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## Addition of waste and introduction of microorganisms after 45 years of soil degradation<sup>1</sup>

Adição de resíduos e reintrodução de microrganismos após 45 anos de degradação do solo

Adriana Avelino Santos<sup>2</sup>, José Antônio Agustini<sup>2</sup>, Kátia Luciene Maltoni<sup>2</sup> and Ana Maria Rodrigues Cassiolato<sup>2\*</sup>

**ABSTRACT** - The construction of hydroelectric power plants (HPP) may result in environmental problems, such as extensive areas of exposed subsoil and conditions of extreme degradation. These areas require alternative that minimize impact and allow partial recovery of their ecosystem functions and vegetation. This study aimed to evaluate the effects of residue addition (organic/macrophytes - OR and inorganic/ash - AR), hydrogel, and inoculation of microorganisms in degraded soil, cultivated with *Jatropha curcas*, through fertility and microbial activity. A conserved Cerrado ("savannah") soil was the source of microorganisms - mainly mycorrhizal fungi. The experiment was conducted for 12 months (during 2010/2011) at the farm of UNESP-School of Engineering/Campus of Ilha Solteira, Selvíria-MS, Brazil, installed in an area where the soil was degraded during the HPP construction, in the 1960s. The experimental design was complete randomized blocks, using a 2×2×4 factorial scheme, i.e., two inoculation treatments (with and without), two hydrogel treatments (with and without), and four residue treatments to introduce the *J. curcas* (OR, AR, OR + AR, and control without residues), with four replicates and five plants evaluated per replicate. The soil fertility analyses, quantification of microbial biomass carbon (MBC), and released C as CO<sub>2</sub> (CO<sub>2</sub>-C), microbial quotient (*q*Mic), and metabolic quotient (*q*CO<sub>2</sub>) were carried out 12 months after planting. The fertility positively responded to the addition of OR and OR + AR, with an increase in pH and SB and reduction in Al and H + Al. The inoculation of soil microorganisms associated with OR and OR + AR residue treatments raised the released CO<sub>2</sub>-C, MBC, and *q*Mic. The addition of hydrogel combined with OR treatment contributed to the increase in the values of MBC and *q*Mic.

**Key words:** *Jatropha curcas*. Macrophytes. Sugar cane ash. Hydrogel. Cerrado. Microbial activity.

**RESUMO** - A construção de usinas hidrelétricas-UHE gera um conjunto de problemas ambientais, dentre estes, extensas áreas de subsolo exposto, condição de extrema degradação. Estas áreas demandam por alternativas que minimizem o impacto e permitam, ao menos, o recobrimento vegetal, para que retomem, parcialmente, suas funções ecossistêmicas. O objetivo deste trabalho foi avaliar os efeitos da adição de resíduos (orgânico/macrófitas - RO e inorgânico/cinza - RA), de hidrogel e da inoculação de microrganismos em solo degradado, cultivado com pinhão manso, por meio da fertilidade e da atividade microbiana. Um solo de Cerrado conservado foi utilizado como fonte de microrganismo, principalmente de fungos micorrízicos arbusculares. O experimento foi conduzido por 12 meses, iniciando em outubro de 2010, na fazenda da UNESP-Faculdade de Engenharia/Campus de Ilha Solteira, em Selvíria-MS. A área em questão, degradada pela construção da UHE, foi originalmente coberta com Cerrado. O delineamento experimental foi em blocos casualizados, em esquema fatorial 2x2x4, com 2 tratamentos de inoculação (com e sem), 2 de hidrogel (com e sem) e 4 de resíduos aplicados na cova para o plantio do pinhão manso (RO, RA, OR + AR), com 4 repetições, avaliando 5 plantas por repetição. Como fonte de microrganismos, especialmente fungos micorrízicos arbusculares, foi utilizado um solo originário de área de Cerrado conservado. Aos 12 meses do plantio foram realizadas análises de fertilidade do solo, quantificação do carbono da biomassa microbiana (CBM) e o CO<sub>2</sub> (C-CO<sub>2</sub>) liberado e calculados os quocientes microbiano (*q*Mic) e metabólico (*q*CO<sub>2</sub>). A adição de RO e RO + RA influenciou positivamente o solo degradado elevando pH e SB, e reduzindo Al e H+Al. Estes tratamentos associados a inoculação elevaram C-CO<sub>2</sub> liberado, CBM e *q*Mic. A adição de hidrogel combinado ao tratamento RO aumentou os teores do CBM e *q*MIC.

