

Contribution to Chemical Study of Stem and Branches of *Trema orientalis* L. (Blum) and *Leucaena leucocephala* (Lam.) De Wit

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Abstract

The use of timber in techniques of soil aggradation helps to preserve the environment by recycling sub products and residues from forestry. However, the nature of timber conditions the degradation process. The present study aimed at characterizing and comparing the chemical characteristics of wood branches, and stems of *Trema orientalis* and *Leucaena leucocephala*. By modified lignin Klason, high amounts of lignin were found in the branches than in the trunk of *Trema*, contradicting the results observed for *Leucaena*. Cellulose concentration, obtained from Kurschner and Hoffner was higher in the branches and lower in the trunk of *Leucaena* than in *Trema*. Moreover, ash contents were higher in the branches than in the trunk. C:N and lignin: N ratios, with N values significantly higher in the branches, were higher in wood trunk than in branches. Moreover, all *Trema* ratios were higher than those found in *Leucaena*. However, branches lignin/N ratio, naturally weaker, would predict a more rapid decomposition of their lignocellulose material once on the ground. Overall, pyrolysis GC-MS of branches and stems identified compounds derived mainly from lignin, followed by polysaccharides.

Keywords: Chemical constituents; *Trema orientalis*; *Leucaena leucocephala*; Aggradation; Degradation; Soil

Introduction

The major problems that modern agriculture is currently facing are land degradation and water scarcity. The use of chemical fertilizers and other artificial fertilizers constitutes an extra solution, but ultimately causes water pollution by nitrogen (N) [1]. For this purpose, change of land use patterns would dictate that we significantly reduce the use of chemical fertilizers in crop patterns. A long-term solution is offered by the "technique of forest" on agricultural soils, in bringing ramial chipped wood (RCW) to develop fertile soils as does the forest [2]. Therefore, several experiments on the application of RCW were performed in agriculture to understand the effects of this matter on the soil and on plants [3-12] and in forestry [13,14].

Chemically composed of various molecules (amino acids, proteins, vitamins, sugars, essential nutrients, cellulose, pentosan, lignin), RCW are a key material for aggradation of degraded soils. Lignin and Polyphenols are at the beginning of humic compounds [1,15-19]. However, the litter decomposition is conditioned by a set of complex interactions between soil organisms, temperature, humidity, substrate quality and physical properties of the litter [20-22]. In fact, the decomposition can be measured by establishing a monitoring loss of litter mass [23]. Under macroclimat uniform conditions, the C: N ratio determines the release rate of N in mineralization and, consequently, the rate of decomposition [24]. In addition, the degradation of lignified substrate is controlled by the mass loss of lignin. However, the lignin degradation is favored by high concentrations of N and is limited by high concentrations of cellulose in the lignified material [25]. In addition, the low initial concentration in lignin negatively impacts the start of timber decomposition. It is done in this case a little bit late because of availing a large amount of carbon (C) by microorganisms [26]. It seems that good quality litter decomposition stimulate the decomposition of lower quality litter; one notes here the interest of mixture of several species in the RCW [27]. The major difficulty encountered when using RCW is low productivity (especially in the first application), resulting from the high N immobilization upon decomposition [9,11,12]. Moreover, the phosphorous (P) would be immobilized during this decomposition process [1]. But, a simultaneous N intake (in mineral or organic form) can limit this phenomenon of immobilization [5-7,28]. In all cases, the nature of the timber is of great importance because it

determines its fate in the soil. Accordingly, the application of RCW enhances forest residues through its contribution to woody lignin, which interacts on soil aggradation. This process contributes in the same time to the environment preservation by recycling sub products and residues from logging.

This study is aiming to characterize and compare the chemical characteristics of wood branches and stems of *Trema orientalis* and *Leucaena leucocephala* in order to lay *in vitro* and *in vivo* basically experiments, from the monitoring of branches decomposition and their effects on crops and soil.

Materials and methods

Study area

Kinshasa geological material is composed of soft formations of recovery (alluvium, sand deposits of Pleistocene and Pliocene aged) and some polymorphous sandstone and soft recovery, while the floor is dominated by the Kalahari sand cover type. The climate of Kinshasa and its surroundings belongs to the type AW4, according to Köppen [29].

