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ORIGINAL PAPER

Morphometry of leaf and shoot variables to assess aboveground biomass structure and carbon sequestration by different varieties of white mulberry (*Morus alba* L.)

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Abstract Mulberry is economically important and can also play a pivotal role in mitigating greenhouse gases. Leaf and shoot traits were measured for *Morus alba* var. Kanmasi, *M. alba* var. Karyansuban, *M. alba* var. Latifolia, and *M. alba* var. PFI-1 to assess aboveground biomass (AGB) and carbon sequestration. Variety-specific and multivariety allometric AGB models were developed using the equivalent diameter at breast height (EDBH) and plant height (*H*). The complete-harvest method was used to measure leaf and shoot traits and biomass, and the ash method was used to measure organic carbon content. The results showed significant ($p < 0.01$) varietal differences in leaf and shoot traits, AGB and carbon sequestration. PFI-1 variety had the greatest leaf density (mean ± SE: 1828.3 ± 0.3 leaves tree^{-1}), Karyansuban had the largest mean leaf area (185.94 ± 8.95 cm^2). A diminishing return was found between leaf area and leaf density. Latifolia had the highest shoot density per tree (46.6 ± 1.83 shoots tree^{-1}), total shoot length (264.1 ± 2.32 m), dry biomass (16.69 ± 0.58 kg tree^{-1}), carbon sequestration (9.99 ± 0.32 kg tree^{-1}) and CO_2 mitigation (36.67 ± 1.16 kg). The variety-specific AGB models *b*(EDBH) and *b*(EDBH)^2 showed good fit and reasonable accuracy with a coefficient of determination (R^2) = 0.98–0.99, standard error of estimates (SEE) = 0.1125–0.3130 and root mean square error (RMSE) = 0.1084–0.3017. The multivariety models *b*ln(EDBH) and (EDBH)$^{0.756}$ showed good-fitness and accuracy with R^2 = 0.85–0.86, SEE = 1.6231–1.6445 and RMSE = 1.609–1.630. On the basis of these findings, variety Latifolia has good potential for biomass production, and allometric equations based on EDBH can be used to estimate AGB with a reasonable accuracy.

Keywords Allometry · Biomass estimation · CO_2 mitigation · Moraceae · Mulberry · Regression models

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Introduction

Global warming is an undeniable fact and has far-reaching, multifaceted effects on planet Earth. Many of the changes observed in climate systems since the 1950s have been unprecedented (IPCC 2014) and pose serious challenges to forest managers to maintain forest ecosystems, biomass production and livelihoods of forest-dependent communities (Bajwa et al. 2015; Guangyi et al. 2017).

The recent climate changes are primarily attributed to a marked rise in greenhouse gases (GHGs) emissions due to human activities. Among GHGs, carbon dioxide (CO_2) is rising at the highest rate. The global concentration of CO_2 has increased from 280 parts per million by volume (ppmv) to 402.2 ± 2.8 ppmv since the 1750s (Peñuelas et al. 2013; Li et al. 2020), mainly due to fossil fuel use and large-scale deforestation. Trees are well known to mitigate atmospheric CO_2 directly through photosynthesis by sequestering it and converting it into biomass.

Plants, through photosynthesis, sequester atmospheric CO_2 and convert it as biomass in different parts of plant and soil organic matter (Zaki et al. 2018). Biomass is a key indicator of the health of an ecosystem, a source of energy and a mitigator of GHGs, both directly and indirectly (Biilgen et al. 2007; Dombroski and Pinto 2019). Accurate and easy methods to measure biomass are, therefore, crucial to determine ecological services of forests and forest plantations (Ablo et al. 2015; Zeng 2015), and necessary for implementing climate change mitigation strategies (Goetz et al. 2015). Because biomass is a function of plant parts, particularly leaves and shoots, the morphometry of these organs and their relationships are important for estimating biomass. There are two types of methods for measuring morphometric variables and biomass: destructive (also called complete harvest) and nondestructive.

Numerous workers have analyzed the morphometry of plant traits, their relationships and estimated biomass using a destructive method or allometric biomass equations for different tree species (Basuki et al. 2009; Meyer et al. 2014; Ali et al. 2015; Galidaki et al. 2017; Guangyi et al. 2017; Škėma et al. 2018; Mahmood et al. 2020). They usually used stem diameter, tree height, stem volume, wood density, and crown length and destructive methods on a small scale to derive allometric equations for biomass. However, the literature is scanty on mulberry in this regard.

Mulberry, native to China, is of great economic importance as the sole food of the mulberry silkworm moth (*Bombyx mori* L.) and as a source of timber, fuelwood, fodder, food, drinks, medicines, constituents of cosmetics, etc. (Qin et al. 2012; Gozlekci et al. 2015; Dimobe et al. 2018). Recently, Li et al. (2020) found that the photosynthetic carbon sink of mulberry is greater compared to the total carbon emission, which indicates a beneficial effect for the environment.

Despite the fact that mulberry has important uses and grows globally in natural forests and forest plantations, too little attention has been paid to investigate morphometric variables and their relationships. Similarly, allometric AGB models are not available to quantify biomass using nondestructive methods. Variety-specific and multivariety models are crucial for quick and accurate quantification of biomass to highlight the ecological services of mulberry, in addition to its traditional uses.

The primary objective of our study was thus to assess carbon sequestration and develop variety-specific and multivariety allometric biomass models for *M. alba*. Specifically, we tested the direction and significance of correlations between leaf and shoot traits, tested two biometric variables, the equivalent diameter at breast height and plant height, as predictors of AGB, and quantified variety-specific carbon sequestration for *M. alba*.

Materials and methods

Research site and plantation management

The study was conducted during 2018–2019 in Peshawar, Pakistan (34°0′57.77″ N, 71°29′15.67″ E and 357 m a.s.l). The climate is subtropical with a mean annual temperature of 22.9 ± 0.09 °C with the lowest mean minimum temperature of 3.14 ± 0.19 °C in January and the highest mean maximum temperature of 39.22 ± 0.45 °C in June. The mean annual precipitation is 462.6 ± 23.7 mm, mostly during January–March, and mean annual evaporation rate (from a free water surface) is > 1600 mm (Bukhari and Bajwa 2008). The soil is clay-loam and crust forming due to a slight salinity and sodicity. The principal land-use is agriculture (84.1%), followed by rangeland (1.1%). The zonal vegetation type is subtropical broadleaf forests.