**Palavras-chaves:** *Jatropha curcas*. Macrófitas. Cinza do bagaço da cana-de-açúcar. Cerrado. Atividade microbiana.

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## INTRODUCTION

Engineering works, such as the construction of hydroelectric power plants (HPP) and mining areas, cause subsoil exposure. This subsequently decreases the resilience of the soil surface, making it difficult to restore vegetation owing to the loss of ecosystem functions (ADHIKARI; HARTEMINK, 2016; LI *et al.*, 2013).

In these areas, soil attributes such as organic carbon, pH, and cation exchange capacity are low, water infiltration is slow, and bulk density and soil temperature are high. Such characteristics compromise the porosity, structure, aggregation, and the associated microbial communities such as arbuscular mycorrhizal fungi (EZEAKU, 2012; O'DELL; CLASSEN, 2011).

In the northeast of São Paulo state, the construction of the Ilha Solteira HPP in the 1960s left extensively degraded areas similar to those described (RODRIGUES *et al.*, 2007). This level of degradation leads to edaphic conditions in which the soil is greatly altered relative to the original soil, hindering the recovery and restoration of the vegetation (O'DELL; CLASSEN, 2011).

The jatropha (*Jatropha curcas* L.) planted in this area was considered an alternative, because it is described as a hardy species with low nutritional demand. It has agricultural use in arid and semi-arid areas for biodiesel production and additionally protect the soil by reducing the erosive processes (PANDEYA *et al.*, 2012).

The combination of vegetation and organic residues in degraded soil can contribute to the reestablishment of microbiological activity, resumption of environmental dynamics, and process of biogeochemical cycles (BARDGETT; STREETER; BOL, 2013; BROWN; MAHONEY; SPRENGER, 2014). On the other hand, microorganisms exhibit low diversity in anthropogenic areas (PAGALING *et al.*, 2013).

The microbiota plays an important role in soil structure, plant establishment, and organic matter (OM) transformation (PAUL, 2016). The quantification of microbial biomass carbon (MBC) and released CO<sub>2</sub>-C and the determination of metabolic and microbial quotients can be considered indicators of soil quality (ARAÚJO; MONTEIRO, 2007), and they are coadjuvant in detecting the recovery of microbiological activity in degraded soil.

The use of residues as a source of OM is necessary in several processes for recovering microbial activity and soil fertility. For example, with abundant growth, aquatic macrophytes reduce the generation of energy in HPP by obstruction of the water from entering the generating units (THOMAZ *et al.*, 2008).

Macrophytes as a source of organic material for degraded soils was recommended by Calgaro *et al.* (2008), who incorporated OM and nutrients into the soil, and this use contributes to solve this waste disposal problem. According to Machado *et al.* (2014), the addition of macrophytes to the soil increased the OM, labile phosphorus (P), exchangeable calcium (Ca<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>), and microbial activity in 1.58 µg CO<sub>2</sub>-C g dry soil day<sup>-1</sup>, and decreased potential acidity (H + Al) and exchangeable aluminum (Al<sup>3+</sup>) compared to degraded soil without residue (control).

The ash produced from sugar cane bagasse in sugar and alcohol plant boilers is another residue that has been used in agriculture and in the recovery of degraded soils. The use of ash in this manner provided macro and micronutrients, retained moisture, and partially corrected the soil acidity (BONI *et al.*, 2017; FERREIRA *et al.*, 2012).

The incorporation of these combined residues plus hydrogel—an absorbent polymer with the capacity to store water and slowly release it into the degraded soil—(COELHO *et al.*, 2008) represents an alternative for situations in which the OM content and water availability are restricted.

The introduction of residues, microorganisms, hydrogel, and the cultivation of a rustic plant (*Jatropha*) may improve the fertility and microbial activity of degraded soil, allowing them to be colonized by microorganisms and plants and re-establish ecosystem functions. Considering the importance of microorganisms, residue availability in the region, and necessity of recovering degraded soils, this study aimed to evaluate the effects of adding organic and inorganic residues, hydrogel, and microorganisms—inoculated into the degraded soil cultivated with *Jatropha*—through soil fertility and microbial activity.

