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A Quantitative Index Based on Leaf Heteroblasty for Predicting Root Biomass in a Frequently Burned Savanna Species: *Cussonia arborea* Hochst. Ex A. Rich. (Araliaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YAGK, MM, TCD and JG designed the study. Author YAGK conducted the field experimentations in Lamto Scientific Reserve and collected the data. Authors YAGK, MM, ABN, AB and TCD wrote the manuscript. Authors YAGK and JG produced the figures. All authors contributed to the refinement of the manuscript and approved the final version. All authors read and approved the final manuscript.

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ABSTRACT

Background: Biotic and abiotic disturbances such as frequent wildfires and herbivory contribute to maintain trees and grasses coexistence in savanna ecosystems. In comparison to stems and leaves, exposed to fire and herbivory, the roots, protected by being belowground, are less affected

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by these disturbances. Therefore, indirect estimation of belowground biomass (BGB) of savanna trees from simple allometric relations based on stem measurements can lead to major biases.

Aims: In this study we explored how the *Leaf ontogenetic change index* (LOCI), a quantitative index based on leaf heteroblastic development, can provide an accurate estimate of BGB in *Cussonia arborea*, a widespread species in West African humid savannas.

Methodology: We examined leaf morphometrics on post-fire resprouts of 40 individuals to assess whether LOCI can inform on plant age. We then analyzed by log-level regressions the variation of LOCI in relation to plant stem volume. Subsequently, we studied the variation of BGB according to stem volume, and as a function of both stem volume and LOCI, which allowed us to evaluate the contribution of LOCI to BGB estimation. BGB was obtained destructively by digging up roots and weighing total dry mass of 25 individuals including small and large trees. Statistical analyses were done with the R software.

Place and Duration of Study: Study was performed in the Lamto Scientific Reserve, Côte d'Ivoire, between May 2020 and June 2021.

Results: Using the stem volume as single explanatory variable of BGB, the regression model provided an adjusted R^2 of 0.71. Association of the stem volume with LOCI increased the adjusted R^2 from 0.71 to 0.90.

Conclusion: Combining LOCI with a measure of stem size provides better estimate of BGB in *C. arborea* compared to estimate based on stem size only. Since a large proportion of woody species in frequently disturbed environments exhibit an overall strategies promoting persistence, future works should evaluate how these strategies are modulated during ontogeny and can explain biomass variation over time.

Keywords: Non-destructive prediction; belowground biomass; Cussonia arborea; disturbance; heteroblasty; ontogenetic traits.

1. INTRODUCTION

Living trees are globally recognized as an important carbon reservoir at the biosphere level, playing a major role in atmospheric carbon dioxide mitigation [1,2]. Indeed, trees capture substantial atmospheric CO_2 during their growth, which they break down during photosynthesis process and store C as aboveground biomass (AGB) in stems, branches, twigs, leaves, reproductive organs, and belowground biomass (BGB) in roots and belowground storage organs [3]. However, deforestation due to human action, as wood harvesting and wildfires, induce losses in AGB and BGB of trees, which causes CO_2 emission in the atmosphere [4,5]. Thus, to better assess the contribution of terrestrial ecosystems to the global carbon budget, both carbon sinks and sources associated to AGB and BGB of trees need to be reliably estimated [6-8]. In comparison with AGB, accurate data on BGB of trees are still lacking [5,9-11]. On the one hand, direct measurements, which are more accurate but involve digging up root systems, are technically difficult to carry out at large spatial scales, and are often disapproved because of the ecological degradation they represent [10]. On the other hand, the use of non-site-specific allometric relationships and root-shoot biomass ratios can lead to significant under- or overestimation of BGB [12]. Investigation for BGB estimation methods, both non-destructive, easy to implement and accurate remains therefore a scientific challenge. This is particularly important for tropical savannas since these ecosystems are disturbance-prone ecosystems, and are widespread in the world, covering nearly one fifth of the terrestrial landscape [13]. In addition, global data indicate that the root-shoot biomass ratios of savannas are among the highest [10,14].

In tropical savannas, wildfire strongly influences the growth dynamic and biomass distribution in above and belowground compartments of woody plants [15-17]. Frequent fires affect the AGB by removing the major part of the stem compartment, especially of the small individuals (young individuals, small resprouts, small-size shrubs) which entirely grow within the flame zone. Meanwhile, the root and storage organ compartment, which is not directly damaged by fire, continues to develop. Thus, for most savanna woody plants, BGB does not necessarily match AGB [18], making hypothetic the use of simple allometric relationships implying only the stem size or a standard value of root-shoot biomass ratio for predicting BGB. Yet, in Africa, where the natural environment is mostly consists in natural savannas [19], allometric equations are systematically used to estimate both AGB and BGB of savanna trees [20-28]. This contributes certainly to the uncertainties in the carbon balance in Africa, which still remain high [29]. In the savanna context, we urgently need to refine non-destructive estimates of BGB using life history traits that change during plant *ontogeny.*

The ontogeny of a plant species describes the progressive development of its individuals in a given environment. Many measurable traits may inform about the ontogenetic stage of the plant. Indicators of ontogenetic stages are primarily morphological changes in the global plant structure, resulting from anatomical, physiological, and biochemical transformations [30]. One of the best documented ontogenetic changes in plants is leaf heteroblasty. Leaf heteroblasty refers to a pattern of plant development in which substantial differences in leaf shape and size are observed between earlier and later ontogenetic stages [31]. Various studies suggested that variation in leaf morphology during plant ontogeny results from combined action of environmental factors (notably light) and the ontogenetic age of plants [32-35]. An architectural analysis of the ontogenetic development of *Dipterocarpus alatus* Roxb. ex G.Don (Dipterocarpaceae) in a tropical rain forest performed by Dang Le et al. [33] found that variation in leaf traits were more strongly correlated with ontogenetic development than with the intensity of a key environmental factor such as light. This conclusion supports that of Allsopp [34] who signified that although leaf morphological changes are modified by environmental stimuli, they are primarily driven by endogenous ontogenetic factors [31]. Therefore, heteroblasty of plant leaves is a good candidate marker of ontogenetic age.

