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Artigos

Microtopographies and soil conditioners on growth and nutrition of *Eucalyptus saligna* in abandoned agricultural field

Microtopografias e condicionadores de solo no crescimento e nutrição de *Eucalyptus saligna* em área agrícola abandonada

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ABSTRACT

Rehabilitation of abandoned agricultural fields is a multidisciplinary task, in pursuit of the development of sustainable productive systems. The objective of this study was to evaluate the effect of microtopography technique and soil conditioners on survival, growth and nutrition of *Eucalyptus saligna* of an abandoned agriculture field. Plots of microtopography and flat plots were established in an abandoned agriculture field (27° 12.08′ S and 49° 07.60′ W). Within plots, we stablished subplots with plants, which received the application of soil conditioners, including phonolite (100 g), mycorrhizal inoculant (200 cm^3), pulp sludge and carbonized rice husk (200 cm^3), phonolite (100 g) and mycorrhizal inoculant (200 cm^3) and control. The experiment monitoring occurred between november 2013 to october 2014 and growth parameters were measured after 30, 90, 150, 210, 270 and 330 days of the experiment onset. From each plant, height, stem diameter and canopy area were measured. At the end of the experiment, 50 leaves were collected from each plant to determine potassium and phosphorus concentrations. The native mycorrhizal community was characterized by bioassay and species identification of field-collected spores. Survival rates of *Eucalyptus saligna* tended to be higher in microtopography plots. The Plant height, stem diameter, canopy area, and shoot P and K concentration were significantly higher in plants at microtopography plots. Soil conditioners influenced significantly the shoot K concentration. The application of microtopography technique improved survival, growth, and nutrition of *Eucalyptus saligna*, and it represents an important strategy to rehabilitate abandoned agricultural fields.

Keywords: Rehabilitation; Heterogeneity; Phonolite; On-farm mycorrhizal inoculum

RESUMO

A reabilitação de campos agrícolas abandonados é uma tarefa multidisciplinar, que busca o desenvolvimento de sistemas produtivos sustentáveis. O objetivo deste estudo foi avaliar o efeito da técnica de microtopografia e condicionadores de solo na sobrevivência, crescimento e nutrição de *Eucalyptus saligna* de uma área agrícola abandonada. Parcelas de microtopografia e parcelas planas foram estabelecidas em um campo agrícola abandonado (27º 12.08’ S e 49º 07.60’ W). Dentro das parcelas, subparcelas com plantas foram estabelecidas e receberam a aplicação de condicionadores de solo, que incluíram fonolito (100 g), inoculante micorrízico (200 cm^3), lodo de celulose e casca de arroz carbonizada (200 cm^3), fonolito (100 g) e inoculante micorrízico (200 cm^3) e controle. O experimento foi monitorado entre novembro de 2013 e outubro de 2014 e parâmetros de crescimento foram mensurados após 30, 90, 150, 210, 270 e 330 dias do início do experimento. De cada planta, foram mensuradas altura, diâmetro do caule e área da copa. Ao final do experimento, 50 folhas de cada planta foram coletadas para determinar a concentração de fósforo e potássio. A comunidade micorrízica nativa foi caracterizada por um bioensaio e as espécies identificadas a partir de esporos coletados em campo. Taxas de sobrevivência de *Eucalyptus saligna* tenderam a ser mais altas em parcelas de microtopografia. Altura da planta, diâmetro do caule, área da copa e concentração de P e K na biomassa foram significativamente maiores em plantas em parcelas de microtopografia. Condicionadores do solo influenciaram significativamente a concentração de K na biomassa. A aplicação da técnica de microtopografia melhora a sobrevivência, crescimento e nutrição de *Eucalyptus saligna* e representa uma importante estratégia para reabilitar áreas agrícolas abandonadas.

Palavras-chave: Reabilitação; Heterogeneidade; Fonolito; Inoculante micorrízico

1 INTRODUCTION

One to six billion hectares of land may be degraded around the world (GIBBS; SALMON, 2015). The topic is a relevant global issue due to its relation to the discontinuity in the provision of ecosystem services (TALLIS *et al*., 2008) and it reflects the emergence in rehabilitation strategies. Among the potential techniques for rehabilitation of degraded areas, we highlight the use of soil conditioners, such as the application of mycorrhizal inoculum produced by the “on-farm” method, and the addition to rock dust as a source of nutrients for plants. It is also possible to highlight the uses of different techniques of physical soil management, such as the creation of microtopographs in the soil.

On-farm mycorrhizal inoculum has been effective in enhancing the benefits of arbuscular mycorrhizal symbiosis (AM) between arbuscular mycorrhizal fungi (AMF) and plants. Studies indicate that "on-farm" mycorrhizal inoculum can promote plant growth and nutrition (DOUDS JUNIOR *et al*., 2016), increase plant resistance to abiotic stresses (LATEF *et al*., 2016) and improve soil stability (LEHMANN; LEIFHEIT; RILLIG, 2017), that led to greater interest in the use of AMF in the rehabilitation of degraded areas.

