










## **Soil attributes as indicators of the stabilization process of erosion in gullies at different formation stages in the southeast region of Brazil**

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### **ABSTRACT**

Water erosion is one of the main forms of soil degradation, causing economic, environmental, and social damage. This study evaluated the effects of different formation stages of gullies (initial - IG; juvenile- JG; mature - MG; and senile - SG) on the chemical, organic carbon and microbiological attributes of soil, using a secondary forest (SF) and two areas of pasture as references in the “Mar de Morros” environment of the Brazilian Atlantic Forest biome. Soil samples (depth 0–0.05 m) were collected at the end of the rainy and dry seasons. Gullies in different stages of formation promote a decrease in soil fertility and modification of microbiological attributes, particularly when compared with secondary forest areas. Reductions of over 60% in total organic carbon (TOC), oxidizable organic carbon (POXC), FDA activity,  $\beta$ -glycosidase, acid phosphatase, C and N from microbial biomass, basal soil respiration (BSR) and glomalin-related soil protein (GRSP) were observed in gullies in early (IG) and intermediate (JG and MG) stages when compared to SF. It was found that the effect of erosion on soil chemical and microbiological attributes is more intense in gullies in the initial and intermediate stages compared with those in the senile stage (SG). Using multivariate PCA, the microbiological and chemical attributes of the soil are discriminated between gullies with distinct formation stages. Chemical attributes, TOC, POXC, FDA activity,  $\beta$ -glycosidase, acid phosphatase, MBC and MBN, BSR, and GRSP are good indicators for evaluating the process of erosion stabilization in gullies.



**Keywords:** enzymatic activity, glomalin, oxisols, soil microbial biomass, soil organic carbon, water erosion.

## **Atributos do solo como indicadores do processo de estabilização da erosão em voçorocas em diferentes estágios de formação na região sudeste do Brasil**

### **RESUMO**

A erosão hídrica é uma das principais formas de degradação do solo, causando danos econômicos, ambientais e sociais. O objetivo deste estudo foi avaliar o efeito de diferentes estágios de formação de voçorocas (inicial - IG; juvenil - JG; maduro - MG; e senil - SG) nos atributos químicos, matéria orgânica e microbiológicos do solo, utilizando uma floresta secundária (SF) e duas áreas de pastagem como referência no ambiente “Mar de Morros” do bioma Mata Atlântica brasileira. Amostras de solo (profundidade 0–0,05 m) foram coletadas no final das estações chuvosa e seca. As voçorocas em diferentes estágios de formação promoveram diminuição da fertilidade do solo e modificação dos atributos microbiológicos, principalmente quando comparados com a área de floresta secundária. Reduções de mais de 60% no carbono orgânico total (TOC), carbono orgânico oxidável (POXC), atividade da FDA,  $\beta$ -glicosidase, fosfatase ácida, C (MBC) e N (MBN) da biomassa microbiana, respiração basal do solo (BSR) e proteína do solo relacionada à glomalina (GRSP) foram observados em voçorocas nos estágios inicial (IG) e intermediário (JG e MG) quando comparados ao SF. Verificou-se que o efeito da erosão sobre os atributos químicos e microbiológicos do solo é mais intenso em voçorocas nos estágios inicial e intermediário em comparação com ao senil (SG). Usando a multivariada PCA, os atributos microbiológicos e químicos do solo discriminaram as voçorocas com distintos estágios de formação. Os atributos químicos, TOC, POXC, atividade FDA,  $\beta$ -glicosidase, fosfatase ácida, MBC and MBN, SBR e GRSP são bons indicadores para avaliar o processo de estabilização da erosão em voçorocas.

**Palavras-chave:** atividade enzimática, biomassa microbiana do solo, carbono orgânico do solo, erosão hídrica, glomalina, latossolos.

### **1. INTRODUCTION**

The Atlantic Forest biome has been subjected to strong anthropic pressure, to include intense deforestation since the discovery of Brazil (SOS Mata Atlântica, 2018). This region is characterized by a variable relief, with flat areas and many valleys and hills. Removal of the original forest intensified erosion and reduced plant-growth capacity (Landeros-Sánchez *et al.*, 2009). In the state of Rio de Janeiro, particularly in the Pinheiral area, there has been a high degree of erosion due to uneven relief, removal of vegetation cover, and inappropriate practices of land use and management in agricultural crops and, mainly, pastures. A study carried out by the Paraíba do Sul River Basin Committee (CEIVAP) indicates that the stretch between the sediment stations of Volta Redonda and Barra do Piraí, in which lies the municipality of Pinheiral, has the second largest specific sediment production ( $588.8 \text{ Mg km}^{-2} \text{ year}^{-1}$ ) (CEIVAP, 2006). Large portions of the soils have a high erosion potential (Gaia-Gomes *et al.*, 2018; 2020), and high levels of rainfall increase water erosion. Water erosion is one of the main forms of soil degradation, causing economic, environmental, and social damage. This type of erosion leads to the emergence of gullies, which are formed by the processes of soil runoff and high soil loss (Vanwalleghem *et al.*, 2005).

Gullies represent the most severe form of erosion and can be classified into their formation stages (initial, juvenile, mature, and senile) based on morphological characteristics. These

include the development of channels, their cross-section, bed profile, walls, branches, and ducts (Oka-Fiore and Soares, 1976), and the presence or absence of vegetation. Although some studies have characterized the formation stages of gullies (Oka-Fiore and Soares, 1976; Dobek *et al.*, 2011), few have evaluated soil attributes (physical, chemical, and biological) in these environments (Machado *et al.*, 2010; Gomide *et al.*, 2011; Gaia-Gomes *et al.*, 2018; 2020), especially in relation to their microbiological attributes.

Soil attributes, such as microbial biomass and enzymatic activity, are influenced by many factors and have been suggested as appropriate indicators for assessing soil quality (Maurya *et al.*, 2020; Tiwari *et al.*, 2019; Zhou *et al.*, 2018). Estimates of soil microbial biomass may indicate changes in the total organic matter of the soil long before changes in total soil C and N levels are detectable (Babur and Dindaroglu, 2020). Moreover, this can provide an index of soil fertility, because it represents an important labile reservoir of soil nutrients, playing an active role in preventing nutrient loss (Yang *et al.*, 2010).

