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Department of Exact and Earth Sciences - *Campus* II
Program of Post Graduation in
Modeling and Simulation of Biosystems

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Leaf litter as a carbon sink in the Atlantic Forest biome (Bahia, Brazil)

Alagoinhas, Bahia (Brazil)
2024

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Dissertation presented to the Post Graduation Program in Modeling and Simulation of Biosystems at the Bahia State University (Universidade do Estado da Bahia - UNEB) as part of the requirements for obtaining the Master's degree in Modeling and Simulation of Biosystems.

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DANIELA KARINE CARVALHO BATISTA DO NASCIMENTO

Dissertation presented to the Post Graduation Program in Modeling and Simulation of Biosystems – PPGMSB, on July 1st., 2024, as a partial requirement for obtaining the degree of Master in Biosystems Modeling and Simulation from the State University of Bahia, as evaluated by the Examining Board:

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ABSTRACT

The Atlantic Forest exhibits a high diversity of species, with biological potential and essential ecosystem services, but it is degraded by forest fragmentation that interferes with nutrient cycling and the trophic network. Among the elements of organic matter, carbon is one of the essential elements for heat absorption and the greenhouse effect, which help in the maintenance of life. Litter accumulates organic matter deposited on the forest floor and is an important carbon sink. The main objective of this study was to evaluate litter as a guarantee of nutrient cycling and carbon sink, through the dynamics of decomposition and quantification of carbon in the remaining leaf fraction, at the edge and inside of the native fragment of the Atlantic Forest in the municipality of Alagoinhas, Bahia (Brazil). Sample plots were marked at the edge and inside the native fragment. From October 2021 to September 2022, decomposition bags were installed and the carbon content was quantified to investigate litter in sequestration and storage as a carbon sink. From December 2021 (spring) to March 2022 (summer), the decay of the dry mass remaining in pockets reflected the decomposition of litter in a similar pattern between the edge (P0) and intermediate (P1) plots, however, this seasonal effect was not observed in the inland plot (P2) in this short period between the consecutive spring and summer weather seasons. With litter decomposition, part of the soluble carbon is released by respiration in the form of CO2, and another part of the structural carbon is immobilized in the biomass of the rhizosphere or edaphic fauna, making the soil also a carbon sink. The seasonal effect of precipitation and temperature influenced on the carbon content in those three plots in the summer (March/2022). In terms of feeding habits, invertebrates could be classified into the six functional groups of predators, detritivores, phytophages, saprophages, coprophages and bioturbators. Among terrestrial invertebrates, there is a rapid recovery (5 days) of prey populations, when the stress factor ceases when the number of predators is small or proportional. In this way, predators can maintain biological control of prey populations without compromising their litter decomposition activity, preserving it as a layer in dynamic renewal between input-mineralization. This protects the soil and acts as a stock in the sequestration of carbon from the atmosphere by plant biomass and a subsequent transfer to the soil. The decomposition dynamics maintain the ecosystem processes of nutrient cycling and nutrition of the trophic network, necessary for the restoration and/or conservation of biodiversity. The litter takes part in carbon sequestration and acts as a reservoir, configuring a carbon sink. This research is relevant for demonstrating the ecosystem service that litter plays as a carbon sink required to maintain nutrient cycling and stabilize the microclimate, minimizing the impacts of environmental degradation and preserving native forests.
An incentive for further investigations!

Keywords: decomposition, invertebrates, restoration, conservation.

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SUMMARY

1. INTRODUCTION

The Atlantic Forest is a biome of high biodiversity, due to its location on the Brazilian coast and association with varied ecosystems such as restinga and mangroves (SOS Fundação Mata Atlântica; INPE, 2021). It is considered one of the largest *hot posts* in the world, due to the occurrence of endemic species threatened with extinction in its areas (Pinto; Hirota, 2022).

Currently, deforestation is one of the main tensioners of the forest for environmental balance. Over the years, predatory anthropogenic actions have devastated this ecosystem, causing forest fragmentation, altering its structural complexity, and threatening the maintenance of biodiversity (Santos *et al*., 2017; Santos *et al*., 2024).

The decomposition of litter ensures the supply of nutrients for the maintenance of the trophic network through cycling in the ecosystem (Ferreira; Magellan; Barbirato, 2020). The action of microorganisms is fundamental for this process due to the release of hydrolytic enzymes. Nutrient contents, such as lignin and the carbon: nitrogen ratio, are fundamental for determining decomposition dynamics (Rebêlo *et al*., 2022).

The carbon cycle includes the soil, through the production of litter and the decomposition of this biomass by the edaphic fauna, which favor the contribution of organic matter and are the main pathways for the transfer of nutrients, such as carbon, from plants to the soil (Costa *et al*., 2010; Bazi, 2019).

The study of litter as a carbon sink contributes to the analysis and suggestions of solutions to environmental problems related to the loss of natural forests and possible reforestation as useful tools for carbon fixation, essential for the production of organic matter in the ecosystem (Batista *et al*., 2020).

The decomposition rate of litter is a determining factor of the feedback mechanism of the organic matter deposited in the soil and the cycling of nutrients (Zhou *et al.*, 2018). Leaf litter is the natural habitat of terrestrial invertebrates, biotic factors that regulate the quality and quantity of the forest floor (Rosa *et al*., 2017).

To relate the dynamics of litter decomposition in inference as a carbon sink in the Atlantic Forest biome, the present quality-quantitative study presented data on remanent dry leaf biomass and its carbon content, aiming to generate information that contributes to the interpretation of environmental dynamics, especially concerning the decomposition process that ensures nutrient cycling, among which is carbon, and its consequent contribution to the trophic network in the native forest of the Atlantic Forest biome.

Therefore, this relationship is of economic and environmental value, since these analyses can collaborate in the planning of public management actions to minimize impacts, enable the restoration of degraded areas, promote the maintenance of biodiversity and ensure a better quality of life for present and future generations.

1.1 Problem

One of the elements that contribute to the Atlantic Forest's ability to act as a carbon sink is leaf litter, composed mainly of decomposing plant material that falls from the trees and covers the forest floor. This layer of leaf litter plays an essential role in the storage of atmospheric carbon fixed by the forest through photosynthesis. In this context, of ecosystem processes and services, does litter contribute as a carbon sink in the Atlantic Forest biome in the municipality of Alagoinhas, North Coast of Bahia (Brazil)?

1.2 Justification

The Atlantic Forest is one of the most exploited and degraded biomes by man, despite its great economic and ecological importance, impacting the loss of biodiversity in addition to the alteration in biogeochemical cycles. Tropical forests contribute largely to the global carbon cycle, storing approximately 76% in soil and 22% in vegetation, functioning as a continuous sink of atmospheric carbon and integrating this element into the soil through leaf litter (Zhou *et al.*, 2018).

The floor of these forest ecosystems contributes to the cycling of organic matter, in addition to acting in the sequestration and storage of carbon (Cunha *et al.*, 2009). This surface layer on the soil is formed by the accumulation of deposited organic material, generally composed of leaves, branches, fruits and seeds, where the microbiota (fungi and bacteria) and the invertebrates that act in the decomposition process, live (Costa *et al.*, 2010).

Decomposition is a determining factor for the role of litter as a sink, especially when considering the composition by the diversity of plant species and the action of microorganisms as the main determinants; in addition to abiotic factors such as precipitation and temperature.

Thus, due to environmental conditions, part of the carbon is incorporated into the soil and another will return to the atmosphere (Carvalho *et al*., 2010).

With the changes that have occurred in soil management, the observation of the litter decomposition dynamics should be seen as an indication of environmental quality. The role of carbon sink guarantees the tropical forest an important ecosystem service of socio-economic scope, requiring research in the area since litter represents a fundamental link in the carbon cycle.

Therefore, this work is justified by the fact that litter decomposition is one of the main means of entry and exit of nutrients in the forest, providing a carbon reservoir in the Atlantic Forest biome and contributing to its resilience.

1.3 Goals

1.3.1 General objective

To evaluate litter as a guarantee of nutrient cycling and carbon sink, through the decomposition and quantification of carbon in the remaining leaf fraction, at the edge and inland of the native fragment of the Atlantic Forest in the municipality of Alagoinhas, Bahia (Brazil).

1.3.1 Specific objectives

To analyze the decomposition of litter in the native forest fragment of the Atlantic Forest.

To evaluate the carbon content in the leaf litter as indicative of litter being an effective carbon sink.

To identify the main functional groups of invertebrates associated with litter and their relationship in the dynamics of decomposition.

To evaluate the influence of seasonality, precipitation and temperature on the dynamics of decomposition, invertebrate populations and carbon content on litter leaf mass.

To establish the relationship between decomposition and nutrient cycling for maintenance and ecosystem balance in the Atlantic Forest fragment of the municipality of Alagoinhas, a reference area for the North Coast and Agreste of Bahia (Brazil), despite being exposed to the intense humidity of the coast.

