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Carbon content in boles of *Tsuga heterophylla* (Raf.) Sarg.

Maria Carolina O. Silva[1], Sabah H. Lamlom[3*] and Rodney A. Savidge[2]
1, Western Forest Products Inc, Planning Department, Holberg, BC, VON 1Z0, Canada
2, University of New Brunswick, Faculty of Forestry and Environmental Management, P.O. Box 4400, Fredericton, NB, E3B 5A3 , Canada
3, University of Omar Al-Mukthar, Faculty of Arts and Science, Tobruk, P.O Box 194, Libya

Abstract

This study investigated the carbon (C) content in boles of twenty western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) trees. Bole samples were obtained from southern Vancouver Island in British Columbia (Conuma River, south of Muchalat Lake, Apple River, and Harris Creek) from a Western Forest Products Inc. management area, during the summer of 2003. Disks (10 centimeters thick) were obtained from the top of live crown (TLC), the base of live crown (BLC), and at breast height (BH) of each tree. By elemental analysis, C content of 20 genotypes had a mean of ~53.5% (w/w), standard error of the mean [SEM] 0.1). Since the variation in each tree was so small, and the SEM for each of the positions within the bole was 0.1 and 0.2, we conclude that there was only slight variation among genotypes at the 99% level of confidence in the three positions within the bole. Considerable additional research is needed in order to have accurate estimates of total C content in any tree, and given that there is variation in C content within tree species, and in order to account for C in any forest stand, total C content should be estimated by integrating each individual tree component.

Key-Words: *Tsuga heterophylla*, Carbon content, Trees, Wood.

Introduction

A common but erroneous practice has been to assume that wood produced by trees of the same species will be identical in characteristics. In fact, wood characteristics within the same tree are never identical. Certain differences, such as weight of wood or diameter growth rate, may be obvious, but they are frequently not appreciated as indicative of the variability that may exist within and between trees of the same species (Panshin and de Zeeuw 1980).

Zobel and Sprague (1998) suggested that within any particular hardwood or softwood species, "juvenile" wood produced in the top of the tree would have different characteristics than more "mature" wood produced below the live crown. They also suggested that differences in wood properties can be detected even when growing the same species in diverse geographical locations. Anatomical and chemical differences in wood formation must be considered in relation to the biological principle that the somatic genotype of the cambium is constant throughout the tree (Savidge 1996, 2000).

*** Corresponding Author**

Within the classic concept of P = G x E, where P is phenotype, E environment, and G genotype, the anatomical and chemical differences occurring in wood arise because E experienced by the cambium and its derivative cells is variable. This results in variable phenotypic expressions at the cellular level (Savidge 2003). If we recognize that a single tree has the same genotype throughout its vegetative tissues, and that wood formed within a tree tends to be heterogeneous, chemically complex, (Panshin and de Zeeuw 1980, Kaar and Brink 1991, Sjöström 1993), and unique (Savidge 2000), we can then hypothesize that C content present in wood of trees is likely to vary in response to changing environmental stimuli (Savidge 2000, 2003).

Wood is a complex structure formed mainly of lignin and polysaccharides (cellulose and hemi-celluloses). Also present in minor amounts are other carbon-containing substances known collectively as extractives (Kirk and Othmer 1998). Variability in C content of a heterogeneous material, including not only lignin and hemicelluloses but also extractives as well as other compounds of wood, can be significant because each component has a unique C percentage and density (Savidge 2000, 2001, 2003). Softwood's chemical composition (based on dry wood) consists of approximately 40%– 50% of cellulose, 15%–20%

hemi-cellulose, 26%–32% of lignin, 0.2%–1% of inorganic matter and usually 1%–3% extractives (Schroeder and Kozlik 1972; Sjöström and Sjastram 1999). Even though wood properties have been intensively studied, only a few studies have acknowledged the possibility of variation in total C content of wood, as it exists in the forest and changes in response to G or E (Cown et al. 1991; Zobel and Jett 1995; Savidge 2000, 2001; Clarke et al. 2004; Lamlom and Savidge, 2007).

