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EVALUATION RELATIONSHIP OF GLOMALIN AND AGGREGATE MORPHOMETRY IN MINED AREAS IN THE RECOVERY PROCESS BRASILIAN AMAZON (RO)

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Abstract: Degraded areas undergoing recovery need to be assessed and monitored through environmental quality indicators in order to describe the real condition of the ecosystem. Therefore, the understanding the microbiological activity associated with the formation of aggregates is a fundamental in assessing soil quality. Thus, the aim this study is to quantify the fractions of glomalin-related soil protein easily extractable (GRSP-EE) and total (GRSP-T), evaluating its relationship with the physical properties of soil aggregates through morphometric analysis. Mined areas submitted to forest restoration in the brazilian Amazon were selected at different land reclamation stages, from 0 to 30 years. The reference ecosystem (Native Forest > 100 years) was also evaluated, providing the identification of association's among physical and microbiological soil properties. The results demonstrate higher concentration of GRSP (T and EE) in advanced stage locations, at the age of 30 years, when compared to the youngest forests, 10 year old. Furthermore, the macro-aggregates showed higher GRSP highlighting as fundamental for the maintenance of aggregate stability. All parameters analyzed (GRSP, aggregate diameter, revegetation age and stage of development) allowed the significance differentiations of reclamation stages. The GRSP-EE, GRSP-T and circularity of soil aggregates showed positive and significant correlations, allowing the identification of associations between soil glomalin and soil aggregates morphometry. The glomalin showed association with soil aggregation, allowing the identification of soil structure improvement with the advance of land reclamation and a promising ecosystem quality indicator.

Keywords: Soil indicators, Soil quality; Recovery of degraded land; Soil aggregate; glomalin.

INTRODUCTION

Mining is one of the pillars of global economic support, in this scenario the abundance of natural resources makes Brazil stand out for having one of the largest ore reserves in the world, annually producing more than 2 billion tons of mineral goods, moving approximately to US$ 40 billion with production/export (IBRAM, 2017). Although mining activities have promoted economic growth and development in the last century, they are also responsible for causing large-scale environmental degradation (MMA, 2019).

During the mining process, the vegetation and the topsoil are removed, modifying the physical, chemical and biological properties, consequently affecting the ecosystem's soil function. Despite all the economic benefits related with the mining, the exploration mineral reserves in important ecosystems, such as the Amazon Forest, requires careful management to minimize environmental impacts and associated problems, restoring the soil structure as close as possible to the original (Ribeiro et al., 2016; Fengler et al., 2017; Carvalho et al., 2019). The immediate restoration areas degraded for mining of these environmental to a self-sustaining is necessary and mandatory in most countries (Kumar et al., 2018), and has main objective of is to achieve the soil quality required for successful reclamation (Luna et al., 2016), analogous to the soil prior their deterioration (reference soil). Thus, long term monitoring of soil quality status during restoration process is important evaluating the capacity of the current state comparing it with the reference soil in natural state, since the reference ecosystem represents ecological conditions of environmental stability (Fengler et al., 2017; Carvalho et al., 2022).

Studies on ecological restoration carried out in mined areas in the Brazilian Amazon demonstrated, the importance of the relationship between the chemical, physical and microbiological properties of the soil with the degree of forest development and in the assessment of ecosystem quality (Longo et al., 2011; Ribeiro et al., 2016; Yada et al., 2016, Fengler et. al., 2017, Carvalho et al., 2019; Carvalho et al., 2022). According to Hermani (2018), it is possible to consider aggregation as a good indicator of health, global state and soil quality, because within a horizon, aggregates of different morphologies can coexist, and each type of aggregate has its own meaning in terms of history, functioning and soil fertility (Peche Filho, 2018). Soil aggregates play a key role in soil quality, since they protect organic material from microbial decomposition and prevent soil structure degradation, thereby ensuring balanced ecosystem (Bronick and Lal, 2005; Luna et al, 2016).

The soil microbial community, such as bacteria and fungi, plays a vital role in nutrient cycling, plant stress reduction, pedogenesis and soil aggregate formation (Six et al., 2004; Singh et al., 2016; Kumar et al. 2018). The arbuscular mycorrhizal fungi (AMF) are dominant in the soil microbial community, responsible for 30% of the soil microbial population and for producing glomalin (glycoprotein) from its structural components (extraradical hyphae and spore wall) (Wright; Upadhyaya, 1996; Rillig, 2002). An estimated 90% of terrestrial plants are believed to associate with some type of mycorrhizal fungi, some 80% of these being Arbuscular Mycorrhizal Fungi (AMF), especially in the humid tropics (Smith and Read, 2008; Reyes et al., 2019).The effective function of AMF depends not only on biological factors, but also on a whole range of abiotic features, including soil structural characteristics (Jamiołkowska et al., 2018). Besides the nutrient provision for plants, AMF also produce glomalin, a glycosylated soil protein (Gillespie et al., 2011;

Sarapakta et al, 2019), influencing soil fertility and plant nutrition (Bedini et al. 2009). Glomalin in the soil acts as a cementing material contributing to the formation and stabilization of soil aggregates, can be used to monitor the recovery of degraded soils (Zhang et al., 2017). The recently produced glomalin, usually quantified as soil protein related to easily extractable glomalin (GRSP-EE), is considered to be rapidly decomposed, while the fraction called total glomalin (GRSP-T) represents the glomalin strongly connected to soil minerals, being considered a relatively persistent fraction (Wu et al., 2012; Singh et al., 2016).

