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Recovering Soils Affected by Iron Mining Tailing Using Herbaceous Species with Mycorrhizal Inoculation

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Abstract The objective of this research was to evaluate a soil recovery strategy in soils that were affected by iron mining tailing using herbaceous species inoculated with *Acaulospora morrowiae* (arbuscular mycorrhizal fungus (AMF)). Tailings were collected on the banks of the Gualaxo do Norte river, one of the places impacted by the Fundão Dam rupture, where tailing layers that were more than one meter were deposited. The experiment was carried out in a greenhouse, using 6 kg pots of non-sterile reject, in a randomized block design in a 4 × 2 factorial scheme, with four cropping systems (*Urochloa ruziziensis* single crop–RS; and intercropping cultivation: *U. ruziziensis* with *Crotalaria spectabilis*–R + C; *U. ruziziensis* with *Guizotia abyssinica*–R + G and *U. ruziziensis* with *C. spectabilis* and *G. abyssinica*–R + C + G), with two AMF inoculation conditions (with 200 *A. morrowiae* spores per pot, and no inoculation), with three replications and 100 days duration. The R + C and R + C + G systems presented the highest shoot dry matter (SDM) yields. Regarding root dry matter production (RDM), a variation of 9.2 g of pot^{-1} roots was observed between the R + C and R + G systems. Mycorrhizal colonization (MC) was higher in the cultivation system with the three herbaceous species, being the R + C + G system 52% higher than RS system. Spore density did not vary among treatments. Microbial carbon biomass was higher in the RS and R + G treatments when not inoculated. Basal respiration was also higher when not inoculated. Overall, the R + C + G system was more efficient than other systems in the accumulation of elements. The cultivation system with three herbaceous plants proved to be efficient in establishing itself initially in the iron mining tailings, being a viable alternative for the rehabilitation process.

Keywords Rehabilitation · *Urochloa ruziziensis* · *Crotalaria spectabilis* · *Guizotia abyssinica*

1 Introduction

Ore beneficiation process generates large waste loads, especially during extraction and processing, and these are stored in containment dams (Santamarina et al. 2019). These dams are designed to contain a large number of materials, but are subjected to failure when inadequately monitored, and can cause major disasters such as those at the mines located in Mariana (2015) and Brumadinho (2019), both in Minas Gerais–Brazil (Santamarina et al. 2019; Vergilio et al. 2020).

In November 2015, the Fundão Dam, responsible for storing tailings from the iron ore extraction of the Alegria Complex, Germano unit, in Mariana–MG, with a total volume of 56.6 million m^3 of tailings, ruptured and caused the spill of 39.2 million m^3 of tailings. From the tailings deposited along the Doce River basin, a Technosol was formed, consisting of the mixture of

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tailings with the local soil and, over time, with the deposition of organic waste from the vegetation. Technosols are the result of human activities and occur predominantly in urban, industrial, road, dump, and mining areas. Mining activities are major contributors to the genesis of Technosols worldwide (Maiti 2013; Echevarria and Morel 2015). In general, these soils have heterogeneous mineralogical, chemical-physical, and microbiological characteristics that are different from those of native soils (Batista et al. 2020; Couto et al. 2021; Jordão et al. 2021; Santos et al. 2019), which makes the need for rehabilitation of these areas predominant. Presence of trace metals in the soil, as well as their effect on organisms, will depend on the type of metal and, mainly, on its bioavailability, which in turn depends on the source of contamination, the type of soil and its characteristics, such as pH, cation exchange capacity, and organic matter (Hamels et al. 2014). Despite much discussion regarding the high content of total metals (not bioavailable) present in the tailings that were deposited alongside the Rio Doce Basin (Segura et al. 2016), as well as the possibility of a time bomb contamination due to the release of these metals with changes in soil pH (Queiroz et al. 2018). Davila et al. (2020), on the other hand, demonstrated that these tailings are probably not a potential source of contamination by metals. However, Andrade et al. (2018) showed that rice plants had little development when grown in tailings, which may be associated with nutrient deficiency and physical properties. But, mineral and/or organic fertilization can provide good conditions for the development of plants in the tailings (Andrade et al. 2018; Esteves et al. 2020; Zago et al. 2019).

Despite not being considered as a chemical pollutant, tailings are still a source of environmental degradation, and the whole basin must be remedied. The most efficient strategy for the remediation of degraded soils is through phytoremediation, which in addition to vegetation cover aims at the reconstruction of soil fertility and ecosystem functions (Stumpf et al. 2014; Ahirwal and Maiti 2018). Establishing a vegetation cover is the best strategy for the rehabilitation of large degraded areas (Maiti 2013; Faucon et al. 2017; Gastauer et al. 2018), such as that impacted by the Fundão dam rupture.

A greater plant diversity is one factor that may favor the rehabilitation process and stabilization of degraded areas, and consequently contribute to the ecological succession throughout the rehabilitation process. However, proper selection and combination of fast-growing species is a critical phase to construct a sustainable ecosystem (Maiti and Maiti 2015; Kumar et al. 2017; Li et al. 2017; Wu et al. 2019). Use of species for the phytoextraction have important alternatives, that include removal of elements from the soil and their accumulation in their tissues, or phytostabilization, that is, immobilization of the elements in the soil which prevents them from being absorbed by their roots (Banerjee et al. 2016; Remigio et al. 2020).

