

## Artigos

# Time effect and agriculture land use on cellulose breakdown process

Efeito do tempo e áreas de uso agrícola no processo de decomposição de celulose

Renan de Souza Rezende<sup>1</sup> 

Bruna Valencio Cavallet<sup>1</sup> 

Alana Maria Polesso<sup>1</sup> 

Edpool Rocha Silva<sup>1</sup> 

Carolina Riviera Duarte Maluche Baretta<sup>1</sup> 

<sup>1</sup>Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brazil

## ABSTRACT

The soil conservation in agriculture may contribute to productivity and sustainable production. The objective was to measure the mass loss rate of cellulose decomposition process in agriculture systems, in different cultivation times (more than 30 years vs. less than 10 years) considering forest in conservation unit and close anthropic forest as control systems. We used substrate bags of two mesh sizes (0.5 mm vs. 10 mm) in soil surface for 30, 60 and 90 days in all systems (agriculture systems vs. close anthropic forest and forest in conservation unit). Cellulose decomposition ecosystem service decreased by a quarter (effect size range  $\pm$  -22 to -26%) in the studied agriculture systems compared to forests systems, highlighting the cotton strip breakdown process as a good ecological indicator. High species richness and plant strata in forest systems increase the mass loss compared to agriculture systems. The difference between 10 and 30 year of agriculture systems ranges from 3% (total decomposition) to 7% (microbial decomposition), lower in 30-year systems. Also, forest fragments near agriculture systems are refuge for detritivore macrofauna and may retain the ecosystem service on these productive areas.

**Keywords:** Cellulose breakdown; Cotton strips decomposition; Standardized methods



## RESUMO

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A conservação do solo em áreas agrícolas pode contribuir para a produtividade e produção sustentável. Nosso objetivo foi avaliar a taxa de perda de massa no processo de decomposição da celulose em sistemas agrícolas, em diferentes épocas de cultivo (mais de 30 anos vs. menos de 10 anos) considerando floresta em unidade de conservação e floresta antropizadas próxima, como sistemas de controle. Foram utilizados sacos de duas malhas (0,5 mm vs. 10 mm) na superfície do solo por 30, 60 e 90 dias em todos os sistemas (agrícolas vs. floresta antrópica e floresta em unidade de conservação). A decomposição diminuiu em um quarto (variação o tamanho do efeito  $\pm$  -22 a -26%) nos sistemas agrícolas estudados em comparação com os sistemas florestais, evidenciando o processo de decomposição como um bom indicador ecológico. A alta riqueza de espécies e estratos vegetais em sistemas florestais aumentaram a perda de massa em comparação com sistemas agrícolas. A diferença entre 10 e 30 anos nos sistemas agrícolas variou de 3% (na decomposição total) a 7% (na decomposição microbiana), diminuindo em sistemas de 30 anos de uso. Além disso, percebemos que fragmentos florestais próximos a sistemas agrícolas podem funcionar como refúgios para macrofauna detritívora e assim ajudar a preservar este serviço ecossistêmico em áreas produtivas.

**Palavras-chave:** Decomposição de celulose; Decomposição de tiras de algodão; Métodos padronizados

## 1 INTRODUCTION

Brazil and agriculture planting, mainly soybean, stand out regarding the relationship between socio-environmental problems and high production of commodities (SILVA JUNIOR; LEONEL-JUNIOR; ROSSI; CORREIA FILHO; SANTIAGO; OLIVEIRA-JÚNIOR; TEODORO; LIMA; CAPRISTO-SILVA, 2020) due to the large and fast extension of these systems (CATTELAN; DALL'AGNOL, 2018). In this way, the socio-environmental issues of agricultural systems have a great relevance in discussions on land use occupation (CATTELAN; DALL'AGNOL, 2018) and contribution to economic issues (PASHAEI KAMALI; MEUWISSEN; DE BOER; VAN MIDDELAAR; MOREIRA; OUDE LANSINK, 2017). This socio-environmental implications of agricultural systems are especially relevant in commodity exporting countries, as Brazil (PASHAEI KAMALI; MEUWISSEN; DE BOER; VAN MIDDELAAR; MOREIRA; OUDE LANSINK, 2017; ROMIG; GARLYND; HARRIS, 1997). Therefore, there arises the need to test new techniques for sustainable development for agriculture production units and ecological indicators that make it possible to evaluate these techniques (BURKHARD; LILL, 2008; ROMIG; GARLYND; HARRIS, 1997).



Suitable indicators (for more see also DALE; BEYELER, 2001; OLIVEIRA; ENGEL; LOIOLA; MORAES; VISMARA, 2021) should consider the complex ecosystems dimensions on temporal and spatial scales (BURKHARD; LILL, 2008; CARDOSO; VASCONCELLOS; BINI; MIYAUCHI; SANTOS; ALVES; PAULA; NAKATANI; PEREIRA; NOGUEIRA, 2013), due to flows of species (BROOKER; GEORGE; HOMULLE; KARLEY; NEWTON; PAKEMAN; SCHÖB, 2021), materials and energy in the ecosystems (HALL; RUSSELL; MOORE, 2019; REZENDE; BERNARDI; GOMES; MARTINS; HAMADA; GONÇALVES, 2021). The environmental interactions in physical and biological compartments drive the ecological processes (FRANZLUEBBERS, 2005; FERREIRA; BOYERO; CALVO; CORREA; FIGUEROA; GONÇALVES; GOYENOLA; GRAÇA; HEPP; KARIUKI; LÓPEZ-RODRÍGUEZ; MAZZEO; M'ERIMBA; MONROY; PEIL; POZO; REZENDE; TEIXEIRA-DE-MELLO, 2019; REZENDE; CARARO; BERNARDI; CHIMELLO; LIMA-REZENDE; ALBENY-SIMÕES; DAL-MAGRO; GONCALVES, 2021), which may allow a systemic and integrated view (BROOKER; GEORGE; HOMULLE; KARLEY; NEWTON; PAKEMAN; SCHÖB, 2021; HALL; RUSSELL; MOORE, 2019). In this way, the ecological processes are appropriate for metrics of ecological indicators (CARDOSO; VASCONCELLOS; BINI; MIYAUCHI; SANTOS; ALVES; PAULA; NAKATANI; PEREIRA; NOGUEIRA, 2013; OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018). Ecological indicators that assess less complex biophysical proxies, such as only community structure and physicochemical characteristics, may be less effective compared to ecological processes (CARDOSO; VASCONCELLOS; BINI; MIYAUCHI; SANTOS; ALVES; PAULA; NAKATANI; PEREIRA; NOGUEIRA, 2013; LUIS; VALDINAR; TALINE; DIEGO; ANGELICA; SIMÓN, 2019). This highlights the importance of ecological processes as indicators, which can be a useful tool to assess the effects of agriculture planting as no-till soybean, such as that on organic matter decomposition (OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018).

