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Leaf litter accumulation and release of nutrients to the soil in *Pinus tropicalis* Morelet stands

Acumulación de hojarasca y liberación de nutrientes al suelo en rodales de *Pinus tropicalis* Morelet

Acumulação de lixo e libertação de nutrientes para o solo em povoamentos de *Pinus tropicalis* Morelet

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ABSTRACT

The research was carried out in the Sumidero Silvicultural Unit, belonging to Empresa Agroforestal Minas, with the objective of evaluating the contribution of leaf litter to the soil in stands of *Pinus tropicalis* Morelet. The collection of leaf litter was carried out in two sites: stand 1 with site quality I, stand 4 with site quality II; rectangular trapscollectors (1X0.5 m) were placed in each stand, in plantations of *P. tropicalis,* with ages between 15 and 24 years. Soil samples were obtained with and without leaf litter, in which the contents of nutrients (phosphorus, potassium, magnesium, calcium) and

organic matter were determined. The results showed significant differences between soils with and without leaf litter, more accentuated in phosphorus and organic matter, with increases higher than 4.9 % and 3.9 % as a consequence of the accumulation of leaf litter; whereas, the highest contribution of nutrients and organic matter is produced in the surface of the soil and decreases, appreciably in the soils extracted from the soil pits made in the stands, maintaining the differences between these in the contents of phosphorus and organic matter.

Keywords: Biomass; organic matter; nutrients.

RESUMEN

La investigación se realizó en la Unidad Silvícola Sumidero, perteneciente a la Empresa Agroforestal Minas, como el objetivo de evaluar el aporte de la hojarasca al suelo en rodales de *Pinus tropicalis* Morelet. La colecta de hojarasca se ejecutó en dos sitios: rodal 1 con calidad de sitio I, rodal 4 con calidad II; para se colocaron trampas-colectoras rectangulares (1X0.5 m) por rodal, en plantaciones de *P. tropicalis,* con edades entre 15 y 24 años. Se obtuvieron muestras de suelo con y sin hojarasca, en las cuales se terminaron los contenidos de nutrientes (fósforo, potasio, magnesio, calcio) y materia orgánica. Los resultados arrojaron diferencias significativas entre suelos con y sin hojarasca, más acentuadas en el fósforo y la materia orgánica, con incrementos superiores a 4,9 % y 3,9 % como consecuencia de la acumulación de hojarasca; mientras que, el mayor aporte de nutrientes y materia orgánica se produce en la superficie del suelo y disminuye, apreciablemente en los suelos extraídos de las calicatas hechas en los rodales, manteniendo las diferencias entre estas en los contenidos de fósforo y materia orgánica.

Palabras clave: *Biomasa; Materia orgánica; nutrientes.*

RESUMO

A investigação foi realizada na Unidade Silvicultural Sumidero, pertencente à Empresa Agroflorestal Minas, com o objectivo de avaliar a contribuição do lixo foliar para o solo em povoamentos de Pinus tropicalis Morelet. A recolha de lixo foliar foi realizada em dois locais: o stand 1 com qualidade de local I, o stand 4 com qualidade de local II; foram colocados coletores de armadilhas retangulares (1X0,5 m) em cada stand, em plantações de P. tropicalis, com idades compreendidas entre os 15 e 24 anos. Foram obtidas amostras de solo com e sem folhagens, nas quais foram determinados os teores de nutrientes (fósforo, potássio, magnésio, cálcio) e matéria orgânica. Os resultados mostraram diferenças significativas entre os solos com e sem ninhada, mais acentuadas no fósforo e na matéria orgânica, com aumentos superiores a 4,9% e 3,9% como consequência da acumulação de ninhada; enquanto que, a maior contribuição de nutrientes e matéria orgânica ocorre à superfície do solo e diminui, sensivelmente, nos solos extraídos das calicatas feitas nos povoamentos, mantendo as diferenças entre estes nos teores de fósforo e de matéria orgânica.

Palavras-chave: Biomassa; Matéria orgânica; Nutrientes.

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INTRODUCTION

Human-induced modification of ecosystems is reaching unknown rates in recent years, with a direct impact on biodiversity, energy flows, production and distribution of goods and services, in some positively and in others negatively (Augustine *et al.,* 2018).