Today C content in dry wood is generally assumed to be 50%; however, few publications on actual C measurements have been reported, often with no reference to methods used (Matthews 1993). Where research on C content has been published, the focus has been on determinations using oven-dried wood (Mingle and Boubel 1968; Reichle et al. 1973; Chow and Rolfe 1989; Yu-Jen et al. 2002; Elias and Potvin 2003; Lamlom and Savidge 2003). Lamlom and Savidge (2003) demonstrated that C content measured in oven-dried wood is significantly lower than in wood powder dried at ambient temperature over a desiccant. Volatile organic compounds within and emitted by trees constitute a substantial component of forest C (Savidge 2001), and data on C content in oven- or kiln-dried woods are therefore inaccurate estimators of true C content of forests.

Parallel to the C content study of softwoods by Lamlom and Savidge (2003) is the C content study of important broadleaved species in Taiwan by Yu-Jen et al. (2002). Yu-Jen et al. found that C content was less than the estimated value of 50%. They also suggested that there is a variation in C content within tree species and within a tree. Other authors concluded that C content should be estimated by considering the proportions of each individual tree component, such as stem, branches, leaves, roots, etc (Birdsey 1992; Lowe et al. 2000; Karjalainen and Makipaa 2000; Gifford 2000).

Lamlom and Savidge (2003) found that C content in wood from North American species varies 46%–55% by dry weight, depending on the species. Lamlom and Savidge (2006) found 1%–2% variation in wood C within the bole depending on the year the wood was produced and the position of the sample within the tree trunk.

To know if any changes in C content occurred due to changing environment and/or genotype, it is necessary to have accurate baseline data (Lamlom 2005). Lamlom and Savidge (2003) developed a standard method for accurate and precise determination of C in wood and began to establish a baseline, noting that it is necessary to investigate each tissue of each species in order to accurately account for total C stored in forest biomass. Investigations in this area will improve accuracy and precision of C estimation in forests (Vogt 1991; Caspersen et al. 2000; Schimel et al. 2000; Elias and Potvin 2003), as well as enhancing overall forest management for C sequestration.

Lamlom and Savidge's (2003) methods were used in our investigation to estimate actual C content of western hemlock. Twenty trees were sampled in order to determine variation in C content in three positions of the bole of western hemlock.

Material and Methods

Sample preparation for boles of western hemlock

In 2003, Western Forest Products Inc. supplied disks from 20 *T. heterophylla* (Raf.) Sarg. trees collected from sites near Harris Creek (48° 38' 57.39" N, 124° 12' 49.96" W), approximately 70 km northwest of Victoria on southern Vancouver Island, in British Columbia, Canada. Disks were 10 centimeters in axial thickness and were obtained from the top of live crown (TLC), the base of live crown (BLC), and at breast height (BH) of the tree (Fig.1).

Disks were labelled and air-dried for approximately 8 months before being sent to the laboratory at the University of New Brunswick (UNB). A band saw was used to obtain 2 consecutive complete slices of wood (1–2 mm thick) from each disk. The thin slices were debarked, and 1 of the 2 slices was reduced to fine particles. To produce fine particles, the slice was first broken into small pieces by hand, then further refined using a coffee grinder and a Wiley mill to obtain fine wood powder.

Finally, to homogenize the resulting particles, the wood powder was placed in liquid nitrogen and ground with a pestle and mortar. In this way, the wood powder produced from an entire cross section of the stem was thoroughly mixed, in effect averaging the biological and chemical variability existing within the area of the cross section.

The fine powder was placed in a glass vial, capped with aluminum foil, weighed using an analytical balance, and dried in a vacuum desiccator over indicator silica gel at ambient-temperature after applying vacuum for 10 minutes and then closing the stopcock. Samples were removed from the desiccator the following day, reweighed, and then returned to the desiccators and re-evacuated. This procedure was repeated for a minimum of 10 days until sample weight stabilized (in order to reach equilibrium dryness). Drying wood powder in this manner ensures water is removed below the fibre saturation point down to the level of the matric potential of the wood particles with minimal loss of volatile organic compounds which are

driven off by heating wood (Lamlom and Savidge 2003). The wood powder from each thin cross-sectional slice was distributed to three containers, from which the mean C content was obtained, and each container was analyzed as three sub-samples, resulting in three separate analyses, each with its standard deviation (SD), from which the standard error of the mean (SEM) was calculated.

