrogen elemental concentrations which were used to determine the carbon to nitrogen ratio (C:N).

Soil Sampling

Forty four soil samples were collected from each cell (Total=132) in 2013 and analyzed for soil carbon, nitrogen and phosphorus concentrations and soil sand, silt and clay percentages, (Table 1). Samples were evenly spaced within each cell and collected 70 cm diagonally from tree base. The top 15 cm of soil were collected using a 50 cm soil probe. Samples were dried in ovens at 60^{o} C until constant mass was obtained. Bulk density was determined by dividing mass of oven dry soil by volume collected (Brady & Weil 2002). Particle size distribution (sand

(>63µm), silt (<63µm-4µm), and clay (<4µm)) was determined by the standard sieve-pipette method.

Carbon and nitrogen concentrations from a subsample of homogenized oven dry samples were measured using a PE2400 CHNS/O Elemental Analyzer (Perkin Elmer, Massachusetts, USA) following standard methods. Phosphorus concentrations were measured using a spectrophotometer following a modified ashing and extraction technique (Chambers & Fourqurean 1991). Carbon, nitrogen and phosphorus concentration are presented as percentages of total soil mass (Table 1).

<u>Biomass Estimation Model Development</u>

The relationship between the above (AGB) and belowground biomass (BGB) was determined for each species from the 2011 samples (AGB and BGB sampled). The 2011 samples were pooled from all three cells due to lack of differences in model coefficients when the AGB to BGB relationship was modeled for each cell separately. A non-linear equation ($Y=aX^b + \varepsilon$, Eq. 1) was used where Y = BGB (kg), X = AGB (kg), a = model estimated intercept, b = model estimated scaling coefficient, and ε = residual error term. Using residual diagnostic plots, a heteroscedastic error structure (variance of BGB increased with greater AGB) was observed. Because of heterogeneous variance, the relationship between AGB and BGB was modeled using a generalized nonlinear least-squares regression (Pinheiro & Bates 2000). The variance structure was modeled using the power of the covariate (Packard 2014; Zuur et al. 2009) since they resulted in homogenization of residuals and lowest Akaike information criterion (AIC) values. Additionally, there is criticism associated with logarithmic transformations and methods associated with back-transforming of these equations (Packard 2014). The resulting model was used to estimate the BGB of the 2014 samples (where only AGB was harvested).

The same non-linear equation ($Y=aX^b + \varepsilon$, Eq. 2) was used to determine the relationship between total woody biomass and equivalent stem cross-sectional diameter at groundline (ESD), where Y= total woody biomass (AGB+BGB), X = ESD, a = model estimated intercept, b = model estimated scaling coefficient, and ε = residual error term. All samples from 2011 and 2014 (*n*=567) were used to develop this relationship. Using residual diagnostic plots, a heteroscedastic error structure was observed. As above, generalized nonlinear least-squares regression was used to fit the original untransformed data and the variance structure was modeled using the power of the covariate structure (Packard 2014; Pinheiro & Bates 2000, Zuur et al. 2009). Tree height and crown diameter were measured but not included in the BEM due to multicollinearity among the predictors, despite recent studies that they suggests they could be included (Picard et al. 2015).

Statistical Analysis

Species specific BEMs were used to determine the biomass of living trees directly following planting and after 6 years. Analysis was limited to trees that were planted in 2009. Pairwise t-tests were used to compare stocktype, species, and cell initial total woody biomass (BGB+AGB directly following outplanting) and woody biomass after 6 years. Variance was estimated separately for each group, the Welch modification to degrees of freedom was used and Holm's *p*-value adjustment was used to control the family-wise error rate. All alpha values were set at 0.05. Data preparation and analysis were completed in R version 3.2.1 (R Core Team 2014).

Results

Biomass Estimation Model (BEM) Development

Since model estimated coefficients (a and b) were not statistically different when models were developed for species within each cell, samples were pooled across cells when the relationship between aboveground (AGB) and belowground (BGB) biomass (coarse roots only) for each species was developed. The low standard error of the regressions showed that species specific models fit the data well (Figure 2). The model derived intercept (a) ranged from 0.544 (*S. nigra*) to 1.334 (*L. styraciflua*) for the early successional species and from 0.595 (*Q. phellos*) to 1.921 (*Q. bicolor*) for the late successional species, mainly resulting from differences in size among species when samples were collected. The model derived exponent (b) ranged from 0.7394 (*S. nigra*) to 0.975 (*P. occidentalis*) for the early successional species and from 0.732 (*Q. phellos*) to 0.942 (*Q. bicolor*) for the late successional species. This range of exponents suggests that the relationship between AGB and BGB differs among the early successional species (Figure 2) and late successional species (Figure 3).