To our best knowledge very few studies such as Climent et al. [36] have explicitly investigated the variation of plant biomass in relation to heteroblastic development of leaves. Given that during their ontogeny trees increase in size (height, diameter, volume) and wood density in both above- and belowground compartments, it is likely relevant that quantitative traits derived from leaves morphological changes can be correlated with variation of AGB and BGB. Furthermore, studies of species having an heteroblastic development have generally been conducted in undisturbed environments, where the stem and root systems of plants remain permanently present in the study environment over time. But, for fire-prone plants such as savanna species, the question is whether the morphological differentiation of leaves continues during the regrowth of stems after fire or whether leaf heteroblastic development is continually reset together with stem growth in non-fire-resistant individuals. If verified, leaf shape could be used to estimate the age (and dimension) of a root stock in disturbed environment and offers a great tool for analyzing plant demographics in these ecosystems.

Cussonia arborea Hochst. ex A.Rich. is a pantropical woody species belonging to the *Araliaceae* Juss. family. The Araliaceae family is one of the plant families where leaf heteroblasty has already been highlighted [37]. *C. arborea* generally grows in savanna areas and this species is abundant in Guinean savannas where it is known that fire frequency and fire intensity are high [16,38,39]. The leaves of *C. arborea* are palmate, 5-10 leaflets lanceolate to oblanceolate with variable size [40] and symmetrically arranged with respect to a central leaflet located in the same plane as the petiole. *In C. arborea*, the leaf heteroblasty corresponds to an increase of blades notches over time, resulting in a gradual transition from a palmately-lobed form with leaflets gently cleft in young stages to a palmately compound form with sessile leaflets in the adult stage. Thus, during the ontogeny of *C. arborea*, the notches of blades become deeper in oldest growth stages until they become completely free in the ultimate stage (Fig. 1).

One of the objectives of our work was to verify in *C. arborea* whether a quantitative index based on the heteroblastic development of leaf, that we named LOCI (Leaf ontogenetic change index), informs on the age of the rootstock even when resprouting after fire. We defined LOCI as the ratio of i) the length of axis formed by the margin of the central leaflet and the margin of one of the leaflets immediately attached to ii) the length of the primary vein of the central leaflet (Fig. 1). The second objective was to examine whether LOCI, alone or in interaction with stem size, provides a good prediction of BGB.

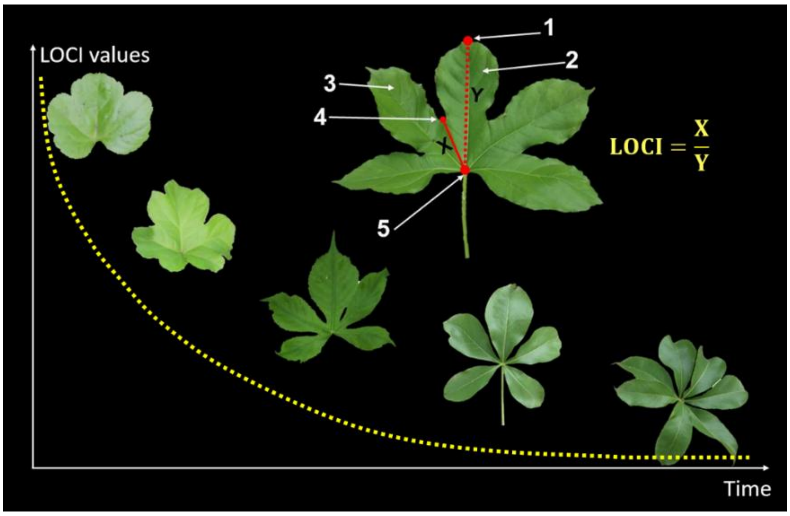

Fig. 1. Heteroblastic development of *Cussonia arborea leaves* over time The LOCI (Leaf ontogenetic change index) is a quantitative index derived from leaf heteroblastic development and varying during the species ontogeny. The smaller the LOCI, the older the individual.
1. Apex of central leaflet; 2. Central leaflet; 3. Leaflet attached to the central leaflet; 4. Corner point between the margin of the central leaflet and the margin of a leaflet attached; 5. Intersection point of the primary veins of all the leaflets.

2. MATERIALS AND METHODS

2.1. Study Site

The study was carried out into the savanna of Lamto Scientific Reserve in the middle Côte d'Ivoire (LSR, 2610 ha, at 6°13′-6°25′N and 5°015′-4°97′W, Fig. 2). The average annual temperature and precipitation estimated over a 20-year period (1998 to 2017) for this region were 28.5°C and 1218 mm, respectively.

Grey levels show *Cussonia arborea* distribution areas. Black points indicate herbarium sampling locations, and black triangles indicate inventory sites. The solid red circle shows the location of Lamto Scientific Reserve in Côte d'Ivoire.

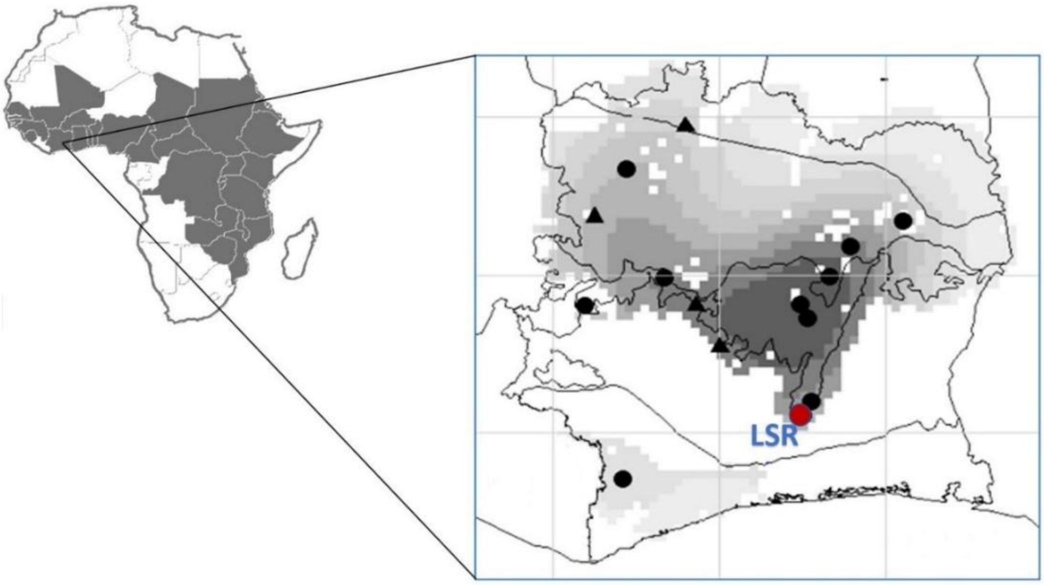

Fig. 2. Distribution areas of *Cussonia arborea* in Africa and in Côte d'Ivoire [40,41]