Therefore, one of the basic requirements for the success of any revegetation technique is to find plants which are tolerant to contaminants generally present in mined areas (Carneiro et al. 2002; Pedroso et al. 2018). There is evidence that herbaceous plants have a greater tolerance for excess soil contaminants than trees (Baker 1987) and plant diversity can act as soil conditioners playing a central role in the success of revegetation by interacting with other components of the soil ecosystem (Ahirwal and Maiti 2018, Jordão et al. 2021; Silva et al. 2018). In this sense, the use of plant species that have availability and easy seed acquisition, such as *Urochloa ruziziensis*, *Crotalaria spectabilis*, and *Guizotia abyssinica*, can be an interesting alternative to begin the process of degraded areas revegetation.

Associated with this, the combined use of these plants with soil microorganisms for the degraded area rehabilitation process is a promising method (Khan 2005; Matias et al. 2009; Maiti et al. 2016; Bandopadhyay et al. 2018; Prado et al. 2019), given that soil microorganisms play a key role in establishing biogeochemical cycles and facilitating the development of vegetation cover (Sinha et al. 2009; Silva et al. 2018). In particular, arbuscular mycorrhizal fungi (AMF) are important components of soil microbiota and contribute to plant nutrition, mitigation of biotic and abiotic stresses, and soil structure stability (van der Heijden et al. 1998; Carneiro et al. 2008; Matias et al. 2009; Mukhopadhyay and Maiti 2011; Dickie et al. 2013; Teixeira et al. 2017). In addition, the AMF helps to remove contaminants from the soil, increasing plant survival (Pedroso et al. 2018). AMF species *Acaulospora morrowiae* belongs to a genus widely found in the Quadrilátero Ferrífero–Brazil (Schneider et al. 2013; Schneider et al. 2017; Teixeira et al. 2017; Vieira et al. 2018), and in areas affected by deposition of Fundão tailings (Prado et al. 2019), being an interesting biotechnological asset to be used as an inoculant.

It is hypothesized that plant diversity and AMF inoculation favor increases in phytomass and the absorption of potentially toxic elements (Na, Cu, Fe, Mn, Zn, Pb, Cr, and Ni) by plants. Therefore, the objective of this research was to evaluate a soil recovery strategy affected by iron mining tailing using herbaceous species with the inoculation of *Acaulospora morrowiae*.

2 Material and Methods

2.1 Study Area and Tailing Sampling

The experiment was conducted in a greenhouse between April and November 2018, using tailings deposited from the banks of the Gualaxo do Norte river, collected two years after the Fundão Dam–Mariana, MG, Brazil dam (20° 16′ 21.97″ S and 43° 12′ 4.32″ W, 486 m altitude). At the time of sampling, the area had been revegetated with *Cynodon dactylan* grass and different legumes (*Cajanus cajan*, *Neonotomia wightii*, and *Mimosa* sp.), with a layer of accumulation greater than one meter of tailings.

Collection was performed in the 0–20 cm tailings layer and the chemical and particle size characteristics of the tailings were clay: 80 g kg^{-1}; silt: 366 g kg^{-1}; sand 554 g kg^{-1}; pH 8.4; MO 13 g kg^{-1}; K 73.1 mg dm^{-3}; P 10.24 mg dm^{-3}; Na 19.72 mg dm^{-3}; Ca 1.21 cmolc dm^{-3}; Mg 0.1 cmol$_c$ dm^{-3}; Al 0.07 cmol$_c$ dm^{-3}; H + Al: 0.73 cmol$_c$ dm^{-3}; SB 1.85 cmol$_c$ dm^{-3}; t 1.88 cmol$_c$ dm^{-3}; T 2.46 cmol$_c$ dm^{-3}; m 1.6%; P-rem 49.57 mg L^{-1}; Zn 1.63 mg dm^{-3}; Fe 167.24 mg dm^{-3}; Mn 130.64 mg dm^{-3}; Cu 0.71 mg dm^{-3}; B 0.04 mg dm^{-3}; S 7.39 mg dm^{-3}.

2.2 Study Design and Conduct

The experiment was performed with a randomized block design in a 4 × 2 factorial scheme, with three replications and duration of 100 days. The treatments were four herbaceous cropping systems and two conditions of arbuscular mycorrhizal fungus (AMF) inoculation, with and without inoculum. Crops consisted of *Urochloa ruziziensis* single crop (RS); *U. ruziziensis* intercropped with *Crotalaria spectabilis* (R + C); *U. ruziziensis* intercropped with *Guizotia abyssinica* (R + G) and *U. ruziziensis* intercropped with *C. spectabilis* and *G. abyssinica* (R + C + G). The treatment scheme is presented in Fig. 1.

Inoculation with AMF was performed at the time of the sowing of herbaceous plants using the species *Acaulospora morrowiae* (UFLA 226, Universidade Federal de Lavras' code) from a cultivation pot with *Urochloa decumbens*as a host plant and trap culture as indicated by Barbosa et al. (2019). Inoculation of *A. morrowiae* was multiplied in 5 kg pots containing a mixture of a substrate composed of soil (Oxisol) and washed sand (in the proportion 1:1, v/v), which was autoclaved for 2 h at 121 °C, sowing the specie *U. decumbens*. Pots were kept in a greenhouse for four months and the spores multiplied in the pots were quantified to determine the amount of inoculum to be applied. The inoculated treatments received 4 g of inoculum soil containing about 200 spores, besides hyphae and colonized roots. Already the uninoculated treatments received the same inoculated soil but sterilized by autoclaving for 1 h at 121 °C, to eliminate AMF propagations. The spore density of native AMF recovered from the tailings before the start of the experiment was 130 spores in 50 g, no morphological or molecular identification of AMF species was made in the tailings. Both in the soil inoculum and the tailings used for the experiment, the spore density was quantified in 50 g of soil or tailings, using the wet sieving method (Gerdemann and Nicolson 1963) and centrifugation in water and sucrose solution (Jenkins 1964). Counting was done directly on corrugated plates under a stereomicroscope.

