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# Carbon Mineralization in Soil Aggregate Classes Under Leguminous Tree Planting in North Fluminense, Brazil

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## Abstract

The physical protection of soil organic matter through its occlusion in aggregates is a mechanism which reduces their accessibility by microorganisms. When the physically protected carbon (C) becomes accessible to the microbiota, it generates a flow of $CO_2$. The objective of this study was to evaluate the C mineralization rate in macro and microaggregates of macerated and non-macerated soils under different leguminous tree, pasture, and secondary forest (capoeira). The $CO_2$ mineralization curves showed that maceration increased the amount of C mineralized in macroaggregates, mainly in leguminous tree. Maceration provided an increase of approximately 50% and 47% in acacia and ingá, respectively, and 35% in sabiá. Capoeira and pasture did not respond to maceration.

**Keywords:** microbial activity, physical protection, carbon lability, organic matter

There are only a few Atlantic Forest massif fragments in the North Fluminense region in Brazil. Part of the deforested area had its soil degraded by livestock farming, with frequent use of fire to renew pastures. Because of this, planting trees is an alternative for recovering these degraded lands (Gama-Rodrigues et al., 2008). Due to the large plant residue deposition on the soil, forest systems are characterized as organic matter accumulator systems, and therefore are considered C reservoirs (Vicente et al., 2019). For example, in addition to producing a significant amount of plant biomass, leguminous tree fix atmospheric $N_2$, which guarantees higher organic matter and nitrogen levels incorporated into the soil (Figueiredo et al., 2016).

Aggregates are secondary particles formed by combining mineral particles with organic and inorganic substances (Bronick and Lal 2005). Depending on their size, they are classified into macroaggregates (2.00-0.25 mm) and microaggregates (0.25-0.053 mm). Macroaggregates are formed by temporary binding agents present in the soil such as carbohydrates released by the roots, fine roots, fungi hyphae, and organic matter (Tisdall and Oades 1982). Conversely, microaggregate formation is related to the interaction between the surface of minerals and organic binders such as carbohydrates of vegetables and microbiological origin (Verchot et al. 2011).

The physical protection represents one of the most important mechanisms for soil organic matter stabilization (Monroe et al., 2022). The C mineralization within the aggregates can be obtained through the $CO_2$ release curve in samples of macerated (submitted physical disaggregation) and non-macerated (did not undergo physical disaggregation) aggregates subjected to incubation. Maceration makes the organic matter that was protected in the aggregates available to the microbiota (Gárcia-Oliva et al., 2004). Furthermore, this procedure can also cause stress to the microbial community, due to the rupture of the aggregate, which can influence their activity. Thus, when the carbon becomes accessible to the microbiota,

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it generates a flow of $CO_2$. The amount of $CO_2$ released varies based on the lability level of organic matter within the aggregates. The objective of this work was to evaluate the C mineralization rate in macro and microaggregates of soils under different leguminous tree, pasture, and secondary forest on a site located in in the Southeast region of Brazil.

The soil was collected in the municipality of Conceição de Macabú, RJ, in an experimental area with five plant covers: *Acacia auriculiformis* (Acácia), *Mimosa caesalpiniifolia* (Sabiá) and *Inga* spp (Ingá), and two additional plant covers adjacent to the plantations used as reference, both approximately 40 years old: a degraded pasture, which represents the vegetation prior to planting tree species, with a predominance of molasses grass (*Melinis minutiflora*), paspalum grass (*Paspalum maritimum*) and satintail grass (*Imperata brasiliensis*); and a forest fragment of the Atlantic Forest in secondary succession (capoeira). Three field replications of each cover were collected at a depth of 0-10 cm. Before the samples were taken, the litter layer was removed. All of the samples were taken in a 1 $m^2$ pit dug to a depth of 0.4 m. To minimize compression and to obtain a representative sample for the aggregation state of the soil, samples were taken using a bricklayer's trowel inserted into the soil at the lower level of the sampling depth. The three composite samples were prepared in each land use system by compositing equal amounts of soils from ten pits for each replicate. The aggregate classes (2.00-0.25 mm; 0.25-0.053 mm) were obtained by dry screening: a set of sieves with different mesh diameters (2.00 mm; 0.25 mm and; 0.053 mm) were used (Gupta and Germida, 1988). 150 g of soil from each composite sample from each plant cover was added to the set of sieves, considering 15 minutes of agitation in a shaking device. A fraction of of macro and microaggregates mass were manually macerated using gral until all aggregates were visibly broken. Sample moisture was standardized to 60% of the soil's maximum saturation capacity (Grisi, 1995).