Production of ground material

Branches and trunk discs were collected in January 2011 from 5 individuals of each species, scattered on sites between 307 m and 396 m. The maximum diameters at the base of branches were 6 cm and 5 cm, while those of the trunk discs were 22 cm and 19 cm, respectively for *Leucaena* and *Trema*. Kamabu and Lejoly [30] described the level of suitable size for trunk discs. The wood milling process is to be found

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in [31]. The extraction of the ground was conducted in two steps: (1) extraction with anhydrous toluene-ethanol mixture and, after air drying, and (2) extraction with hot water [32].

Chemical analysis

Lignin was determined by modified lignin Klason method [32], of which the particularity is the transferring wood samples extracted in an autoclave at 125 °C for 1 hour after impregnation in 3 ml of 72% H₂SO₄ and a bath at 30 °C for 1 hour, successively. Cellulose index has been based on Kurschner and Hoffner works [32] after transformation of the lignin in nitro phenolic products following the attack of sawdust by the acetic acid and ethanol mixture, which was carried in a water bath with reflux condenser for 1 hour then filtered and mixed with 3 times alcohol and nitric acid. Finally, filtering under vacuum followed by washing three times (with ethanol, cold water and finally with hot water), and drying in an oven for 24 hours was made to determine the cellulose index. Hemicellulose, in turn, from the standard CPPA-G-12 [32], which consists for submitting crush in the attack of concentrated hydrochloric acid (azeotropic 131 ± 0.5 g/L) and dosage of furfural obtained by the action of the 2-4-dinitro-phenylhydrazine (DPNH) on the aldehyde group. Minerals have been treated by complete digestion, assisted by microwaving in a Teflon bomb using an acid solution, followed by elemental analysis [32]. An elemental analyzer Perkin Elmer 2400, calibrated to acetanilide was conditioned to analyze concentrations of C, H and N. The selected atomization method was heating in inductive coupling plasma (ICP) and the detection method was by atomic emission spectroscopy (AES). Everything was done on optimum machine DV 4300 (Perkin Elmer) with a Scott type of nebulizer. Finally, ashes analysis was performed according to its standard method, known as D 1102-84, which consists by determining the percentage residue remaining after dry oxidation (that is to say oxidation at 580-600 °C) of crushed wood (ASTM International, 1984) [33]. Pyrolysis combined with gas chromatography, coupled simultaneously to mass spectrometry (Py-GC/MS), allowed the separation and identification of organic fragments of the timber [31].

Results

Wood humidity

Woody material humidity decreased after extraction of *Trema* and *Leucaena* samples. Some samples had deteriorated and an approximate value of 5% was considered. This situation did not allow the comparison of two used extraction processes. Table 1 shows results of extraction process.

Lignin, pentosan, cellulose, extract and ash contents

The extract contents are largely high in the branches, and in the wooden sticks for both species. The percentage of extracts obtained by using hot water is greater than that obtained with EtOH-toluene for both species for both branches and trunks.

The fiber content did not seem to vary significantly in both species, except for the branches of *Trema* compared to the *Leucaena* trunk wood content, as well as that of the *Trema* trunk wood.

The variation of lignin concentration was not unidirectional especially; as it changes according to the nature of species (26.1 and 23.7 vs 23.0 and 25.3 for *Leucaena* and *Trema* stems and branches, respectively). The insoluble lignin rates were higher than those of the soluble lignin for all samples.

The pentosans content was generally low into the branches than the stem wood, but higher in the trunk of wood (19.4, 23.0 vs 18.7, 21.1 for *Leucaena* and *Trema*, respectively). This result showed a high

concentration of pentosans in *Trema* than in *Leucaena* when respective concentrations between same fractions were listed.

Furthermore, the ash content was high in the branches than in the trunk. Meanwhile, higher ash contents (especially in the branches) are found in *Trema* samples than in *Leucaena*. Table 2 shows the chemical clues found on *Trema* and *Leucaena*.

Elementary chemical composition of stem wood and branches of *T. orientalis* and *L. leucocephala*

The N content is much higher in the branches than in the trunk for both species. Moreover, in two compartments of the shaft, *Leucaena* appeared to have higher N levels than those obtained in *Trema*.

For both species, C content is higher in trunk than in branches. In addition, C contents of *Leucaena* are slightly greater than those of *Trema*. Contrasts revealed highly significant differences (P<0.05) regarding N and C between species.