Previous studies have shown that changes in the soil environment such as climate, age of the site and vegetation characteristics can alter the responses of these two glomalin fractions (GRSP-EE and GRSP-T) (Fokom et al., 2012; Singh et al., 2016), acting as determining factors in the landscape restoration process (Ahirwal and Maiti, 2017; Kumar et al. 2018). Thus, considering that glomalin correlates with various physical, chemical and biochemical attributes of the soil, it can be considered useful within the system of indicators for assessing soil quality both in sustainable agriculture, as well as in assessing soil impact and monitoring of environments in recovery process (Gao et al., 2019; Sarapakta et al., 2019; Liu et al., 2020).

According to Lovelock et al. (2014), there is little explored field with regard to the identification of new indicators for the assessment of soil quality. Especially in the case of soils that make up the different Brazilian biomes, are required studies on the use of physical-hybrid attributes as a way of measuring their ecosystem sustainability. Thus, considering the importance of glomalin in the restoration of degraded areas (Vasconcellos et al., 2013; Luna et al., 2016; Reyes et al 2019) and its strong relationship with soil aggregation, it is possible to establish new indicators assessing the ecosystem and the ecological recovery process of degraded areas (Carvalho et al., 2020).

Thus, the present work aims to quantify the fractions of soil protein related to glomalin and to evaluate its relationship with the physical properties of soil aggregates through morphometric analysis, in an area degraded by cassiterite mining, located in tropical forest in the Brazilian Amazon (RO). Five mine were selected at different levels of the recovery process, namely: zero, intermediate and advanced management stages, in addition to the reference ecosystem (Native Forest), in order to identify interrelationships between these soil properties as parameters of soil quality.

MATERIALS AND METHODS

STUDY LOCATION

The experimental area is located in the Jamari National Forest, managed by the Brazilian Institute for the Environment and Renewable Mineral Resources (IBAMA), located 90 km from the city of Porto Velho - RO, by Br-36. The location has an area of approximately 225,000 ha, of which 90% is covered by Tropical Forest. It is characterized by high biodiversity of flora and fauna and abundant mineral reserves, where species of high commercial value are found for wood exploitation and mineral (Longo et al., 2011).

The soils are predominantly of the class dytropic Red-Yellow Latosoil texture (Kandiuand) and dytropic Red-Yellow Latosoil (Paleudult) covered by open rainforest with small patches of tropical rainforest, characterized by a high richness of spaced arboreal individuals and a diverse wildlife, including several species threatened with extinction. The natural soil pH is acid, ranging from 3.4 to 5.0. The climate is hot and humid, with average temperatures of 24 °C,

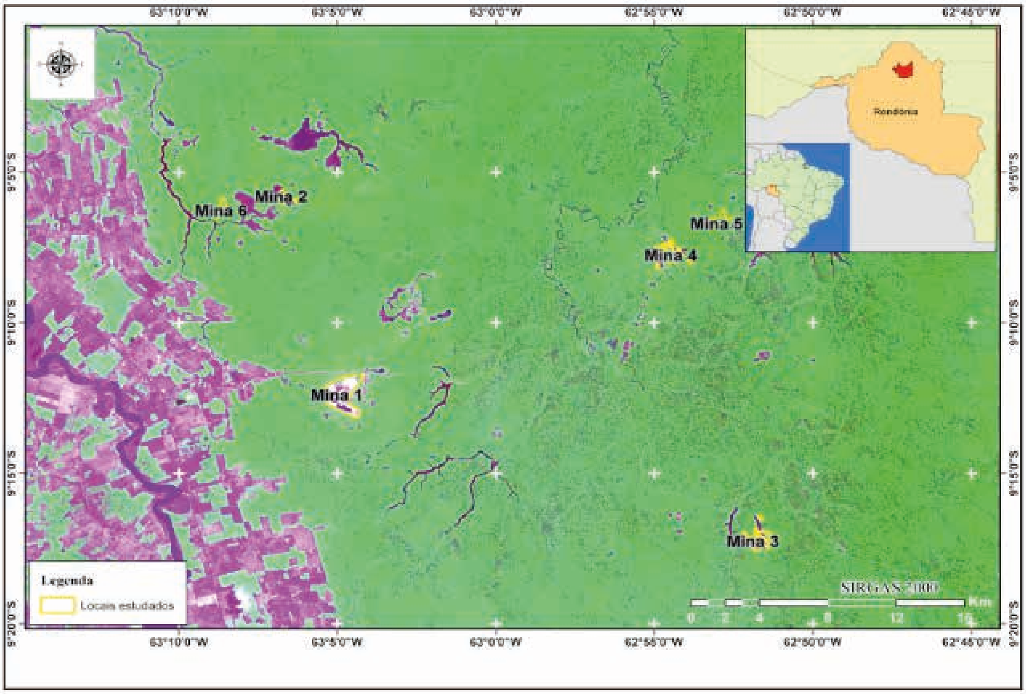

Figure 1: Experimental area location of the Jamari National Forest

Font: INPE, 2015 (Carvalho, 2022).