Vegetation of the LSR is about 80% Guinean savanna, with variable tree densities, supporting the development of a dense, diversified and tall grass layer thanks to the high-water availability [42]. The 20% remaining of LSR consist in gallery forests and forest islands. The woody layer of LSR's savanna is composed to about 90% of adult trees of four species: *Crossopteryx febrifuga* (Afzel. ex Don G.) Benth. (Rubiaceae), *Piliostigma thonningii* (Schum.) Milne-Redh. (Fabaceae), *Bridelia ferruginea* Benth. (Phyllanthaceae) and *C. arborea* [42]. Regular burning of vegetation by a prescribed yearly mid-dry season fire is used for the conservation of LSR's savanna [38,42]. This prescribed fire exerts a stabilizing effect on the LSR's vegetation by preventing massive tree invasion [42,43]. Fire severity on woody vegetation closely varies with many factors such as fire season, fuel load, fuel moisture content, wind speed, fire intensity, fire residence time, fire spread rate etc. [38,44,45]. Based on N'Dri *et al*. studies [38,44,45] run on three different sites of LSR during four fire cycles, the average spread rate and average intensity of mid-dry season fire were 0.14 ± 0.03 ms^{-1} and 3920 ± 740 kWm^{-1}, respectively. The maximum fire temperature was about 10 times higher than the commonly accepted lethal temperature for plant cells, which is 60°C [46]. The average maximum temperature of fire was 645 ± 2°C while the average of the residence time of fire above 60 °C was approximately 1.8 s [38].

2.2 Analysis of Leaves Architecture in Post-fire Resprouts

We examined, four to six months after the fire of the year 2020, the leaf architecture in basal resprouts of 40 individuals with different stump surface area. The stump area represents the sum of basal surfaces of all living stems and old dead stems consumed by fire, and was used as a proxy for the age of the root system. The stump surface area was estimated from the measure of the total circumference of the stump surface using Eq. 1.

$$SS = \frac{CS^2}{4\pi} \qquad (1)$$

where S_S and C_s represent the stump surface area and the stump surface circumference, respectively.

We randomly collected five of the recently emerged leaves of each resprout that were photographed under a white background with a graduated ruler as a scale. Each leaf photography was then incorporated into MESURIM software [47] to measure distances used to estimate the LOCI (Fig. 1). Individual's LOCI was taken here as the average of each value obtained for the five leaves collected.

2.3 Sampling and Measurements of LOCI, Biometric Parameters and Belowground Biomass

A total of 25 individuals selected in three classes of basal diameter (D_b): D_b < 10 cm, 10 cm ≤ D_b ≤ 15 cm and D_b > 15 cm, were described. We collected five vigorous leaves on one annual shoot of each individual and determined individual's LOCI following the same procedure outlined in Section 2.2.

The total height of individuals (H) was measured using interlocking graduated stakes reaching a total height of 12 m. Basal diameter (D_b, diameter at 10 cm height from ground level) was directly measured for small-sized stems (D_b ≤ 5 cm), using a caliper. For individuals which basal diameter was greater than 5 cm, we first measured the stem basal circumference also at 10 cm height from ground level using a measuring tape, and then converted the circumference to diameter.

The root system of each individual was manually excavated using a pickaxe, a daba (a kind of hoe with a reduced handle) and a machete (Appendix 1), and then was removed from the soil after felling the aerial part. Fine roots (diameter < 2 mm) were not sampled. The belowground compartment was divided into taproot and lateral roots. The entire root system of each sample was weighed to obtain the total fresh biomass, and then five subsamples of the taproot and lateral roots, 100 g each, were collected by weighing to obtain fresh mass. The total fresh biomass of root system of large trees (with root systems weighing more than 5 kg) was obtained on the field to the nearest 25 g using a mechanical scale (Appendix 2), while the total fresh biomass of small-size individuals and that of all subsamples were obtained in laboratory to the nearest 0.1 g using an electronic scale (Appendix 2). All the subsamples were oven-dried at 105 °C until a constant mass was obtained after 3 to 5 days of drying. After drying, the average water content of the sub-samples was estimated, using eq.2. The total dry biomass of taproot and lateral roots were deduced from eq.3, and then the total

BGB of each sample was estimated by summing the dry masses of taproot and lateral roots.

$$WC = \left(\frac{FB-DB}{FB}\right)100 \quad (2)$$

$$DB = \left(\frac{1-WC}{100}\right)FB \quad (3)$$

where WC (%) is the water content of one subsample. FB and DB are respectively the total fresh biomass and total dry biomass of taproot or lateral roots.

2.4 Statistical Analysis

All the length measurements (height, diameter, and circumference) were converted to meters (m) prior to data processing. Similarly, biomass measurements were converted to kilograms (kg). Statistical analyses and graphics were performed using the version 4.0.2 of the R software [48].

We assessed the relationship between LOCI and the stump surface area in post-fire resprouts using the Spearman correlation test. We used the Spearman's correlation rather than Pearson's as the relationship between the two variables are not linear.

Univariate linear regression was used to test whether LOCI can predict stem volume. The stem volume V was calculated from the formula V= D_b^2H, corresponding to a function of the stem base cross-sectional area (represented by D_b^2) and the stem length (represented by H) [49]. D_b^2H is a volume index that has been shown to be highly relevant for predicting tree stem volume of trees in tropical ecosystems [50]. Since, on the one hand, the values of stem volume are strictly positive (so not normally distributed), and on the other hand, the values of LOCI are theoretically between 0 and 1, we used a log-level model to estimate stem volume from LOCI.

We assessed BGB variation according to the stem volume using an allometric equation derived from the linearized logarithmic form of the power function. It is known that the power function or its linearized logarithmic form (eq.4 and eq.5 respectively) provide better fits for plant biomass prediction models [51].

$$B = aD^b \quad (4)$$

$$lnln(B) = a + bln(D) \quad (5)$$

where B is the response variable (plant biomass), D is the explanatory variable (generally stem diameter, a stem height or stem diameter at breast height), and a and b are the coefficients of the model. In our case B= BGB and D= D_b^2H.

