DANIEL RODRIGUES DA SILVA

MINING TAILINGS EFFECTS FROM MARIANA DAM DISASTER IN MINAS GERAIS, BRAZIL, ON GERMINATION, BIOMETRIC PARAMETERS, MINERAL CONTENT, PHOTOSYNTHETIC METABOLISM, MORPHOANATOMY AND MYCORRHIZAL COLONIZATION IN *Brachiaria decumbens* Stapf. (POACEAE)

Thesis presented to the Universidade Federal de Viçosa, as part of the Botany Graduate Program requirements, to obtain the title of *Doctor Scientiae*.

Adviser: Luzimar Campos da Silva

VIÇOSA - MINAS GERAIS
2020

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
Silva, Daniel Rodrigues da, 1991-
S586m Mining tailings effects from Mariana dam disaster in Minas
2020 Gerais, Brazil, on germination, biometric parameters, mineral
content, photosynthetic metabolism, morphoanatomy and
mycorrhizal colonization in *Brachiaria decumbens* Stapf.
(Poaceae) / Daniel Rodrigues da Silva. – Viçosa, MG, 2020.
57 f. : il. (algumas color.) ; 29 cm.

Orientador: Luzimar Campos da Silva.
Tese (doutorado) - Universidade Federal de Viçosa.
Referências bibliográficas: f.29-41.

1. Gramínea. 2. Degradação do solo. 3. Doce, Rio, Bacia
(MG e ES) . I. Universidade Federal de Viçosa. Departamento de
Biologia Vegetal. Programa de Pós-Graduação em Botânica.
II. Título.

CDD 22 ed. 584.9

DANIEL RODRIGUES DA SILVA

MINING TAILINGS EFFECTS FROM MARIANA DAM DISASTER IN MINAS GERAIS, BRAZIL, ON GERMINATION, BIOMETRIC PARAMETERS, MINERAL CONTENT, PHOTOSYNTHETIC METABOLISM, MORPHOANATOMY AND MYCORRHIZAL COLONIZATION IN *Brachiaria decumbens* Stapf. (POACEAE)

Thesis presented to the Universidade Federal de Viçosa, as part of the Botany Graduate Program requirements, to obtain the title of *Doctor Scientiae*.

APPROVED: April 23, 2020.

Assent:

Daniel Rodrigues da Silva

Daniel Rodrigues da Silva
Author

Luzimar Campos da Silva
Adviser

DEDICATÓRIA

Dedico este trabalho à minha mãe Fátima, a meu pai José Gomes, à minha avó Dona Clara aos meus tios Lourdes e Cláudio, a meus amigos e professores de Viçosa, Ubá e Guidoval e às cabeças pensantes por trás deste trabalho.

AGRADECIMENTOS

Agradeço ao meu pai José Gomes e minha mãe Fátima pelo apoio e suporte dado durante todos esses anos e pela compreensão na minha ausência durante todo esse período. Um agradecimento especial para minha vó dona Clara que é uma pessoa muito especial na minha vida.

Agradeço à Renata e à família Garcia/Diogo (Esmeralda, Nanda, Virgínia, Fernando Diogo, Ivanir, Marcelo, Léo, Gu, Daniel e Guilherme) que me acolheram de braços abertos nessa minha estadia aqui em Viçosa.

Ao Programa de Pós-Graduação em Botânica e ao laboratório de Anatomia Vegetal do Departamento de Biologia Vegetal da Universidade Federal de Viçosa. À minha orientadora Luzimar Campos por acreditar no meu trabalho sempre e por sempre incentivar e se preocupar com seus inúmeros orientados. Agradeço também às técnicas do laboratório de anatomia vegetal Aurora e Rosana.

Agradeço ao professor Carlos Ernesto Schaefer por ter dado a ideia do trabalho e por ser uma fonte inesgotável de conhecimento dessa Universidade, à pesquisadora Kacilda Naomi Kuki por ser uma das pessoas que mais contribuem com ideias geniais para a execução e aprimoramento dos trabalhos e aos professores do DBV (Aristea, Marília, Renata, Cléberson, Wagner Otoni, João Meira e Jeferson) pelos ensinamentos ao longo desses anos. Aos funcionários do DBV Ângelo, Mauro, Marcele, Luciene, Chiquinho, Marreco e Guilherme. Ao professor Wagner Araújo, da Fisiologia Vegetal, por disponibilizar espaço na Unidade de Crescimento de Plantas (UCP).

Aos meus amigos irmãos de república que são mesmo que ser da família Ivanilson (Recife), Álvaro (Colômbia) pela parceria ao longo desses anos, muito obrigado pelos momentos de descontração, apertos, risos, brigas, festas e centenas de casos para contar que serão lembrados para sempre. Como diria o Colômbia: “Tá fácil non Recife”, “Yo no queria estar em sus pantalones” e realmente não foi fácil, mais na convivência com vocês as dificuldades eram amenizadas e por vezes esquecidas.

Um agradecimento especial ao Michel, grande amigo e irmão científico e de república por ter me ajudado ao longo desses anos, seja carregando uma tonelada de solo para experimento, ou seja, contando prosa, tocando violão, etc. Agradeço imensamente ao Gustavão pela amizade e pelo suporte na parte estatística do trabalho e ao Pedrão também pela amizade ao logo desses tempos. Agradeço aos meus amigos/irmão do “Las Peladas G.O” pelo futebol semanal que não falta, pelos churrascos sempre animados e pelas resenhas fantásticas que sempre geram memes

e divertimento e ao Luiz Augusto que foi meu professor e hoje é um amigo que me incentivou a vir fazer a pós-graduação aqui na UFV.

Agradeço a amizade sempre sincera da Regiane, Rafa, Juliane Luz e Manu. Ao grupo de estudos da professora Luzimar, ao pessoal do laboratório e à agora professora Larisse pela amizade e por sempre que vem à Viçosa me presentear com uma boa conversa no café! Agradeço ao grupo “Seletão” de pessoas (Analu, Luana, Ivan, Katy, Ana Paula e Cristielle) pelos momentos divertidos de descontração nessa Viçosa. Por fim, agradeço imensamente ao pássaro negro que abre as portas do conhecimento com a chave que ele traz no bico.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

BIOGRAFIA

Daniel Rodrigues da Silva, filho de José Gomes da Silva e Maria de Fátima Rodrigues da Silva, nasceu em Mauá/SP, em 18 de maio de 1991. Cursou o ensino médio na Escola Estadual Mariana de Paiva em Guidoval - MG, concluindo em 2008. Ingressou na Universidade do Estado de Minas Gerais - Campus Ubá em fevereiro de 2009, onde concluiu o curso de licenciatura plena em Ciências Biológicas, quatro anos mais tarde. Em abril de 2013 iniciou o Mestrado em Botânica, pelo Departamento de Biologia Vegetal na Universidade Federal de Viçosa, concluindo o curso com a defesa da dissertação intitulada "Efeitos da poluição atmosférica de Ipatinga - MG sobre *Joannesia princeps* Vell. (Euphorbiaceae) com ênfase nos nectários extraflorais", em 31 de agosto de 2015. Em fevereiro de 2016, iniciou seu doutorado em Botânica, pelo Departamento de Biologia Vegetal da Universidade Federal de Viçosa.

“*Sometimes science is more art than science, Morty.*

A lot of people don't get that.”

(Rick Sanchez)

ABSTRACT

SILVA, Daniel Rodrigues da, D.Sc., Universidade Federal de Viçosa, April, 2020. **Mining tailings effects from Mariana dam disaster in Minas Gerais, Brazil, on germination, biometric parameters, mineral content, photosynthetic metabolism, morphoanatomy and mycorrhizal colonization in *Brachiaria decumbens* Stapf. (Poaceae)**. Adviser: Luzimar Campos da Silva.

After more than four years, the country still remembers what was one of the worst socio-environmental disasters in history. The catastrophic Fundão dam rupture in Mariana - MG, southeastern Brazil, which occurred in November 2015, launched more than 50 million cubic meters of iron mining tailings Technosols (IMTT) into the environment. The tailings quickly followed the drainage of Gualaxo do Norte, Carmo and Rio Doce rivers in Minas Gerais, arriving at the mouth of Rio Doce in Atlantic Ocean, leaving a trail of destruction along the way. This IMTT "tsunami" caused death of people, destruction of villages and urban infrastructure, displacement of hundreds of families, riparian forest suppression, impact on water bodies, among others. Currently, in the pastures affected by IMTT, the species *Brachiaria decumbens* Stapf. (Poaceae) is one of the most common plants, therefore, the work objective was to evaluate the effects of IMTT released in Rio Doce basin on ecophysiology, morphoanatomy and mycorrhizal colonization in *B. decumbens*, in addition, we seek to test the effects on affected soil quality when mixing the IMTT launched in Rio Doce basin with the soil unaffected by IMTT. For this, the unaffected soil by IMTT (Ref), the Technosol with a thick deposition of iron mining tailings (Tec) was sampled in Barra Longa - MG and we made a mixture of 50 % of each soil type (Ref/Tec) for a greenhouse experiment that lasted for 110 days, cultivating *B. decumbens* in these three soil types. Our results showed that the iron mining tailings Technosols (Tec) are poor in nutrients, particularly in zinc and organic matter and caused iron toxicity which resulted in decrease in growth, development, mineral content and photosynthetic metabolism of *B. decumbens*. Corroborating previous studies, it was observed in IMTT a poor nutritional status. The Ref/Tec soil for having superior quality to Tec proved to be an suitable approach for use in areas where Technosols of iron mining tailings were deposited, representing an important step for a quick recovery of these soils, for agricultural, pasture production and for environmental restoration.

Keywords: Rio Doce. Forage grass. Nutritional deficit.

RESUMO

SILVA, Daniel Rodrigues da, D.Sc., Universidade Federal de Viçosa, abril de 2020. **Efeitos dos rejeitos de mineração do desastre da barragem de Mariana em Minas Gerais, Brasil, sobre a germinação, parâmetros biométricos, conteúdo mineral, metabolismo fotossintético, morfoanatomia e colonização micorrízica em *Brachiaria decumbens* Stapf. (Poaceae)**. Orientadora: Luzimar Campos da Silva.

Passados mais de quatro anos, o país ainda se lembra daquele que foi um dos piores desastres socioambientais da história. O catastrófico rompimento da barragem de Fundão em Mariana - MG, sudeste do Brasil, ocorrido em novembro de 2015, lançou no meio ambiente mais de 50 milhões de metros cúbicos de Tecnosolos de rejeitos de mineração de ferro (TRMF) no ambiente. Os rejeitos rapidamente seguiram as drenagens dos rios Gualaxo do Norte, Carmo e Rio Doce, em Minas Gerais, chegando na foz do Rio Doce no Oceano Atlântico, deixando um rastro de destruição pelo caminho. Esse "tsunami" de TRMF causou a morte de pessoas, destruição de vilarejos e de infraestrutura urbana, desalojamento de centenas de famílias, supressão de floresta ripária, impacto em corpos de água, dentre outros. Atualmente, nas pastagens afetadas pelo TRMF, a espécie *Brachiaria decumbens* Stapf. (Poaceae) é uma das plantas mais comuns, diante disso, o objetivo do trabalho foi avaliar os efeitos do TRMF lançados na bacia do Rio Doce na ecofisiologia, morfoanatomia e colonização micorrízica em *B. decumbens*, além disso, buscamos testar os efeitos na qualidade do solo afetado ao se misturar o TRMF lançado na bacia do Rio Doce com o solo não afetado por TRMF. Para isso, foi coletado em Barra Longa - MG o solo não afetado pelo TRMF (Ref), o Tecnosolo com uma deposição espessa de rejeitos de mineração de ferro (Tec) e fizemos uma mistura de 50 % de cada tipo de solo (Ref/Tec) para um experimento em casa de vegetação que durou 110 dias no qual foi realizado o cultivo de *B. decumbens* nesses três tipos de solo. Nossos resultados mostraram que os Tecnosolos de rejeitos de mineração de ferro (Tec) são pobres em nutrientes, principalmente em zinco e matéria orgânica e causaram toxicidade por ferro o que resultou na diminuição de crescimento, desenvolvimento, teor mineral e metabolismo fotossintético de *B. decumbens*. Corroborando estudos anteriores, foi observado no TRMF um pobre status nutricional. O solo Ref/Tec por ter tido qualidade superior ao Tec se mostrou uma abordagem adequada para uso em áreas onde foram depositados os Tecnosolos de rejeitos de mineração de ferro, representando um passo importante para uma rápida recuperação desses solos, para produção agrícola, de pastagens e para recuperação ambiental.

Palavras-chave: Rio Doce. Gramínea forrageira. Déficit nutricional.

SUMMARY

1. INTRODUCTION 11
2. MATERIAL AND METHODS 13
    2.1. Soil sampling site 13
    2.2. Greenhouse experiment 13
    2.3. Soil and plant analysis 13
    2.4. Seed germination tests 14
    2.5. Biometrics parameters 14
        2.5.1. Plant height 14
        2.5.2. Specific leaf area determination 15
        2.5.3. Tillers count and weight of fresh and dried plant material 15
    2.6. Photosynthetic pigment content 15
    2.7. Gas exchange 15
    2.8. Anatomical and histochemical analyzes 16
    2.9. Plant root system architecture and mycorrhizal colonization analysis 16
    2.10. Statistical analysis 17
3. RESULTS 17
    3.1. Soil fertility analysis 17
    3.2. Foliar chemical analysis 18
    3.3. Germination tests 19
    3.4. Biometrics parameters 19
        3.4.1. Plant height 19
        3.4.2. Specific leaf area 20
        3.4.3. Number of tillers 20
        3.4.4. Weight of fresh and dry plant material 20
    3.5. Photosynthetic pigment content 20
    3.6. Gas exchange 21
    3.7. Leaf anatomical characterization and histochemical analysis 21
    3.8. Plant root system architecture, anatomy and mycorrhizal colonization 22
4. DISCUSSION 23
5. CONCLUSION 29
6. REFERENCES 29

1. INTRODUCTION

On November 5th, 2015, the Fundão iron ore dam at Mariana (Minas Gerais state) collapsed, resulting in the biggest environmental disaster in Brazilian history (Cordeiro et al., 2019; IBAMA, 2015; Valeriano et al., 2019). After this event, a wave with more than 50 million m^3 of iron mining tailings Technosols were dumped into the environment (Guerra et al., 2017; Marta-Almeida et al., 2016; Segura et al., 2016), following the drainages of Gualaxo do Norte, Carmo and Rio Doce Rivers, in Minas Gerais, arriving at Rio Doce mouth in Atlantic Ocean, totaling 663.2 km of directly impacted water bodies and 36 affected municipalities (Hatje et al., 2017; IBAMA, 2015; Santos et al., 2019).

The disaster caused a loss of 457.6 ha of riparian forest and covered a riverside area of 1176.6 ha (Omachi et al., 2018). The social impacts were considerable, with the interruption of basic urban services such as electricity supply and drinking water, burial of buildings at Bento Rodrigues and Paracatu de Baixo district, displacement of over 600 families and 19 confirmed deaths (Fernandes et al., 2016; IBAMA, 2015; Valeriano et al., 2019).

The foreseeable impacts will last for years affecting the lives of about 1 million people living in the affected riverside communities, due to the reduced fish stocks, compromised agricultural and indigenous lands (Fernandes et al., 2016; Garcia et al., 2017; IBAMA, 2015; Marta-Almeida et al., 2016). According to Garcia et al. (2017), the estimated loss of environmental services is about US$ 521 million per year.

In Minas Gerais the jointing of Piranga and Carmo rivers forms the Rio Doce (IGAM, 2005) in the Quadrilátero ferrífero region, one of the most important mining regions of Brazil, explored since the early seventeenth century for the extraction of gold, iron and aluminum (Borba et al., 2002; Hatje et al., 2017). The total historic gold extraction in the region is estimated to be over 1,300 tonnes (Borba et al., 2002) and iron and gold mineral deposits in the region are associated with potentially toxic elements such as manganese, arsenic from arsenopyrite and the mercury that comes from old-times gold mining activities after 300 years of intense mineral exploitation, leading air, soil and water pollution (Borba et al., 2002; Hatje et al., 2017; Matschullat et al., 2000). For example, gold mining process alone is estimated to have generated 390,000 tonnes of arsenic in water bodies (Borba et al., 2002).

According to Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Brazilian Institute of Environment and Renewable Natural Resources) - IBAMA reports carried out in 2014, the nature of the dam tailings was basically iron oxide and quartz (IBAMA, 2015). According to Almeida et al. (2018) and Guerra et al. (2017) the mining tailings dumped in the Rio Doce do not present risks to human health because of low concentration of toxic metallic elements.

Although iron mining tailings has been considered have no risk of contamination (Davila et al., 2020; Figueiredo et al., 2020), the extraordinary volume of mining tailings dumped into the environment may have caused the reworking and spreading of bottom sediments in rivers that historically hold heavy metals from previous mining activities, making them exposed to biological systems (IBAMA, 2015).

Deposits of mining tailings from ruptured dam affected fluvial terraces and floodplains with riparian forests areas and pastures (Aires et al., 2018; Schaefer et al., 2015). According to Leal et al. (2016) and Moreira et al. (2009), 85 % of Brazilian pastures are occupied by grasses of *Brachiaria* genus, so these forage grasses are extremely important for national livestock. The genus *Brachiaria* has about 100 species that occur in tropical environments, especially in Africa (Renvoize et al., 1996). These species have adapted well to Brazilian conditions since they tolerate acid soils with low nutrient availability such as phosphorus and calcium. Moreover, they are tolerant to high concentrations of aluminum in the soil, require low use of agricultural inputs, simple management practices and have a reasonable drought tolerance (Crispim et al., 2003; Rao et al.,1996).

Among the species of the *Brachiaria* genus, the species *Brachiaria decumbens* Stapf. (Poaceae), native to eastern Africa stands out in Brazilian pastures (Alvim et al., 2002; Macedo, 2004; Renvoize et al., 1996; Seiffert, 1980). This grass occupies between 50 to 55 % of the *Brachiaria* grass pastures in Brazil and presents better mineral composition than some native foraging plants (Alvim et al., 2002; Crispim et al., 2003; Macedo, 2004).