Incubation was performed by placing each vial of soil in a glass jar that was kept hermetically sealed. Soil respiration was estimated by the amount of $CO_2$ released initially every three days (in the first 10 days), and then every 5 days, for a total period of 40 days. Samples containing 40 g of different aggregate classes (macerated and non-macerated) + 1 g of fresh soil (representing the inoculum with a new population of microorganisms) were weighed. Each 3L glass jar contained a bottle with 10 mL of 1M NaOH and a bottle with water in order to maintain constant humidity until the end of incubation. At the end of each incubation period, the bottles with NaOH were titrated with 0.5 M HCl and 2 mL of barium chloride ($BaCl_2$) was added to complete $CO_2$ precipitation. The NaOH bottles were replaced for the new incubation period until the end time. The soil was weighed and the humidity was maintained at 60% of the saturation capacity to maintain the humidity of each treatment until the end of incubation (Azuaje et al., 2012).

The C mineralization process was tested using several models such as the Gompertz, Logistic, and Mitscherlich models including linear, quadratic, with and without intercept. The decision about the goodness of fit of the models was made based on information theory, as suggested by Burnham and Anderson (2004). The goodness of fit was evaluated using the initial criterion of Akaike (1974) corrected for the sample size, as recommended by Sugiura (1978). Next, the goodness-of-fit measures derived from the corrected Akaike criterion were computed. These measures were calculated for all the models evaluated with or without random effects on the parameters. The models that challenged the traditional assumptions of homoscedasticity and independence of random errors were also evaluated. The data was analyzed using the SAS tool (Statistical Analysis System) and R Software.

The Gompertz model was considered the best choice to represent the reality contained in the data and also because it is efficient in biologically interpreting its parameters (Table 1). The curves of mineralized C in macro and microaggregates, macerated and non-macerated, of the different vegetation covers showed an exponentially increasing slope in the initial incubation period, followed by a regular and progressive increase in C mineralization from the 10th day onwards. The amount of mineralized C was greatest between the 35th and 40th incubation days (Figures 1 and 2). These results were similar to Araújo et al. (2001), Gonçalves et al. (2001) and Barreto et al. (2010), who found that the amount of mineralized C was lower during the first incubation days. In the same vegetation cover as in the present study, Nunes et al. (2015) showed that the C mineralization curve fit the exponential model, and that there was still C available for mineralization by microorganisms after 20 weeks of incubation.

**Table 1.** Model adjustment coefficients.

| | Models | | | |
|---|---|---|---|---|
| | **Linear** | **Logistics** | **Mitscherlich** | **Gompertz** |
| Random Effects Matrix | D[b0,b1] | D[a,k] | D[a,k] | D[a,k] |
| Variance | VP+corr | VP+corr | VP+corr | VP+corr |
| D.F. | 65 | 65 | 64 | 66 |
| Llike | -2741.08 | -2805.71 | -2770.02 | -2720.58 |
| $AIC_c$ | 5630.3 | 5759.5 | 5685.6 | 5591.9 |
| Δ AIC | 38.4 | 167.6 | 93.7 | 0 |
| $W_r$ | 4.58718E-09 | 4.03759E-37 | 4.50094E-21 | ≈ 1.0 |
| ER | 217998774.7 | 2.47672E+36 | 2.22176E+20 | 1 |

D[,] = diagonal matriz; VP = power function for variance; corr = correlação autorregressiva contínua; D.F.= degrees of freedom; Llike= log likelihood function; $AIC_c$ = Akaike information criterion corrected for sample size; Δ AIC= difference between the Akaikes and the lowest value in the evaluated set; $W_r$= model probability; RE= fit evidence ratio