For plant nutrition, the addition of rock dust is also relevant (STAMFORD *et al*., 2011; THEODORO *et al*., 2013), although there are challenges in applying it (FYFE; LEONARDOS; THEODORO, 2006). The main benefits of this technique include (i) the reduction of the dependency of external commodities during crop production, (ii) the possibility of using mining tillings as source of rocks, (iii) the decrease of pollution due to nutrient leaching after application of soluble fertilizers and (iv) the recovery improvement of degraded lands (FYFE; LEONARDOS; THEODORO, 2006).

Finally, in degraded areas, the microtopography technique, determined by the heterogeneity in the terrain, formed by mounds (elevations) and pools (depressions) (GILLAND; MCCARTHY, 2014), helps to minimize the erosion, the retention of water, the sediments, the seeds and nutrients in the soil and promotes the establishment of a diverse plant community (AUMOND; LOCH; COMIN, 2012). Considering that these techniques are complementary strategies during the rehabilitation process, the objective of this study was to evaluate the interaction of use soil physical management and soil conditioner on growth and nutrition of *Eucalyptus saligna* during recovery of an abandoned agricultural field.

2 MATERIALS AND METHODS

2.1 Study site

This study was conducted in an abandoned agricultural field, located in Botuverá (27º 12.08' S and 49º 07.60' W), state of Santa Catarina, South of Brazil, within the Itajaí-Açu river Valley, where Argissols are the dominant soil type. The study site had a slope ranging from 15 to 30º, situated at 85m above sea level. The place was previously used for pasture and crop production, since 2003, and abandoned in 2012. Soil samples from the study site had the following properties: pH 5.05; P 8.6 mg/dm^3; K 170 mg/dm^3; organic matter 1.6%; Al 0.65 cmolc/dm^3; Ca 2.0 cmolc/dm^3; Mg 1.55 cmolc/dm^3; H+Al 4.41 cmolc/dm^3 and CEC 8.6 cmolc/dm^3. The climate is type Cfa of Köppen – subtropical humid, with average annual temperature of 20ºC and during the experimental period (November 2013 to October 2014) the precipitation was 1.869 mm and the temperature ranged from 13.7ºC to 33.9ºC. Native vegetation adjacent to the study area is typical of the Ombrophilous Dense Forest, Brazilian Atlantic Forest floristic domain.

2.2 Plant and fungal isolates

The seedlings of *Eucalyptus saligna* Sm were purchased from a commercial nursery and transplanted to the field when they were 3-months old.

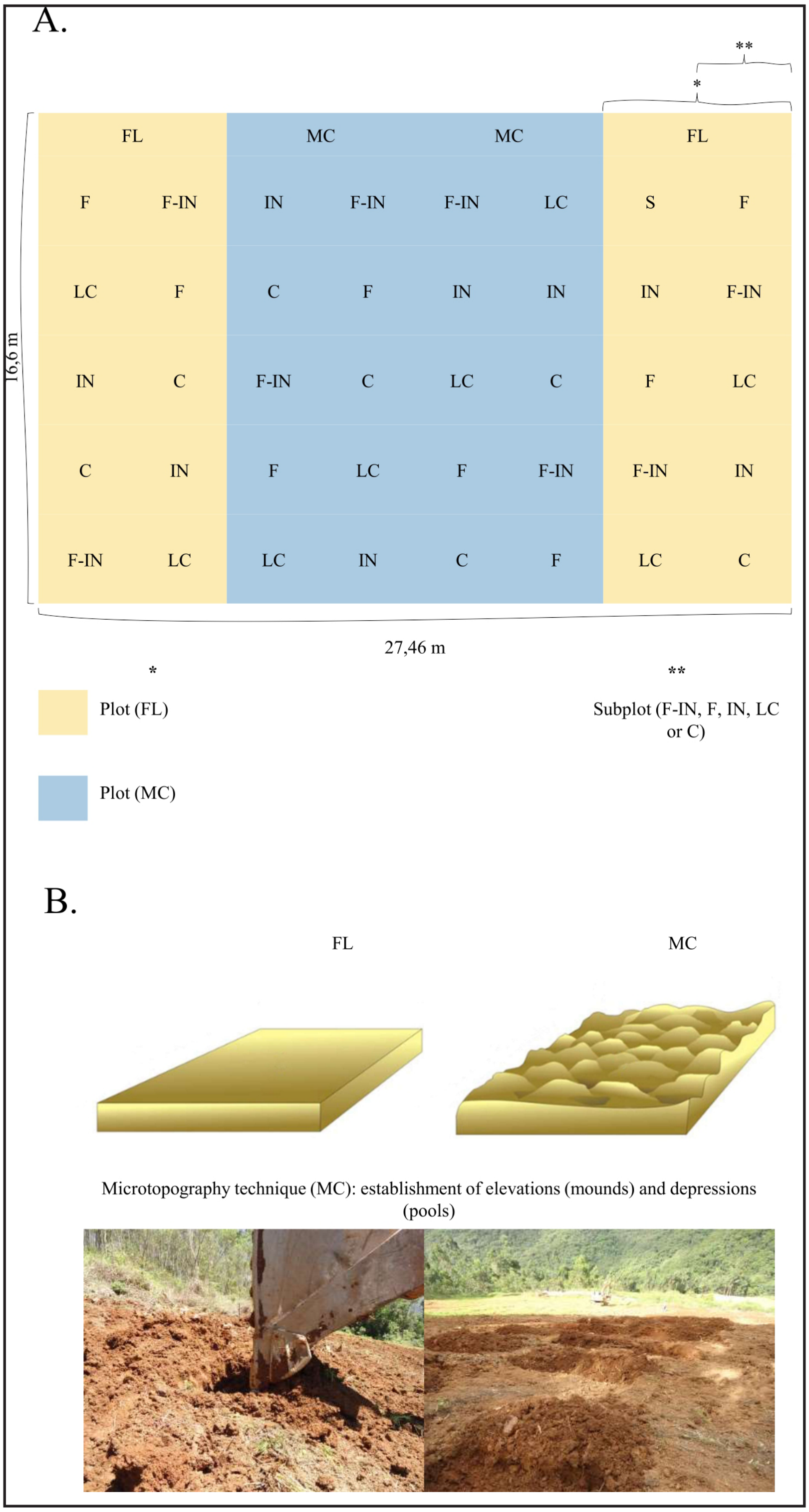
The AMF isolates used in the experiment were *Claroideoglomus etunicatum* MGR288A and *Dentiscutata heterogama* PNB102A, obtained from the International Culture Collection of Glomeromycota (CICG) (http://www.furb.br/cicg). On-farm mycorrhizal inoculant of both fungi was produced separately, according to procedures described by Czerniak and Stürmer (2014) and it was composed of the mixture of pulp sludge, carbonized rice husk and soil (1:1:1), containing 23 to 28 AMF infective propagules cm^{-3} of substrate.

