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*Article*

# **Establishment of Alleycropped Hybrid Aspen "Crandon" in Central Iowa, USA: Effects of Topographic Position and Fertilizer Rate on Aboveground Biomass Production and Allocation**

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**Abstract:** Hybrid poplars have demonstrated high productivity as short rotation woody crops (SRWC) in the Midwest USA, and the hybrid aspen "Crandon" (*Populus alba* L.  $\times$ *P. grandidenta* Michx.) has exhibited particularly promising yields on marginal lands. However, a key obstacle for wider deployment is the lack of economic returns early in the rotation. Alleycropping has the potential to address this issue, especially when paired with crops such as winter triticale which complete their growth cycle early in the summer and therefore are expected to exert minimal competition on establishing trees. In addition, well-placed fertilizer in low rates at planting has the potential to improve tree establishment and shorten the rotation, which is also economically desirable. To test the potential productivity of "Crandon" alleycropped with winter triticale, plots were established on five topographic positions with four different rates of fertilizer placed in the planting hole. Trees were then harvested from the plots after each of the first three growing seasons. Fertilization resulted in significant increases in branch, stem, and total aboveground biomass across all years, whereas the effects of topographic position varied by year. Allocation between branches and stems was found to be primarily a function of total aboveground biomass.

**Keywords:** agroforestry; biofuels; marginal land; *Populus*; triticale; woody biomass

# **1. Introduction**

Using baseline scenarios, the U.S. Department of Energy estimates that forestlands in the contiguous United States have the capability to produce 298 million dry Mg of biomass annually by the year 2030 [1]. Likewise, the baseline estimate for perennial crops (woody and herbaceous) on agricultural lands was 346 million dry Mg of biomass annually, with estimates for high-yield scenarios reaching 705 million dry Mg annually. Production from both land cover types will be vital to meet the nation's demands for biofuels, bioenergy, and bioproducts. For example, adequate woody feedstock supply is necessary for achieving our national goal of 16 billion gallons of cellulosic biofuels by 2022, established under the U.S. Energy Independence and Security Act of 2007. Short rotation woody crops (SRWC) are purpose-grown trees that are a vital component of this potential woody biomass supply. Comparison with other perennial energy crops shows that SRWC have superior energy use efficiency and similar productivity [2]. In fact, biomass yields of up to 10 Mg ha<sup>-1</sup> yr<sup>-1</sup> are common in the Midwest and those approaching 20 Mg ha<sup>-1</sup> yr<sup>-1</sup> are attainable when growing adapted genotypes at sites with optimal environmental conditions [3,4]. To become competitive as a feedstock for cellulosic biofuels, however, the economic performance of SRWC must be improved [5]. One approach is targeting marginal agricultural lands upon which traditional crops often perform poorly [6]. The hybrid aspen "Crandon" (*Populus alba* L. × *P. grandidenta* Michx.) appears especially promising, due to both its high productivity and its adaptability to sloping marginal lands [7].

In addition, economic performance may be improved by growing SRWC in conjunction with agricultural crops in the form of alleycropping systems. These alternating strips of agricultural crops and trees offer the opportunity to harvest an annual crop and generate revenue early in the rotation while the trees become established. Furthermore, alleycropping systems have been shown to provide numerous benefits over agricultural monocropping in temperate regions including enhanced erosion control [8], more efficient nutrient cycling [9], improved water quality [10], greater carbon sequestration [11], improved pest control [12], and higher productivity when tree and row crops are properly matched to minimize competition with one another [13]. Hybrid poplars have been used in alleycropping systems with corn [14], soybeans [14–16], canola [17], and various other crops [18,19] with mixed success. For hybrid poplars, which experience peak growth during mid- to late-growing-season [20], it is logical that winter triticale (*Triticum* spp. × *Secale* spp.) which completes its growth cycle by mid-season would be a better match in alleycropping systems than crops with similar peak-growth periods as hybrid poplars (e.g., corn). Winter triticale has proven to be productive in double-cropping systems with corn [21,22] and sorghum [23], but the published literature appears to lack studies on winter triticale alleycropped with hybrid poplars.