The combined effects of stem volume and LOCI on BGB variation were evaluated using multiple linear regressions. We hypothesized a potential interaction effect between stem volume and LOCI in predicting BGB. We therefore tested whether the interaction and the individual effects of each variable were statistically significant. The purpose of these analyses was test whether combining stem volume with LOCI improves the BGB estimate made only from stem volume.

The validation assumptions of the two BGB regression models (the univariate model with stem volume as a predictor, and the multivariate model with stem volume and LOCI as explanatory variables) were tested by examining:

1) residuals versus fitted values, to check for homogeneity of variance;
2) the normality of residuals;
3) the independence of the variables.

For the multivariate model in particular, we checked for multicollinearity between the predictor variables (stem volume and LOCI), by estimating the variance inflation factor (VIF) of each variable. Variables are generally considered not inducing multicollinearity when VIFs are less than 10; VIFs greater than 10 meaning of severe multicollinearity that requires correction of the model [52]. In the specific case of full models including interaction term between predictor variables, the VIFs can be insignificant if the variables are not previously centered [53], often falsely indicate multicollinearity [54]. To avoid this and obtain reliable FIVs, we used, here, the results of the regression summary statistics to create mean-centered version of LOCI. The stem volume scores were not transformed again since we used their logarithmic transformation to perform the regression. To transform LOCI into its mean-centered version, the arithmetic mean of the distribution of LOCI in the dataset was first determined. Subsequently, the mean value was subtracted from each original score of LOCI to obtain a new value of LOCI that represents its centered score.

3. RESULTS

3.1 LOCI Status in Post-fire Resprouts

The Spearman's correlation test revealed a strong decreasing non-linear relationship (S= 21063, r= -0.97, $P < 0.05$) between LOCI and the stump surface area. The leaf architecture of resprouts with small stumps surface areas was close to that of seedlings with a high LOCI (Fig. 3A), and the larger the stump surface area, the more the architecture of resprouts leaves close to that of mature trees with a smaller LOCI (Fig. 3E, Appendix 3).

3.2 Influence of LOCI on Prediction of Stem Volume and Belowground Biomass

Our results showed a significant decreasing relationship (F = 71.68; $P < 0.05$) between the stem volume and LOCI (Fig. 4). This observation statistically confirms the relation between heteroblastic development of leaves and the individual growth during *C. arborea* ontogeny, as the stem grows over time with LOCI decrease. LOCI variation explained about 75% (Adjusted R-squared= 0.75) of the stem volume variation. The normal probability plot and other quantile distributions of the regression model of stem volume by LOCI are presented in Appendix 4.

The univariate regression model of BGB by stem volume revealed a positive correlation between the two variables, meaning that BGB increases with increasing stem volume (Fig. 5A). About 70% of the BGB variation was significantly (F = 60.34; $P < 0.05$) explained by the stem volume variation (Table 1). Based on the plots of the normal probability (Q-Q) and other quantile distributions (Appendix 5), the validation assumptions of the goodness of fit of the model were verified.

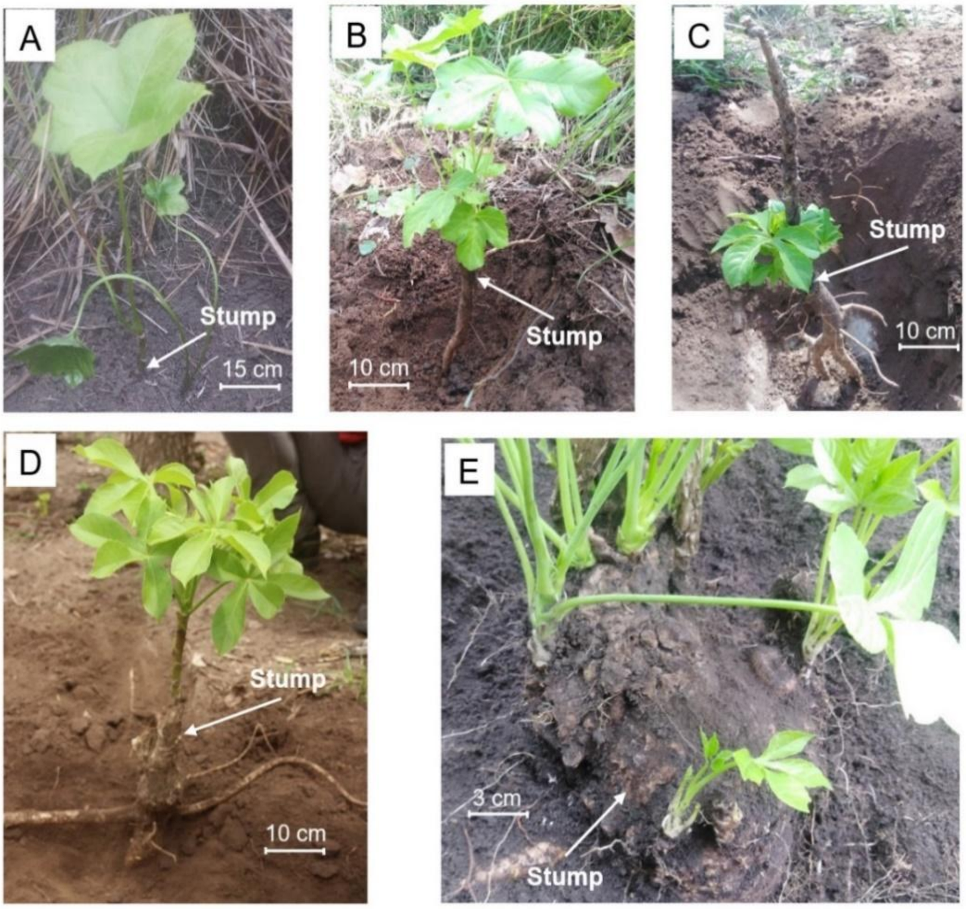

Fig. 3. Basal resprouts of *Cussonia arborea* with different LOCI according to the size of the stump