1.4 Hypothesis

In forest environments, climate change affects the temperature and available humidity, which is essential to the litter decomposition process. In this way, seasonality can regulate the rate of litter decomposition, which contributes to the dynamics of carbon sequestration and storage.

2. THEORETICAL FOUNDATION

2.1 Decomposition dynamics

The Atlantic Forest is one of the richest forests in biodiversity in the world, with approximately 35% of the species existing in Brazil found in this biome, and represents the second largest tropical rainforest in Brazilian territory. Currently, it ranks third in extension in Brazil, behind the Amazon and the Cerrado, representing approximately 12.4% of its original extension (Fundação SOS Mata Atlântica; INPE, 2021).

One of the main functions of the Atlantic Forest is the provision of a set of environmental services, with direct and indirect contributions from ecosystems to serve man (Campos, 2022). The provision of ecosystem services, such as soil protection, nutrient cycling, food and medicine production, pest control, pollination, leisure, and climate regulation, ensures the maintenance of life on the planet (Cabral; Bustamante, 2016). In addition, it contributes to minimizing climate change by sequestering carbon (Azevedo *et al.*, 2018).

Some studies bring us the importance of litter acting as a fundamental component of ecosystem elements, whose diversity of plant species influences the dynamics of decomposition (Andrade *et al.*, 2020). The contribution of material ensures litter renewal and maintains the nutrient cycling process (Scoriza; Pinã-Rodrigues, 2014).

Decomposition represents a key process in maintaining soil fertility, being one of the limiting factors in the establishment and development of forest ecosystems (Peixoto Neto, 2017; Brumatti; Silva; Oliveira, 2023). The dynamics of these flows in developing ecosystems constitute the main route of nutrient supply, and their knowledge is fundamental (Parron *et al.*, 2015). In the composition of leaf litter, the leaves stand out among the other components, such as branches, flowers, fruits and seeds, all of which contain carbon, the main nutrient of the greenhouse effect in the biogeochemical cycle (Gama-Rodrigues; Gama-Rodrigues; Barros, 2008; Silva *et al.,* 2022).

Leaves are the largest representative fraction in the composition of litter in the Atlantic Forest (Gomes *et al.*, 2010; Menezes *et al.*, 2010). The diversity of species, due to the varied physicochemical composition, influences the functioning of ecosystem processes (Parsons *et al.,* 2014; Nascimento *et al*., 2018). Lignified leaves, which take longer to decompose, accumulate a higher concentration of nutrients (Pérez-Harguindeguy *et al*., 2000).

The fragmentation of forests interferes with the decomposition of litter. According to Xulux-Tolosa *et al.* (2003), Menezes *et al.* (2010) and Machado (2011), the speed of litter decomposition will vary according to succession in secondary forests. In the early stages of succession, the plant material produced is more rigid than forests in the advanced stage (Pereira *et al.,* 2013). Under these conditions, the action of the invertebrates is compromised and may constitute a slower process of litter decomposition (Toledo; Pear tree; Menezes, 2002; Câmara *et al.,* 2018; Bazi, 2019).

A higher amount of nutrients, such as lignin and tannin, also allows for a longer shelf life of the leaves (Giebelmann *et al.*, 2013; Peixoto Neto, 2017). Therefore, litter that has a variety of lignin-rich leaves will decompose more slowly than the one with a higher amount of starch (Laskowskil; Berg, 2006). It is also of great importance to relate the microbiota present in litter, since these microorganisms ensure nutrient cycling (Chapman; Koch, 2007; Sayer *et al*., 2020).

Knowing the dynamics of litter decomposition is necessary, since land use in forests changes its management, favoring changes in biogeochemical processes and their relationship in the regulation of the microclimate in the interior of the forest (Toledo; Pear tree; Menezes, 2002; Breaks; Bond, 2020). Vegetation, which suffers edge effects, regresses to the initial stages of succession; the vegetation in the interior, on the other hand, remains in more advanced stages (Silva, 2010; Cherulli, 2018). Thus, early-stage forests, because they produce more rigid leaves with a high degree of sclerification, have delayed decomposition dynamics (Gafta; Roman; Ursu, 2018).

Litter regulates nutrient cycling through its decomposition, with fauna as one of its main actors, while variables such as temperature, rainfall and soil quality are the most striking abiotic factors (Yu *et al.*, 2019). The preserved forest is structurally better and harbors a high diversity of decomposer organisms. Terrestrial saprophagous invertebrates participate in litter decomposition, redistributing organic waste and promoting nutrient cycling in the ecosystem (Neves, 2023; Sá, 2023).

Decomposition represents a key process in maintaining soil fertility, being one of the limiting factors in the establishment and development of forest ecosystems (Peixoto Neto, 2017; Brumatti; Silva; Oliveira, 2023). When litter decomposition occurs more slowly, probably caused by the edge effect on the forest fragment, the diversity and functionality of the microfauna are altered, contrary to the preserved area (Laurance *et al.*, 2011; Cherulli, 2018; Gafta; Roman; Ursu, 2018). Considering the high concentration of carbon present in the forest floor, the dynamics of litter decomposition require greater attention as a carbon sink in this biome. Organic matter contains about 58% carbon and is one of the indicators of soil quality, as it interacts with its physical, chemical and biological aspects (Vezzani *et al*., 2009; Silva, 2018).

Therefore, the evaluation of the dynamics of litter decomposition in the Atlantic Forest in the municipality of Alagoinhas, a reference for the North Coast and Agreste of Bahia (Brazil), is necessary to contribute with data on this nutrient cycling process, emphasizing the importance of this process for the role of the forest as a carbon sink, and its relevance for the maintenance of the ecosystem and the microclimate.

2.2 Carbon dynamics

In general, forests are the largest carbon sinks, since plants and soil, the latter in greater percentage, are the major carbon sinks and stores (Silveira *et al*., 2008; Cunha *et al*., 2009). Forest areas absorb carbon from the atmosphere through photosynthesis and in large proportions act as carbon sinks due to their size and longer lifespan (Treehugger, 2022). Forests sequester approximately twice as much carbon dioxide as they emitted between 2001 and 2019, constituting a carbon sink with a net absorption of 7.6 billion tons of CO2 annually (Wri Brasil, 2021). However, it is worth noting that influences from extreme heat waves, deforestation, drought, forest fires, and tree deaths have increased, particularly in semi-arid regions, representing approximately 41% of the earth's surface (Dass *et al.*, 2018).

Biogeochemical cycles are important regulators for the functioning of tropical forests. By covering extensive areas, tropical forest ecosystems naturally become large carbon reservoirs also due to their high productivity, with great potential in their ecosystem functions as a carbon sink (Malhi; Grace, 2000; Dass *et al.*, 2018). Through photosynthesis, photosynthesizing organisms fix the carbon in the atmosphere, whose dynamic balance also depends on the balance between primary production and decomposition. Therefore, deforestation and fires contribute to the release of carbon that is immobilized mainly in tree biomass into the atmosphere (Bettiol *et al.*, 2023).In light of this, it is extremely important to quantify carbon in tropical forests in order to understand the stock of the element in forests, given the worsening environmental crisis caused by the activities of international corporations (Mohanraj *et al.*, 2011; Dass *et al*., 2018).

It should be noted that the process of deforestation and fires in tropical forests contributes to their destruction and fragmentation. In this way, it goes from being a sink to emitting the carbon stored back into the atmosphere (Paiva, 2018; Souza; Mesquita Junior, 2022). The process of forest fragmentation due to the advance of large estates and invasive species in eucalyptus trees and pastures in the tropics can also decrease and interfere with the carbon cycle each year, leading to the imbalance of the local climate initially in a more direct way (Piva *et al.*, 2021).

In The Paris Agreement in 2015, Brazil committed to reducing deforestation in the Amazon and Cerrado, as well as net CO2 emissions by 37% by 2025 and by 43% by 2030 (United Nations, 2015). As the Brazilian economy has agriculture as one of its pillars, forest areas have been suffering from direct human interference through deforestation, fires, and changes in land, used to replace pastures, monocultures, and eucalyptus trees (Paiva, 2018). The Greenhouse Gas Emissions and Removals Estimates System (SEEG), developed by the Climate Observatory in 2023, recorded deforestation in tropical forests driven by agricultural activities and representing 75% of all Brazilian climate pollution (SEEG, 2023).

The Global Carbon Project indicated a 1.1% increase in greenhouse gas emissions in 2023, equivalent to 37.5 billion tons of CO2, with the burning of fossil fuels being the main emission. The Climate Action Tracker also published a new estimate of global temperature in 2023 and concluded that there has been no change in the scenario since the Glasgow Climate Conference in 2021. These studies revealed that countries, in general, are not fully complying with the agreements signed (Climate Observatory, 2023a).