## MATERIAL AND METHODS

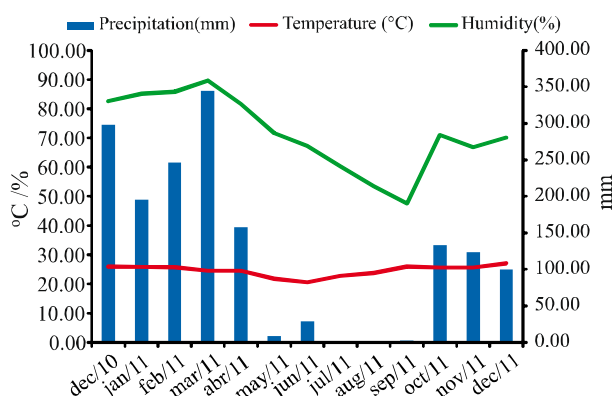
The experiment was conducted over 12 months (October 2010 to 2011) in degraded soil without vegetation cover. The soil of the study area was originally covered by Cerrado vegetation. The construction of Ilha Solteira HPP degraded extensive areas through deforestation and the removal of soil and subsoil up to 12 m in depth.

The experimental site was located at the Farm of Unesp, on the Ilha Solteira Campus, on the right side of the Paraná River, downstream of the HPP in the city of Selvíria, MS (20°22'45" S and 51°22'33" W). In this region, dystrophic Red Latosols predominate. The climate is classified as Aw (tropical climate with a dry winter and rainy summer) according to the Köppen system; the

local average altitude is 335 m, with an average annual temperature and precipitation of 23.7 °C and 1,300 mm, respectively (DEMATTE, 1980).

The soil temperature, evaluated at 0.05 m depth, indicated variations from 23–37 °C. The humidity ranged from 9.5–7.6% at a depth of 0.00–0.15 m. The climatic conditions that characterized the area during the experimental period are presented in Figure 1.

**Figure 1** - Averages of temperature (°C), precipitation (mm), and humidity (%) during the experimental period (Unesp Farm of Research and Extension)



The experiment was conducted in a complete randomized block design, in a 2×2×4 factorial scheme. The treatments were: inoculation of microorganisms (with and without soil inoculum), hydrogel (with and without), and residues in a hole for the planting of *Jatropha* (macrophytes, macrophyte + ash, and control, without residues), randomly distributed within 4 blocks (replications) and 5 plants evaluated per treatment, per block. Each block occupied an area of 960 m<sup>2</sup> (30 × 32 m), the planting holes were spaced 3 × 2 m, and the spacing between blocks was 5 m.

Prior to the start of the experiment, the soil was characterized using a sample composed of 4 simple samples, which were collected from a depth of 0.0–0.1 m. The sample was air dried and sieved (2 mm) for granulometric analysis (clay = 450, silt = 128, sand = 422 g kg<sup>-1</sup>) using the pipet method, as described by Donagema *et al.* (2011), and for fertility (RAIJ *et al.*, 2001). The fertility analysis presented the following results: P = 4.0 mg dm<sup>-3</sup>; OM = 7.0 g dm<sup>-3</sup>; pH (CaCl<sub>2</sub>) = 4.2; K<sup>+</sup> = 0.4 mmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>2+</sup> = 1.0 mmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup> = 1.0 mmol<sub>c</sub> dm<sup>-3</sup>; H+Al = 13.0 mmol<sub>c</sub> dm<sup>-3</sup>; Al<sup>3+</sup> = 2.0 mmol<sub>c</sub> dm<sup>-3</sup>, and sum of bases (SB) = 2.4 mmol<sub>c</sub> dm<sup>-3</sup>.

The experimental area was chiseled and harrowed at a depth of 0.40 m. Holes measured 0.3 m diameter × 0.9 m depth. The bottom half of the holes (0.45 m) was filled with soil that had been turned upside down, and dolomitic limestone (36 g hole<sup>-1</sup>), ammonium sulfate (24 g hole<sup>-1</sup>), simple superphosphate (14 g hole<sup>-1</sup>), and potassium chloride (1.4 g hole<sup>-1</sup>) were added to the upper half. The low amount of corrective and fertilizers were chosen to avoid interference with the inoculation.