In forest soils different materials are deposited, coming from different strata of vegetation, such as leaves, branches, inflorescences, whose set is called leaf litter; of this, the leaf is the most important fraction in these ecosystems (Barreto *et al*., 2018). The production and speed of decomposition of organic residues provided by the forest canopy determine the thickness of leaf litter accumulated on the ground. In forest ecosystems, leaf litter decomposition is the main way for nutrients to enter the soil and one of the key points in the recycling of organic matter and nutrients (Molina *et al.,* 2018).

The rate of decomposition of plant debris depends on environmental factors such as: temperature, rainfall and the different forest or plant species (Moreno Valdez *et al.,* 2018)**.** Therefore, the effect of decomposition on soil quality involves determining the values of physical, chemical and biological indicators that allow differentiation according to soil type, vegetation cover, climate, time of sampling and use.

According to Kuruvilla *et al*., (2016), research on leaf litter decomposition is useful in determining the amount of nutrients passing from soil-reservoir to mineral soil and how they influence soil fertility.

The great variety of tropical ecosystems that develop on different edaphoclimatic conditions, raises the need to apply harvesting technologies according to the characteristics of each plant formation, where the behavior of leaf litter is an indicator to be taken into account, because of its importance in the stability and functioning of ecosystems, in the recycling of nutrients and the improvement of soil conditions. Therefore, this research aims to evaluate the contribution of leaf litter to the soil in stands of *Pinus tropicalis* Morelet*.*

MATERIALS AND METHODS

Study locality

The Sumidero Forestry Unit, which belongs to the Empresa Agroforestal Minas has a forestry patrimony of 29,696 ha. It is located in the municipality of Minas de Matahambre in the province of Pinar del Río, bordered to the north by the Ezequiel Candelaria Farm, to the south by the Empresa Agroforestal Minas, to the east by the Sumidero Stream and to the west by the Canta Rana hamlet.

It is located in an intramountainous valley with fertile soils, which makes agriculture its main economic base, together with forestry. Due to the extension of the territory, its patrimony presents great variability of relief. In the last year the average annual temperature in the area was 24.38ºC, July was the warmest month, with 28.60ºC and January the coldest, with 20.6ºC. Maximum temperatures reached 33.48ºC in August and minimum temperatures dropped to 14.24ºC in January. The average annual rainfall

sum is 1 331.18 mm, with the highest value in June (235.88 mm) and the lowest in February (only 27.48 mm). Rainfall during the rainy season (May-October) represents on average 79.80% of the total annual volume. Data obtained from the meteorological and hydrological station of Santa Lucía, Minas de Matahambre, Pinar del Río.

Study Site

The study was conducted between January 2020 and January 2021. Two stands of *Pinus tropicalis* Morelet were selected with ages ranging from 15 to 24 years and taking into account the quality of the site, these were: Stand 1, with site quality I; Stand 4, with site quality II. Stand 1 was subjected to greater silvicultural intervention, while Stand 4 was not subjected to forest management. In each stand, three 30 x 30 m plots were established for litterfall collection.

Field work Leaf litter collection

To estimate the amount of litter entering the system, rectangular traps of 1 X 0.5 m were randomly placed. Four replicates (traps) were placed per stand, the traps were placed from January 1, 2020 to January 30, 2021, and the intercepted material was collected every 30 days (González *et al*., 2011).

The samples collected in the field were placed in plastic bags duly labeled and then transferred to the chemistry laboratory of the University of Pinar del Río for processing. Once in the laboratory, they were separated into their components (branches, needles, leaves of other plants, flowers and fruits). These fractions were dried in a *Yamato Scientific America Inc*. DNE910 forced air oven at 70 ^{0}C to a constant weight (Krishna and Mohan, 2017). The dry mass was determined on a KERN ABJ-NM analytical balance of 0.1 mg accuracy. The results obtained were expressed in grams per square meter.

Soil sample extraction

A stratified random sampling with extraction of soil samples at different points distributed in zigzags (Martín and Cabrera, 1987) over the stand surface was proposed, obtaining five representative samples in each one for the two soil conditions (with leaf litter and without leaf litter) and sampling depths (0-10, 11-20 cm and 21-30 cm) in which agrochemical characteristics were determined: P, K, Ca, Mg and MO.