Currently, after more than four years of the Mariana dam disaster, *B. decumbens* has become the main grass in pasturelands affected by mining tailings, growing on Technosols either naturally or managed. Understanding how this plant is adapted to the local affected by Technosols can provide knowledge about the effects of Fe-rich mining tailings on plant-soil relationships, helping developing recovery strategies of the impacted environments in Rio Doce basin. Therefore, we hypothesized that the iron mining tailings Technosols from Fundão iron ore dam collapse affect the development of *B. decumbens* and that the mixture between unaffected soil by mining tailings and Technosol with a thick deposition of iron mining tailings can be beneficial for improving the quality of these affected soils. The aim of this study was to analyze the influence of iron mining tailings Technosols (IMTT) on *B. decumbens* ecophysiology, morphoanatomy and mycorrhizal colonization and testing whether a mixture of

IMTT with local regional soils can enhance the affected soil properties seeking a novel approach for Technosols recovery for agricultural, pasture production and environmental restoration.

2. MATERIAL AND METHODS

2.1. Soil sampling site

Soil samples were collected for a greenhouse experiment and for chemical analysis in a cattle pasture area whose soil is classified as red yellow Argisol, in Gesteira district of Barra Longa, Minas Gerais, Brazil (Lat: 20°15'28.61"S and Long: 43°07'24.48"O) (Figures 1A and 1B). Soil samples were collected approximately at 60 km downstream from the ruptured dam site and had part of the ground covered by the ruptured iron mining tailings (Figure 1C). A 20 to 30 cm layer of unaffected soil without iron mining tailings (Lat: 20°15'37.15"S and Long: 43°7'23.08"O) and a 20 to 30 cm layer of a Technosol with a thick deposition of iron mining tailings (Lat: 20°15'34.61"S and Long: 43°7'22.32"O) were collected (Figure 1D). The sampling was performed during the summer, on November 29th, 2018.

2.2. Greenhouse experiment

Were planted seeds of *Brachiaria decumbens* Stapf. (Poaceae) (Bioseeds® seeds, cultivar Basilisk) in 25-liter plastic pots. 50 seeds were planted randomly in each pot, in seven pots containing unaffected soil without iron mining tailings (Ref), seven pots containing Technosol with a thick deposition of iron mining tailings (Tec) and seven pots containing a mixture of 50 % of each soil type (Ref/Tec) were used, totaling 21 pots and 3 treatments (Figure 2). In each pot 50 seeds were planted, and these were periodically irrigated with water and grown in a greenhouse. The experiment lasted 110 days, starting on January 21st, 2019 (summer season) and ending on May 11th, 2019 (fall season).

2.3. Soil and plant analysis

The soil samples were dried at room temperature for analyzing the fertility and physical soil characteristics. For that, the Remaining P (Rem-P), P, K, Na, Ca, Mg, S, B, Cu, Mn, Fe, Zn, Cr, Ni, Cd, Pb, exchangeable Al (Al^{3+}), exchangeable acidity (H + Al), pH in H_2O, organic matter (OM), sum of exchangeable bases (SB), effective cation exchange capacity (t), cation exchange capacity at pH 7.0 (T), base saturation (V) and aluminum saturation (m) were estimated and soil texture was classified as coarse sand, fine sand, and the clay and silt contents was estimated.

For leaf chemical analysis, all leaves were collected at the end of the experimental period in five pots of all treatments. The leaves of each pot were placed in plastic bags, mixed and an aliquot of approximately 100 g of leaves were taken for foliar chemical analysis. These were washed with running water, followed by distilled water. The samples were dried in an oven and ground to determine the levels of N, P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B, Ni, Pb, Cd and Cr.

For the analysis of plant tissue and soil, methods based on internationally recognized protocols were used, according to the Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Corporation) - EMBRAPA (EMBRAPA, 2009, 1997).

2.4. Seed germination tests

The seed germination tests were performed on the same seeds planted for the greenhouse experiment. The seedlings count was performed until the 28th day and the final germination percentage (FGP) of structurally normal seedlings was estimated (Brasil, 2009). The following parameters and indexes were calculated: T10 (Time spent to 10 % germination), T50 (Time spent to 50 % germination), T90 (Time spent to 90 % germination) (Farooq et al., 2005) and MGT (Mean Germination Time) (Labouriau, 1983), which take into account seed germination time. GSI (Germination Speed Index) (Maguire, 1962), MGR (Mean Germination Rate) (Labouriau, 1983) and CVG (Velocity of Germination Coefficient) (Nichols and Heydecker, 1968) which take into account the germination speed. CVt (Germination Time Coefficient of variation) (Carvalho et al., 2005), VarGer (Variance of Germination Time) (Labouriau, 1983), Unc (Germination Uncertainty) (Labouriau and Valadares, 1976) and Sinc (Germination Synchrony) (Labouriau and Valadares, 1976) which take into account the homogeneity of germination and UnifG (Germination Uniformity Index) (Castan et al., 2018; Sako et al., 2001).

2.5. Biometrics parameters

2.5.1. Plant height

Plant height was measured with a manual measuring tape and was made between the soil surface and the curvature of the last expanded leaf of each pot in each treatment (Araujo et al., 2011; Silva et al., 2020). The measurements were taken weekly, from the 16th day to the 106th day of the experiment.

2.5.2. Specific leaf area determination

The specific leaf area (SLA) is the ratio between leaf area to the dry leaf mass (Fleck et al., 2016). For this, the leaves of 10 tillers cut close to the soil surface of the pots were collected at the end of the experiment. Sampling was performed in 5 pots of each treatment. Leaf area was measured using an Area Meter LI-3100C (LI-COR® Biosciences). Subsequently, the leaves were dried in an oven at 65 °C for 72 hours until they reached constant weight to determine the weight of dry plant material. The SLA was estimated per g/cm^2 (Fleck et al., 2016).

2.5.3. Tillers count and weight of fresh and dried plant material

To determine the number of tillers, all 5-pot tillers of each treatment were collected and counted at the end of the experiment. For the weight of fresh plant material and weight of dried plant material, the plants were cut close to the soil surface of the pots at the end of the experimental period and bagged. Leaves, culms and inflorescences were separated for individual weighing of each fresh material. Subsequently, the samples were dried in a forced air oven at 65 ºC until they reached constant weight to determine the weight of dried plant material of leaves, culms and inflorescences.

2.6. Photosynthetic pigment content

To estimate the levels of photosynthetic pigments, leaf squares of 0.25 cm^2 were collected. Two leaves per pot were used to collect two leaf squares per leaf and samplings were performed in 5 pots for each treatment, totaling 30 samples in total. The samples were incubated at room temperature for 24 h in 7 ml of dimethyl sulfoxide (DMSO) in flasks covered with aluminum foil. The samples absorbance was determined using the Thermo Scientific ™ Multiskan ™ GO Microplate Spectrophotometer equipment. For this, the following wavelengths were used: 665 nm (chlorophyll *a*), 649 nm (chlorophyll *b*) and 480 nm (carotenoids) and the pigment concentrations per area (µg/cm^2) were calculated according to Wellburn (1994).

2.7. Gas exchange

Gas exchange analysis: CO_2 net photosynthetic assimilation rate (A, µmol (CO_2) m^{-2} s^{1}), stomatal conductance (g_s, mol (H_2O) m^{-2} s^{-1}), transpiratory rate (E, mmol m^{-2} s^{-1}), ratio of internal and external concentration of CO_2 (Ci/Ca, µmol (CO_2) m^{-2} s^{-1}) and carboxylation efficiency estimation of the Rubisco enzyme (A/Ci, µmol (CO_2) m^{-2} s^{-1}) were performed with the aid of a Infrared gases, model LI-6400XT (LI-COR®, Lincoln, USA) equipped with blue /

red source model LI-6400-02B (LI-COR®). Gas exchange readings were taken on fully expanded second node, 5-pot leaf in all treatments. The measurements were performed between 8:00 and 12:00 a.m., with light intensity of 1500 μmol m^{-2} s^{-1}.

2.8. Anatomical and histochemical analyzes

Samples from the middle region of fully expanded second node leaves and root apexes (that have been washed) of *B. decumbens* were collected at the end of experiment and fixed in 2.5 % Glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and in Neutral Buffered Formalin - NBF (formaldehyde solution (37 %), dibasic sodium phosphate heptahydrate, monobasic sodium phosphate monohydrate and distilled water) (Clark, 1981), followed by dehydration in an ethyl series. Subsequently, the samples were included in methacrylate (Historesin, Leica Instruments®, Heidelberg, Germany), sectioned at 5 μm thick, (leaves were sectioned transversely and roots were sectioned longitudinally) in a rotating microtome of automatic advance (model RM2155, Leica Microsystems® Inc., Deerfield, USA), using glass razors. After the microtomy, part of the slides with anatomical cuts were stained with toluidine blue (O'Brien et al., 1964) and part was used for the following histochemical tests: Reaction with periodic acid - Schiff (PAS) with adequate control, for detection of total polysaccharides (McManus, 1948) and Xylidine ponceau (XP) 1 % (pH 2.5) (Vidal, 1970), for total proteins. Samples of leaves fixed in NBF were sectioned transversely in a table microtome (model LPC, Rolemberg and Bhering Comércio e Importação LTDA, Belo Horizonte, Brazil) for the following histochemical tests: Sudan Black B (O'Brien and McCully, 1981), for lipids and Ferric chloride (Johansen, 1940) for total phenolic compounds. Slides were mounted on synthetic resin (Permount®, Fisher Scientific®, Pittsburgh, USA) and in water. The observations and the recording of the images were made in a photomicroscope (model AX70TRF, Olympus Optical®, Tokyo, Japan) equipped with the image capture system (model AxioVision Release® 4.8.1, Carl Zeiss Vision® GmbH, Germany).

2.9. Plant root system architecture and mycorrhizal colonization analysis

The root system architecture was determined with roots that were sampled, washed and photographed with digital camera at the end of the experimental period.

For the mycorrhizal colonization analysis, part of the *B. decumbens* roots collected for anatomical analysis were used. From these roots, about 1 g of fine roots of approximately 2 mm were collected and broken up into 1 to 2 cm pieces. The samples were cleared with a 10 % KOH solution (w/v) for 24 hours, then the KOH solution was drained, and the roots were washed. The samples were placed in 2 % HCl (v/v) solution for 10 minutes, after this procedure, the HCl was drained from the samples and these were placed in Trypan blue in 0.05 % lactoglycerol (w/v) and heated to approximately 70ºC for 30 minutes. After reaching room temperature, the samples were transferred to a lactoglycerol solution (Phillips and Hayman, 1970). Roots were mounted on slides for observation in the same photomicroscope of anatomical analyzes.

2.10. Statistical analysis

The statistical program RStudio version 1.2.1335 was used in all analyzes (RStudio, 2019). In greenhouse experiment, the experimental unit was a pot containing *B. decumbens* plants. To assess the effect of soil type on *B. decumbens*, we applied Analysis of variance (ANOVA) from package "easyanova" (Arnhold, 2013) followed by the post-hoc Tukey test at 5 % probability for data with normal distribution. For data whose distribution was not normal we used Kruskal-Wallis test from the package "agricolae" (Mendiburu, 2019), followed by the post-hoc Nemenyi test. Data normality was analyzed by Shapiro-Wilk test, along with exploratory qqPlot analyzes and homogeneity of variances were tested by Levene's test from package "car" (Fox and Weisberg, 2019). Soil attributes data were summarized by principal component analysis (PCA) from package 'FactoMineR' (Lê et al., 2008), for this, data were standardized and Pearson correlation was calculated. Seed germination tests were calculated using the SeedCalc package (Silva et al., 2018). In analyzing the effect of time on plant height it was observed that the data followed a sigmoid pattern, and therefore we perform a nonlinear mixed regression with vase as a random effect, using the package "nlme" (Pinheiro et al., 2019). We got the initial parameters of asymptote (asym), inflection point of x-value (xmid), and angular coefficient of the tangent at point of inflection (scal) using the function “SSlogis”, from package ‘stats’ (R Core Team, 2020).

3. RESULTS

3.1. Soil fertility analysis

It was observed that Ref, Ref/Tec and Tec are acidic soils with pH ranging from 4.57 - 5.09 and have low fertility (low P, Ca, Mg, B, sum of exchangeable bases (SB), effective CEC (t) and base saturation (V)) (Table 1). One factor that differentiates Ref and Ref/Tec from Tec is the low content of organic matter (OM) and Zn in Tec. Ref has more available Al^{3+} and the exchangeable acidity (H + Al) is high in this soil, along with Cu and Zn levels.

The soil fertility analyzes carried out before the experiment (B.E.) showed that zinc content in Ref was 84.7 % higher than in Tec and 52.9 % higher than in Ref/Tec, whereas Ref/Tec contained 67.5 % more Zn than Tec. The same was observed for OM content in soils before the experiment. The OM content in Ref was 72.2 % higher than in Tec and 30.8 % higher than in Ref/Tec, whereas Ref/Tec presented 59.8 % more OM than Tec (Table 1).

For available iron and manganese, it was observed that the three soil types presented high levels of these elements but the iron content in Ref was about nine times higher than in Tec. The CEC at pH 7.0 (T) and the aluminum saturation (m) in Ref were higher than in Ref/Tec and Tec and the S content in all soils was considered adequate. For Cr, Ni, Cd and Pb content was observer low values of this elements in the three soil types (Table 1). Regarding the soil physical attributes (Table 2), it was observed that Ref has more coarse sand. Clay contents were higher in Ref/Tec and Tec and in Tec, more fine sand and silt were observed. The three soil types are clayey (type 3).

The principal component analysis (PCA) of soil attributes before the experiment showed that the two principal components explained 86.4 % of the total dataset variability (Figure 3A). The first axis was positively correlated with Cu, T, H+Al, OM, Pb, Coarse sand, Zn, Fe, t, Rem-P, Al^{3+}, Ni, B and Cr and was negatively correlated with Fine sand, pH, Clay, Silt and S. The K content was highly correlated with both PCA1 and PCA2. The second axis was positively correlated with V, Ca, SB, Mg, P and Mn and was negatively correlated with m. The PCA of soil attributes in the soil collected after the experiment showed that the two principal components explained 72.7 % of the total dataset variability (Figure 3B). The first axis was positively correlated with T, H+Al, N, Cu, OM, Fe, Pb, Al^{3+}, m, t, Zn, Ni, Rem-P and Mg and was negatively correlated with V, pH and Mn. The Mg content was highly correlated with both PCA1 and PCA2. The second axis was positively correlated with SB, Ca, K, S, V and B.

3.2. Foliar chemical analysis

In the chemical analysis of leaves, low levels of N, P, and Cu were observed in plants cultivated in all tested soils (Table 3). Adequate levels of K, Ca, Mg, S, Mn, B and Al were observed in the three treatments. It is worth noting that although Mg levels are adequate, it is possible to observe that the content of this element in leaves of plants grown in Ref was 1.7 times higher than in plants grown in Ref/Tec and Tec (Kruskal-Wallis p-value < 0.001). For leaf Fe levels, it was possible to observe adequate levels only in plants grown in Ref. The plants grown in Ref/Tec and Tec showed levels above that recommended. For the levels of Mn and leaf B, it is possible to observe that the plants grown in Tec showed higher leaf concentrations of these elements compared to the plants grown in Ref and Ref/Tec but the leaf levels of these two elements in all treatments are within the range of adequate mineral sufficiency for *B. decumbens*. For Zn, adequate levels of this element were found only in the leaves of plants grown in Ref, with significant low levels of this element in Ref/Tec and Tec (p-value < 0.001). No significant leaf levels of Ni, Pb, Cd and Cr were detected in *B. decumbens* (Table 3).

The PCA of foliar chemical analysis showed that the two principal components explained 55.2 % of total dataset variability (Figure 4). The first axis was positively correlated with Mg, Cu, Zn, N, S, P and was negatively correlated with Mn, Fe and Al. The second axis was positively correlated with B, Ca, K, Cu, Fe and Mn.

3.3. Germination tests

The seed germination tests showed statistical differences only in T10, MGT, MGR and CVG (Table 4), the ANOVA was statistically significant between treatments (p-value < 0.05). At T10 of seeds planted in Tec was observed a value 33.3 % higher than in Ref/Tec and the MGT in Tec was more than 20 % higher than Ref and Ref/Tec. However, the MGR and CVG in Ref and Ref/Tec showed that seeds germination was more than 20 % faster than in Tec (Table 4). For FGP, GSI, T50, T90, UnifG, VarGer, CVt, Sinc and Unc it was observed no difference (Table 4).

3.4. Biometrics parameters

3.4.1. Plant height

For *B. decumbens* height, it was observed that plants grown in Ref had better performance compared to plants grown in Tec and plants grown in Ref/Tec had a medium development (Figure 5). At the end of the experiment plants grown in Ref presented inflorescences, showing that these plants were in different phenological stage than those grown in Ref/Tec and Tec (Figure 6C).

The asym values by the nonlinear model (nlm) show the predicted maximum height of *B. decumbens* cultivated in all soil types (Table 5). It is possible to observe that the height of plants grown in Ref were approximately 40 % higher than plants grown in Tec and approximately 22 % higher than plants grown in Ref/Tec. The height values of plants grown in Ref/Tec were approximately 20 % higher than in plants grown in Tec. In addition, the height of *B. decumbens* in Tec decrease before the height of plants grown in Ref and Ref/Tec, as can be seen by the inflection points (Table 5).

3.4.2. Specific leaf area

Regarding the specific leaf area (SLA), it was observed that plants grown in Ref had an 8.4 % higher SLA than plants grown in Ref/Tec and 5 % higher than plants grown in Tec (Figure 7A). Kruskal-Wallis statistical test did not show differences between Ref/Tec and Tec treatments, but in comparison between Ref/Tec and Tec with Ref, differences were observed (p-value = 0.001).

3.4.3. Number of tillers

Plants grown in Ref developed almost twice tillers than plants grown in Tec (p-value = 0.001; Figure 7B).