For the inoculation, 200 g of soil-inoculum was mixed with the soil of the upper half of the holes to provide, in addition to the microorganisms, approximately 600 arbuscular mycorrhizal fungi (AMF) spores. The inoculum was prepared in the greenhouse using soil collected from a preserved Cerrado fragment that was previously cultivated with *Urochloa decumbens*.

The hydrogel used was Stockosorb (Degussa-Hüls Ltd.), which is made of polymers formed from acrylamide and acrylic acid on potassium-based salt. The hydrogel was prepared by diluting 3 g of the product with 700 mL of water, applied immediately after planting the seedlings and covered with a thin layer of soil.

The residue treatments were 480 g of dry mass of macrophytes (RO), added to the soil of the upper part of the holes. The macrophytes were collected at the Engenheiro Souza Dias HPP (Jupia) in Três Lagoas-MS. According to Thomaz *et al.* (2008), the most frequent macrophytes are *Egeria densa* Planch, *Egeria najas* Planch, *Ceratophyllum demersum* L., *Eichhornia azurea* Kunth, *Eichhornia crassipes* (Mart.) Solms., *Pistia stratiotes* L., and *Typha latifolia* L. The residue was air-dried and triturated (1 cm) prior to adding it to the holes. For characterization purposes, samples were analyzed as described by Malavolta *et al.* (1997). The material contained, respectively: N = 26; P = 3; K = 9.5; Ca = 25 g kg<sup>-1</sup> dry mass, and S = 33; B = 52; Zn = 96; Cu = 51; Fe = 248, and Mn = 127 mg kg<sup>-1</sup>.

The ash (AR) originated from the burning of sugarcane bagasse in boilers, was 980 g mixed with the soil from the upper parts of the holes. This residue was collected at the ALCOVALE Company (Aparecida do Tabuado-MS). A sample was submitted to a chemical analysis of the available elements (RAIJ *et al.*, 2001), which resulted in: P = 54 mg dm<sup>-3</sup>; MO = 15 g dm<sup>-3</sup>; pH = 5.0 CaCl<sub>2</sub>; K = 5.6 mmol<sub>c</sub> dm<sup>-3</sup>; Ca = 8 mmol<sub>c</sub> dm<sup>-3</sup>; Mg = 6 mmol<sub>c</sub> dm<sup>-3</sup>; H+Al = 40 mmol<sub>c</sub> dm<sup>-3</sup>; Al = 2 mmol<sub>c</sub> dm<sup>-3</sup>, and SB = 19.6 mmol<sub>c</sub> dm<sup>-3</sup>. The total ash analysis resulted in: C = 570 g kg<sup>-1</sup>; ammoniacal N = 220 mg N kg<sup>-1</sup>; Kjeldahl N = 6.1 g kg<sup>-1</sup>; nitrate-nitrite N = 421 mg of N kg<sup>-1</sup>, where 570 g of C/6.471 g of N makes the C:N ratio of this material 85:1.

The *Jatropha* seedlings were prepared in commercial substrate and tubes. Seedlings with 2 pairs

of leaves were transplanted into plastic bags (2 kg) with soil from the experimental area (0.0–0.1 m depth). They remained in a covered nursery for 4 months, followed by 15 d outside of the nursery, before being transported to the field to adapt to the present environmental conditions.

Twelve months after the introduction of *Jatropha* seedlings to the field, soil samples were collected (0.0–0.1 m) to evaluate the fertility (RAIJ *et al.*, 2001), quantify the microbial biomass carbon (MBC) by the fumigation-extraction method (VANCE *et al.*, 1987), and quantify the released CO<sub>2</sub>-C (ANDERSON; DOMSCH, 1989). The microbial quotient (*q*Mic) was calculated by the expression MBC/soil organic carbon (SOC) (SPARLING, 1992), and the metabolic quotient (*q*CO<sub>2</sub>) represents the amount of released CO<sub>2</sub>-C per unit of MBC. The data were subjected to analysis of variance, and means were compared by Tukey's test ( $p \leq 0.05$ ) using the SISVAR software (FERREIRA, 2011).