Analytical techniques used in soil analysis

Humidity

Humidity was calculated using the methodology described by Paneque (2010). Where the results of the analysis are expressed based on the oven-dried sample and the percentage humidity content of the air-dried sample is determined before performing the analysis (Equation 1).

$$\text{Humedad (\%)} = \frac{C-A}{B-A} \times 100 \quad (1)$$

Where:
A = Constant weight of the crucible or crucible weight at 105°C.

B = crucible weight + air dry sample.
C = Crucible weight + dry sample at 100°C.

Factor to correct the analytical results in (fch) is calculated by:

Humidity correction factor (Equation 2).

$$Fch = \frac{100 + \% \, humedad}{100} \quad (2)$$

Organic matter and ashes

Equipment and utensils

- Analytical balance of 0.1 mg accuracy.

- Muffle.

- Porcelain crucibles of 25 cm^3.

- Desiccator.

- 100 ml volumetric flask • 10 and 100 ml test tubes.

- 10 ml pipette.

- Wash bottle with distilled water

Procedure

- Weigh 2 g of the air-dried material in 25 cm^3 crucibles, previously tared at 550°C.

- Take the crucible to the muffle, raising the temperature gradually (from 50 to 50°C) to avoid loss in the combustion. Once the temperature of 550°C is reached, keep the crucible in the muffle for three hours.

- Switch off the muffle and when the temperature in the muffle is below 100 °C, remove the crucible and if carbonized substances that are difficult to burn have formed, allow the crucible to cool and moisten the contents with 6 to 8 drops of concentrated nitric acid and/or the same quantity of hydrogen peroxide (H_2O_2).

- Evaporate the added solution in a burner and under a hood until dry and ash again at 550 °C, place in the desiccator for 30 minutes.

- Weigh on an analytical balance to the nearest 0.1 mg.

- Moisten the ashes with distilled water and dissolve them with 5 mL of hydrochloric acid 1+1 (distributed in 2 additions of 2.5 mL) and weigh

quantitatively into a 100 mL volumetric flask. Make up to volume with distilled water, shake and leave to stand for 18 hours (solution A).

• Take 10 mL of solution A and transfer to a 100 mL volumetric flask. Make up to the mark with distilled water and shake (solution B).

Calculate the % ash, and indirectly the % organic matter by (Equation 3).

$$\text{Cenizas (\%) (abs. seca)} = \frac{(B - A)}{M} * 100 * fch \quad (3)$$

Where:
A = Constant crucible weight at 550°C.
B = Constant crucible weight + ash.
M = Weight of air dry sample.
Fch = Humidity correction factor.

Calculate % M.O. by (Equation 4).

$$\text{Materia Orgánica (\%) (abs. seca)} = 100 - \% \text{ cenizas} \quad (4)$$

Total Phosphorus

Equipment and utensils

• Analytical balance 0.1 mg accuracy.

• Technical balance 0.01 g precision.

• Spectrophotocolorimeter with a wavelength of 410 nm.

• Photocolorimeter with blue filter.

• Volumetric flasks of 50 ml.

• 1000 ml volumetric flasks

• 100 ml Erlenmeyer flask.

• Volumetric pipettes with central bulb of 10 mL.

• 25 or 50 ml burettes.

• Beaker of 400 ml. **Procedure**

• Take an aliquot of 5 - 10 ml of the mineralized sample (solution B) and place in a 50 mL volumetric flask or 100 ml Erlenmeyer flask.

• Add 25 mL of dye solution and make up to the mark with distilled water (if working in volumetric flask) or 20 or 15 ml of distilled water (depending on

the aliquot taken) to complete the final volume of the erlenmeyer flask to 50 mL. Shake and let stand for 15 minutes to achieve the maximum color development.

- Read the spectrophotocolorimeter at 410 nm wavelength or in photocolorimeter with blue filter.

Calculate the % of total P by (Equation 5).

$$\text{Fosforo (\%) (abs. seca)} = \frac{mgP}{1(\text{gráfico})} * \frac{100}{M} * \frac{100}{10} * \frac{50}{a} * fch * 10^{-4} \tag{5}$$

Where:
M = Weight of air-dried sample taken to incineration in g.
100/10 = Dilution factor of solution
B. a = mL of the aliquot for
colorimetry. 50 = Final volume of
colorimetry. Fch = Humidity
correction factor 10-4 = Factor to
take to %.