As a consequence of this phenomenon, extreme weather events arise, such as heat waves, droughts, storms, and floods, which impact food security and the migratory process in more economically vulnerable countries. The World Meteorological Organization (WMO) report pointed out that the decade 2011-2020 recorded higher temperatures on the earth's surface (Climate Observatory, 2023b).

The fragmentation of tropical forests also affects biodiversity and not only the global carbon balance, as animal species need connected forest areas to survive (Fischer *et al*., 2021). Therefore, it is necessary and urgent to seek a balance between conservation, restoration and responsible socioeconomic development (Martins; Miranda; Batista, 2021). In this context, some studies are currently emerging that aim to create alternatives to reduce the current atmospheric carbon concentrations, but these solutions do not come close to or replace the power of natural carbon sinks in forests (Vanali; Tavares; Miranda, 2023; Valencia; Ribeiro, 2023).

3. MATERIAL AND METHODS

3.1 Study area

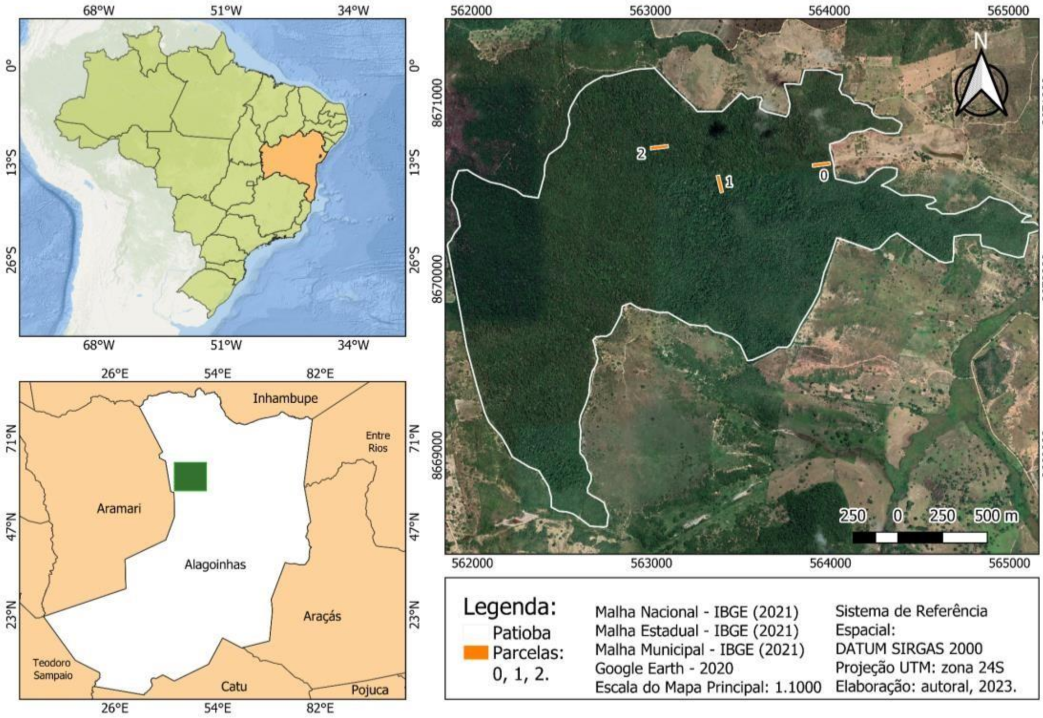
The study was carried out in the municipality of Alagoinhas, located on the North Coast and Agreste of Bahia, in a native forest fragment of the Atlantic Forest at Patioba Farm (Figure 1). The extension of the native fragment is approximately 343 hectares, the largest in the municipality of Alagoinhas.

The vegetation of the Patioba is described as a Dense Ombrophilous Forest, composed of evergreen trees (medium and large) with dense crowns, shrubby and subshrubby sizes, as well as lianas, characterized by vines and vines that grow towards the light (Evangelista; Almeida, 2020; Dantas, 2021).

Details of the collection points in plots 0, 1 and 2 of the forest fragment (Figure 2). The decomposition bags were placed on the litter in Sep/2021, along a transect in each plot, with a random monthly collection of 5 bags to record initial and remaining leaf mass data, after the period in the field bag, and calculation of the decomposition dynamics.

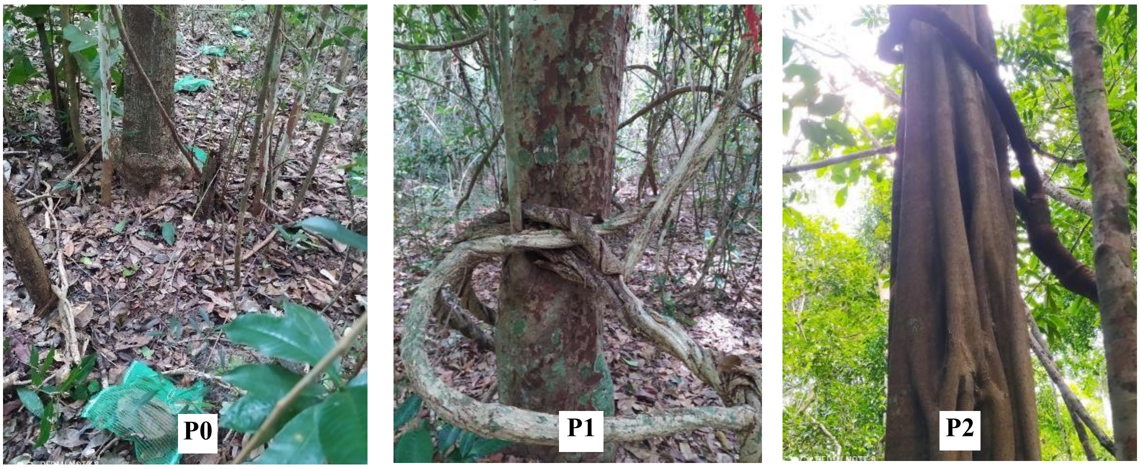
Precipitation and temperature data from the Weather Spark website (2022) were used to investigate a possible correlation and seasonal effect on the dynamics of the decomposition process (Table 1).

Figure 1. Study areas with indications of the collection sites at the edge (P0), intermediate (P1) and inland (P2) of the native fragment of Atlantic Forest at Patioba Farm. Alagoinhas, Bahia (Brazil). Oct/2021 to Mar/2022.



Source: José Gabriel Ferreira dos Santos, 2023.

Figure 2. Details of the vegetation on the edge (P0), intermediate (P1) and inland (P2) of the Atlantic Forest fragment at Patioba Farm. Alagoinhas, Bahia (Brazil). October/2021.



Source: authorial, 2022.

Table 1. Average monthly rainfall (mm) and temperature (ºC) in Alagoinhas, Bahia (Brazil).

Abiotic factor	Oct/ 2021	Nov	Dec	Jan/ 2022	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Precipitation (mm)	47.3	54.5	53.3	40.8	50.1	53.2	91.7	131.1	122.5	97.4	67.6	47.2
Temperature (ºC)	22.7	27.1	26.9	28.5	28.2	27.8	25.3	25.0	21.4	21.7	24.0	24.4

Source: adapted from the Weather Spark website (2022).

3.2 Sorting of litter decomposition pockets

Initially, in September, 20 cm x 20 cm decomposition bags were made with 1 mm^2 green nylon mesh and hot glue (Figure 3). In the field, plots P0, P1 and P2 were marked with nylon rope, samples of whole leaves with good litter appearance were collected in the plots. From these leaves, 10g were weighed and placed in decomposition bags. This method was adapted from Anderson and Ingram (1993).

A total of 100 decomposition bags were positioned on the litter, allowing access to the terrestrial invertebrate fauna for the decomposition process, along a transect in the three plots with a surplus for loss prevention. Each bag containing about 10g of whole leaves and/or in good condition, that is, not yet degraded by the activity of microorganisms or terrestrial invertebrates in the forest fragment (Figure 3).

Figure 3. Decomposition bags of 0.20 m^2 made of nylon mesh, thrown into the fragment of Atlantic Forest at the Patioba Farm. Alagoinhas, Bahia (Brazil). December/2021 to July/2022.

Source: authorial, 2022.

Monthly, 5 bags were collected in each plot (P0, P1 and P2) and transported to the laboratory for manual screening of the plant material, with capture of terrestrial invertebrates preserved in 70% alcohol, with 5 drops of concentrated glycerin for better preservation, and cleaning of excess sediment. The samples were weighed for wet mass registration, then placed in paper envelopes and taken to the oven at 60ºC for 72 hours for subsequent dry mass registration. The dry masses of the December/2021 and March/2022 exchanges were used for carbon quantification. The higher the concentration of organic mass, the lower the amount of water and the more accurate the carbon analysis (Mohanraj *et al.*, 2011).

As the months passed, the bags were covered with new depositions of plant material, requiring attention to be found. As expected, some pockets were lost, probably buried by edaphic fauna, such as bioturbators, ants and beetles.

