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Environmental drivers of shifts on microbial traits in sites disturbed by a large-scale tailing dam collapse

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Conflict interests

The authors declare no conflict interests

ABSTRACT: This study aimed to assess the most affected traits related to microbial ecophysiology and activity and investigate its relationships with environmental drivers in mine tailings spilled from the Fundão dam at disturbed sites across Gualaxo do Norte river, Minas Gerais, Brazil. The mine tailings are characterized by increased pH value, silt percentage, and bulk density, while clay percentage, organic carbon (C_{org}), total nitrogen (Nt), and moisture contents are reduced. Microbial biomass, enzymatic activities (arylsulfatase, β-1,4-glucosidase, acid and alkaline phosphatases), and the total microbial activity potential (FDA hydrolysis) were generally lower in tailings compared to undisturbed reference soil (Und). Enzyme-based indexes (GMea, WMean, and IBRv2) showed microbial communities with significantly lower degradative efficacy in the tailings than Und in all sites ($R^2 \geq 0.94$, $p < 0.001$). Non-metric multidimensional scaling and distance-based redundancy analysis revealed that microbial communities exhibited significant differentiation (R^2 adjusted = 0.73, $p = 0.0001$) between mine tailings and Und over the different studied sites, which was strongly influenced by changes on physicochemical properties (pH, C_{org} and Nt contents, the predominance of small-sized particles of silt, and bulk density) and the presence of Se, Cr, Fe, and Ni, even at low concentrations. Our study suggests that the physicochemical properties and the presence of low bioavailable concentrations of heavy metals in dam tailings promote shifts on microbial communities through reductions in the C storage and biogeochemical cycling of nutrients by these communities compared to those in undisturbed reference soils surrounding and, therefore, has negative implications for the ecosystem functioning.

Keywords: Microbial community changes; Enzymatic activity; Microbial activity; Ecosystem functioning; Mine tailings; Soil quality index.

1. Introduction

Recent researches have indicated several environmental impacts since Fundão dam has collapsed on November 2015, near to municipality of Mariana, Minas Gerais, Brazil, including the native vegetation loss (Aires et al., 2018; Carmo et al., 2017), increase on suspended solid material along the impacted rivers (Hatje et al., 2017), changes on estuarine macrofaunal assemblages (Pires et al., 2017), freshwater microbiomes (Cordeiro et al., 2019) and arbuscular mycorrhizal fungi diversity at disturbed soils (Prado et al., 2019). However, we still have a limited understanding of the main environmental factors modulating shifts on traits related to microbial ecophysiology and activity and its implications for the ecosystem functioning at disturbed sites. Microbial communities are critical to biogeochemical cycles and crucial for the functioning and sustainability of the ecosystem (Bardgett and Van Der Putten, 2014; Nielsen et al., 2015). Microbes play significant roles in maintaining multiple ecosystem functions, including organic matter (OM) decomposition, nutrient cycling, primary productivity and climate regulation (Bardgett and Van Der Putten, 2014; Schimel and Schaeffer, 2012; Sinsabaugh et al., 2009). Hence, the quick response of microbes to environmental changes makes it possible to know changes in ecosystem functioning, particularly in the environment of mine tailings (Li et al., 2016; Silva et al., 2018).

Mine tailings constitute a new environment for microbes due to small particle size, has no aggregate structure, low contents of OM and nutrient, particularly nitrogen (N), and generally present high levels of heavy metals (Mendez et al., 2008; Santos et al., 2016). For instance, the availability of the substrates is thought to be the basis for building up microbial biomass and enzymes (Delgado-Baquerizo et al., 2016; Mooshammer et al., 2014). Consequently, decreases in the OM content reduces nutrient availability, leading to stoichiometric and energetic constraints on the growth and activity of microbial communities (Silva et al., 2018; Sinsabaugh et al., 2009; Waring et al., 2014). This, in turn, might alter metabolic indexes that function as proxies for the microbial carbon use efficiency (metabolic quotient, $q\mathrm{CO}_2$) and microbial conversion of organic substrates into biomass (microbial quotient, *q*MIC) (Rui et al., 2016; Silva et al., 2018).