The relationship between equivalent stem cross-sectional diameter at groundline (ESD) and total woody biomass (BGB of trees harvested in 2014 was estimated using the above model) was determined for each species also using pooled samples across cells. Standard error of regressions was small and showed that the models described the data well for all species (ranging from 0.522 for *P. occidentalis* to 1.891 for *Q. phellos*) (Figure 4 & Figure 5). Model derived intercept (a) ranged from 0.028 (*P. occidentalis*) to 0.032 (*L. styraciflua*) for the early successional species (Figure 4) and from 0.047 (*Q. palustris*) to 0.055 (*Q. phellos*) for the late successional species (Figure 5), mainly resulting from differences in size among species when harvested. The differences in harvested size among species were dependent upon species specific growth rates and initial planting size. The model derived exponent (scaling coefficient (b)) ranged from 2.442 (*B. nigra*) to 2.788 (*P. occidentalis*) for early successional (Figure 4) and 2.455 (*Q. phellos*) to 2.735 (*Q. bicolor*) for late successional species (Figure 5). Species with larger exponents accumulated more total biomass per centimeter stem diameter than those with lower exponents. This may have resulted from differences in wood density, branching and rooting architecture or biomass allocation patterns.

Biomass Estimation Model Implementation

There was no difference in initial biomass at the time of planting (calculated using species specific BEM) between the bare root (BR) and tubeling (TB) stocktypes for all species. Gallon stocktype (GAL) had greater initial biomass than TB for all species except *L. styraciflua* and *Q. bicolor* and greater initial biomass than BR for all species except *Q. bicolor*.

Stocktype Comparison

In the AMB cell, *B. nigra*, *Q. palustris*, *Q. phellos*, and *S. nigra* saplings planted as GAL had significantly greater final biomass than those planted as BR (Table 2). In contrast, *P. occidentalis* BR and TB final biomass was significantly greater than the GAL final biomass in AMB cell and *P. occidentalis* TB final biomass was greater than GAL in the SAT cell. In the SAT cell *B. nigra*, *P. occidentalis*, and *Q. phellos* saplings planted as GAL had significantly greater final biomass than those planted as BR (Table 2). While in the FLD cell, only *L. styraciflua* saplings planted as GAL had significantly greater final biomass than those planted as BR. However, for all species planted in the FLD, GAL generally had greater survival than BR and TB (Table 2).

Additionally, GAL final biomass was significantly greater than TB final biomass for *L. styraciflua*, *Q. palustris*, and *Q. phellos* in the AMB and *B. nigra*, *L. styraciflua*, *Q. bicolor*, and *Q. phellos* in the SAT (Table 2). There was no difference in final biomass between BR and TB stocktypes for *B. nigra*, *P. occidentalis, Q. bicolor, Q. phellos* in AMB and all species (excluding *P. occidentalis*) in the SAT (Table 2).

Cell Comparison

Total biomass accumulated by all living trees after 6 years in the ambient (AMB), saturated (SAT) and flooded cell (FLD) was 20,077.2 kg, 9035.2 kg, and 195.8 kg respectively, suggesting that the increasingly stressful conditions had a significant impact on the ability of these species to accumulate biomass. In particular, *B. nigra, P. occidentalis, Q. bicolor, Q. palustris* and *Q. phellos* had significantly less final biomass in the SAT than in the AMB and all species had significantly less biomass in the FLD than in the AMB and SAT (Table 3).

Discussion

Biomass Estimation Model Development

The power-law relationship between BGB and ABG provide a means for non-destructive estimation of coarse root BGB of the seven species used in our study that is more robust than r:s ratios. Scaling coefficients from this relationship provide insights into how biomass allocation changes as biomass increases. A scaling coefficient of less than 1 (as for most species in this study) suggests that the r:s ratio decreases as saplings accumulate AGB. Departure from an isometric relationship between AGB and BGB have been reported across a range of habitats, species, spatial scales and time scales (Cheng & Niklas 2007; Niklas 2005; Niklas 2004; Enquist & Niklas 2002; Yang & Luo 2012). Hui et al. 2014 showed that as dbh of trees increased, the r:s ratio decreased and the scaling coefficient increased to approximately 1. Results from the current study suggest that inclusion of saplings when determining biomass and carbon accumulation and allocation in restored/created wetlands is important because they may be allocating more resources belowground before shifting allocation of resources aboveground. *L. styraciflua*, *P. occidentalis* and *Q. bicolor* have scaling coefficients approaching 1 suggesting they are able to quickly allocate resources belowground following outplanting and then shift allocation aboveground. These species may be appropriate for returning these important ecosystem functions to restored/created wetlands.

The BEM developed in this study describing the relationship between stem diameter and total biomass provides a non-destructive estimate of sapling biomass that is useful for determining the development of this ecosystem function in restored/created wetlands and other forested systems (riparian buffers, uplands, natural forested wetlands etc.). Development of BEMs for saplings is valuable because they can be a major component of understory (Gemborys 1974) and canopy gaps of mature forests (Ehrenfeld 1980) and can be significant components of the vegetative structure in early successional stages of many ecosystems, including abandoned agricultural fields (Monette and Ware 1983) and recently restored (Hudson 2010) and created wetlands (DeBerry & Perry 2012). BEMs for seedlings and saplings allow more accurate prediction of biomass during tree development, which may allow for improved characterization of factors that affect ecosystem development. Accurate quantification of tree biomass, particularly saplings, is an important step in understanding carbon dynamics across a range of forested ecosystems (including wetlands) as saplings play a large, but yet unquantified, role in the global carbon cycle (Temesgen et al. 2015).