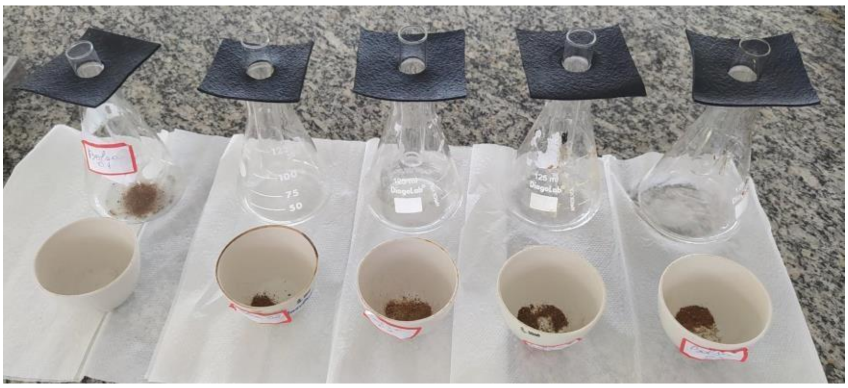
3.3 Total organic carbon (TOC) analysis

Samples of litter remaining in the decomposition pockets were collected in December 2021 (spring) and March 2022 (summer) for analysis and quantification of organic carbon in dry leaf mass.

Of the three analytical methods proposed to quantify total organic carbon (TOC), namely: dry, wet and chemical combustion, wet combustion was selected and adapted. Dry combustion presents the most accurate results (Bisutti; Hilke; Raessler, 2004), but with high costs, and it is convenient to apply the adapted wet method which, even with the need for certain equipment and chemical substances, made the experiment possible. In the wet combustion method, CO2 is oxidized by $K_2Cr_2O_7$ (potassium bichromate) under acidic conditions (Anderson; Ingram, 1993) and the C content can be determined by spectrometry.

The samples collected from the decomposition bags were dehydrated in a drying oven at 60ºC for 72 hours to collect a subsample for manual maceration (crucible and mortar) and obtain 0.05g of the spray for analysis. The black rubberized material was placed externally on the top of the glass container for condensation (Figure 4).

Figure 4. Plant material remaining from the decomposition bag macerated by hand.

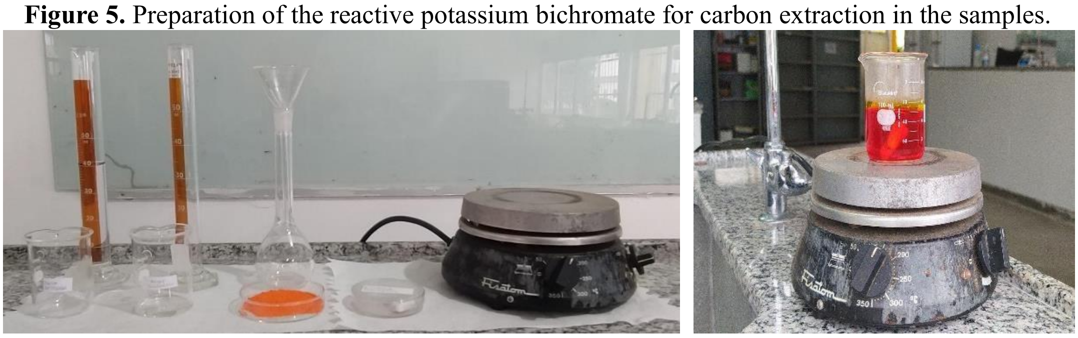


Source: authorial, 2023.

Some previous tests were carried out in order to analyze the effectiveness of the experiment and the need for adaptation of materials. The preparation of a potassium bichromate-based reagent initiated these reactions. After preparation, the reagent was isolated and the flask was covered with aluminum foil to keep it protected from light. No repeatability test was performed, but the experiment was repeated three times to ensure the reliability of the method.

For the measurement with potassium bichromate (27.2 mM), the reactive carbon was quantitatively determined in subsamples of the litter remaining from the decomposition pockets, using as a basis the reaction principles originally developed by Walkley and Black in 1934. It is a reaction where the plant material is digested in an acidic medium, and heated in a digesting plate. This method consists of reacting the organic carbon of the sample with excess potassium bichromate, causing carbon oxidation and reduction of potassium bichromate to Cr^{+3}. In this reaction, the heat emitted in the addition of the reactants involved in the reaction (bichromate and sulfuric acid solution) and also from the heating plate, serves as energy for the catalyzation of the reaction. Initially, in this quantitative carbon process, chromium (valence +6) before the reaction, gives the solution its orange color. At the end of the reaction with the carbon, the chromium is reduced to Cr^{+3}, which gives the solution its green color, which is then measured in the spectrophotometer at a wavelength of 585 nm (Figure 5).

Figure 5. Preparation of the reactive potassium bichromate for carbon extraction in the samples.



Source: authorial, 2023.

After the carbon-bichromate oxidoreductive reaction, the value of the spectrophotometric reading was converted to the amount of carbon in the sample. This procedure was performed with the aid of a standard glucose curve at different concentrations. Thus, the following glucose solutions were made: 3.8 mM, 19.2 mM, 48 mM and 80 mM, proceeding to the same reaction performed with the litter subsample, and the line of the graph equation showed an R2 of 0.989.

For a more accurate quantification of carbon, a previous test was performed with a standard carbon curve using anhydrous glucose to prove the presence of carbon in the reaction, ensuring the safety of the results with the analytical method of quantification of total organic carbon (TOC). Fractionated into five parts with different volumes into solutions 1, 2, 3, 4 and 5, and the white solution as a water-based control to serve as a reading in the spectrophotometer.

Through the reaction of potassium bichromate with sulfuric acid, the presence of the element carbon was found in the analyzed samples. The green color confirms the presence of carbon and orange indicates zero or almost no carbon (Figure 6, Table 2).

Figure 6. Test to quantify the carbon content in diluted solutions with standard glucose curve. Green color for the presence of carbon (left) and orange absence of carbon (right).

Source: authorial, 2023.

Table 2. Concentrations of the standard glucose solution for the elaboration of the reference standard curve in the quantification of carbon content.

Carbon standard solution (10 g)	
Anhydrous glucose solution:	600 ml distilled water and 30 ml sulfuric acid.
Solution 1	10 g of glucose in 100 ml of anhydrous glucose solution
Solution 2	80 ml of solution 1 diluted in 100 ml of distilled water
Solution 3	60 ml of solution 2 diluted in 100 ml of distilled water
Solution 4	40 ml of solution 3 diluted in 100 ml of distilled water
Solution 5	20 ml of solution 4 diluted in 100 ml of distilled water
Solution 6	potassium bichromate

Source: authorial, 2023.

3.4 Identification of invertebrates in the pouches and their functional groups

The litter samples remaining from the decomposition pockets, from May to July 2022, were manually sorted to collect terrestrial invertebrates and classify them into functional groups according to Parron *et al.* (2015). Specimens were preserved in 70% alcohol with 5 drops of pure glycerin. The identification was made by Neves (2023) based on the specific literature of Paoletti and Hassall (1999), Baccaro (2006), and Cardoso (2017), and in comparison with virtual herbarium collections.

Regarding feeding habits, six functional groups of predators, detritivores, phytophagous, saprophagous, coprophagous and bioturbators were considered, according to Parron *et al.* (2015) for the classification of terrestrial invertebrates captured in decomposition pockets.

3.5 Model by Lotka - Volterra

The simple mathematical model of two nonlinear, first-order differential equations, to describe the population dynamics of two predator-prey species, was developed by mathematician Vito Volterra and biophysicist Alfred James Lotka in 1920. The classical mathematical modeling of ecological systems was done with the expanded application of the simple Lotka-Volterra model to the case where there is trophic competition between carnivores (predators) and detritivores (prey) (Souza, 2017). The dynamics of predator-prey interaction were modeled in continuous flow and prey dependence (prey-dependent) for edge (P0) and inland (P2) over 100 days, a period used as a comparative standard for monthly collections over 12 months. The quarterly data were not modeled by incipient or nonexistent abundance.

3.6 Statistical analysis

The data were used in ANOVA ($p<0.05$) and Pearson's linear correlation ($p<0.05$) analyses using the *open-source* software *PAST Analyst* (version 4.17).