Excess of heavy metals is known to be toxic to soil microbes since are more sensitive than soil fauna or plants (Santos et al., 2016). Although levels of heavy metal in areas affected by Fundão dam tailings were expected to be high, it is now recognized that such levels are low along the Doce River basin due to being associated with Fe crystalline fractions (Guerra et al., 2017; Queiroz et al., 2018; Silva et al., 2016). In some studies, however, soil microbes were shown to be more influenced by other environmentally unfavorable factors than the excess of heavy metals itself. For instance, in some cases, reductions in microbial biomass were revealed to be more reliant on organic C, total nitrogen, or clay contents (Chodak et al., 2013; Stefanowicz et al., 2012), whereas in other cases, microbial respiration was more influenced by the moisture or organic C/sulfur ratio (Stefanowicz et al., 2012; Woch et al., 2018).

Likewise, the extracellular enzymes produced by microbes to degrade organic substrates are also affected by several environmental factors, including the availability of the substrates, pH, heavy metals, as well as the physical structure and texture of soils and sediments (Burns et al., 2013; Rui et al., 2016; Silva et al., 2018). For instance, the soil pH and resource limitation (e.g. OM) might strongly decline enzymatic activity (Sinsabaugh et al., 2009, 2008). Heavy metals have an important role on the partial or total inhibition of enzymes by reacting with integral parts of the active catalytic sites or groups involved in maintaining the correct structure of the enzyme (Fließbach et al., 1994; Li et al., 2009). On the other hand, physical structure and texture of soil might influence the microbial respiration rates, decomposition of organic matter, retention of nutrient and water, and consequently has an effect on the microbial biomass and enzyme activity through the supply of air, moisture, and nutrients for microbes (Bonan et al., 2013; Jiang et al., 2013; Rui et al., 2016; Wardle, 1992).

Besides microbial communities being sensitively altered by environmental disturbances, the assessment of environmental quality based on responses of individual traits of these communities may be challenging. For instance, the activities of different enzymes may differently respond to the fluctuations in environmental conditions, which difficult decision making based on appropriate conclusions about the effects of disturbances (Gao et al., 2013). A solution to this challenge may be the use of enzyme-based numerical indexes to assess the response of enzymatic activities in soils under environmental disturbances (Paz-Ferreiro and Fu, 2016). For instance, the geometric mean (GMea) and weighted mean (WMean) indexes have been widely accepted approaches to assess metal-contaminated soils (Hinojosa et al., 2004; Lessard et al., 2014; Lu et al., 2015). The integrated biological response index (IBRv2 index), proposed by Sanchez et al. (2013), integrates the response of multiple biomarkers and have been used as a measurement on the environmental quality in aquatic systems (Serafim et al., 2012), oil-contaminated marine ecosystems (Marigómez et al., 2013), and chlorpyrifos-treated soils (Sanchez-Hernandez et al., 2017).

In this study, we hypothesized that mine tailings would significantly shift microbial ecophysiology and activity in areas disturbed by the Fundão dam collapse. To test this hypothesis, we investigated several microbial traits, namely microbial biomass, basal respiration, metabolic (qCO$_2$) and microbial (qMIC) quotients, and enzymatic activity. Additionally, we investigated the main environmental factors modulating shifts on microbial traits and applied enzyme-based indexes of soil quality to evaluate the impacts of mine tailings on the biochemical processes mediated by microbes.