The enzymatic activity of the soil, in turn, plays a critical role in the cycles of soil elements and how they are synthesized by organisms. The conditions that favor microbial activity, such as the presence of vegetation (rhizosphere), also lead to higher enzyme activity (Zhang *et al.*, 2018). Enzymes participate in the catabolism of organic and mineral soil components, and their activity correlates with organic matter, physical and chemical properties, and with the microbial biomass (Maurya *et al.*, 2020; Tiwari *et al.*, 2019; Yuan and Yue, 2012).

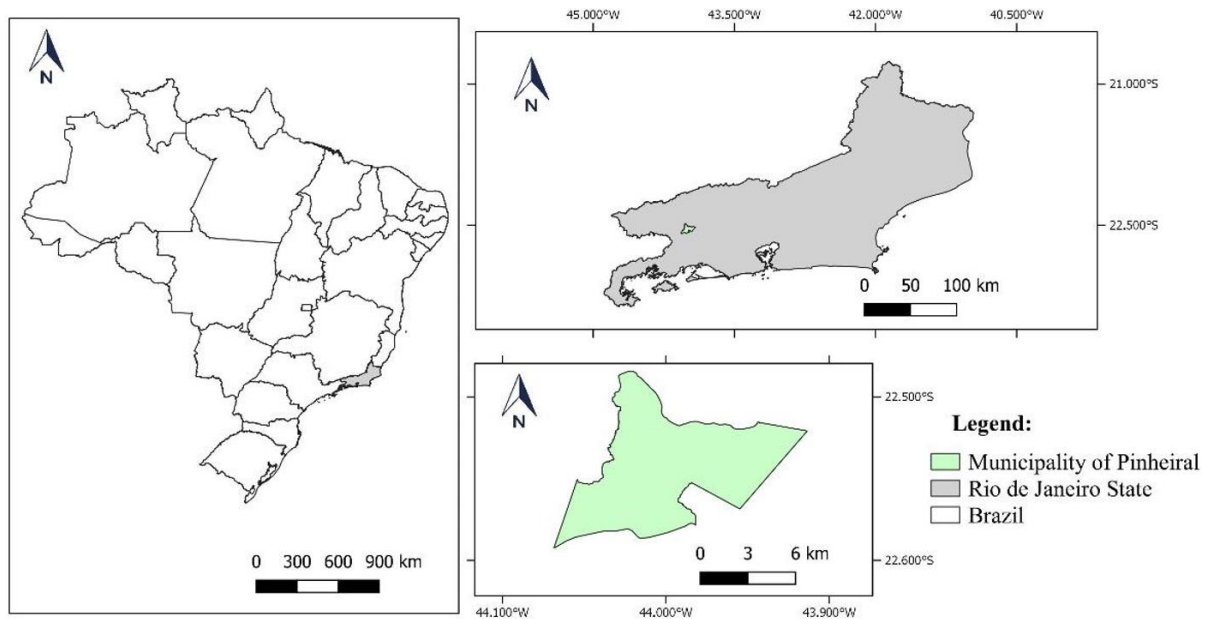
The soil microbial community has long been recognized for its involvement in the biogeochemical transformation of carbon, nitrogen, phosphorus, and sulfur in soil, which directly influences the structure and productivity of the plant community (Elliot *et al.*, 2015). Furthermore, bacteria and fungi promote improvements in soil structure that favor aggregation and porosity (Hashim *et al.*, 2020; Muchane *et al.*, 2018). For example, when associated with plant roots, arbuscular mycorrhizal fungi (AMF) improve soil aggregation via hyphae and the production of glomalin (Parihar *et al.*, 2020). This glycoprotein is important for the aggregation and storage of soil carbon and nitrogen (Sekaran *et al.*, 2021, Wang *et al.*, 2017). Glomalin is positively correlated with the stability of aggregates and carbon. The response of these components to changes in land use suggest that glomalin may be used as an indicator of ecosystem degradation/recovery (Liu *et al.*, 2020).

The hypothesis of this study was that the chemical attributes, organic carbon, and soil microbiological attributes change in gullies at different stages of formation (initial, juvenile, mature, and senile) and can be used as indicators of erosion stabilization. Thus, the objective of this study was to evaluate the effect of gully formation stage (initial, juvenile, mature, and senile) on soil chemical, organic carbon, and soil microbiological attributes using a secondary forest area and two areas of regenerating pasture (initial and medium stage) as references in "Mar de Morros" in the Atlantic Forest biome, Brazil.

## 2. MATERIAL AND METHODS

The study was conducted in the municipality of Pinheiral - RJ, in the Sub-basin of the Ribeirão Cachimbal, which is located in the region of the Middle Paraíba Fluminense and comprises the hydrographic basin of the Paraíba do Sul River (Figure 1). It is located between latitudes 22°29'03" S and 22°35'27" S, and longitudes 43°54'49" W and 44°04'05" W. The climate of the region, according to Köppen (1936) is Am, with a tropical rainy climate, monsoon, and dry winter. The area forms part of the ecological area of the Atlantic Forest, whose original vegetation is identified as Baixo Montana Atlantic Rainforest, and is characteristic of areas with altitudes of 300–800 m. The region presents various forms of relief with different degrees of dissection. The altitude varies from 360 m in the large floodplain of Paraíba do Sul, at the mouth of the Cachimbal Stream, to 720 m in the Arrozal mountain range in the south of the Cachimbal River Basin (Machado *et al.*, 2010). In the area covered by the

Cachimbal Stream, hillsides with varied slopes predominate; 35% are classified as strong wavy, 28% wavy, 17% soft wavy, 10.5% flat, 9% mountainous, and 0.5% steep (Santos *et al.*, 2017).



**Figure 1.** Study area map, municipality of Pinheiral – RJ, Brazil.

The current soil coverage of the region is composed by pastures, both managed (mainly *Brachiari* sp.) and non-managed (molasses grass – *Melinis minutiflora*, *Hyparrhenia rufa*, sapê, *Imperata brasiliensis*, *Andropogon bicornis*; bahia grass - *Paspalum notatum*, other grasses; and six native legumes). These pastures are in various stages of degradation, level of use and/or abandonment, and give rise to other forms of vegetation in the area, such as shrubby pastures and shrubs. The distribution of land in the sub-basin is distinct in its different portions of the slopes, with a predominance of large (over 150 ha) and medium properties on its upper and middle thirds, where the main activities are dairy farming and cattle breeding.