2.3 Experimental design

To evaluate the interaction between microtopography technique and soil conditioners, four plots were established, with 114 m^2 each, and ten subplots established within each plot. Soil physical management treatments were established within each plot, which consisted of plots with microtopographies (MC) or flat plots (FL) (Figure 1 A, B). The microtopography technique (MC) included the establishment of mounds (elevations) and pools (depressions). In two plots, hills and pools were established on the ground, in parallel to the contour lines and equidistant from each other for 2-3 m. The creation of mound and pools involved a hydraulic excavator and each unit was 50 cm deep and 1.5 m wide. The remaining two plots were left undisturbed and referred herein as flats (FL).

Treatments of soil conditioners were established in each subplot and included: 100 g phonolite (F), 200 cm^3 of mycorrhizal inoculant (IN), 200 cm^3 of pulp sludge and carbonized rice husk (LC), 100 g phonolite and 200 cm^3 of mycorrhizal inoculant (F-IN) and control (C). Part of the soil (*ca.* 200 cm^3) removed from the hole was mixed with the amount of F, IN and LC for each treatment, homogenized and returned to the hole previously to seedling transplanting. The control (C) treatment did not receive any amount of phonolite, mycorrhizal inoculant, pulp sludge or carbonized rice husk. After the establishment of soil conditioner treatments, seedlings of *Eucalyptus saligna* were planted and a period of 30 days of adaptation was determined until the first evaluation. The investigation had an experimental area of 455 m^2 and 200 experimental units (five plants in each subplot and 50 plants in each plot).

Figure 1 – Experimental design with plots with microtopographies (MC) or flat plots (FL) and subplots that received soil physical management treatments: phonolite and mycorrhizal inoculant (F-IN), phonolite (F), mycorrhizal inoculant (IN), pulp sludge and carbonized rice husk (LC) and control (C)



Source: Authors (2020)

The addition of 100 g of phonolite was based on the application of 12 ton/ha of calcareous, commonly used in agricultural systems. The phonolite came from the locality of Chapada dos Indios, Distrito Alcalino of Lages, SC, and had the following chemical characteristics SiO_2 56.6%; Al_2O_3 22%; Fe_2O_3 2.3%; TiO_2 0.2%; CaO 0.9%; MgO 0.1%; K_2O 6%; Na_2O 9.8% e MnO 0.2%. The phonolite was processed through mill grinding and passed sieved over a 100 mesh sieve, to obtain the particle size of 0.149 mm. Due to the fact the mycorrhizal inoculant was produced using pulp sludge and carbonized rice husk as the substrate - which is used as a soil conditioner and influences the nutrition of plants -, we decided to establish a control treatment without inoculation. It consists on the addition of sterilized pulp sludge and carbonized rice husk, which had the following chemical characteristics: pH 6.9, P_2O_5 0.21%, K_2O 0.16%, Ca 0.17%, Mg 0.03% and N 1.01% (CZERNIAK; STÜRMER, 2014).