Four mulberry varieties were planted with 1.5 m between rows and 1.0 m between plants spacing in 1995. The trees were pollarded 0.5 m above the ground in January 2018. The leaves were harvested once, in March–April 2018; up to 50% of the foliage was removed for rearing the mulberry silkworm moth. The plantation was hoed and weeded in February and fertilized once with di-ammonium phosphate (206 kg ha^{-1}) and twice with urea (113 kg nitrogen ha^{-1}, before and after the leaf harvest). The plantation was irrigated every 2 weeks during the summer and every 4 weeks during the spring and autumn.

Leaf and shoot traits

Destructive sampling was used to assess the leaf and shoot traits, and AGB. Leaves from 15 trees of each of four varieties, *Morus alba* var. Kanmasi, *M. alba* var. Karyansuban, *M. alba* var. Latifolia, and *M. alba* var. PFI-1 (60 total) were harvested randomly 0.5 m aboveground to collect the complete growth for 1 year. Data were recorded for (1) leaf and shoot traits including: number of leaves $tree^{-1}$, mass of each leaf (using a digital balance), area of each leaf (using leaf area measuring meter), number of shoots $tree^{-1}$, total shoots length $tree^{-1}$, diameter of multiple barked stems with diameter ≥ 3.0 cm at breast height (using a digital Vernier caliper) to two decimals, (2) standing tree height (using a stadia rod), and (3) leaf and shoot biomass.

After harvest, the leaves and shoots were separated tree wise in the field and weighed using a bipod anchor rope on the tree and a block and tackle. All trees had multiple stems due to the pollarding; therefore, diameter of all multiple stems with diameter ≥ 3.0 cm at breast height (1.3 m aboveground level; DBH) was measured. The diameter data for the multiple stems was used to estimate the tree equivalent diameter at breast height (in cm) using the formula of Cienciala et al. (2013) in Eq. 1.

$$\text{EDBH} = 2\sqrt{\frac{(\text{DBH}_{s1})^2}{2} + \frac{(\text{DBH}_{s2})^2}{2} + \ldots + \frac{(\text{DBH}_{sn})^2}{2}}, \quad (1)$$

where DBH_{s1}, DBH_{s2}, … DBH_{sn} is stem diameter at breast height of *n*-stems of a given tree (in cm).

Carbon estimation

The organic carbon contents were estimated in leaf and barked shoot by the ash-method described by Allen et al. (1986). The shoots (15 total) and leaves (75 total) of each variety were washed separately with distilled water, dried using tissue paper, and weighed. The leaves and shoots were then oven dried at 80 °C until constant mass and ground finely. A 5-g sample of ground material of each leaf and shoot was placed separately in pre-weighed crucibles and ignited in the Muffle furnace at 450 °C for 3 h. The experiment was repeated five times. The ash and organic carbon content as follows:

$$\%\ \text{Ash} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100, \quad (2)$$

$$\%\ \text{Carbon} = 0.58(100 - \%\ \text{Ash}), \quad (3)$$

where W_1 is the mass of the crucible, W_2 is the mass of the oven-dried ground sample + crucible weight, and W_3 is the total mass of the ash and crucible.

The harvested leaves and shoots of each tree were packed separately in plastic bags in the field and placed into the solar kiln for a fortnight. The kiln-dried leaves and shoots were oven dried at 80 °C and 105 °C, respectively, until constant mass, then organic carbon content was estimated using Eq. 4.

$$\text{Carbon content} = \text{Dry biomass}/\text{CTV}, \quad (4)$$

where CTV = organic carbon content (kg) in the respective tree part estimated using Eq. 3.

The organic carbon content in the leaves and shoots, estimated using Eq. 4, was used to calculate variety-specific CO_2-equivalent mitigation using Eq. 5:

$$\text{Carbon dioxide (in kg)} = \text{Organic carbon content (in kg)} \times 3.67, \quad (5)$$

Statistical analyses

The overall difference (hypothesis: all means were equal) among four varieties was tested for significance using one-way analysis of variance (ANOVA). The difference between individual varieties was tested applying Tukey's honestly significant difference (HSD) test ($p = 0.05$) using Minitab version 17 (Minitab, State College, PA, USA). The correlation was tested between (a) leaf area and leaf mass, (b) leaf area and number of leaves tree^{-1}, and (c) the equivalent diameter at breast height and shoot length using a bivariate scatterplot with least squares regression and groups. The fitness and reasonability of the model was determined using the coefficient of determination and standard error of estimates, respectively. The strength of the correlation was assessed using Pearson's correlation coefficient (r^2). Six each variety-specific and multivariety (including trees from four varieties, $n = 60$) allometric models were developed for AGB. The allometric equations used were (1) $M = a + b(\text{EDBH})$ (linear, single variable), (2) $M = a + b(\text{EDBH})^2$ (stem volume), (3) $M = a + b\ln(\text{EDBH})$ (exponential), (4) $M = a + b(\text{EDBH})^b$ (power law), (5) $M = a + b(\text{EDBH})^2H$ (stem volume and tree height, double variable), and (6) $M = a + b(\text{EDBH}^2\text{H})^b$ (power law, double variable), where M = dry AGB tree^{-1} in kg, EDBH = equivalent diameter at breast height in cm, H = tree height in m, ln = natural logarithm, a = regression constant, and b = regression coefficient.

The statistical validity of the models was judged on the basis of indices of best-fit (R^2) and F-values and reasonability using the standard error of estimates (SEE) and root mean square error (RMSE). The model b (EDBH) was further validated by testing for a difference between observed and calculated dry AGB using a t-test. The models used were based on three assumptions: independent residuals, normal distribution, and constant variance.