Mine	Revegetation Age	Textural Class	Reclamation stages
Mine 1	0	Silty	freshly mined
Mine 2	10	sandy	Intermediate
Mine 3	15	clayey	Intermediate
Mine 4	20	clayey	Advanced
Mine 5	30	sandy clay	Advanced
Secondary forest (SF)	50	sandy clay	Reference System
Primary forest (PF)	> 100	clayey	Natural Forest

Table 1 - General description of the Study area

Glomalin (mg g^{-1})	**Mine 1**	**Mine 2**	**Mine 3**	**Mine 4**	**Mine 5**	**SF**	**PF**
	0 years	**10**	**15**	**20**	**30**	**50**	**<100**
GRSP-EE	0.40d	3.38c	3.94c	5.95b	6.21b	6.87b	7.06a
GRSP-T	0.21e	6.84d	8.51d	10.37c	14.42b	17.42a	18.10a

Table 4 – Soil protein related to easily extractable (GRSP-EE) and total (GRSP-T) glomalin in mined and unmined soil aggregates depending on the area's forest development stage (ANOVA).

relative humidity varies around 85% (MMA/ IBAMA, 2015; Fengler et al., 2017, Carvalho et al., 2021).

The program for the recovery of mining areas in the Jamari National Forest was started in 1990, with the signing of a Term of Conduct Adjustment Commitment with the IBAMA (Yada et al., 2015), aiming at the regulation of measures to correct the damage caused to the environment by cassiterite mining. In 1997, the mined sites were subdivided into plots and the recovery actions started, with topographic reconstruction, terracing, soil correction actions, mineral and organic fertilization, followed by revegetation with the cultivation of legumes and planting of native forest species.

Approximately 40,000 seedlings of 22 different species were planted in each mine. For planting forest species, the main species used were: *Leucena lencocephala* (Leucena); *Inga* sp. (Ingá xixica); *Ingá edulis* (Ingá de metro); *Anadenanthera macrocarpa* (Angico); *Syzgium jambolanum* (Jambolão); *Vismia* sp. (Lacre branco); *Mangifera indica* (Mangueira); *Jacaranda copaia* (Para-pará); *Ochroma pyramidale* (Pau-de-balsa); *Parkia multijuga* (Paricá); *Schizolobuim janeireme* (Bandarra); *Tabebuia* sp (Ipê) (RIBEIRO, 2005).

Different types of management were developed in the experimental area throughout the recovery process, thus, it is necessary to characterize the area in relation to the soil textural class, age of revegetation and level of development (Table 1).

The study was performed using five areas in recovery process with different management and revegetation ages ranging from 10 to 30 years and Native Forest with closed canopy was adopted as reference ecosystems and used to assess the performance of the forest restoration (Tab. 1).

AGGLOMERATE MORPHOMETRY

Soil collections were performed following the sampling procedure, in three 10 m x 10 m subplots. In each sub-plot, five sub-samples of approximately 1.5 kg, at a depth of 0-10 cm were performed. After collection, the samples were destined for analysis, where they remained for 15 days at room temperature (30 to 35 ° C) for drying in the open, then approximately 200 g of soil was sieved in a set sieves of 5 different diameters, the first with 6.0 mm, the second with 4.0 mm, the second with 2.0 mm, the fourth with 1.0 mm and the fifth with 0.71 μm. Then were randomly selected 100 aggregates of each study area to be photographed through the digital microscope (Dino Lite model AM-211), processed in the ImageJ® program for soil aggregate morphometric analysis. The images were converted to black and white, eliminating noise to determine the morphometric parameters of the soil aggregate: area, roundness, circularity, Feret diameter and Feret Max/Min, following standardized methodology of the Brazilian Agricultural Research Corporation (EMBRAPA, 2017).

The aggregate area was evaluated through the number of pixels contained in each processed image and the values were converted into millimeters (mm^2) after calibration, for the processing of data.

The rounding of the particles of aggregate was calculated by comparing their area as a function of their perimeter (Eq. 1) (COX, 1927; RIBEIRO; BONETTI, 2013).

Equation* 1** $\quad$ ***Round $= 4 \pi A/p^2$

Where,

A is the area of the aggregate; P is the perimeter of the aggregate.

The circularity of aggregate expresses how close the aggregate morphology is to perfect sphericity, comparing the particle area as a function of its major axis, calculated from the equation 2 (PERTLAND, 1917; RIBEIRO; BONETTI, 2013).

***Equation* 2** $Circ = 4A/\pi(L)^2$

Where,

A is the area of the aggregate,

L is the major axis of the aggregate.

The Feret's diameter of aggregate were measures by calculating the largest distance between any two points along the limit of the processed image of the soil aggregate, (Eq. 3) (WANG et al., 2014).

***Equation* 3** $DF = 4Ar/\pi$

Where,

Ar is the rounding of the aggregate

The Max/Min is the ratio between smallest and largest diameters based on Feret measurements and can be used for the study of irregular particles, as they represent the diameter that crosses the center of a given object taking some arbitrary direction (Eq. 4) (Wang et al., 2014).