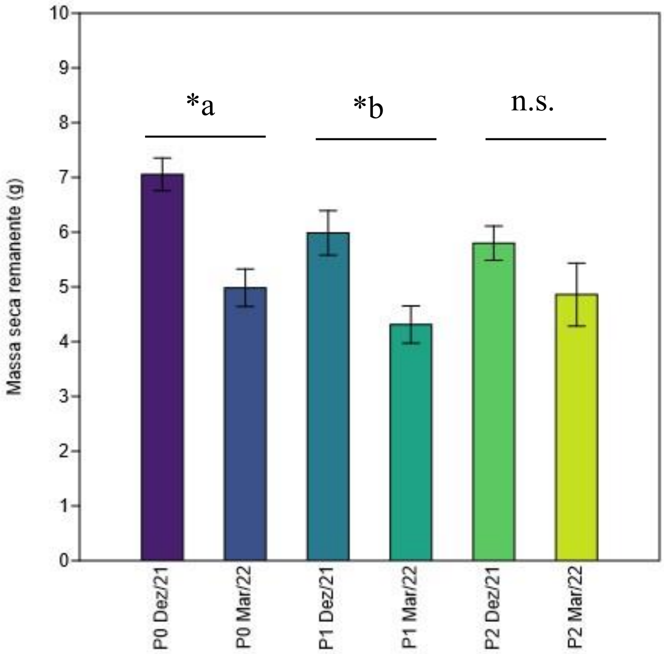
4. RESULTS AND DISCUSSION

4.1 Litter decomposition and effect of seasonality

The decrease in dry mass indicates litter decomposition, which was faster in the edge (P0) and intermediate (P1) plots in the lower part of the fragment, but slower in the inland plot (P2) at 262 meters of altitude. The P1 and P2 plots are more preserved and more humid, with a greater diversity of invertebrates, which fragment the litter and thus contribute to decomposition.

The remaining dry mass in the pockets decreased ($p<0.05$) in the edge (P0) ($F_{1.8}=20.81$; $p=0.002$) and intermediate (P1) ($F_{1.8}=9.94$; $p=0.014$) plots between December 2021 and March 2022, with the most pronounced effect on the edge plot (P0). The seasonal effect was not observed ($p<0.05$) for the leaf dry mass in the interior portion (P2) of the fragment ($F_{1.8}=2.06$; $p=0.189$) between the consecutive spring and summer seasons. The remaining dry mass differed between the three plots in December/2021 (spring) ($F_{2.12}=3.91$; $p=0.049$), but not in March/2022 (summer) ($F_{2.12}=0.68$; $p=0.524$) (Figure 7, Table 3).

Figure 7. Remanent dry leaf mass (g) ($p<0.05$) in the decomposition pockets collected in December/2021 (spring) and March/2022 (summer) in the edge (P0) and interior (P1 and P2) plots of the native forest fragment of Fazenda Patioba, Alagoinhas - Bahia (Brazil).



Source: authorial, 2024.

Table 3. Samples of burlap from bags collected in December/2021 (spring) and March/2022 (summer) in the native forest fragment of Fazenda Patioba, Alagoinhas - Bahia (Brazil).

Month	Portion	Purse	Dough initial (g)	Fresh pasta remanent (g)	Remaining dry mass (g)	MF -MS (g)	Carbon content (g C/Ms)
December 2021	P0	B01	10	6.82	6.60	0.22	0.333
		B02	10	7.13	6.95	0.18	0.438
		B03	10	6.56	6.38	0.18	0.343
		B04	10	7.39	7.26	0.13	0.461
		B05	10	8.43	8.09	0.34	0.463
	P1	B01	10	7.39	6.81	0.58	0.441
		B02	10	4.76	4.53	0.23	0.426
		B03	10	7.08	6.61	0.47	0.434
		B04	10	5.93	5.74	0.19	0.344
		B05	10	6.63	6.24	0.39	0.422
	P2	B01	10	5.35	5.16	0.19	0.434
		B02	10	6.03	5.86	0.17	0.431
		B03	10	5.88	5.62	0.26	0.439
		B04	10	5.58	5.39	0.19	0.418
		B05	10	7.41	6.96	0.45	0.408
March 2022	P0	B01	10	9,88	5,17	4,71	0,438
		B02	10	8,98	4,65	4,33	0,453
		B03	10	9,36	5,07	4,29	0,385
		B04	10	8,28	6,06	2,22	0,429
		B05	10	9,58	3,97	5,61	0,419
	P1	B01	10	5,97	3,30	2,67	0,461
		B02	10	9,71	5,36	4,35	0,471
		B03	10	7,45	4,66	2,79	0,401
		B04	10	7,25	4,07	3,18	0,444
		B05	10	7,31	4,17	3,14	0,452
	P2	B01	10	9,37	4,42	4,95	0,412
		B02	10	7,01	3,45	3,56	0,435
		B03	10	9,22	6,00	3,22	0,397
		B04	10	7,36	4,02	3,34	0,410
		B05	10	9,18	6,41	2,77	0,428

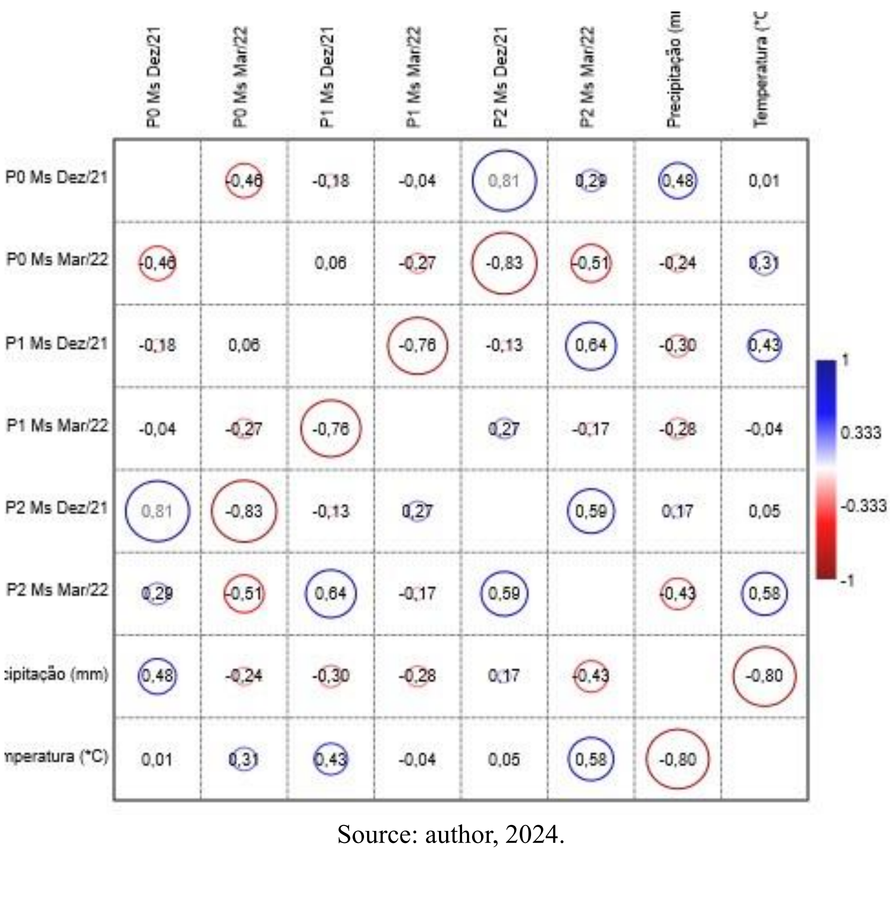
Source: author, 2024.

The decomposition rate is determined by the quality of the substrates, as well as the quantity of decomposers found in the litter (Perez-Suarez *et al.*, 2009; Giebelmann *et al.*, 2011). In areas with the presence of litter for a longer time for decomposition, the organic matter protects the soil from erosion and retains moisture that solubilizes the plant material and nourishes the soil (Caldeira *et al.*, 2019). One of the factors that contribute to the accumulation of litter is rainfall (Facelli; Pickett, 1991; Sanches *et al.,* 2009). The quality and quantity of litter depend a lot on the environment, as the surroundings can favor the deposition process and, thus, alter its decomposition (Xiong; Nilsson, 1999).

Precipitation and temperature in the months of November 2021 to March 2022 did not have direct effects on decomposition ($p<0.05$), through the dry mass remaining in the pockets, although it was present to ensure the retention of the moisture necessary for the process (Figure 8).

In an annual period, it is possible to record the effects of the precipitation and temperature variables, influencing the decomposition rate and consequently the remaining mass in the pockets under the action of invertebrates and microbiota (Carvalho *et al*., 2010).

Figure 8. Pearson's correlation ($p<0.05$) between precipitation (mm), temperature (ºC) and remanent dry mass (g) in the decomposition pockets collected in December/2021 (spring) and March/2022 (summer) in the decomposition plots in edge (P0) and inland (P1 and P2) of the native forest fragment at Patioba Farm, Alagoinhas - Bahia (Brazil).