3.4.4. Weight of fresh and dry plant material

For the weight of fresh and dry plant material it was observed that plants cultivated in Ref presented the highest values, plants cultivated in Ref/Tec presented median values and plants cultivated in Tec presented the lowest values (Figure 8). Kruskal-Wallis statistical test showed significant statistical differences between treatments (p-value = 0.001). It is possible to evidence that plants grown in Ref reached the reproductive phase with the development of inflorescences, a fact that was not observed in plants cultivated in Ref/Tec and Tec. A clear example of the best performance in the production of plant material from plants grown in Ref was the weight of leaves and culms. It was observed that in these plants the weight of fresh and dry plant material was higher than in plants grown in Tec. Plants grown in Ref had leaf weight (fresh and dry) seven times greater than plants grown in Tec and had culm weight (fresh and dry) more than ten times greater than plants grown in Tec.

3.5. Photosynthetic pigment content

It is possible to observe that the levels of chlorophyll *a* and *b* in plants grown in Ref were higher than the levels found in plants grown in Ref/Tec and Tec (Figure 9A – 9B). The contents of chlorophyll *a* were significantly different from each other (p-value < 0.01) and shows that the content of this pigment in plants grown in Ref was 47.6 % higher than in plants grown in Tec and 34.9 % higher than in plants grown in Ref/Tec (Figure 9A). The chlorophyll *a* content in plants grown in Ref/Tec was 19.5 % higher than that of plants grown in Tec (Figure 9A). For chlorophyll *b* it is possible to observe that the content of this pigment in plants grown in Ref was significantly different from the values found in Ref/Tec and Tec (p-value < 0.01), with the content of this pigment in plants grown in Ref approximately 50 % higher than in plants grown in Ref/Tec and Tec (Figure 9B). For the levels of carotenoids, the values found in plants grown in Ref and Ref/Tec were statistically equal to each other and different from plants grown in Tec (p-value < 0.01), and the content of this pigment in the plants grown in Ref and Ref/Tec was approximately 30 % higher than in plants grown in Tec (Figure 9C). The ratio between chlorophyll *a* and *b* did not differ statistically between the three types of treatments (p-value > 0.05) (Figure 9D).

3.6. Gas exchange

The results of gas exchange showed that the net photosynthetic assimilation rate (A) of plants grown in Ref was more than 50 % higher than in plants grown in Tec (Figure 10A). A similar pattern was observed for stomatal conductance (g_s) and transpiratory rate (E) (Figure 10B - 10C). It was registered in plants grown in Ref/Tec greater A, g_s and E than in plants grown in Tec. The ratio of internal and external concentration of CO_2 (Ci/Ca) of plants grown in Tec was approximately 20 % higher than values found in plants grown in Ref (Figure 10D). The estimation of the carboxylation efficiency of the rubisco enzyme (A/Ci) of plants grown in Ref was more than 30 % higher than that of plants grown in Ref/Tec and more than 60 % higher than that of plants grown in Tec (Figure 10E). Kruskal-Wallis statistical test showed significant statistical differences between the three treatments for all gas exchange parameters analyzed (p-value < 0.01).

3.7. Leaf anatomical characterization and histochemical analysis

Anatomical analyzes of *B. decumbens* leaves grown in Ref, Ref/Tec and Tec revealed the typical structure of leaves that show the C_4 photosynthetic metabolism, with the ordered arrangement of mesophyll cells around of the bundle sheath. The mesophyll and bundle sheath form concentric layers around the vascular bundle (Kranz anatomy) (Figure 11A – 11D). Anatomical differences were observed in the shape of plant cells grown in Ref, Ref/Tec and Tec. The cells of mesophyll and bundle sheath of plants grown in Ref, are rounded, presenting a healthy aspect (Figure 11A). The cells of plants grown in Ref/Tec and Tec have a flaccid appearance, with deformed cells and exhibiting sinuous and angular walls (Figure 11B - 11C).

In the histochemical analysis, the test with periodic acid - Schiff (PAS) the total polysaccharides are stained with magenta and it's possible to observe the centrifugal orientation of chloroplasts with accumulation of transitory starch in the vascular bundle sheath and mesophyll cells that reacted positively to this test (Figure 12A - 12C). The difference between leaves of plants grown in Ref, Ref/Tec and Tec is evident. Chloroplasts with transitory starch accumulation are more evident in vascular bundle sheath cells of plants grown in Ref than in bundle sheath cells of plants grown in Ref/Tec and Tec (Figure 12A - 12C).

For Xylidine ponceau test, orange-stained proteins were observed in the same position as the chloroplasts, in the mesophyll and in stomata (Figure 12D - 12F). The presence of proteins in bundle sheaths chloroplasts, mesophyll and stomata was more evident in plants grown in Ref than in other treatments (Figure 12D). It is possible to observe that the presence of proteins in bundle sheath chloroplasts of plants grown in Ref/Tec was more evident than in plants grown in Tec (Figure 12E - 12F).

For Sudan Black B test, blue to black stained lipids were observed in bundle sheath chloroplasts and mesophyll (Figure 12G - 12I). It is possible to observe that lipids were more evident in bundle sheath chloroplasts of plants grown in Ref than in bundle sheath cells of plants grown in Ref/Tec and Tec (Figure 12H - 12I).

For the ferric chloride test, it was observed that the phenolic compounds were stained brown to black in sclerenchyma cell walls and in chloroplasts of bundle sheath cells (Figure 12K - 12L). No differences were observed for this test between treatments.

3.8. Plant root system architecture, anatomy and mycorrhizal colonization

The root system architecture showed that there was a mischaracterization in fasciculate structure of plant roots grown in Tec (Figure 13), in addition, these roots had fewer branches and visually presented less thickness than roots of plants grown in Ref and Ref/Tec. The color was a differential factor between roots. The roots of plants grown in Tec have a darker color and roots of plants grown in Ref and Ref/Tec showed lighter colors and similar root architectures (Figure 13).

In the anatomical analyzes of roots and the root tissue colonization by mycorrhizae, it was possible to observe in plants grown in Ref, mycorrhizal association evidenced by the large number of septate hyphae and microsclerotia of the dark septate endophytic fungi (DSEF) (Figure 14A - 14F). Nematodes were observed in these roots (not shown).

In roots of plants grown in Ref/Tec (Figure 14G - 14K), mycorrhizal associations were observed with the presence arbuscular mycorrhizal fungi (AMFs) within cells, septate fungi hyphae, DSEF microsclerotia and the presence of nematodes in the process of elimination by cell digestion. It is possible to observe cells with cellular content for the nematode degradation (Figure 14H and Figure 14K).

In plants grown in Tec (Figure 14L - 14N), roots with thin cell walls and presence of nematodes were observed, some of which are being eliminated by cell digestion activities. In Figure 14N it is possible to see a cord of cells with cellular content for digestion and remains of the parasite. No mycorrhizal associations were observed in the roots of plants grown in this soil.

4. DISCUSSION

Iron mining tailings Technosols from Fundão ruptured dam are acids, extremely poor in nutrients and organic matter and some studies show that mining tailings have low fertility as well as the mining tailings dumped in Rio Doce basin (Forján et al., 2019; Juwarkar et al., 2009; Wang et al., 2017; Young et al., 2015) and can form a barrier of hundreds of years for the colonization and establishment of plants in this soils (Mendez and Maier, 2008).

The fact that *B. decumbens* is one of the most common plants occurring on IMTT shows that grasses tolerate and adapt to environments whose edaphic conditions are unfavorable (Shu et al., 2005) but the IMTT launched in Rio Doce basin is so inhospitable for plants that even *B. decumbens* species that tolerates acidic, low-fertility soils with high concentrations of Al (Crispim et al., 2003; Rao et al., 1996) has had its development impaired. Other plants that do not have the adaptive capacity of *B. decumbens* would have difficulty establishing in these environments.

Even with two recent catastrophic disasters involving rupture of mining dams in the country (Fundão dam rupture in Mariana, Minas Gerais in 2015 and rupture of the B1 dam in Córrego do Feijão mine complex in Brumadinho, Minas Gerais in 2019), the Sistema Brasileiro de Classificação de Solos (Brazilian Soil Classification System) - SiBCS 2018 does not have a classification for mining tailings (Ruiz et al., 2020). Tec is classified as a Technosol due to its anthropogenic origin since this type of soil is characterized by the presence of artefacts and technic hard material, originated from the modifications that humans make to the soil (IUSS Working Group WRB, 2015; Schad, 2018).

Instead of contamination, the Fundão dam rupture spread thousands of kilometers of a degraded, depleted and infertile soil, which can be very damaging, as changes in soil quality can be problematic for the provision of ecosystem services for nutrient cycling, water infiltration and retention in soil, native and agricultural plant production, causing alterations in environmental quality (FAO and ITPS, 2015; Schröder et al., 2016).

Regarding the Argisol used as reference soil (Ref) in the experiment, the low fertility of this soil was observed but with higher levels of organic matter and zinc than in the Technosol with a thick deposition of iron mining tailings (Tec) and in the mixture of the two soil types (Ref/Tec). According to the Sistema Brasileiro de Classificação de Solos (Brazilian Soil Classification System) - SiBCS, 2018, Argisols are the second most abundant soil type in the country falling behind only oxisols, they are present in 24 % of the territorial surface, being found in all Brazilian regions and are characterized due to the high degree of weathering, depth, acidity, low natural fertility and with saturation of aluminum in some cases.

The organic matter content in soils considered ideal is in the range between 4.01 and 7.0 dag/kg and above of this value (Neto and Costa, 2012). In Ref and in Ref/Tec the content found was considered average, between 2.01 - 4.0 dag/kg (Neto and Costa, 2012), but it was responsible for the better development of the plants cultivated in these soils in comparison with plants grown in Tec. The mixture of Argisol with iron mining tailings Technosols (Ref/Tec) in equal proportions proved to be a method that provides a significant improvement in organic matter contents, improving the tailings quality.

Low levels of organic matter were observed in Tec (value between 0.71 and 2.0 dag/kg) (Neto and Costa, 2012) and this finding is worrisome when we talk about the ability to recover environments affected by disturbances like what happened in Rio Doce basin, since the organic matter in soils is important for processes to recover soil quality (Nieder and Benbi, 2008).

Organic matter provides energy that is fundamental to the biological processes of living beings in the soil, being a fundamental attribute for environmental quality since it increases the cation exchange capacity of soils, improves structural quality and reduces soil erosion, it is essential for nutrient cycling when it is mineralized and nutrients become available as well as being important for the nutrition of the soil microbiota (Bot and Benites, 2005; Nieder and Benbi, 2008).

Due the low organic matter content in the iron mining tailings Technosols spilled in Rio Doce, Machado et al. (2019) and Figueiredo et al. (2020) recommend the possibility of using this soil in civil construction as a non-structural cementitious product and for making blocks.

Regarding seed germination, it was observed that the seeds planted in Tec took longer to germinate, a fact due to the low content of organic matter in this soil, since it is responsible for water retention in soil that will be absorbed by the seeds in the process known as imbibition, activating several metabolic processes indispensable for the germination process (Bewley et al., 2013; Mondo et al., 2012; Önemli, 2011).

Zinc is an essential micronutrient for plants which available form for absorption by plants is the Zn^{2+} cation and the concentration of this element in soil varies between different types of soil around the world (Barker and Pilbeam, 2007; Broadley et al., 2012; Kabata-Pendias, 2011). According to Neto and Costa (2012), adequate soil zinc values are those greater than 1.5 mg/dm^3 (as the values observed in the Ref and in the Ref/Tec) and values considered low are those less than 1 mg/dm^3 (as the values observed in Tec).

For *B. decumbens*, the appropriate zinc leaf concentration range is between 20 - 50 mg/kg (Werner et al., 1996) and values below this concentration can promote the deficiency symptoms of this micronutrient in plants as seen more drastically in plants grown in Tec.

Zinc deficiency in plants can interfere with several important biological processes, because this essential micronutrient is part of the structure of ribosomes and participate in the protein synthesis process, besides that, more than 300 proteins require the participation of zinc (Broadley et al., 2012; McCall et al., 2000; Storey, 2006) like zinc metalloproteins related with DNA replication and transcription and genetic regulation (Storey, 2006).

Zinc is extremely important in processes of detoxification of reactive oxygen species (ROS) since it is part of the most abundant superoxide dismutase enzyme in plants, CuZn superoxide dismutase (Broadley et al., 2012) and evidence suggests the participation of this micronutrient in the expression processes of genes that encode the enzymes ascorbate peroxidase and glutathione reductase, which act to combat oxidative stress (Cakmak, 2000). Zinc deficiency can affect leaf growth and plant growth, as this micronutrient is associated with the metabolic route of auxin (Indole-3-acetic acid - IAA) (Martins et al., 2015).

Regarding the leaf iron content, the adequate levels of this micronutrient in *B. decumbens* are between 50 - 250 mg/kg (Werner et al., 1996). The leaf iron content of plants grown in Ref proved to be adequate, whereas in plants grown in Ref/Tec and Tec levels were observed above what is recommended for the species. Critical levels of iron that cause toxicity to plants are above 500 mg/kg as found in plants grown in Tec (Barker and Pilbeam, 2007; Broadley et al., 2012).

The iron excess in plants causes toxicity through its participation in the Fenton reaction whose product is the reactive oxygen species (ROS) that cause oxidative damage in biological membranes, DNA and proteins (Briat et al., 2010; Nikolic and Pavlovic, 2018; Schieber and

Chandel, 2014). About 90 % of the leaf iron is in chloroplasts and the ROS produced cause losses in photosynthetic activity (Jeong et al., 2008; Pereira et al., 2013). Although we observed values that demonstrate leaf iron toxicity in plants grown in Tec, the literature establishes that the leaf iron levels found in the present study do not pose a risk for animal nutrition whose maximum tolerable iron limit for cattle nutrition is 1000 mg/kg (National Research Council, 1980).

The fact that plants grown in Tec had high levels of leaf iron can be justified by the low leaf renewal and lesser leaf development observed in plants grown in that soil, even with the randomization performed in the selection of samples for the foliar chemical analyzes of the leaves of plants grown in Tec they were older than the leaves of plants grown in other treatments that showed greater leaf renewal.

Zinc deficiency and iron toxicity in plants can interfere with the production of photosynthetic pigments. In zinc deficient plants is common the occurrence of leaf chlorosis due to the decrease in the production of chlorophyll *a* and *b* (Mukhopadhyay et al., 2013; Storey, 2006) and the oxidative stress induced by iron toxicity can damage chlorophyll biosynthesis reactions (Aarti et al., 2006).

The photosynthetic impairment observed in *B. decumbens* grown in Tec occurred due to the dependence of zinc on C4 photosynthetic metabolism of grasses and by the iron toxicity. Zinc is part of the structure of the carbonic anhydrase (CA) enzyme located in mesophyll cells whose function is the conversion of CO_2 into bicarbonate (HCO_3^-) that will be combined with phosphoenolpyruvate in a reaction catalyzed by the enzyme phosphoenolpyruvate carboxylase (PEPC) to form oxalacetate and subsequently malate or aspartate (depends of the plant species), a 4-carbon acid that flows into the bundle sheaths where it will be decarboxylated by the NAD-malic enzyme, releasing CO_2 that will enter us chloroplasts and will be fixed by Ribulose bisphosphate carboxylase/oxygenase - RUBISCO in the Calvin-Benson cycle (Broadley et al., 2012; Taiz et al., 2014). Depending on the species and the degree of zinc deficiency, photosynthetic assimilation is reduced by between 50 and 70 % and iron toxicity can reduce the photosynthetic rate by 40 % (Alloway, 2008; Kampfenkel et al., 1995).

Lower values of stomatal conductance and lower rates of transpiration were observed in plants cultivated in Tec, which indicates a greater number of closed stomata in plants grown in this soil compared to plants grown in other soil types, being a mechanism that prevents excessive absorption of iron by plants (Dufey et al., 2009; Pereira et al., 2013). Zinc deficiency is also related to these symptoms, as this fact contribute to the reduction of stomatal opening as zinc can play an important role in the absorption of potassium (K^+) by stomatal guard cells, in addition to contributing to the maintenance of the integrity of the plasma membrane (Broadley et al., 2012; Khan et al., 2004; Sharma et al., 1995). The deficiency of this micronutrient also causes a decrease in transpiratory rates and interferes with the water balance of the plants, compromising the growth and responses of the plants to water stress (Khan et al., 2004).

In some species of algae and plants, the chemical ethoxyzolamide acts by inhibiting carbonic anhydrase, which tends to decrease the amount of CO_2 in the active site of Rubisco, favoring the reaction of this enzyme with O_2, stimulating photorespiration (Igamberdiev and Roussel, 2012; Riazunnisa et al., 2006; Shiraiwa and Schmid, 1986). Iron toxicity can harm the electron transport chain, inhibiting the Calvin cycle, decreasing Rubisco's carboxylation rate and stimulating photorespiration (Kampfenkel et al., 1995). In this work the zinc deficiency and iron toxicity interferes with photosynthetic metabolism indicating that *B. decumbens* under the stresses imposed by the iron mining tailings Technosols were not able to metabolize the carbon, in addition, this interference in the carbon concentrating mechanism in the active site of Rubisco in *B. decumbens* tend to increase the concentration of O_2 in the active site of Rubisco, favoring photorespiration and partial loss of fixed carbon, being responsible for the lesser development of the leaf area, tiller development and biomass production.

Few anatomical alterations were observed in plants grown in Ref/Tec and Tec, while histochemical tests showed that in the vascular bundle sheath, chloroplasts with accumulation of transitory starch, proteins and lipids in leaves of *B. decumbens* grown in Ref/Tec e Tec, were less evident, previously justified by the impairment of carbohydrate metabolism in plants, caused by zinc deficiency and iron toxicity.

It is estimated that 70 % of the leaf protein is located in the chloroplasts, being largely represented by Rubisco and the proteins that form the thylakoid membranes light-harvesting complex (Buchanan-Wollaston, 2003) and a low protein accumulation was observed in Tec-grown plants. Rubisco is probably one of the most abundant proteins on earth along with actin (Ellis, 1979; Raven, 2013) and its decrease in an ecosystem can have serious consequences for the primary productivity of an ecosystem like the Rio Doce basin.