Tailings were air-dried and then sieved in a 2-mm-mesh sieve and placed in 6 kg pots. It was chosen not to use any method of sterilization of the tailings so that the microcosm could mimic the natural environment, where inoculation would be done under conditions without the control of the local microbial community. During this period there were three fertilizers, the first at the time of the herbaceous implantation, applied via nutrient solution 25.0 mg kg^{-1} of N; 55.3 mg kg^{-1} of P; 5.5 mg kg^{-1} of K; 33.8 mg kg^{-1} of S; 1.0 mg kg^{-1} of B; 0.10 mg kg^{-1} of Co; 5.0 mg kg^{-1} of Cl; 0.10 mg kg^{-1} of Mo and 25.0 mg kg^{-1} of Mg. The other fertilizers were performed at 48 and 85 days of implantation, both containing 300 mg kg^{-1} K and 300 mg kg^{-1} N. Six plants were kept in each pot, of each herbaceous plant, according to the proposal for each treatment. Irrigation was performed daily with distilled water, always following the field capacity of the tailing.

The experiment was conducted for 100 days from the date of planting. At the end of the experiment, the total

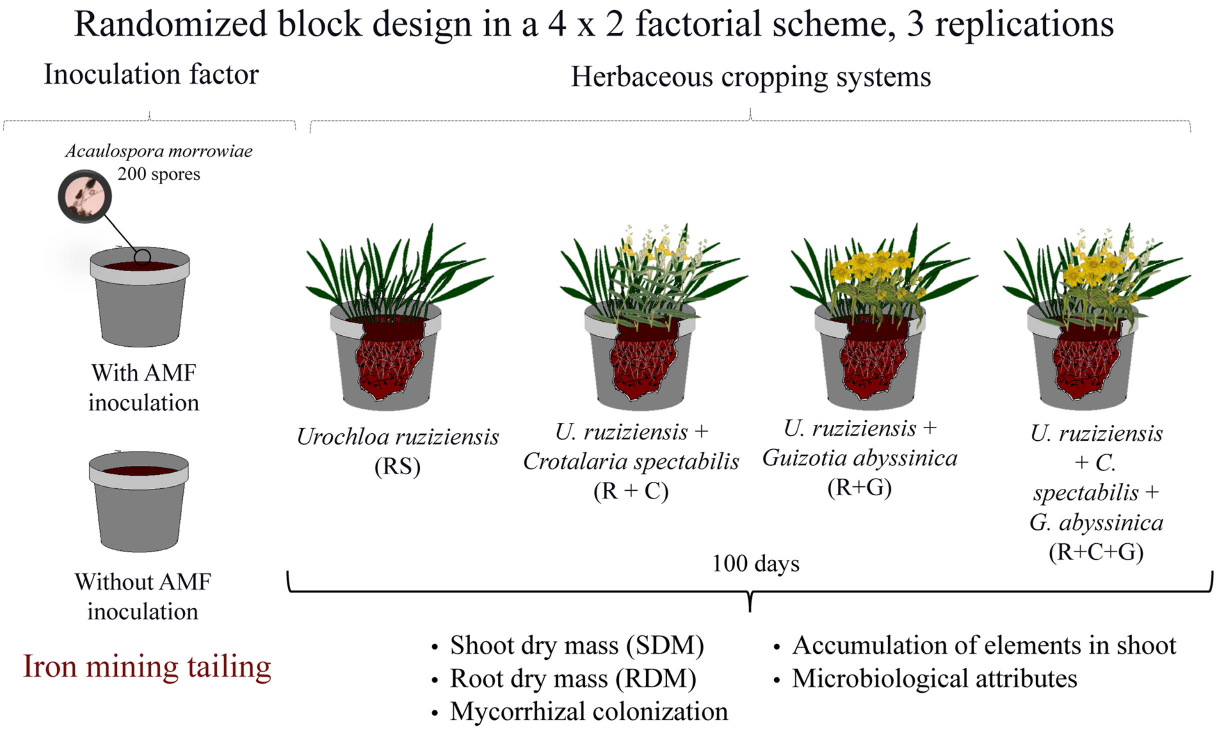

Fig. 1 Scheme of experimental design. The treatments were four herbaceous cropping systems and two conditions of arbuscular mycorrhizal fungus (AMF - *Acaulospora morrowiae*) inoculation (with and without) cultivated in tailings deposited on the banks of the Gualaxo do Norte river two years after the Fundão Dam rupture. Herbaceous Crop Systems:. *Urochloa ruziziensis* single crop (RS); *U. ruziziensis* intercropped with *Crotalaria spectabilis* (R + C); *U. ruziziensis* intercropped with *Guizotia abyssinica* (R + G) and *U. ruziziensis* intercropped with *C. spectabilis* and *G. abyssinica* (R + C + G)

dry matter of the root (RDM) was determined, as well as the total dry matter of the shoot (SDM), and the rate of mycorrhizal colonization. The potential of plant phytoextraction was determined for Na, Cu, Fe, Mn, Zn, Pb, Cr, and Ni. As a way of quantifying the microbial community in treatments, spore density, microbial biomass carbon (MBC), basal respiration (BR), and metabolic coefficient ($q\mathrm{CO}_2$) were evaluated.