2. Materials and methods

2.1 Study area and sampling

This study was carried out in sites located along the Gualaxo do Norte River in the state of Minas Gerais, Brazil (Fig. 1). The region climate is classified as Cwa according to the Köppen-Geiger climate classification, with a rainy season between June and December, the annual average temperature of 19 °C and an annual average rainfall of 1,375 mm (Alvares et al., 2013). We chose the following three disturbed sites across Gualaxo do Norte river: site A (20°16´0.034" S; 43°18'14.177" W) is a *Eucalyptus* plantation, while the sites B (20°17'47.916" S; 43°12'18.584" W) and C (20°16'22.272" S; 43°12'4.293" W) are native forests (Fig. 1). These sites were chosen as being representative of the impacts of dam tailings deposition on the river banks and background soil. At each site samples were collected in three conditions according to disturbance level (Directly impacted – Di; Partially impacted – Pi; and Undisturbed reference soil – Und) such as characterized in Table 1. Sampling was carried out in December 2017, two years after the disaster. A 25-m transect was established at each studied condition. At five equally spaced sampling points along the transect (5 m from each other), five subsamples of soil and tailings were randomly taken from the surface layer (0-10 cm) and pooled to obtain one composite sample per point. This produced 15 composite samples for each site, which totaled 45 samples. In the laboratory, field moist samples to physicochemical characterization were air-dried and sieved at 4 mm mesh, and the samples for biological analyses were freshly sieved at 2 mm mesh and stored under refrigeration in sterile, hermetically sealed plastic bags until analyses.

2.2 Physicochemical properties of samples

Soil and tailings samples pH was measured in deionized water solution (1: 2.5 v/v) using a glass electrode and moisture was determined using the gravimetric method by drying samples to constant weight at 65 °C as described in Embrapa (2011).

Extractable iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni), and selenium (Se) were extracted from samples by Mehlich-1 extractor and determinated in an ICP-OES analyzer, except for Se, which was measured using atomic absorption spectrometry. Organic carbon (C_{org}) content was determined by the dichromate digestion, followed by colorimetric analysis (Sparks et al., 1996). Total N (Nt) content was determined by the Kjeldahl digestion method after sample extraction with KCl (Bremner, 1960). Bulk density was determined according to the core method (Grossman and Reinsch, 2002). The texture corresponding fraction (sand, 2.0–0.02 mm; silt, 0.02–0.002 mm; clay, <0.002 mm) was determined by the Bouyoucos method after removal of organic matter with H_2O_2 and dispersion of samples with hexametaphosphate sodium (Bouyoucos, 1962). Three blank samples only containing the dispersing solution were used for each batch of samples for quality control purposes and the temperature was corrected accordingly (Bouyoucos, 1962).

2.3 Ecophysiological traits of microbial community

The microbial biomass C (MBC, µg C g^{-1}) was determined following the fumigation-extraction method using samples equivalent to 20 g fresh weight shaken in 100 ml of 0.5 M K_2SO_4 for 30 min (Vance et al., 1987). Unfumigated samples were also extracted following the same procedure and used as controls. The oxidisable carbon in the K_2SO_4 extracts was estimated by titration of potassium dichromate excess with 33.3 mM ammonium ferrous sulfate. MBC was calculated from the difference between the organic C extracted from fumigated and control samples, multiplied by a k_{EC} factor of 2.64 (Vance et al., 1987). Microbial basal respiration (BR, µg C–CO_2 g^{-1} 72 h^{-1}) was determined by measuring the evolved CO_2 from samples (Alef, 1995). Briefly, samples of 20 g (equivalent to fresh weight) were placed in glass bottles of 1 L in the presence of 0.05 M NaOH for 72 h at 25 °C, and the cumulative CO_2 was determined by titration of

NaOH solution with 0.05 M HCl. Metabolic quotient (qCO$_2$, µg C–CO$_2$ µg^{-1} MBC 72 h^{-1}) was calculated from the BR: MBC ratio (Insam and Haselwandter, 1989). Microbial quotient (qMIC, %) was calculated from the MBC: C$_{org}$ ratio (Anderson and Domsch, 1989).