The two species do not show significant differences in their respective levels of H%. At the same time, one noted the absence of S in both species.

Table 3 shows C, N, H, P, K, Ca, Mg, Na, Fe and Mn contents and C: N ratio for *T. orientalis* and *L. leucocephala*.

At first glance, the following model can be observed when the concentrations of nutrients in stems and branches wood of *Leucaena* are considered: K>Ca>Mg>P>Fe>Na>Mn. K is more concentrated element in different parts of the shaft, while Mn is least concentrated one. On the contrary, model: Ca>K>P>Mg>Fe>Na>Mn is observed for *Trema*. In this light, the contrasts were most significant for Ca, Mg, and K than for P, Na, Fe, and Mn between both species.

The calculation of C: N ratio for both species provides globally higher values for trunk compared to those obtained for branches (e.g., 211.48 opposed to 92.35).

In addition to C: N ratio, quality study of an organic substrate should be completed by that of lignin: N ratio for each studied species to better understand the parameters involved during the material degradation. Table 4 shows the results related to lignin: N ratio.

	<i>L. leucocephala</i>		<i>T. orientalis</i>	
	Trunk	Branch	Trunk	Branch
Crushed not extracted, %	6.1	6	9.3	8.2
Crushed extracted to EtOH-toluene, %	5	5	5	5
Crushed extracted to water, %	4	5.9	5	6.2

Table 1: Moisture of extracted and not extracted crushed.

Clues	Sample			
	L	RCW-L	T	RCW-T
Extraction				
EtOH-toluene, %	2.3	3.8	2.3	1.3
Hot water, %	4.6	5.3	4.0	7.1
Total, %	6.9	9.1	6.3	8.4
Fiber, %	45.7	45.5	46.4	39.8
Insoluble lignin, %	24.5	21.7	20.4	22.9
Soluble lignin, %	1.6	2.0	2.6	2.4
Total, %	26.1	23.7	23.0	25.3
Pentosans, %	19.4	18.7	23.0	21.0
Ashes, %	0.9	1.6	1.3	2.9

T and L related to *T. orientalis* and *L. leucocephala*, respectively; RCW-L and RCW-T related to ramial chipped wood from *L. leucocephala* and *T. orientalis*, respectively.

Table 2: Chemical clues on *T. orientalis* and *L. leucocephala*.

Ech.	%N	%C	C/N	%H	P	K	Ca	Mg	Na	Fe	Mn
							mg kg ⁻¹				
L1	0.52	49.32	94.85	6.17	380	2370	2445	589	155	250	2,2
L2	0.47	49.23	104.74	6.21	240	2305	2123	640	127	290	0,98
L3	0.52	49.18	94.58	6.24	297	1968	2672	616	136	247	1,36
L4	0.45	49.3	109.56	6.24	148	2484	2152	642	142	202	1,72
L5	0.38	49.28	129.68	6.29	310	2196	2448	735	155	221	2,35
LM	0.47 ± 0.06	49.26 ± 0.06	106.68 ± 14.38	6.23 ± 0.04	275 ± 86.73	2264.6 ± 195.93	2368 ± 229.91	644.4 ± 55.02	143 ± 12.19	242 ± 33.29	1.72 ± 0.57
LB	0.6	48.37	80.62	6.22	611	4342	3861	724	151	279	4,19
T1	0.23	48.64	211.48	6.25	817	3024	966	371	257	207	2,04
T2	0.4	48.69	121.7	6.24	1157	5878	1899	387	381	215	3,15
T3	0.26	48.74	187.46	6.2	326	3945	1873	444	183	200	4,92
T4	0.26	48.57	186.73	6.15	121	4501	1187	547	308	334	2,33
T5	0.39	48.21	123.62	6.13	476	4491	1185	516	70	230	3,01
TM	0.31 ± 0.08	48.57 ± 0.21	166.21 ± 40.98	6.19 ± 0.05	579.4 ± 410.83	4367.8 ± 1036.60	1422 ± 433.09	453 ± 77.40	239.8 ± 119.31	237.2 ± 55.25	3.09 ± 1.12
TB	0.53	48.02	92.35	6.04	1045	5533	7214	864	217	266	7.95

L1-L5 and T1-T5: trunk wood samples collected from 5 different individuals of *Leucaena* and *Trema*, respectively. LM and TM are means with their standard deviations, while LB and TB are related to branches wood of the same species, respectively.