2.3 Evaluations Performed at the End of the Experiment

After 100 days, the herbaceous plants were collected, the roots were washed with distilled water and 1 g of thin roots separated to evaluate the rate of mycorrhizal colonization. The procedure for quantifying mycorrhizal colonization included separation of 1 g of roots smaller than 2 mm in diameter, which was cleaned and stained with Trypan Blue (0.05%), according to the method of Phillips and Hayman (1970). Counting was carried out on a checkered plate under a stereomicroscope, and the colonization rate was quantified by the intersection method of Giovannetti and Mosse (1980).

Other roots were dried off at 65 °C in one air circulation oven and stored in paper bags. Oven was kept heated until samples reached constant weight to determine the total dry matter of the roots (RDM) of each pot. For determination of the total dry matter of the shoot (SDM) for each treatment, the plants were washed in distilled water and the aerial part was separated to quantify the SDM of each species, dried in an oven at 65 °C in one air circulation oven and stored in paper bags.

In order to evaluate the phytoextraction potential of chemical elements (Na, Cu, Fe, Mn, Zn, Pb, Cr, and Ni) in the shoot, the plants were ground separately. Determination of metals was performed according to USEPA method 3051A (USEPA - United States Environmental Protection Agency 1998), using 0.5 g of vegetable material crushed in a batch type mill. All weights were recorded to ensure the proper conversion of the metal concentration at the end of the analysis, as recommended by the USEPA method. Concentrations of chemical elements were determined by argon-induced plasma emission spectrophotometry (ICP-OES).

The spore density of AMF was determined according to Gerdemann and Nicolson (1963). The microbial biomass carbon (MBC) determination was made according to the fumigation-extraction method proposed by Vance et al. (1987). Basal respiration (BR) was determined using the NaOH to capture CO_2 method proposed by Alef (1995). The metabolic quotient (qCO_2) was calculated from the relationship between BR and MBC, according to the methodology proposed by Anderson and Domsch (1993).

2.4 Statistical Analysis

The results were submitted to data normality (Shapiro-Wilk), as well as to the analysis of variance (ANOVA), and, when significant, the Tukey test at 5% significance was applied using the RStudio version 3.6 (R Core Team, 2020).

3 Results

The analysis of variance showed that the culture systems influenced almost all variables, except spore density and metabolic quotient (qCO_2) (Table 1). However, the mycorrhizal fungus inoculation factor (AMF) only influenced the root dry matter (RDM) and basal respiration (BR) (Table 1). As for the interaction between the factors, significance was observed for microbial biomass carbon (MBC) and qCO_2 (Table 1).

The R + C and R + C + G systems presented the highest shoot dry matter (SDM) yields on average 10.5 g pot^{-1} higher than the other systems (RS and R + G) (Fig. 2a), not differing as to the inoculation factor (Table 1). Root yield (RDM) ranged from 9.4 to 18.6 g pot^{-1}, with higher values for R + G and R + C systems, respectively (Fig. 2b). The RDM was the only variable of plant growth/development that had a significant effect on the inoculation factor (Table 1), observing that the plants not inoculated with AMF showed a 23% increase in root yield compared to inoculated plants (Fig. 2c).

Mycorrhizal colonization (MC) varied among systems, with a greater value for R + C + G treatment (Fig. 3). The MC was increased due to the increase in herbaceous diversification, ranging from 24% for single *Urochloa ruziziensis* (RS) cultivation to 51% in the system of higher intercropping (Fig. 3).

Table 1 Summary of the variance analysis table of the variables of the cultivated herbaceous preculture phase established in Tailings deposited on the banks of the Gualaxo do Norte River, cultivation with herbaceous plants, two years after the Fundão dam rupture

	Cultivation systems	Inoculation	Cultivation × inoculation	
Variables	Test *F* significance			CV%
SDM	13.517**	0.25^{ns}	0.872^{ns}	10.57
RDM	6.164**	5.539**	0.56^{ns}	26.63
MC	7.198**	2.540	0.707^{ns}	27.27
Spores	1.943^{ns}	2.319^{ns}	1.241^{ns}	37.09
MBC	3.858**	6.511^{ns}	2.852**	17.09
BR	4.838**	10.492**	2.403^{ns}	29.28
qCO_2	1.819^{ns}	3.977^{ns}	3.714**	17.58
Accumulation				
Na	6.013**	1.512^{ns}	0.483^{ns}	42.58
Cu	6.205**	4.037^{ns}	0.222^{ns}	43.65
Fe	4.068**	0.632^{ns}	2.145^{ns}	46.64
Mn	6.147**	0.07^{ns}	0.044^{ns}	58.22
Zn	6.437**	0.23^{ns}	1.245^{ns}	36.33
Pb	5.547**	0.207^{ns}	0.255^{ns}	29.20
Cr	3.159**	0.451^{ns}	0.007^{ns}	33.17
Ni	1.310**	2.265^{ns}	0.754^{ns}	72.21

Cultivation × *inoculation* interaction of factors cultivation systems and inoculation of arbuscular mycorrhizal fungi. Variables: *SDM* total shoot dry matter, *RDM* total root dry matter, *MC* mycorrhizal colonization, *Spores* spore density, *MBC* microbial biomass carbon, *BR*: basal respiration, qCO_2 metabolic quotient. **, and ^{ns} significant at 5%, and not significant, respectively, by the F test

Crop systems have significantly accumulated chemical elements in the shoots (Fig. 4). In general, the RS system presented the smallest accumulation of elements, among others. In turn, the R + C + G system stood out with the highest accumulations for all evaluated chemical elements. The contents of each element are shown in Figure S1.