Before elemental analysis could take place, tin containers were pre-washed in a beaker with double-distilled (all glass distillation) water, followed by two washes with analytical grade acetone following the methods of Lamlom and Savidge (2003). During this process, it was important to ensure the tin capsules were open. The tin capsules were drained of excess acetone, and placed in the vacuum desiccators under continual vacuum overnight to remove all residual liquid.

Approximately 1 mg of each sample of dry wood powder was weighed into a dry, clean, tin container (5×3.5 mm, CE Elantech, Inc.) using a Cahn C-30 microbalance (precision 0.001 mg). Wood powder weights ranged between 0.500 mg and 1.500 mg. The tin containers were then closed. The wood powder was analyzed for C, H, and N content using a Carlo Erba CHN 1500 elemental analyzer. Baseline gas chromatographic resolution of CO_2, N_2, and H_2O were carried out under the same conditions as Lamlom and Savidge (2003): column length, 3 meters; column diameter, 6 x 4 mm (OD/ID); packing material, Porapak QS, 50-80 mesh; UHP helium flow rate, 85 ml/min; helium reference flow rate, 40 ml/min; gas chromatograph oven temperature 90°C, filament temperature (thermo conductivity detector) 190°C. In the oxidation furnace, the combustion products passed through a 12 cm layer of chromium trioxide (CrO_3) followed by a 6 cm layer of silver coated cobalt oxide separated by a few mm of quartz (silica) wool, all packed within a vertical clear quartz tube (45 cm long, 14 mm i.d., 18 mm o.d., ThermoQuest). In the reduction furnace the mixture of combustion products (CO_2, N_2, NOx and water) passed through a second quartz tube fully packed with metallic copper to scrub oxygen and reduce any nitrous oxides to nitrogen (N_2).

The analyzer was calibrated according to Lamlom and Savidge's methods (Lamlom and Savidge, 2003).

Estimating instantaneous error associated with the Carbon estimates

Statistical methods for calculating standard deviations and standard errors of the mean attempt to provide meaningful indications of error associated with an estimate based on the variation found from a number of measurements. However, in contrast to statistical error estimation, when the identical object is repeatedly measured, there is always an error associated with the final estimate, even when each of the replicate measurements yields the identical estimate of the true value (SD=0, SEM=0). This real uncertainty is referred to as the instantaneous error, otherwise known as the precision of the measurement. The instantaneous error of an estimate is obtained through estimation of the precision of each methodological step in the analysis (Eurachem 2000). If the instantaneous error assessment reveals the statistically calculated error to be overly optimistic, then in support of solid science it is responsible to report the more conservative instantaneous error.

At least three sub-sample estimations were provided for each C estimate. Each estimate required several procedural steps, and each step had an instantaneous error associated with it. The instantaneous error of each C estimate was calculated as follows:

1. Sampling positions in the tree were obtained from three positions as shown in figure 1. The BH samples were taken at ~135 cm, the BLC samples were taken between 217 cm and 1992 cm, and TLC samples were taken between 1167 cm and 2978 cm.

2. The percentage error of the moisture content at the time of weighing. Because C as determined is based on the starting mass of the wood matter, variation in moisture content in the wood matter could greatly affect the end results. Keeping the samples in a vacuum desiccator over indicator silica gel for at least 10 days or until equilibrium dryness was reached eliminated this error. At time of weighing samples an analytical balance was used; the precision of the balance was known to be 0.001 mg and the linearity stated by the suppliers was ± 0.0001mg. The balance was calibrated using reference masses at the beginning of every day, and the balance was zeroed before every sample was weighed. The uncertainty associated with this step was 0.0001mg.

3. The percentage contribution of the air to the C estimate while placing the sample for each run was zero for C, H, and N. Since the system was closed and nothing had been added to it, we are assuming the system was clean. When an empty capsule was placed into the system as a blank analysis, the integrated peak area units for C, H and N were negligible; therefore, the error-was calculated as 0.