Additionally, fertilization may improve SRWC economics by increasing productivity and/or shortening rotations. For example, broadcast fertilization at agronomic rates around the time of canopy closure (typically at mid-rotation) may substantially increase biomass growth rates [24]. However, such fertilization may also stimulate the allocation of biomass to branches and thereby reduce the value of the trees for certain markets (e.g., lumber). In contrast, fertilization early in the rotation has been observed to stimulate growth while having minimal effects on biomass allocation [25]. While broadcast applications are not environmentally or economically desirable early in the rotation because the trees have not yet fully occupied the site and therefore take up little of the applied nutrients, much lower rates of well-placed fertilizer have been shown to significantly increase the early growth of hybrid poplars [26,27].

In this study, we sought to evaluate the productivity of SRWC under a combination of management practices aimed at improving economic performance. Specifically, the productivity of the hybrid aspen ―Crandon‖ grown in an alleycropping system with winter triticale was evaluated at multiple topographic positions with different rates of fertilizer placed in the planting hole. Subsets of trees were then harvested after each of the first three growing seasons to determine the effects of topographic position and fertilizer rate on aboveground dry biomass (branch, stem, and total) and allocation (branch:stem ratio). The results are expected to be useful for informing researchers, landowners, and natural resource professionals about the roles that placement in the landscape and early fertilization play on the establishment of "Crandon" in alleycropping systems. The economic performance of the alleycropping system, including yields for winter triticale, has been described by Manatt *et al*. [28].

# **2. Materials and Methods**

# *2.1. Tree Materials*

The trees used in this study were established in the greenhouse during spring 2009 using 10-cm long dormant hardwood cuttings grown in 236-cm<sup>3</sup> Accelerator<sup>®</sup> containers (Nursery Supplies Inc., Chambersburg, PA, USA). They were continuously sub-irrigated until reaching a height of approximately 10 cm, after which they were watered twice daily with an automated overhead sprinkler system. Two weeks prior to field planting, the trees were pruned back to a height of 20 cm and placed outdoors to harden off.

# *2.2. Study Site and Experimental Design*

The study site is located at Iowa State University's Uthe Research and Demonstration Farm approximately 20 km southwest of Ames, IA, and is situated on an east-facing hillside adjacent to Big Creek and ranging in elevation from 305 to 325 m above sea level. Soil surveys indicate that the floodplain (previously in mixed grass) consists of Coland clay loam, whereas the rest of the study area (previously in row crops) consists primarily of Clarion loam  $(\sim 75\%)$  along with smaller areas of Nicollet loam, Spillville loam, and Zenor sandy loam. Plots measuring  $18.3 \times 24.5$  m were established at each of five topographic positions (floodplain, toe slope, back slope, shoulder slope, and summit), with three plots placed along the north-south axis of each topographic position, for a total of 15 plots. Within each plot, two sets of trees were planted: 48 trees spaced at  $3.0 \times 3.65$  m for long-term evaluation of growth and environmental impacts relative to other perennial and annual biomass cropping systems, and 24 trees placed at half-spacing  $(1.5 \text{ m} \times 3.65 \text{ m})$ ; Figure 1) which were harvested over the first three years and are the subject of this paper. These short-term trees were randomly assigned to one of the three harvest years and one of four fertilizer rates  $(0, 10, 20, 0r 40 g$  tree<sup>-1</sup> of 20-10-5 NPK tablets; Henry Field's Seed and Nursery Co., Aurora, IN, USA), with two trees independently assigned to each combination of fertilizer rate and harvest year (*i.e*., 5 topographic positions × 3 plots × 4 fertilizer rates × 3 years × 2 trees year<sup>-1</sup> fertilizer rate<sup>-1</sup> = 360 trees). Welsh [29]

and Wilson *et al*. [30] provide more-detailed descriptions of the long-term study and the additional biomass cropping systems evaluated therein.

Figure 1. Overhead view of plot layout showing long-term study trees (white circles), short-term study trees (green circles), and winter triticale (gold strips). The short-term trees are the subject of this paper. Tree spacing and plot dimensions are given in meters.