In the R + C + G system, there was a greater accumulation of Na, 64% more than the RS system, highlighting the high phytostabilization potential of *Guizotia abyssinica*. This fact was also observed for Cu, in which the R + G and R + C + G systems presented the largest accumulations, 56 and 63%, respectively, more than the RS system (Fig. 4).

Observing Fe accumulations, the R + C + G system presented an accumulation equivalent to 57% more than

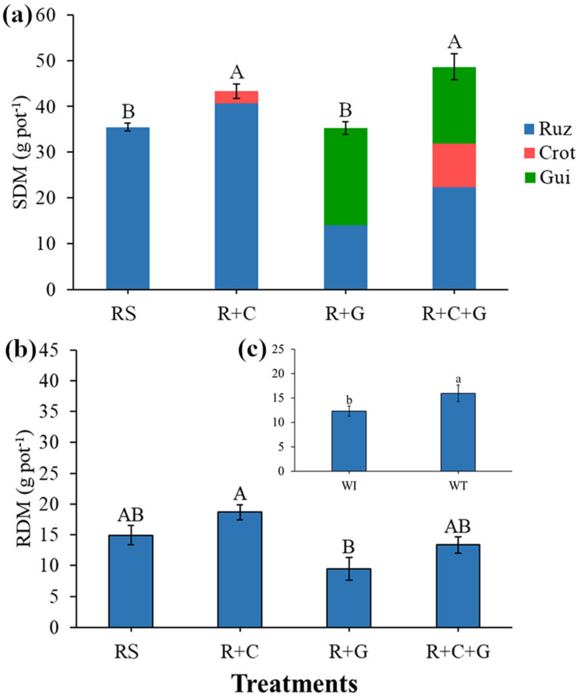

Fig. 2 Total shoot dry mass (SDM) production (**a**), root dry mass (RDM) (**b**) and influence of arbuscular mycorrhizal fungi inoculation on root dry matter production (**c**) of herbaceous cultivated in tailings deposited on the banks of the Gualaxo do Norte river two years after the Fundão Dam rupture. Herbaceous Crop Systems: Ruz: *Urochloa ruziziensis* (*Ruz*) single; R+C: *U. ruziziensis* intercropped with *Crotalaria spectabilis* (*Crot*); R+G: *U. ruziziensis* consortium with *Guizotia abyssinica* (*Gui*); and R+C+G: *U. ruziziensis* consortium with *C. spectabilis* and *G. abyssinica*. Means followed by the same capital letter in (a and b), and lowercase in (c) do not differ from each other by the Tukey test at 5% probability

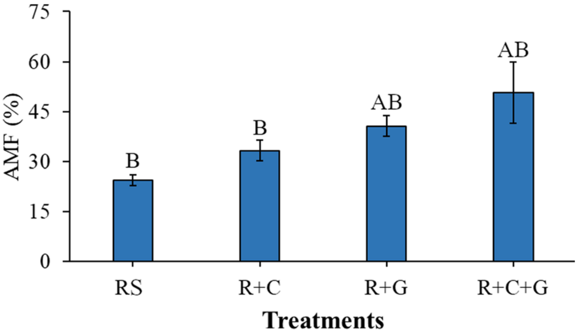

Fig. 3 Mycorrhizal colonization rate of herbaceous roots grown in tailings deposited on the banks of the GualaxoGualáxo do Norte river 2 years after the Fundão Dam rupture. Herbaceous Crop Systems: Ruz: *Urochloa ruziziensis* single; R + C: *U. ruziziensis* intercropped with *Crotalaria spectabilis*; R + G: *U. ruziziensis* consortium with *Guizotia abyssinica*; and R + C + G: *U. ruziziensis* consortium with *C. spectabilis* and *G. abyssinica*. WI: with arbuscular mycorrhizal fungi inoculation; WT: without arbuscular mycorrhizal fungi inoculation. Means followed by the same letter do not differ from each other by the Tukey test at 5% probability

the RS system (Fig. 4), while the R + C and R + G systems presented intermediate accumulations to the other systems, respectively, 5521.2 and 8879.1 mg pot^{-1}. For Mn, it was observed that the system composed with the largest number of herbaceous species (R + C + G) phytoextracted 8345.4 mg pot^{-1}, which is 71.65 and 56% higher than the accumulations observed in the RS, R + C, and R + G, respectively. Zn accumulation varied from 496.4 to 1203.8 mg pot^{-1} in the RS and R + C + G systems, respectively. In turn, accumulation of Pb in the R + C, R + G, and R + C + G systems were 14, 20, and 46% higher respectively than those observed in the RS system. Regarding Cr, the RS system presented an accumulation of 4.5 mg pot^{-1}, lower than that observed in the R + C + G system, with the highest accumulation observed. Ni showed 42% more accumulation in the system R + C + G compared to the RS system. For Na, Fe, Zn, Cr, and Ni elements, the R + C, and R + G systems presented intermediate accumulations to the RS and R + C + G systems, respectively (Fig. 4), smaller, and larger accumulations for the mentioned chemical elements.

Spore density did not vary between treatments (Fig. 5a, b). The MBC was higher in the RS and R + G treatments without inoculation with AMF (Fig. 5c). The RB was higher in the R + G and R + G + G treatments, and when not inoculated (Fig. 5d, e). $q\text{CO}_2$ was higher in non-inoculated RS treatments and in R + C, R + G and R + C + G treatments when inoculated (Fig. 5f).