4. The integrated peak area values for CO_2, N_2, and H_2 were manually delimited on the chromatogram based on known retention times Lamlom and Savidge (2003), and the error associated with this step is 0.

5. The variation of the pulse of pure oxygen fed into the oxidation chamber following sample drop was

controlled by setting the Carlo Erba NA 1500 elemental analyzer at the time of the calibration. Every time the analysis was set up to start, the oxygen was set at the same pressure level. A calibration with a known material (high purity crystalline L-leucine, Sigma-Aldrich Chemical Co.) was performed every morning to make sure the conditions set in the starting calibration were still the same. The error associated with this step is included in the error attending the standard curve based on use of crystalline L- leucine; the errors attending all aspects of the operation, such as the percentage of variation in the temperature chamber, and the percentage in the electrical signal generated by the wheatstone bridge thermo-conductivity detector of the elemental analyzer, were embodied in the repeatability of integrated peak area output using L-leucine as calibration standard.

The statistical error attending the expression of the standard curve for L-leucine was determined, hence the error attending interpolation of integrated peak area values into the standard curve and deduction of the actual amount of C present. This error was associated with the calculation of the linear regression: $Y_C = 6.492 \times(10^6) X + 65809$ ($R^2 = 0.996$), where Yc is C peak and X is unknown C content in mg, and R^2 is the linear correlation coefficient. The error associated with the C estimate was obtained from the square root of the mean squared differences between the observed C and predicted C. The error associated with this step was estimated at ± 0.0345 mg of C.

Assuming the errors listed above are independent, then the instantaneous error rate is estimated as the sum of the errors at each step (Castrup, 2004); for example, if our estimate X is the result of a series of steps (a, b, c, etc), which has the associated error ΔX then:

$$X = a + b + c \ldots \text{etc.}$$

$$\Delta X = \sqrt{\{(\Delta a)^2 + (\Delta b)^2 + (\Delta c)^2\}\ldots}$$

$$= \sqrt{\{(0.0001 \text{ mg})^2 + (0.0345 \text{ mg})^2}$$

Associated error ΔX = 0.0345 mg

Overall the combined uncertainties in the methodology are ± 0.0345 mg. For example in the top live crown where the samples contain in average 53.5% (w/w) of C and approximately 1 mg was the weight of the dry substance investigated, the sample will contain 0.535 mg of C. Consequently the overall combined uncertainties in the methodology are 0.0345mg / 0.535mg = 0.064mg * 100 = 6.4% error.

Statistical error

For statistical analysis, the mean and standard deviation of at least three subsamples per sample were calculated. During the course of analysis, when the standard deviation was greater than 0.7 %, more sub-samples were performed. Averages of C content per tree were plotted with their respective standard deviation. The standard error of the mean (SEM) at 99% of confidence was included to show the dispersion of the sampling errors while trying to estimate a population mean from a sample mean for the three positions in the bole (Fig. 2).

Results and Discussion

The mean C content for TLC was ~53.5% (w/w) with a standard deviation of ± 0.4%. The mean C content for BLC sub-samples was ~ 53.4% (w/w) with a standard deviation of ± 0.5%. The mean C content for BH sub-samples was ~ 53.7% (w/w) with a standard deviation of ± 0.7%. For the three positions in the bole (TLC, BLC, and BH), the standard error of the mean was included to show the dispersion of the sampling errors. The SEM for TLC and BLC was 0.1 (n = 20), while the SEM for BH was 0.2 (n = 20). The C content ranged from 52.6% ± 0.1% (SD) to 54.3% ± 0.6% (SD) (w/w) in the TLC, from 52.6% ± 0.2% (SD) to 54.4% ± 0.1% (SD) (w/w) in the BLC, and from 52.7% ±0.3% (SD) to 55.5% ± 0.7% (SD) (w/w) in the BH of the bole (Fig. 2). Hydrogen contents ranged from 7.7% ± 0.1% (SD) to 8.3% ± 0.1% (SD). Nitrogen was also analyzed, but its content never exceeded trace levels. Hydrogen and nitrogen were not further investigated in this study.