## *2.3. Site Preparation, Planting, and Harvest*

Plots were tilled and planted to winter triticale in the fall of 2008, and tree rows were prepared by spraying glyphosate herbicide in 1-m wide strips prior to planting in spring of 2009. Trees were planted into the strip-killed triticale at the end of May, using a tractor-mounted auger (20 cm diameter) to dig the planting holes. Fertilizer tablets were placed at a distance of approximately 10 cm from the trees, and at depth of approximately 10 cm below the ground. The triticale was harvested from the plots in early July, and was similarly grown in the alleys between the tree rows in the following two years (planted with a no-till drill in the fall and harvested in early July). Fertilizer (30 kg N ha<sup>-1</sup> as urea) was broadcast in the alleys of triticale each spring. Glyphosate was applied to the plots twice during the first growing season; once in mid-summer using wick applicators in the immediate vicinity  $(-0.5 \text{ m} \text{ radius})$  of the trees, and once in early-fall using a shielded backpack sprayer for spot-treatment (primarily on the floodplain where weed pressure was high). Surviving trees were harvested during the dormant season following each of the first three growing seasons ( $n = 332$  trees). For trees having good stem form (*i.e*., straight distinct leader; n = 256), height and diameter at the base of the harvestable stem (10 cm above ground level) were recorded for use in developing allometric biomass equations. For all harvested trees, aboveground biomass was separated into components (stem and branch) and oven-dried at 100 °C until stable, at which time dry mass was measured.

# *2.4. Data Analysis*

The experiment was analyzed as a split-plot design with fixed blocks (*i.e*., three blocks [north, central, and south] each containing one plot per topographic position; randomized complete block design). Topographic position was the main plot effect (experimental unit = plot), with position  $\times$  plot as a random effect. The split-plot effects (experimental unit = tree) included the two-way factorial of fertilizer and year (completely randomized design). Analysis of variance (ANOVA) was conducted using PROC MIXED (method = type3) in  $SAS^{\mathcal{B}}$  (SAS Institute Inc., Cary, NC, USA) for aboveground dry biomass components (branch, stem, and total) and allocation (branch:stem biomass ratio). The biomass components were log-transformed (base = 10) prior to analysis due to the variance being proportional to the mean, which increased substantially (by a factor of approximately 40) from the first year to the third year. For the branch:stem biomass ratio, log-transformed total aboveground dry biomass was used as a covariate, based on previous research showing tree size to be an important factor in biomass allocation [25]. Denominator degrees of freedom were determined via the Kenwood-Rogers method and significant treatment effects were further evaluated using multiple comparisons analysis with Tukey adjustment [31]. In the event of a significant interaction between main effects, multiple comparisons were conducted on the interaction rather than the main effects. To produce allometric biomass equations, linear regression was conducted using PROC GLM in SAS<sup>®</sup> to model each of the three components (branch, stem, and total aboveground biomass) based on tree height and diameter at the base of the harvestable stem.

# **3. Results and Discussion**

#### *3.1. Analysis of Variance (ANOVA)*

Significant ( $p < 0.05$ ) block, fertilizer, year, and position  $\times$  year effects were observed for all three aboveground biomass parameters, whereas allocation (branch:stem biomass ratio) was not significantly impacted by any of the treatment factors (Table 1). Based on the significant interaction between position and year, discussion of year effects is hereafter limited to the position  $\times$  year interaction. For describing treatment effects, geometric means (calculated by converting least squares means of log-transformed values to the original units of measure) are reported; however, because geometric means are systematically lower than arithmetic means calculated from untransformed data [32], final (third-year) productivity is reported in terms of arithmetic means scaled to a per-hectare basis.

Multiple comparisons analysis showed that, across all three years, the geometric mean of total aboveground dry biomass for the north block (94 g tree<sup>-1</sup>) was significantly lower than that of the south block (169 g tree<sup>-1</sup>) and central block (191 g tree<sup>-1</sup>). The lower biomass productivity was likely attributable to deer damage (*i.e*., browsing of growing points and rubbing/breaking stems during the rutting season), as deer and their tracks were most frequently observed at the north end of the study site. The physical condition of the trees was surveyed after the first growing season, and it was found that approximately 13% of the trees in the north block had been damaged by deer, as compared to rates of 7% and 8% for the south and central blocks, respectively. By the end of the second growing season, the trees had grown sufficiently tall (mean height  $= 2.2$  m) that the main stems were less susceptible to browsing. However, girdling of the stems as a result of deer rub continued throughout the three years

in all plots, which reduced harvestable biomass via dieback and breakage of stems. Despite these challenges, overall survival was high (92%) and varied by topographic position (from 86% on the floodplain to 97% on the back slope) and, to a lesser extent, by fertilizer rate (from 88% at 20 g tree<sup>-1</sup> to 95% at 40 g tree<sup>-1</sup>).