4 Discussion

The increase in the amount of herbaceous plants species is a positive strategy for the revegetation of tailings, since most diverse cultures presented the highest yield of phytomass as observed in the R + C and R + C + G systems, which, on average, they presented more than 10.5 g of pot^{-1} compared to the less diversified RS system (Fig. 2a). The increase in the phytomass production in the systems that have the legume *Crotalaria spectabilis* shows the importance of a legume in the consortium, as they are species with high biomass production and mainly fixing atmospheric nitrogen, essential for the increase of soil organic matter (Maiti and Maiti 2015).

Reduction in phytomass of the brachiaria in the presence of *Guizotia abyssinica* (Fig, 2a) may be due to a possible interspecific competition between the two

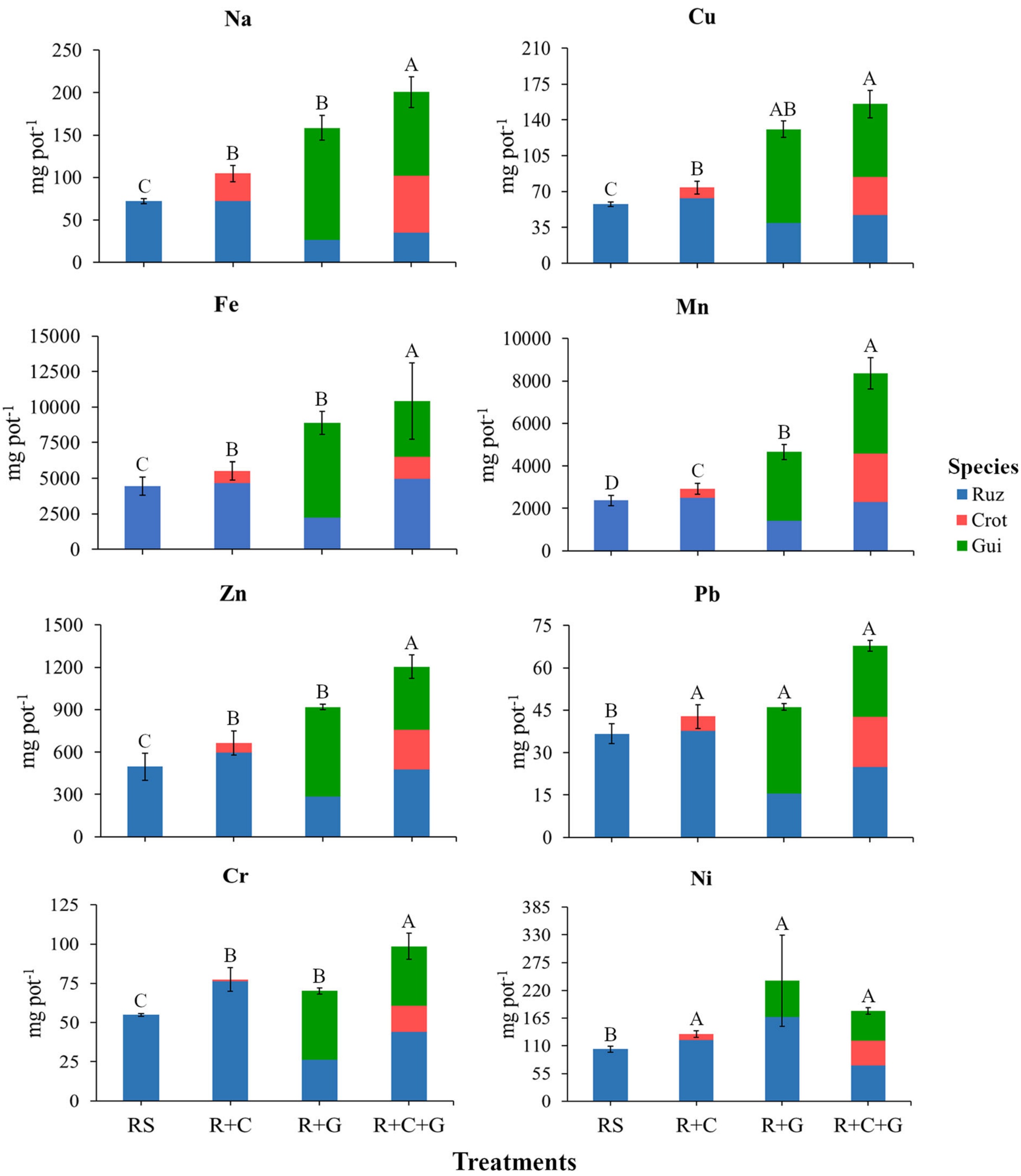

Fig. 4 Accumulation of elements in the aerial part of herbaceous cultivated in tailings deposited on the banks of the Gualáxo do Norte river two years after the Fundão Dam rupture. Herbaceous Crop Systems: Ruz: *Urochloa ruziziensis (Ruz)* single; R+C: *U. ruziziensis* intercropped with *Crotalaria spectabilis (Crot)*; R+G: *U. ruziziensis* consortium with *Guizotia abyssinica (Gui)*; and R+C+G: *U. ruziziensis* consortium with *C. spectabilis* and *G. abyssinica*. Means followed by the same letter within the same element do not differ from each other by the Tukey test at 5% probability

crops for nutrients or to an allelopathic effect, which exerts a high pressure of suppression on the forages when intercropped with oilseeds, as reported by Silva et al. (2010). These results demonstrated the importance