Result

Morphological variables

Means for variety-specific leaf and shoot variables are presented in Table 1. A highly significant ($p < 0.01$) variation in number of leaves tree^{-1} was found among the varieties. PFI-1 was the most prolific leafing variety, while Karyansuban was the least leafing variety. PFI-1, Latifolia and Kanmasi produced 2.5, 2.3 and 2.2 times, respectively, greater leaves compared to Karyansuban. Contrarily, Karyansuban produced the largest and heaviest single leaves, while PFI-1 produced the smallest and lightest single leaves. The Karyansuban leaf was 1.5 and 1.9 times larger and heavier, respectively, compared to PFI-1 leaf. The leaf area and single leaf mass of Latifolia and Kanmasi did not differ significantly ($p > 0.05$). Latifolia produced the most shoots tree^{-1} and longest total shoots tree^{-1}, while Karyansuban produced the fewest shoots tree^{-1} and shortest shoots tree^{-1}. Number of shoots tree^{-1} of Kanmasi and PFI-1 did not differ significantly ($p > 0.05$). Latifolia attained the highest tree height, which was about 1.3 times greater compared to Karyansuban. The thickest and the thinnest equivalent diameter

Table 1 Mean ± SE leaf and shoot variables for varieties of mulberry

Variety	Leaf variables				Shoot variables			
	NLT (No)	LA (cm^2)	GLM (g)	DLM (g)	NST (No)	TSLT (m)	Height (m)	EDBH (cm)
Kanmasi	1662.1 ± 35.08 a	163.78 ± 8.93 a	2.94 ± 0.13 b	0.93 ± 0.14 b	32.2 ± 1.51 b	179.5 ± 2.79 b	2.42 ± 0.12 a	13.53 ± 0.42 b
Karyansuban	741.7 ± 23.14 b	185.94 ± 8.95 ab	4.01 ± 0.18 a	1.33 ± 0.20 a	20.5 ± 0.30 c	118.0 ± 1.99 d	1.93 ± 0.08 b	10.14 ± 0.46 c
Latifolia	1720.6 ± 30.32 a	158.81 ± 7.77 a	2.88 ± 0.14 b	0.90 ± 0.13 b	46.6 ± 1.83 a	264.1 ± 2.32 a	2.50 ± 0.10 a	16.20 ± 0.54 a
PFI-1	1828.3 ± 28.10 a	124.99 ± 4.28 b	2.16 ± 0.09 c	0.73 ± 0.11 c	28.5 ± 0.72 b	133.6 ± 2.55 c	2.43 ± 0.11 a	14.50 ± 0.78 ab
$F_{3, 56}$; (p)	564.1; (0.00)	10.46; (0.00)	31.04; (0.00)	32.23; (0.00)	76.8; (0.00)	846.2; (0.00)	6.46; (0.00)	20.26; (0.00)

Notes: Means in a column that do not share same letter differ significantly (Turkey's HSD test, $p = 0.05$); NLT = number of leaf tree^{-1}; LA = leaf area; GLM = single green leaf mass; DLM = single dry leaf mass; NST = number of shoots tree^{-1}; TSLT = total shoots length tree^{-1}; EDBH = equivalent diameter at breast height

at breast height was found in Latifolia and Karyansuban, respectively. The equivalent diameter at breast height of Kanmasi and PFI-1 did not differ significantly ($p > 0.05$).

The results further showed a positive slope between leaf area and single leaf mass (Fig. 1A). The correlation was highly significant ($p < 0.01$) and strong with a Pearson correlation coefficient between 0.88 and 0.94 (Table 2). The coefficient of determination with significant F-value and standard error of estimates (0.14–0.23) showed a good-fit of the model and reasonable accuracy, respectively. Contrarily, a negative slope was found between leaf area and number of leaves tree^{-1} (Fig. 2B). The negative correlation was highly significant ($p < 0.01$) and strong with a Pearson correlation coefficient varying from − 0.94 to − 0.99 (Table 2). Good-fit of the model was indicated by $R^2 = 0.89$–0.97 with highly significant ($p < 0.01$) F-values.