***Equation* 4**

$$Dr = \frac{Dmín}{Dmáx}$$

GLOMALIN

Glomalin extractions of soil samples (1.0 g) were carried out as described by Wright and Upadhyaya (1998), estimating contents of easily extractable glomalin (GRSP-EE) (extraction with 20 mM citrate, pH 7.0 at 121 °C for 30 min), and of total glomalin (GRSP-T) (extraction with 50 mM citrate, pH 8.0 at 121 °C). The protein contents were subsequently determined using the Bradford dye-binding assay with bovine serum albumin as standard (Wright and Upadhyaya, 1998, Carvalho, 2021).

STATISTICAL ANALYSIS

The differences between the parameters of glomalin content, texture, and development stage, analysis of variance (ANOVA) was performed, the averages were compared by Test Tukey (valor-p < 0,05), to verify the degree of correspondence between the levels of glomalin and the morphometry of the aggregates.

Correlation analysis and Principal Components Analysis were used to assess how the levels of glomalin and the morphometry parameters of the aggregates could be summarized and employed in monitoring and assessment the long-term success of reclamation in Amazonia mined soils. Both analyses were performed adopting the Kendall correlations for $p \leq 0.001$. The z-scores standardization was performed before the PCA, in order to equalize the differences arising from the dimensional greatness of the parameters evaluated (Eq. 5).

***Equation* 5**

$$Z_{ij} = \frac{(X_{ij} - Xm_j)}{S_i}$$

where X_{ij} is the original data value, Xm_j is the mean for the ith variable, and S_i is the standard deviation for the ith variable.

The Kaiser-Meyer-Olkin (KMO) test was employed to confirm the suitability of the sample data before performing PCA (Hair et al., 1987; Kaiser, 1974). Only principal components (PCs) with eigenvalues ≥1 and explaining at least 5% of the total variation were examined (Mukhopadhyay et al., 2014). The OBLIMIN rotation was employed to redistribute the variance in the retained PCs, and only variable loadings that achieved at least ±0.30 were evaluated (Hair Jr. et al., 2010).

The statistical analyses were performed using R statistical software (R Core Team, 2017), using the packages ggplot2 (Wickham, 2009), Hmisc (Harrell Jr. and Dupont, 2016), FactoMineR (Le et al., 2008), factoextra (Kassambara and Mundt, 2017), and the routines STHDA (2017, 2017a, and 2017b) and Kabacoff (2012).

RESULTS

ANALYSIS OF THE QUANTIFICATION OF GLOMALIN

The contents of GRSP-EE and GRSP-T varied significantly comparing the land reclamation stages and the reference ecosystems. The locations with 20 and 30 years of forest development, showed no significant differences with the reference ecosystem. The locations with intermediate ages (10 and 15 years) showed significant differences with older locations, the secondary forest, and the primary forest (reference ecosystems).

The soil aggregates size showed significant differences, allowing the differentiation of glomalin fractions in aggregates of 6 mm, 1-4 mm, and 0.71 mm (Table 5). The ANOVA showed a trend of increase in glomalin fractions with the increase of soil aggregate size. For GRSP-EE the values ranged from 3.52 mg g^{-1} (0.71 mm) to 5.19 mg g^{-1} (6.0 mm) and GRSP-T from 8.48 mg g^{-1} (0.71 mm) to 11.56 mg g^{-1} (6.0 mm).

MULTIVARIATE ANALYSIS OF VARIANCE RELATED TO MORPHOMETRIC DATA (MANOVA)

A MANOVA evaluating the morphometric variables of the recovery areas in relation to the reference ecosystem, considering age, substrate and their interactions, made it possible to differentiate between the employed variables. The analysis of the interactions between morphometry and developmental stage resulted in significant differences. However, the variables that will stand out allow to clearly differentiate and separate the reference ecosystem between two younger, intermediate and older locations: Area, Circularity, Feret, MinFeret and Max/Min Feret, presenting expressive results in the difference between the areas in recovery process in concordances with the raw data (Table 6).

Considering the interactions between morphometry and aggregate diameter, it was possible to identify a significant difference ($p < 0.05$) between the penile diameters for Area, Circularity, Feret and Max/Min Feret (Table 7).

CORRELATION ANALYSIS RELATED TO GLOMALIN CONTENT AND MORPHOMETRY

The GRSP-EE and GRSP-T showed significant correlations with morphometric soil aggregates parameters. The glomalin was negative correlated with soil aggregates circularity. One explanation to this result was the existence of mineral particles in the samples. The soils were collected mined spoils submitted to forest restoration. Even after forest restoration, the development phenomenon is only slower than the process of forest formation, such as delay, or the place still presents many mineral particles that can present a rounded shape. Such a pattern, combined with the results of Table 8, suggests that the process could be clearer only at peneira 6, being only circularity an inadequate parameter for the analysis of the aggregation, mainly in degraded areas, or with little organic action.The Feret diameter, MinFeret and soil aggregate area were positively correlated to the Total Glomalin. These morphometric parameters showed high positive correlations, indicating redundancy for these variables.