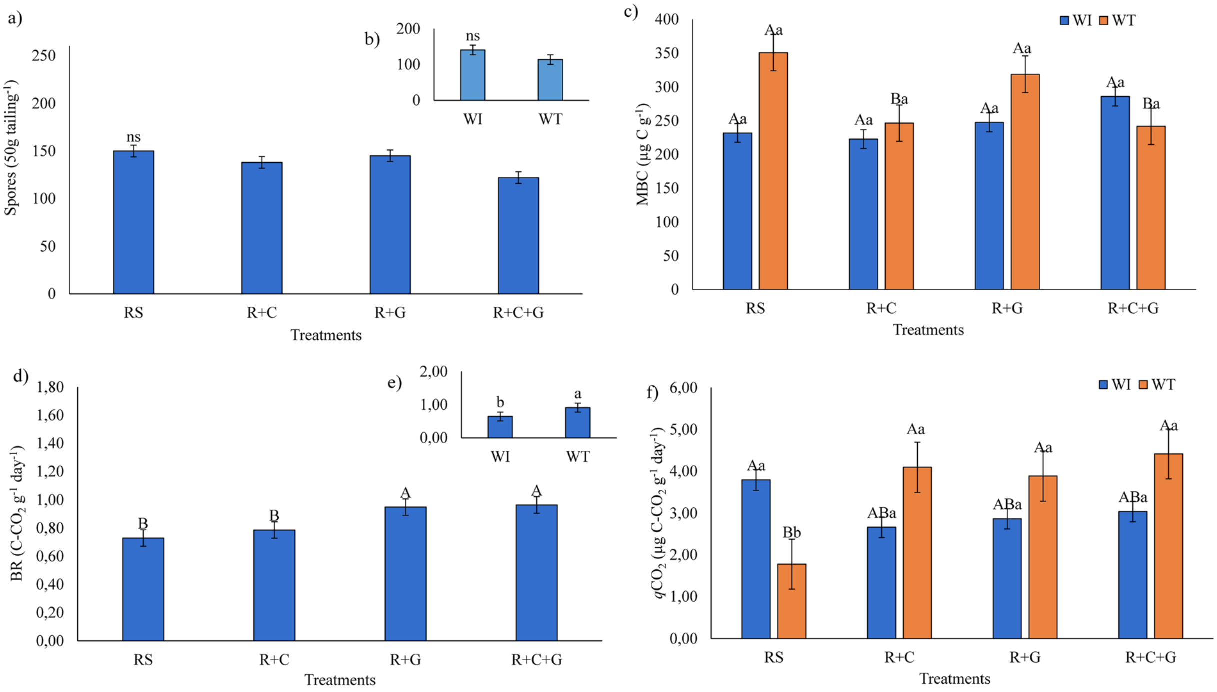

Fig. 5 Spores density (Spores), microbial biomass carbono (MBC), basal respiration (BR), metabolic quotient (qCO2) of tailings deposited on the banks of the Gualáxo do Norte river two years after the Fundão Dam rupture cultivated with herbaceous. Herbaceous Crop Systems: Ruz: *Urochloa ruziziensis* single; R+C: *U. ruziziensis* intercropped with *Crotalaria spectabilis*; R+G: *U. ruziziensis* consortium with *Guizotia abyssinica*; and R+C+G: *U. ruziziensis* consortium with *C. spectabilis* and *G. abyssinica*. Means followed by the same letter within the same element do not differ from each other by the Tukey test at 5% probability

of understanding the complexity of the floristic composition when designing a revegetation system, especially in an environment as specific as the iron mining tailing studied.

The lower yield of the root system (RDM) in the R + G consortium (Fig. 2b) can also be related to the negative effect of the *Guizotia abyssinica* consortium with *Urochloa ruziziensis*, a fact not observed in the R + C consortium. Roots are important factors in the process of rehabilitation of degraded areas, as they stimulate the biological activity of the soil, introduce sources of different organic compounds (rhizodeposition), increase the concentration of organic carbon in the soil and act in the structuring of the soil (Vilela et al. 2014; Carneiro et al. 2015; Silva et al. 2018). *U. ruziziensis* has an abundant root system, with rapid and continuous development, providing significant improvements in the soil, especially in aspects related to microbial activity, structure, and accumulation of organic matter in the soil (Salton and Tomazi 2014). In this sense, considering the results obtained in the present study, it is possible to estimate the production of 5 t of roots ha^{-1} in *Urochloa ruziziensis* and that, when intercropped with *Crotalaria spectabilis*, an increase of 1.2 t was observed, totaling 6.2 t of roots ha^{-1}. Although the RS system showed low production of phytomass (SDM), its root system was well developed (14.9 g pot^{-1}), which did not occur in the R + G system (Fig. 2b).

In addition to the root production capacity, which increases the exudation of carbon compounds through rhizodeposition and stimulates the microbiota, it also favors physical conditions of the tailings, which is one of the main problems, since the high density of tailings associated with low porosity favors the thickening and sealing of the surface and consequently decreases water infiltration. Thus, it increases the flow and the transport of sediments to the riverbeds, causing silting and increased turbidity as well as the presence of chemical elements in the river water.

The stimulation of microbial activity in the Technosol of the iron mining tailing area can be favored by the presence of AMF; however, in this study, there was a reduction in MCB and BS (Fig. 5). The low response of inoculation with *Acaulospora morrowiae* in the production of roots may have influenced the decrease in root exudation, and thus reduce the density in the rhizosphere microbiota in tailing. However, mycorrhizal colonization, which can be considered high, was observed in both treatments (with or without inoculation), demonstrating that native fungi are already adapted to the condition of Technosol as shown in studies by Prado et al. (2019). Several studies reported the null effect of inoculation with exotic AMF on plant growth compared to native AMF (Doubková and Sudová 2016; Rydlová and Vosátka 2003).