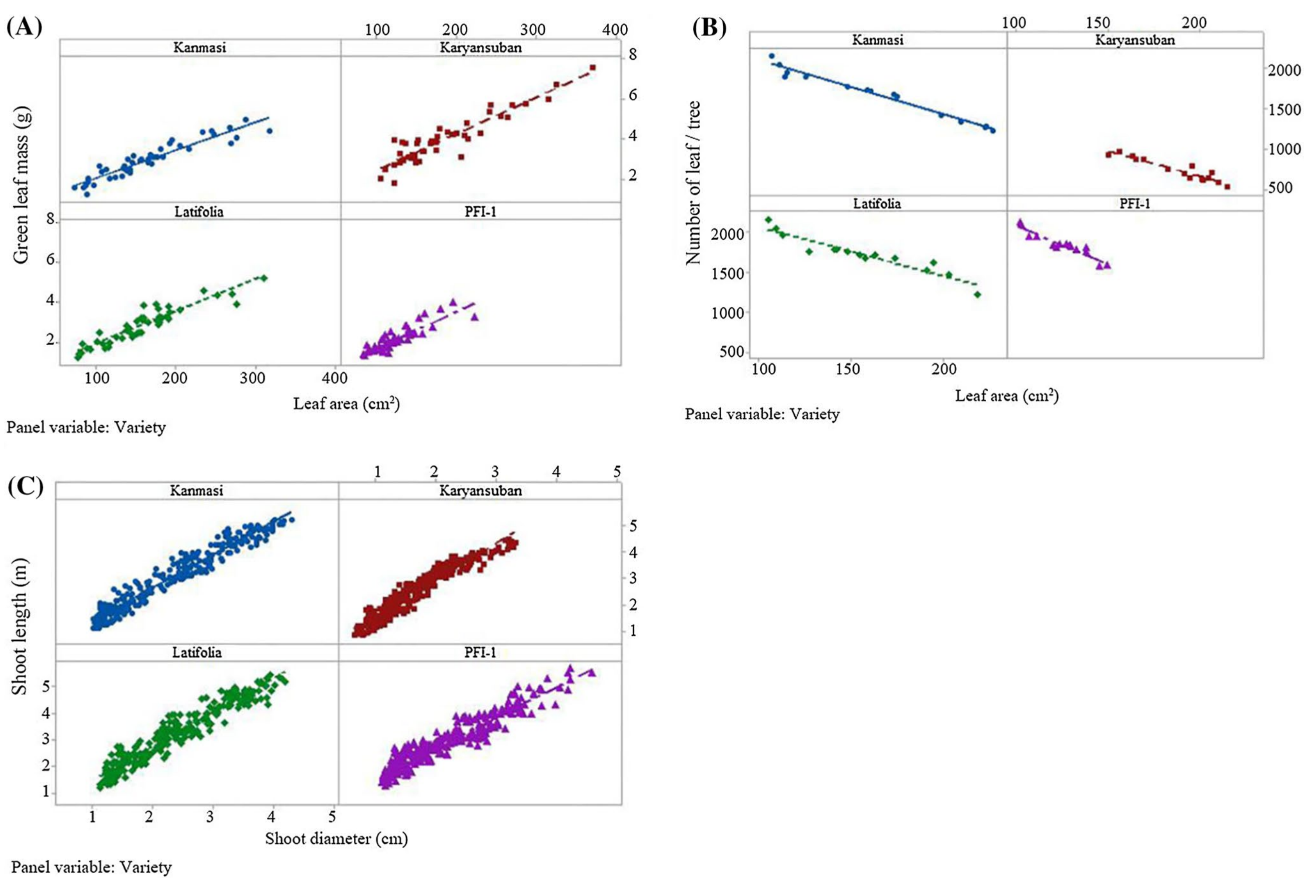

Fig. 1 Bivariate plot of correlation between (A) leaf area (cm^2) and single leaf mass (g), (B) leaf area (cm^2) and number of leaves tree^{-1}, and (C) shoot diameter (cm) and shoot length (m)

Table 2 Summary of correlation statistics between leaf area and single leaf mass; leaf area and number of leaves tree^{-1}, and shoot diameter and shoot length for varieties of mulberry

Response variable	*a*	*b*	R^2	SEE	*F*	r^2
Kanmasi						
Single leaf mass	−21.13	62.98	0.88	0.21	333.1 *	0.94 *
No. of leaves tree^{-1}	2758	−6.651	0.97	0.51	461.7	−0.99
Shoot length	0.13	1.25	0.94	0.03	4951.5	0.97
Karyansuban						
Single leaf mass	−3.58	47.3	0.86	0.23	263.5	0.93
No. of leaves tree^{-1}	1903	−6.21	0.89	0.47	107.7	−0.95
Shoot length	0.20	1.37	0.91	0.03	3039.9	0.95
Latifolia						
Single leaf mass	6.59	52.89	0.86	0.20	270.0	0.93
No. of leaves tree^{-1}	2664	−6.04	0.89	0.45	110.0	−0.95
Shoot length	0.16	1.29	0.93	0.03	3656.3	0.96
PFI-1						
Single leaf mass	34.68	41.87	0.77	0.14	144.7	0.88
No. of leaves tree^{-1}	3033	−9.62	0.89	0.52	104.7	−0.94
Shoot length	0.41	1.15	0.90	0.03	2804.6	0.95

*All values in the column are highly significant ($p<0.01$). Notes: a = y-intercept; b = slope of line; R^2 = Coefficient of determination; SEE = standard error of estimates; r^2 = Pearson correlation coefficient

The standard error of estimates (0.45–0.52) showed reasonable accuracy.

The relationship between shoot diameter and shoot length was positive (Fig. 3C), highly significant ($p<0.01$) and strong ($r^2=0.95$–0.97) in all the tested varieties (Table 2). A good-fit of the model was indicated by $R^2=0.90$–0.94 with highly significant ($p<0.01$) F-values. The standard error of estimates (0.03) showed a high accuracy of the model.

Aboveground biomass and organic carbon contents

The results of leaf and shoot dry biomass are presented in Table 3. Dry leaf biomass tree^{-1} and dry shoot biomass tree^{-1} varied significantly ($p<0.01$) among the tested varieties. Latifolia produced the highest dry leaf mass tree^{-1}, as well as dry shoot biomass tree^{-1}, followed by PFI-1, while Karyansuban produced the lowest dry leaf and shoot biomass tree^{-1}. Latifolia and PFI-1 produced about 2.4 and 2.3 times, respectively, greater dry biomass tree^{-1} compared to Karyansuban. The difference in dry biomass tree^{-1} between Latifolia and PFI-1 was not significant ($p>0.05$). The overall variation in organic carbon contents, both in leaf and shoot did not differ significantly ($p>0.05$).

Allometric aboveground biomass models

The variety-specific and multivariety allometric dry AGB models are presented in Fig. 2 and a summary of statistics in Table 4. The variety-specific AGB models showed good-fit of models with a coefficient of determination from 0.96 to 0.99 with highly significant ($p<0.01$) F-values. The standard error of estimates (0.1125–0.4503) and RMSE (0.1084–0.4340) values showed good reasonability and accuracy; however, model b(EDBH) and $b(\text{EDBH})^2$ showed relatively better accuracy. The allometric models based on one biometric variable were more efficient and practicable compared to models based on two biometric variables, $b(\text{EDBH})^2H$ and $b(\text{EDBH}^2\text{H})^{0.756}$. The difference between observed dry AGB tree^{-1} and calculated dry biomass tree^{-1}, calculated using model b(EDBH), was not significant ($p>0.05$).

The three multivariety AGB models based on one biometric variable, b(EDBH), $b\ln$(EDBH) and $b(\text{EDBH})^{0.756}$ showed good-fit of models ($R^2=0.85$–0.86) with highly significant ($p<0.01$) F-values. The standard error of estimates (1.6231–1.6759) and RMSE (1.609–1.662) showed good reasonability and accuracy of these multivariety AGB models. The difference between observed multivariety dry AGB tree^{-1} and calculated dry biomass tree^{-1}, calculated using multivariety linear model b(EDBH), was not significant ($p>0.05$).