PRINCIPAL COMPONENT ANALYSIS RELATED TO GLOMALIN CONTENT AND MORPHOMETRY

The value obtained in the Kaiser-Meyer-Olkin (KMO) test (0.6) indicated as acceptable for the application of PCA (Hair et al., 1987; Kaiser, 1974). Four principal components (PCs) showed eigenvalues ≥1, accounting for 88% of the total data variability (Fig. 2). The first principal component (PC1) included

Glomalin (mg g⁻¹)	Aggregate (mm)									
	0.71		1.0		2.0		4.0		6.0	
GRSP-EE	3.52	c	4.18	b	4.48	b	4.52	b	5.19	a
GRSP-T	8.48	c	9.92	b	10.07	b	10.43	b	11.56	a

*Averages followed by the same letter on the line do not differ by Teste Tukey a 5%

Table 5 – Soil protein related to easily extractable (GRSP-EE) and total (GRSP-T) glomalin in aggregates of different diameters (ANOVA).

MINE Age	MINE 1 0		MINE 2 10		MINE 3 12		MINE 4 15		MINE 5 20		MINE 6 30		SF 50		PF <100	
Area	**0.001**	**e**	**0.105**	**de**	**0.169**	**de**	**0.241**	**d**	**0.254**	**c**	**0.269**	**b**	**0.271**	**b**	**0.291**	**a**
Circularity	**0.013**	**e**	**0.096**	**de**	**0.142**	**d**	**0.166**	**c**	**0.169**	**c**	**0.247**	**b**	**0.262**	**b**	**0.302**	**a**
ROUND	0.012	c	0.039	b	0.054	ab	0.076	ab	0.115	a	0.119	**a**	0.112	a	0.122	**a**
D. Feret	**0.032**	**e**	**0.145**	**de**	**0.208**	**d**	**0.234**	**d**	**0.289**	**de**	**0.258**	**c**	**0.301**	**b**	**0.333**	**a**
MaxMin	**0.017**	**e**	**0.053**	**d**	**0.061**	**d**	**0.076**	**d**	**0.113**	**c**	**0.153**	**b**	**0.157**	**b**	**0.194**	**a**

*Variables in bold indicate significant differences between treatment methods (MANOVA, p ≤ 0,05). The means followed by the same letter, on the line, do not differ significantly from each other (p ≤ 0,05).

Table 6 - Morphometric variables of aggregates only from mines in relation to different stages of recovery and natural ecosystem (MANOVA).

Sieves	0.71 mm		1.0 mm		2.0 mm		4.0 mm		6.0 mm	
Area	**0.044**	**d**	**0.074**	**d**	**0.133**	**c**	**0.894**	**ab**	**0.912**	**a**
Circularity	**0.030**	**cd**	**0.082**	**d**	**0.135**	**c**	**0.873**	**ab**	**0.898**	**a**
AR	0.154	ab	0.139	c	-0.052	ab	-0.234	ab	-0.050	**a**
Feret	**0.040**	**d**	**0.039**	**d**	**0.141**	**c**	**0.818**	**b**	**0.890**	**a**
MaxMin	**-0.138**	**c**	**-0.153**	**c**	**0.040**	**b**	**0.081**	**a**	**0.233**	**a**

*The variables in bold will show significant differences between the treatment methods (MANOVA, p ≤ 0.05). The means followed by the same letter are not significantly different (p ≤ 0.05).

Table 7 - MANOVA results to assess the differences between the penises and the mean values of the morphometric variables.

Variables	GRSP-EE	GRSP-T	Area	Circularity	Rounding	Feret	Max/min	Clayey	Silty	Sandy	Sandy Clay
GFE	**1.00**	**0.61**	0.15	**-0.22**	-0.14	0.17	-0.13	0.18	-0.07	0.00	-0.02
GT		**1.00**	**0.24**	**-0.21**	-0.08	**0.25**	-0.04	0.15	-0.10	0.03	0.07
Area			**1.00**	0.04	0.08	**0.92**	0.10	0.12	-0.06	-0.04	-0.11
Circul				**1.00**	**0.25**	-0.02	**0.31**	-0.01	0.18	-0.10	-0.16
Arred					**1.00**	0.02	**0.83**	-0.19	0.06	0.10	0.22
Feret						**1.00**	0.15	0.11	-0.08	-0.03	-0.01
Max/min							**1.00**	0.08	-0.05	-0.01	-0.08
Clayey								**1.00**	**0.40**	**-0.80**	**0.72**
Silty									**1.00**	**-0.60**	**-0.81**
Sandy										**1.00**	**0.90**
Sandy Clay											**1.00**

*Os valores em negrito são diferentes de 0 com um nível de significância alfa=0.001

** GRSP-EE (soil protein related to glomalin easily extractable), GRSP-T (soil protein related to glomalin total), ROUND (rounding), CIR (circularity) e DF (diameter of feret).