Chloroplasts and endoplasmic reticulum are responsible for the synthesis of fatty acids in plants (Boudière et al., 2014; Hölzl and Dörmann, 2019; Rawsthorne, 2002). Membrane glycerolipids are important in the stabilization of PSII photosynthetic proteins (Boudière et al., 2014; Hölzl and Dörmann, 2019) and plants grown in Ref/Tec and Tec had their carbohydrate metabolism impaired by alterations in chloroplasts evidenced in the histochemical tests by alterations in the accumulation of lipids, starch and proteins. The role of lipids in plants is not only linked to the constitution of membranes but also to the signaling role of these substances in response to environmental stresses by activating defense mechanisms of plants and acting to alleviate the intensity of the stressor (Okazaki and Saito, 2014). Epicuticular wax, cutin and suberin are derived from lipids and make up an important a physical barrier protecting plants from environmental stresses decreasing water loss to the environment and controlling the flow of gases entering and leaving plants (Okazaki and Saito, 2014; Pollard et al., 2008) thus, plants grown in Tec would be less adept at responding to lipid-mediated environmental stresses.

According to Rao et al. (1996), one of the adaptive characteristics of *B. decumbens* to acidic and nutrient-poor soils is the maintenance of root growth at the expense of the aerial part growth and the development of several root branches for the Ca requirement. The roots of plants grown in Tec showed just the opposite of that, showing how inhospitable for plants can be these iron mining tailings Technosols.

Dark septate endophytic fungi (DSEF) are part of a large and widely distributed polyphyletic group of fungi that maintain intracellular association with plant roots and whose ecological role is not yet fully understood (He et al., 2019; Jumpponen and Trappe, 1998; Piercey et al., 2004). According to Jumpponen and Trappe 1998, it is estimated that there is the occurrence of DSEF in about 600 plant species, 320 genera and 100 families.

Morphologically these fungi are characterized by the presence of septate hyphae of black pigmentation thanks to the presence of melanin in these structures (Jumpponen and Trappe, 1998; Piercey et al., 2004) and can act in helping to absorb soil macro-and micronutrients, producing phytohormones gibberellins (GAs), indoleacetic acid (IAA) and volatile organic compounds that can stimulate plant growth (Berthelot et al., 2016; He et al., 2019; Vergara et al., 2019; Waqas et al., 2012). DSEF can act facilitating the growth of plants in stressful places, favoring the tolerance of plants to these environments and protecting the roots against pathogens (Reininger and Sieber, 2012; Waqas et al., 2012).

Arbuscular mycorrhizal fungi (AMF) belong to the phylum Glomeromycota and are characterized by the filamentous structures called arbuscules that form within root cells (Schüβler et al., 2001; Winagraski et al., 2019). These endomycorrhizal fungi establish symbiotic relationships with plants, benefiting from the carbohydrates produced by plant photosynthesis and providing the plant with nutrients absorbed from soil (Winagraski et al., 2019). According to estimates, between 80 and 90 % of terrestrial plants are colonized by AMF (Schüβler et al., 2001; Smith and Read, 2008).

AMFs offer important ecological services, being considered natural biofertilizers since they promote an increase in absorption of mineral nutrients and water, with a consequent improvement in plant productivity, help in tolerating environmental stresses and in protecting against pathogens (Begum et al., 2019; Bencherif et al., 2019; Berruti et al., 2016; Chen et al., 2018; Ingraffia et al., 2019; Liao et al., 2018; Rouphael et al., 2015).

The DSEF and AMF can degrade organic matter as well as P and N compounds in the soil, helping plant growth (Hodge et al., 2001; Hodge and Fitter, 2010; Surono and Narisawa, 2017). In Tec, the activity of these fungi was not observed in our analyzes due to the low content of organic matter in this soil, which was reflected in the low plant development and in the lesser capacity to deal with the attack of parasites. In a study conducted in the Rio Doce basin, Prado et al. (2019) showed that the process of revegetation of areas affected by iron mining tailings Technosols tends to increase the diversity of arbuscular mycorrhizal fungi (AMF) in these soils, being a promising tool for future recovery programs in affected areas by this impact.

5. CONCLUSION

Our work demonstrated how the iron mining tailings Technosols from Fundão iron ore dam collapse is poor in zinc, organic matter and causes iron toxicity in *B. decumbens*, compromising the ecophysiology, morphoanatomy and symbiotic relationships with mycorrhizal fungi of a species well adapted to stressful environments but that resisted the inhospitable conditions offered by iron mining tailings Technosols precisely because it has a high adaptive capacity. The mixture of unaffected soil by iron mining tailings with Technosol with a thick deposition of iron mining tailings (Ref/Tec) promoted an improvement in the quality of this tailings, being recommended as an suitable approach to be used in areas were Technosols with iron mining tailings are formed, representing an important step towards a rapid recovery of Technosols, for agricultural, pasture production and for environmental restoration.

Acknowledgments The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico - National Council for Scientific and Technological Development (CNPq), Brazil, for the Research Productivity Scholarships granted to L.C. Silva 309308/2018-6. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

6. REFERENCES

Aarti, P.D., Tanaka, R., Tanaka, A., 2006. Effects of oxidative stress on chlorophyll biosynthesis in cucumber (Cucumis sativus) cotyledons. Physiol. Plant. 128, 186–197. https://doi.org/10.1111/j.1399-3054.2006.00720.x

Aires, U.R.V., Santos, B.S.M., Coelho, C.D., da Silva, D.D., Calijuri, M.L., 2018. Changes in land use and land cover as a result of the failure of a mining tailings dam in Mariana, MG, Brazil. Land use policy 70, 63–70. https://doi.org/10.1016/j.landusepol.2017.10.026

Alloway, B.J., 2008. Zinc in soils and crop nutrition, 2nd ed. International Zinc Association - IZA and International Fertilizer Industry Association - IFA, Brussels, Belgium and Paris, France.

Almeida, C.A., Oliveira, A.F. de, Pacheco, A.A., Lopes, R.P., Neves, A.A., Lopes Ribeiro de Queiroz, M.E., 2018. Characterization and evaluation of sorption potential of the iron mine waste after Samarco dam disaster in Doce River basin – Brazil. Chemosphere 209, 411–420. https://doi.org/10.1016/J.CHEMOSPHERE.2018.06.071

Alvim, M.J., Botrel, M. de A., Xavier, D.F., 2002. As principais espécies de Brachiaria utilizadas no País. Comun. Técnico 22.

Araujo, L.C., Santos, P.M., Mendonça, F.C., Mourão, G.B., 2011. Establishment of Brachiaria brizantha cv. Marandu, under levels of soil water availability in stages of growth of the plants. Rev. Bras. Zootec. 40, 1405–1411. https://doi.org/10.1590/S1516-35982011000700002

Arnhold, E., 2013. Pacote em ambiente R para análise de variância e análises complementares. Brazilian J. Vet. Res. Anim. Sci. 50, 488. https://doi.org/10.11606/issn.1678-4456.v50i6p488-492

Barker, A. V., Pilbeam, D.J., 2007. Handbook of plant nutrition. CRC Taylor & Francis, Boca Raton. https://doi.org/10.1093/treephys/tpq048

Begum, N., Qin, C., Ahanger, M.A., Raza, S., Khan, M.I., Ashraf, M., Ahmed, N., Zhang, L., 2019. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.01068

Bencherif, K., Djaballah, Z., Brahimi, F., Boutekrabt, A., Dalpè, Y., Lounès-Hadj Sahraoui, A., 2019. Arbuscular mycorrhizal fungi affect total phenolic content and antimicrobial activity of Tamarix gallica in natural semi-arid Algerian areas. South African J. Bot. 125, 39–45. https://doi.org/10.1016/j.sajb.2019.06.024

Berruti, A., Lumini, E., Balestrini, R., Bianciotto, V., 2016. Arbuscular Mycorrhizal Fungi as Natural Biofertilizers: Let's Benefit from Past Successes. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01559

Berthelot, C., Leyval, C., Foulon, J., Chalot, M., Blaudez, D., 2016. Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. FEMS Microbiol. Ecol. 92, fiw144. https://doi.org/10.1093/femsec/fiw144

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013. Seeds Physiology of Development, Germination and Dormancy. Springer New York, New York, NY. https://doi.org/10.1007/978-1-4614-4693-4

Borba, R.P., Figueiredo, B.R., Matschullat, J., 2002. Geochemical distribution of arsenic in waters, sediments and weathered gold mineralized rocks from Iron Quadrangle, Brazil. Environ. Geol. 44, 39–52. https://doi.org/10.1007/s00254-002-0733-6

Bot, A., Benites, J., 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production. Food and Agriculture Organization of the United Nations (FAO), Rome.

Boudière, L., Michaud, M., Petroutsos, D., Rébeillé, F., Falconet, D., Bastien, O., Roy, S., Finazzi, G., Rolland, N., Jouhet, J., Block, M.A., Maréchal, E., 2014. Glycerolipids in photosynthesis: Composition, synthesis and trafficking. Biochim. Biophys. Acta - Bioenerg. 1837, 470–480. https://doi.org/10.1016/j.bbabio.2013.09.007

Brasil, 2009. Regras para análise de sementes, 1ª edição. ed. Ministério da Agricultura, Pecuária e Abastecimento, Brasília.

Briat, J.-F., Ravet, K., Arnaud, N., Duc, C., Boucherez, J., Touraine, B., Cellier, F., Gaymard, F., 2010. New insights into ferritin synthesis and function highlight a link between iron homeostasis and oxidative stress in plants. Ann. Bot. 105, 811–822. https://doi.org/10.1093/aob/mcp128

Broadley, M., Brown, P., Cakmak, S., Rengel, Z., Zhao, F., 2012. Function of Nutrients: Micronutrients, 3rd ed, Marschner's Mineral Nutrition of Higher Plants. Elsevier, London. https://doi.org/10.1016/C2009-0-63043-9

Buchanan-Wollaston, V., 2003. POSTHARVEST PHYSIOLOGY | Senescence, Leaves, in: Encyclopedia of Applied Plant Sciences. Elsevier, pp. 808–816. https://doi.org/10.1016/B0-12-227050-9/00078-8

Cakmak, I., 2000. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytol. 146, 185–205. https://doi.org/10.1046/j.1469-8137.2000.00630.x

Carvalho, M.P., Santana, D.G., Ranal, M.A., 2005. Emergência de plântulas de Anacardium humile A. St.-Hil. (Anacardiaceae) avaliada por meio de amostras pequenas. Rev. Bras. Botânica 28, 627–633. https://doi.org/10.1590/S0100-84042005000300018

Castan, D.O.C., Gomes-Junior, F.G., Marcos-Filho, J., 2018. Vigor-S, a new system for evaluating the physiological potential of maize seeds. Sci. Agric. 75, 167–172. https://doi.org/10.1590/1678-992x-2016-0401

Chen, M., Arato, M., Borghi, L., Nouri, E., Reinhardt, D., 2018. Beneficial Services of Arbuscular Mycorrhizal Fungi – From Ecology to Application. Front. Plant Sci. 9, 16. https://doi.org/10.3389/fpls.2018.01270

Clark, G., 1981. Staining procedures, 4th ed. Williams & Wilkins, Baltimore.

Cordeiro, M.C., Garcia, G.D., Rocha, A.M., Tschoeke, D.A., Campeão, M.E., Appolinario, L.R., Soares, A.C., Leomil, L., Froes, A., Bahiense, L., Rezende, C.E., de Almeida, M.G., Rangel, T.P., De Oliveira, B.C.V., de Almeida, D.Q.R., Thompson, M.C., Thompson, C.C., Thompson, F.L., 2019. Insights on the freshwater microbiomes metabolic changes associated with the world's largest mining disaster. Sci. Total Environ. 654, 1209–1217. https://doi.org/10.1016/j.scitotenv.2018.11.112

Crispim, S.M.A., Barioni Júnior, W., Branco, O.D., 2003. Valor Nutritivo de Brachiaria decumbens e Brachiaria humidicola no Pantanal Sul-Mato- Grossense. Embrapa Pantanal 1–4.

Davila, R.B., Fontes, M.P.F., Pacheco, A.A., Ferreira, M.S., 2020. Heavy metals in iron ore tailings and floodplain soils affected by the Samarco dam collapse in Brazil. Sci. Total Environ. 709, 136–151. https://doi.org/10.1016/j.scitotenv.2019.136151

Dufey, I., Hakizimana, P., Draye, X., Lutts, S., Bertin, P., 2009. QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. Euphytica 167, 143–160. https://doi.org/10.1007/s10681-008-9870-7

Ellis, R.J., 1979. The most abundant protein in the world. Trends Biochem. Sci. 4, 241–244. https://doi.org/10.1016/0968-0004(79)90212-3

EMBRAPA, 2009. Manual de análises químicas de solos, plantas e fertilizantes, 2nd ed. Embrapa Informação Tecnológica, Brasília, DF.

EMBRAPA, 1997. Manual de métodos de análise de solo. Empresa Brasileira de Pesquisa Agropecuária / Centro Nacional de Pesquisa de Solos, Rio de Janeiro.

FAO, ITPS, 2015. Status of the World's Soil Resources (SWSR) - Main Report. Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome, Italy.

Farooq, M., Basra, S.M.A., Ahmad, N., Hafeez, K., 2005. Thermal hardening: A new seed vigor enhancement tool in rice. J. Integr. Plant Biol. 47, 187–193. https://doi.org/10.1111/j.1744-7909.2005.00031.x

Fernandes, G.W., Goulart, F.F., Ranieri, B.D., Coelho, M.S., Dales, K., Boesche, N., Bustamante, M., Carvalho, F.A., Carvalho, D.C., Dirzo, R., Fernandes, S., Galetti, P.M., Millan, V.E.G., Mielke, C., Ramirez, J.L., Neves, A., Rogass, C., Ribeiro, S.P., Scariot, A., Soares-Filho, B., 2016. Deep into the mud: ecological and socio-economic impacts of the dam breach in Mariana, Brazil. Nat. Conserv. 14, 35–45. https://doi.org/10.1016/j.ncon.2016.10.003

Figueiredo, M.D., Lameiras, F.S., Ardisson, J.D., Araujo, M.H., Teixeira, A.P. de C., 2020. Tailings from Fundão tragedy: Physical–chemical properties of the material that remains by Candonga dam. Integr. Environ. Assess. Manag. 1–7. https://doi.org/10.1002/ieam.4227

Fleck, S., Raspe, S., Cater, M., Schlappi, P., Ukonmaanaho, L., Greve, M., Hertel, C., Weis, W., Rumpf, S., Thimonier, A., Chianucci, F., Beckschäfer, P., 2016. Part XVII: Leaf Area Measurements, in: Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests. UNECE ICP Forests Programme Co-ordinating Centre, Eberswalde Germany, p. 34.

Forján, R., Rodríguez-Vila, A., Covelo, E.F., 2019. Increasing the nutrient content in a mine soil through the application of technosol and biochar and grown with Brassica juncea L. Waste and Biomass Valorization 10, 103–119. https://doi.org/10.1007/s12649-017-0027-6

Fox, J., Weisberg, S., 2019. An R companion to applied regression, 3rd ed. SAGE Publications, Inc.

Garcia, L.C., Ribeiro, D.B., de Oliveira Roque, F., Ochoa-Quintero, J.M., Laurance, W.F., 2017. Brazil's worst mining disaster: Corporations must be compelled to pay the actual environmental costs: Corporations. Ecol. Appl. 27, 5–9. https://doi.org/10.1002/eap.1461

Guerra, M.B.B., Teaney, B.T., Mount, B.J., Asunskis, D.J., Jordan, B.T., Barker, R.J., Santos, E.E., Schaefer, C.E.G.R., 2017. Post-catastrophe analysis of the Fundão tailings dam failure in the Doce River system, Southeast Brazil: Potentially toxic elements in affected soils. Water, Air, Soil Pollut. 252, 1–12. https://doi.org/10.1007/s11270-017-3430-5

Hatje, V., Pedreira, R.M.A., de Rezende, C.E., Schettini, C.A.F., de Souza, G.C., Marin, D.C., Hackspacher, P.C., 2017. The environmental impacts of one of the largest tailing dam failures worldwide. Sci. Rep. 7, 1–13. https://doi.org/10.1038/s41598-017-11143-x

He, C., Wang, W., Hou, J., 2019. Characterization of dark septate endophytic fungi and improve the performance of liquorice under organic residue treatment. Front. Microbiol. 10, 1–14. https://doi.org/10.3389/fmicb.2019.01364

Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413, 297–299. https://doi.org/10.1038/35095041

Hodge, A., Fitter, A.H., 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc. Natl. Acad. Sci. 107, 13754–13759. https://doi.org/10.1073/pnas.1005874107

Hölzl, G., Dörmann, P., 2019. Chloroplast Lipids and Their Biosynthesis. Annu. Rev. Plant Biol. 70, 51–81. https://doi.org/10.1146/annurev-arplant-050718-100202

IBAMA, 2015. Laudo Técnico Preliminar. Impactos ambientais decorrentes do desastre envolvendo o rompimento da barragem de Fundão, em Mariana, Minas Gerais.

IGAM, 2005. Instituto Mineiro de Gestão das Águas - IGAM - Bacia hidrográfica do Rio Doce [WWW Document]. URL http://www.igam.mg.gov.br/index2.php?option=com_content&do_pdf=1&id=155 (accessed 2.2.18).

Igamberdiev, A.U., Roussel, M.R., 2012. Feedforward non-Michaelis–Menten mechanism for CO2 uptake by Rubisco: Contribution of carbonic anhydrases and photorespiration to optimization of photosynthetic carbon assimilation. Biosystems 107, 158–166. https://doi.org/10.1016/j.biosystems.2011.11.008

Ingraffia, R., Amato, G., Frenda, A.S., Giambalvo, D., 2019. Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. PLoS One 14, e0213672. https://doi.org/10.1371/journal.pone.0213672

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Jeong, J., Cohu, C., Kerkeb, L., Pilon, M., Connolly, E.L., Guerinot, M.L., 2008. Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions. Proc. Natl. Acad. Sci. 105, 10619–10624. https://doi.org/10.1073/pnas.0708367105

Johansen, D., 1940. Plant microtechnique. McGraw-Hill Book Co. Inc., New York.