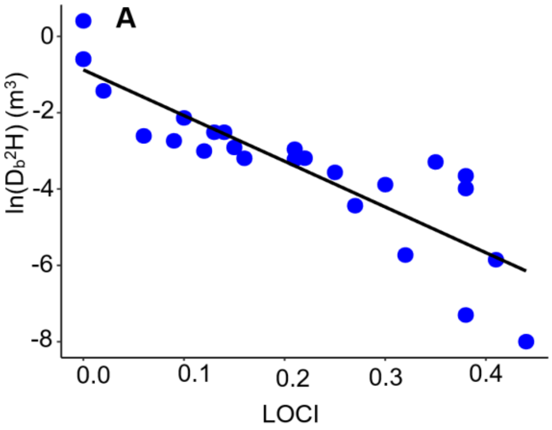

Fig. 4. Plots of the variation of LOCI in relation to stem volume expressed by $D_b{}^2H$

The association of stem volume and LOCI improved the quality of BGB regression (Table 2). Indeed, nearly 90% of the variation of BGB was significantly ($P < 0.05$) explained by both the variation of stem volume, variation of LOCI and variation of their interaction. The slope of the stem volume was positive, while the slope of LOCI and that of the interaction between stem volume and LOCI were negative. This suggests that, of two individuals having the same stem volume, the one with the smaller LOCI is potentially the one with the larger BGB; conversely, of two individuals having the same LOCI, the one with the bigger stem volume is the one with the larger BGB (Fig. 5B). Analysis of the normal probability plot and other quantile distributions described in Appendix 6 provided validation of the assumptions of BGB prediction model by both the stem volume and LOCI under the model we used. In addition, the VIFs of predictive variables, ranging from 4.11 to 4.70, did not reveal presence of multicollinearity (Table 2).

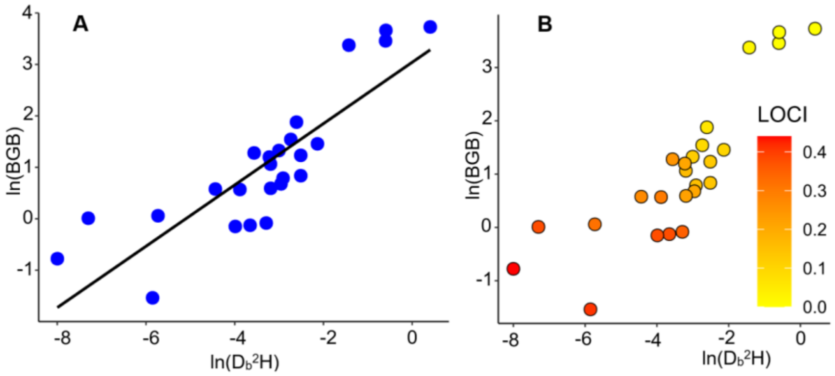

Fig. 5. Variation of belowground biomass (BGB): A) in relation to stem volume ($D_b{}^2H$); B) according to stem volume and LOCI

Table 1. Descriptive statistics and regression coefficients of prediction model of belowground biomass (BGB) by the stem volume ($D_b{}^2H$)

ln(BGB)	Coef.	Stand. Err.	t. value	*P*>| t |	[95% Conf. Interval]	
Intercept	3.04	0.29	10.32	.00***	2.43	3.65
$\ln(D_b{}^2H)$	0.6	0.08	7.71	.00***	0.44	0.76

Number of observations: 25
F stat. (1, 23): 59.4
R-squared: 0.72
Adjusted R-squared: 0.71

Table 2. Descriptive statistics and regression coefficients of prediction model of belowground biomass (BGB) by the stem volume (D_b^2H) and LOCI. Regression was performed with a mean-centered version of LOCI

ln(BGB)	Coef.	Stand. Err.	t. value	*P*>| t |	[95% Conf. Interval]		VIF
Intercept	1.70	0.33	5.17	*.00****	1.01	2.38	
$\ln(D_b^2H)$	0.26	0.10	2.62	*.02**	0.05	0.47	4.70
LOCI	-9.52	1.46	-6.5	*.00****	-12.57	-6.48	5.23
$\ln(D_b^2H)$: LOCI	-1.09	0.32	-3.45	*.00***	-1.75	-0.43	4.11

Number of observations: 25
F stat. (3, 21): 68.84
R-squared: 0.91
Adjusted R-squared: 0.89

4. DISCUSSION

Our results clearly established that the inclusion of LOCI (Leaf ontogenetic change index) in prediction models of stem volume and belowground biomass (BGB) in *C. arborea* increases the accuracy of the estimates. Combining LOCI and stem volume provided a best model for explaining BGB variation and explained better BGB variation than univariate model based only on stem volume (Tables 1 and 2).

When the aerial parts of plants are not killed and renewed over time, the existing interrelation between the ontogenetic age, the size of aerial compartment and the size of belowground compartment is conserved during ontogeny. In this case, estimating BGB based only on aerial dimensions can be relatively accurate, since both the above and belowground compartments have the same age. For undisturbed plants, the carbon resources needed for plant growth are essentially derived from current photosynthesis if leaves are not damaged [55] or storage in the stem xylem’s parenchyma tissues [56]. In resprouters, where plants aerial parts are consumed and regrow repeatedly, it is difficult to estimate exactly the individual age. For these plants, contrary to the aboveground compartment, the belowground compartment is protected from fire and keeps growing, leading to an apparent disruption in the initial relation between the individual age, the size of the aerial compartment and the size of the belowground compartment. However, our results indicate that even if the aboveground part is removed, the belowground part that was protected from fire keeps ageing in terms of physiology. The newly produced resprouts show leaf characters corresponding to an older individual.

Our results suggest that despite the fire-induced disruption on growth, the link between the size of the aboveground and belowground compartments of C. arborea plants is not totally broken, since stem volume explained BGB variation with a relatively acceptable level of accuracy (Table 1). This could be explained by the fact that regrowth of new stems during the non-fire-resistant stages always depends on carbon reserves stored in the root compartment [57]. Thus, the stem volume of recently emitted stem may be proportional to the size of the carbon reserves, which itself depends on the size of the root compartment. More robustly, taking into account the physiological age of individuals [58], as expressed by LOCI, the predicting model of BGB provides more accurate estimate (Table 2). LOCI helps to bridge the relatively important gap between individual age and the relation between the size of aboveground compartment and the size of belowground compartment. LOCI could therefore be readily used to both study the demographics of *C. arborea* in frequently disturbed areas and get a more accurate estimate of belowground biomass accumulation in savannas.