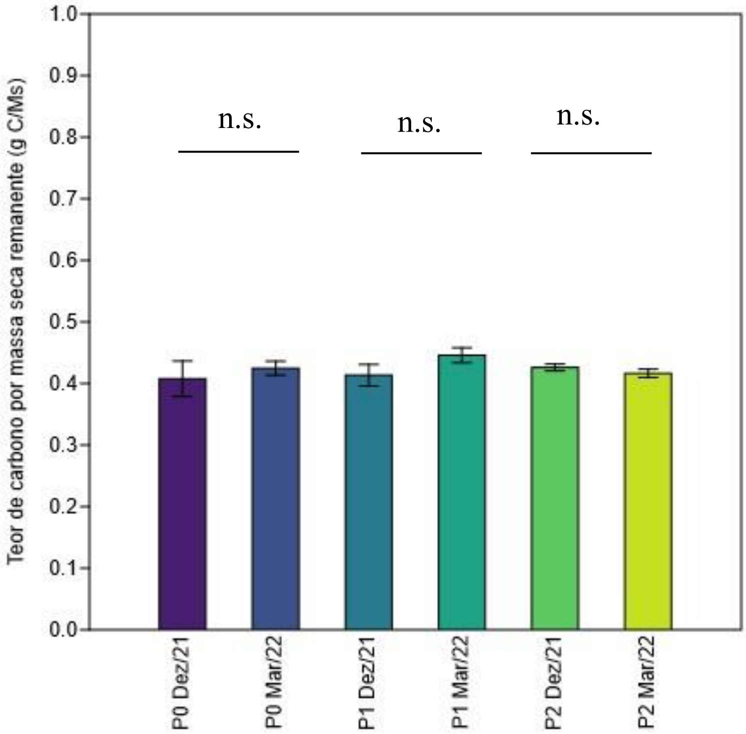


Source: author, 2024.

4.2 Carbon content in litter as sink

The carbon content in the remaining dry leaf mass (g C/Ms) of the decomposition pockets did not differ ($p<0.05$) in the plots at the edge (P0) ($F1.8 = 0.31$; $p = 0.594$), intermediate (P1) ($F1.8=2.30$; $p=0.168$) and inland (P2) ($F1.8=1.18$; $p=0.309$) between December 2021 and March 2022. The seasonal effect was not observed ($p<0.05$) for the carbon content between the consecutive spring and summer seasons. The carbon content in the remaining dry mass did not differ between the three plots in December/2021 (spring) ($F2.12=0.23$; $p=0.801$) or in March/2022 (summer) ($F2.12=2.14$; $p=0.161$) (Figure 9).

Figure 9. Carbon content ($p<0.05$) in the dry leaf mass remaining in the bags collected in December/2021 (spring) and March/2022 (summer) in the edge (P0) and interior (P1 and P2) plots of the native forest fragment at Fazenda Patioba, Alagoinhas - Bahia (Brazil).



Source: authorial, 2022

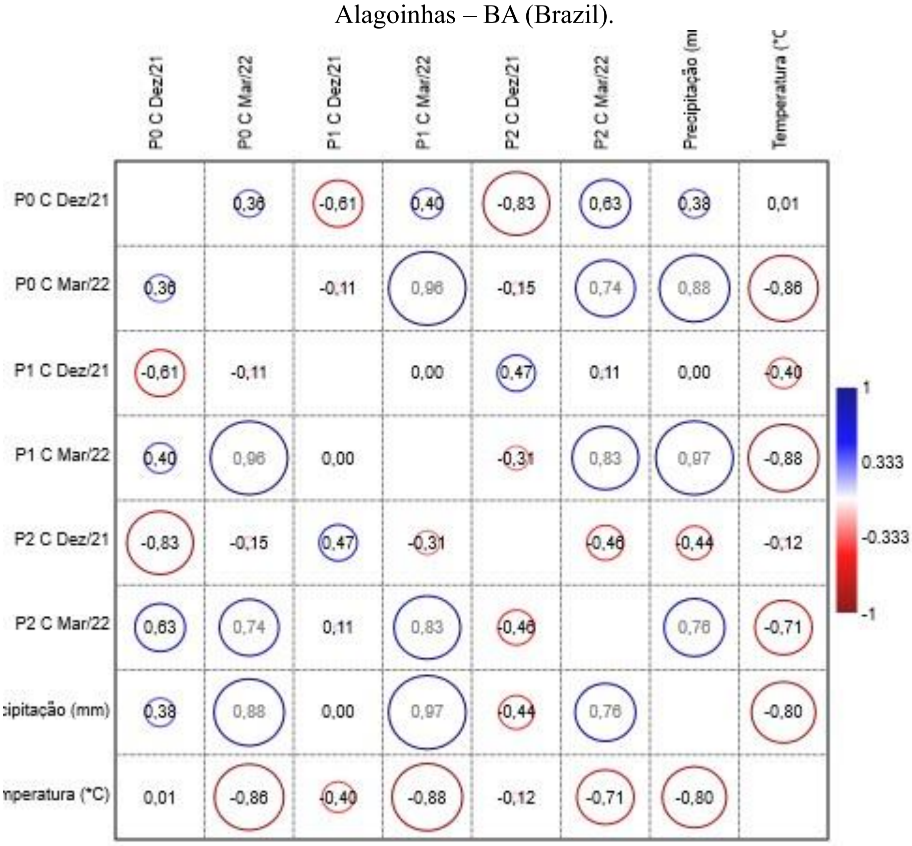
The carbon present in the remnant dry leaf mass is justified by the decomposition-resistant lignin. Areas with anthropogenic interference, such as the edge, receive more sunlight, which also contributes to the photo degradation of organic matter. The carbon analysis must be done over a longer period of time that includes the four climatic seasons to investigate possible seasonal effects. The litter of the plots works as a carbon sink, which guarantees for a certain time the fixation of carbon in the biogeochemical cycle in the forest fragment. In the preserved forest, with greater diversity and abundance of decomposers, the litter deposited on the ground, also protected by the tree canopy, increases moisture retention, contributing to the leaching of the leaf mass and accelerating the decomposition process.

The leaf litter has its peculiarities and its organic constituents reflect the variability of the plant species that make up the local native flora. Yue *et al*. (2016) showed that the decomposition of litter in a forest depends on the different decomposition rates of the biopolymers present in the species that make up the litter, showing greater resistance to degradation by lignin and greater degradation facility for cellulose in a humid place. Boerjan, Ralph and Baucher (2003) point out that the compound lignin makes up about 30% of the carbon found in litter residue after 2 years or more of decomposition.

Leaf litter is made up of different parts of vegetables. What is present in this material are components rich mainly in cellulose, hemicellulose and lignin. The action of lignin likely has a great influence on the result because its branched carbon chain has a higher amount of carbon (Yue *et al*., 2016). Lignin is a polymer made up of phenylpropanoid units, which have a hydrophobic character, therefore more difficult to retain water molecules for hydration and leaching. Furthermore, longer-lived litter samples may contain proportionally more lignin than more hydrophilic structural polymers (Boerjan; Ralph; Baucher, 2003).

Rainfall and temperature exerted promoting and inhibiting effects ($p<0.05$), respectively, on the carbon content in the dry leaf mass of litter in the three plots (P0, P1 and P2) in March 2022 (summer), thanks to the moisture retained in the previous spring (Figure 10).

Figure 10. Pearson's correlation ($p<0.05$) between precipitation (mm), temperature (ºC) and carbon (g C) in the remaining dry leaf mass of the decomposition pockets, collected in December/2021 (spring) and March/2022 (summer), in the edge (P0) and inland (P1 and P2) plots of the native forest fragment at Patioba Farm, Alagoinhas – BA (Brazil).



Source: author, 2024.

4.3 Terrestrial invertebrates and their functional groups

The importance of carbon lies in the production of body biomass of terrestrial invertebrates, which inhabit the litter, and also in some calcium carbonate structures, such as the shells of Gastropoda.

Specimens were collected, mainly of the orders Isopoda and Scolopendridae, occurring in the 3 plots, but in the juvenile phase, showing the decomposition pocket as an additional protection niche in the litter (Table 4).

Table 4. *Checklist* of invertebrates in the decomposition pockets of the native forest fragment at Patioba Farm, Alagoinhas - Bahia (Brazil). P0 and P2: May, June and July/2022; P1: June/2022.

Class	Order	Family/Subfamily	Genus/Species	Plot P0	Plot P1	Plot P2
Malacostraca	Isopoda	Philoscidae	*Philoscia muscorum*	7	2	10
Insecta	Dictyoptera/Isoptera	Blattaria/Blaberidae				1
	Lepidoptera Larva		Larva	1		
	Collembola			1		
	Coleoptera					1
			Sp. 1			1
			Sp. 2			1
	Diptera					1
	Hemiptera	Aphididae		2		2
	Dermaptera				1	
	Hymenoptera				6	
Araneae	Araneae		Sp. 3		1	
			Sp. 4			1
	Opiliones					1
Diplopoda	Scolopendridae			4	1	5
			Young Sp. 5			3
Oligochaeta				7		
Gastropoda			Sp. 6	5		
			Sp. 7	2		
			Sp. 8			
			Shell Sp. 9		1	1
			Sp. 10			1
			Sp. 11			2
	Abundance in decomposition pockets			**29**	**13**	**30**

Source: author, 2024.