Jumpponen, A., Trappe, J.M., 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol. 140, 295–310. https://doi.org/10.1046/j.1469-8137.1998.00265.x

Juwarkar, A.A., Yadav, S.K., Thawale, P.R., Kumar, P., Singh, S.K., Chakrabarti, T., 2009. Developmental strategies for sustainable ecosystem on mine spoil dumps: a case of study. Environ. Monit. Assess. 157, 471–481. https://doi.org/10.1007/s10661-008-0549-2

Kabata-Pendias, A., 2011. Trace elements in soils and plants, 4th ed. CRC Press Taylor & Francis Group, Boca Raton. https://doi.org/10.1201/b10158-25

Kampfenkel, K., Van Montagu, M., Inze, D., 1995. Effects of Iron Excess on Nicotiana plumbaginifolia Plants (Implications to Oxidative Stress). Plant Physiol. 107, 725–735. https://doi.org/10.1104/pp.107.3.725

Khan, H.R., McDonald, G.K., Rengel, Z., 2004. Zinc fertilization and water stress affects plant water relations, stomatal conductance and osmotic adjustment in chickpea (Cicer arientinum L.). Plant Soil 267, 271–284. https://doi.org/10.1007/s11104-005-0120-7

Labouriau, L.G., 1983. Uma nova linha de pesquisa na fisiologia da germinação das sementes.

Labouriau, L.G., Valadares, M.E.B., 1976. On the germination of seeds Calotropis procera (Ait.) Ait.f. An. Acad. Bras. Cienc. 48, 263–284.

Lê, S., Josse, J., Husson, F., 2008. FactoMineR : An R Package for Multivariate Analysis. J. Stat. Softw. 25, 1–18. https://doi.org/10.18637/jss.v025.i01

Leal, E.S., Ítavo, L.C.V., Valle, C.B. do, Ítavo, C.C.B.F., Dias, A.M., Barbosa-Ferreira, M., Soares, C.M., Melo, G.K.A. de, Ferreira, V.B.N., 2016. Anti-nutritional potential of protodioscin and kinetics of degradation in Urochloa grasses. Semin. Ciências Agrárias 37, 2247. https://doi.org/10.5433/1679-0359.2016v37n4p2247

Liao, D., Wang, S., Cui, M., Liu, J., Chen, A., Xu, G., 2018. Phytohormones Regulate the Development of Arbuscular Mycorrhizal Symbiosis. Int. J. Mol. Sci. 19, 13754–13759. https://doi.org/10.3390/ijms19103146

Macedo, M.C.M., 2004. Análise comparativa de recomendações de adubação em pastagens. In: SIMPÓSIO SOBRE MANEJO DA PASTAGEM, 21.,.

Machado, M.S.M.M., Santos, A.M.M., Freire, C.B., Guimarães, A.C.P.D., Lameiras, F.S., 2019. Blocks for civil construction made with the sediment deposited in the Candonga dam. REM - Int. Eng. J. 72, 105–111. https://doi.org/10.1590/0370-44672017720198

Maguire, J.D., 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 2, 176–177.

Marta-Almeida, M., Mendes, R., Amorim, F.N., Cirano, M., Dias, J.M., 2016. Fundão Dam collapse: Oceanic dispersion of River Doce after the greatest Brazilian environmental accident. Mar. Pollut. Bull. 112, 359–364. https://doi.org/10.1016/j.marpolbul.2016.07.039

Martins, L.E.C., Monteiro, F.A., Pedreira, B.C., 2015. Photosynthesis and leaf area of Brachiaria brizantha in response to phosphorus and zinc nutrition. J. Plant Nutr. 38, 754–767. https://doi.org/10.1080/01904167.2014.939758

Matschullat, J., Borba, R.P., Deschamps, E., Figueiredo, B.R., Gabrio, T., Schwenk, M., 2000. Human and environmental contamination in the Iron Quadrangle, Brazil. Appl. Geochemistry 15, 181–190. https://doi.org/10.1016/S0883-2927(99)00039-6

McCall, K.A., Huang, C., Fierke, C.A., 2000. Function and mechanism of zinc metalloenzymes. J. Nutr. 130, 1437S-1446S. https://doi.org/10.1093/jn/130.5.1437s

McManus, J.F.A., 1948. Histological and histochemical uses of periodic acid. Stain Technol. 23, 99–108. https://doi.org/10.3109/10520294809106232

Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid environments—An emerging remediation technology. Environ. Health Perspect. 116, 278–283. https://doi.org/10.1289/ehp.10608

Mendiburu, F., 2019. Statistical procedures for agricultural research.

Mondo, V.H.V., Gomes Junior, F.G., Pinto, T.L.F., Marchi, J.L. de, Motomiya, A.V.A., Molin, J.P., Cicero, S.M., 2012. Spatial variability of soil fertility and its relationship with seed physiological potential in a soybean production area. Rev. Bras. Sementes 34, 193–201. https://doi.org/10.1590/S0101-31222012000200002

Moreira, L.M., Martuscello, J.A., Fonseca, D.M., Mistura, C., Morais, R.V., Júnior, J.I.R., 2009. Perfilhamento, acúmulo de forragem e composição bromatológica do capim-braquiária adubado com nitrogênio. Rev. Bras. Zootec. 38, 1675–1684.

Mukhopadhyay, M., Das, A., Subba, P., Bantawa, P., Sarkar, B., Ghosh, P., Mondal, T.K., 2013. Structural, physiological, and biochemical profiling of tea plantlets under zinc stress. Biol. Plant. 57, 474–480. https://doi.org/10.1007/s10535-012-0300-2

National Research Council, 1980. Mineral Tolerance of Domestic Animals. National Academies Press, Washington, D.C. https://doi.org/10.17226/25

Neto, J.C.P., Costa, J.O., 2012. Análise do solo: determinações, cálculos e interpretação. Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG Sul de Minas Gerais, Lavras.

Nichols, M.A., Heydecker, W., 1968. Two approaches to the study of germination data. Proc. Int. Seed Test. Assoc. 33, 531–540.

Nieder, R., Benbi, D.K., 2008. Organic matter and soil quality, in: Carbon and Nitrogen in the Terrestrial Environment. Springer Netherlands, pp. 113–135. https://doi.org/10.1007/978-1-4020-8433-1_4

Nikolic, M., Pavlovic, J., 2018. Plant Responses to Iron Deficiency and Toxicity and Iron Use Efficiency in Plants, in: Plant Micronutrient Use Efficiency. Elsevier, pp. 55–69. https://doi.org/10.1016/B978-0-12-812104-7.00004-6

O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59, 368–373. https://doi.org/10.1007/BF01248568

O'Brien, T.P., McCully, M.E., 1981. The study of plants structure principles and select methods. Termarcarphi Pty. Ltda, Melbourne.

Okazaki, Y., Saito, K., 2014. Roles of lipids as signaling molecules and mitigators during stress response in plants. Plant J. 79, 584–596. https://doi.org/10.1111/tpj.12556

Omachi, C.Y., Siani, S.M.O., Chagas, F.M., Mascagni, M.L., Cordeiro, M., Garcia, G.D., Thompson, C.C., Siegle, E., Thompson, F.L., 2018. Atlantic Forest loss caused by the world´s largest tailing dam collapse (Fundão Dam, Mariana, Brazil). https://doi.org/10.1016/j.rsase.2018.08.003

Önemli, F., 2011. The effects of soil organic matter on seedling emergence in sunflower (Helianthus annuus L.). Plant, Soil Environ. 50, 494–499. https://doi.org/10.17221/4064-PSE

Pereira, E.G., Oliva, M.A., Rosado-Souza, L., Mendes, G.C., Colares, D.S., Stopato, C.H., Almeida, A.M., 2013. Iron excess affects rice photosynthesis through stomatal and non-stomatal limitations. Plant Sci. 201–202, 81–92. https://doi.org/10.1016/j.plantsci.2012.12.003

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161. https://doi.org/10.1016/S0007-1536(70)80110-3

Piercey, M.M., Graham, S.W., Currah, R.S., 2004. Patterns of genetic variation in Phialocephala fortinii across a broad latitudinal transect in Canada. Mycol. Res. 108, 955–964. https://doi.org/10.1017/S0953756204000528

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D.R., 2019. nlme: Linear and nonlinear mixed effects models.

Pollard, M., Beisson, F., Li, Y., Ohlrogge, J.B., 2008. Building lipid barriers: biosynthesis of cutin and suberin. Trends Plant Sci. 13, 236–246. https://doi.org/10.1016/j.tplants.2008.03.003

Prado, I.G.O., Silva, M.C.S., Prado, D.G.O., Kemmelmeier, K., Pedrosa, B.G., Silva, C.C., Kasuya, M.C.M., 2019. Revegetation process increases the diversity of total and arbuscular mycorrhizal fungi in areas affected by the Fundão dam failure in Mariana, Brazil. Appl. Soil Ecol. 141, 84–95. https://doi.org/10.1016/j.apsoil.2019.05.008

R Core Team and contributors, 2020. 'stats.'

Rao, I.M., Kerridge, P.C., Macedo, M.C.M., 1996. Nutrition requirements of Brachiaria and adaptation to acid soils, in: Miles, J.W., Maass, B.L., Valle, C.B., Kumble, V. (Eds.), Brachiaria: Biology, Agronomy and Improvement. Centro Internacional de Agricultura Tropical, Tropical Forages Program and Comunications Unit; Campo Grande Brasil, Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Gado de Corte, Cali, Colômbia, pp. 53–71.

Raven, J.A., 2013. Rubisco: still the most abundant protein of Earth? New Phytol. 198, 1–3. https://doi.org/10.1111/nph.12197

Rawsthorne, S., 2002. Carbon flux and fatty acid synthesis in plants. Prog. Lipid Res. 41, 182–196. https://doi.org/10.1016/S0163-7827(01)00023-6

Reininger, V., Sieber, T.N., 2012. Mycorrhiza reduces adverse effects of Dark Septate Endophytes (DSE) on growth of conifers. PLoS One 7, e42865. https://doi.org/10.1371/journal.pone.0042865

Renvoize, S.A., Clayton, W.D., Kabuye, C.H.S., 1996. Morphology, taxonomy, and natural distribution of Brachiaria (Trin.) Griseb., in: Brachiaria: Biology, Agronomy, and Improvement. Centro Nacional de Pesquisa de Gado de Corte, Cali, pp. 1–15.

Riazunnisa, K., Padmavathi, L., Bauwe, H., Raghavendra, A.S., 2006. Markedly low requirement of added CO2 for photosynthesis by mesophyll protoplasts of pea (Pisum sativum): possible roles of photorespiratory CO2 and carbonic anhydrase. Physiol. Plant. 128, 763–772. https://doi.org/10.1111/j.1399-3054.2006.00803.x

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., Pascale, S. De, Bonini, P., Colla, G., 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci. Hortic. (Amsterdam). 196, 91–108. https://doi.org/10.1016/j.scienta.2015.09.002

RStudio, 2019. RStudio | Open source & professional software for data science teams - RStudio.

Ruiz, F., Perlatti, F., Oliveira, D.P., Ferreira, T.O., 2020. Revealing tropical technosols as an alternative for mine reclamation and waste management. Minerals 10, 1–11. https://doi.org/10.3390/min10020110

Sako, Y., Mcdonalds, M.B., Fujimura, K., Evans, A.F., Bennett, M.A., 2001. A system for automated seed vigour assessment. Seed Sci. Technol. 29, 625–636.

Santos, O.S.H., Avellar, F.C., Alves, M., Trindade, R.C., Menezes, M.B., Ferreira, M.C., França, G.S., Cordeiro, J., Sobreira, F.G., Yoshida, I.M., Moura, P.M., Baptista, M.B., Scotti, M.R., 2019. Understanding the environmental impact of a mine dam rupture in Brazil: Prospects for remediation. J. Environ. Qual. 48, 439–449. https://doi.org/10.2134/jeq2018.04.0168

Schad, P., 2018. Technosols in the World Reference Base for Soil Resources – history and definitions. Soil Sci. Plant Nutr. 64, 138–144. https://doi.org/10.1080/00380768.2018.1432973

Schaefer, C.E.G.R., Santos, E.E., Souza, C.M., Neto, J.D., Filho, E.I.F., Delpupo, C., 2015. Historical scenario, physiography and strategies for environmental rehabilitation of the landscape affected by the Fundão Dam breaking accident at Mariana, Minas Gerais State. Arq. do Mus. História Nat. e Jard. Botânico 24, 104–135.

Schieber, M., Chandel, N.S., 2014. ROS Function in Redox Signaling and Oxidative Stress. Curr. Biol. 24, R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. Soil Use Manag. 32, 476–486. https://doi.org/10.1111/sum.12288

Schüβler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105, 1413–1421. https://doi.org/10.1017/S0953756201005196

Segura, F.R., Nunes, E.A., Paniz, F.P., Paulelli, A.C.C., Rodrigues, G.B., Braga, G.Ú.L., 2016. Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). Environ. Pollut. 218, 1–13. https://doi.org/10.1016/j.envpol.2016.08.005

Seiffert, N.F., 1980. Gramíneas forrageiras do gênero Brachiaria. Circ. Técnica n° 1, Embrapa Gado Corte 1–14.

Sharma, P.N., Tripathi, A., Bisht, S.S., 1995. Zinc requirement for stomatal opening in cauliflower. Plant Physiol. 107, 751–756. https://doi.org/10.1104/pp.107.3.751

Shiraiwa, Y., Schmid, G.H., 1986. Stimulation of photorespiration by the Carbonic Anhydrase inhibitor Ethoxyzolamide in Chlorella vulgaris. Zeitschrift für Naturforsch. C 41, 564–570. https://doi.org/10.1515/znc-1986-5-613

Shu, W.S., Ye, Z.H., Zhang, Z.Q., Lan, C.Y., Wong, M.H., 2005. Natural colonization of plants on five Lead/Zinc mine tailings in Southern China. Restor. Ecol. 13, 49–60. https://doi.org/10.1111/j.1526-100X.2005.00007.x

SiBCS, 2018. Sistema Brasileiro de Classificação de Solos., 5. ed. ed. Embrapa, Brasília, DF.

Silva, C.T.R., Bonfim-Silva, E.M., Silva, T.J. de A., Pinheiro, E.A.R., José, J.V., Ferraz, A.P.F., 2020. Yield Component Responses of the Brachiaria brizantha Forage Grass to Soil Water Availability in the Brazilian Cerrado. Agriculture 10, 13. https://doi.org/10.3390/agriculture10010013

Silva, L.J., Medeiros, A.D., Oliveira, A.M.S., 2018. SeedCalc: Seed Germination and Seedling Growth Indexes.

Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis, 3rd Editio. ed. Elsevier, New York. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

Storey, J.B., 2006. Zinc, in: Handbook of Plant Nutrition. Taylor & Francis, Boca Raton, pp. 411–436.

Surono, Narisawa, K., 2017. The dark septate endophytic fungus Phialocephala fortinii is a potential decomposer of soil organic compounds and a promoter of Asparagus officinalis growth. Fungal Ecol. 28, 1–10. https://doi.org/10.1016/j.funeco.2017.04.001

Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., 2014. Plant Physiology and Development, 6th ed. Sinauer Associates, an imprint of Oxford University Press, Oxford.

Valeriano, C.M., Neumann, R., Alkmim, A.R., Evangelista, H., Heilbron, M., Neto, C.C.A., Souza, G.P., 2019. Sm–Nd and Sr isotope fingerprinting of iron mining tailing deposits spilled from the failed SAMARCO Fundão dam 2015 accident at Mariana, SE-Brazil. Appl. Geochemistry 106, 34–44. https://doi.org/10.1016/j.apgeochem.2019.04.021

Vergara, C., Araujo, K.E.C., Sperandio, M.V.L., Santos, L.A., Urquiaga, S., Zilli, J.É., 2019. Dark septate endophytic fungi increase the activity of proton pumps, efficiency of 15N recovery from ammonium sulphate, N content, and micronutrient levels in rice plants. Brazilian J. Microbiol. 50, 825–838. https://doi.org/10.1007/s42770-019-00092-4

Vidal, B.C., 1970. Dichroism in collagen bundles stained with Xylidine-Ponceau 2R. Ann. Histochim. 15, 289–96.

Wang, L., Ji, B., Hu, Y., Liu, R., Sun, W., 2017. A review on in situ phytoremediation of mine tailings. Chemosphere 184, 594–600. https://doi.org/10.1016/j.chemosphere.2017.06.025

Waqas, M., Khan, A.L., Kamran, M., Hamayun, M., Kang, S.-M., Kim, Y.-H., Lee, I.-J., 2012. Endophytic fungi produce Gibberellins and Indoleacetic Acid and promotes host-plant growth during stress. Molecules 17, 10754–10773. https://doi.org/10.3390/molecules170910754

Wellburn, A.R., 1994. The spectral determination of Chlorophylls a and b, as well as total Carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2

Werner, J.C., Paulino, V.T., Cantarella, H., 1996. Recomendação de adubação e calagem para forrageiras, in: Recomendação de Adubação e Calagem Para o Estado de São Paulo. Instituto Agronômico; Fundação IAC, Campinas, pp. 263–271.