The ecological processes of organic matter decomposition may be a good alternative as ecological indicators to traditional methods (BLEICH; PIEDADE; MORTATI; ANDRÉ, 2015; REZENDE; CARARO; BERNARDI; CHIMELLO; LIMA-REZENDE; ALBENY-



SIMOES; DAL-MAGRO; GONCALVES, 2021; REZENDE; CARARO; CHIMELLO; LIMA-REZENDE; MORETTO; GONCALVES, 2023; TIEGS; CLAPCOTT; GRIFFITHS; BOULTON, 2013; TIEGS; COSTELLO; ISKEN; WOODWARD; MCINTYRE, 2019) and can be applied, as example, in no-till soybean systems (for more see also CAVALLET; SILVA; BARETTA; REZENDE, 2022). Substrate breakdown process shows a high effects on organic matter cycle and soil properties (PAINII-MONTERO, 2020; POKHREL; KINGERY; COX; SHANKLE; SHANMUGAM, 2021). Organic matter breakdown and decomposition may increase the mineral particles in soil and the soil water retention (LUIS; VALDINAR; TALINE; DIEGO; ANGELICA; SIMÓN, 2019; MARTIN-RUEDA; MUÑOZ-GUERRA; YUNTA; ESTEBAN; TENORIO; LUCENA, 2007). Consequently, the soil water retention may increase the availability of nutrients such as N, P and S, which also increases the ion retention capacity in soil (HALL; RUSSELL; MOORE, 2019; LUIS; VALDINAR; TALINE; DIEGO; ANGELICA; SIMÓN, 2019; MARTIN-RUEDA; MUÑOZ-GUERRA; YUNTA; ESTEBAN; TENORIO; LUCENA, 2007). Organic matter decomposition also decreases soil degradation over time, due to presence of some compounds at soil mixtures such as humus concentration (LUIS; VALDINAR; TALINE; DIEGO; ANGELICA; SIMÓN, 2019). Finally, normal rates of substrate decomposition may stabilize and preserve the good soil structure and soil biological properties over time (HALL; RUSSELL; MOORE, 2019; POKHREL; KINGERY; COX; SHANKLE; SHANMUGAM, 2021).

Substrate decomposition is a temporal continuous process in trophic webs and it may drive by substrate and soil chemical relations with the biological communities (COTRUFO; GALDO; PIERMATTEO, 2010). Higher nutrient concentrations in plant organic matter and soil may accelerate the decomposition process, as well as lower concentrations of recalcitrant organic compounds (e.g. fibers, lignin and cellulose) and secondary compounds (e.g. tannins and polyphenols) (CAPELLESSO; SCROVONSKI; ZANIN; HEPP; BAYER; SAUSEN, 2016; COTRUFO; GALDO; PIERMATTEO, 2010). Also, healthy microbial and invertebrate communities are essential for organic matter decomposition and, consequently, for the yield in agriculture systems (PEARSONS;



TOOKER, 2021). Detritivore or decomposer invertebrates directly use the organic matter tissue, and geophagists or bioturbators may improve soil structure, accelerating the decomposition process (BURGHARDT; BRADFORD; SCHMITZ, 2018). The decomposer invertebrate activity decreasing organic matter size may also increase organic matter incorporation into the soil and facilitate microbial activity (Joly and others 2020). Microbial activity in decomposition processes contributes to soil nutrient cycling and to the fertility and production of agroecosystems (COONAN; KIRKBY; KIRKEGAARD; AMIDY; STRONG; RICHARDSON, 2020). Previous studies have compared organic matter breakdown in different sized bags showing a decrease of the rates in micromesh and increase in macromesh by indirect effects of meso- and macrofauna (BRADFORD; TORDOFF; EGGERS; JONES; NEWINGTON, 2002). The imbalance in biological communities caused by microbial pathogens and pest insects may result in agricultural production limitations (COTRUFO; GALDO; PIERMATTEO, 2010).

However, despite consensus around the general merit of accounting for ecological processes as ecological indicators (OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018), this is less used in subtropical systems (CARDOSO; VASCONCELLOS; BINI; MIYAUCHI; SANTOS; ALVES; PAULA; NAKATANI; PEREIRA; NOGUEIRA, 2013; OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018; CAVALLET; SILVA; BARETTA; REZENDE, 2022). The use of ecological processes as ecological indicators is more frequent in aquatic systems in subtropical zones (FERREIRA; BOYERO; CALVO; CORREA; FIGUEROA; GONÇALVES; GOYENOLA; GRAÇA; HEPP; KARIUKI; LÓPEZ-RODRÍGUEZ; MAZZEO; M'ERIMBA; MONROY; PEIL; POZO; REZENDE; TEIXEIRA-DE-MELLO, 2019; TAYLOR; LIZOTTE; TESTA, 2019; TIEGS; COSTELLO; ISKEN; WOODWARD; MCINTYRE, 2019). In literature are several tropically studies focused on organic matter breakdown in terrestrial systems (CAPELLESSO; SCROVONSKI; ZANIN; HEPP; BAYER; SAUSEN, 2016; COTRUFO; GALDO; PIERMATTEO, 2010), and several more comparing tropical and temperature regions (HENEGHAN; COLEMAN; ZOU; CROSSLEY; HAINES,



1999). In this way, the organic matter decomposition process may offer great potential to ecological indicators for agriculture, as soybean production management over time (OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018), mainly through the detection of management techniques which favor the conservation of soil physicochemical and biological conditions over long-term use (ROMIG; GARLYND; HARRIS, 1997) in subtropical systems. Our objective was to measure the mass loss rate of cotton decomposition process (standardizing organic matter quality) in agriculture systems with different cultivation times (more than 30 years vs. less than 10 years) considering forest in conservation unit and close anthropic forest as control.