Biomass estimation provides an additional way to evaluate planted sapling performance. Current BEMs for the species used in this study (see Jenkins et al. 2003; 2004, Chojnacky et al. 2014) were constructed for large trees (dbh >2.5 cm) and only model AGB. The few studies developing BEMs for saplings focused at the genus taxonomic level for *Salix*, *Betula* and *Quercus* and most only sampled AGB (Tefler 1969; Roussopoulos & Loomis 1979, Smith & Brand 1983, Williams & McClenahem 1984). Comparisons with these studies are further compounded by the methodological differences in stem measurements (several sampled at 15 cm above ground level) and model fitting (log base 10 transformations of biomass). Despite these differences, the estimated scaling coefficients from this study were marginally higher than literature values for these species. The inclusion of BGB in the present study is a potential reason for this but the BEMs developed in this fashion provide a more robust model for estimating total biomass for these species and for evaluating this ecosystem function in restored/created wetlands.

<u>Accumulated Biomass</u>

Stocktype comparison

In the FLD, the choice of stocktype for all species (except *L. styraciflua*) did not affect the biomass accumulated after 6 years. This suggests that larger stocktypes are not able to return this ecosystem function in very stressful environmental conditions; however, it should be noted that the GAL stocktype did have increased survival compared to the BR and TB for most species. Based on survival, the TB stocktype is not recommended when planting *P. occidentalis, Q. bicolor, Q. palustris,* and *Q. phellos* in similar stressful conditions and GAL stocktype can be recommended when planting *B. nigra*, *L. styraciflua, P. occidentalis, S. nigra, Q. bicolor*, and *Q. phellos* due to increased survival

In the SAT, the larger stocktype (GAL) should be used and the smaller stocktype (BR) should be avoided when planting *B. nigra*, *P. occidentalis*, and *Q. phellos* due to the significantly greater final biomass attained by the GAL stocktype for these species. The BR stocktype can be recommended for the remaining species (*L. styraciflua*, *S. nigra*, *Q. bicolor,* and *Q. palustris*) because it accumulated a similar amount of biomass over 6 years as the GAL. However, due to the lower survival of the BR stocktype, greater density plantings should be used. The TB stocktype should be avoided when planting *B. nigra*, *L. styraciflua*, *Q. bicolor*, and *Q. phellos* due to the significantly lower final biomass accumulated and reduced survival.

In the AMB *B. nigra*, *Q. palustris*, *Q. phellos*, and *S. nigra* saplings planted as GAL had significantly greater final biomass than those planted as BR, suggesting that for these species in these conditions, GAL may be a better choice of planting stocktype, while for the other species (*L. styraciflua*, *Q. bicolor*) BR or GAL may be appropriate. GAL may not be an appropriate choice for *P. occidentalis* in AMB conditions since BR and TB final biomass was significantly greater than the GAL final biomass. Both BR and TB are appropriate for planting *B. nigra*, *P. occidentalis*, *Q. bicolor*, *Q. phellos*, since there was no difference in final biomass between these stocktypes, while the BR should be selected as opposed to TB for *L. styraciflua* and *Q. palustris.*

Species Comparison

In addition to the stocktype, species selection was shown to influence biomass accumulated after 6 years. Within the FLD, *S. nigra* accounted for 55.6% of the total amount of biomass accumulated and is the only species that did not experience substantial mortality. This suggests that in very stressful created and restored wetland conditions *S. nigra* may be a more appropriate choice to replace or return woody biomass to the system. The only other species to have moderate success in the FLD was *B. nigra.*

Cell Comparison

The reduction in biomass in the SAT compared to AMB resulted from the increased hydrologic stress (saturation within the root zone). The substantial reduction in biomass production in the FLD resulted from flooding above the root collar, reduced soil nitrogen and phosphorus concentrations, increased clay concentrations, herbaceous competition and poor survival of planted trees.

Reduction in tree survival and biomass accumulation can be attributed to prolonged saturated or flooded soil conditions that remove the plant available oxygen from the soil pore space. The reduction in oxygen leads to a lack of aerobic respiration in roots, which decreases the energy available for trees to maintain functions of existing tissues (Hale & Orcutt 1987; Brady & Weil 2002). Many growth chamber, greenhouse, mesocosm and field experiments have investigated the effect of hydrology on a multitude of responses across many species of trees. While species specific responses may vary (e.g. *Taxodium distichum*, mangroves) most species exhibit decreased survival and biomass accumulation when grown under prolonged inundation. Niswander & Mitch (1995) planted ten tree species (three of which were used in this study, *B. nigra, L. styraciflua, Q. palustris)* across a hydrologic gradient in a created wetland. Similar to the results in this study, they found that trees planted in shallow water died or were severely stressed, and that trees planted in the wet meadow portion were able to survive and grow, while trees planted in the upland section were the largest and had the densest foliage. Pennington & Walters (2006) investigated growth and survival four species (two of which were used in this study, *Q. palustris* and *Q. bicolor*) planted in three hydrologic zones (wetland, transition, upland) of created perched wetlands. Again, similar to the present study, trees grown in the transitions zone (high soil water availability with oxidized root zone) had greater height growth and survival after 5 years than those planted in the wetland zone (reduced oxygen in the root zone). This suggests that the environmental conditions during and following restoration/creation of wetlands can greatly influence the return of ecosystem functions such as biomass accumulation.