Total Potassium

Equipment and utensils

- Analytical balance 0.1 mg accuracy.

- Flame photometer.

- 1000 ml volumetric flask

- 250 ml volumetric flasks

- 50 ml burette.

- Wash bottle with distilled water

- Stove.

Procedure

Take a portion of the mineralized sample (solution B) and read in the flame photometer.

Determine the K concentration on the calibration graph.

Calculate the % of total K by (Equation 6).

$$\text{Potasio (\%) (abs. seca)} = mgK/1(\text{gráfico}) * \frac{100}{M} * \frac{100}{10} * fch * 10^{-4} \tag{6}$$

Where:
M = Weight of air-dried sample taken to incineration in g.
100/10 = Dilution factor of solution
B. Fch = Humidity correction factor
10-4 = Factor to take to %.

Total calcium and magnesium

Equipment and utensils

- Analytical balance 0.1 mg accuracy.

- Thermo-adjustable stove.

- Volumetric pipettes of 3, 5, 10, 20 and 25 mL.

- 1000 mL volumetric flask

- Beaker of 100 mL.

- 200 mL Erlenmeyer flask

- Graduated burette of 25 mL.

- Mortar and 100 cm^3 porcelain hand.

- Spatula.

Procedure Calcium

- Pipette a 20 mL aliquot of the incinerated sample (solution B) and place in a 200 mL Erlenmeyer flask.

- Dilute with distilled water to approximately 100 mL.

- Add 3 mL of 1 N sodium citrate solution and 10 mL of 10% KOH solution (to bring to pH 12.3-12.5).

- Add a pinch of Murexide indicator with the tip of a spatula and titrate with 0.02 N EDTA solution in the presence of a control until the colour changes from pink to lilac.

Calculate the % total Ca by (Equation 7).

$$\text{Calcio (\%) (abs.seca)} = a * N * 0.02 * \frac{100}{M} * \frac{100}{10} * \frac{100}{b} * fch \quad (7)$$

Where: a = Volume of EDTA consumed in the
titration in mL N = molar concentration in EDTA
equivalent.
0,02 = Weight of the Ca me expressed in g.

M = Weight of air-dried sample taken to incineration in g.
100/10 =Dilution factor of solution B.
100 = To express in %.
B = mL of solution B taken to titrate.
Fch =Moisture correction factor

Magnesium

- To the already titrated calcium solution 10 mL of 3 N hydrochloric acid is added, which changes the solution to a colourless one.

- Add 5 mL of concentrated ammonium hydroxide to raise the pH of the extract to approximately 9.

- Add a pinch of Eriochrome Black T or 7 to 9 drops of indicator solution.

- Titrate with 0.02 N EDTA until the colour changes from mauve to blue.

Calculate the % total Mg by (Equation 8).

$$\text{Magnesio (\%) (abs. seca)} = a * N * 0.032 * \frac{100}{M} * \frac{100}{10} * \frac{100}{b} * fch \tag{8}$$

Where: a = Volume of EDTA consumed in the
titration in mL N = EDTA Normality.
0.032 = Weight of the Ca me expressed in g.
M = Weight of air-dried sample taken to incineration in g.
100/10 = Dilution factor of solution B.
100 = To express in %.
B = mL of solution B taken to titrate.
Fch = humidity correction factor

Statistical analysis

With the data obtained, an analysis of variance (ANOVA) of simple classification was performed. The Tuckey test was used, in order to know if there were significant differences between the means.

RESULTS AND DISCUSSION

Physical determination of leaf litter

The average volume of branches, leaves and fruits in the collected litterfall showed that the highest proportion of necromass was represented by leaves 2762.3 g/m^2 with 90.9 %, followed by branches 217.5 g/m^2 with 7.1 % and fruits 59.8 g/m^2 with 2 %, which showed the lowest values (Table 1), showing that the mass of leaves occupies a higher volume in the production of plant matter in the studied ecosystems. When the different

components that make up the litterfall per stand are averaged together, accumulation levels are obtained for stand 1 of approximately 1094.6 g/m ^{2}per month and for stand 4 (1945 g/m^2). When extrapolating these values to the year, the total amount for stand 1 was 2.2 t/ha and for stand 4 (3.6 t/ha), which shows that the latter is the one with the highest litterfall production. It should be noted that the values obtained exceed the annual accumulation reported by other authors for natural forests (Fuentes *et al.*, (2018) (Table 1).