## 2 MATERIAL AND METHODS

### 2.1 Study systems

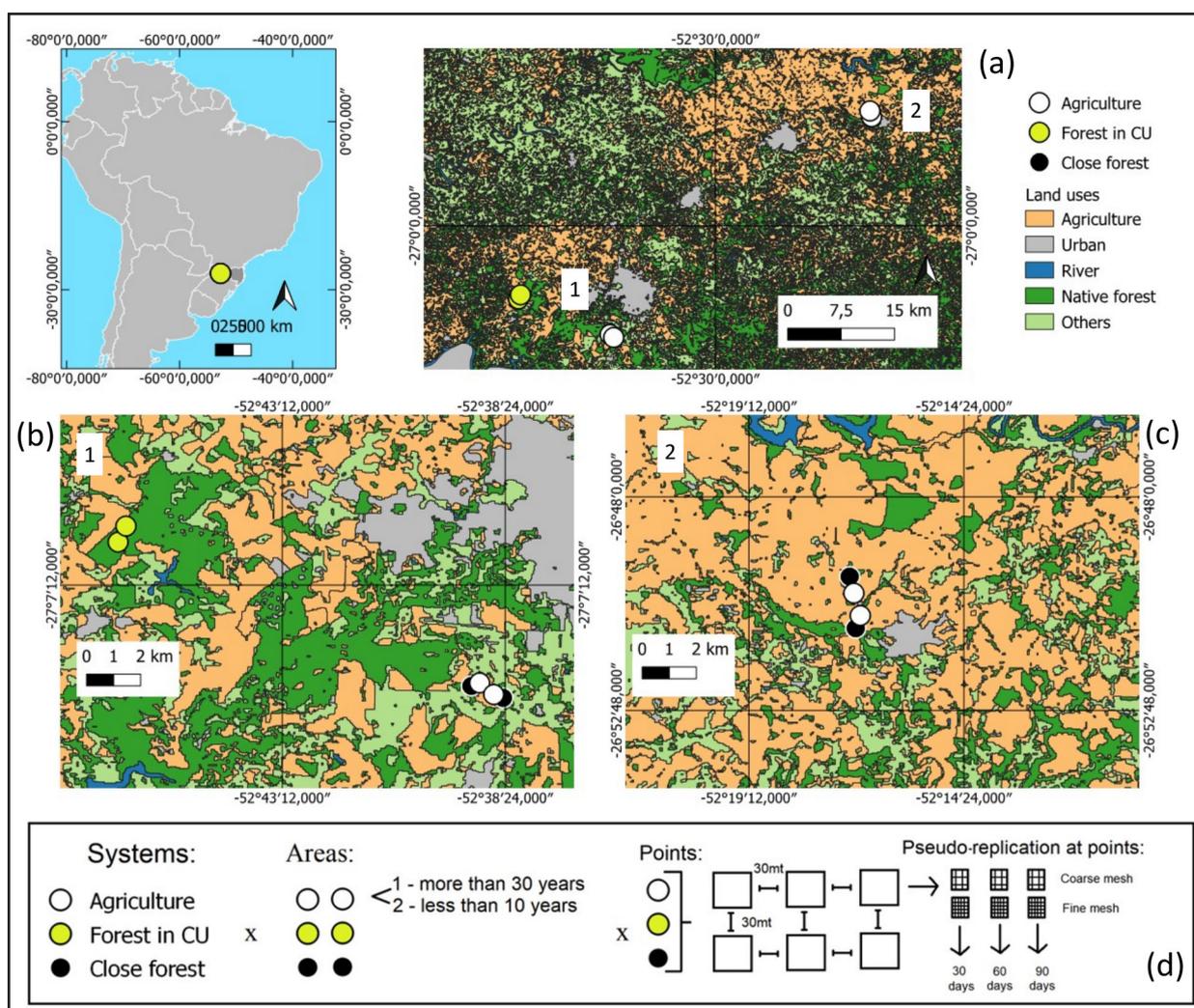
The study of agriculture systems with more than 30 years ( $n = 2$  areas) and close forest 1 ( $n = 2$  areas in a paired design) was conducted in Faxinal dos Guedes city (Fig 1a and b), in the west of Santa Catarina state ( $26^{\circ}51'10''S$  and  $52^{\circ}15'36''W$ , mean altitude of 1005 m). The region's climate of agriculture systems with more than 30 years is characterized as humid temperate (*Cfb*) with an average annual temperature ranging from 10 to 16°C and precipitation of 2255 mm (PEEL; FINLAYSON; MCMAHON, 2007) (Table 1; Fig 1a and b).

The study of agriculture systems with less than 10 years ( $n = 2$  areas) and close forest 2 ( $n = 2$  areas in a paired design) was carried out in Chapecó city ( $27^{\circ}9'19.50''S$  and  $52^{\circ}38'58.40''W$ , mean altitude of 661 m). The climate of agriculture systems with less than 10 years was Subtropical *Cfa* type (mesothermal, humid and with hot summer), according to Köppen's classification, with annual average temperature range of 18-19 °C and annual average precipitation of 2000 mm (PEEL; FINLAYSON; MCMAHON, 2007). The soil was Red Latosol (Oxisol) with occurrence on gently undulating relief and clayey texture (Table 1; Fig 1a and b).



The control forest in conservation unit (n = 2 areas) was in Guatambú city (27° 6'11.12"S and 52°46'43.97"W, mean altitude of 599 m), with same climate, annual average temperature, and annual average precipitation that Chapecó (previous description) in the west of Santa Catarina State and South of Brazil (Table 1; Fig 1 a and b).

Figure 1 – The study systems at land use distribution areas studied (a, b and c). Also, the simplified experimental design between systems, areas, points, and pseudo-replicas at points (d)



Source: Authors (2023)

In where: The study systems at land use distribution areas studied (a). Forest system in conservation unit are the yellow circles, close forest system are the black circles and agriculture system are the white (a, b and c). In the agriculture system, the areas with less than 10 years represented by 1 (b) and with more than 30 years are circles are represented by 2 (c). Also, the simplified experimental design between systems, areas, points, and pseudo-replicas at points (d).



Table 1 – Description of systems evaluated regarding the main land use between the years 2019 and 2020

System	Areas	Soil management
The agriculture systems with more than 30 years	Agriculture area 1	The cultivation has been carried out under no-till for 39 years, with a high plant diversity used in crop rotation and combinations of winter cover species [turnip ( <i>Raphanus sativus</i> ) + rye ( <i>Secale cereale</i> ) + oats ( <i>Avena strigosa</i> ) + millet ( <i>Pennisetum glaucum</i> )], and soybeans ( <i>Glycine max</i> ), corn ( <i>Zea mays</i> L.) and beans ( <i>Phaseolus vulgaris</i> ) in summer. The grain yield obtained in the 2019/2020 harvest was 4.283 kg per hectare of soybeans.
	Agriculture area 2	The cultivation has been carried out for more than 32 years, with a smaller contribution and plant diversity, also using crop succession in winter [oat ( <i>A. strigosa</i> ) + ryegrass ( <i>Lolium multiflorum</i> ) + wheat ( <i>Triticum aestivum</i> )] and soybean ( <i>G. max</i> ) in summer. The grain yield obtained in the 2019/2020 harvest was 4.326 kg per hectare of soybean.
The agriculture systems with less than 10 years	Agriculture area 3	Consolidated no-tillage system less than 9 years in the system. There is crop rotation with <i>Avena sativa</i> L., <i>Lolium multiflorum</i> L. and other species that have established themselves in the local seed bank with soybean ( <i>G. max</i> ) in summer. The grain yield obtained in the 2019/2020 harvest was 3.240 kg per hectare of soybean.
	Agriculture area 4	The vegetation cover was <i>Lolium multiflorum</i> L. and other species that established themselves from the local seed bank with soybean ( <i>G. max</i> ) in summer. In this same system there is a history of use for beef cattle and the addition of organic fertilizer from poultry substrate in pastures. The grain yield obtained in the 2019/2020 harvest was 3.422 kg per hectare of soybean. Consolidated no-tillage system less than 9 years in the system.
Forest areas close to Agriculture area		The four fragments of Mixed Ombrophilous Forest vegetation located near agricultural systems have some anthropization (abandoned 5 years ago) and had been used in the past for the extraction of yerba mate or for animal grazing for at least 3 years. Vegetation is composed of species native to the region, the main ones being: <i>Araucaria angustifolia</i> (Bertol.) Kuntze, <i>Cedrela fissilis</i> Vell., <i>Apuleia leiocarpa</i> (Vogel), <i>Nectandra megapotamica</i> (Spreng.) Mez, <i>Ilex paraguariensis</i> A. ST.-Hil, <i>Casearia sylvestris</i> Sw., <i>Prunus myrtifolia</i> (L.) Urb..
Native forest areas far from Agriculture area		Two fragments of native forest were composed of Mixed Ombrophilous Forest, with minimal human intervention in the conservation unit of Chapecó National Forest.