Carbon sequestration

The per tree sequestered carbon varied significantly ($p<0.01$) among the tested varieties. Latifolia sequestered the most carbon both in leaf tree^{-1} and shoot tree^{-1}, while Karyansuban sequestered the least carbon (Table 5). The difference in carbon sequestered tree^{-1} between Latifolia and PFI-1 was not significant ($p>0.05$). The carbon sequestered tree^{-1} by Latifolia and PFI-1 was 2.6 and 2.5 times, respectively, greater compared to Karyansuban. The estimated

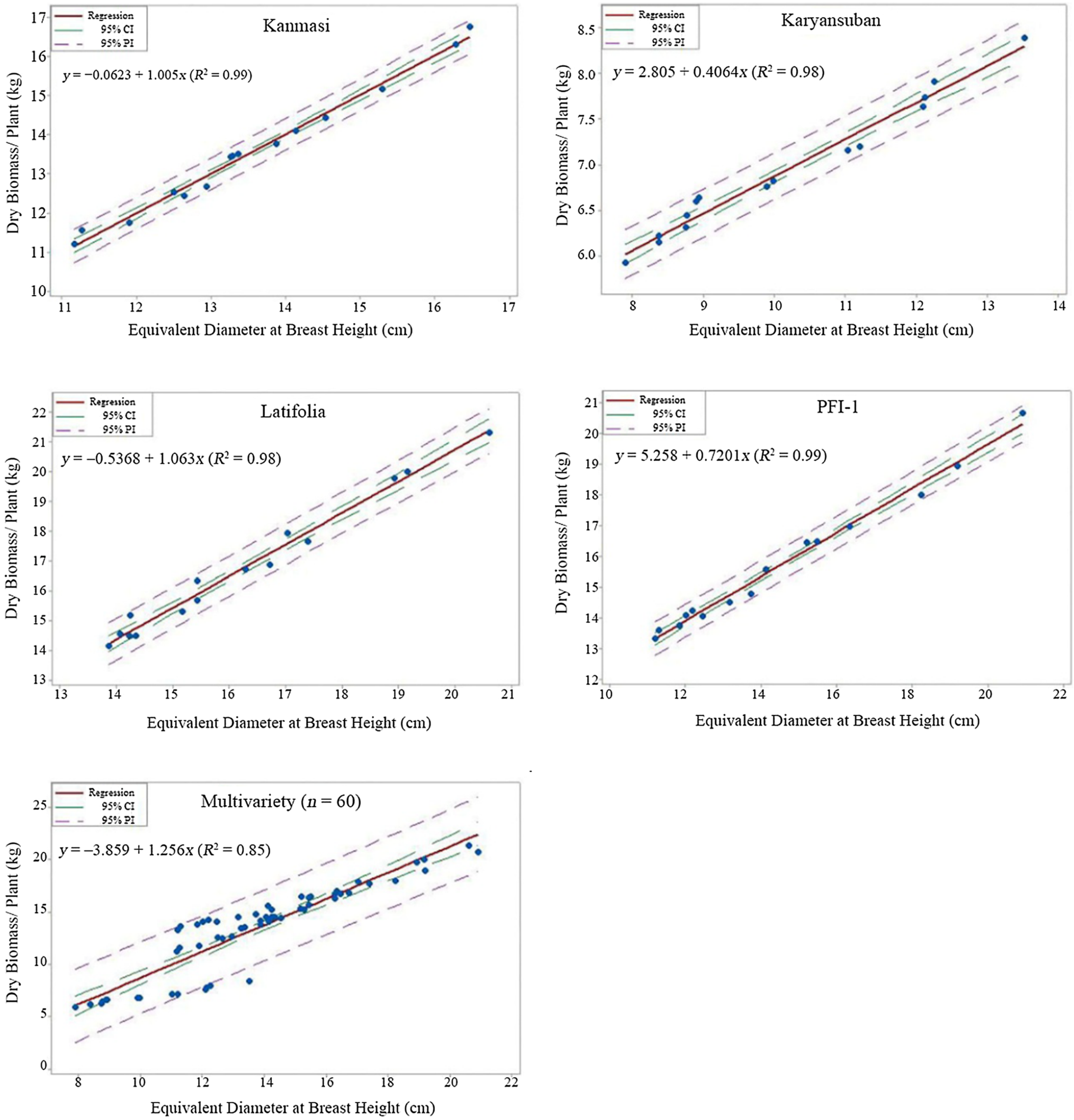

Fig. 2 Variety-specific and multivariety aboveground dry biomass models developed using equivalent diameter at breast height as the predictor variable

carbon sequestered ha^{-1} was between 25.56 ± 0.66 MT and 66.93 ± 1.99 MT. Latifolia and PFI-1 sequestered about 33.8% and 28.3%, respectively, more carbon ha^{-1} compared to Kanmasi, while Kanmasi sequestered almost double the carbon sequestered by Karyansuban.

The atmospheric CO_2 mitigated by the four varieties varied significantly ($p < 0.01$). Latifolia mitigated the most CO_2, followed by PFI-1 (Table 5), while Karyansuban mitigated the least. The difference in mitigated CO_2 between Latifolia and PFI-1 was not significant ($p > 0.05$). Latifolia and PFI-1 mitigated 2.6 and 2.5 times, respectively, more CO_2 than Karyansuban did.

Table 4 Summary of statistics of variety-specific and multivariety dry AGB models developed using equivalent diameter at breast height and plant height