Models relating total biomass and stem cross-sectional diameter at groundline are needed for saplings since most prior models excluded saplings and belowground biomass. Models developed for larger diameter trees underestimate biomass of smaller trees even with inclusion of sapling adjustments (Nelson et al. 2014). Additionally, accurate BEMs are needed to make predictions about carbon storage and exchange in forest ecosystems. The investigation of r:s ratios also provides important information about how saplings transition from allocating biomass belowground to aboveground as they grow.

The results of investigating the biomass accumulated after 6 years for saplings planted under different environmental conditions provides important information for those attempting creation and restoration of any forested system, particularly for those involving wetland conditions. When planting in stressful hydrologic, soil, and competitive conditions, based on the results from the FLD, *S. nigra* appears to be the best choice among these species for returning woody biomass (followed by *B. nigra*). In these conditions, the choice of stocktype influenced the survival but did not appear to influence the biomass accumulated after 6 years as all stocktypes were heavily stressed. When planting in less stressful wetland environmental conditions (moderate hydrologic and competitive stress), the early successional species used in this study are a better choice to return woody biomass than the late successional species. The late successional species (oaks) may be important for additional ecological functions (such as acorn production) but may not be an appropriate planting choice when rapid accumulation of biomass is the goal. The choice of stocktype between GAL and TB appears to be important in areas of moderate environmental stress, where larger stocktypes are a better choice for replacing biomass, even after 6 years. However, in these conditions the BR was able to accumulate similar amounts of biomass as the GAL, suggesting that it may be an acceptable alternative to the larger more expensive stocktype. In areas with low environmental stress, the smaller stocktype are able to equal and exceed the biomass accumulated by the larger stocktype.

In conclusion, the recommended species and stocktypes will help improve the practice of wetland restoration and creation by enhancing the return of lost ecosystem structure and functions which can be better evaluated using these new sapling biomass estimation models.