In the entire region of the Médio Vale of the Paraíba do Sul River, the first type of use was extractivism, which was subsequently replaced by coffee cultivation in the colonial period. Progressively, the coffee plantations were replaced by livestock farming, and inadequate management has contributed to the formation of the landscape that dominates the region today. The remaining forest fragments are small and located on the interfluves of the main hydrographic basins and in areas that are difficult to access, whose relief has prevented agricultural use.

Four gullies were selected (Figure 2), which were previously classified based on their stage of formation (initial, juvenile, mature, and senile) as described by Oka-Fiori and Soares (1976) and Dobek *et al.* (2011), from Google Earth images, and validated through field trips. The initial stage (IG) gully has channels and the bed has an irregular profile with a “V” cross-section and rectilinear walls, without ramifications. The juvenile stage (JG) gully has been subjected to a more intense erosive process with the formation of ducts, causing elongation, broadening, and deepening of the main channel. The mature gully (MG) has ramifications with jagged lines and a “U” cross section, with movement of earth on the walls, enlargement, vertical erosion in the ramifications, development of vegetation (grasses) at the bottom and consolidation of erosive processes in the channel. Finally, the senile gully (SG) presents a “U” cross section, a slightly irregular contour line, smooth sloping walls, vegetation cover, predominantly *Embaúba* (*Cecropia* sp.), grasses, and bamboo (*Phyllostachys* sp.) throughout the area, and a bed covered with alluvial deposits. Three areas were selected next to the gullies, one area under secondary



forest (SF), one area under initial regeneration pasture (ISR), and one area under regeneration in the medium stage (MSR), which was adopted as a reference. In all study areas, the soil was identified as Latossolo Vermelho-Amarelo (Oxisol).



**Figure 2.** Gullies in different stages of formation. (a) Gully in the initial stage; (b) gully in the juvenile stage; (c) gully in the senile stage; (d) gully in the mature stage.

**Source:** Gaia-Gomes *et al.* (2020) adapted.

To evaluate the chemical, physical, and microbiological attributes of the soil, samples were collected at the 0–5 cm depth in two distinct seasons (end of the rainy season and dry season) in each gully and in the SF, MSR, and ISR areas. The samples were collected within the gullies in an irregular grid covering its extension. For SF, PRI, and PRM, 20 × 20 m plots were selected in each area, and 16 simple samples were collected to make four composite soil samples.

Physical analysis (granulometry), chemical analysis (pH, Ca, Mg, Ca, P, H+Al, K, N), and soil organic carbon determination were performed according to Teixeira *et al.* (2017). Oxidizable carbon (POXC) was evaluated with the use of permanganate as described by Weil *et al.* (2003).

To assess enzyme activity, collected samples were stored at 4°C until processing. Activities of  $\beta$ -glucosidase and acid phosphatase were assessed according to the method described by Eivazi and Tabatabai (1988); and fluorescein diacetate (FDA) hydrolysis as describe by Schnürer and Rosswal (1982).

Total soil microbial activity was quantified immediately after sampling using the basal soil respiration method, as described by Jenkinson and Powlson (1976), while the microbial biomass carbon (MBC) was assessed via fumigation-incubation as described by Vance *et al.* (1987) and Tate *et al.* (1988); microbial biomass nitrogen (MBN) was determined as described by Brookes *et al.* (1985).

Glomalin-related soil protein (GRSP; easily extractable-GRSP and total-GRSP) was extracted from the samples as described by Wright and Updahyaya (1998). GRSP-easily extractable fractions (EE-GRSP) were obtained via autoclave extraction using 20 mM sodium citrate solution, pH 7.4, at a temperature of 121°C for 30 min. The total-GRSP fraction (T-GRSP) was obtained using 50 mM sodium citrate, pH 8.0, at 121°C, for 60 min. When necessary, more than one autoclave cycle was performed to extract this fraction until samples

were light-yellow in color. Both fractions were quantified by the Bradford method (1976) modified by Wright *et al.* (1996).

### 2.1. Statistical analysis

The results were analyzed by Lilliefors test for normality (test of Lillifors/ STATISTIC 8.0) and homogeneity of variances (Levene/STATISTIC 8.0). When this premise was met, the averages were compared by the Bonferroni t parametric test and, when this did not occur, the means were compared by the non-parametric Kruskal Wallis test ( $P < 5\%$ ). To better understand the different environmental quality conditions of the study units, some parameters of each study unit were selected for multivariate statistical analysis by way of principal component analysis (PCA) and clustering (Paired group; Similarity measure: Gower), in addition to a correlation analysis with the PAST statistical program. For these analyses, the variables were grouped according to the number of replicates used for the statistical analysis (ANOVA and means test).

## 3. RESULTS AND DISCUSSION

There was no significant difference in the levels of clay and sand (coarse and total) in gullies at different formation stages (initial - IG; juvenile - JG; mature - MG; and senile - SG) and areas of secondary forest (SF), initial-stage regenerating pasture (PRI), and medium-regeneration stage pasture (MSR); however, variation was observed in the textural class (SF - clay-loam; ISR - sandy-loam; MSR, IG, JG, MG, SG - sandy clay loam) (Table 1).

Higher levels of fine sand were observed in ISR, in contrast to SF, MSR, and SG. No differences were observed between the gullies, nor between the SF and MSR areas. The highest levels of silt were observed in the SF and differed only from those in MSR and IG. Variable silt plus fine sand, an indicator of soil erodibility (Silva *et al.*, 2003), was higher in SF and ISR, differing from MSR and IG, indicating that these fractions were removed by the erosive process in the latter two areas. This may be because superficial and subsurface water flow is more intense during the initial stage of gully formation. The other gullies (JG, MG, SG) presented intermediate values of silt plus fine sand (Table 1).

**Table 1.** Soil granulometric analysis in gullies at different formation stages (IG – initial; JG - juvenile; MG – mature; SG - senile), secondary forest (SF), mid-stage regeneration pasture (MSR), and initial-stage regeneration (ISR), at a depth of 0–0.05 m, in Pinheiral (RJ).

Areas	Sand			Clay	Silt	Silt + Thin sand	Textural class
	Thin	Coarse	Total				
	$\text{g kg}^{-1}$						
SF	129 b	283 a	412 a	283 a	304 a	434 a	Clay-loam
MSR	106 b	427 a	533 a	279 a	188 b	294 b	Sandy clay loam
ISR	179 a	418 a	597 a	185 a	217 ab	397 a	Sandy-loam
IG	141 ab	506 a	647 a	202 a	151 b	292 b	Sandy clay loam
JG	144 ab	403 a	547 a	238 a	212 ab	359 ab	Sandy clay loam
MG	131ab	323 a	454 a	328 a	218 ab	349 ab	Sandy clay loam
SG	109 b	387 a	496 a	295 a	208 ab	317	Sandy clay loam

Means followed by equal letters in the column do not differ by the Bonferroni T or Kruskal Wallis test at 5%.