## RESULTS AND DISCUSSION

After 12 months, the treatments with inoculation (INC) had increased levels of released CO<sub>2</sub>-C and MBC. These observations were accompanied by *q*Mic but not *q*CO<sub>2</sub>, apart from the lower levels of P and SB (Table 1).

The lower levels of P and SB can be attributed to the increased microbial activity and to the partial and temporary P and bases immobilization in the microbial biomass, owing to the low levels of P and SB in the degraded soil (P = 3 mg dm<sup>-3</sup>; SB = 2.4 mmol<sub>c</sub> dm<sup>-3</sup>). Bardgett *et al.* (2003) reported the competition between soil microorganisms and plants through N addition (organic and inorganic source) in low productivity soils, while N was temporarily immobilized in the biomass thus reducing its availability. The authors reported that, compared to plants, microorganisms were strong drivers of competition. This behavior also applies to other

**Table 1** - Averages, F values, and coefficients of variation (CV) for soil fertility attributes, released CO<sub>2</sub> carbon (CO<sub>2</sub>-C, mg g soil day<sup>-1</sup>), microbial biomass (MBC, mg C g<sup>-1</sup> (dry soil), microbial quotient (*q*Mic, mg C g soil<sup>-1</sup>), and metabolic quotient (*q*CO<sub>2</sub>, mg released CO<sub>2</sub>-C/mg C g<sup>-1</sup> dry soil day<sup>-1</sup>), for degraded soil and treatments including the addition of inoculation (INC), hydrogel (HYD), and residues (RES) for holes

Treatments	P mg dm <sup>-3</sup>	OM g dm <sup>-3</sup>	pH CaCl <sub>2</sub>	H+Al -----	Al mmol <sub>c</sub> dm <sup>3</sup> -----	SB	CO <sub>2</sub> -C	MBC	<i>q</i> Mic	<i>q</i> CO <sub>2</sub>
Inoculation (INC)										
Y INC	5.7b	9.2	4.5	24.3	5.7	8.1 b	8.6 a	18.56 a	0.34 a	0.46 b
N INC	6.4a	9.5	4.6	23.4	5.2	10.2 a	6.8 b	10.75 b	0.19 b	0.64 a
Hydrogel (HYD)										
Y HYD	6.1	9.2	4.5	24.0	5.3	9.3	7.6	15.21 a	0.28 a	0.52 b
N HYD	6.1	9.5	4.6	23.6	5.6	9.1	7.8	14.09 b	0.26 b	0.58 a
Residues (RES)										
OR	6.4 a	9.4 a	4.6 a	21.6 b	3.6 b	11.4 a	9.0 a	16.93 a	0.31 a	0.55 b
AR	5.8 a	9.3 a	4.4 b	25.9 a	7.2 a	7.5 b	6.4 b	12.81 b	0.24 b	0.51 b
OR+AR	6.5 a	9.8 a	4.7 a	21.8 b	3.5 b	11.2 a	9.6 a	17.06 a	0.30 a	0.62 a
Control	2.0 b	6.9 b	4.4 b	25.9 a	7.5 a	6.2 b	5.8 c	11.81 b	0.22 b	0.51 b
F values										
INC	5.75*	1.60 <sup>ns</sup>	2.47 <sup>ns</sup>	2.80 <sup>ns</sup>	2.61 <sup>ns</sup>	9.44**	111.54**	633.9**	361.29**	169.65**
HYD	0.00 <sup>ns</sup>	0.40 <sup>ns</sup>	2.47 <sup>ns</sup>	0.64 <sup>ns</sup>	0.44 <sup>ns</sup>	0.05 <sup>ns</sup>	1.23 <sup>ns</sup>	13.14**	4.79*	16.34**
RES	3.67*	3.78*	18.74**	23.35**	44.38**	13.84**	137.01**	77.83**	28.58**	12.57**
INC x HYD	0.76 <sup>ns</sup>	0.10 <sup>ns</sup>	5.97**	0.09 <sup>ns</sup>	3.27 <sup>ns</sup>	0.39 <sup>ns</sup>	0.29 <sup>ns</sup>	1.46 <sup>ns</sup>	0.22 <sup>ns</sup>	0.65 <sup>ns</sup>
INC x RES	1.85 <sup>ns</sup>	0.73 <sup>ns</sup>	1.16 <sup>ns</sup>	1.76 <sup>ns</sup>	1.53 <sup>ns</sup>	2.70*	11.09**	26.87**	12.50**	11.86**
HYD x RES	4.53**	0.87 <sup>ns</sup>	1.49 <sup>ns</sup>	0.43 <sup>ns</sup>	0.75 <sup>ns</sup>	1.58 <sup>ns</sup>	5.350**	5.41**	2.79*	5.02**
CV (%)	18	8	3	8	24	29	8	8	12	10