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Tables and Figures

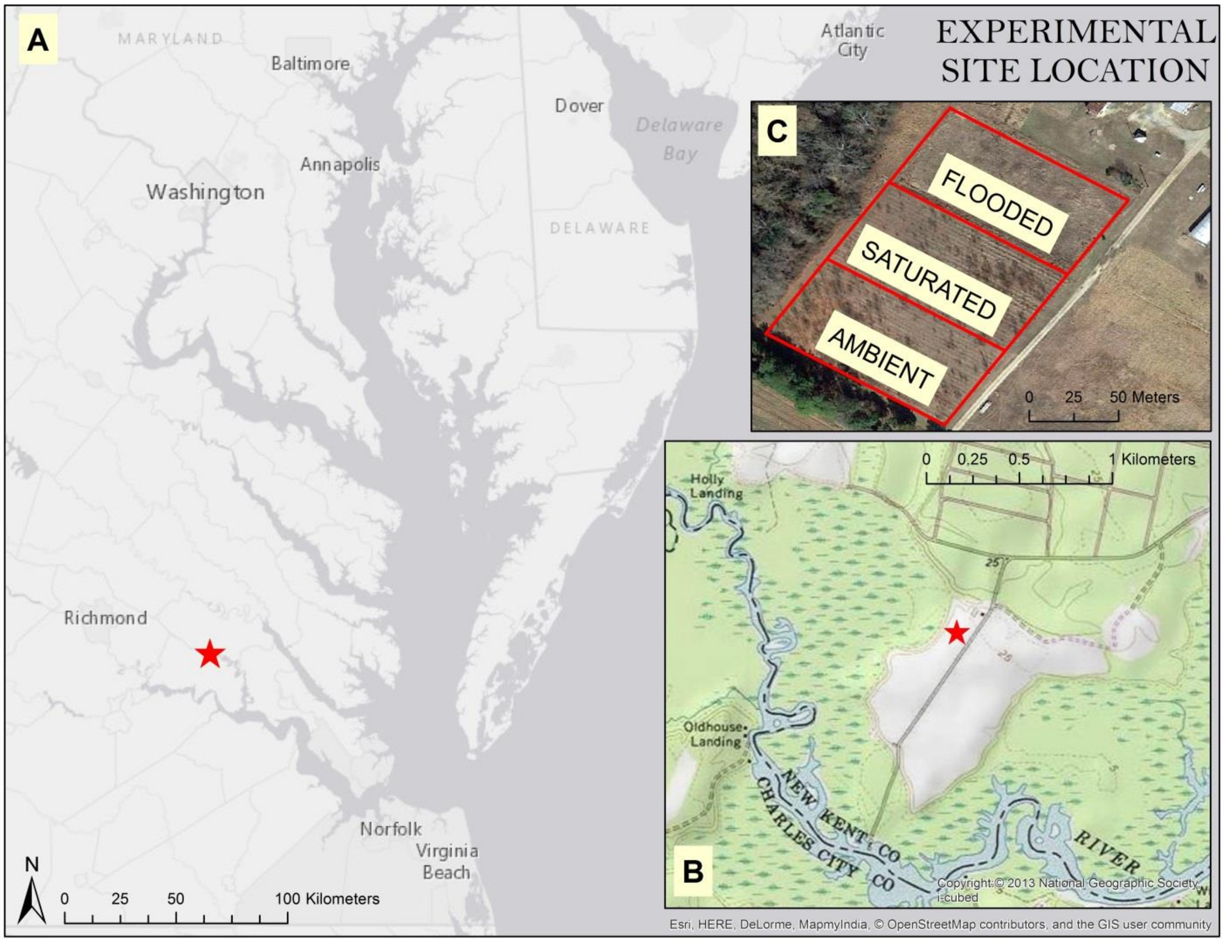

Figure 1. The experimental site is located in the Mid-Atlantic region of the United States of America, in the Coastal Plain of Virginia (**A**). The site is on an upland terrace in the Virginia Department of Forestry, New Kent Forestry Center (**B**). The hydrologically distinct cells (ambient (AMB), saturated (SAT) and flooded (FLD)) are 49 m x 144 m in size (**C**).

Table 1. Description of environmental parameters for the three experimental cells. Values represent averages with associated standard error.

Environmental Parameter	Ambient	Saturated	Flooded
Hydrology	Recevied only precipitation	Kept saturated for a minimum of 90% of the growing season within the root-zone (10cm) of the plantings and irrigated as needed	Inundated above the root crown for a minimum of 90% of each year
Soil Preparation	Disked and Tilled	Disked and Tilled	Excavated to a depth of 1m to an existing clay layer
Herbaceous Vegetation Control	Riding Lawnmower, Push mower, weedwacker, Glyphosate application	Riding Lawnmower, Push mower, weedwacker, Glyphosate application	None
Soil Bulk Density (g/cm^3)	1.03 (0.02)	1.1 (0.02)	1.38 (0.02)
Soil Percentage Sand	85.16 (0.93)	88.35 (0.66)	63.74 (1.52)
Soil Percentage Silt	10.22 (0.83)	7.57 (0.47)	17.27 (0.97)
Soil Percentage Clay	4.62 (0.19)	4.08 (0.23)	18.99 (1.00)
Soil Percentage Carbon	1.47 (0.06)	1.2 (0.06)	0.34 (0.02)
Soil Percentage Nitrogen	0.17 (0.01)	0.15 (0.01)	0.08 (0.01)
Soil Percentage Phosphorus	0.29 (0.01)	0.26 (0.01)	0.18 (0.01)