In relation to soil chemical attributes, the pH was lowest in the SF areas (dry season and rainy season) compared with the gullies and the pasture (MSR and ISR) (Table 2). This pattern can be explained by the greater deposition of plant litter in the SF area when compared with the gullies and pasture. This leads to a higher rate of decomposition and the release of high levels of acid, which reduces the soil pH. Rukshana *et al.* (2011) reported that decomposing organic matter, depending on its chemical nature, can provide H<sup>+</sup>, which acidifies the soil.

**Table 2.** Chemical attributes and soil organic carbon in gullies at different formation stages (IG – initial; JG - juvenile; MG – mature; SG - senile), secondary forest (SF), mid-stage regeneration pasture (MSR), and initial-stage regeneration (ISR), at a depth of 0–0.05 m in two seasons (rainy season; dry season), in Pinheiral (RJ).

Areas	pH		Ca		Mg		Al		H+Al	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
	cmol <sub>c</sub> Kg <sup>-1</sup>									
SF	4.06 b*	3.68 c	0.75 b	1.00 ab*	0.42 a	1.27 ab*	2.36 a*	1.68 ab	8.95 a	9.98 a
MSR	4.41 a*	4.10 b	1.32 a	1.17 a*	0.50 a	0.85 b*	2.18 ab	2.23 a	8.70 a	11.11 a*
ISR	4.57 a*	4.59 a	0.67 bc	1.07 ab*	0.62 a	1.50 a*	1.05 b*	0.50 b	5.16 b	5.53 b
IG	4.55 a*	4.30 b	0.40 d	0.65 cd*	0.62 a	0.95 b*	1.19 ab	1.40 ab	3.88 b	4.29 b
JG	4.38 a	4.31 b	0.47 cd	0.63 cd*	0.60 a	1.00 b*	1.53 ab	1.60 ab	4.83 b	5.28 b
MG	4.52 a*	4.28 b	0.47 cd	0.60 d	0.60 a	0.97 b*	1.55 ab	1.52 ab	4.66 b	5.86 b*
SG	4.49 a*	4.21 b	0.90 b	0.87 bc	0.77 a	1.20 ab*	1.50 ab	1.52 ab	4.46 b	5.73 b*

Areas	P		K		N		TOC		POXC	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
	mg L <sup>-1</sup>									
	g kg <sup>-1</sup>									
SF	7.94 a*	1.17 b	2.07 a	8.20 ab*	2.65 a*	1.53 a	29.21 ab	38.00 a	0.68 a	0.57 a
MSR	6.92 ab*	2.33 a	2.23 a	6.70 b*	2.42 ab*	1.13 b	30.58 a	24.28 ab	0.63 ab*	0.54 a
ISR	6.43 ab*	2.43 ab	2.17 a	11.75 a*	1.58 bc*	0.70 c	21.24 abc	17.76 ab	0.44 abc	0.39 ab
IG	6.33 ab*	1.15 b	2.22 a	6.23 b*	1.14 c*	0.26 d	5.91 bc	5.95 b	0.36 abc*	0.18 b
JG	6.38 ab*	1.47 ab	1.27 b	5.30 b*	1.15 c*	0.39 cd	12.51 abc	10.07 ab	0.27c	0.31 ab
MG	6.26 b*	1.84 ab	1.03 b	4.37 b*	0.89 c*	0.48 cd	4.12 c	6.50 b	0.26 bc	0.35 ab
SG	6.40 ab*	1.28 ab	2.33 a	11.07 a*	1.02 c	0.59 cd	16.95 abc	11.27 ab	0.49 abc	0.39 ab

Means followed by equal letters in the column do not differ by the Bonferroni T or Kruskal Wallis test at 5%.

\* Indicates a significant difference between seasons by the Bonferroni T or Kruskal Wallis test at 5%. TOC – Total organic matter; POXC – oxidizable carbon.

For H+Al content, the gullies in the different formation stages followed the same pattern observed in the ISR, in both seasons (Table 2). For the Al, it was verified that the gullies presented intermediate values between the ISR area and the SF and MSR areas, with higher values in the last two areas (Table 2). Lower pH value in SF may have contributed to the increase in Al solubility.

The nutrients most affected by the erosive process were Ca, K, and N, with the lowest values observed in gullies in the initial (IG) and intermediate (JG and MG) stages of formation (Table 2). In general, there was no difference in Mg and P content between the gullies and the SF and pasture areas (Table 2). No significant differences were observed between SG and the forest in relation to the nutrients Ca, Mg, P and K. It can be inferred that this pattern is related to the process of erosion stabilization in the gully, due, among other factors, to colonization by plant species (spontaneous regeneration) throughout its extension (Figure 2c), thus promoting the addition of plant litter and organic matter and the incorporation and maintenance of soil nutrients. Vegetation influences erosion by reducing its intensity and improving the capacity of soil infiltration (Jianbo *et al.*, 2018).

Gomide *et al.* (2011) noted that the reduced soil fertility in gullies was due to the decreased



organic matter contents resulting from the removal of vegetation in these environments, thus leading to reduced cycling of nutrients and increased losses through leaching. This pattern was confirmed by the analysis of total organic carbon (TOC) and oxidizable carbon (POXC) in the study areas. There were high positive correlations ( $r = >0.75$ ,  $p < 0.5$ ) between TOC and Ca, P and N in the rainy season. Significant decreases in TOC (approximately 80% in IG) and POXC (approximately 67% in IG) were observed in gullies in the initial and mature stages (environments in which larger reductions in nutrient levels were observed) compared with pasture and/or SF areas. POXC is a labile fraction of organic carbon that is sensitive to soil use and management. It is highly informative for total soil organic matter, nutrients, soil structure, and microbial pools and activity; parameters commonly used as indicators of soil function, such as C sequestration, nutrient cycling, formation of soil structure, and biodiversity (Bongiorno *et al.*, 2019).