The edge (P0) and inland (P2) plots had accumulated invertebrate diversity records for 3 months (May, June, and July/2022), while the intermediate plot (P1) presented only 1-month data (June/2022) (Table 5).

The Simpson and Shannon diversity indices were high but did not differ ($p<0.05$) between the plots of the forest fragment (Tables 5 and 6). The richness indices of Chao and iChao were higher in the internal plots (P1 and P2) of the fragment (Table 5).

The high diversity indices of Simpson and Shannon indicated that the forest fragment plots have a rich variety of species, being a positive sign for the quality of the ecosystem. As these indices did not differ ($p<0.05$) between the plots, it can be inferred that the diversity of species is relatively homogeneous throughout the fragment regardless of the location of the plots. On the other hand, the richness indices of Chao and iChao, which measure the number of species present in a given environment, were higher in the internal plots (P1 and P2) of the fragment. Indoor areas are more protected from disturbance compared to the edge, offering favorable conditions for the survival and reproduction of species, such as less direct exposure to sunlight, prolonged moisture retention, and protection from predators.

Table 5. Indices of diversity and richness of terrestrial invertebrates in the decomposition pockets of the native forest fragment at Patioba Farm, Alagoinhas - Bahia (Brazil). P0 and P2: May, June and July/2022; P1: June/2022.

Indexes	P0 Edge	Intermediate P1	Iland P2
Taxa_S	8	7	13
Individuals	29	13	30
Dominance_D	0,1478	0,2051	0,1379
Simpson_1-D	0,8522	0,7949	0,8621
Shannon_H	1,9840	1,8620	2,3630
Evenness_e^H/S	0,9092	0,9196	0,8173
Brillouin	1,5470	1,1750	1,7200
Menhinick	1,4860	1,9410	2,3730
Margalef	2,0790	2,3390	3,5280
Equitability_J	0,9542	0,9569	0,9213
Fisher_alpha	3,6520	6,1820	8,7210
Berger-Parker	0,2414	0,4615	0,3333
Floor-1	8,32	11,62	22,02
iChao-1	8,97	18,54	30,06
ACE	9,17	22,21	33,49
Squares	8,72	15,40	25,06

Source: author, 2024.

The Simpson and Shannon diversity indices of the invertebrates showed no differences between the plots (Table 6).

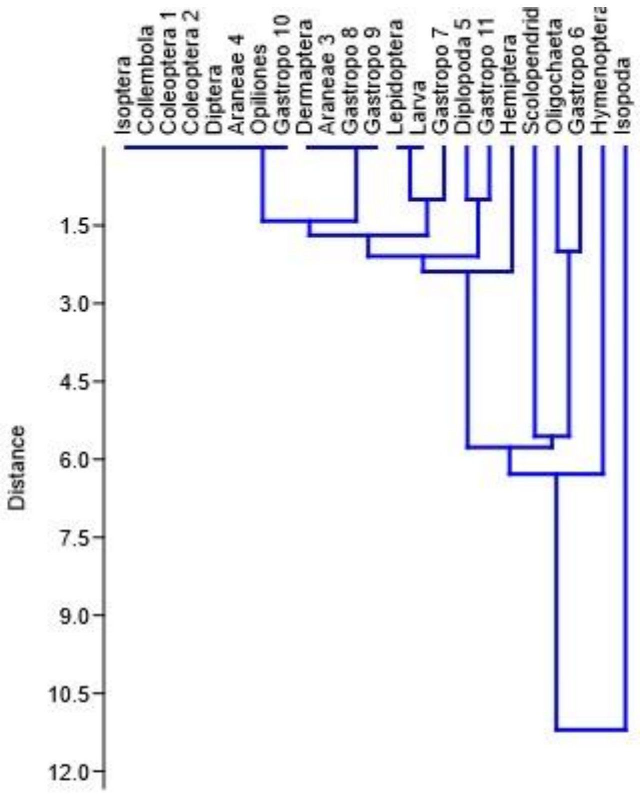
Table 6. Test ($p<0.05$) of the Simpson and Shannon diversity indices of terrestrial invertebrates in the decomposition pockets of the native forest fragment at Patioba Farm, Alagoinhas - Bahia (Brazil). P0 and P2: May, June and July/2022; P1: June/2022.

	Plots		
Indexes	**Edge (P0) X Intermediate (P1)**	**Edge (P0) X Inland (P2)**	**Intermediate (P1) X Inland (P2)**
Simpson	p = 0.4457	p = 0.8578	p = 0.4149
Shannon	p = 0.4392	p = 0.1908	p = 0.1133

Source: author, 2024.

Considering the study area, the *cluster analysis* by taxonomic groups showed the predominance of Isopoda, followed by Hymenoptera and Gastropoda/Oligochaeta, Scolopendridae and other groups (Figure 11).

Figure 11. Cluster analysis (taxonomic groups) of invertebrates captured in the decomposition pockets of the native forest fragment at Patioba Farm, Alagoinhas - Bahia (Brazil). P0 and P2: May, June and July/2022; P1: June/2022.



Source: author, 2023.

The decomposition pockets showed an invertebrate fauna similar in diversity to the fauna associated with litter, but with the occurrence of young individuals in the P2 inland plot in the months of May and June/2022, both detritivorous prey (Isopoda) and predators (Diplopoda, Scolopendridae) (Figure 12).

Figure 12. Invertebrates captured in the decomposition pockets of the native forest fragment on the Farm Patioba, Alagoinhas - Bahia (Brazil). Legend: 1. Isopoda (young), 2. Coleoptera, 3. Diptera, 4. Araneae, 5. Diplopoda (young), 6 and 7. Oligochaeta, 8 to 10. Gastropoda, 11. Scolopendridae, 12. Lepidoptera (larva).

Source: authorial, 2023.

Litter and soil invertebrates are influenced by environmental factors, such as humidity and temperature. However, edge effects, which cause environmental variations, can influence the dynamics of edaphic fauna (Correia; Oliveira, 2000; Moço *et al.*, 2005; Menezes *et al.*, 2010; Machado, 2011). The relatively higher amount of lignin in a sample is also based on the difficulty of its decomposition by invertebrates, due to its recalcitrant characteristic.

Sayer *et al.*, 2020 indicated that litter quality depends on its organic and inorganic composition, influencing interactions with soil fauna and consequently the decomposition rate. The action of microorganisms can be repressed by the presence of lignin.

Terrestrial invertebrates occurred among the six functional groups of predators, detritivores, phytophages, saprophages, coprophages and bioturbators. The taxonomic levels were considered at the order level to contemplate the diversity found in the decomposition pockets (Table 7). Similar data were recorded by Neves (2023), Sá (2023), and Silva (2023) concerning the invertebrates inhabiting the litter.

Table 7. Functional groups of invertebrates collected with litter in the plots of the Atlantic Forest fragment at Patioba Farm. Alagoinhas, Bahia (Brazil).

Order	Predator	Phytophagus	Detritivore	Saprophage	Coprophage	Bioturbador
Isopoda			X	X	X	
Isoptera			X	X		
Lepidoptera		X	X			
Larva (Insecta)			X			
Collembola			X			X
Coleoptera	X	X	X	X	X	X
Diptera	X					X
Hemiptera (Aphididae)		X				
Dermaptera	X					
Hymenoptera	X					
Araneae	X					
Opiliones	X					
Scolopendridae	X					
Oligochaeta						X
Gastropoda			X			

Source: authorial, 2023.

Exposure to light contributes to the reduction of certain organisms that assume the role of decomposers. In the inland area, the treetops protect the soil and favor a more humid and protected environment for a greater diversity of niches and their occupants, accelerating decomposition.

Invertebrates make up an abundant taxon in forest ecosystems, playing an essential role in nutrient decomposition and cycling (Rosa *et al*., 2017). Organisms act in functional groups and perform primordial functions for the ecosystem, detritives act in the fragmentation of plant material; predators regulate the populations of other invertebrates; bioturbators promote soil fertilization (Parron *et al*., 2015). These plant-animal interactions contribute to the natural regeneration of the forest environment (Araújo, 2012).

Predatory organisms, such as beetles, ants and spiders, stand out for controlling the populations of other invertebrates, helping to maintain balance in the food chain (Melo *et al*., 2009).