Model	Allometric equation	R^2	SEE	RMSE	F	p
Kanmasi ($n = 15$)						
b(EDBH)	$y = -0.0623 + 1.005x$	0.99	0.1767	0.1703	1183.73	0.00
b(EDBH)^2	$y = 6.806 + 0.03639x^2$	0.99	0.1658	0.1598	1346.65	0.00
b(EDBH)^2H	$y = 9.702 + 0.0082x^2H$	0.98	0.2154	0.2075	792.69	0.00
$b$ln(EDBH)	$y = -22.01 + 13.68\ln x$	0.98	0.2394	0.2307	638.93	0.00
b(EDBH)$^{0.756}$	$y = -4.485 + 2.518x^{0.756}$	0.99	0.1884	0.1815	1039.85	0.00
b(EDBH^2H)$^{0.756}$	$y = 8.461 + 0.0494(x^2H)^{0.756}$	0.99	0.1986	0.1914	934.29	0.00
Karyansuban ($n = 15$)						
b(EDBH)	$y = 2.805 + 0.4064x$	0.98	0.1152	0.1111	544.19	0.00
b(EDBH)^2	$y = 4.889 + 0.01933x^2$	0.98	0.1125	0.1084	571.99	0.00
b(EDBH)^2H	$y = 5.550 + 0.0064x^2H$	0.97	0.1320	0.1272	411.50	0.00
$b$ln(EDBH)	$y = -2.718 + 4.188\ln x$	0.97	0.1345	0.1296	396.13	0.00
b(EDBH)$^{0.756}$	$y = 1.456 + 0.9518x^{0.756}$	0.98	0.1187	0.1144	512.48	0.00
b(EDBH^2H)$^{0.756}$	$y = 5.094 + 0.0323(x^2H)^{0.756}$	0.98	0.1160	0.1117	537.33	0.00
Latifolia ($n = 15$)						
b(EDBH)	$y = -0.5368 + 1.063x$	0.98	0.3013	0.2903	763.40	0.00
b(EDBH)^2	$y = 8.335 + 0.0314x^2$	0.98	0.3130	0.3017	706.12	0.00
b(EDBH)^2H	$y = 11.61 + 0.0073x^2H$	0.96	0.4503	0.4340	334.61	0.00
$b$ln(EDBH)	$y = -32.61 + 17.75\ln x$	0.98	0.3435	0.3310	584.38	0.00
b(EDBH)$^{0.756}$	$y = -6.265 + 2.800x^{0.756}$	0.98	0.3071	0.2959	734.45	0.00
b(EDBH^2H)$^{0.756}$	$y = 9.823 + 0.0497(x^2H)^{0.756}$	0.97	0.4126	0.3980	400.89	0.00
PFI-1 ($n = 15$)						
b(EDBH)	$y = 5.258 + 0.7201x$	0.99	0.2343	0.2258	1210.18	0.00
b(EDBH)^2	$y = 10.70 + 0.0229x^2$	0.99	0.2462	0.2372	1095.01	0.00
b(EDBH)^2H	$y = 12.29 + 0.006x^2H$	0.98	0.3344	0.3223	587.40	0.00
$b$ln(EDBH)	$y = -13.27 + 10.91\ln x$	0.98	0.3516	0.3388	530.08	0.00
b(EDBH)$^{0.756}$	$y = 1.740 + 1.856x^{0.756}$	0.99	0.2546	0.2453	1022.96	0.00
b(EDBH^2H)$^{0.756}$	$y = 11.08 + 0.0393(x^2H)^{0.756}$	0.99	0.2556	0.2463	1015.02	0.00
Multivariety ($n = 60$)						
b(EDBH)	$y = -3.859 + 1.256x$	0.85	1.6759	1.662	316.00	0.00
b(EDBH)^2	$y = 4.822 + 0.0432x^2$	0.80	1.8956	1.879	234.34	0.00
b(EDBH)^2H	$y = 7.626 + 0.0115x^2H$	0.71	2.2827	2.263	143.58	0.00
$b$ln(EDBH)	$y = -30.06 + 16.75\ln x$	0.86	1.6231	1.609	340.73	0.00
b(EDBH)$^{0.756}$	$y = -9.343 + 3.152x^{0.756}$	0.85	1.6445	1.630	330.43	0.00
b(EDBH^2H)$^{0.756}$	$y = 5.582 + 0.0735(x^2H)^{0.756}$	0.75	2.1148	2.097	176.87	0.00

ln = natural logarithm; R^2 = coefficient of determination; SEE = standard error of estimates; RMSE = root mean square error

Table 5 Variety-specific mean ± SE leaf, shoot and total carbon sequestered tree^{-1} ± SE and CO_2 mitigated tree^{-1}

Variety	LSC kg tree^{-1}	SSC kg tree^{-1}	Total sequestered carbon		Mitigated CO_2 kg tree^{-1}
			kg tree^{-1}	MT ha^{-1} *	
Kanmasi	0.76 ± 0.02 b	6.71 ± 0.21 b	7.47 ± 0.23 b	50.02 ± 1.47 b	27.41 ± 0.74 b
Karyansuban	0.39 ± 0.01 c	3.43 ± 0.09 c	3.82 ± 0.10 c	25.60 ± 0.66 c	14.02 ± 0.38 c
Latifolia	0.92 ± 0.03 a	9.07 ± 0.26 a	9.99 ± 0.32 a	66.93 ± 1.99 a	36.67 ± 1.16 a
PFI-1	0.89 ± 0.03 a	8.69 ± 0.18 b	9.58 ± 0.31 a	64.20 ± 1.98 a	35.16 ± 0.99 a
$F_{3,19}$; (p)	28.5; (0.00)	225.0; (0.00)	233.0; (0.00)	87.66; (0.00)	86.63; (0.00)

Means in a column that do not share same letter are significantly different (Tukey's HSD test, $p = 0.05$); LSC = leaf sequestered carbon, SSC = shoot sequestered carbon; * estimated based on 6,700 trees ha^{-1}

Discussion

The accurate quantification of tree biomass is important for appraising ecosystem carbon storage and understanding ecological processes like wood production and nutrients cycling (Ali et al. 2015). The complete-harvest method is a reliable approach for measuring accurate aboveground biomass. This method is efficient and precise because no subsampling is involved (Ritson and Sochacki 2003). However, this method has its own limitations, i.e., complete-harvesting of trees is ecologically undesirable operation; hence, sampling size has to be kept as small as possible. The small sampling size may result in greater variability and consequently affect the reliability of the results.

The leaf is an important part of a tree because it is the primary solar light-harvesting organ. The present findings show a significant varietal variation in leaf and shoot variables and in their relationships. The leaf variables, therefore, may affect growth performance of a tree species. This study shows that leaf size in terms of area increases leaf mass but reduces leafing intensity. This trade-off between leaf size and leafing intensity is assigned to the fact that a smaller, but more leaves are helpful in maintaining more supernumerary axillary buds. Previously, Dombroskie and Aarssen (2012) found a leaf size-leafing intensity trade-off at the plant level in 16 broadleaf tree species in Canada. Similarly, Sun et al. (2019) found a diminishing return between leaf size and leafing intensity in bamboo species.

Our findings further highlight that leafing intensity is positively related to number of shoots tree^{-1}, total shoot length tree^{-1}, and the equivalent diameter at breast height. Latifolia produces smaller and lighter leaves but more shoots trees^{-1}, and longer and thicker shoots. The positive effect of smaller but more leaves on shoot variables may be explained in terms of a larger "bud bank" generated by shoots, supporting higher leafing intensity. The larger bud bank provides more meristems for strategic deployment, i.e., in the expression of growth-form including branching intensity or as a reserve for survival and compensation after tissue loss to herbivores or physical disturbance (Aarssen 2012). Moreover, the high leafing intensity indicates greater cumulative leaf surface area tree^{-1}. The size of shoot variables of Latifolia, especially total shoot length tree^{-1} indicates a large tree canopy. The large cumulative leaf surface area combined with the large tree canopy can intercept more solar radiation, which increases the rate of photosynthesis and, consequently, biomass. Biomass production, however, depends upon several factors including genetic variation (Weraduwage et al. 2015); mature tree size, tree lifespan and growth rate (Nowak et al. 2002), and plantation management practices (McPherson 1998; Bajwa and Khan 2015). Apart from genetic function and plantation management practices, biomass production also depends on climatic and edaphic factors. For instance, AGB increases along a temperature gradient but decreases with potential evapotranspiration, clay and sand soil contents across 12 forest sites in moist temperate, semi-humid, and semi-arid zones in Iran (Ali et al. 2020). The variation in AGB in the present study may primarily be assigned to genetic function because site-specific environmental conditions and plantation management practices were the same for the four varieties.