In the senile stage gully, with natural regeneration occupying its entire extension, TOC and POXC levels were observed that were statistically similar the reference areas (SF, MSR, and ISR). Although TOC values below those in the reference areas were observed, especially in relation to SF and MSR, TOC was approximately 180% higher in IG. Shi *et al.* (2019) noted that the restoration of vegetation promotes the addition of plant litter and increases the amounts of small roots, which results in the addition of higher levels of carbon to the soil. In addition, it can improve soil infiltration and reduce runoff and sediment transport, which reduces TOC losses. According to Rumpel *et al.* (2018), plants, bacteria, fungi, and soil fauna, such as earthworms, contribute to the addition of organic matter and nutrients, which promote soil aggregation, making it resilient to erosion and increasing its retention capacity.

Regarding the influence of the sampling period on soil chemical attributes, there was greater variation in pH, Ca, Mg, P, K, and N. While high values for pH, P, and N were found in the rainy season, the highest levels of Ca, Mg, and K were generally recorded in the dry season. Of the evaluated organic matter fractions, the TOC did not vary between seasons in the reference areas and in the gullies. On the other hand, higher POXC values were observed in the rainy season compared to the dry season in the MSR area and in the initial gully. Significant change in TOC content in response to climate change is usually difficult to detect in a short time. In contrast, POXC is considered a labile fraction of SOM, and therefore may have an advantage over the TOC in detecting differences due to variations in temperature and precipitation (Culman *et al.*, 2012).

Changes in the microbiological attributes of the soil were observed across the gully formation stages (Table 3). Total enzyme activity, evaluated by fluorescein diacetate (FDA) hydrolysis, was 69% less in the mature stage gully, compared with the SF in the rainy season. In the dry season, there was a 90% reduction in gullies in the initial, juvenile, and mature formation stages. In the senile gully, during rainy and dry periods, FDA activity was intermediate (110.13 and 44.07  $\mu\text{gFluoresc g}^{-1} \text{SS hour}^{-1}$ , respectively) between the reference areas (SF, MSR, IRS) (average = 154.05 and 108.45  $\mu\text{gFluoresc g}^{-1} \text{SS hour}^{-1}$ , respectively) and the other gullies (IG, JG, MG) (average = 59.95 and 9.21  $\mu\text{gFluoresc g}^{-1} \text{SS hour}^{-1}$ , respectively) (Table 3). A similar pattern was observed for acid phosphatase and  $\beta$ -glucosidase activity (Table 3). The reductions in acid phosphatase reached 77% in MG in the rainy season, and 62% in IG in the dry season compared with that in the SF area.  $\beta$ -Glucosidase activity decreased by around 71 and 85% in IG in the rainy and dry seasons, respectively, compared with that in the SF.

Thus, the effect of erosion on soil enzymatic activity is more intense in gullies in the initial (IG) and intermediate (JG and MG) stages compared with those in the senile stage (SG). This is possibly related to greater stability of erosive processes in SG, in addition to the positive effect of natural regeneration on enzymatic activity in SG. Silva *et al.* (2018) emphasized that the quantity and the quality of substrate added to the soil was important for increasing



enzymatic activity. This allows for greater incorporation of organic matter, soil protection (physical barrier) against erosive processes, and increased stimulation of microbial activity. The enzymatic activity has been considered a good indicator of soil quality, reflecting the degree of quality achieved by a solo in the rehabilitation process (Silva *et al.*, 2018).

**Table 3.** Soil microbiological attributes in areas of gullies at different formation stages (IG – initial; JG - juvenile; MG – mature; SG - senile), secondary forest (SF), mid-stage regeneration pasture (MSR), and initial-stage regeneration (ISR), at a depth of 0–0.05 m in two seasons (rainy season, dry season), in Pinheiral (RJ).

Areas	FDA		Acid phosphatase		$\beta$ -Glucosidase		BSR	
	$\mu\text{gFluoresc g}^{-1} \text{SS hour}^{-1}$		$\mu\text{mols pNP g}^{-1} \text{SS hour}^{-1}$		$\mu\text{mols pNP g}^{-1} \text{SS hour}^{-1}$		$\text{mg CO}_2 \text{ g}^{-1} \text{SS d}^{-1}$	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
SF	159.80 a*	115.81 a	7.19 a*	5.33 a	4.96 a	6.04 a	9.47 a	12.40 a
MSR	152.92 ab*	119.05 a	5.67 ab	4.06 b	4.48 ab*	3.69 ab	7.74 a	8.45 abc
ISR	149.43 ab*	90.49 b	4.15 ab*	3.04 bcd	2.23 cd	3.51 ab*	9.39 a	11.51 ab
IG	64.86 b*	10.90 d	2.49 bc	2.03 d	1.43 d	0.92 b	8.99 a	5.23 abc
JG	66.37 b*	7.28 d	4.15 ab*	2.33 cd	2.31 bcd	1.35 b	8.32 a	6.65 abc
MG	48.61 b*	9.46 d	1.63 c	2.34 cd	1.62 cd	1.89 ab	4.37 a	4.45 c
SG	110.13 ab*	44.07 c	2.63 ab	3.31 bc	3.66 abc*	2.28 ab	11.76 a*	6.73 abc

Areas	MBC		MBN		EE-GRSP		T-GRSP	
	$\text{mg Cmic kg}^{-1}$		$\text{mg Nmic kg}^{-1}$		$\text{mg g}^{-1} \text{soil}$		$\text{mg g}^{-1} \text{soil}$	
	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
SF	211.04 a	536.65 a*	211.04 a	536.65 a*	0.94 ab	1.86 a*	1.66 a	3.85 a*
MSR	111.89 ab	312.33 ab*	111.89 ab	312.33 ab*	1.38 a	1.01 ab	2.84 a	1.77 ab
ISR	48.46 ab	121.67ab	48.46 ab	121.67ab	0.63 abc	0.60 ab	1.44 ab	1.03 ab
IG	45.54 b	79.29ab	45.54 b	79.29ab	0.31 abc	0.20 b	0.52 ab	0.22 b*
JG	76.92 ab	52.31b	76.92 ab	52.31b	0.32 abc	0.22 b	0.68 ab	0.31 b*
MG	37.86 b	67.13 ab*	37.86 b	67.13 ab*	0.08 c	0.28 b	0.16 b	0.35 b
SG	66.74 ab	71.30 ab	66.74 ab	71.30 ab	0.19 bc	0.42 ab	0.78 ab	0.79 ab

Means followed by equal letters in the column do not differ by the Bonferroni T or Kruskal Wallis test at 5%.