Since the variation in each tree was so small, and the standard error of the mean for each of the positions within the bole was 0.1 and 0.2, on the basis of statistical errors it can be concluded that there was only slight variation among genotypes at the 99% level of confidence in the three positions within the bole (Fig. 3). On the other hand, an instantaneous error of 6.4% (see above) indicates that the breadth of variation could have been significant. The variation found in each position of the tree suggested that C content in each of those positions of each tree was not constant.

The mean and the SEM were included in figures 2 and 3 to show the variation of the measurements in regards to the actual C content and the dispersion of sampling errors of the population. The error bars attached to each result represent the dispersion of each measurement around the mean.

Eurachem (2000) defines uncertainty of a measurement as the range of values that describe the dispersion of the value that could be attributed to the measurement, and such knowledge provides increased confidence in the validity of a result. The analytical methods generate information on factors that can influence independently or on overall performances, and can be applied to the estimation of uncertainty associated with the results of the method. To estimate the overall uncertainty, it was

necessary to identify and analyze each source of uncertainty separately as shown above.

This study found that the mean C content of western hemlock bole was ~ 53.5% (w/w). The tendency to date has been to focus on C content in wood using procedures based on oven-dried wood, ignoring any volatile matters present in wood. In addition, it has been usual for wood to be mixed with bark rather than investigating each tissue separately. Therefore, past data on C content may be imprecise in relation to the actual C contents estimated for forests.

Based on the assumption of 50% C content of wood, Houghton et al. (1985) estimated that uncertainty attending the actual C content of forests could be as much as 10%. Nevertheless, 50% C content has been the most broadly promulgated value in forest modeling (Wenzel 1970, Atjay et al. 1979, Karchesy and Koch 1979, Sedjo 1989, Dewar and Cannell 1992, Hollinger et al. 1993, Matthews 1993, Thuille et al. 2000). One of the biggest limitations of determining C content in forest, is that volume tables used in forest inventory are in general only valid for inside-bark volume of merchantable-sized logs. There are no data for the non-merchantable components (Savidge 2001). Chard (2005) attempted to investigate non-merchantable biomass of western hemlock by comparing the amount of above ground tree volume by combining with known growth rates and merchantable volumes. The derived yield curves could be used to calculate the C content of western hemlock trees, and compared to forest inventories to estimate the amount of C in forested stands. For instance, biomass for mature western hemlock wood commonly is based on the assumption of a specific gravity of 0.440 g/cm^3. Estimates of biomass volume could then be multiplied by the C content 53.5% (w/w) and the specific gravity of western hemlock to determine the total C content of a stand (Lamlom and Savidge 2006).

Considerable additional research is needed in order to have accurate estimates of total C content in any tree, and given that there is variation in C content within tree species, C content should be estimated depending on each individual tree component. This will be the path to follow in order to account accurately for C in any forest stand.