Table 3: Contents of trunks (T1-T5 and L1-L5) and branches (TB and LB) C, N, H, P, K, Ca, Mg, Na, Fe and Mn, and C: N ratios.

<i>Leucaena</i>			<i>Trema</i>
N%	Bs	0.47	0.31
	Brs	0.60	0.53
Lignin %	Bs	26.1	23.0
	Brs	23.7	25.3
Lignin : N	Bs	55.53	74.19
	Brs	39.5	47.74

Bs and Brs relate to wood and branches respectively

Table 4: Lignin: N ratio in *Leucaena* and *Trema*.

The results of lignin: N ratio reflected a higher ratio for the trunk than those the branches, valid result for both species. It also emerges from the above mentioned table that the *T. orientalis* ratios is significantly higher than that of *L. leucocephala*.

The products of the pyrolysis GC-MS branches were identical to those identified for trunk: globally, 44 compounds for *T. orientalis*, including 11 corresponding derived products from polysaccharides, and 33 from lignin; while 46 compounds in total, of which 13 are derived from polysaccharides and 33 from lignin, in *L. leucocephala*. The programs (attached) show a chemical similarity between both species.

Discussion

The trend to reduce moisture of ground material after successive solvent extractions and hot water indicates normal behavior of wood when subjected to different treatments (chemical, physical). However, it is not possible to completely remove water from the timber, because of different forms (water content, impregnating water, and capillary water) in which it is present [34]. Wood humidity is very important, because it determines the variations in volume and form, but also in calculation of shrinkage [34].

Background lignin is one of the most abundant polymers in nature [35]. Lignin rates found in this study differ not only according to the relevant parts, but also by species. Indeed, changes are established when considering different species [25], but also within the same individual [1,2,32,36-38]. Moreover, Mendieta-Araica et al. [39] reported lignin levels, in the fine fraction of *Moringa oleifera*, in significant increase (from 11.5 to 106.5%) when soil was amended in N. These levels were higher when the amount of N increased from 0 to 261 kg N ha⁻¹ yr⁻¹ for a density of 100.000 to 167.000 plants ha⁻¹. Close values (24.1%, 21.5 and 20.7%) have still been found by Lourenço et al. [40]

on *L. leucocephala*, and *Acacia melanoxylon* woods, respectively. The latter species is of course for the *Mimosaceae* family. [41] Observed, however, that the lignin content in the *A. melanoxylon* wood was similar in sapwood and heart wood. Nevertheless, these results confirm the fact that the lignin content of woody species can vary within the tree and depends on the species, and the environment considered. However, the lignin content found in this study do not appear to differ significantly from the average percentage (25.0 ± 3.5%) fixed for Mayumbe woods (Congo Kinshasa) [42], provided that the diameter is less than 25 cm. The high concentration of insoluble lignin in wood, and even in the branches is justified to the extent that this material gives the cell hydrophobicity, and resistance, allowing plants to have an upright growth habit. Moreover, this resistance is against biological degradation, and penetration of various chemicals [32].

It seems that the percentage in pentosan, for tropical hardwoods, would be related to the degree of woodiness [42]. Thus, woods with high lignin contents generally have pentosan low percentage. The result is that our samples are more or less lignified, and have pentosan content higher than the average (11-12%) of tropical timber, according to the levels reported by the aforementioned authors. Hemicelluloses occur predominantly in the strength of wood properties, but also represent the first product to be degraded during heat treatment of wood [43,44].

The concentrations of cellulose (45.7 and 45.5% for *Leucaena* and 46.4 and 39.8% for *Trema*) are broadly consistent with the literature data (45 ± 2%), except for *Trema* branches where a low value (39.8%) was found. Close results (42-48%) were found by [45] on characterization of 25 tropical hardwoods. Ferial et al. observed equally a grade of 41.1% in fiber in *L. leucocephala* wood, corroborating with the results of this study. Istaş et al. [41] have considered the fact that percentage increased with age of tree, mineral elements, and tropical wood extracts is inversely correlated with the decrease of their celluloses contents, corrected cellulose and alpha-cellulose. Thus, the corrected average cellulose content of 40 deciduous woods of Congo-Kinshasa, determined on subjects of 30 cm in diameter, was 48% while it gets only 44% for the average of the results provided by 80 elderly. Moreover, these values are close to those proposed by Jing-shuang et al. [46], quoted by ASTM International [32], for some hardwood species (45% for *Acer rubrum*; 45% for *Fagus grandifolia*) of the North American region. However, they are significantly higher than the average (42 ± 2%), provided for softwoods of temperate zone [32].