Source: Authors (2023)

## 2.2. Cotton breakdown process

The experiment used coarse-mesh bags (5 mm; 30 x 30 cm), which can be accessed by both microorganisms and invertebrate detritivores and allow quantifying total breakdown, while fine-mesh bags (0.05 mm; 30 x 30 cm) exclude detritivores and allow quantifying microbial breakdown. Each bag contained 1 g ( $\pm 0.1$ ) of cotton strip then dried in an oven at 50°C. The cotton strip shows composition of  $\pm 95\%$  cellulose with 12 x 6 cm in size and average weight of  $\pm 2.25$  grams. The samples were incubated on soil



surface. Standardized substrates provide a comparable pattern among the different systems studied, due to low chemistry complexity compared to leaf litter (COLAS; WOODWARD; BURDON; GUÉROLD; CHAUVET; CORNUT; CÉBRON; CLIVOT; DANGER; DANNER; PAGNOUT; TIEGS, 2019; TIEGS; CLAPCOTT; GRIFFITHS; BOULTON, 2013; TIEGS; COSTELLO; ISKEN; WOODWARD; MCINTYRE, 2019). Standardized substrates are used to decrease organic matter effect due to the variation of labile and recalcitrant compounds (COLAS; WOODWARD; BURDON; GUÉROLD; CHAUVET; CORNUT; CÉBRON; CLIVOT; DANGER; DANNER; PAGNOUT; TIEGS, 2019; TIEGS; CLAPCOTT; GRIFFITHS; BOULTON, 2013; TIEGS; COSTELLO; ISKEN; WOODWARD; MCINTYRE, 2019). Also, according to these authors, the nutrients use that are not initially present in the standardized substrates (e.g. N and P) by the decomposing community is provided by the environment (dissolved or particulate). This process increases the environment characterization by decomposition of the standardized substrates and reinforces its effectiveness with an environmental impact assessment tool. Another advantage of using cotton strips as an environmental assessment tool is the quick assessment (days-months) compared to litter (months-years).

The samples were incubated at two different areas for each system, by six points for each area being spaced by 30 m (Fig 1c). At each point, triplicates were used for each mesh (fine and coarse), with three bags incubated on the surface in contact with the soil. The incubation period started during the soybean crop germination and ended after 30, 60 and 90 days, totalizing 360 bags (10 area x 6 points per area x 2 meshes x 3 times; Fig 1c). On removal from the treatments, the bags were placed individually into insulated plastic bags and transported in thermal containers ( $\pm 4$  °C) to the laboratory. In the laboratory, detritus from cotton strip from the bags were washed with distilled water.

A disk (1.2 cm in diameter) of cotton strip detritus from each bag was extracted for determining remaining ash-free dry mass (AFDM; calculated after incineration in a muffle furnace at 550°C for 4 h). The remaining material was oven-dried at 60°C for 72 h to determine its dry mass (GRAÇA; BARLOCHER; GESSNER, 2005). In addition, a set of bags remained in the field to estimate the loss by transport and moisture in the



organic matter (correction factor). Initial mass was corrected by multiplication by the correction factor and the final mass by the AFDM. Cotton breakdown was quantified by proportion of mass loss (LML) = [(initial mass – final mass)/ initial mass].

### 2.3 Data analysis

Cotton breakdown rates ( $k$ ) were calculated using a negative exponential model ( $W_t = W_0 e^{-kt}$ ;  $W_t$  = remaining weight;  $W_0$  = initial weight;  $-k$  = decay rate;  $t$  = time) of the percent of mass lost over time (GRAÇA; BARLOCHER; GESSNER, 2005).

We compared the total cotton strip mass loss proportion between systems (forest in conservation unit vs. close forest vs. agriculture systems), mesh size (coarse vs. fine) and interaction between these factors by two-way factorial generalized linear mixed-effects analysis (GLMM; glmer function in lme4 package). We considered one random effect on areas replication nested with temporal replication (30, 60 and 90 days) for removing spatial and temporal pseudoreplication (CRAWLEY, 2007). "Systems" (forest in conservation unit vs. close forest vs. agriculture systems) is used to designate the average values of the areas, while "areas" (e. g. agriculture areas 1, 2, 3 and 4) is used to designate each individual location of a system. The p-values were obtained by likelihood ratio tests (Chi-square distribution) of the full model against a partial model without the explanatory variable (CRAWLEY, 2007).

All models were tested for error distribution by hnp function and package and corrected for over or under dispersion. Differences among the categorical variables (systems and mesh treatments) were assessed through orthogonal contrast analysis (CRAWLEY, 2007), which ordered (increasingly) and tested pairwise (with the closest values) and sequentially, by adding to the model the values with no differences and testing with the next (i.e., stepwise model simplification).

The control effect size was analyzed in a way analogous to the response ratio commonly used in meta-analysis (KORICHEVA; GUREVITCH; MENGENSEN, 2013) and recently used to evaluate ecological processes such as detritus decomposition (CORREA-ARANEDA; TONIN; PÉREZ; ÁLVAREZ; LÓPEZ-ROJO; DÍAZ; ESSE; ENCINA-MONTOYA; FIGUEROA; CORNEJO; BOYERO, 2020; LÓPEZ-ROJO; PÉREZ; POZO; BASAGUREN;



APODAKA-ETXEBARRIA; CORREA-ARANEDA; BOYERO, 2020b; LÓPEZ-ROJO; PÉREZ; BASAGUREN; POZO; RUBIO-RÍOS; CASAS; BOYERO, 2020a). In the effect size analysis, we tested the mass loss from cotton strips in “agriculture systems” of different managements treatments by ratios between each treatment and the control (forest in conservation unit and close forest) for the respective bag mesh (coarse and fine) and system (forest in conservation unit and close forest of each agriculture system). After, for consistent estimation of the magnitude of change from the null value, the values of cotton mass loss were log-transformed.