2.4 Extracellular enzymatic activity assays

Enzymatic activities were determined colorimetrically based on ρ-nitrophenol release after incubation of buffered substrates. The pH of the buffers was adjusted to match the pH of each enzyme assay. The substrates of tested enzymes included ρ-nitrophenyl sulfate for arylsulfatase (Aryl, EC 3.1.6.1) (Tabatabai and Bremner, 1970), ρ-nitrophenyl-phosphate for both acid (AcP, EC 3.1.3.2) and alkaline (AlkP, EC 3.1.3.1) phosphatases (Eivazi and Tabatabai, 1977), and ρ-nitrophenyl-β-D-glycoside for β-1,4-glucosidase (BG, EC 3.2.1.21) (Eivazi and Tabatabai, 1988). For each enzyme assay, soil and tailings samples of 1 g (equivalent to fresh weight) were mixed with 4 ml of buffer and 1 ml of the substrate following a 1 h incubation at 37 °C. After incubation, 1 ml of 0.5 M CaCl$_2$ and 4 ml of 0.1 M Tris (hydroxymethyl) aminomethane (THAM, pH 12) was added to the supernatant to stop the reaction of BG, whereas, for the other enzymes, the same amount of CaCl$_2$ was added with 4 ml of NaOH and the absorbance was measured on a spectrophotometer at 410 nm after samples filtering.

Total microbial activity potential was determined based on the hydrolysis of 3',6'-diacetyl fluorescein (FDA) using samples of 2 g (equivalent to fresh weight) shaken with 40 ml buffer in 50 ml Falcon conical tubes (Corning, Tamaulipas, Mexico) for 24 h at 35 °C (Schnürer and Rosswall, 1982). After incubation, 2 ml of solution acetone: water (50% v/v) was added to the supernatant to stop the reaction and samples were filtered after tubes centrifugation at 3500 rpm for 6 min. The fluorescein released in supernatant was measured on a spectrophotometer at 490 nm. Controls were performed for each assay as described above, except for the addition of the substrate, that was made after termination of reactions. Three technical replicates were used for the slurry in all assays. Extracellular enzymatic activities were quantified in the unit of µg ρ-nitrophenol g^{-1} h^{-1} and the total microbial activity potential in mg fluorescein g^{-1} 24 h^{-1}.

2.5 Enzyme-based indexes of soil quality

We applied three numerical indexes to assess the impact of tailings on integrated responses of enzymatic activities of microbial communities: the geometric mean of enzyme activity (GMea index) (Hinojosa et al., 2004), the weighted mean index (WMean index) (Lessard et al., 2014), and the integrated biological response index (IBRv2 index) (Sanchez et al., 2013).

GMea index was calculated as follows:

$$\text{GMea} = \left(\prod_{i=1}^{n} y_i\right)^{\frac{1}{n}}$$

where y_i is the enzymatic activity, n is the total number of studied enzymes.

WMean index calculation was performed as follows:

$$\text{WMean} = \sum_{i=1}^{n} w_i \times y_\text{i}$$

where y_i is the enzymatic activity, n is the total number of studied enzymes, and w_i is the weight of each enzyme calculated as follows:

$$w_i = \frac{\text{Ʊ}_i}{\sum_{i=1}^{n} \text{Ʊ}_i}$$

where Ʊ_i is the eigenvector for each enzymatic activity associated with the first principal component obtained from the principal component analysis. GMea and WMean indexes were expressed in units of µg product g^{-1} h^{-1}.

IBRv2 index was calculated in three steps. In the first step, the individual enzymatic activity values (X_i) are compared to mean reference values (X_0) and log-transformed to reduce variance:

$$Y_i = \log\left(\frac{X_i}{X_0}\right)$$

The second step follows the standardization of Yi based on the general mean (μ) and standard deviation (s) for each enzymatic activity:

$$Zi = \frac{(Y_i - \mu)}{s}$$

The variation in enzymatic activity is then represented as a basal line centered on 0 using the standardized response of enzymatic activity (Z_i) and the mean of reference enzyme data (Z_0):

$$A_i = Z_i - Z_0$$

Compared to reference values, positive A_i–scores indicate an increase of enzymatic activity and negative A_i–scores indicate inhibition of enzymatic activity. We plotted the A_i–scores using sunray plots for a visual inspection of all enzymatic responses at each studied site. Finally, the IBRv2 index was obtained from the sum of variation (A_i) in enzymatic activity between the undisturbed reference soils and the tailings as follows:

$$IBRv2 = \sum_{i=1}^{n} |A_i|$$

where A_i is the deviation of ith enzymatic activity and n is the total number of studied enzymes.