Concerning the ability of some woody species to subsist in fire-prone ecosystems through resprouting strategy, many authors consider that the post-fire regrowth of stems is mainly driven by the existence and dimension of a belowground bud bank [59-61]. These buds would have been preserved from fire thanks to their location in the basal part of stems, i.e., at ground level or underground, where fire is less severe [57,59-61]. In woody plant species, resprouting from belowground organs can involve a certain degree of preformation with buds containing the leaves of the future resprout, but most frequently new shoots are developed either from the smallest and longest-lived buds without preformation meristematic strands, or develop as adventitious buds [58,62]. Situations with buds that do not contain preformed structure but develop structure with higher physiological age (for example with higher LOCI) question how and where the information determining the physiological age is stored. We dispose of very limited information about the mechanisms involved in plant ageing especially when it is not related to the existing stem structure, here removed by fire. Resprouting can allow plants to replicate the structure or architecture of the parent stem from which a preexisting bud originated and, in some situation, confer clonality [60,61,63]. Even if not stated explicitly, most studies analyzing clonal plants consider that the subsequent ramets of a clone should have similar functioning. Our results in *C. arborea* suggest otherwise as even when the previously developed stems are removed (here by fire), the plant keeps ageing and develop different morphological characters over time, relatively independently from a size effect.

We urgently need similar information as obtained for *C. arborea* for other savanna species to refine our assessment of belowground biomass and to evaluate more accurately the contribution of savannas to the carbon budget, for example in the context of REDD+ projects [64].

5. CONCLUSION

In this study, we show that heteroblastic development of *C. arborea* leaves continues after post-fire regrowth. The recently produced shoots on large stumps showed LOCI corresponding to older individuals. LOCI can therefore be used to discriminate *C. arborea* individuals between different ages even if their stumps are not visible. It is therefore a very fast and nondestructive method that can be applied over large populations as a proxy for plant age in demographic studies and help refine (in association with stem size) a more accurate estimation of belowground organ sizes. These results highlight three major points: i) Indirect estimation of belowground biomass of fire-prone trees requires knowing age of individuals; ii) Some ontogenetic traits can be expressed in aboveground compartment despite fire-induce effects and can provide estimate of individual age; iii) Consideration of both ontogenic traits and size of aboveground compartment allows to accurate estimation of belowground biomass. Since a large proportion of woody species in frequently disturbed environments exhibit various traits related to their overall survival strategy in these environments, future work should evaluate how these traits are modulated during ontogeny and can explain biomass variation over time. Assessing the physiological basis of stem meristem ageing in post-fire resprouts offers an interesting field of study for future work.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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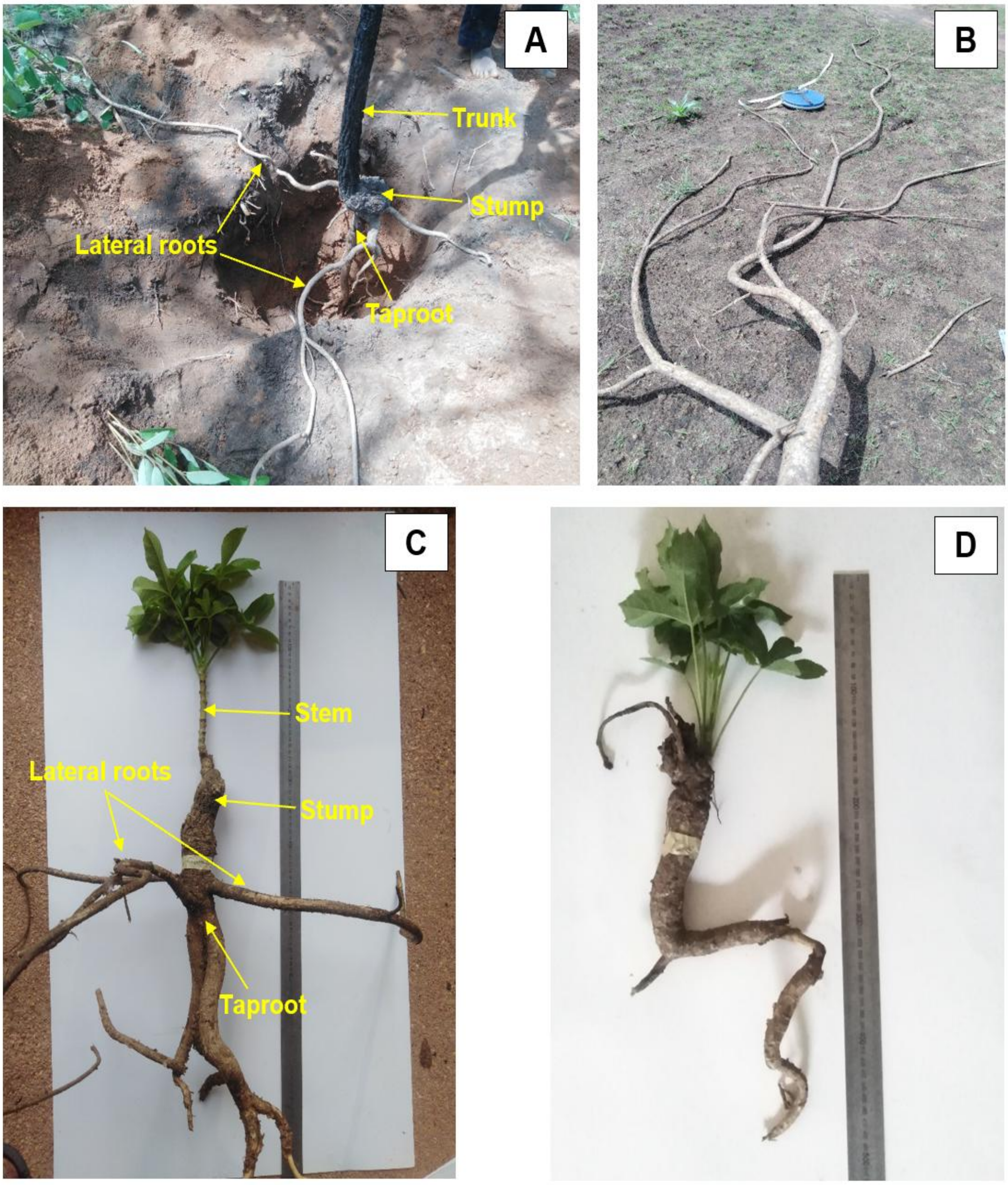
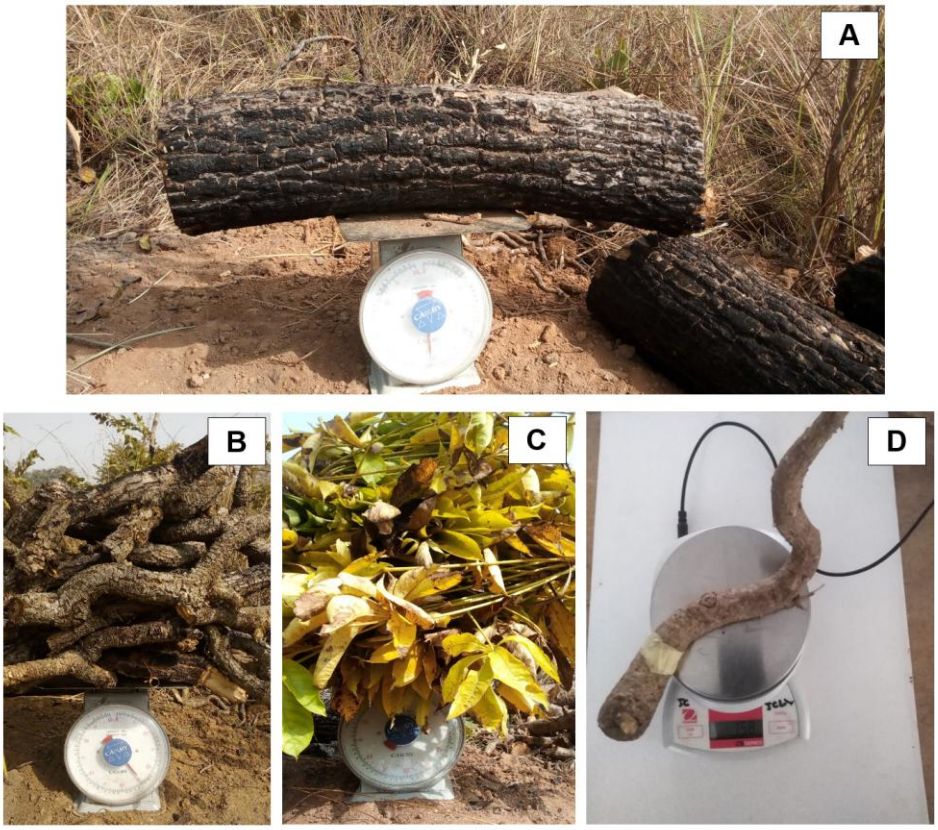
APPENDIX 1