The ratios were calculated by the respective bag mesh (coarse vs. coarse and fine vs. fine) and systems (agriculture vs. close forest of each location and agriculture vs. mean values of forest in conservation unit) at points (CORREA-ARANEDA; TONIN; PÉREZ; ÁLVAREZ; LÓPEZ-ROJO; DÍAZ; ESSE; ENCINA-MONTOYA; FIGUEROA; CORNEJO; BOYERO, 2020; LÓPEZ-ROJO; PÉREZ; POZO; BASAGUREN; APODAKA-ETXEBARRIA; CORREA-ARANEDA; BOYERO, 2020b; LÓPEZ-ROJO; PÉREZ; BASAGUREN; POZO; RUBIO-RÍOS; CASAS; BOYERO, 2020a). Posteriorly, nonparametric bootstrapped 95% confidence intervals (1000 bootstrap replicates) were used (DAVISON; HINKLEY, 1997) to test whether the magnitude and direction for each treatment was different from those of the control by BCa method (in *boot* function and package from R software; R version 3.6.2) (CANTY; RIPLEY, 2016). All analyses were performed using R software (R Core Team 2022).

## 3 RESULTS

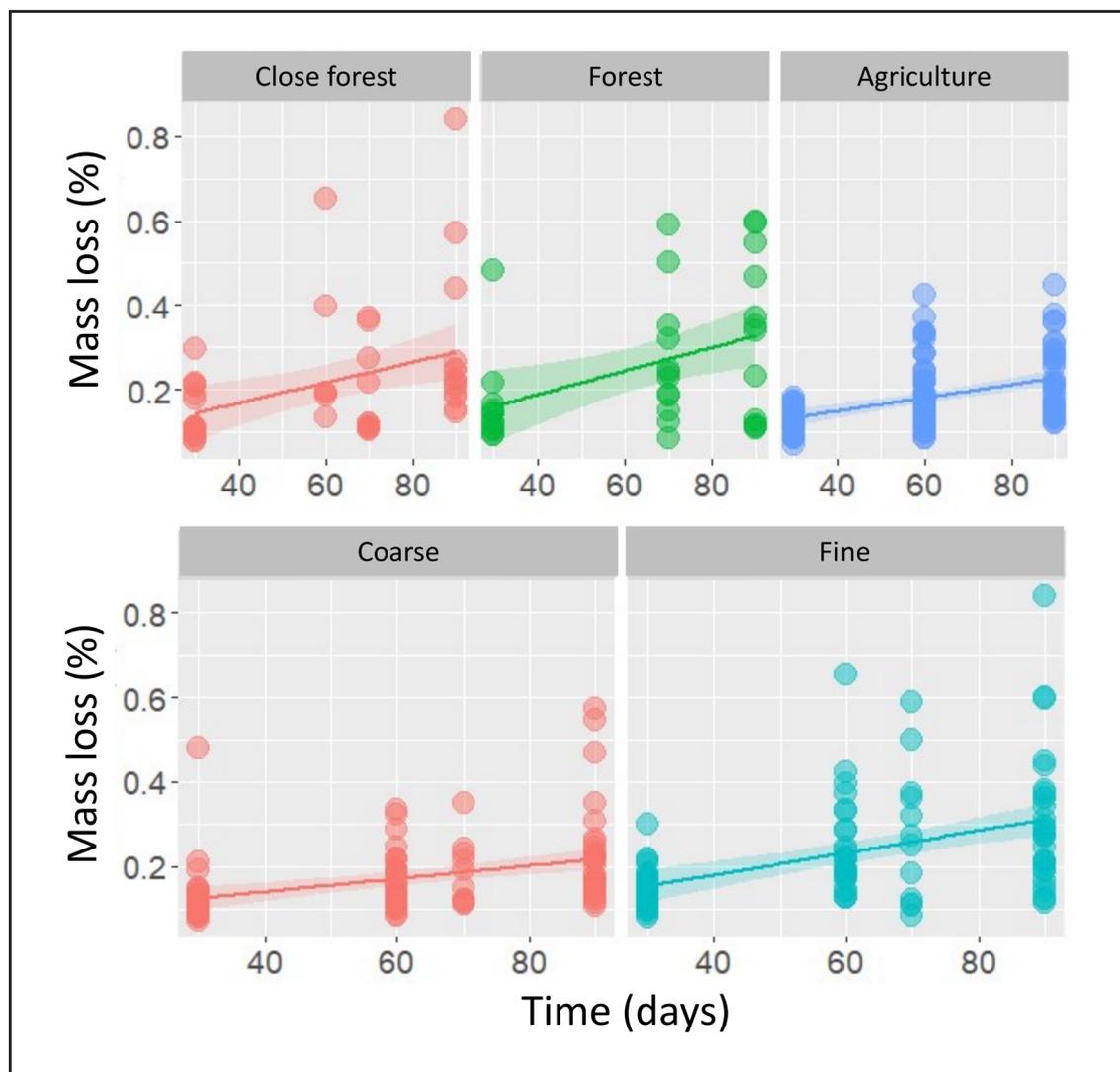
### 3.1 Mass loss

The forest in conservation unit ( $0.30 \pm 0.04$ ;  $k = -0.0088 \text{ d}^{-1}$ ) shows the highest cotton strip mass loss compared to close forest systems ( $0.28 \pm 0.04$ ;  $k = -0.0082 \text{ d}^{-1}$ ) followed by agriculture systems ( $0.19 \pm 0.01$ ;  $k = -0.0076 \text{ d}^{-1}$ ; Table 1; Figure 1). The cotton strip mass loss proportion was high in fine mesh ( $0.32 \pm 0.04$ ;  $k = -0.0082 \text{ d}^{-1}$ ) compared to coarse mesh ( $0.29 \pm 0.02$ ;  $k = -0.0079 \text{ d}^{-1}$ ; Table 1; Figure 2).



In coarse mesh, forest in conservation unit shows the highest mass loss ( $0.29 \pm 0.03$ ;  $k = -0.0087 \text{ d}^{-1}$ ) of cotton strip in soil surface compared to close forest ( $0.27 \pm 0.02$ ;  $k = -0.0079 \text{ d}^{-1}$ ), followed by agriculture systems ( $0.17 \pm 0.01$ ;  $k = -0.0075 \text{ d}^{-1}$ ). The high cotton strip mass loss in coarse mesh in soil surface was observed in forest in conservation unit A ( $0.40 \pm 0.04$ ;  $k = -0.0106 \text{ d}^{-1}$ ), with the lowest value in agriculture system with less than 10 years of use ( $0.14 \pm 0.01$ ;  $k = -0.0075 \text{ d}^{-1}$ ; Figure 3).