Winagraski, E., Kaschuk, G., Monteiro, P.H.R., Auer, C.G., Higa, A.R., 2019. Diversity of arbuscular mycorrhizal fungi in forest ecosystems of Brazil: A review. CERNE 25, 25–35. https://doi.org/10.1590/01047760201925012592

Young, I., Renault, S., Markham, J., 2015. Low levels organic amendments improve fertility and plant cover on non-acid generating gold mine tailings. Ecol. Eng. 74, 250–257. https://doi.org/10.1016/j.ecoleng.2014.10.026

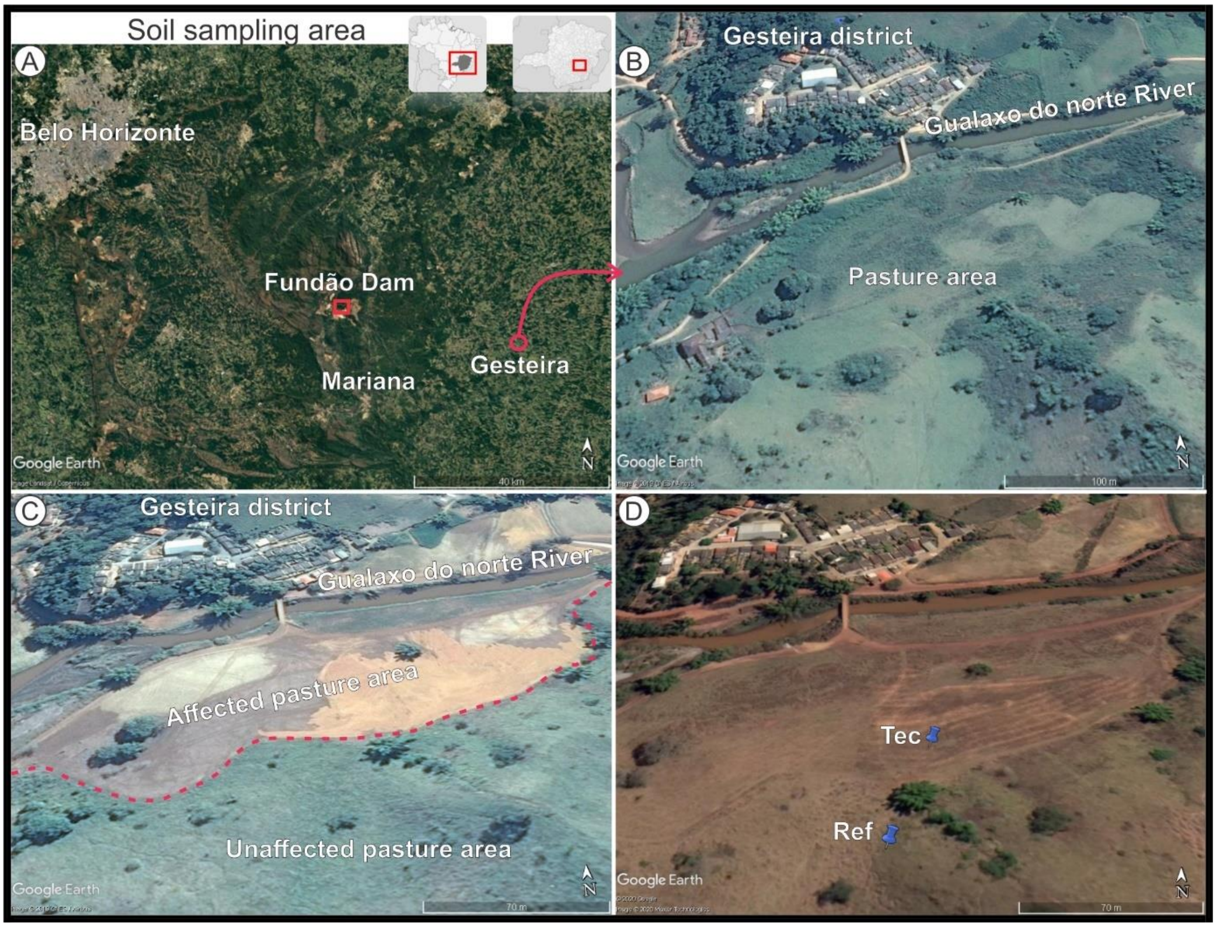

Figure 1: Soil sampling area. A and B – Localization of Gesteira district in Barra Longa, Minas Gerais, Brazil about 2 and a half years before the Fundão dam rupture (aerial image of July 5, 2013). C - Gesteira about 10 months after dam rupture showing pasture area affected by mining tailings and unaffected pasture area (aerial image of September 8, 2016). D - Places where soil was collected for the experiment (aerial image of August 25, 2017). Aerial images from Google Earth Pro 7.3.2 version.

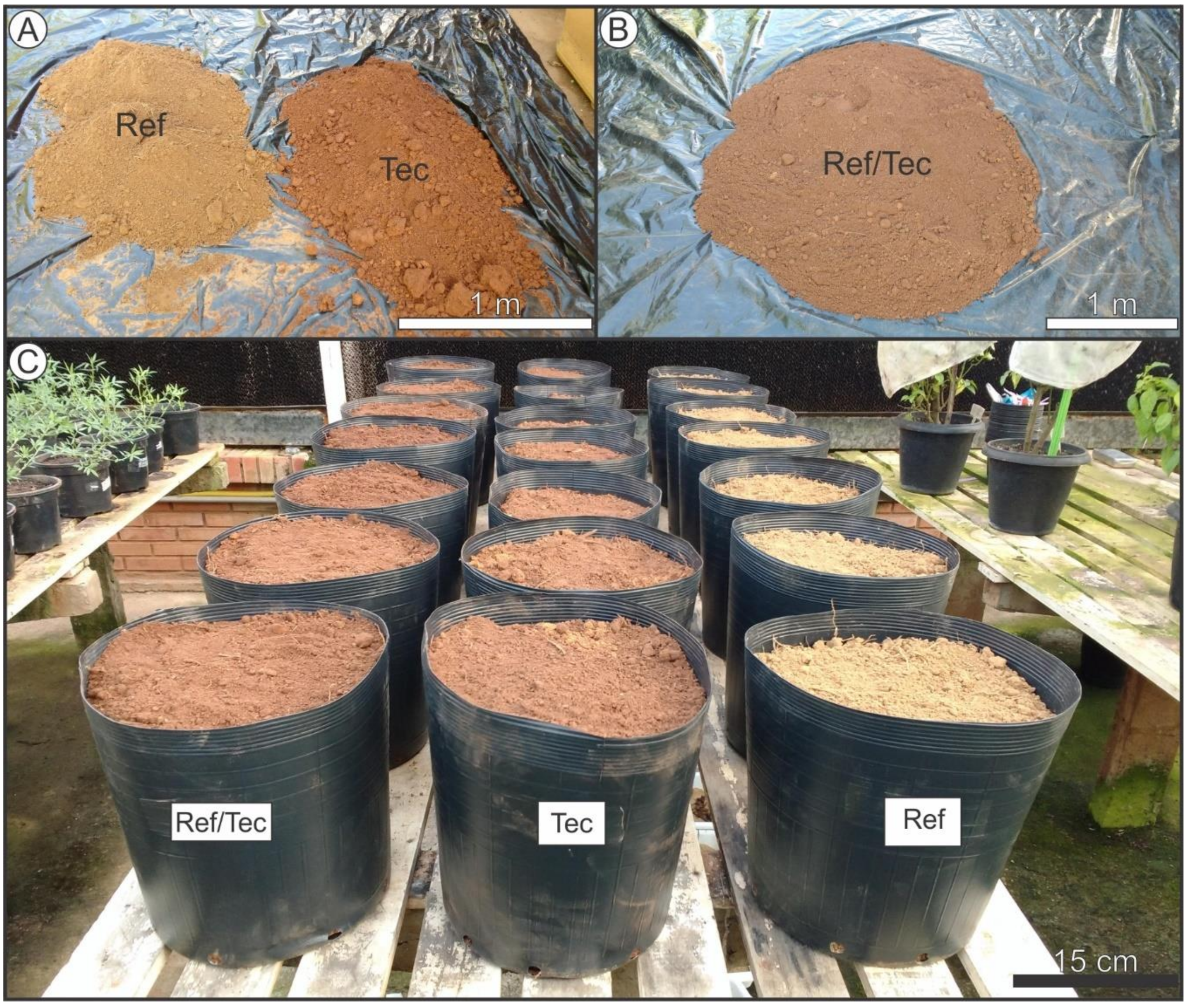

Figure 2: Greenhouse experiment. A - Ref (red yellow Argisol unaffected by iron mining tailings) and Tec (Technosol with a thick deposition of iron mining tailings). B - Ref/Tec (Mixture of 50 % Ref plus 50 % Tec). C - Treatments and repetitions of the experiment.

Table 1. Descriptive statistics of chemical soil attributes in Ref, Ref/Tec and Tec. Significant differences ($p < 0.05$) are shown in bold

Soil Parameter	Ref		Ref/Tec		Tec		p-value	
	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.
pH H_2O	4.57±0.03 B	4.82±0.05 c	4.74±0.004 A	4.9±0.05 b	4.72±0.05 A	5.09±0.04 a	**0.0001**	**3.09x10^{-08}**
Rem-P (mg/l)	$^{(N)}$23.3±0.61 A	$^{(N)}$22.87±0.86 a	$^{(N)}$21.8±0.2 AB	$^{(N)}$22.48±0.92 a	$^{(N)}$15.9±0.94 B	$^{(N)}$19±1.47 b	**0.007**	**0.001**
P (mg/dm^3)	1.76±0.04 AB	2.1±0.32 a	1.9±0.14 A	1.94±0.21 a	1.6±0.14 B	2.25±0.32 a	**0.01**	0.15
K (mg/dm^3)	$^{(N)}$51.3±1.69AB	33.2±5.34 ab	$^{(N)}$68±4.32 A	41±5.16 a	$^{(N)}$16.3±2.62 B	30.7±10.35 b	**0.007**	**0.04**
Ca (cmol$_c$/dm^3)	$^{(N)}$0.32±0.03 AB	0.86±0.18 a	$^{(N)}$0.49±0.01 A	0.89±0.21 a	$^{(N)}$0.22±0 B	0.77±0.2 a	**0.006**	0.53
Mg (cmol$_c$/dm^3)	0.12±0.004 B	0.25±0.05 a	0.2±0.01 A	0.26±0.02 a	0.04±0.009 C	0.17±0.03 b	**9.84x10^{-08}**	**0.0002**
S (mg/dm^3)	10.3±0.2 C	21.32±6.56 b	19.06±0.7 B	36.67±6.42 a	30.5±1.71 A	29.3±7.6 ab	**2.97x10^{-09}**	**0.002**
B (mg/dm^3)	0.22±0.03 A	$^{(N)}$0.02±0 a	0.07±0.02 B	$^{(N)}$0.04±0.07 a	0.07±0.03 B	$^{(N)}$0.04±0.07 a	**8.51x10^{-05}**	0.58
Cu (mg/dm^3)	3.49±0.23 A	$^{(N)}$2.47±0.18 a	2.16±0.11 B	$^{(N)}$1.71±0.15 ab	0.87±0.05 C	$^{(N)}$0.89±0.07 b	**6.59x10^{-09}**	**0.0001**
Mn (mg/dm^3)	45.5±6.97 B	35.78±2.84 c	77±18.45 A	45.81±4.99 b	65.5±3.78 AB	56.9±5.7 a	**0.01**	**5.19x10^{-07}**
Fe (mg/dm^3)	780.4±149.2 A	$^{(N)}$248.65±28.9 a	350.2±132 B	$^{(N)}$158.9±41 ab	85.6±12.5 C	$^{(N)}$49.6±6.3 b	**4.65x10^{-05}**	**0.0001**
Zn (mg/dm^3)	4.12±0.71 A	2.51±0.36 a	1.94±0.13 B	1.17±0.18 b	0.63±0.07 C	0.95±0.45 b	**3.22x10^{-06}**	**2.3x10^{-07}**
Cr (mg/dm^3)	$^{(N)}$0.16±0.11 A	0.18±0.07 a	$^{(N)}$0.09±0.13 A	0.05±0.03 b	$^{(N)}$0±0 A	0.11±0.08 ab	0.1	**0.009**
Ni (mg/dm^3)	0.49±0.16 A	$^{(N)}$0.44±0.22 a	0.1±0.12 B	$^{(N)}$0.06±0.1 b	0.07±0.06 B	$^{(N)}$0±0 b	**0.001**	**0.0004**
Cd (mg/dm^3)	$^{(N)}$0.006±0.009 A	$^{(N)}$0.02±0.05 a	$^{(N)}$0±0 A	$^{(N)}$0±0 a	$^{(N)}$0±0 A	$^{(N)}$0±0 a	0.11	0.12
Pb (mg/dm^3)	3.58±0.18 A	2.98±0.18 a	2.9±0.11 B	2.5±0.12 b	1.71±0.14 C	1.72±0.25 c	**8.2x10^{-08}**	**2.55x10^{-09}**
Al^{3+} (cmol$_c$/dm^3)	$^{(N)}$0.78±0.04 A	$^{(N)}$0.59±0.11 a	$^{(N)}$0.27±0 B	$^{(N)}$0.24±0.05 ab	$^{(N)}$0.31±0.03 AB	$^{(N)}$0.05±0.09 b	**0.008**	**0.0001**
H+Al (cmol$_c$/dm^3)	4.73±0.18 A	$^{(N)}$5.4±0.28 a	3.26±0.12 B	$^{(N)}$3.8±0.1 ab	2.46±0.2 C	$^{(N)}$2.32±0.24 b	**7.12x10^{-08}**	**0.0001**
OM (dag/kg)	2.95±0.26 A	3.02±0.29 a	2.04±0.22 B	1.84±0.11 b	0.82±0.06 C	0.65±0.32 c	**4.56x10^{-07}**	**7.37x10^{-12}**
SB (cmolc/dm^3)	0.58±0.04 B	1.22±0.22 a	0.87±0.03 A	1.28±0.23 a	0.31±0.01 C	1.03±0.24 a	**8.08x10^{-09}**	0.153
t (cmol$_c$/dm^3)	1.36±0.08 A	1.82±0.14 a	1.14±0.03 B	1.52±0.21 b	0.5±0.12 C	1.09±0.22 c	**1.2x10^{-06}**	**7.76x10^{-06}**
T (cmol$_c$/dm^3)	5.31±0.2 A	6.62±0.23 a	4.14±0.12 B	5.08±0.22 b	2.7±0.2 C	3.36±0.3 c	**4.09x10^{-08}**	**2.44x10^{-14}**
V (%)	$^{(N)}$10.9±0.71 B	18.5±3.37 b	$^{(N)}$21.1±1.06 A	25.1±3.57 a	$^{(N)}$11.2±0.8 AB	30.6±5.75 a	**0.02**	**0.0002**
m (%)	$^{(N)}$57.3±0.61 A	33.05±8.25 a	$^{(N)}$23.6±0.73 B	16.52±4.43 b	$^{(N)}$40.9±12.7 AB	4.97±8.49 c	**0.01**	**4.73x10^{-06}**

B. E.: Before the experiment, A. E.: After the experiment, pH: H_2O (relation 1:2,5), P: phosphorus; K: potassium; Fe: iron; Zn: zinc; Mn: manganese; Cu: copper; Cd: cadmium; Pb: lead; Ni: nickel; Cr: chrome (Mehlich extractor 1); Ca: calcium; Mg: magnesium; Al^{3+} (KCl extractor 1 mol/L); H + Al: acidity potential (calcium acetate extractor 0,5 mol/L pH 7,0); SB: sum of exchangeable bases; t: effective cation exchange capacity; T: cation exchange capacity at pH 7.0; V: base saturation; Rem-P: Remaining P; m: aluminum saturation; OM: organic matter. Mean±SD followed by capital letters compare the B. E. values. Mean±SD followed by small letters compare the A. E. values. Means followed by the same letter do not differ statistically from each other at 5 % of probability by the Tukey test. (N)Means followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test.

Table 2. Descriptive statistics of physical soil attributes in Ref, Ref/Tec and Tec. Significant differences ($p < 0.05$) are shown in bold

Soil Parameter	Ref	Ref/Tec	Tec	p-value
Coarse sand	0.37±0.01 A	0.24±0.03 B	0.15±0.01 C	**6.15×10^{-07}**
Fine sand	0.19±0.01 B	0.19±0.01 B	0.24±0.01 A	**0.003**
Clay	0.35±0.004 B	0.41±0.01 A	0.43±0.03 A	**0.0009**
Silt	0.07±0.004 C	0.13±0.007 B	0.17±0.02 A	**2.6×10^{-05}**
Texture	Sandy clay	Clay	Clay and Sandy clay	-
Soil type	3	3	3	-

Mean±SD followed by the same letter do not differ statistically from each other at 5 % of probability by the Tukey test.

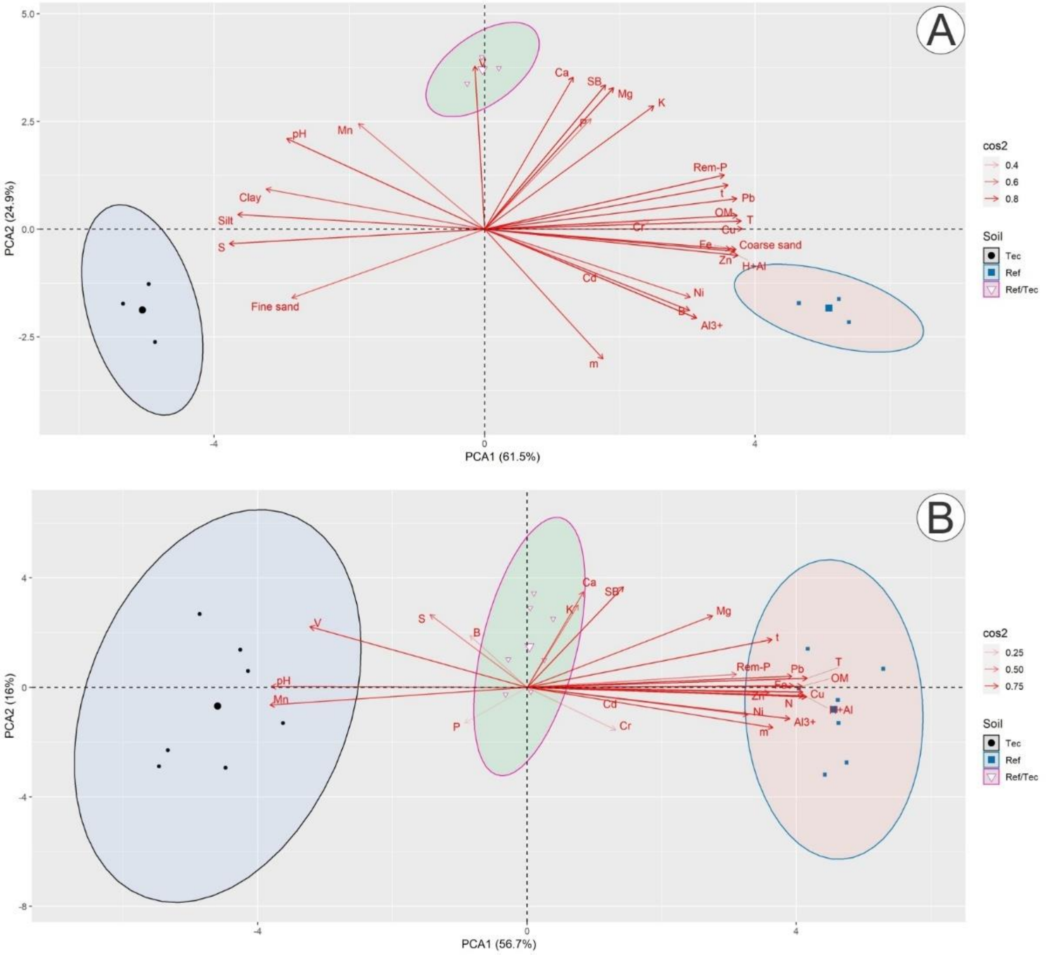

Figure 3: Principal component analysis (PCA) of soil attributes in Ref, Ref/Tec and Tec. A - Before the experiment. B - After the experiment. The level of Pearson correlation of each vector was indicated by (cos2).