**Table 1.** Results of ANOVA for total biomass  $(B_T)$ , branch biomass  $(B_B)$ , stem biomass  $(B<sub>S</sub>)$ , and branch: stem biomass allocation ratio  $(A<sub>B-S</sub>)$  for the hybrid aspen "Crandon". Statistically significant effects ( $p < 0.05$ ) are shown in bold.

<b>Effects</b>	$B_T$	$B_B$	$B_{S}$	$A_{B:5}$
<b>Block</b>	0.0044	0.0082	0.0044	0.3147
<b>Position</b>	0.7234	0.7362	0.5854	0.1968
Fertilizer	< 0.0001	< 0.0001	0.0012	0.1244
Year	< 0.0001	< 0.0001	$0.0001$	0.2658
Fertilizer $\times$ Year	0.2515	0.4008	0.3552	0.7438
Position $\times$ Fertilizer	0.7287	0.8617	0.6711	0.9083
Position $\times$ Year	0.0005	0.0018	0.0074	0.5097
Position $\times$ Fertilizer $\times$ Year	0.6691	0.7393	0.9712	0.7721

For the fertilizer effects, total aboveground dry biomass was significantly higher at 20 and 40 g tree<sup>-1</sup> than at 0 g tree<sup>-1</sup>, and at 40 g tree<sup>-1</sup> compared to 10 g tree<sup>-1</sup> (Table 2). The same pattern was observed for branch biomass, whereas the differences for stem biomass were limited to the fertilizer rate of 40 g tree<sup>-1</sup> being significantly higher than 0 and 10 g tree<sup>-1</sup>. These results demonstrate that relatively small amounts of well-placed fertilizer at planting (e.g., 40 g fertilizer tree<sup>-1</sup> = 14 kg N ha<sup>-1</sup>, compared with 120 to 240 kg N ha<sup>-1</sup> annually for corn [2]) can significantly improve aboveground biomass production of "Crandon" during tree establishment. In fact, total biomass after the third year was 0.6 and 1.2 Mg ha<sup>-1</sup> higher for trees receiving 20 g tree<sup>-1</sup> and 40 g tree<sup>-1</sup> of fertilizer, respectively, compared to trees receiving no fertilizer (Figure 2). This increase of 41 to 86% is similar to the response observed by Guillemette and DesRochers [27], who reported first- and second- year increases in tree volume of approximately 20 to 70% for hybrid poplars supplied with 20 to 25 g tree<sup>-1</sup> of fertilizer at planting on former agricultural sites.

**Table 2.** Geometric means for aboveground total  $(B_T)$ , branch  $(B_B)$ , and stem  $(B_S)$  dry biomass per tree by fertilizer rate. Significant differences ( $p < 0.05$ ) are denoted by different letters within the column.

<b>Fertilizer Rate</b> $(g \text{ tree}^{-1})$	$\mathbf{B_{T}}$ (g tree $^{-1})$		$B_B$ $(g \text{ tree}^{-1})$		$\mathbf{B}_{\rm S}$ (g tree $^{-1}$	
	102	c	43	c	54	c
10	132	bc	60	bc	62	bc
20	162	ab	81	ab	71	abc
40	200	a	98	a	89	a

**Figure 2.** Arithmetic means for above ground total  $(\Box)$ , branch  $(\Diamond)$ , and stem  $(\Diamond)$  dry biomass per hectare after the third year, by fertilizer rate and adjusted for survival.



The results of the multiple comparisons analysis of position  $\times$  year are shown in Table 3. Significant within-year differences between positions were only observed at year 1, in which the trees growing on the floodplain had lower branch biomass than those on the shoulder slope and lower stem biomass than those on the back slope. The remaining significant differences were among years, in association with the trees increasing in size over time. Also, a lack of significant differences was observed between the most productive position at year 2 and the least productive positions at year 3. Specifically, the trees growing on the toe slope did not differ significantly at year 2 from other positions at year 3 in terms of stem biomass (summit, shoulder, and back slope), branch biomass (summit and shoulder slope), and total biomass (summit).