2.4 Characterization of arbuscular mycorrhizal fungal community

Previously to transplanting the seedlings in the field, ten soil samples (200 cm^3 each) were randomly collected in each plot and pooled to evaluate the mycorrhizal inoculum potential and to identify AMF species native to the area. Mycorrhizal inoculum potential (MIP) was estimated by a bioassay, following the methodology of Moorman and Reeves (1979). A mixture of field soil and sterile sand (1:1) was distributed in five plastic cones and seeded with *Sorghum bicolor*. For comparison, a positive control was established with a culture of *Rhizophagus clarus* RJN102A, obtained at the CICG. After 30 days under greenhouse conditions, the root system was stained (KOSKE; GEMMA, 1989) and the mycorrhizal root colonization determined by the grid line intersect method (GIOVANNETTI; MOSSE, 1980).

The AMF spores were extracted from a 100cm^3 aliquot of soil by wet sieving (GERDEMANN; NICOLSON, 1963), followed by sucrose gradient (20%/60%) centrifugation, proposed by Jenkins (1964). The spores were separated by morphotypes, under a dissecting microscope, mounted on permanent slides and identified under a microscope.

2.5 Plant data sampling

Biometric measurements (stem diameter and plant height) and canopy area were obtained for each plant at 30, 90, 150, 210, 270 and 330 days after seedling transplantation. Stem diameter was measured using adigital caliper at 5 cm above the soil-stem interface and plant height measured up to the highest apical meristem with a tape measure. The canopy area was estimated by averaging canopy diameters length, which were measured on the East-West and North-South position for each plant, according to the following Equation (1):

$$A = \frac{\pi \text{ x } D^2}{4} \tag{1}$$

In which: A = canopy area and D = averaging canopy diameters length.

After 330 days of the experiment onset, 50 leaves of each plant were randomly collected, pooled and sent to a commercial laboratory (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina - EPAGRI, at Caçador-Santa Catarina) to determine potassium (K) and phosphorus (P) concentrations.

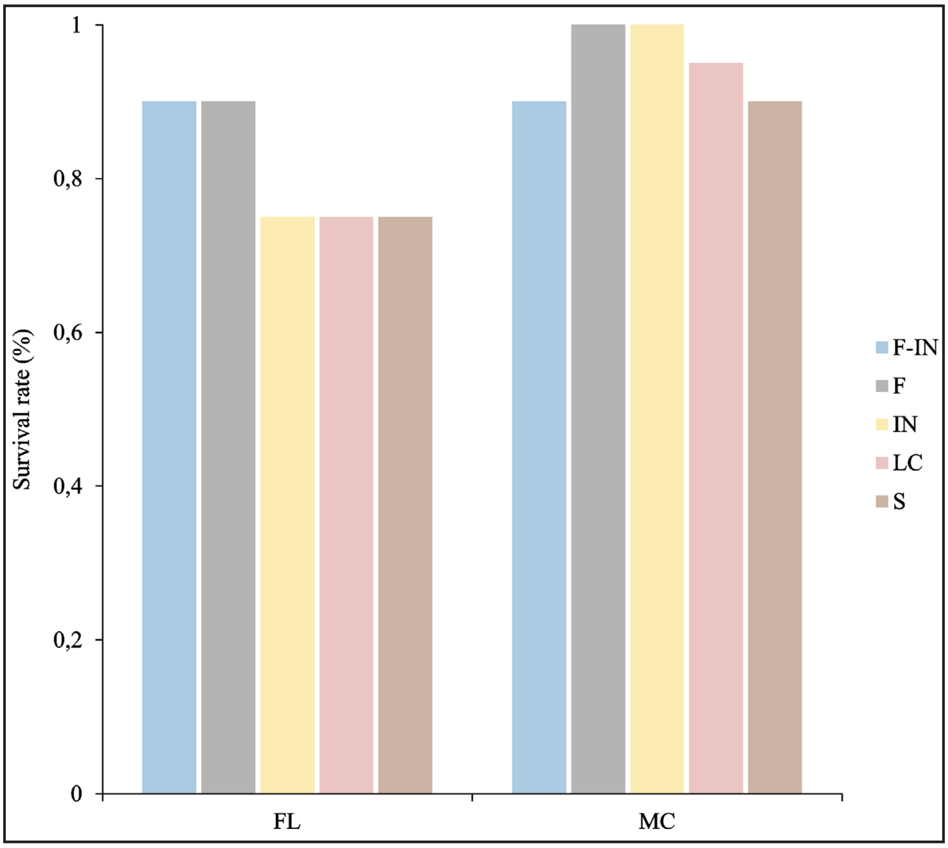
2.6 Statistical analysis

The data were checked for normality using the Shapiro-Wilk test and they were transformed and normalized using the Box and Cox method (power transformation). After that, data were submitted to an analysis of variance (ANOVA) for two factors, followed by the Tukey test (5% probability). Mycorrhizal colonization data were transformed using arcsine square root of colonization previously to comparison using an analysis of variance (ANOVA) followed by Tukey test (5% probability). Height and stem diameter data compared to 30, 90, 150, 210, 270 and 330 days after experiment onset, between MC and FL plots were analyzed by Student's t-test (5% probability). Multiple regression analyses were used to investigate increase on height and stem diameter over sampling time. Analyses were carried out using the statistical software Statistica, version 7.0 (StatSoft Co.).