In each column, averages followed by the same letter do not differ among themselves, according to the Tukey test at a significance level of 0.05. \*\* and \*: significant at 0.01 and 0.05, respectively; <sup>ns</sup>: non-significant. OR = macrophytes; AR = ash, and control = without residues; Y = with; N = without). The block variable showed no significant difference among treatments

nutrients, justifying the reductions in P and SB observed in the present study.

In the degraded soil with hydrogel addition, more moisture retention was expected; combined with the addition of residues, this would favor the microbiota activity. However, the applied hydrogel provided a small increase in MBC and, consequently, in  $q_{\text{Mic}}$ . This led to a decrease in  $q_{\text{CO}_2}$ , but did not influence the released  $\text{CO}_2\text{-C}$  (Table 1). Soil moisture is among the most important variables in the environment for microorganisms (MENDES *et al.*, 2012). In the present study, the benefits of applying hydrogel far outweighed the costs of the product and the application operations.

After 12 months, the addition of residues caused an increase in P and OM in all treatments compared to the control (Table 1). However, Bao *et al.* (2017) considered OM levels between 8.6–9.6 g kg<sup>-1</sup> to be low, even in recovering mining areas. These values are close to those reported in the present study, but the natural Cerrado can reach 45.2 g kg<sup>-1</sup> OM. Together, this information indicates the need to search for other sources to introduce and maintain OM in the soil.

The use of OR—isolated or combined with AR—increased pH and SB and decreased H + Al and Al<sup>3+</sup> (Table 1). These observations can be attributed to the possible complexation of Al<sup>3+</sup> by OM added via residues, which also contributed to the increase in SB because of the addition of Ca, Mg, and K present in the OM composition (SILVA; MENDONÇA, 2007). Examining a degraded Cerrado area with exposed subsoil cultivated with *Stryphnodendron polyphyllum* Mart., Calgaro *et al.* (2008) reported the use of water hyacinth and sugarcane bagasse as sources of OM. They also observed significant increases in P and SB levels and decreases in H + Al and Al<sup>3+</sup>, compared to the initial characterization of the area. The results of Calgaro *et al.* (2008) further corroborate the observations of the present study.

The treatments with OR exhibited increased microbial activity, i.e., released  $\text{CO}_2\text{-C}$ , MBC,  $q_{\text{Mic}}$ , and  $q_{\text{CO}_2}$  (Table 1). Hydrogel associated with OR contributed to the increased microbial activity (Table 2). However, there was no change in the behavior of these variables where AR was associated with hydrogel, which should increase humidity. In addition, increased microbial activity was also observed in the treatments associated with hydrogel as OR and OR + AR, again emphasizing the importance of organic residue for microbial activity (BELO *et al.*, 2012; MENDES *et al.*, 2012).

The inoculum and hydrogel application significantly affected pH; however, the values exhibited small variation, i.e., from 4.5–4.7 (Table 2). As reported by Ilunga Wa Ilunga *et al.* (2015), such slight variation

implied that the inoculum and hydrogel did not contribute to or influence pH. The treatment without hydrogel and inoculation presented the highest pH (4.7), while the others (inoculation × hydrogel, inoculation × without hydrogel, without inoculation × with hydrogel) had lower pH values (4.5). The lower pH in these treatments can be attributed to the temporary immobilization of bases through the microorganisms' and plants' consumption. This immobilization resulted in the accumulation of acidic cations such as H<sup>+</sup> in the soil, explaining the decreased pH through the increased H<sup>+</sup> activity (SILVA; MENDONÇA, 2007).