Table 1. - Percentage of litter biomass by components and total obtained in stands of *Pinus tropicalis* Morelet in the Sumidero Silvicultural Unit

Biomass flux (g/m^2)	Stand 1	Stand 2	Total (g/m^2)	%
Sheets	994,7 [b]	1767,6 [a]	2762,3	90,9
Fruits	14,4 [b]	45,4 [a]	59,8	2
Woody Material	85,5 [b]	132 [a]	217,5	7,1
Monthly concentration	1094,6 [b]	1945 [a]	3039,6	
Total annual contribution (t/ha)	2,2 [b]	3,6 [a]		

Different letters between rows indicate statistical differences for ($p < 0.05$) according to Tuckey's test.

The differences found between stands for total accumulation levels and estimated litterfall components correspond to a greater vegetation cover in stand 4, while stand 1 was subjected to harvesting and silvicultural treatments, which resulted in the removal of large and small trees for different forest uses, which may have influenced the decrease in the estimated litterfall contents. The lower litter production in stand 1 could also be explained by the poor structural development of the trees, which directly influences a lower contribution of leaves, twigs, flowers and fruits Montoya *et al.*, (2019).

This study can be comparable to those conducted in other tropical forests, such as those of (Aryal *et al.,* 2015), where it is reported that the leaf litter was mainly composed of leaves with 69 % and 91 %, which allows us to affirm that leaves are the major source of energy exchange and recycling of nutrients in these ecosystems, fulfilling an important role in the dynamics and stability of the ecosystem.

The production and accumulation of leaf litter can be determined by the structural conditions of the ecosystems evaluated and the agro-climatic characteristics of the area, the contributions of leaf litter can show variations, for example, in soils with erosion problems, depending on the time of year and climatic variations that may occur. It is proposed that in any type of forest, the highest litterfall occurs at a certain time of the year, so the behavior of a species, or group of species, is determined by the occurrence of phenological phases as a result of the stimuli of climatic elements, mainly temperature and precipitation (Krishna and Mohan, 2017).

The values obtained are an estimation of what is really produced in stands, because the litter is collected directly from the soil and the samples are exposed to the action of decomposing agents and the weather, therefore considerable percentages of disintegrating material are always obtained.

Humidity content of leaf litter

The humidity content in leaf litter (Table 2) ranged between 40.5 and 70.4 %, with higher values in stand 4. The difference in humidity between stands is due to the characteristics of the ecosystems, greater population and crown of pines, in stand 4 limits solar radiation on the soil surface where the litter is located and favors the humidity content. Garcia (2017) and Montoya *et al*., (2019), state that a lower density of foliage is equivalent to a greater opening of the canopy, which allows greater entry of sunlight, consequently increasing soil temperature and, therefore, lower humidity contents (Table 2).

Table 2. - Humidity content of litterfall obtained in *Pinus tropicalis* Morelet stands in the Sumidero Silvicultural Unit

Stand	A	B	% Humidity	Fch
1	20.7	35.5	40.5 [b]	1.4
4	28.6	40.4	70.4 [a]	1.7

Measures with different letters are statistically different for ($p < 0.05$) according to Tuckey's test

It is essential that plantations have adequate foliage and that silvicultural activities are as non-aggressive as possible, in order to achieve less disturbance, which can result in greater accumulations of leaf litter and therefore in soil humidity content. Fallen leaf litter and its decomposition in the soil is one of the ways of maintaining soil humidity and recycling mineral nutrients. The leaf litter, by its sponge action, maintains the humidity conditions that allow the action of microorganisms in a regulated manner. This produces a balance between the canopy, leaf litter, decomposers and nutrients, and perhaps this is the basic balance for tropical forest ecosystems to remain intact over time (Hernández and Hernández, 2005).