3 RESULTS AND DISCUSSION

After 330 days of transplanting to field conditions, plants growing in plots MC tended to have a higher survival rate (90 to 100%) compared to plants in plots FL (70 to 90%) (Figure 2).

Figure 2 – Survival rate (%) of *Eucalyptus saligna* at 330 days after experiment onset, grown on different soil conditioners: phonolite and mycorrhizal inoculant (F-IN), phonolite (F), mycorrhizal inoculant (IN), pulp sludge and carbonized rice husk (LC) and control (C) at flat plots (FL) and microtopography plots (MC)



Source: Authors (2020)

Biometric measurements, canopy area and P and K shoot concentrations were influenced by soil physical management treatments while soil conditioner influenced only shoot K concentration (Table 1). There was no significant interaction between physical management and soil conditioners in the measured parameters.

Table 1 – Summary of bifactorial ANOVA results (*F* statistics and p-value) for growth parameters and shoot phosphor and potassium of *Eucalyptus saligna* at 330 days after experiment onset, grown under distinct soil physical management and soil conditioners

Parameters	**P value (F value)**		
	M	**C**	**M x C**
Height (cm)	<0.001 (22.66)	0.36 (1.09)	0.17 (1.61)
Stem diameter (mm)	<0.001 (41.54)	0.69 (0.56)	0.13 (1.77)
Canopy Area (m^2)	<0.001 (19.34)	0.47 (0.89)	0.25 (1.35)
P (g Kg^{-1})	<0.001 (11.38)	0.68 (0.57)	0.25 (1.34)
K (g Kg^{-1})	<0.001 (67.92)	0.004 (3.99)	0,14 (1,71)

Source: Authors (2020)

In which: P = phosphorus; K = potassium; M = soil physical management and C = soil conditioners.

Plant height, stem diameter, canopy area and shoot P and K concentration were significantly higher in *Eucalyptus saligna* plants growing in MC compared to FL plots (Figure 3). Height and stem diameter of *Eucalyptus saligna* plants were significantly higher in FL only at 30 days after planting (Table 2). Increase on height and stem diameter had a significant relationship with the periods analyzed in FL and MC (Figure 4).

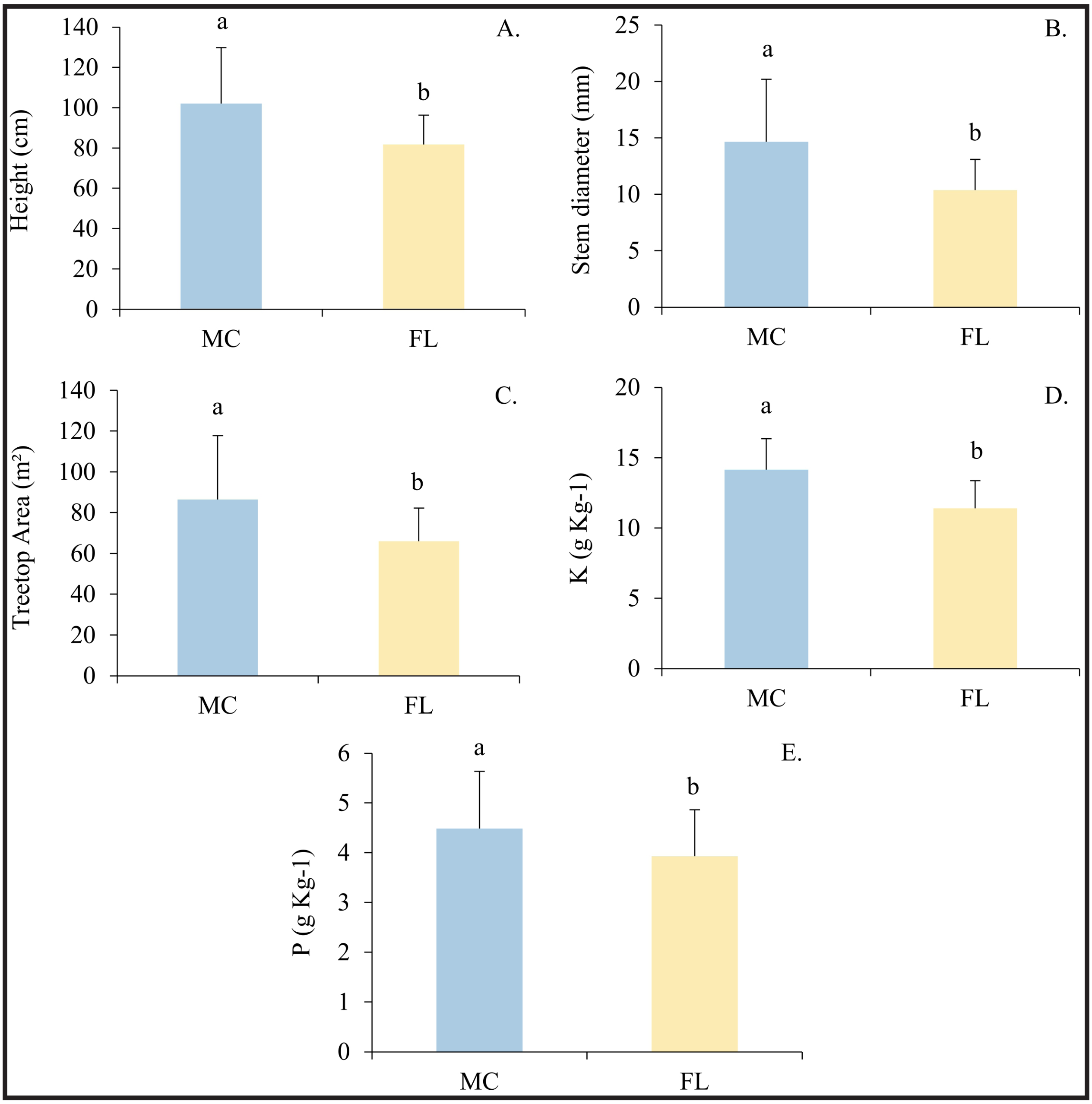
Table 2 – Height and stem diameter of *Eucalyptus saligna* at 30, 90, 150, 210, 270 and 330 days after experiment onset, grown at flat plots and microtopography plots