Belowground compartment with or without the aboveground compartment of some sampled *Cussonia arborea* individuals. A) Trunk and root system of a young tree; B) Lateral root of a mature tree; C and D) Aboveground and belowground compartments of two annual resprouts.



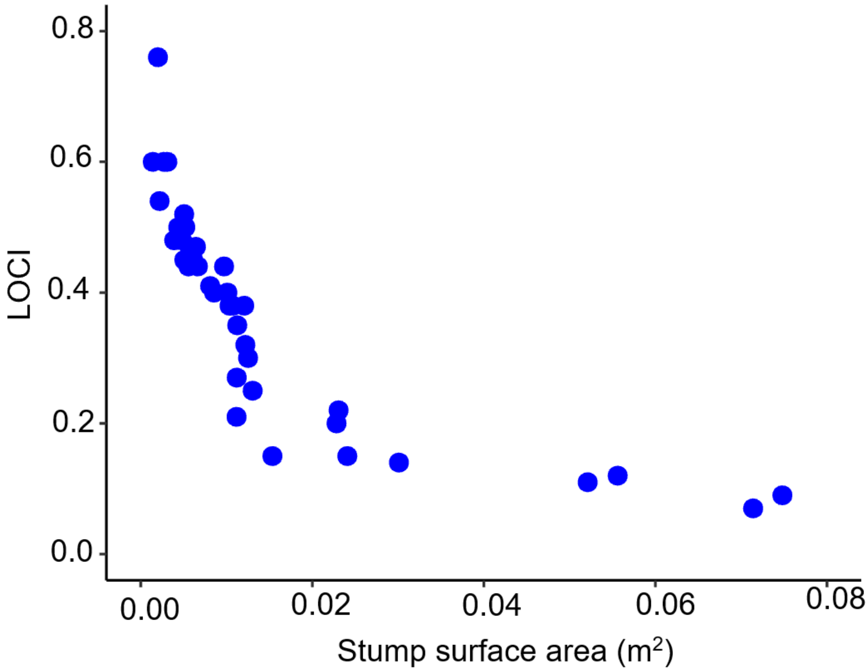
APPENDIX 2

Measurement of aboveground and belowground fresh biomass of some sampled *Cussonia arborea* individuals; A) Weighing of a trunk part of a mature tree on mechanical scale; B) Weighing of the branches of a mature tree; C) Weighing of the leaves of a mature tree; D) Weighing of a sample of lateral root of a young tree on electronic scale.



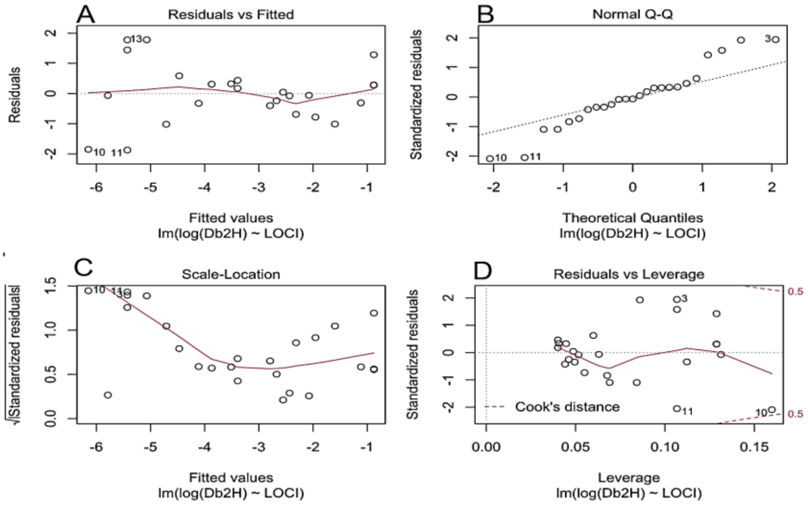
APPENDIX 3

Variation of LOCI (Leaf ontogenetic change index) in relation to the stump surface area of annual post-fire resprouts of *Cussonia arborea*