Table 3. Descriptive statistics of *Brachiaria decumbens* leaf chemical analysis in plants grown for 110 days in Ref, Ref/Tec and Tec. Significant differences ($p < 0.05$) are shown in bold

Element	Ref	Ref/Tec	Tec	p-value
N (dag/kg)	0.88±0.1 a	0.74±0.07 b	0.85±0.1 ab	**0.03**
P (dag/kg)	0.07±0.01 a	0.06±0.008 a	0.06±0.01 a	0.62
K (dag/kg)	1.29±0.2 b	1.46±0.18 ab	1.69±0.27 a	**0.01**
Ca (dag/kg)	0.43±0.05 a	0.36±0.04 a	0.43±0.04 a	**0.04**
Mg (dag/kg)	$^{(N)}$0.31±0.01 a	$^{(N)}$0.18±0.01 b	$^{(N)}$0.18±0.01 b	**0.001**
S (dag/kg)	0.09±0.009 a	0.08±0.01 a	0.08±0.01 a	0.3
Cu (mg/kg)	3.12±0.3 a	2.38±0.23 b	2.82±0.3 a	**0.0006**
Fe (mg/kg)	131.5±55.6 b	331.01±174.5 a	599.6±400.9 a	**0.002**
Zn (mg/kg)	22.5±4.5 a	8.65±1.67 c	14.24±5.16 b	**2.21x10^{-05}**
Mn (mg/kg)	125.4±45.05 b	171.21±49.3 b	239.26±49.9 a	**0.001**
B (mg/kg)	$^{(N)}$11.08±1.44 b	$^{(N)}$13.84±3.72 b	$^{(N)}$25.32±7.84 a	**0.001**
Al (mg/kg)	$^{(N)}$55.5±28.58 b	$^{(N)}$214.79±122.65 a	$^{(N)}$236.30±152.46 a	**0.002**
Ni (mg/kg)	0	0	0	-
Pb (mg/kg)	0	0	0	-
Cd (mg/kg)	0	0	0	-
Cr (mg/kg)	0	0	0	-

N: Nitrogen, P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Cu: copper; Fe: iron; Zn: zinc; Mn: manganese; B: boron; Al: Aluminum; Ni: nickel; Pb: lead; Cd: cadmium; Cr: chrome. Mean±SD followed by the same letter do not differ statistically from each other at 5 % of probability by the Tukey test. $^{(N)}$Mean±SD followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test.

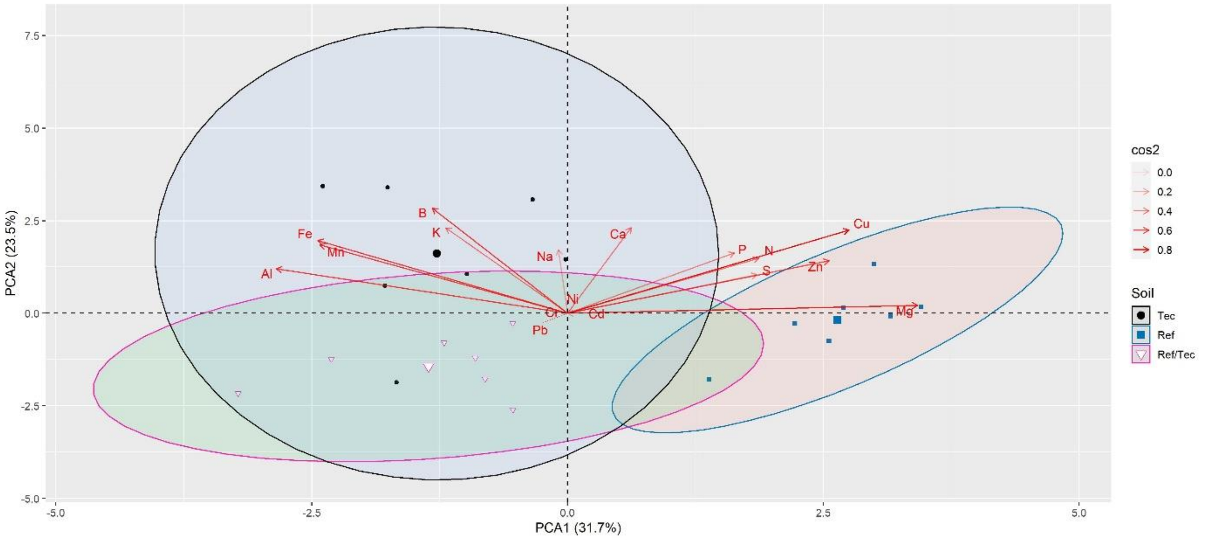

Figure 4: Principal component analysis (PCA) of *Brachiaria decumbens* chemical analysis of leaves in plants grown for 110 days in Ref, Ref/Tec and Tec. The level of Pearson correlation of each vector was indicated by (cos2).

Table 4. Seed germination tests of *Brachiaria decumbens* planted in Ref, Ref/Tec and Tec. Significant differences ($p < 0.05$) are shown in bold.

Parameters and indexes	Ref	Ref/Tec	Tec	p-value
FGP	58.5±4.7 a	61.7±12.2 a	63.14±3.4 a	0.54
GSI	5.2±0.86 a	6.12±1.53 a	4.59±0.84 a	0.06
T10 (days)	3.15±0.46 a	2.66±0.5 b	3.99±1 a	**0.007**
T50 (days)	5.58±1.5 a	5.38±0.82 a	6.69±1.4 a	0.34
T90 (days)	11.34±1.82 a	10.9±2.44 a	12.9±1.88 a	0.24
UnifG	8.18±1.6 a	8.24±2.06 a	8.9±0.94 a	0.68
MGT (days)	6.85±0.95 b	6.59±0.89 b	8.57±0.93 a	**0.001**
MGR	0.14±0.01 a	0.15±0.02 a	0.11±0.01 b	**0.004**
VarGer	10.09±4.72 a	16.15±9.55 a	14.52±4.18 a	0.23
CVt*	45.24±10.1 a	58.08±13.51 a	44.26±7.08 a	0.057
Sinc*	0.11±0.03 a	0.12±0.04 a	0.10±0.01 a	0.72
Unc	2.99±0.2 a	2.95±0.35 a	3.23±0.18 a	0.12
CVG	14.8±1.9 a	15.43±2.3 a	11.7±1.3 b	**0.004**

T10: Time spent to 10 % germination, T50: Time spent to 50 % germination, T90: Time spent to 90 % germination, MGT: Mean Germination Time, GSI: Germination Speed Index, MGR: Mean Germination Rate, CVG: Velocity of Germination Coefficient, CVt: Germination Time Coefficient of variation, VarGer: Variance of Germination Time, Unc: Germination Uncertainty, Sinc: Germination Synchrony, UnifG: Germination Uniformity Index. Mean±SD. Means followed by the same letter do not differ statistically from each other at 5 % of probability by the Tukey test. (*): Means followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test.

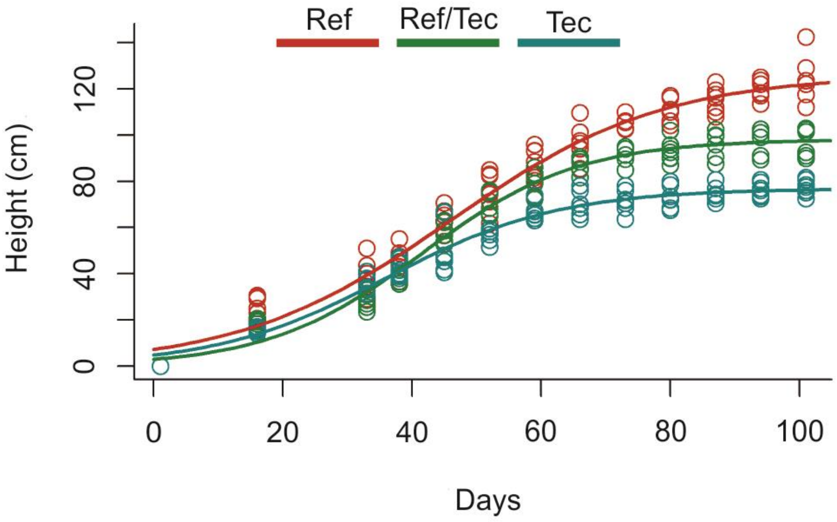

Figure 5: Fitted sigmoid curves of *Brachiaria decumbens* height and growth of plants grown for 110 days in Ref, Ref/Tec and Tec.

Table 5. Sigmoid curve parameters of height of *Brachiaria decumbens* grown for 110 days in Ref, Ref/Tec and Tec.

Parameter	Treatment	asym	xmid	scal
Height	Ref	126.08±5.6	45.9±1.5	16.3±1.48
	Ref/Tec	98.07±4.41	41.6±1.9	12±1.48
	Tec	76.7±2.5	36.1±1.21	13.3±1.3

Sigmoid curve parameters ± 95 % Confidence interval; asym - Asymptote; xmid - inflection point of x-value; scal - angular coefficient of the tangent at point of inflection.

Figure 6: Development of *Brachiaria decumbens* cultivated in Ref, Ref/Tec and Tec after 110 days of experiment in greenhouse. A – Plants cultivated in Ref/Tec. B - Plants cultivated in Tec. C - Plants cultivated in Ref. D - Treatments and repetitions at the end of the greenhouse experiment.

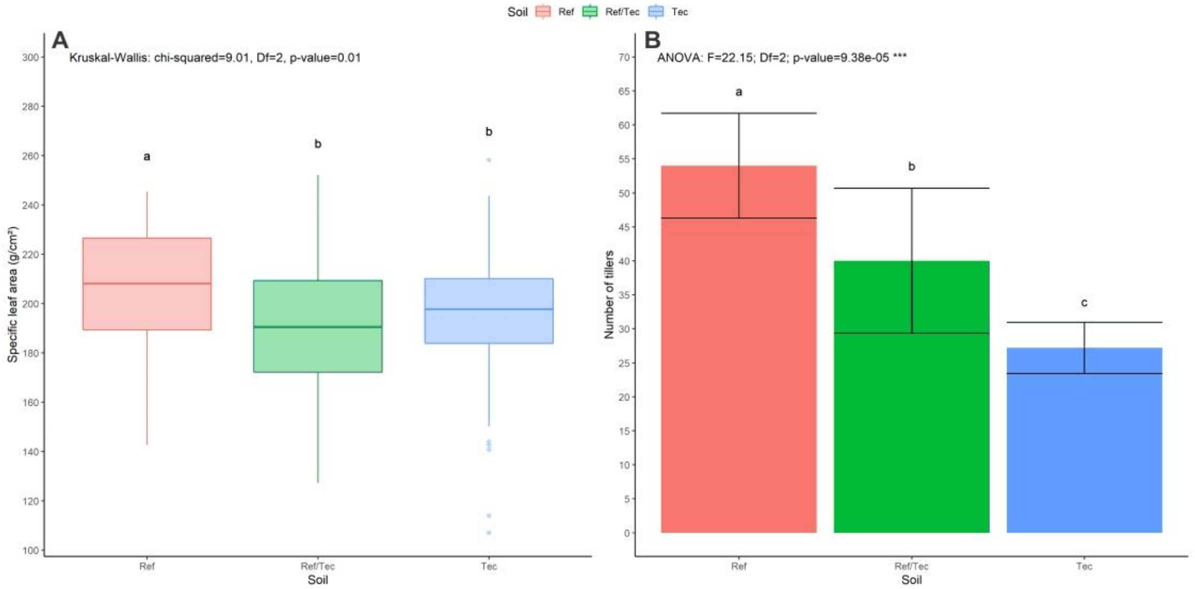

Figure 7: Specific leaf area determination and number of tillers in *Brachiaria decumbens* grown for 110 days in Ref, Ref/Tec and Tec. A - Specific leaf area boxplots. Lower and upper box boundaries represents 25th and 75th percentiles and the lines inside box represents median; lower and upper error lines represents 10th and 90th percentiles. Medians followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test. B - Number of tillers column chart. The vertical bars indicate 95 % confidence intervals. Means followed by the same letter do not differ statistically from each other at 5 % of probability by the Tukey test.

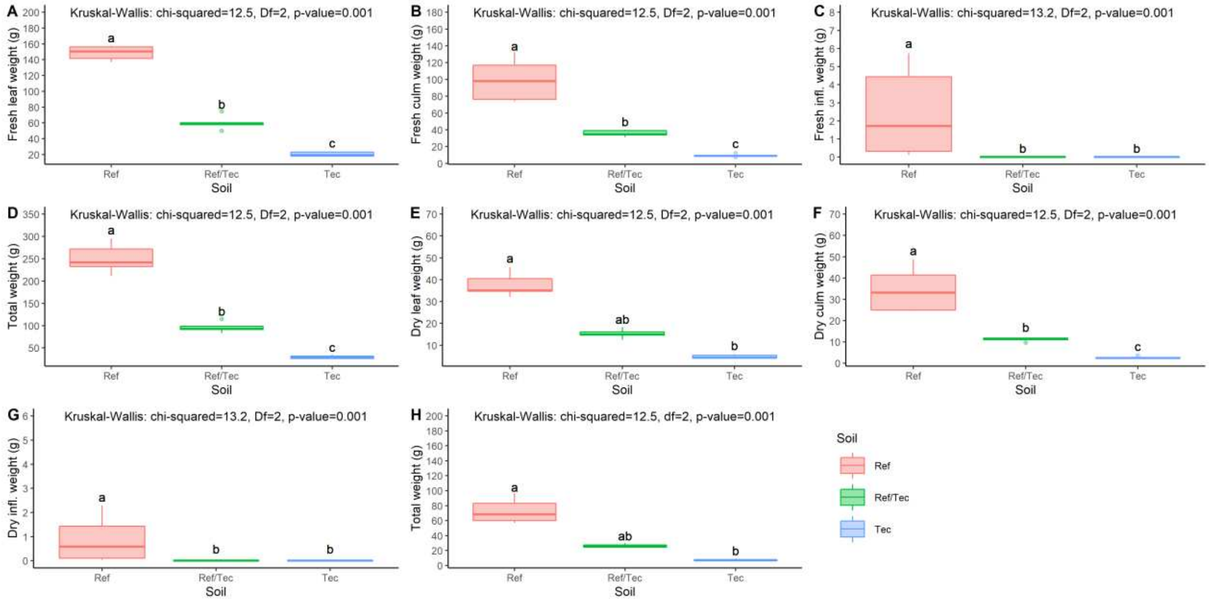

Figure 8: Boxplots of *Brachiaria decumbens* weight of fresh and dried leaf, culm and inflorescence grown for 110 days in Ref, Ref/Tec and Tec. Lower and upper box boundaries represents 25th and 75th percentiles and the lines

inside box represents median; lower and upper error lines represents 10th and 90th percentiles. Medians followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test. In C and G – Infl. (Inflorescence).

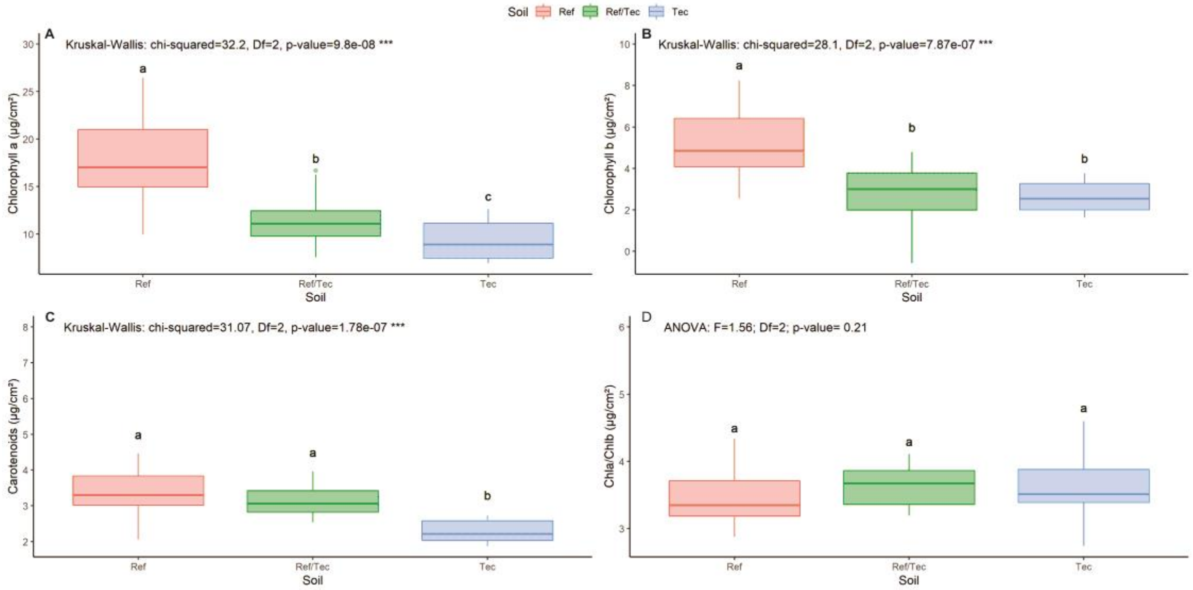

Figure 9: Boxplots of *Brachiaria decumbens* photosynthetic pigment content in plants grown for 110 days in Ref, Ref/Tec and Tec. A - Chlorophyll *a* concentration. B - Chlorophyll *b* concentration. C – Carotenoids concentration. D - Ratio between Chlorophyll *a* and Chlorophyll *b* concentration. Lower and upper box boundaries represents 25th and 75th percentiles and the lines inside box represents median; lower and upper error lines represents 10th and 90th percentiles. Medians followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test and ANOVA.