Days after experiment onset	**Average ± standard deviation**			
	Height (cm)		**Stem diameter (mm)**	
	FL	**MC**	**FL**	**MC**
30	27.91 ± 4.31 a	24.38 ± 2.01 b	2.84 ± 0.17 a	2.63 ± 0.15 b
90	40.28 ± 4.31 b	43.40 ± 2.67 a	3.77 ± 0.42 b	4.19 ± 0.42 a
150	59.89 ± 4.43 b	63.26 ± 13.28 a	5.69 ± 0.51 b	6.87 ± 0.89 a
210	60.83 ± 3.85 b	70.58 ± 5.37 a	6.89 ± 0.84 b	8.53 ± 1.26 a
270	67.67 ± 5.26 b	84.57 ± 7.39 a	8.18 ± 0.82 b	10.74 ± 1.71 a
330	81.55 ± 6.93 b	102.15 ± 11.98 a	10.17 ± 1.09 b	14.61 ± 2.51 a

Source: Authors (2020)

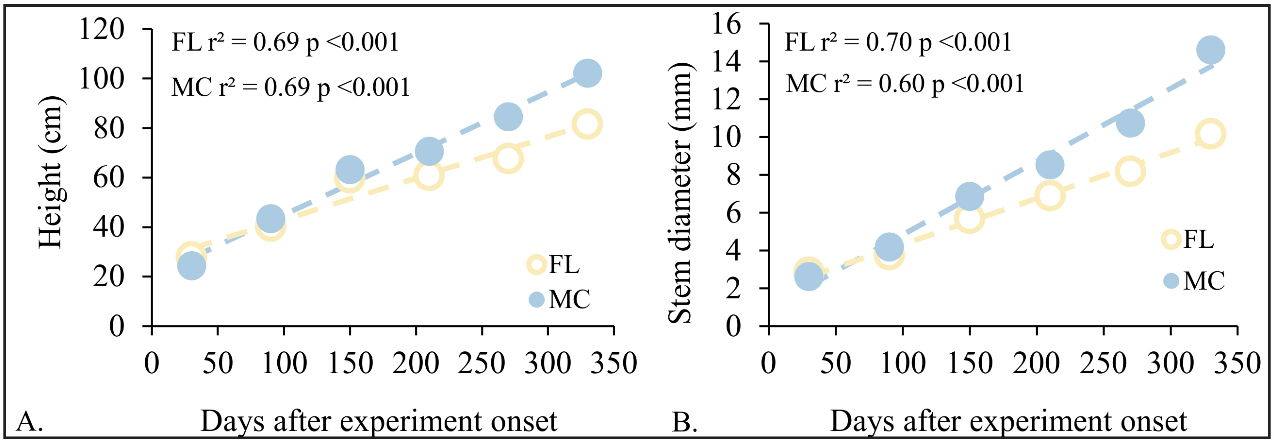
In which: FL = flat plots; MC = microtopography plots (MC); *Means followed by the different letters are significantly different ($p < 0.05$), by Student's t-test, within each sampling day.