The broader lack of significant within-year effects for topographic position may be due in large part to the adaptability of "Crandon" to a variety of site conditions, although the reduced statistical power associated with main plot effects in split-plot designs may also be a contributing factor. While the use of fixed blocks and a single study site precludes inference to a larger geographic area, previous research at multiple sites in Iowa has shown that "Crandon" produces less variable (and often higher) yields relative to other woody species. Specifically, Goerndt and Mize [7] found that for 10-year-old plantations "Crandon" productivity varied by a factor of approximately two (16 to 30 Mg ha<sup>-1</sup> year<sup>-1</sup>) between site types (upland, sloping, or bottomland), whereas silver maple (*Acer saccharinum* L.) varied by a factor of more than four (4 to 18 Mg  $ha^{-1}$  year<sup>-1</sup>) and the hybrid cottonwood "Eugenei" (*P. deltoides* Bartr. ex Marsh.  $\times$  *P. nigra* L.) varied by a factor of more than eight (2 to 17 Mg ha<sup>-1</sup> year<sup>-1</sup>). In the current study, "Crandon" yields similarly varied by a factor of approximately two between the best and worst topographic positions, thus providing general support for the hypothesis that "Crandon" produces relatively consistent yields across topographic positions.

Position $\times$ Year	$B_T$ $(g \text{ tree}^{-1})$		B <sub>B</sub> $(g \text{ tree}^{-1})$		$B_{S}$ $(g \text{ tree}^{-1})$	
<b>Combinations</b>						
Year 1						
Summit	25.7	d	10.7	de	13.5	de
Shoulder Slope	25.1	d	11.5	d	12.6	de
<b>Back Slope</b>	25.7	d	7.8	de	15.8	d
Toe Slope	24.0	d	8.5	de	13.2	de
Floodplain	9.8	d	3.0	e	6.2	e
Year 2						
Summit	178	$\mathbf c$	93	$\mathbf{c}$	76	$\mathbf{c}$
Shoulder Slope	158	$\mathbf c$	76	$\mathbf{c}$	74	$\mathbf{c}$
<b>Back Slope</b>	129	$\mathbf c$	52	$\mathbf{c}$	72	$\mathbf{c}$
Toe Slope	240	bc	110	bc	115	bc
Floodplain	195	$\mathbf c$	93	$\mathbf{c}$	85	$\mathbf{c}$
Year 3						
Summit	646	ab	372	ab	251	ab
Shoulder Slope	676	a	380	ab	288	ab
<b>Back Slope</b>	813	a	479	a	316	ab
Toe Slope	891	a	537	a	331	a
Floodplain	1202	a	724	a	447	a

**Table 3.** Geometric means from position  $\times$  year interaction for above ground total  $(B_T)$ , branch ( $B_B$ ), and stem ( $B_S$ ) dry biomass per tree. Significant differences ( $p < 0.05$ ) are denoted by different letters within the column.

Based on visual observations, the lower initial productivity on the floodplain was likely attributable to greater weed competition. The prevalence of weeds on the floodplain was believed to be due to a combination of factors including a large seed bank built up under the previous land cover of mixed grasses, as well as greater availability of resources to stimulate weed growth (e.g., higher soil N content, organic matter content, and water conductivity than the other topographic positions at the site [32]). Notably, the trees growing on the floodplain eventually surpassed the other positions and had 1.3 Mg ha<sup>-1</sup> higher total biomass than the summit and shoulder slope after the third year (Figure 3). Similarly, the lack of significant differences between the toe slope at age 2 and the summit position at age 3 highlights the trend of faster growth at lower-lying topographic positions. Goerndt and Mize [7] observed similar trends with three-year-old "Crandon" producing biomass of 1.8 and 5.9 Mg ha<sup>-1</sup> yr<sup>-1</sup> for upland and bottomland sites, respectively. The yields in the current study are somewhat lower than this, and while the exact cause is unclear it may be related to the use of smaller planting stock, less favorable site conditions, negative interactions with winter triticale, or some combination thereof. Thus, additional research is recommended, especially with respect to evaluating different types of planting stock and the possibility of allelopathic effects with winter triticale.

**Figure 3.** Arithmetic means for above ground total  $(\Box)$ , branch  $(\Diamond)$ , and stem  $(\Diamond)$  dry biomass per hectare after the third year, by topographic position and adjusted for survival.