Table 8 – Correlation Matrix Person - glomalin and morphometry of soil aggregates with 2.0 mm in diameter. Person Correlation Matrix - glomalin and morphometry of soil aggregates with 2.0 mm in diameter.

three morphometric variables with significant loading values (Area, Feret, MinFeret), satisfying the criterion of ±0.55 proposed by Hair Jr. et al. (2010) for our sample size. These variables explained over 99% of the variability of PC1 and around 31.2% of the total data variation. The other variables showed very low loading values, below the minimum value proposed by Hair Jr. et al. (2010) (±0.3) to provide explanatory power in PCA.

The second principal component (PC2) was dominated by two morfometric variables, namely Arred and Max/Min. These variables explained 96.5% of retained variation of PC2, accounting for 24.1% of total data variation. Almost all retained variation (99.9%) of PC3 can be explained by the soil texture variables, percentage of clay, silt and sand (Fig. 2). These variables accumulated 20.6% of the total data variation. The PC4 showed 97.8% of total variation retained in the variables related to glomalin content (GFE and GT), accounting for 10.6% of total data variation.

DISCUSSION

ANALYSIS OF THE QUANTIFICATION OF GLOMALIN

In our research, both as fractions of the GRSP increased as a function of the mine recovery stage, considering the results obtained for each class of aggregates (Table 2 and 3), demonstrating degree of positive development and growing biological activity in these mined areas. These results are in line with those of Kumar et al. (2019), who evaluated a chronosequence of recovered coal mines, in tropical conditions, and observed that the areas in the most advanced stage of recovery had higher GRSP contents in relation to the younger areas. Silva et al. (2012), in clay mining areas, they observed that the revegetation of the pits with tree legumes, either in monoculture or in intercropping, increases the concentration of the GRSP fractions in relation to the pit area with spontaneous vegetation (Carvalho, 2021).

Study carried out in the eastern periphery of the Amazon in a cultivated region, in a young area (3 to 4 years), middle age (6 to 8 years), degraded secondary forests (50) and in mature tropical forests (> 120 years), demonstrated that GRSP-EE and GRSP-T values did not differ significantly over the succession (Reyes et al. 2018). The general averages were 1.14 ± 0.32 g kg^{-1} (GRSP-T) and 0.40 ± 0.04 g kg^{-1} (GRSP-EE), diverging from the averages of the present study, carried out in a recovered mined area in the southern region of the Amazon (10 to 50 years), which varied between 2.44 ± 7.64 mg g^{-1} (GRSP-EE) and 4.59 ± 19,56 mg g^{-1} (GRSP-T), however, the highest averages were observed in areas in more advanced stages of recovery and reference ecosystem.

In general, the levels of soil protein related to easily extractable glomalin and total glomalin found in the present study are close to those found by Purin et al., (2006) (1.08 to 5.85 mg g^{-1} soil), while in the search for Thuber; Fernandes (2014) in different types of management and planting, the results had small variations for GRSP-EE (0.88 à 1.54 mg g^{-1}) e GRSP-T (3.20 à 4.34 mg g^{-1}). Fokom et al. (2012), found values of GRSP-EE and GRSP-T 6.51 and 8.45 mg g^{-1}, respectively in managed soil, in the humid tropical forest zone in Southern Cameroon. In secondary forest soil, these authors found values of 10.56 mg g^{-1} for GRSP-EE and 15.67 mg g^{-1} for GRSP-T. Wright; Upadhyaya (1998), believe that glomalin fractions can vary from 1 to 20 mg g^{-1} of soil, however, there may be a wide variation in the levels found in different types of soils and different regions. In forest soils, Rillig et al. (2001) observed values of glomalin> 60 mg g^{-1} soil. However, this value may exceed 100 mg g^{-1} of GT in tropical soils in Hawaii with an estimated age of 4.1 million