This result might indicate the presence of autochthonous microorganisms. The pH decreased where no inoculation occurred but where hydrogel was added, which indirectly suggests the presence of microbial activity (Table 2), because the surface layers of soil were removed in this area. O'Dell and Classen (2011) reported that, on the surface layers where the soil was removed, the autochthonous microbial communities were reduced because of low soil resilience. However, these microbial communities did not disappear, corroborating the indirect observation on microbial activity in the present study.

The lowest P content occurred in the presence of hydrogel and AR or OR + AR. In this case, the C:N ratios may explain the observed behavior. According to Corrêa, Velini and Arruda (2003) reported that the evaluated macrophytes (also from the Jupiá lake) had C:N values lower than 20. Hadas *et al.* (2004) considered this ratio low enough to allow the rapid mineralization of the residue, releasing nutrients for the microorganisms. However, when C:N is higher than 20, as was the case of AR (C:N = 85:1), slow decomposition might promote the immobilization of P or other elements in microbial biomass. These results demonstrate that increasing the humidity is not sufficient to obtain greater microbial activity, and other factors need to be considered, such as the C:N ratio of the material and soil fertility.

Khanna *et al.* (2004) reported a reduction of released  $\text{CO}_2\text{-C}$  after sugarcane bagasse ash was applied to soil, which corroborates the findings in the present study. The high C:N ratio in soils with low OM contents highlight the importance of the application of organic residue with a low C:N ratio. Similarly, Gatiboni *et al.* (2011) reported higher microbial activity during the initial phase of OM decomposition owing to a more balanced nutrient availability. This information suggests that upon sampling, 12 months after OR incorporation, the microbial activity still responded positively to the addition of residue (Table 1).

The behavior of SB in the interaction residues and inoculation (with and without) (Table 2), corroborates the verified for P. The high C:N ratio of AR (85:1), associated

**Table 2** - Statistical unfolding of significant interactions for P, sum of bases (SB), pH, carbon of CO<sub>2</sub> released (CO<sub>2</sub>-C - mg g soil<sup>-1</sup> h<sup>-1</sup>), microbial biomass carbon (MBC, mg C g<sup>-1</sup> dry soil), microbial quotient (*q*Mic, mg C g<sup>-1</sup> soil), and metabolic quotient (*q*CO<sub>2</sub>, mg released CO<sub>2</sub>-C/mg C g<sup>-1</sup> dry soil day<sup>-1</sup>) for degraded soil and treatments including the addition of inoculation (INC), hydrogel (HYD), and residues (RES) for holes

Treatments	OR	AR	OR+AR	Control
----- P (mg dm <sup>-3</sup> ) -----				
Y HYD	7.25 aA	5.50 aB	5.87 bAB	5.50 aB
N HYD	5.62 bA	6.00 aA	7.12 aA	5.62 aA
----- SB (mmol <sub>c</sub> dm <sup>-3</sup> ) -----				
Y INC	8.96 bA	7.02 aA	9.92 aA	6.68 aA
N INC	13.85 aA	7.96 aB	12.46 aA	6.52 aB
----- released CO <sub>2</sub> -C (mg CO <sub>2</sub> g <sup>-1</sup> dry soil day)				
Y INC	10.37 aA	6.58 aB	10.92 aA	6.42 aB
N INC	7.72 bA	6.21 aB	8.35 bA	5.10 bC
Y HYD	8.51 bB	6.15 aC	9.73 aA	6.09 aC
N HYD	9.58 aA	6.64 aB	9.55 aA	5.44 aC
----- MBC (mg C g <sup>-1</sup> dry soil) -----				
Y INC	20.62 aA	15.25 aB	23.25 aA	15.12 aB
N INC	13.25 bA	10.39 bB	10.87 bB	8.50 bC
Y HYD	18.37 aA	12.50 aB	17.50 aA	12.50 aB
N HYD	15.50 bA	13.12 aB	16.62 aA	11.12 bC
----- <i>q</i> Mic (%) -----				
Y INC	0.39 aA	0.29 aB	0.41 aA	0.29 aB
N INC	0.23 bA	0.19 bB	0.18 bB	0.16 bB
Y HYD	0.33 aA	0.23 aB	0.31 aA	0.24 aB
N HYD	0.29 bA	0.25 aBC	0.29 aA	0.21 bC
----- <i>q</i> CO <sub>2</sub> (mg CO <sub>2</sub> -C / mg C g <sup>-1</sup> dry soil) -----				
Y INC	0.51 bA	0.43 bB	0.47 bAB	0.42 bB
N INC	0.58 aB	0.60 aB	0.77 aA	0.60 aB
Y HYD	0.47 bB	0.51 aB	0.60 aA	0.50 aB
N HYD	0.62 aA	0.52 aB	0.64 aA	0.52 aB
Treatments	Com H	Sem H		
----- pH (CaCl <sub>2</sub> ) -----				
Y INC	4.53 aA	4.50 bA		
N INC	4.50 aB	4.65 aA		