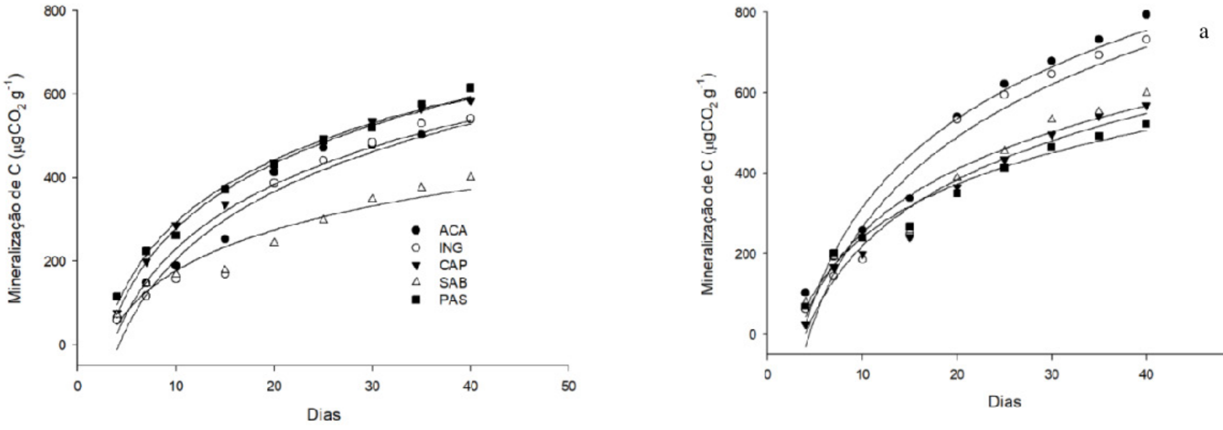

**Figure 1.** Observed (symbols) and expected (lines) values of mineralized C according to incubation time in non-macerated macroaggregates (a) and macerated (b) of soils under different vegetation covers. ACA, acacia; ING, inga; CAP, capoeira; SAB, sabia; e PAS, pasture.

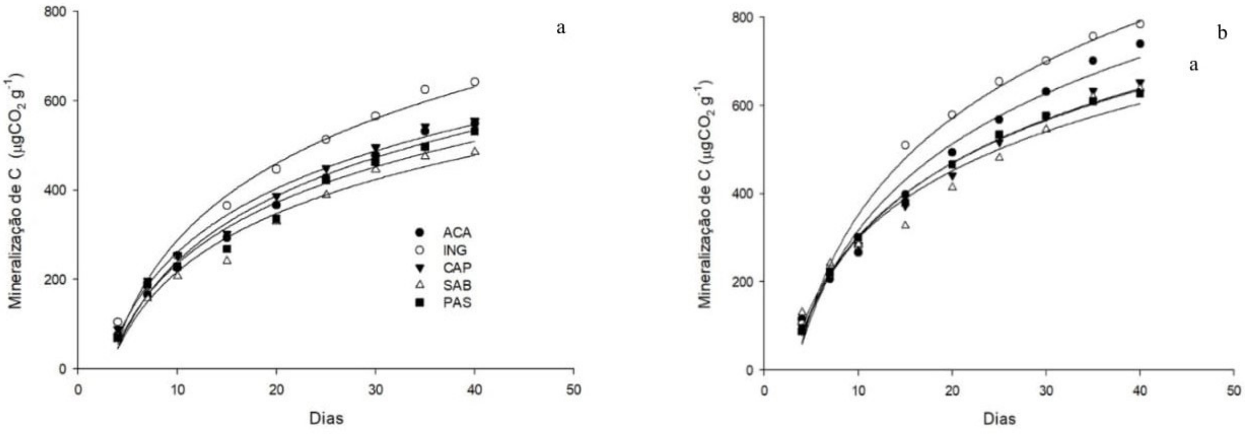

**Figure 2.** Observed (symbols) and expected (lines) values of mineralized C according to incubation time in non-macerated microaggregates (a) and macerated (b) of soils under different vegetation covers. ACA, acacia; ING, inga; CAP, capoeira; SAB, sabia; e PAS, pasture.