\* Indicates a significant difference between seasons by the Bonferroni T or Kruskal Wallis test at 5%. FDA - fluorescein diacetate hydrolysis; Basal soil respiration (BSR), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN); EE-GRSP = Easily extractable - Glomalin-related soil protein; T= Total - Glomalin-related soil protein.

For all enzymes evaluated, there were strong positive correlations ( $r > 0.80$ ,  $p < 0.01$ ) with TOC and POXC. In general, the soil enzyme activities are strongly related to the content of organic matter and MBC (Maurya *et al.*, 2020; Tiwari *et al.*, 2019; Yuan and Yue, 2012; Gispert *et al.*, 2013). Positive correlations were found between MBC ( $r > 0.70$ ,  $p > 0.02$ ) and the enzymes FDA, acid phosphatase, and  $\beta$ -glucosidase in both seasons. Silva *et al.* (2012), evaluated carbon and soil enzyme activity in soils under native vegetation, pasture, and agriculture in Pinheiral (RJ), and reported a significant positive correlation between the  $\beta$ -glucosidase activity and the TOC of the soil; this pattern was confirmed in the present study.  $\beta$ -Glucosidase activity is fundamental for the release of nutrients from organic matter, acting in the early stages of degradation of organic compounds, reducing molecular size and producing smaller organic structures (Tiwari *et al.*, 2019; Tabatabai, 1994).  $\beta$ -Glucosidase activity has been used to evaluate soil quality under different management practices (Bhattacharyya *et al.*, 2021; Tiwari *et al.*, 2019).

Regarding the effect of the sampling period, FDA activity was higher in the rainy season compared with the dry season in all study units. Acid phosphatase activity varied between

seasons in the reference areas (SF and ISR) and in JG, with higher activity observed in the rainy season. Variation in  $\beta$ -glucosidase activity was found in the MSR and SJ area, with higher activity in the rainy season, and ISR with higher activity in the dry season. Some studies have demonstrated that enzyme activity is also regulated by temperature, precipitation, and soil moisture content (Yang *et al.*, 2017; Kittredge *et al.*, 2018).

Basal soil respiration (BSR), indicative of total microbial activity, did not vary between the gullies and reference areas during the rainy season (Table 3), while in the dry season, sediment production decreased the BSR by around 60% in the MG compared with the SF. The carbon (MBC) of soil microbial biomass was higher in the SF area compared with IG and MG in the rainy season, and JG in the dry season (Table 3). The nitrogen (MBN) of soil microbial biomass was higher in the SF area compared with IG and MG in the rainy season (Table 3). This can be explained by the higher intake of plant material in this area, providing greater soil coverage. This results in the accumulation of more organic material and nutrients (Table 2) for development of the microbial community (Alves *et al.*, 2011). In contrast, in the initial stage gully, the absence of vegetation (Figure 2) together with the low carbon content and N (Table 2) may have contributed to the lower values of MBC and MBN in both seasons.

Generally, similar MBC and MBN values were observed between the SF and the pasture areas (MSR and ISR) and the senile gullies (Table 3). In the pastures, the organic matter content and possible dense root mass may favor the presence of microbial biomass in the rhizosphere (Lopes *et al.*, 2010). In the senile gully (SG), the presence of vegetation may improve the incorporation of carbon and nitrogen into microbial biomass, with minor losses by erosion.

No variations were observed in the levels of MBC, MBN, and basal soil respiration between the rainy and dry seasons in most study units (Table 3).

Regarding the glomalin-related soil protein, no significant differences were observed between areas, for both fractions (easily extractable and total) in the rainy season, except the MG, which presented values lower than SF and MSR (Table 3). In the dry season, the highest values of GRSP (EE and Total) were observed in the reference areas, followed by SG with intermediate values, and with lower values the other gullies (IG, JG and MG) (Table 3). Significantly reduced levels of GRSP were found in gullies at different stages of formation (IG, JG, MG) (Table 3), with a 94% reduction in T-GRSP observed in the initial stage gully compared with the SF. This pattern could be related to the negative impact of erosion on AMF structures (hyphae and spores: structures that present glomalin in their composition). Conversely, higher values of GRSP in SF may be related to higher microbial activity, which may have impacted the rate of hyphae and arbuscular mycorrhizal fungi spore decomposition, and the consequent higher deposition of glomalin fractions. High correlations were observed between the GRSP fractions and variables related to soil microbial activity, such as the FDA (GRSP -T:  $r = 0.84$ ,  $p = 0.03$ ; GRSP -FE:  $r = 0.81$ ,  $p = 0.02$ ) (dry and rainy season) and BSR (GRSP -T:  $r = 0.79$ ,  $p = 0.03$ ; GRSP -FE:  $r = 0.79$ ,  $p = 0.03$ ) (dry season). According to Lutgen *et al.* (2003), the decomposition of hyphae should be considered for the increase in GRSP.

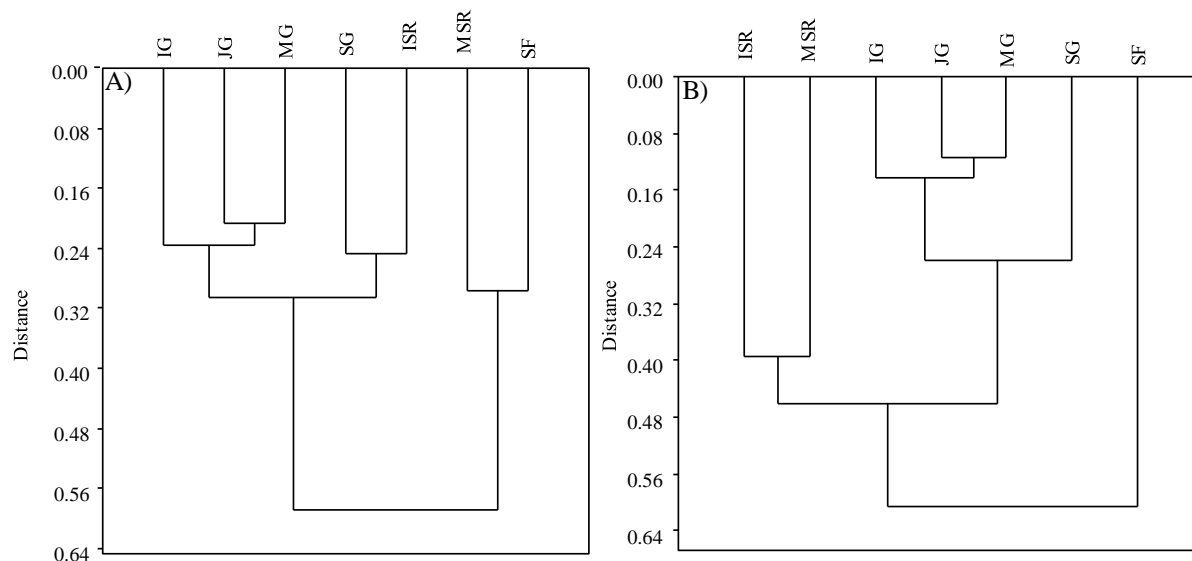
Positive and highly significant correlations were found between the GRSP and TOC fractions and N (GRSP -T:  $r = 0.98$ ,  $p = 0.00$ ; GRSP -FE:  $r = 0.96$ ,  $p = 0.00$ ) in soil in the dry season. GRSP is an important form of accumulated carbon and nitrogen in soil, since it contains high levels of these elements and is strongly correlated with soil aggregation (Sekaran *et al.*, 2021). Thus, reductions in the soil content of this protein lead to reduced soil quality. Positive correlations between glomalin and carbon, nitrogen, and soil aggregation have been found in several studies, which assessed different conditions of soil use and management, as well as degraded areas and the recovery process (Sekaran *et al.*, 2021; Liu *et al.*, 2020; Dai *et al.*, 2013).