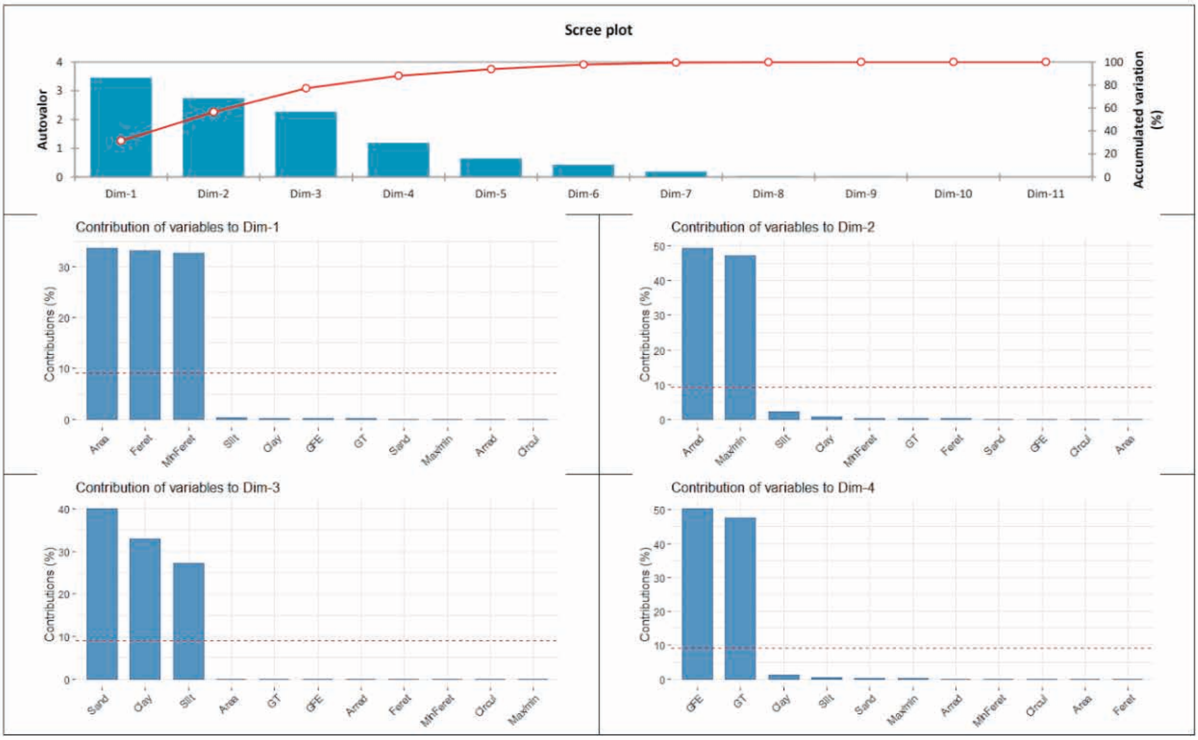

Figure 2: Contribution of variables in the dimension of PCA

years (Rillig et al., 2003).

ANALYSIS OF VARIANCE RELATED TO GLOMALIN CONTENT

Our results showed that the increase in GRSP was 12 times at 30 years after revegetation in relation to the mine area in stage 0 of recovery (table 4). Besides that, observed high increases in the GRSP, in macroaggregates of soils (table 5), similar to the study of Liu et al. (2020), also observed greater concentration in macroaggregates after a long period (>22 years) in pasture recovery. Such increases in PSRG at 30 years after revegetation and the similarity with the secondary forest area, allow us to infer that the process of mine recovery, using native forest species and legumes, is being effective in restoring the biological activity of the soil, when PSRG is used as an indicator (Carvalho, 2021).

Kumar et al, (2019), significant differences were observed in the areas undergoing recovery at the age of 1 to 26 years after revegetation, in relation to the area of natural forest. With variation in the content of the GRSP-EE between 0.3 e 3.2 g kg^{-1} and GRSP-T between 0.7 and 7.3 g kg^{-1}, the lowest values observed in the mine at a young stage of recovery, moderate in areas of intermediate age and higher in soils with a more advanced stage of recovery, as well as data from Table 4. Such a pattern demonstrate that the state of maximum aggregation occurs in soils under mature forest and natural vegetation, where microbiological activity is active, positively influencing soil aggregation and production of glomalin (Bedini et al., 2007; Fokon et al., 2012; Truber, 2014).

In most of the mines and reference areas, there was a tendency to increase the concentrations of GRSP-T and GRSP-EE in soil samples with larger diameter aggregates, and this same pattern can be observed when assessing the contents of GRSP in the aggregates, regardless of the development stages of the areas (Table 5), confirming that each type and size of aggregate has its own significance in terms of soil functioning and fertility (Peche Filho, 2018). This fact is consistent with previous studies (Fokom et al., 2012; Zhang et al., 2014; Zhu et al., 2019; Silva et al., 2019; Liu et al., 2020), in which macroaggregates have the greatest capacity for accumulation of glomalin and are highly correlated with aggregate stability due to the contribution of soil microbiota (Deng et al., 2018; Yao et al., 2019; Zhu et al., 2017; Liu et al, 2020).

Silva et al., (2019) discuss that in macroaggregates, the action of fungal mycelium provides greater stability of aggregates in water (Zou et al., 2015), and this fact may be an indication of greater synthesis of PSRG (Kohler-Milleret et al., 2013). GRSP is reported as one of the main sources of C in the soil, playing a crucial role in the capture and COS (Singh et al., 2017; Kumar et al., 2018), as well as maintaining soil fertility (Preger et al. 2007). In addition, and not least, the GRSP functions as a cementing agent in the soil (Rillig, 2004; Thuber et al, 2014; Wright; Upadhyaya, 1998), contributing to aggregation through the bonding of soil particles and different sizes of aggregates, which promotes greater soil stability (Gao et al., 2019).

The interaction of the glomalin fractions in relation to the type of texture was also positive in differentiating the mines (Table 6). The areas represented by mines 4, 5 and 6 throughout all physical and microbiological analyzes showed similar characteristics of soil with a clay texture, always very close to the natural areas considered as reference ecosystems, demonstrating that the type of management and type of Soil directly contributes to the condition of glomalin production and consequently favors the aggregation and structural stability of the areas under recovery, due to the positive correlation of glomalin with the clay content and negative with the sand content (De Gryze et al., 2010).

The present study suggests that the variation in the production of GRSP may have occurred due to the difference in the textural class, since the clay may act to protect the glomalin, reducing the action of decomposing microorganisms. In addition, according to the data presented (table 6), the model and management carried out in the mined areas was fundamental in the development of the recovering ecosystem, contributing to the production of soil protein related to glomalin and improving the condition of the mined soil throughout the process recovery.