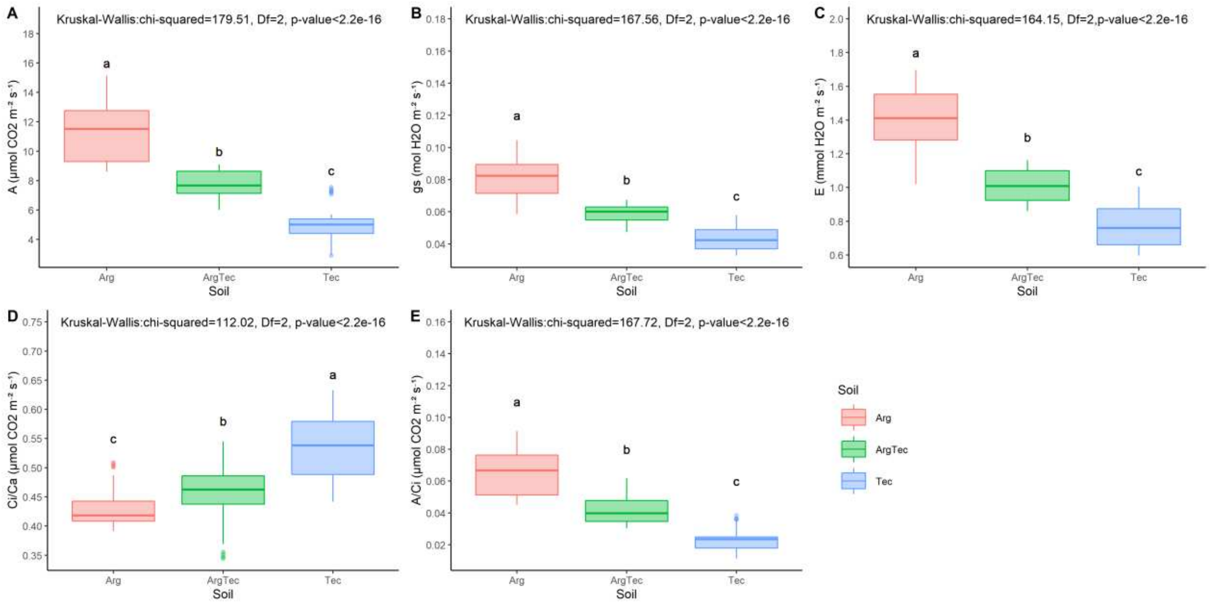

Figure 10: Boxplots of *Brachiaria decumbens* gas exchange cultivated in Ref, Ref/Tec and Tec. A - CO_2 net photosynthetic assimilation rate (*A*). B - Stomatal conductance (*gs*). C - Transpiratory rate (*E*). D - Ratio of internal

and external concentration of CO_2 (*Ci/Ca*). E - Carboxylation efficiency estimation of the rubisco enzyme (*A/Ci*). Lower and upper box boundaries represents 25th and 75th percentiles and the lines inside box represents median; lower and upper error lines represents 10th and 90th percentiles. Medians followed by the same letter do not differ statistically from each other at 5 % of probability by the Nemenyi test.

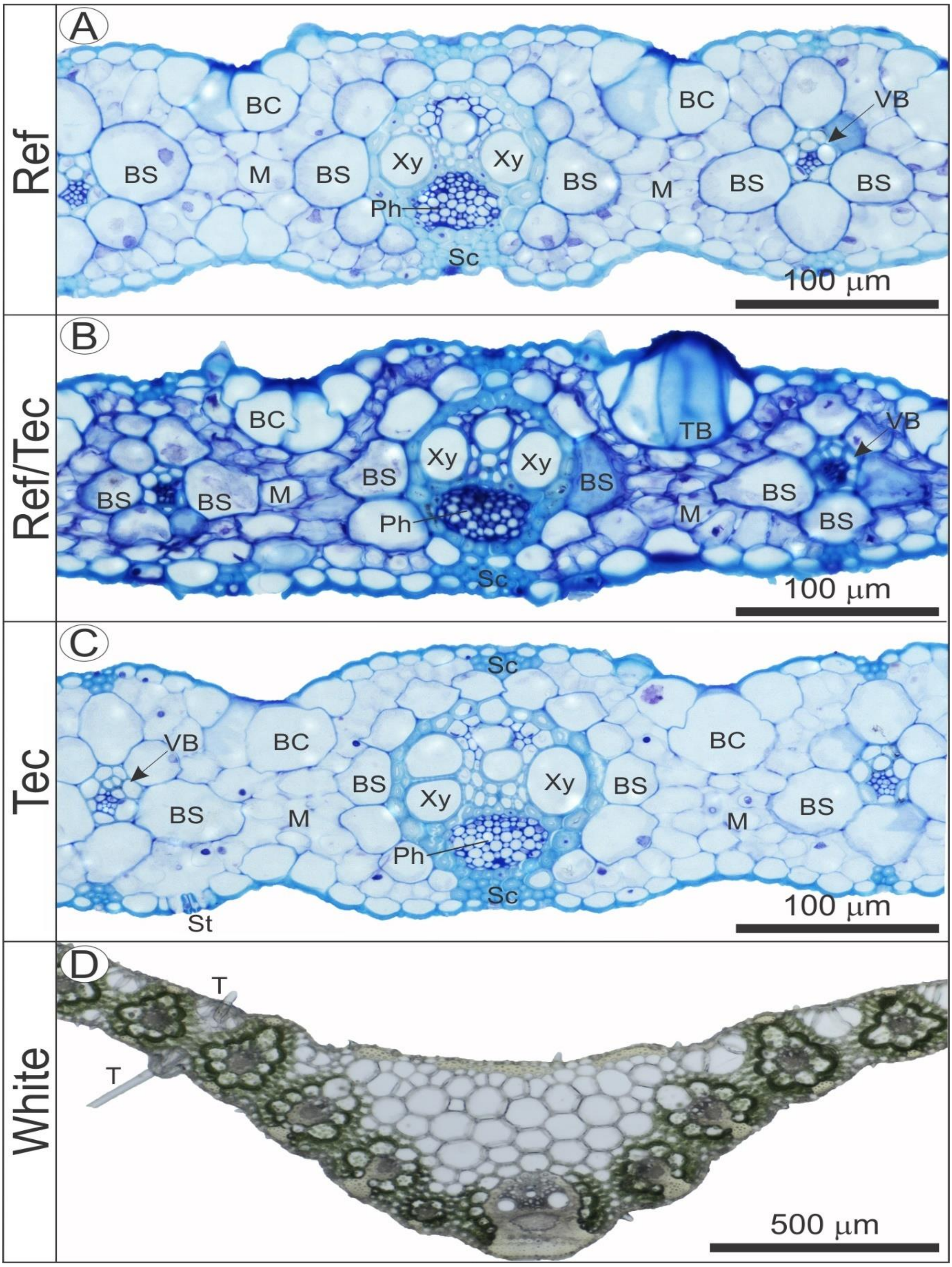

Figure 11: Leaf anatomy of *Brachiaria decumbens* cultivated in Ref, Ref/Tec and Tec. A - C. Cross sections stained with toluidine blue. The mesophyll and bundle sheath cells form concentric layers around the vascular bundle (Kranz anatomy). A. Leaf of plant grown in Ref where it is possible to observe cells with a round shape and a healthy aspect. B - C. Plants grown in Ref/Tec and Tec respectively, it is possible to observe the presence of flaccid and deformed cells with sinuous and angular cell walls. D - Sample of leaf sectioned transversely in a table microtome and not submitted to reagents (White), note the natural leaf color. BS, Bundle Sheath; BC, Buliform Cell; M, Mesophyll; Xy, Xylem; Ph, Phloem; VB, Vascular Bundle; TB, Trichome Base; St, Stomata, T, Trichome.

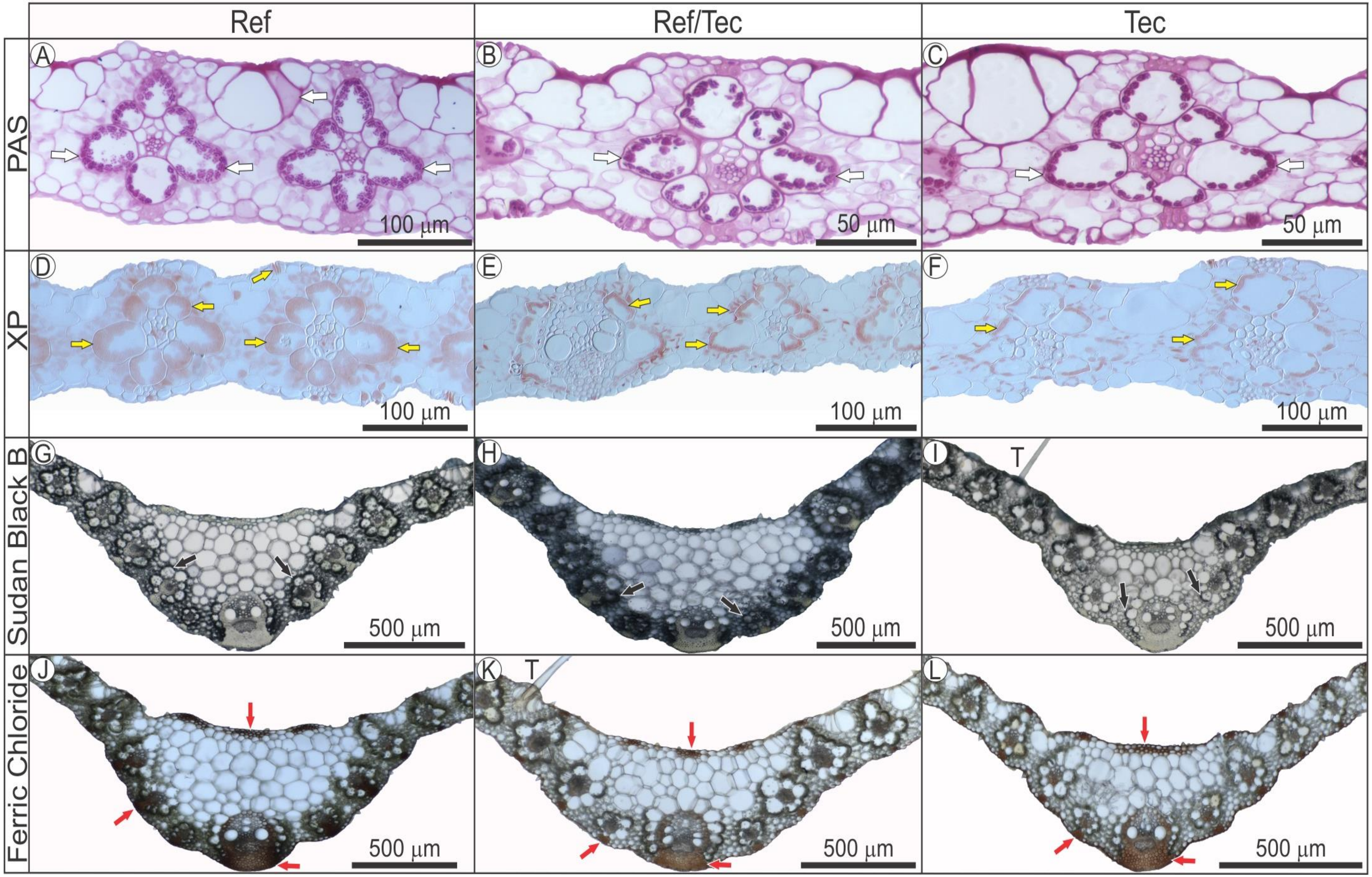
Ref
Ref/Tec
Tec
PAS
A
100 µm
B
50 µm
C
50 µm
XP
D
100 µm
E
100 µm
F
100 µm
Sudan Black B
G
500 µm
H
500 µm
I
T
500 µm
Ferric Chloride
J
500 µm
K
T
500 µm
L
500 µm

Figure 12: Leaf histochemical tests in *Brachiaria decumbens* cultivated in Ref, Ref/Tec and Tec. A - C. Periodic acid test - Schiff (PAS) with magenta-stained total polysaccharides. The centrifugal orientation of chloroplasts with transitory starch accumulation can be observed in bundle sheath cells (white arrows) and it is possible to observe the accumulation of polysaccharides in buliform cell (white arrow in A). D - F. Xylidine ponceau test (XP) with orange-stained proteins in bundle sheath chloroplasts (yellow arrows) and in stomata (yellow arrow in D). G - I. Sudan Black B test. The lipids stained from blue to black in chloroplasts of bundle sheath cells (black arrows). J - L. Ferric Chloride test. The phenolic compounds appear stained brown and black in sclerenchyma cell walls (red arrows) and in chloroplasts of bundle sheath cells. T, Trichome.

Figure 13: Root system architecture of *Brachiaria decumbens* roots cultivated in Ref, Ref/Tec and Tec after 110 days in a greenhouse.

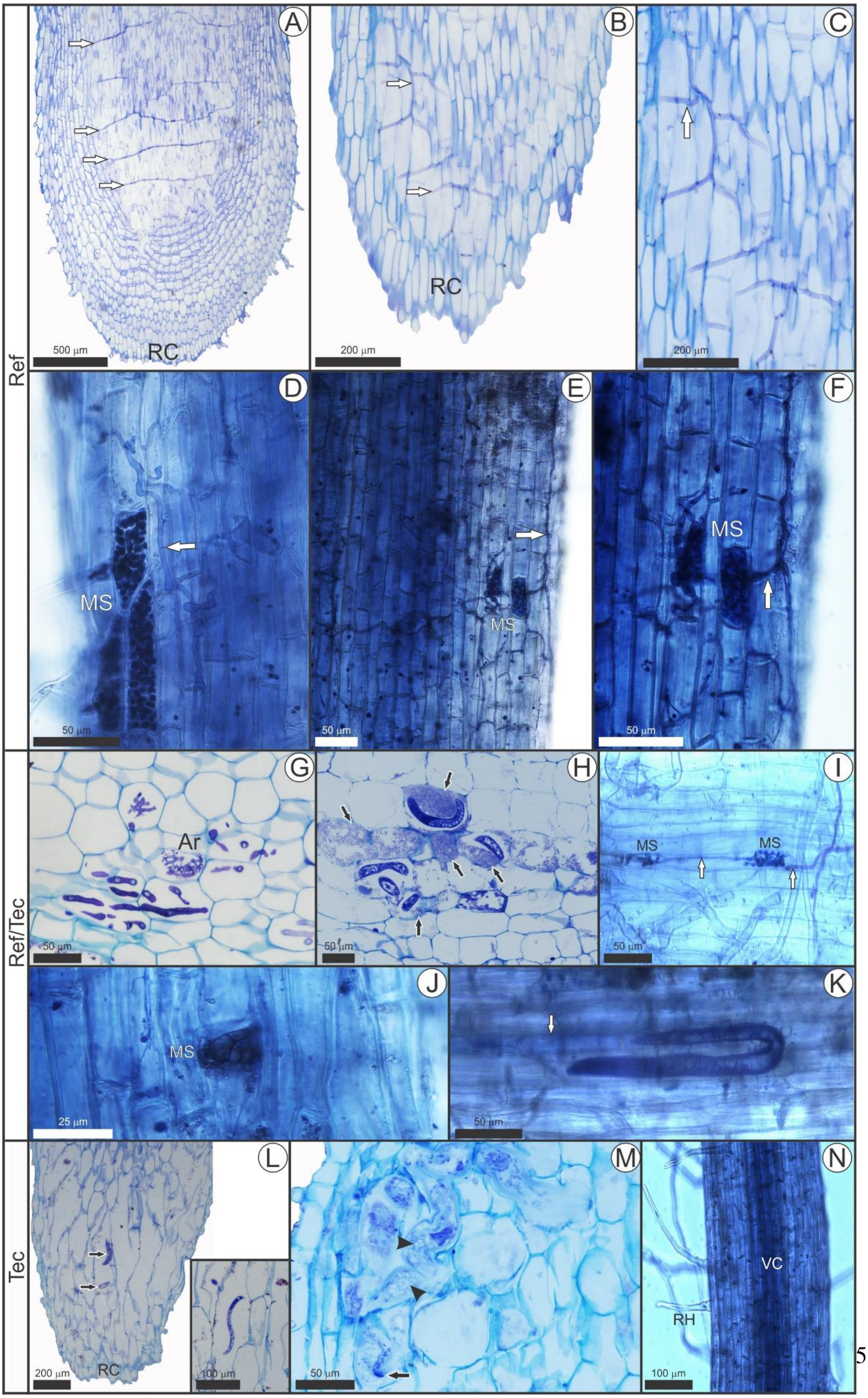
Ref
A
500 µm
RC
B
RC
200 µm
C
200 µm
D
MS
50 µm
E
MS
50 µm
F
MS
50 µm
Ref/Tec
G
Ar
50 µm
H
50 µm
I
MS
MS
50 µm
J
MS
25 µm
K
50 µm
Tec
L
RC
200 µm
100 µm
M
50 µm
N
VC
RH
100 µm

Figure 14: Plant root anatomy and mycorrhizal colonization analysis in *Brachiaria decumbens* cultivated in Ref, Ref/Tec and Tec. A - C. Longitudinal sections of root apex stained with toluidine blue from plants grown in Ref. It is possible to observe septate hyphae (white arrows). D - F. Roots cleared and stained with Trypan blue. It is possible to observe the presence of dark septate endophytic fungi (DSEF) associated with *B. decumbens* roots grown in Ref. It is possible to observe the septate hyphae (white arrows) and the presence of microsclerotia (MS). G - H. Longitudinal sections of root apex stained with toluidine blue from plants grown in Ref/Tec. It is possible to observe in G the presence of fungal arbuscles within a cell (Ar) and in H cells with cellular content for nematodes degradation (black arrows). I - K. Roots cleared and stained with Trypan blue from plants grown in Ref/Tec. In I and J it is possible to observe DSEF with septate hyphae (white arrows) and the presence of microsclerotia (MS). In K, it is possible to observe next to the nematode a cell with content for parasite degradation (white arrow). L - N. Longitudinal sections of root apex stained with toluidine blue from plants grown in Tec. In L and M the presence of nematodes within cells is observed (black arrows in L). In M it is possible to observe the moment when a nematode undergoes the degradation process (black arrow), note the cells full of content for the parasite's degradation (arrowheads). N - Root cleared and stained with Trypan blue from a plant grown in Tec. RC, Root Cap; MS, Microsclerotia; RH, Root Hair; VC, Vascular Cylinder; Ar, Arbuscle.