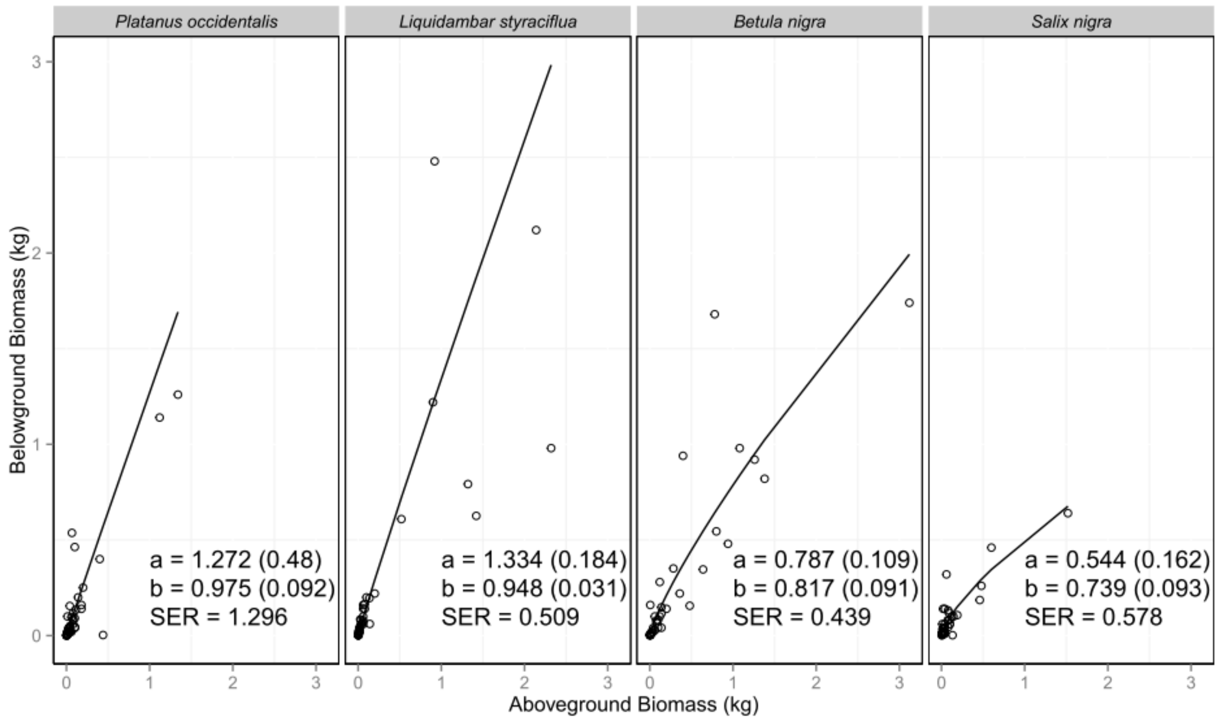

Figure 2. Results of fitting Eq. 1 ($Y=aX^b + \varepsilon$) for early successional species destructively harvested in 2011. Y=Total dry belowground biomass (coarse roots) (kg) and X= Total dry aboveground biomass (kg) (excluding leaves). Model derived intercept (a) and exponent (b) with standard errors in parentheses are presented with standard error of the regression (SER) for each species.

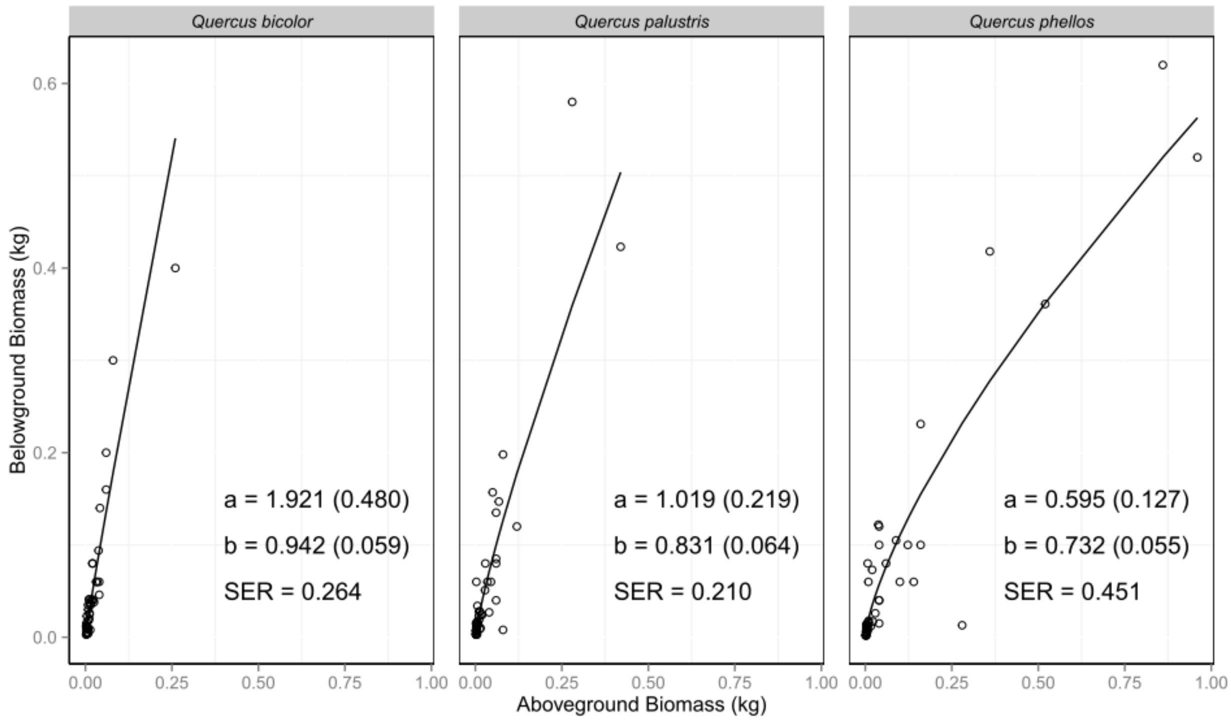

Figure 3. Results of fitting Eq. 1 ($Y=aX^b + \varepsilon$) for late successional species destructively harvested in 2011. Y=Total dry belowground biomass (coarse roots) (kg) and X= Total dry aboveground biomass (excluding leaves) (kg). Model derived intercept (a) and exponent (b) with standard errors in parentheses are presented with standard error of the regression (SER) for each species.