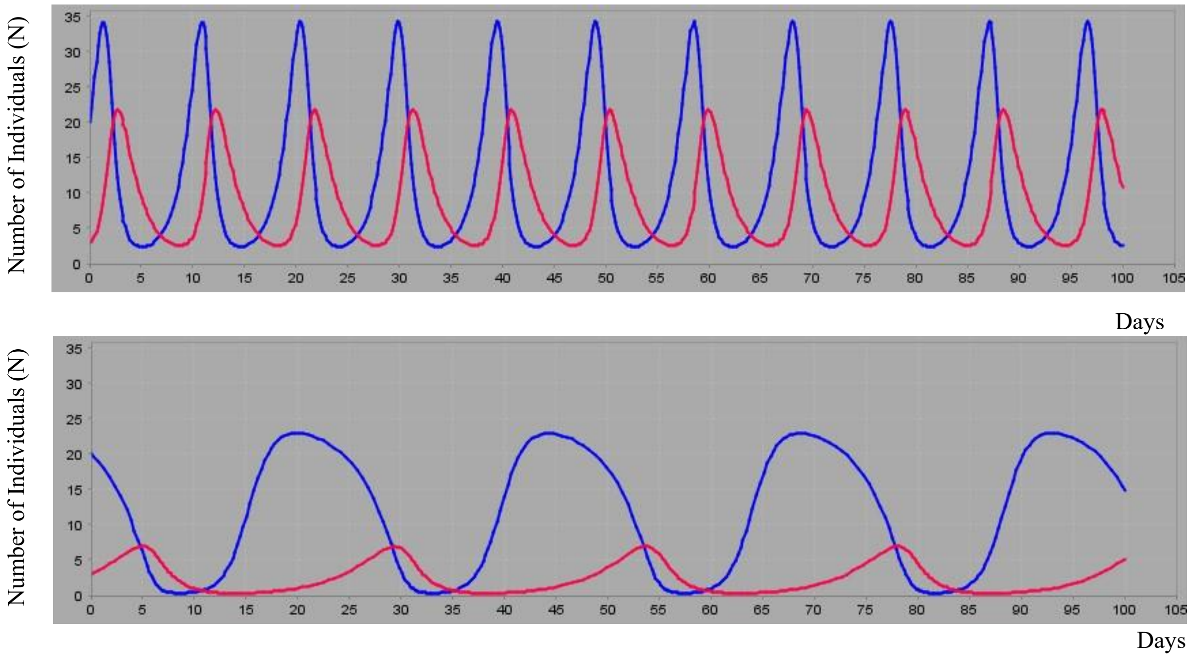
Detritivores, such as isopods, cockroaches, ants, and gastropods, have the crucial role of fragmenting the organic remains of leaf litter, feeding on decomposing plant matter, animal feces, and other waste, facilitating the process of decomposition of organic matter on the forest floor (Quadros, 2010; Parron *et al*., 2015).

Cockroaches and ants act as bioturbators by moving leaf litter and soil, accumulating organic material that serves as a nutrient reserve, promoting the recirculation of organic matter, and making the soil more fertile (Offenberg et al., 2019). In addition, these organisms contribute to the increase in soil porosity, aeration and water input, contributing to the maintenance of the survival of various plant and animal organisms (Brown *et al*., 2015).

In the months of May to July 2022, in the decomposition pockets, there were fewer predators (N=4) than prey (N=25) from the edge plots (P0); more predators (N=9) than prey (N=4) in the intermediate portion (P1) and fewer predators (N=13) than prey (N=17) in the inland portion (P2) of the forest fragment (Figures 13 and 14).

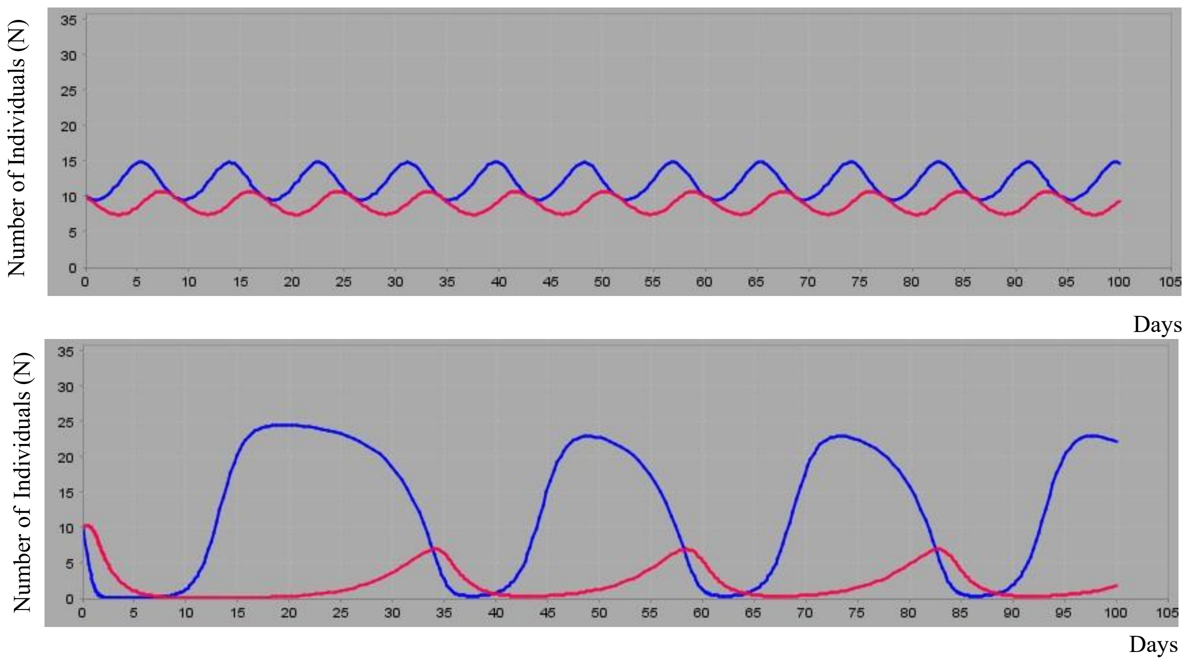
The population dynamics of terrestrial invertebrates were represented in simple Lotka-Volterra simulations showing rapid recovery (5 days) of prey populations when the stress factor ceases when the number of predators is small or proportional (Figures 13 and 14). In this way, predators can maintain biological control of prey populations without compromising their litter decomposition activity, keeping it as a layer in dynamic renewal between mineralization contribution that protects the soil and acts as a store to sequester carbon from the atmosphere through plant biomass and subsequent transfer to the soil.

Figure 13. Simple Lotka-Volterra simulation model for the predator-prey ratio (4:25) in continuous independent (top) and prey-dependent (bottom) flow at the edge (P0 plot) of the native fragment.



Source: authorial, 2023.

Figure 14. Simple Lotka-Volterra simulation model for the predator-prey ratio (13:17) in continuous independent (upper) and prey-dependent (lower) flow in the interior (P2 plot) of the native fragment.



Source: authorial, 2023.

In the simple Lotka–Volterra model, the two first-order nonlinear differential equations describe the interaction between two species, prey and predator. This model assumes that species have homogeneous populations, disregarding factors such as age and sex; The predator feeds exclusively on the prey, and the prey obtains its nutrients from the resources available in the environment. In addition, external factors that may limit population dynamics are not considered (Souza, 2017).

The decomposition dynamics carried out by the microbiota and invertebrates maintain the ecosystem processes of nutrient cycling and nutrition of the trophic web, necessary for the restoration and/or conservation of biodiversity. In a preserved forest environment, the continuous process of litter decomposition transforms the forest fragment into a carbon sink.

5. CONCLUSIONS

The leaf litter of the native Atlantic Forest at Patioba Farm, in Alagoinhas - Bahia (Brazil), works as a carbon sink.

From December 2021 to March 2022, there was a decay of the dry mass remaining in the pockets, which reflected the decomposition of litter in a similar pattern between the edge (P0) and intermediate (P1) plots, however, this seasonal effect was not observed in the inland plot (P2) between consecutive spring and summer weather seasons.

There was a seasonal effect on the carbon content in the plots between December 2021 and March 2022.

With the decomposition of litter, part of the soluble carbon is released by respiration in the form of CO_2, and another part of the structural carbon is immobilized in the biomass of the rhizosphere or edaphic fauna, making the soil another carbon sink.

Among terrestrial invertebrates, there is a rapid recovery (5 days) of prey populations, when the stress factor ceases when the number of predators is small or proportional. In this way, predators can maintain biological control of prey populations without compromising their litter decomposition activity, keeping it as a layer in dynamic renewal between input-mineralization that protects the soil and functions as a stock in the sequestration of carbon from the atmosphere by plant biomass and a subsequent transfer to the soil.

The decomposition dynamics carried out by the microbiota and invertebrates maintain the ecosystem processes of nutrient cycling and nutrition of the trophic web, necessary for the restoration and/or conservation of biodiversity. In a preserved forest environment, the continuous process of litter decomposition transforms the forest fragment into a carbon sink.

This research is relevant for demonstrating the ecosystem service that litter plays as a carbon sink required to maintain nutrient cycling and stabilize the microclimate, minimizing the impacts of environmental degradation and preserving native forests. An incentive for new investigations.

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