CORRELATION ANALYSIS RELATED TO GLOMALIN CONTENT AND MORPHOMETRY

The significant positive correlation between GRSP-T, GRSP -EE with the stability of aggregates evaluated through the morphometric variable MWD (table 7). Demonstrating strong influence of glomalin and stabilization of soil aggregates in pasture restoration, at all stages of the recovery process (Liu et al., 2020). In a study on the relationship between the concentration of microbial biomass, organic C associated with the aggregate and glomalin in agricultural soils, there was a positive correlation between the MWD, MCB, CO, GRSP-T and GRSP-EE, showing greater stability of soil aggregate through the positive effects of soil binding agents, including glomalin-related soil protein (Zang et al., 2015). Such positive correlation indicates the important role of the microbiota in the formation and stability of the aggregate, due to variable decomposition rates of hyphae and spores resulting in the production of glomalin, as published by Bedini et al. (2007). Zhang et al., (2017) states that the glomalin produced (GRSP-FE e GRSP-T) correlates positively with soil factors and AMF infections and can be used to monitor the recovery of degraded soils.

Other studies in differentiated conditions also report a positive and high correlation with MWD and variables such as diversity of FMAs (Bedini et al., 2007), highlighting that due to glomalin being closely related to the main aggregation indicators, its great contribution directly results in the structuring of the soil (Demenois et al., 2018; Zhijie et al., 2019). Furthermore, according to Jamiołkowska et al. (2018), soil translocation can cause changes in the population and abundance of arbuscular mycorrhizal fungi in the soil profile and these processes can also influence glomalin content and aggregation-related soil properties (Gispert et al., 2017).

According to Rillig et al. (2001), the main factors that are involved in controlling the production of glomalin in the soil are not yet clear. However, according to these authors the combination of nutrient concentration, climate and diversity of FMAs influences the deposition of proteins in the soil. In addition, the amount of glomalin found can also vary according to the type of soil (Wu et al., 2012). However, it is known that the clay concentration influences aggregation through the expansion and dispersion of soil particles, reflecting in a soil with greater aggregate stability.

PRINCIPAL COMPONENT ANALYSIS RELATED TO GLOMALIN CONTENT AND MORPHOMETRY

Removal of coal through surface mining resulted in formation of mine spoiled landscapes. The afforestation and natural colonization of vegetation on these degraded act as tool of reclamation because of intrinsic engineering of plants that have capacity to alter soil structure and function with time (Ahirwal et al., 2017).

To better understand the comprehension of the relationship among GRSP and other important soil properties as well as relationship of these soil properties with mines chronosequence, we plotted the whole measured soil variable on two principal components of PCA (Fig. 2). The first PCA component is responsible for major variation of the data total (31%) and showing significant loadings three morphometric variables with significant loading values (Area, Feret, MinFeret). And the second PCA component is responsible for variation of the data total (25%) and showing significant loadings of variables with significant loading values (Around and Max/Min Feret).

The PCs clearly demonstrate how the variability of the data can be subdivided as well as its magnitude. The main component having a greater or lesser eigenvalue, or retaining more variability, does not mean that it explains the phenomenon better or worse, but that it retained a greater portion of variability, that is, differences. This means that the soil aggregates show greater variations in the area, feret and Min Feret. That is, these morphometric variables demonstrate differences between the sample aggregates. In general a The area and Ferets measurements can be related to the intragroup variation, reflecting the differences in shape in the same soil.

In general, the age of forest development showed a similar trend, with older recovered forests and the reference ecosystem, or indicating an increase in glomalin with the increase in forest age, similar results were obtained by (Kumar et al. 2016 Luna et al., 2016; Fokom et al., (2012). Thus, based on the results of the present study, it is possible to state that the quantification of glomalin consists of a potential indicator of changes caused by land use, by fully meeting the factors and by being correlated with important soil attributes (Rillig et al., 2003).

CONCLUSION

Soil protein related to GRSP-T and GRSP-EE increased in areas with more advanced stages of recovery, while in the area with zero stage of recovery the concentrations of these fractions were negative, thus showing themselves sensitive to environmental changes and differentiating the degree of development of mined soil.

The GRSP varied significantly in relation to soil texture and age of forest development in recovered mined soil, resulting in greater incorporation of GRSP in the sandy-clay and clayey texture. In addition, the larger aggregates showed higher index of GRSP compared to micro-aggregates, demonstrating an important function of macro-aggregates in the accumulation of GRSP, which has been highlighted as fundamental for the maintenance of aggregate stability.

The concentrations of fractions of soil protein related to glomalin (easily extractable and total) correlated positively with the MWD, circularity and area of soil aggregates, which shows its contribution to the stability of aggregates. The similar correlation of GRSP and MDW with several measured important soil properties suggest that, increasing GRSP content resulted in multi-level improvement in soil characteristics. Thus, increasing GRSP content with age of management is good sign of recovery of soil properties after disturbance due to mining.

Based on the results of the present study, considering that the glomalin content correlating with a number of these parameters, as we have proven, thus it is believed that glomalin can be considered useful in the indicator system in the assessment of soil biological quality, monitoring of degradation factors and their impact on the soil in forest ecosystems.

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