Figure 3 – Height (a), stem diameter (b), canopy area (c), shoot K concentration (d) and shoot P concentration (e) of *Eucalyptus saligna* at 330 days after experiment onset, grown at flat plots (FL) and microtopography plots (MC). The means followed by the different letters are significantly different among FL and MC (p < 0,05)



Source: Authors (2020)

Figure 4 – Multiple regression of height (a) and stem diameter (b) of *Eucalyptus saligna* at 30, 90, 150, 210, 270 and 330 days after experiment onset, grown at flat plots (FL) and microtopography plots (MC)



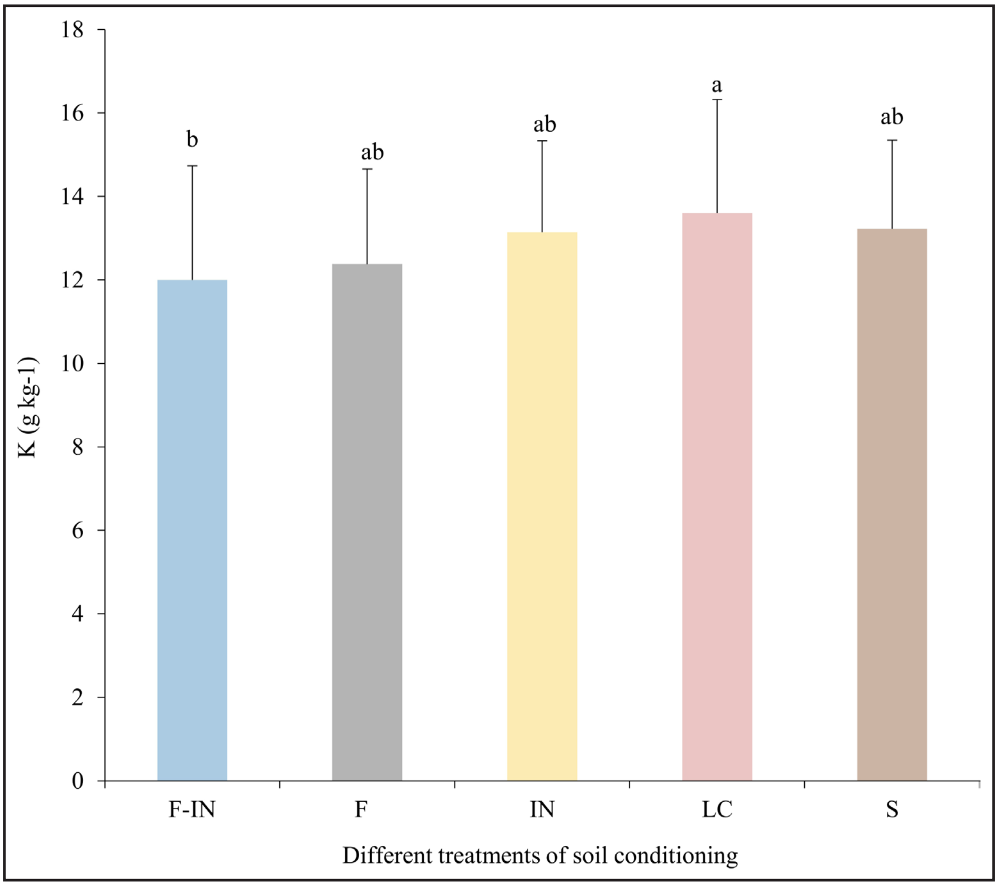
Source: Authors (2020)

The higher averages in MC plots compared to FL plots are consistent with other studies that also found areas with microtopography showed a higher survival rate (SIMMONS; WU; WHISENANT, 2012) and higher plant growth (MAÇANEIRO; SEUBERT; AUMOND, 2013). These results can be explained due to some attributes provided by microtopography, such as greater diversity and productivity of plants, preservation of soil moisture, the decreased on the impact of plants from unpredictable hydrological regimes during extreme rain conditions and the decrease on erosion and runoff of the soil (BRULAND; RICHARDSON, 2005; MOSER; AHN; NOE, 2009). Aumond, Loch and Comin (2012) validated the efficiency of the microtopography technique for recovering areas degraded by mining activities. They observed that this technique increased water retention, sediments, organic matter and nutrients reduced solar radiation and soil surface runoff. Consequently, plant growth and survival were higher in areas where the microtopography technique has been implemented.

Among the benefits of the microtopography technique, it is possible that our experimental conditions were more strongly influenced by the reduction of surface runoff in MC areas. The process of surface runoff can lead to soil loss, which reduces the productivity of agricultural and forestry systems as nutrient and organic matter are removed from the area, soil biota communities are decreased and water retention capacity is diminished (ZUAZO; PLEGUEZUELO, 2008). The experimental area has a declivity of 15-30% and the creation of mounds and pools systems (microtopography) may have retained more water and soil in pools (KAMPHORST et al., 2000), determining higher plant growth. This factor is even more relevant in *Eucalyptus* spp. whose productivity is determined by adequate supply of nutrients and water (STAPE et al., 2010).