Considering that data on biomass and nutrient contents of branches depend on the diameter at breast height (DBH), the season, the diameter of branches, age, and possible interactions between these factors [47], difficulties arise with regard to the comparison with similar studies. In addition, the contents of various elements in the plant extremely differ because of their different physiological functions. In this study, the comparison is made only in order to inform changes in concentrations of possible elements between shaft regions.

N concentration is generally more important in the twigs than in the bark, but remains very low in the trunk [27]. The N concentrations are lower than those found by Ostryko et al. [10] (0.6 to 0.8%, composed only of hardwood), [7] (0.72% for a mixture of 86% deciduous and conifer 14%). On the contrary, the concentrations are of the same order of magnitude as those found by Zongo [27] (0.209 to 0.578% for hardwood), [48] (from 0.29 to 0.45%) for 5 species of the *Dipterocarpaceae* family and [6] for the sugar maple (0.36%). Relatively low values (0.19 and 0.20%, 0.20 and 0.18%) were observed by Luxmoore et al. [49] for branches and trunks of *A. mangium* and *A. auriculiformis* (*Mimosaceae*), respectively. The barks were analyzed separately for wood and branches. The leguminous nature of *Leucaena* explains the differences in N concentrations observed between both species. Indeed, the *Leucaena* fixed more N than the *Trema*.

As it is the case for N, the P content is generally higher in the branches than in the bark, and the trunk. P soil deficiency requires trees to develop a recycling mechanism to limit losses [50]. This strategy explains the interest of its high concentration in the branches. In addition, the high concentration of P as N in the branches, compared to that of wood trunk, is linked to their high capacity in living cells [1].

K concentration differs depending on the considered fraction in the shaft. Indeed, Tremblay and Beauchamp [7] found similar results while Pettigrew [14] found low levels (1900 mg kg⁻¹). Nevertheless, K contents can vary with altitude, and season [51].

Calcium (Ca)

According to Van den Driessche [52], Ca does not undergo translocation from the leaves to the branches, because of its low mobility and therefore tends to accumulate in the bark; hence, it's high concentration in this tissue. Moreover, Tissaux [26] and Ostryko and Page [10] found Ca low concentrations in the branches, and even less in the trunk, while it was high in the bark. On the contrary, the results of present study have shown higher concentrations in the branches (3861 and 7314 mg kg⁻¹ for *Leucaena* and *Trema*, respectively) than in the trunk (2368 and 1422 mg kg⁻¹) of both species.

Magnesium (Mg)

Magnesium is one of the mobile elements of which a part is concentrated in the branches, while the other part would be lost by leaching [52]. In general, the Mg concentration is higher in the bark than in the branches [27]. According to Tissaux [26], birch and oak can have a quite different situation with higher concentrations in the bark as they remain low in the trunk. With that, the average Mg concentration of branches can range from 420 to 1200 mg kg⁻¹, according to Tremblay et al. [7].

Carbon (C) and hydrogen (H)

C concentrations as those in H found here (48.21 to 48.74% against 48.37 to 49.32% C, respectively for *Trema* and *Leucaena*; 6.04 to 6.25% against 6.17 to 6.29% H, respectively for *Trema* and *Leucaena*) are close to the values (carbon (49.5%), hydrogen (6%)), characterizing the tropical timber [53,54]. However, one can notice the slightly

higher values in the branches, and in the trunk for both species. Castaño-Santamaría and Bravo [55] reported higher C percentages in the bark than in the heart wood and sapwood. Therefore, the authors suggested the following model for C wood concentration: bark > heart wood > sapwood.

Sodium (Na), iron (Fe) and manganese (Mn)

Represent a portion of sufficiently heavy elements in the timber. They do not seem to play an active role, but their presence in the wood may oppose their use [32]. However, it is obviously seen that these minerals are generally more concentrated in the branches than in the trunk. This is probably due to the fact that early wood tracheids have larger lumens with over punctuations, thus constituting the main routes for the fluids transport, rich in minerals from the roots [32]. The final timbers, of which the tracheids, having stronger walls and thicker lumens, act essentially as mechanical support.