Nutrient content in soils with and without leaf litter according to their depth

The agrochemical analysis of the soils with and without leaf litter according to their depth (Table 3), shows significant differences between them for the contents of phosphorus, potassium and organic matter, as well as with the microelements evaluated, which increased in the soils with leaf litter on the surface and decreased in the soils with litter in the depth, being much more significant the decrease of the components in the soils that did not contain leaf litter both on the surface and in the depth, which shows that the greater contribution of the leaf litter is more accentuated in the superficial part of the soils with leaf litter, highlighting in both cases that there are significant differences in the phosphorus and organic matter components, it is clear that this happens, because the organic matter is not a stable mixture of chemical

substances, it is rather a dynamic mixture in constant change, which represents each stage of the decomposition of the dead organic matter, from the simplest to the most complex (Gaspar *et al.,* 2015).

These results are comparable with those reported by Moreno *et al.,* (2018) who report significant differences for the components between surface and depth. Fernandez *et al*., (2016), also state that as depth increases, organic matter content decreases (Table 3).

Table 3. - Nutrient content (%) of soils with and without leaf litter, as well as surface and depth in *Pinus tropicalis Morelet* stands of the Sumidero Silvicultural Unit

	Nutrients (%)				
	P_2O_5	K_2O	M.O	Ca	Mg
Leaf litter-surface soil	4,9 [a]	4,1 [a]	3,9 [a]	1,6 [a]	2,5 [a]
Soil with leaf litter-depth	3,3 [b]	3,4 [ab]	2,4 [b]	1,4 [a]	1,9 [ab]
Soil without leaf litter-surface	2,5 [c]	3,5 [ab]	1,3 [c]	1,3 [a]	1,5 [b]
Soil without leaf litter-depth	0,2 [d]	1,6 [c]	0,9 [cd]	0,7 [ab]	0,9 [bc]

Different letters between columns indicate statistical differences for ($p < 0.05$) according to Tuckey's test

Similar results are reported by Molina *et al.,* (2018); while they describe higher values of Ca accumulations and lower values of K (Fernández *et al.,* 2016). It is also known that in *Munrochloa ritchei* plants the highest accumulation is achieved in magnesium (Kuruvilla *et al.,* 2016).

The greater contribution of organic matter produced by leaf litter to the soil is a matter of vital importance for the enrichment of soils, showing a better nutritional content in the forest species evaluated. The canopy through the leaf litter not only supplies organic matter, but also regulates soil temperature, so that the decomposition of organic matter and the supply of nutrients occurs continuously and gradually, which counteracts, in turn, factors such as soil erosion, land degradation and desertification, which make the health of the soil increases FAO (2017).

In this sense, it can be said that litter plays an important role in relation to the dynamics and stability of these ecosystems, as it constitutes an important source of energy and nutrients for edaphofauna and plants. The production of leaf litter and its decomposition are essential for the transfer of energy and nutrients to the soil, which constitutes a flow that supplies an important fraction of rapidly mineralizable nutrients in deciduous forests Doll *et al.,* (2018). The low contents of nutrients found in the soils without leaf litter in the surface as well as in the depth, can bring less production, observed in the little structural development of the trees, on the other hand in the soils with leaf litter were observed better conditions of the plants, as well as in the physical, chemical and biologic properties of the soil, when were observed higher contents of accumulation of nutrients, increase the rate of accumulation of organic matter. In these systems, soil organic matter is maintained at satisfactory levels for its fertility; the recycling of bases in tree

residues can reduce or slow down the acidification process, in addition to controlling erosion (Montoya *et al*., 2019).

CONCLUSIONS

Necromass, consisting of litter and dead wood, has several ecological functions in forests, such as providing habitats for wildlife, acting as a reservoir for water and nutrients. Therefore, changes in the amounts and composition of necromass have important effects on ecosystem functioning. The findings of this study allow us to broaden our knowledge about the physical composition of leaf litter, as well as the contribution of nutrients and organic matter to the soil. It is demonstrated that the majority fraction of the leaf litter is made up of leaves (90.9 %) compared to woody material (7.1 %) and flowers that only accounted for 2 %; and how necromass generates a considerable contribution of nutrients and organic matter on the soil surface, which decreases appreciably in depth.

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Authors' contribution:
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