The MC increased with the increase of herbaceous species, with values of up to 51% in the R + C + G (Fig. 3). The genus *Urochloa* grasses is efficient in forming symbiosis of AMF (Miranda et al. 2010), and its simultaneous cultivation with other plants allows the colonization of different plant species by the same fungus (Carrenho et al. 2018). It is also reported that the *Urochloa* consortium increases the potential for inoculum and AMF colonization, which favors the soil infectious capacity and the activity of these symbionts in the plant (Moraes et al. 2019). Although no response to AMF inoculation was observed, this greater mycorrhizal colonization demonstrates that the native AMF community of the Technosol of the iron mining tailing area is efficient and, mainly, adapted to the conditions of this formed environment, as reported in other studies as well (Kemmelmeier 2018; Prado et al. 2019).

Tailing has high pH values due to the high levels of sodium hydroxide applied in the iron ore separation process (Santos et al. 2019). This environmental characteristic may be responsible for inhibiting the activity, development, and effective symbiosis of *Acaulospora* (Jordão et al. 2021), as it is a genus widely found in soils with a pH below 6.2 (Stürmer and Bellei 1994; Stürmer et al. 2006). Although no effects of inoculation with *Acaulospora morrowiae* were observed, this species was isolated in soils contaminated with heavy metals (Klauberg-Filho et al. 2002; Schneider et al. 2013). It is widely distributed around the accident site and across the Quadrilátero Ferrífero-Brazil (Prado et al. 2019; Schneider et al. 2013; Schneider et al. 2017; Teixeira et al. 2017; Vieira et al. 2018). And this species has already been cited as efficient in promoting plant growth in soils with a high metal content (Schneider et al. 2017). Eventually, it is believed that with the natural decrease in pH in tailings, this, and other species more adapted to higher acidic environments may be able to perform effective symbiosis. Mycorrhizal colonization also contributes to absorption of contaminants by fungal hyphae, reducing their transport to plant tissues (Miransari 2011), and can be considered as one of the most important factors for the rehabilitation of soils contaminated with heavy metals. Pedroso et al. (2018) observed positive effects of AMF on plant growth for the rehabilitation of soils contaminated with Zn, Cu, Pb, and Cd, promising the use of plants associated with these fungi to contribute to the supply of organic matter and litter formation that favors soil biological activity. However, in our study this increase in microbial activity was not observed (Figure 5).

In addition, tailings already had a high amount of native AMF spores (130 spores in 50 g), demonstrating that these fungi have already started the colonization process of this degraded environment, which justifies the poor response to inoculation. According to Prado et al. (2019), the revegetation process of the tailings deposited on the banks of the Rio Doce, as well as the dispersion of AMF spores from undisturbed areas, serves as a repository for inoculating native fungi to the affected areas, showing their adaptation to the environment, and that the number of spores and the richness of AMF species increased along with the revegetation process. As reported in Teixeira et al. (2017) and Vieira et al. (2018), the higher spore density and AMF diversity indicated that degraded areas after iron mining activity are in the process of recovery.

As for the ability to accumulate elements, treatments with the greater number of species were the most efficient (Fig. 4). In general, the R + C + G system was more efficient in presenting the highest accumulations of elements, with an emphasis on the Pb accumulation where the R + C, R + G, and R + C + G systems did not differ (Fig. 4). According to Zu et al. (2017), the increase in metal absorption can be induced by the composition and proportions of organic compounds secreted in the rhizosphere of different intercropped plants, through interspecific interactions of the roots on single plants. According to Lin et al. (2018), only suitable combinations of intercropped plants can increase the absorption of heavy metals. In this present study, this is evident by observing the potential for absorption and accumulation of these metals in the aerial part of the three cultures when combined, contributing to the initial tailings stabilization, which can be an interesting strategy for the impacted areas.

The plants used in the revegetation process must be effective in covering the soil to reduce erosion, in addition to assisting in the restoration of main ecosystem processes, which drives rehabilitation (Thijs et al. 2017;

Carrenho et al. 2018; Kumari and Maiti 2019; Remigio et al. 2020). With regard to the iron mining tailings deposited on the banks of the rivers of the Rio Doce Basin, the rehabilitation processes become even more complex, as there is a heterogeneity in the physico-chemical constitution of the tailings, especially in relation to their greater density, lack of structure and high pH, high Fe and Mn, making it difficult to establish plants (Esteves et al. 2020; Matos et al. 2020; Scotti et al. 2020). Thus, the use of intercropped plants that produce high phytomass and roots, which can accumulate potentially toxic elements in their tissues, produce greater volume of roots, stimulate the biological activity of the soil, and assist efficiently in the rehabilitation process, providing the necessary edaphological conditions for ecological succession.

5 Conclusion

The cultivation system with three herbaceous plants proved to be efficient in the production of phytomass and in establishing itself initially in the iron mining tailings. Therefore, systems with a greater number of herbaceous plants are a viable alternative for the process of rehabilitation of areas impacted by the accident. The inoculation with *Acaulospora morrowiae* did not influence dry matter production by the aerial part nor the accumulation of elements in the herbaceous plants grown in the tailings, but decreased baseline breathing.

Accumulation of chemical elements was higher in the cultivation of *Urochloa ruziziensis* in association with *Crotalaria spectabilis* and *Guizotia abyssinica*, indicating the potential of these species in the extraction of chemical elements contained in iron mining tailings.

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