2.5 Statistical analyses

All analyses were performed using the R statistical environment (R Core Team, 2019). Linear mixed-effects models (LMM) were estimated with a random intercept for each transect to take account of the dependence among sampling points within transect and included condition, site, as well as their interaction terms as fixed effects using the *nlme* package (Pinheiro et al., 2019). We verified model assumptions by visual inspection of the residual plots for homogeneity of variance and quantile-quantile for normality (Kozak and Piepho, 2018). Data were root- or log-transformed when necessary to meet the model assumptions. To evaluate the model accuracy, the marginal R^2 (variance explained by the fixed effects) and the conditional R^2 (variance explained by the entire model, including fixed and random effects) were calculated for each microbial trait using the *MuMIn* package (Barton, 2019). After verifying the significance of the model parameters, the significance of treatment contrasts was assessed with the Tukey *post-hoc* tests ($p < 0.05$) in the *emmeans* package (Lenth, 2019). Tukey tests were also applied to GMea and WMean indexes to comparing tailings to the respective undisturbed reference soils within each site. To investigate the relationships between environmental factors and microbial traits, a correlation matrix on the scaled variables was constructed using the Spearman rank-order correlation coefficient (ρ) in the *corrplot* package (Wei and Simko, 2017). Gower dissimilarity matrices were used to perform non-metric multidimensional scaling (NMDS) and visualize the differences in microbial traits among the studied conditions in each site using the *vegan* package (Oksanen et al., 2019). Permutational analysis of variance (PerMANOVA) determined the significance of these differences after 9,999 permutations in the *vegan* package (Anderson, 2001). The *p*-values were subjected to Bonferroni adjustment for multiple comparisons and reported only if $p < 0.05$ after correction. Euclidean matrices were used to perform a distance-based redundancy analysis (db-RDA) to identify the most important environmental drivers promoting shifts on microbial traits in the *vegan* package based on Monte Carlo tests with 9,999 full permutations (Legendre and Anderson, 1999).

3. Results

3.1 Physicochemical properties of samples

The physicochemical properties and extractable heavy metal contents for the surface layer (0-10 cm) are available in Table 2. Overall, all the tailings analyzed samples have presented a pH value near to neutrality, varying between 6 and 8, while undisturbed reference soils (Und) had acidic values between 3 and 5. The tailings have substantially reduced C_{org} and Nt contents, while the C:N ratio is variable. Moisture percentage is low in tailings and vary between 8 and 12%. The mine tailings were characterized by low and variable extractable contents of Fe, Mn, and Ni. Extractable Cu is higher in mine tailings than in Und at sites A and C, while Zn and Pb are lower in all the studies sites. Se and Cr are low in Und, except for Cr, that was not detected in samples of sites B and C. In all sites, the mine tailings are characterized by the predominance of small-sized particles of silt, which is at least 4-fold that in Und at sites B and C and 1.5-fold at site A (Fig. S1). The sand percentage in the tailings is generally $\geq$ 50%, while clay percentage is reduced (Fig. S1). Mine tailings present high bulk density at sites A and B (Table 2). The high predominance of small-sized particles increases the bulk density in tailings over the different sites compared to its respective Und ($R^2 = 0.02$, $p = 0.002$, Fig. S2).

3.2 Ecophysiological traits of microbial community

The most of microbial traits were significantly influenced by both condition and site factors (R^2_m values ≥ 0.5, Table S2). In all sites, microbial biomass C was lower in disturbed conditions (tailings) than undisturbed reference soils (Und), but Pi and Und of sites B and C exhibited higher values compared to that in site A (Fig. 2a). The highest microbial respiration rates were observed in both disturbed conditions of site A compared to Und and the same conditions at other sites, whereas decreased rates were observed in disturbed conditions at sites B and C compared to Und (Fig. 2b). A similar pattern was observed for the $q\mathrm{CO}_2$ in both disturbed conditions of site A, which was also higher in Di of site C (Fig. 2c). No effect of condition or site was observed for the *q*MIC (Fig. 2d).