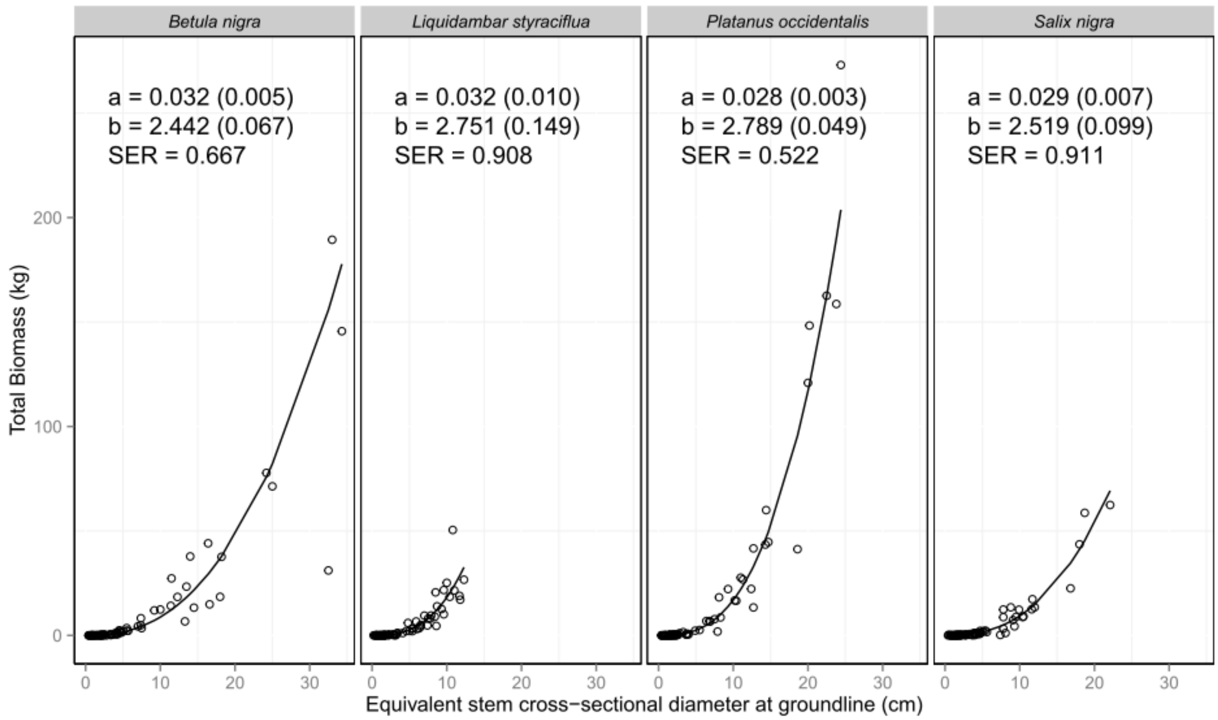

Figure 4. Results of fitting Eq. 1 ($Y=aX^b + \varepsilon$) for early successional species destructively harvested in 2011 and 2014. Y=Total biomass (kg) and X=Equivalent stem-cross sectional diameter at groundline (cm). Model derived intercept (a) and exponent (b) with standard errors in parentheses are presented with standard error of the regression (SER) for each species.

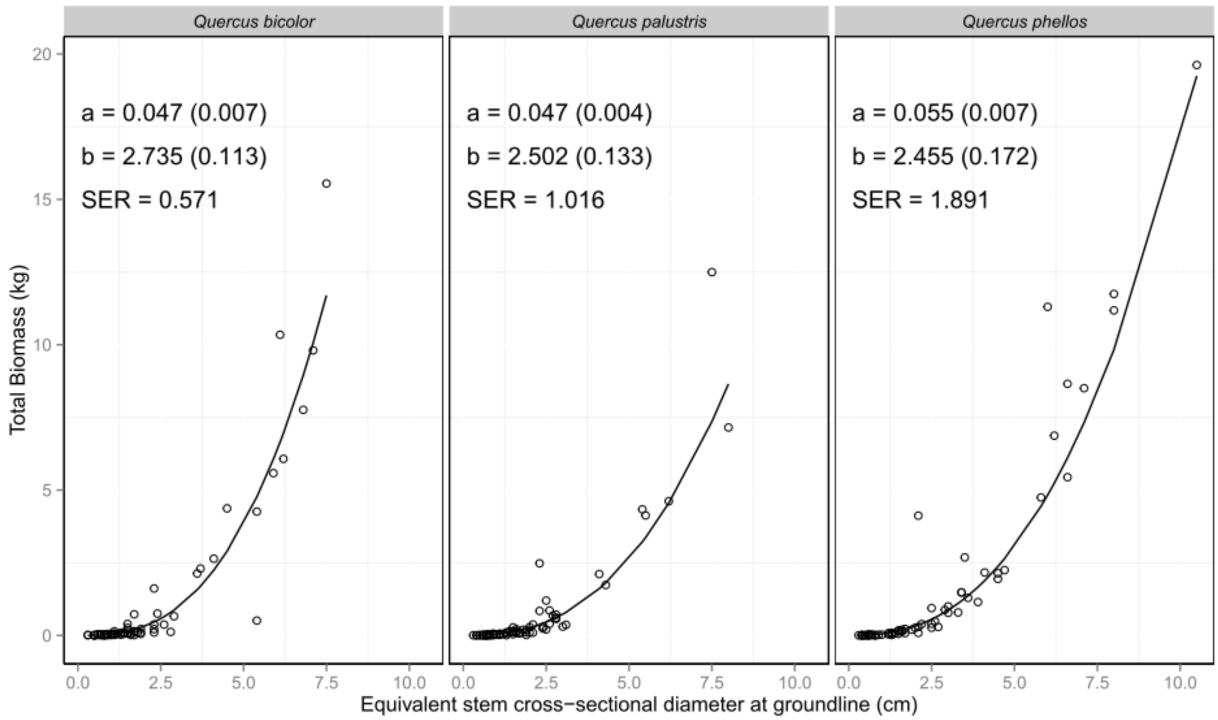

Figure 5. Results of fitting Eq. 1 ($Y=aX^b + \varepsilon$) for late successional species destructively harvested in 2011 and 2014. Y=Total biomass (kg) and X=Equivalent stem-cross sectional diameter at groundline (cm). Model derived intercept (a) and exponent (b) with standard errors in parentheses are presented with standard error of the regression (SER) for each species.