According to Dommergues and Mangenot [56] and Davy [12], C: N ratio of a biologically active soil is usually less than 12, or more generally between 10 and 15 [57]. However, our results provide an increased C: N ratio. The C: N ratio calculated (from 94.58 to 129.68 for wood stems and branches 80.62 of *Leucaena* and 121.7 to 211.48 for *Trema*) is generally higher than that found in the literature: 84% for Beauchemin et al. [4], 86% for Dayegamiye et al. [5], 47-117% for Tremblay [8], or even 62, 69 and 64% found successively in 1995, 1997 and 1999 by Ostryko [10]. However, critical threshold of N immobilization is achieved with C: N ratios > 100 for branches and C: N > 300 for trunk in a forest soil [21]. In all cases, the immobilization phenomenon would benefit linked to the overall stimulation of the trophic chain rather than an unbalanced intake of C and N [6].

The lignin: N ratio is logically higher (74.19 and 55.53, respectively for *Trema* and *Leucaena*) for trunk than branches (47.5 and 39.5, respectively for *Trema* and *Leucaena*). The interest in using of lignin: N ratio to predict more efficiently the rate of substrate decomposition resides in that lignin has control over the decomposition rate both by its resistance to enzymatic attacks, and the physical protection of cell components against degradation. However, Moore et al. [21] noted that following decomposition process, the residual mass is strongly correlated with lignin: N ratio of original material; therefore, high lignin content slows down the decomposition rate. Therefore, values related to lignin: N ratios, found here predict that decomposition rate of branches for both studied species would be faster than wood trunk. In addition, decomposition rate of based-*Leucaena* organic material would be even faster than *Trema*.

Differences in observed extract concentrations may reflect combinations of genetic factors, conditions and places of growth, and the age of tree [32]. However, it is noted in this study that high levels of extracts are found more in branches than in trunk. This part of the shaft is generally composed of living cells that in turn contain a variety of molecules (amino acids, sugars, proteins, hormones, ...) indispensable for the development of the plant. Many of the above molecules belong to the category of pentosans. Moreover, values found are logically higher than those (5 ± 3%) of hardwoods in temperate regions [32], but also to those (0.6 to 6.6%) found by [45] on 25 species of tropical hardwoods. In general, Congo hardwoods contain 3 to 8% of extracts soluble with ether and alcohol-benzene [42]. In addition, extracts are generally present in low concentrations, making it difficult to determine their distribution in the wood. However, higher concentrations can be observed in juvenile woods [32], as it is the case of concentrations found in this study.

The ash contents found in this study (0.9% and 1.6% for *Leucaena*, 1.3% and 2.9% for *Trema*) ranged in the average (0.08 to 3%) for Congo hardwood, according to Mburu et al. [42]. The values found are so weak in the trunk, but high in the branches, thereby confirming the ash distribution rule within the same tree.

The multitude of compounds identified in this study (44 and 46, respectively for *Trema* and *Leucaena*) demonstrates the wood heterogeneity. The compounds thus identified are the same regardless of the considered tree region. However, these compounds are present in the lumen, and cell walls pores where they can react with the structural components of the timber and thereby, become inaccessible by common extraction techniques [32]. Perhaps acetic acid, catechol, eugenol, vanillin are extracts, but identified as lignin derivatives, may be invoked to require illustration. Furthermore, the literature is almost silent on the pyrolysis-GC-MS of *Trema* and *Leucaena* woods, which does not allow a good discussion of these results.

Conclusion

It can be seen that branches have a clear advantage over the trunk, and can serve as an amendment product for tropical soils. Some precautions are essential, such as the amount (kg.m^{-2}) per hectare, fragment size, spreading period for a satisfactory result. However, to better understand its effects on the soil and/or crop yield, it is essential to establish an experimental design *in vitro* and *in vivo* using the same species in order to verify, or maybe confirm or deny advanced findings in this study. Moreover, future experiments could allow the identification of wood material decomposition to better understand which of the ratios (lignin: N or C: N) would influence the weight loss, but also to predict the characterization effects, such as based materials of branches with mixed and unmixed species.

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