The dry biomass tree^{-1} varies from 6.93 ± 0.20 kg to 16.69 ± 0.58 kg. The standard error (0.20 to 0.58) among 15 trees of each variety shows a little variation in the data. This result indicates that dry biomass ha^{-1}, calculated based on 6700 trees ha^{-1} (row and plant spacing: 1.5 m and 1.0 m), may give an error of 1.34 MT ha^{-1} to 3.89 MT ha^{-1}, which is reasonably < 5%. The results of dry biomass ha^{-1} (46.43 ± 1.26 MT–111.82 ± 3.88 MT) are broadly comparable with Boschini (2002) who obtained about 40 MT ha^{-1} dry biomass of mulberry in Costa Rica, where mulberry was planted 60 cm apart and harvested at 30 cm aboveground level at an interval of 120 days. This comparison is in broader terms because the present varieties, plantation management and ecological conditions are different from those in the study by Boschini (2002).

The variety-specific and multivariety AGB models indicate that the equivalent diameter at breast height is a reliable biometric variable for deriving efficient and accurate allometric equations. The coefficient of determination, *F*-values, standard error of estimates and root mean square error; however, show that the variety-specific models based on a single biometric variable, EDBH are reasonably more accurate and effective compared to double variables EDBH-*H*. Two multivariety models, $b$ln(EDBH) and (EDBH)$^{0.756}$ show relatively better reasonability and accuracy with $R^2 = 0.85$–0.86, SEE = 1.6231–1.6445 and RMSE = 1.609–1.630. The tree height does not improve accuracy and efficiency of the models, perhaps due to the low precision in tree height measurement compared to the equivalent diameter at breast height. The bush type growth with a round canopy of the varieties supports this argument. Previously, stem diameter has been reported a reliable variable for deriving allometric biomass equations for 23 tree species and multi-stemmed shrubs in the savannah ecosystem in Botswana (Meyer et al. 2014), 14 shrub and small tree species in eastern China (Ali et al. 2015), three underbrush tree species in Lithuania (Škėma et al. 2018), China-fir (*Cunninghamia lanceolata*) in south-eastern China (Guangyi et al. 2017) and 14 tree species in a hill zone in Bangladesh (Mahmood et al. 2020).

Generally, half of the dry biomass is thought to be organic carbon content (Thomas and Martin 2012), while the present study showed about 55%–56% organic carbon in dry shoot biomass and 47%–48% in dry leaf biomass. Variation in carbon contents in different parts of a tree has also been recorded by Mahmood et al. (2020). The results of organic carbon content in different parts of tree warrant estimation of organic carbon contents separately for each part of tree for accurate quantification of carbon sequestration. Trees absorb atmospheric CO_2 through stomata and fix it as carbon during photosynthetic process. The sequestered carbon varied from 3.82 ± 0.10 kg tree^{-1} to 9.99 ± 0.32 kg tree^{-1}, indicating an absorption of atmospheric CO_2 up to 36.67 ± 1.16 kg tree^{-1} year^{-1}. The variation in absorption of atmospheric CO_2 level can be explained in terms of variation in morphological variables attributed to genetic potentiality, tree age, leaf area, photosynthetic efficiency, edaphic and climatic conditions, and management of the mulberry plantation (Jana et al. 2009). Mulberry varieties have also been reported to differ in their absorption of atmospheric CO_2 (Qin et al. 2012; Jiang et al. 2017); however, the methodology and details of results were not published.

Our study is the first attempt to quantify variety-specific AGB of *M. alba* and carbon sequestration. Similarly, first time variety-specific and multivariety allometric AGB models, were developed using one and two biometric variables. The biometric variable EDBH was a strong predictor of AGB.

Conclusions

M. alba var. Latifolia had higher values for leaf and shoot variables except for leaf area and single leaf mass. A diminishing return was found between leaf area and number of leaves tree^{-1}, whereas a positive correlation was found (1) between leaf area and single leaf mass and (2) between shoot diameter and shoot length. The variety-specific and multivariety AGB models based on EDBH showed goodfitness and reasonable accuracy. The Latifolia variety mitigated 36.67 ± 1.16 kg tree^{-1} CO_2. Based on these findings, it is concluded that Latifolia has good potential of biomass production, secondly EDBH is a strong predictor of AGB and can be used for variety-specific and multivariety AGB models.

References

Aarssen LW (2012) Reducing size to increase number: a hypothesis for compound leaves. Ideas Ecol Evol 5:1–5

Ablo PIH, Mathieu J, Claude N, Laurent SA, Quentin P (2015) Improving the robustness of biomass functions: from empirical to functional approaches. Ann For Sci 72:795–810

Ali A, Sanaei A, Li M, Nalivan OA, Ali KA, Pour MJ, Valipour A, Karami J, Aminpour M, Kaboli H, Askari Y (2020) Impacts of climatic and edaphic factors on the diversity, structure and biomass of species-poor and structurally-complex forests. Sci Total Environ 706:1–12

Ali A, Xu MS, Zhao YT, Zhang QQ, Zhou LL, Yang XD, Yan ER (2015) Allometric biomass equations for shrub and small tree species in subtropical China. Silva Fenn 49:1–10

Allen SE, Grimshaw HM, Rowland AP (1986) Chemical Analysis. In: Moore PD, Chapman SB (eds) Methods in Tree Ecology, 2nd edn. Blackwell Scientific Publications London, UK, pp 285–304

Bajwa GA, Khan MA (2015) Management of macro-and micro nutrients in soil and mulberry foliage in Peshawar, Pakistan. Sarhad J Agri 31:151–158