Figure 2 – Overall cotton strip mass loss percentage among systems (forest in conservation unit, close forest, and agriculture system) and bag mesh (coarse and fine)



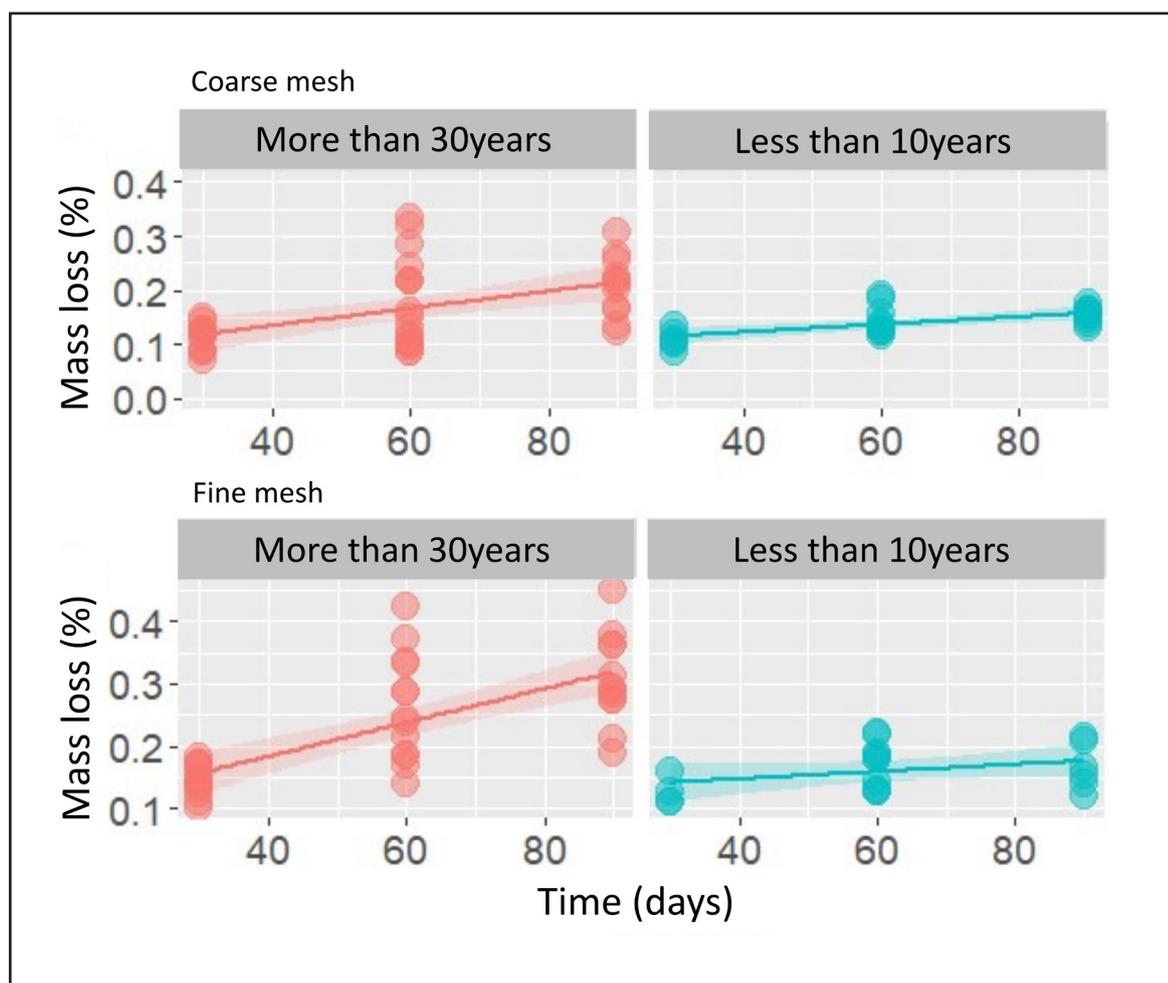
Source: Authors (2023)

In where: Lines represent the smoothers of variables and systems the 95% confidence intervals of the models.



The same pattern was also observed in fine mesh, with high mass loss in forest in conservation unit ( $0.31 \pm 0.02$ ;  $k = - 0.088 \text{ d}^{-1}$ ) followed by close forest systems ( $0.28 \pm 0.03$ ;  $k = - 0.0084 \text{ d}^{-1}$ ) and agriculture systems ( $0.20 \pm 0.02$ ;  $k = - 0.0077 \text{ d}^{-1}$ ). The high cotton strip mass loss in fine mesh in soil surface was observed in close forest 2 (30 years;  $0.45 \pm 0.09$ ;  $k = - 0.0106 \text{ d}^{-1}$ ) and forest in conservation unit A ( $0.40 \pm 0.04$ ;  $k = - 0.0090 \text{ d}^{-1}$ ), with the lowest value in close forest 3 (10 years;  $0.18 \pm 0.02$ ;  $k = - 0.0076 \text{ d}^{-1}$ ) and agriculture system with less than 10 years of use ( $0.15 \pm 0.01$ ;  $k = - 0.0074 \text{ d}^{-1}$ ; Figure 3).

Figure 3 – Cotton strip mass loss percentage in agriculture system with more than 30 years vs. less than 10 years in coarse and fine bag mesh



Source: Authors (2023)

In where: Lines represent the smoothers of variables and systems the 95% confidence intervals of the models.



### 3.2 Effect size and direction of mass loss

The cotton strip mass loss process in soil surface was negatively affected, with significant results in agriculture system when compared to forest in conservation unit (mean of 26% less in agriculture system) and close forest (mean of 22% less in agriculture system) as control for all treatments and bag meshes (from log of mass loss in agriculture/ mass loss in control [the control can be the forest in conservation unit or the close forest in the paired design] tested by nonparametric bootstrapped 95% confidence intervals from BCa method) by effect size analyzes (fine and coarse; Figure 4). The cotton strip mass loss ranged from 15% lower in agriculture system with less than 10 years to 36% in agriculture system with 30 years of use in fine mesh for forest in conservation unit as control by effect size analyzes (Figure 4a). In coarse mesh, these values ranged from 22% lower in agriculture system with less than 10 years of use to 32% in agriculture system with more than 30 years of use compared to forest in conservation unit as control by effect size analyzes (Figure 4a). Also, the cotton strip mass loss ranged from 19% lower in agriculture system with less than 10 years of use to 26% in agriculture system with more than 30 years of use in fine mesh for close forest as control by effect size analyzes (Figure 4b). In coarse mesh, these values ranged from 20% lower in agriculture system with less than 10 years of use to 23% in agriculture system with more than 30 years of use compared to close forest as control by effect size analyzes (Figure 4b).