Allocation (branch:stem ratio) was not significantly impacted by any treatment factors (see Table 1); however, the covariate was found to be a significant factor in predicting allocation ( $p = 0.0155$ ), with branch:stem ratio increasing as total aboveground biomass increased. Thus, our results suggest the increases in branch:stem ratio commonly associated with fertilization and/or topography-related resource availability may actually be indirect effects realized via increasing total biomass (Figure 4). This finding agrees with previous research by Coyle and Coleman [24], in which biomass allocation for two *P. deltoides* clones was dictated primarily by tree size rather than nutrient or water availability.

**Figure 4.** Relationship between branch:stem ratio and the covariate, log-transformed (base = 10) aboveground total biomass (dotted lines), along with least-squares means for (a) fertilizer treatments by year  $(4 \text{ fertilizer rates} \times 3 \text{ years} = 12 \text{ points})$ ; and (**b**) topographic position by year (5 positions  $\times$  3 years = 15 points). Error bars represent +/− 1 standard error of the means.



#### *3.2. Linear Regression*

Based on the ANOVA results, which indicated aboveground biomass allocation was primarily a function of tree size, each of the three biomass components (branch, stem, and total) was modeled using an equation equivalent to the Schumacher and Hall volume equation [33]:

$$
\log B_{(y)} = a \log D_{10} + b \log H + c \tag{1a}
$$

which can be re-written as:

$$
B_{(y)} = (D_{10})^a \times H^b \times 10^c \tag{1b}
$$

where  $B_{(y)}$  is the dry biomass in g tree<sup>-1</sup> for a given aboveground component (y),  $D_{10}$  is the diameter of the tree in cm at harvestable height (10 cm aboveground), and H is the tree height in cm.

The results of the linear regression are presented in Table 4. The model was found to have a strong fit for all three aboveground biomass components, with coefficients of determination  $(R^2)$  ranging from 0.90 (branch) to 0.98 (stem). These results demonstrate that the aboveground biomass of young ―Crandon‖ trees may be reliably estimated based on readily-obtained measurements (*i.e*., height and diameter at harvestable height).

**Table 4.** Coefficient values for Equation 1 based on linear regression of aboveground total biomass (B<sub>T</sub>), branch biomass (B<sub>B</sub>), and stem biomass (B<sub>S</sub>), along with  $R^2$  values reflecting model fit.

Component	a	Ŋ	c	${\bf R}^2$
$\rm B_T$	2.040	0.769	$-0.174$	0.97
$\rm B_B$	2.985	0.055	0.820	0.90
Bs	1.548	1 1 5 3	1 202	0 98

It should be noted that the allometric relationships observed during establishment (when trees are open-grown) are not likely to be maintained after canopy closure is reached. Particularly, branch biomass is expected to level off after canopy closure, as horizontal crown expansion is curtailed and continued vertical crown expansion is met with shading out of the lower branches. However, we expect that the changes to these allometric relationships will correlate with certain metrics of competition. A metric of particular interest is crown competition factor (CCF), which has been previously described as a tool for optimizing clone selection and spacing for SRWC [34]. For closed stands, CCF represents the ratio of the crown area of an open-grown tree to that of a tree of the same diameter in a closed stand [35], and therefore may similarly reflect the ratio of branch biomass predicted by allometric equations for open-grown trees to that of trees in a closed stand. Thus, we suggest CCF may be useful as an additional predictor variable in allometric equations for aboveground biomass, and merits further investigation with "Crandon" and other SRWC species.

# **4. Conclusions**

The results of this study demonstrate that low rates of fertilizer at planting can nearly double the productivity of the hybrid aspen "Crandon" during establishment (*i.e.*, the first 3 years of growth). Further research should be done to determine whether these early gains in aboveground biomass are maintained throughout the rotation and/or allow for a shorter rotation length, as well as to evaluate whether winter triticale may have any negative (e.g., allelopathic) effects on tree productivity. The current study also reinforces previous research suggesting that "Crandon" is a versatile clone capable of producing relatively consistent yields across a variety of topographic positions. Aboveground biomass allocation (branch:stem ratio) was found to be primarily a function of tree size (*i.e*., total dry biomass), and all three tree components (branch, stem, and total aboveground biomass) were found to be strongly correlated with tree height and diameter at harvestable height (*i.e*., 10 cm aboveground).

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# **Conflict of Interest**

The authors declare no conflict of interest.

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