The treatments of soil conditioners influenced only the shoot K concentration and significant difference was observed between treatments F-IN and LC, with the latter presenting the highest average (Figure 5).

Figure 5 – Shoot K concentration of *Eucalyptus saligna* at 330 days after experiment onset, grown with different soil conditioners



Source: Authors (2020)

In where: *Phonolite and mycorrhizal inoculant (F-IN), phonolite (F), mycorrhizal inoculant (IN), pulp sludge and carbonized rice husk (LC) and control (C). Means followed by the different letters are significantly different ($p < 0.05$).

Lack of response to phonolite application was unexpected, considering this rock is a potential source of K. There are three possible explanations for this result. First, the slow K solubility from this type of rock considering the short term that the experiment was conducted (SICHEL *et al*., 2012). The second possible explanations refers to the need of using a more finely grounded phonolite, preferably with particle size < 0.002 mm, that would accelerate K solubility (THEODORO; LENARDOS, 2006). The third one is that the amount of K in the soil attended the nutritional demand of *Eucalyptus saligna* for K. Considering that the experimental area was an abandoned agricultural field, the soil chemical analysis showed 170 $mg.kg^{-1}$ of K, a level well above that recommended for seedling transplanting (10 $mg.kg^{-1}$ of K) and maintenance (60 $mg.kg^{-1}$ of K) of *Eucalyptus* spp. (NOVAIS; BARROS; NEVES, 1986).

Inoculation of on-farm mycorrhizal inoculum also had no influence on *Eucalyptus saligna* growth and nutrition. Our results corroborate with studies of Adjoud et al. (1996) that observed no growth response in five out 11 species of *Eucalyptus* inoculated with AMF indicating that mycorrhizal response of *Eucalyptus* might be species-dependent. The low or absence of growth response of *Eucalyptus* to AMF inoculation might be explained by the dual mycorrhizal association established by this genus. Seedlings of *Eucalyptus* species are typically colonized by arbuscular mycorrhizal fungi up to 9 months old when the ectomycorrhizal association becomes dominant (CHEN; BRUNDRETT; DELL, 2000). Considering that growth parameters in our study were measured in plants between 3 and 16 months old, it is possible that plants benefited more of the ectomycorrhizal association rather than the AMF on-farm inoculant applied.

On the other hand, the arbuscular mycorrhizal inoculum potential of the experimental area was relatively high. The result of the mycorrhizal inoculum potential bioassays shows that mycorrhizal colonization of *Sorghum bicolor* plants growing in soils collected from MC (34.58% ± 7.77, of root colonization) and FL (34.22 ± 3.76, percentage of root colonization) areas did not differ, but it was significantly lower compared to *Rhizophagus clarus* (84.08% ± 8.74, of root colonization). In addition, the spore collection in the experimental area indicated the presence of five different AMF species (*Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler, *Diversispora spurca (*C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schüßler, *Acaulospora scrobiculata* Trappe, *Septoglomus viscosum* (T.H. Nicolson) C. Walker, D. Redecker, D. Stille & A. Schüssler, *Acaulospora morrowiae* Spain & N.C. Schenck, and *Glomus* sp.).

For comparison, Moorman and Reeves (1979), using the same mycorrhizal inoculum potential bioassay of this study, detected 1% of mycorrhizal colonization after 30 days in soils from perturbed areas. Inoculum potential, as observed in our research, was found in areas of natural ecosystems or in environments of anthropic exploration with subsequent revegetation (VIEIRA *et al*., 2018). Therefore, the relatively high inoculum potential detected in the soil of the experimental area suggests that the native mycorrhizal fungal community in the experimental area could have rapidly colonized eucalyptus plants in all treatments, resulting in the lack of effect of mycorrhizal inoculation treatment.

In this study, the application of pulp sludge was added as control treatment without inoculation and influenced K shoot concentration of *Eucalyptus saligna* compared to treatment with the addition of inoculant mycorrhizal and phonolite. These results were expected as theapplication of pulp sludge has been widely used as a soil conditioner in agricultural and forestry systems and positive results on the growth of *Eucalyptus grandis, Eucalyptus citriodora* and *Eucalyptus urograndis* have been observed (TOLEDO *et al*., 2015).

4 CONCLUSION

The results of this study support the use of microtopography technique to improve early plant growth and nutrition during the process of rehabilitation of abandoned agriculture fields.

The efficiency of microtopography technique suppressed the effect of distinct soil conditioners, which are known for its influence on plant growth and nutrition and should be incorporated in programs aiming to rehabilitate degraded areas.

Among the soil conditioners used, the application of pulp sludge and carbonized rice husk contributed to increased K concentration in plants compared to the addition of mycorrhizal inoculant on farm.

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