No differences in GRSP were observed between the rainy and dry seasons in most study units (Table 3). Variations were observed only in SF, IG and JG. In the SF area, the highest GRSP levels (both fractions) were observed in the dry season, and in IG and JG, in the rainy

season for T-GRSP fraction (Table 3). Nogueira *et al.* (2016) in a forest system in the Atlantic Forest biome observed a pattern similar to that of the present study for the EE-GRSP, whose contents were higher in the dry season compared to the rainy season. On the other hand, these authors, in the same area, observed that the T-GRSP was higher in the rainy season compared to the dry season, as observed in IG and JG. This shows that the dynamics of the GRSP fractions can vary depending on climate changes within the same environments, and also in different environments, as observed in the present study.

Based on dissimilarity between study units in terms of chemical attributes, total and oxidizable organic carbon, and the microbiological attributes of soils in gullies of different formation stages (IG, JG, MG, and SG) and the reference areas (SF, MSR, and ISR) in the rainy and dry seasons, a multivariate cluster analysis was performed (Figure 3). Three large distinct groups were formed in both seasons, which were separated at a maximum distance of about 58% in the rainy and dry seasons (Figure 3). In both seasons, a greater distance between SF (58%) and the other study units was observed. In both rainy and dry seasons, greater similarity was observed between early, juvenile, and mature gullies, with a distance of up to 24% in the rainy season and 15% in the dry season, i.e. 76 and 85% similarity, respectively (Figure 3a and 3b).

The senile stage gully, in turn, formed a group with the ISR area during the rainy season, with a distance of 32% from the other gullies (Figure 3a). In the dry season, the distance between the SG and the other gullies was 25% (Figure 3b). Thus, SG is more differentiated from other gullies in relation to these soil attributes, which may be due to their format (“U” cross section, slightly regular contour line, walls with gentle slope) and the presence of vegetation cover in its entire extension (Oka-Fiore and Soares, 1976).

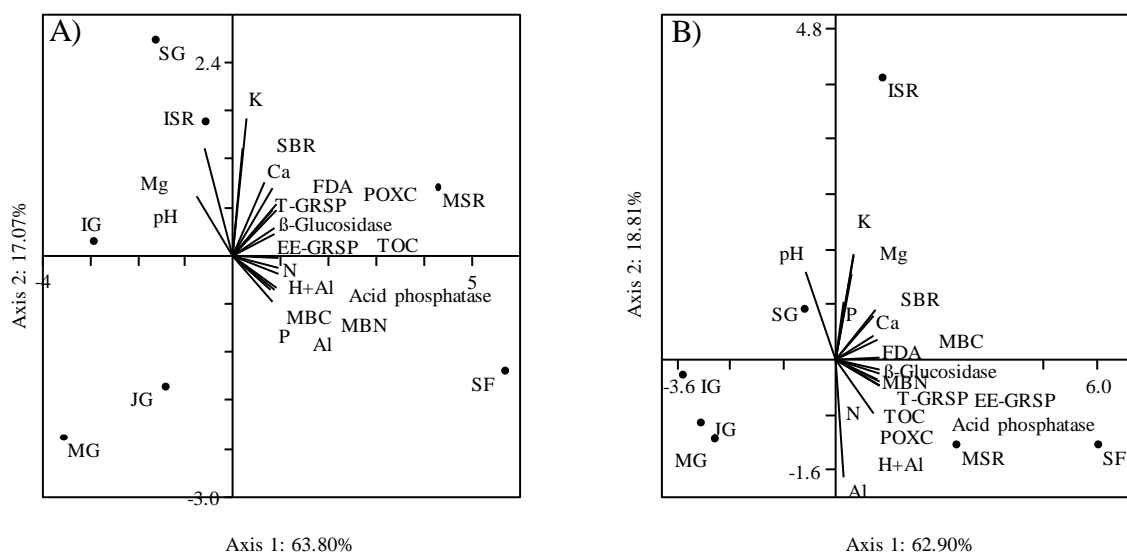


**Figure 3.** Dendrogram of cluster analysis integrating the chemical attributes, total organic carbon, oxidizable organic carbon, and the soil microbiological attributes of gullies in different stages of formation (IG - initial; JG - juvenile; MG - mature; SG - senile), secondary forest (SF), mid-stage regeneration pasture (MSR), and initial-stage regeneration (ISR), at a depth of 0–0.05 m in two seasons (rainy season – ch; dry season - sc), in Pinheiral (RJ).

Principal component analysis (PCA) revealed which variables contributed most to the grouping/separation of areas under study (Figure 4). Two main components for the chemical attributes, TOC and POXC, and the microbiological attributes (BSR, MBC, FDA, acid phosphatase, and  $\beta$ -glucosidase) of the soil were generated for both the rainy and dry seasons (Figure 4).

In the rainy season, the distribution of the selected variables showed an accumulated

variance of 80.87% for axes 1 and 2; axis 1 was able to explain 63.80% and axis 2, 17.07% of this variance (Figure 4a). In the dry season, the accumulated variance was 81.71% for axes 1 and 2. The first axis explained 62.90% and the second axis 18.81% of the variance (Figure 4b).