Averages followed by the same letter, lowercase in the column and upper case in the row, for each variable, do not differ from each other by the Tukey test at 0.05 probability. (OR = macrophytes; AR = ash, and control without residues; Y = with; N = without)

with low soil base contents (2.4 mmol<sub>c</sub> dm<sup>-3</sup>), contribute to the immobilization of the bases in the microbial biomass (CORRÊA; VELINI; ARRUDA, 2003; HADAS *et al.*, 2004). The addition of OR in the soil without inoculation generated high levels of SB, showing less interference from the microorganisms on the exchangeable bases, leaving them available.

The interaction between the inoculation × residue demonstrated that the microbial activity increased with the addition of OR and inoculum (Table 2). In the absence of the inoculum, OR also enhanced microbial activity, which can be verified by the values of released CO<sub>2</sub>-C, MBC, and *q*Mic, with unclear results for *q*CO<sub>2</sub>. It can be said that the evaluated microbial activities were influenced by

the high C:N ratio of AR (85:1) (HADAS *et al.*, 2004), as their values for the treatments in which only AR was applied were similar to those of the control (Table 2).

Soil microorganisms are directly responsible for soil functioning, as they play major roles in processes such as the decomposition of organic residues, nutrient cycling, and OM formation (MENDES *et al.*, 2012). The substrate chemical composition and other soil nutrient factors are considered responsible for the increases or decreases in microbial activity (BROWN; MAHONEY; SPRENGER, 2014). In the present study, the importance of the OR application was to ensure faster recovery of the soil and microbial activity in the area.

The differences among treatments with the addition of residues and the control allow us to highlight the positive contribution of these factors in microbial activity (Table 1). The increased respiratory rate indicates OR transformation due to biological activity with the release of nutrients (BELO *et al.*, 2012).

The inoculation and the hydrogel, combined with the residues (OR and OR + AR), resulted in the highest levels of MBC—that is, microbial multiplication and consequent C incorporation into its biomass (Table 2). Increases in microbial biomass are linked to nutrient cycling upon the release of C and other phytonutrients. Some nutrients are temporarily immobilized on the microbial biomass during multiplication. This becomes a labile reserve of nutrients—particularly C and N, which are rapidly released upon the death of microorganisms (HADAS *et al.*, 2004).

The highest values of  $q_{\text{Mic}}$  indicate the maintenance of C in the soil. Thus, the most favorable conditions for the microorganisms were verified in the treatments with OR + AR or OR only, associated with the inoculation (Table 2). The higher efficiency of the microbial biomass is related to the lower values of  $q_{\text{CO}_2}$ . If less C is lost through respiration, more C can be incorporated into the microbial biomass, contributing to the increased soil carbon content (BELO *et al.*, 2012).

On the other hand, high values of  $q_{\text{CO}_2}$  indicate that, because of stressful conditions, the microbial population oxidizes C for its maintenance and adaptation to the soil. The microbial population thereby directs more energy for cellular maintenance than for growth, such that a proportion of the C from  $\text{CO}_2$  will be lost or not incorporated in the soil (ARAÚJO; MONTEIRO, 2007).

## CONCLUSIONS

1. The addition of OR and OR + AR to degraded soil increased pH and SB and reduced H + Al and  $\text{Al}^{3+}$ ;

2. The soil inoculated with microorganisms associated with OR and OR + AR addition increased the levels of released  $\text{CO}_2$ -C, MBC, and  $q_{\text{Mic}}$ ;
3. The hydrogel combined with RO treatment increased the MBC and  $q_{\text{Mic}}$ ;
4. The residues (OR and OR + AR), associated with the inoculation of microorganisms and hydrogel, present some positive results but are still insufficient for maintaining the vegetal cover.

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