## 4 DISCUSSIONS

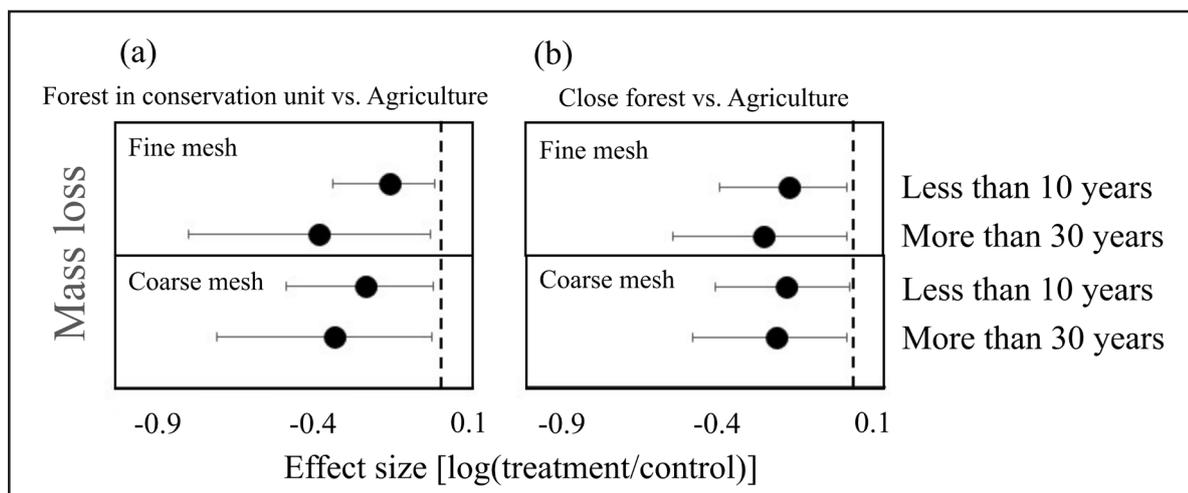
### 4.1 Overview of results

In general, we observed a cotton decomposition rate loss of a quarter in the agriculture systems studied. The land use of agriculture decreases the cellulose breakdown process over time. In this way, we found that the greater the native forest age, higher the conservation and faster the cellulose decomposition processes. The



agriculture age increases the ecosystem service loss (microbial and total decomposition), but the ecosystem service loss was not high in agriculture system compared to close forest. However, we must consider that a slower decomposition rate might not mean that nutrients are not available, and it could decrease leaching. The benefits of a quick decomposition are context-dependent. In fine mesh (microbial decomposition), the ecosystem service loss was 7% in agriculture systems with more than 30 years compared to agriculture systems with less than 10 years. On the other hand, in coarse mesh (total decomposition) the ecosystem service loss was 3% in agriculture systems with more than 30 years compared to agriculture systems with less than 10 years. Finally, the forest fragments near agricultural systems, despite being alter, may retain the ecosystem service in agriculture systems, being refuge for detritivore macrofauna. All these results, associated with the low cost and ease application, highlighting that the cellulose decomposition process (measured by cotton strips) as a good ecological indicator for subtropical agriculture systems.

Figure 4 – Effect size and direction of cotton strip mass loss in, forest in conservation unit (a) and close forest (b) controls (95% bootstrapped confidence intervals)



Source: Authors (2023)

In where: Effect size and direction of cotton strip mass loss in the two agriculture systems (more than 30 years vs. less than 10 years) between bag meshes (coarse and fine) expressed as log-ratios between treatments (agriculture system) and respective controls, forest in conservation unit (a) and close forest (b); Circles are means, and whiskers denote upper and lower bounds of 95% nonparametric bootstrapped



confidence intervals. Closed circles will represent intervals that do reject the null hypothesis (i.e., do not contain the value of 0) and open circles will represent intervals that do not reject the null hypothesis.

## 4.2 Effect of agriculture management changes

The decay rate in cellulose decomposition (by cotton strip) was 22-26% slower in agriculture systems compared to forested systems. Also, forest systems show similar nutrient cycling rates (between different forest systems), with high mass loss compared to agriculture systems. Habitat structure in monoculture of agriculture systems differs to the high species richness and plant strata in forest systems and may change the microclimate between these systems (SU; GABRIELLE; MAKOWSKI, 2021; ZAPATA; RAJAN; MOWRER; CASEY; SCHNELL; HONS, 2021), and consequently, the decomposing community and decomposition rates (COTRUFO; GALDO; PIERMATTEO, 2010; FOUR; CÁRDENAS; DANGLES, 2019). Habitat structure in monoculture of agriculture systems may also increase soil temperature on local scale (SCHWERZ; CARON; ELLI; STOLZLE; MEDEIROS; SGARBOSSA; ROCKENBACH, 2019, p. 1) and decrease soil moisture compared to forest systems (KRAFT; OLIVEIRA FILHO; CARNEIRO; KLAUBERG-FILHO; BARETTA; BARETTA, 2021; TIEGS; COSTELLO; ISKEN; WOODWARD; MCINTYRE, 2019), which can directly influence the decomposition process (POKHYLENKO; DIDUR; KULBACHKO; BANDURA; CHERNYKH, 2020). Also, high plant diversity may increase the quality of organic matter (REZENDE; SALES; HURBATH; ROQUE; GONÇALVES; MEDEIROS, 2017; TONIN; LIMA; BAMBI; FIGUEIREDO; REZENDE; GONÇALVES, 2021) in soil of forest systems compared to agriculture systems. Additionally, the complementarity of these two factors may affect the cotton decomposition.

The agriculture system with less than 10 years of use (15% lower vs. forest in conservation unit and 19% lower vs. close forest) increases the dependence on microbial decomposition. The mass loss was high in fine mesh compared to coarse mesh, which highlights the dominance of microorganisms in decomposer community of recently fertilized systems such as agriculture systems (BANI; PIOLI; VENTURA; PANZACCHI;