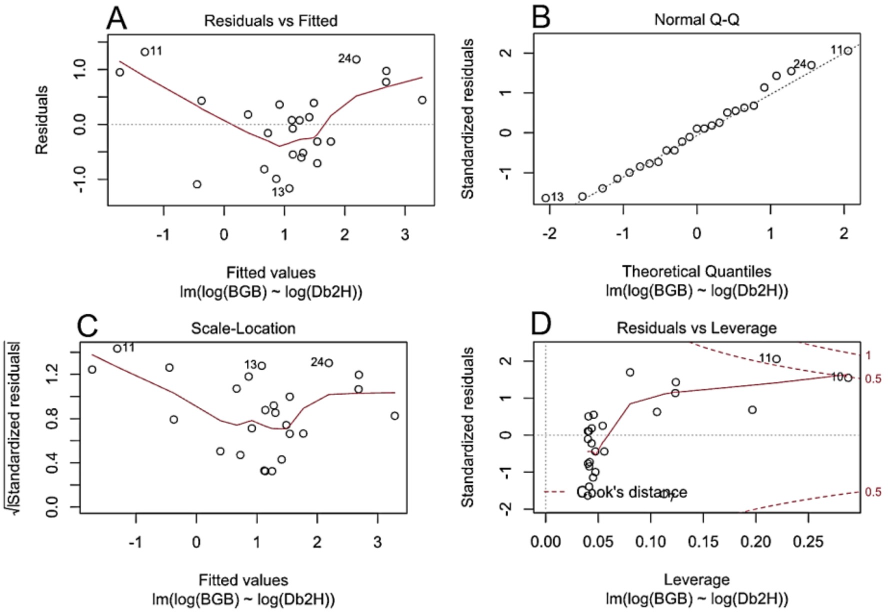
APPENDIX 4

Model validation graphs obtained by applied the regression model of stem volume ($D_b{}^2H$) by LOCI (Leaf ontogenetic change index). Panel A and C show residuals versus fitted values and both can be used to assess variance homogeneity. Panel B is a QQ-plot for normality, and Panel D shows the standardized residuals versus leverage and the Cook statistic is superimposed as contour plots [1]. In case of a good fit, as is the case here, the residual quantiles in panels A and C should be symmetrically distributed (i.e., with equal variance) around an average = 0. Based on the QQ-plot in panel B, the residuals look normally distributed since most of the points are in a line. Panel D shows that there are no observations with a Cook distance greater than 1, which represents the threshold value at which the validity of the regression model would be critical [1].



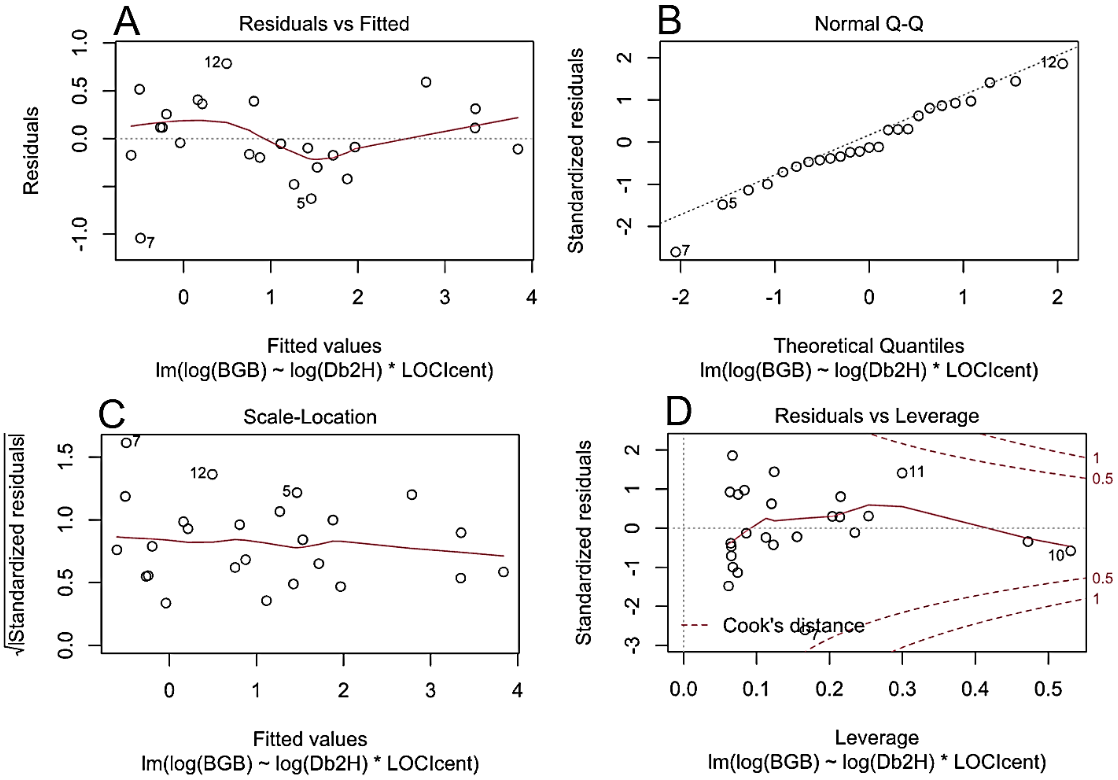
APPENDIX 5

Model validation graphs obtained by applied the regression model of belowground biomass (BGB) by LOCI. The graphs were examined as describing in Appendix 4. The results allowed to validate the assumptions of the regression model.



APPENDIX 6

Model validation graphs obtained by applied the regression model of belowground biomass (BGB) by both the stem volume (D_b^2H) and LOCI. The graphs were examined as describing in Appendix 4. The results allowed to validate the assumptions of the regression model.



REFERENCE RELATED TO THE APPENDIX SECTION

1. Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health, Springer Science+Business Media, LLC; 2009.

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