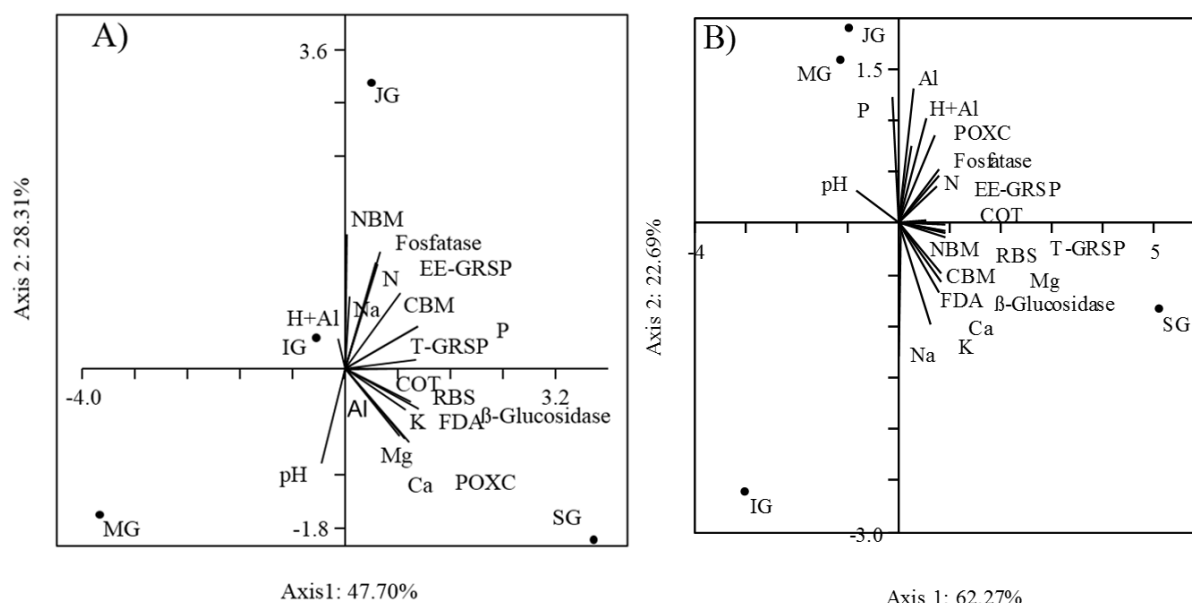
**Figure 4.** Ordination diagram (A) rainy season; B) dry season; produced by principal component analysis of microbiological and chemical attributes of soil from gullies at different stages of formation (IG – initial; JI– juvenile; MG - mature, SG - senile), at a depth of 0–0.05 m in two seasons, in Pinheiral (RJ). MBC - microbial biomass carbon; MBN - microbial biomass nitrogen; BSR - basal soil respiration; T-GRSP – Total - glomalin-related soil protein; EE-GRSP – easily extractable - glomalin-related soil protein; FDA - fluorescein diacetate hydrolysis; TOC - total organic carbon; POXC - oxidizable carbon.

As shown in Figure 4a and 4b (rainy and dry seasons, respectively), the areas presented different separations. In the rainy season the gullies and pasture were arranged in the lower (JG and MG) and upper (ISR, SG, and IG) left quadrants, while the SF and MSR areas were arranged in the lower and upper right quadrants, respectively. Most variables were positively correlated ( $r > 0.70$ ) with axis 1, which was strongly related to the reference areas (MSR and SF). In the dry season, the IG, JG, and MG formed a group in the lower left quadrant, while the pastures and SF were arranged on the opposite side, correlating with most of the analyzed variables; this represents the changes that occurred in the chemical and microbiological properties of the soil during erosion. SG was close to axis 0, highlighting an intermediate pattern between the gullies in the earliest stages and the reference areas, suggesting that the rehabilitation of soil properties is more advanced.

PCA was used to evaluate the distribution of gullies at different formation stages (Figure 5) based on their chemical variables and the fractions of carbon and soil microbiological attributes evaluated in this study. During the rainy season, the distribution of the selected variables showed an accumulated variance of 76.01% for axes 1 and 2; axis 1 was able to explain 47.70% and axis 2, 28.31% of variance (Figure 5a). In the dry season, the accumulated variance was 84.96% for axes 1 and 2. The first axis explained 62.27% and the second explained 22.69% of the variance (Figure 5b). In both seasons, the senile stood out from the other gullies and was located in the lower right quadrant. Conversely, gullies IG, JG, and MG were generally located in the left upper and lower quadrants (Figure 5a, b). Axis 1 was responsible for the separation between gullies, while most variables were positively correlated ( $r > 0.70$ ) with this axis as well as the senile gully (SG). Thus, one can observe that SG is more stable, and thus the microbiological and chemical attributes of the soil differ from those in gullies at previous stages. This is probably related to the development of dense vegetation in SG, enabling



increased protection, soil carbon incorporation, and improved temperature and humidity conditions, which may stimulate the soil microbiota and the cycling of nutrients.



**Figure 5.** Ordination diagram (a - rainy season; b - dry season) produced by principal component analysis of microbiological and chemical attributes of soil from gullies at different stages of formation (IG - initial; JG - juvenile; MG - mature, SG - senile), at a depth of 0–0.05 m in two seasons, in Pinheiral (RJ). MBC - microbial biomass carbon; MBN - microbial biomass nitrogen; BSR - basal soil respiration; T- GRSP – Total - glomalin-related soil protein; EE- GRSP - easily extractable - glomalin-related soil protein; FDA - fluorescein diacetate hydrolysis; TOC - total organic carbon; POXC – oxidizable carbon.

## 4. CONCLUSIONS

Gullies in different stages of formation promote a decrease in soil fertility and modification of microbiological attributes, particularly when compared with secondary forest areas.

Using multivariate PCA, the microbiological and chemical attributes of the soil were discriminated between gullies with distinct formation stages. Stabilization of erosion in SG favors the regeneration of these attributes when compared with gullies in the initial (IG) and intermediate (JG and MG) formation stages.

The chemical attributes of total organic carbon; oxidizable organic carbon; FDA,  $\beta$ -glucosidase, and acid phosphatase activity; MBC and MBN; BSR; and GRSP are good indicators for evaluating erosion stabilization in gullies.

## 5. ACKNOWLEDGEMENTS

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