Bajwa GA, Shahzad MK, Satti HK (2015) Climate change and its impacts on growth of Blue Pine (*Pinus wallichiana*) in Murree Forest Division, Pakistan. Sci Tech Dev 34:27–34

Basuki TM, van Laake PE, Skidmore AK, Hussin YA (2009) Allometric equations for estimating the above-ground biomass in tropical lowland *Dipterocarp* forests. Forest Ecol Manag 257:1684

Biilgen S, Keles S, Kaygusuz K (2007) The role of biomass in greenhouse gas mitigation. Energy Source Part A 29:1243–1252

Boschini FC (2002) Establishment and management of mulberry for intensive forage production. Pp: 115–122 In: Sânchez MD (ed.) Proceed. Mulberry for Animal Production, May–August 2000, FAO Electronic Conference

Bukhari SSB, Bajwa GA (2008) Temporal temperature rise and its effects on other climatic factors. Pak J For 58:1–18

Cienciala E, Centeio A, Blazek P, Soares M, Russ R (2013) Estimation of stem and tree level biomass models for *Prosopis juliflora/ pallida* applicable to multi-stemmed tree species. Trees 27:1060–1070

Dimobe K, Tondoh JE, Weber JC, Bayala J, Oue´draogo K, Greenough K, (2018) Farmers' preferred tree species and their potential carbon stocks in southern Burkina Faso: implications for bio-carbon initiatives. PLoS ONE. https://doi.org/10.1371/journal.pone.0199488

Dombroski JLD, Pinto JRS (2019) Crown area as a parameter for biomass estimation of *Croton sonderianus* Müll. Arg FLORAM 26(4):e20150247

Dombroskie SL, Aarssen LW (2012) The leaf size/number trade-off within species and within trees for woody angiosperms. Plant Eco Evol 145:38–45

Galidaki G, Zianis D, Gitas I, Radoglou K, Karathanassi V, Tsakiri-Strati M, Woodhouse I, Mallinis G (2017) Vegetation biomass estimation with remote sensing: focus on forest and other wooded land over the Mediterranean ecosystem. Int J Remote Sens 38:1940–1966

Goetz SJ, Hansen M, Houghton RA, Walker W, Laporte N, Busch J (2015) Measuring and monitoring needs, capabilities and potential for addressing reduced emissions from deforestation and forest degradation under REDD+. Environ Res Letts. https://doi.org/10.1088/1748-9326/10/12/123001

Gozlekci S, Uzun HI, Yegin A (2015) Some physico-chemical characteristics of mulberry (*Morus alba, Morus laevigata, Morus nigra*) species grown in western Turkey. Acta Hortic 1106:191–198

Guangyi M, Yujun S, Saeed S (2017) Models for predicting the biomass of *Cunninghamia lanceolata* trees and stands in Southeastern China. PLoS ONE 12(1):e0169747. https://doi.org/10.1371/journal.pone.0169747

IPCC (2014) Climate Change: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC Geneva

Jana BK, Biswas S, Majumder M, Roy PK, Mazumdar A (2009) Carbon sequestration rate and above-ground biomass carbon potential of four young species. J Ecol Nat Environ 1:15–24

Jiang YB, Huang RZ, Yan XP, Jia CH, Jiang SM, Long TZ (2017) Mulberry for environmental protection. Pak J Bot 49:781–788

Li Y, Wang Y, He Q, Yang YL (2020) Calculation and evaluation of carbon footprint in mulberry production: A case of Haining in China. Int J Environ Res Public Health 17: 1339; doi: https://doi.org/10.3390/ijerph1704133917

Mahmood H, Siddique MRH, Islam SMZ, Abdullah SMR, Matieu H, Iqbal MZ, Akhter M (2020) Applicability of semi-destructive method to derive allometric model for estimating aboveground biomass and carbon stock in the Hill zone of Bangladesh. J For Res 31:1235–1245

McPherson EG (1998) Atmospheric carbon dioxide reduction by Sacramento's urban forest. J Arboric 24:215–223

Meyer T, D'Odorico P, Okin GS, Shugart HH, Caylor KK, O'Donnell FC, Bhattachan A, Dintwe K (2014) An analysis of structure: biomass structure relationships for characteristic species of the western Kalahari. Afr J Ecol 52(1):20–29

Nowak DJ, Stevens JC, Sisinni SM, Luley CJ (2002) Effects of urban tree management and species selection on atmospheric carbon dioxide. J Arbori 28:113–122

Peñuelas J, Sardans J, Estiarte M, Ogaya R, Carnicer J, Coll M, Barbeta A, Rivas-Ubach A, Llusià J, Garbulsky M, Filella I, Jump AS (2013) Evidence of current impact of climate change on life: A walk from genes to the biosphere. Glob Change Biol 19:2303–2338

Qin J, He NJ, Wang Y, Xiang ZH (2012) Ecological issues of mulberry and sustainable development. J Resour Ecol 3:330–339

Ritson P, Sochacki S (2003) Measurement and prediction of biomass and carbon content of *Pinus pinaster* trees in farm forestry plantations, South-Western Australia. For Eco Manag 175:103–117

Škėma M, Mikšys V, Aleinikovas M, Šilinskas B, Varnagirytė K (2018) Biomass structure and morphometric parameters for non-destructive biomass estimation of common forest underbrush species in Lithuania. Pol J Environ Stud 27:325–333

Sun J, Wang MT, Lyu M, Niklas KJ, Zhong QL, Li M, Cheng DL (2019) Stem and leaf growth rates define the leaf size vs. number trade-off. AoB Plants 11: plz063; doi: https://doi.org/10.1093/aobpla/plz063

Thomas CS, Martin AR (2012) Carbon content of tree tissues: a synthesis. Forests 3:332–352

Weraduwage SM, Chen J, Anozie FC, Morales A, Weise SE, Sharkey TD (2015) The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Fron Pla Sci 6:167–187

Zaki NAM, Latif ZA, Suratman MN (2018) Modelling above-ground live trees biomass and carbon stock estimation of tropical lowland dipterocarp forest: integration of field-based and remotely sensed estimates. Int J Remote Sens 39:2312–2340

Zeng WS (2015) Integrated individual tree biomass simultaneous equations for two larch species in Northeastern and Northern China. Scand J For Res 30:594–604