Table 2. Percentage survival and average biomass accumulated 6 years following planting estimated for each stocktype/species combination. Standard error is reported in parentheses. Same letter represent no significance difference among stocktype for each species within each cell (p>0.05). NA represents species/stocktype combinations with fewer than one surviving individual.

Species	Stocktype	Ambient		Saturated		Flooded	
		% Survival	Final Biomass (kg)	% Survival	Final Biomass (kg)	% Survival	Final Biomass (kg)
Betula nigra	Gallon	100.0	64.26 (6.88) a	97.2	40.45 (4.09) a	78.4	1.24 (0.42) a
	Bare root	33.3	36.88 (7.14) b	55.8	18.43 (2.5) b	9.4	1.11 (0.85) a
	Tubeling	25.8	43.12 (11.08) ab	68.8	27.51 (3.79) b	66.7	0.41 (0.21) a
Liquidambar styraciflua	Gallon	92.3	36.68 (4) a	100.0	35.76 (3.42) a	70.3	0.55 (0.16) a
	Bare root	68.3	41.64 (4.41) a	69.4	31.23 (4.16) ab	28.6	0.32 (0.17) ab
	Tubeling	13.9	14.4 (4.58) b	35.0	19.77 (3.28) b	47.1	0.12 (0.05) b
Platanus occidentalis	Gallon	82.1	71.13 (14.04) b	92.1	20.12 (4.02) b	10.8	0.31 (0.12) NA
	Bare root	55.8	151.74 (25.18) a	0.0	NA	0.0	NA
	Tubeling	96.7	219.11 (28.44) a	64.5	54.77 (15.35) a	0.0	NA
Salix nigra	Gallon	89.2	18.16 (3.58) a	86.8	19.34 (4.47) a	94.7	1.22 (0.31) a
	Bare root	0.0	NA	27.9	39.77 (10.83) a	85.0	1.28 (0.29) a
	Tubeling	30.0	28.47 (8.28) a	28.3	32.87 (12.49) a	77.8	0.76 (0.16) a
Quercus bicolor	Gallon	100.0	5.31 (0.81) a	100.0	3.72 (0.84) a	30.6	0.29 (0.11) a
	Bare root	80.9	4.32 (0.71) a	89.7	2.53 (0.45) ab	17.1	0.59 (0.27) a
	Tubeling	44.7	3.11 (0.69) a	61.0	1.45 (0.31) b	0.0	NA
Quercus palustris	Gallon	97.2	6.61 (1.41) a	95.0	3 (0.46) a	4.8	1.27 (1) a
	Bare root	71.1	3.17 (0.51) b	77.8	1.97 (0.34) a	4.1	0.02 (0) a
	Tubeling	16.1	0.56 (0.43) c	43.8	1.25 (0.54) a	0.0	NA
Quercus phellos	Gallon	85.7	10.07 (1.48) a	94.1	6.68 (0.75) a	17.9	0.83 (0.29) NA
	Bare root	45.3	2.72 (0.67) b	55.7	2.65 (0.42) b	1.5	0.09 (NA) NA
	Tubeling	25.0	2.97 (1.54) b	51.1	2.99 (0.62) b	0.0	NA

Table 3. Average biomass accumulated 6 years following planting estimated for each species (incorporating all stocktypes). Standard error is reported in parentheses. Same letters represent no significant difference in final biomass among cells for each species ($p<0.05$).

Cell	Species	Final Biomass (kg)
Ambient	*Betula nigra*	54.74 (5.21) a
Saturated	*Betula nigra*	30.41 (3.91) b
Flooded	*Betula nigra*	0.9 (1.93) c
Ambient	*Liquidambar styraciflua*	37.08 (3.91) a
Saturated	*Liquidambar styraciflua*	31.27 (3.58) a
Flooded	*Liquidambar styraciflua*	0.37 (1.05) b
Ambient	*Platanus occidentalis*	144.38 (11.26) a
Saturated	*Platanus occidentalis*	32.72 (8.38) b
Flooded	*Platanus occidentalis*	0.31 (0.43) c
Ambient	*Salix nigra*	20.91 (5.06) a
Saturated	*Salix nigra*	26.81 (6.83) a
Flooded	*Salix nigra*	1.11 (1.5) b
Ambient	*Quercus bicolor*	4.41 (2.06) a
Saturated	*Quercus bicolor*	2.7 (2.22) b
Flooded	*Quercus bicolor*	0.41 (0.84) c
Ambient	*Quercus palustris*	4.66 (2.96) a
Saturated	*Quercus palustris*	2.34 (1.6) b
Flooded	*Quercus palustris*	0.65 (1.36) c
Ambient	*Quercus phellos*	6.42 (2.82) a
Saturated	*Quercus phellos*	4.19 (1.85) b
Flooded	*Quercus phellos*	0.73 (0.88) c