The curves also showed that maceration generally increased the amount of C mineralized in the macroaggregates. Maceration provided an increase of approximately 50% and 47% in acacia and ingá, respectively, and 35% in sabiá. Capoeira and pasture did not show responses to maceration (Figures 1a and 1b). The C mineralization curves after maceration suggest that leguminous tree modified the chemical composition of C in the soil aggregates studied and favored C mineralization, unlike the reference covers. Maceration increased the specific surface area of the aggregates and the accessibility to microbial attack by unprotecting the inaccessible soil organic C reservoir (Cadisch et al., 2006). Studies by Gupta and Germida (1988) and Beare et al. (1994) also found a significant increase in C mineralization in aggregates after maceration. Additionally, maceration can potentially cause an imbalance in the microbial community due to the destruction of soil aggregates. This can lead to stress in this community, similar to what occurs in areas with conventional tillage systems. In these areas, management practices break up the aggregates, exposing organic matter to microbial and enzymatic attack, ultimately causing an imbalance. Furthermore, maceration can mix different organic matter fractions with varying levels of lability and transformation. This effect can be intensified in macroaggregates that arise from areas with leguminous tree species.

Maceration of microaggregates also promoted an increase in mineralized C during the 40 days of incubation in all vegetation covers (Figures 2a and 2b), which suggests the presence of organic matter available for action by the soil microbiota. The curves of mineralized C in non-macerated microaggregates varied little between vegetation covers (Figure 2a). When evaluating the chemical composition of C in different soil fractions (coarse fractions: air-dried fine earth;

macro and micro-aggregates and fine fractions: occluded fractions of macro and micro-aggregates) using Fourier transform infrared spectroscopy, Lisboa et al. (2022) showed that aromatic material was predominant in coarser fractions (less C protection from the action of the microbiota) and the fine fractions had a more aliphatic character. Due to the lower complexation of organic matter to the mineral fraction in the coarser fractions, the labile forms of C were the first used by microorganisms, which led to a proportional enrichment of aromatic C. This is in contrast to the fine fractions, which protect organic matter from microbiota action, in which there was an accumulation of more labile fractions. Likewise, when analyzing the chemical composition of C also using infrared spectroscopy, Vicente et al. (2023) observed that the organic matter fraction associated with minerals (Cm < 53 µm) was the main reservoir of labile compounds.

In turn, Faustino et al. (2022) observed that cultivation with leguminous tree favored the distribution of labile and moderately labile fractions along the soil profile when studying the lability of C using the chemical fractionation method of C by an oxidation gradient in the same areas of the present study. On the other hand, the reference covers contributed with a recalcitrant fraction below 20 cm deep.

The organic matter added to the soil after 13 years of conversion of degraded pasture into plantations with leguminous tree favored soil microbiota activity through the diversity of compounds with different lability levels. Although, its physical protection particularly in macroaggregates is a strategy for preserving C in the soil. The results also showed that the larger aggregate fraction was more responsive to changes provided by maceration. Thus, tree-based production systems which promote a significant input of organic matter and no-tillage are alternatives for maintaining C in the soil, and consequently for reducing $CO_2$ emissions into the atmosphere.

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Emanuela Forestieri Gama-Rodrigues: conceptualization (lead), data curation (equal), formal analysis (supporting), funding acquisition (equal), investigation (supporting), methodology (supporting), project administration (lead), resources (lead), supervision(lead), writing – original draft (supporting), writing – review & editing (lead).

Antonio Carlos Gama-Rodrigues: funding acquisition (lead), project administration (supporting), resources (equal), writing – original draft (supporting), writing – review & editing (supporting).

Ricardo Augusto M. Vieira: data curation (supporting), formal analysis (supporting), methodology (supporting), writing – original draft (equal), writing – review & editing (supporting).

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