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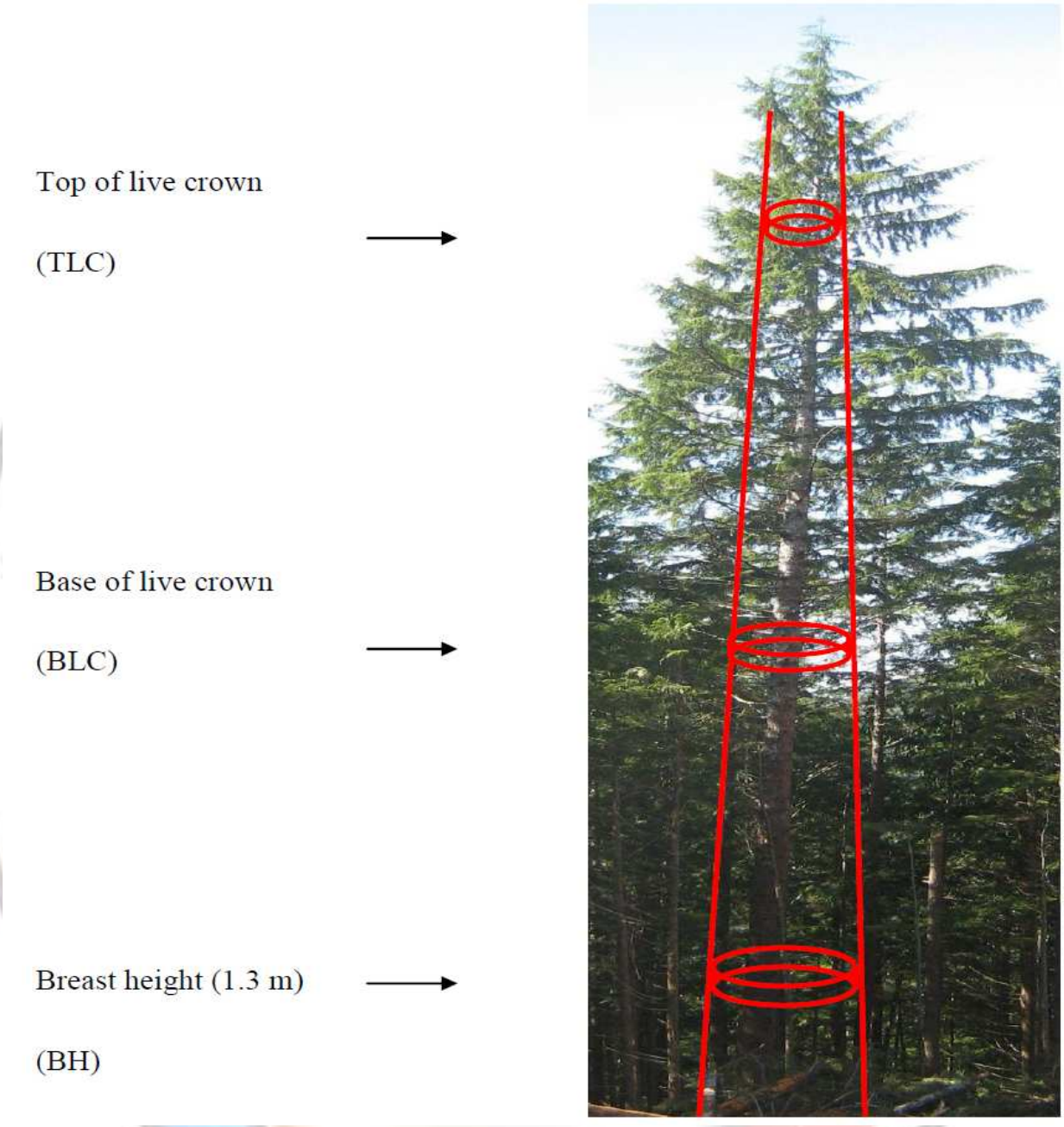

Fig. 1: Tree diagram shows sample locations at three positions along tree trunks. Three disks were obtained from each tree trunk: 1 disk from near the top of live crown (TLC), 1 disk from base of live crown (BLC), and 1 disk from breast height (BH).