BORRUSO; TOGNETTI; TONON; BRUSETTI, 2018; DUARTE; PASCOAL; GARABÉTIAN; CÁSSIO; CHARCOSSET, 2009). In this way, the microbial decomposers had access to all bags (ALVIM; MEDEIROS; REZENDE; GONÇALVES, 2015; MEDEIROS; CALLISTO; GRAÇA; FERREIRA; ROSA; FRANÇA; ELLER; REZENDE; GONÇALVES JUNIOR, 2015; QUINTÃO; REZENDE; GONÇALVES JÚNIOR, 2013) and, additionally, macroinvertebrates had access only to the coarse bags, but with low participation and activity of invertebrate's community on deposition process (NAVARRO; REZENDE; GONÇALVES JÚNIOR, 2013). The recent fertilization may increase the microbial decomposition activity through the availability of nutrients (BANI; PIOLI; VENTURA; PANZACCHI; BORRUSO; TOGNETTI; TONON; BRUSETTI, 2018; DUARTE; PASCOAL; GARABÉTIAN; CÁSSIO; CHARCOSSET, 2009). High microbial decomposition activity, mainly bacteria and fungi, may be explained by substrate homogenization (NAKATSUKA; KARASAWA; OHKURA; WAGAI, 2020; YARWOOD, 2018) and pesticide use (CAVALLET; SILVA; BARETTA; REZENDE, 2022) in productive agricultural systems. The microorganisms may have a low requirement for substrate consumption in agriculture systems (CAMPANELLA; BERTILLER, 2008; SEKARAN; SAGAR; DENARDIN; SINGH; SINGH; ABAGANDURA; KUMAR; FARMAHA; BLY; MARTINS, 2020) and cotton may be a recalcitrant substrate compared to dominant litters at the sites. In this way, high microbial decomposition activity may be leveraged by the high capacity of this community to metabolize refractory molecules (e.g., cellulose and lignin) and to decompose them (NAKATSUKA; KARASAWA; OHKURA; WAGAI, 2020; XIAO; CHEN; KUMAR; CHEN; GUAN, 2019). Also, pesticide use may limit the decomposer invertebrate community in the system (CORNEJO; PÉREZ; LÓPEZ-ROJO; GARCÍA; PÉREZ; GUERRA; NIETO; BOYERO, 2021), favoring microorganism community (GUNSTONE; CORNELISSE; KLEIN; DUBEY; DONLEY, 2021).

The agriculture system with more than 30 years of use (32% lower vs. forest in conservation unit) increases the loss of substrate decomposition by macrofauna. The macrofauna of decomposer / detritivore trophic groups may directly utilize substrate tissues for feeding (REZENDE; CARARO; BERNARDI; CHIMELLO; LIMA-REZENDE; ALBENY-SIMÕES; DAL-MAGRO; GONCALVES, 2021; TIEGS; COSTELLO; ISKEN; WOODWARD;



MCINTYRE, 2019). The food activity of detritivore macrofauna may increase the biological fragmentation and accelerate the decomposition rates (FROUZ, 2018; TORRES; ABRIL; BUCHER, 2005). Also, high plant diversity systems increase substrate quality (REZENDE; SALES; HURBATH; ROQUE; GONÇALVES; MEDEIROS, 2017; TONIN; LIMA; BAMBI; FIGUEIREDO; REZENDE; GONÇALVES, 2021) and may stimulate substrate decomposition by food activity of detritivore macrofauna (HUANG; GONZÁLEZ; ZOU, 2020; POKHYLENKO; DIDUR; KULBACHKO; BANDURA; CHERNYKH, 2020), besides accelerating nutrient cycling (BROADBENT; ORWIN; PELTZER; DICKIE; MASON; OSTLE; STEVENS, 2017; SENA; GONÇALVES JÚNIOR; MARTINS; HAMADA; REZENDE, 2020). On the other hand, a long-term use of no-till system (more than 30 year) may compromise the edaphic fauna (KRAFT; OLIVEIRA FILHO; CARNEIRO; KLAUBERG-FILHO; BARETTA; BARETTA, 2021). This result highlights the importance of close forest in increasing plant diversity in conservation of ecosystem services (BROOKER; GEORGE; HOMULLE; KARLEY; NEWTON; PAKEMAN; SCHÖB, 2021), mainly for food activity of detritivore macrofauna (OLANDER; JOHNSTON; TALLIS; KAGAN; MAGUIRE; POLASKY; URBAN; BOYD; WAINGER; PALMER, 2018; REZENDE; SALES; HURBATH; ROQUE; GONÇALVES; MEDEIROS, 2017). Also, the close forest conservation stage may an important factor to quantity and quality of ecosystem services (CAVALLET; SILVA; BARETTA; REZENDE, 2022), that can be studied in the future works.

## 5 CONCLUSIONS

The land use of agriculture decreases the cellulose breakdown process over time. We observed an ecosystem service rate (by cotton strips decomposition) loss of a quarter in the agriculture systems studied compared to forest systems. Forest systems (by high species richness and plant strata) shows high ecosystem service rate of cotton strips mass loss due to differences in habitat structure and microclimate (temperature and humidity) compared to agriculture systems. Also, the agriculture use time increases the ecosystem service loss, mainly by less microbial and total decomposition. In this way, also due to low cost and ease application of the method,



the cellulose decomposition (mainly by cotton strips measurement) can be considered a good ecological indicator for subtropical agriculture systems. Finally, the forest fragments near agricultural systems may retain the ecosystem service, being refuge for detritivore macrofauna.

## ACKNOWLEDGEMENTS

RSR (projects number 403945/2021-6 and 302044/2022-1) and CRDMB are grateful to National Council for Scientific and Technological Development (CNPq). We thank the support from the Foundation to Support the Research and Innovation of State of Santa Catarina (FAPESC; TO 2021TR001802) and the Community University of the Chapecó Region (LabEntEco).

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## Authorship Contribution

### 1 Renan de Souza Rezende

Biologist, Dr. in Ecology, Professor

<https://orcid.org/0000-0002-4129-0863> • renanrezende30@gmail.com

Contribution: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft

### 2 Bruna Valencio Cavallet

Agronomist, Mr. in Environmental Sciences

<https://orcid.org/0009-0008-2469-7574> • brunacavallet@gmail.com

Contribution: Data curation; Investigation; Writing – review & editing

### 3 Alana Maria Polesso

Agronomist, Mr. in Environmental Sciences

<https://orcid.org/0000-0002-2232-5231> • alana.polesso@unochapeco.edu.br

Contribution: Data curation; Investigation; Writing – review & editing

### 4 Edpool Rocha Silva

Zootechnist, Dr. in Environmental Sciences

<https://orcid.org/0000-0002-1776-4790> • edpoolrs@unochapeco.edu.br

Contribution: Data curation; Investigation; Writing – review & editing

### 5 Carolina Riviera Duarte Maluche Baretta

Agronomist, Dr. in Agronomy, Professor

<https://orcid.org/0000-0001-7131-1517> • carolmaluche@unochapeco.edu.br

Contribution: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing

## How to quote this article

Rezende, R. S.; Cavallet, B. V.; Polesso, A. M.; Silva, E. R.; Baretta, C. R. D. M. Time effect and agriculture land use on cellulose breakdown process. *Ciência Florestal*, Santa Maria, v. 33, n. 2, e70837, p. 1-25, 2023. DOI 10.5902/1980509870837. Available from: <https://doi.org/10.5902/1980509870837>.