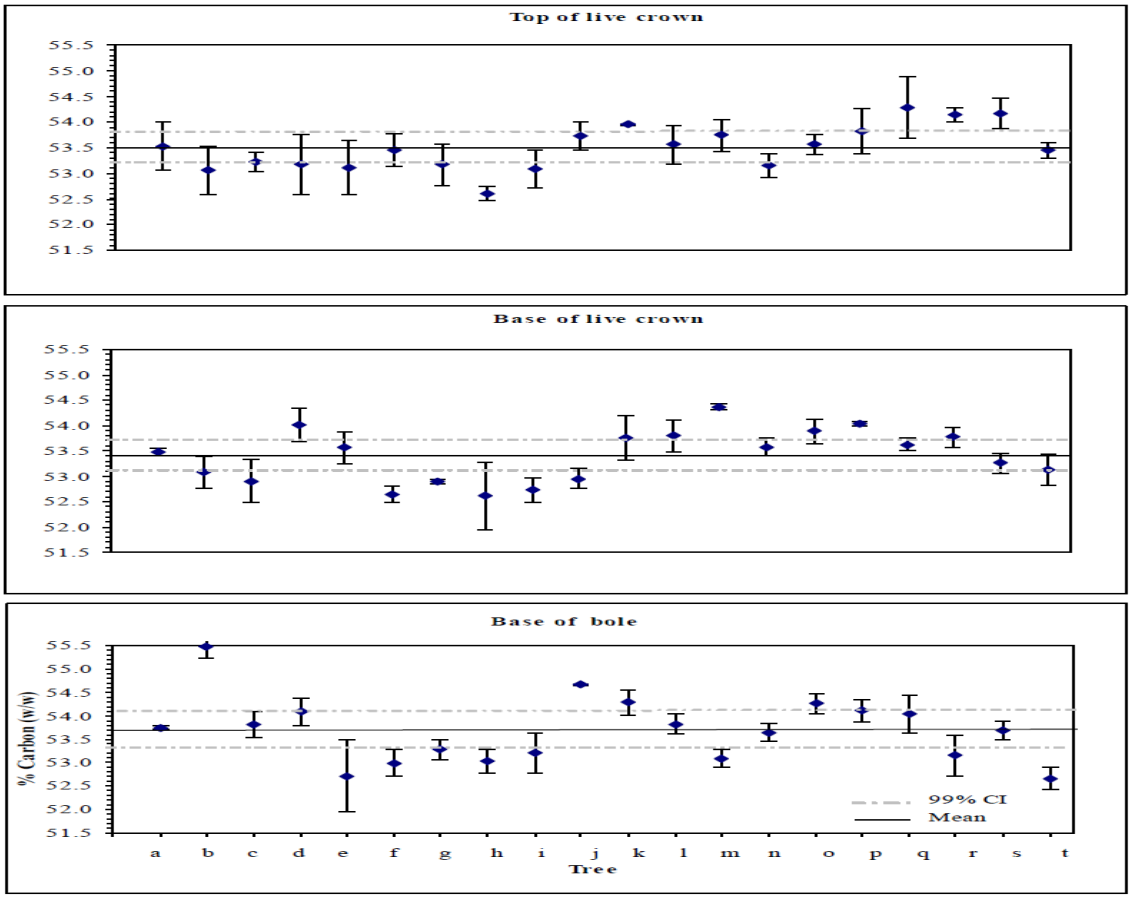

Fig. 2: Mean C content of TLC, BLC, and BH positions and standard deviations (error bars) based on at least three replicate analyses. The horizontal solid line represents the overall mean. To show the dispersion of the sampling errors, the 99% confidence interval from the standard error of the mean is shown as a dotted line.

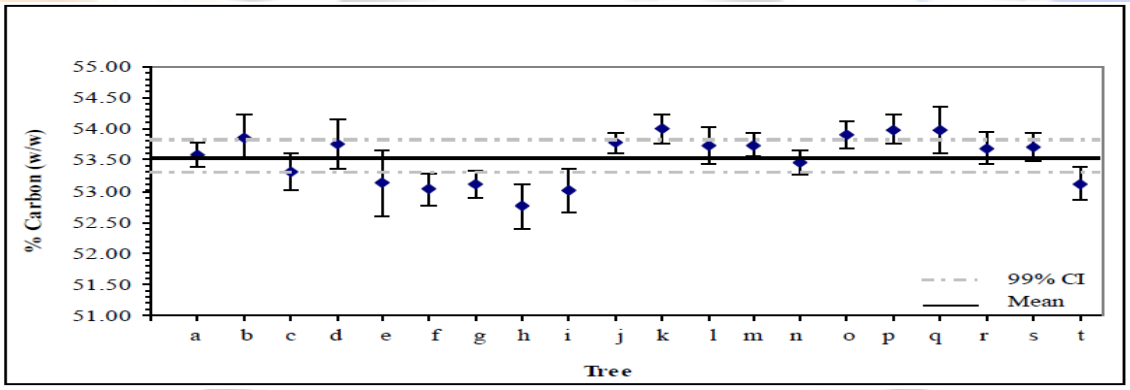

Fig. 3: Mean C content per tree based on C contents at TLC, BLC and BH positions, with standard deviations (error bars, at least 9 analyses). The horizontal solid line represents the overall mean. To show the dispersion of the sampling errors, the 99% confidence interval from the standard error of the mean is shown as a dotted line.