3.3 Extracellular enzyme activities

Arylsulfatase activity was invariable in site A and declined in disturbed conditions of sites B and C (Fig. 3a). Besides, its activity in disturbed conditions of sites A and B were higher than that in site C. β-1,4-glucosidase activity reduced in disturbed conditions of sites A and B but did not differ in site C (Fig. 3b). However, site C presented higher activity in the disturbed conditions compared to site B. Acid phosphatase activity reduced in disturbed conditions of all sites, although it has been higher in Di of the sites A and C compared to the same condition in site B (Fig. 3c). Alkaline phosphatase activity was lower in the disturbed condition of site A compared to Und, and compared to the same condition of sites B and C (Fig. 3d). However, compared to site A, the sites B and C presented the higher activity of alkaline phosphatase in Pi condition. Total microbial activity potential, measured as FDA hydrolysis, was lower in Di of site B and both disturbed conditions of site C, but Di at site A, whereas Und at site C was higher compared to the same condition of site A and B (Fig. 3e).

3.4 Enzyme-based indexes of soil quality

The integrated response of enzymatic activities was assessed by three enzyme-based numerical indexes, GMea, WMean, and IBRv2. The GMea index decreased significantly in the mine tailings at all sites (Fig. 4a, Table S2). The principal component analysis explained 75% of the variance in the data (48% in PC1 and 27% in PC2) and its associated eigenvectors were used to calculate the WMean index (Table S3). Similarly, this index decreased significantly in mine tailings relative to Und over the different sites (Fig. 4a). The IBRv2 index exhibited high values [site A (Di = 19.36, Pi = 20.27), site B (Di = 17.26, Pi = 12.71), and site C (Di = 13.90, Pi = 12.46)], indicating a strong inhibition of all enzyme activities in the mine tailings (Ai–scores values below the zero line, Fig. 4b). Sunray plots of Ai–scores calculated from the enzymatic activities provided a visual illustration of the overall effect of Fundão dam tailings on enzyme activities. For both disturbed conditions at site A (i.e. Di and Pi), all enzymes presented high inhibition patterns varying from 6-fold (BG, AcP, AlkP, and FDA) to 2-fold (Aryl) lower than in Und. On the other hand, in Di condition of site B, all enzymes exhibited inhibition pattern 4-fold lower relative to Und, while in Pi the higher inhibition patterns were observed for both AcP and FDA (5-fold compared to Und). Site C presented a strong inhibition for Aryl, AlkP, and FDA in both disturbed conditions (> 4-fold lower than in Und), whereas the activities of BG and AlkP had little inhibition (Fig. 4b).

3.5 Correlations between environmental factors and microbial traits

The most traits related to microbial ecophysiology and activity were positively correlated with each other, C_{org}, Nt, and moisture (Fig. 5). Despite these positive correlations, all the microbial traits were strongly negatively influenced by the pH, extractable Ni, Cu, Mn, Fe, Se, and Cr contents, bulk density, and silt percentage. However, extractable Ni and Cu had a weak positive effect on β-1,4-glucosidase activity. The C:N ratio positively correlated to the β-1,4-glucosidase, alkaline phosphatase, and FDA hydrolysis, but negatively with *q*MIC. The $q\mathrm{CO}_2$ was negatively correlated with microbial biomass, acid and alkaline phosphatases activities, C_{org} and Nt contents. The *q*MIC was negatively correlated with arylsulfatase, β-1,4-glucosidase and FDA hydrolysis. Both qCO_2 and *q*MIC were negatively influenced by organic C and total N contents.

3.6 Changes on traits of microbial community

The non-metric multidimensional scaling (NMDS) ordination of samples based on all the microbial traits showed a clear separation of samples among the studied conditions (Fig. 6). PerMANOVA showed significant differences in microbial traits among mine tailings and undisturbed reference soils in all the studied sites across the Gualaxo do Norte river (Table 3). The distance-based redundancy analysis (db-RDA) indicated that changes in physicochemical properties and extractable heavy metals contents (R^2 adjusted = 0.73, $p = 0.0001$) were the main dimension to the microbial traits (Fig. 7), showing a close relationship between the physicochemical properties (i.e. pH, silt, and BD) and extractable content of heavy metals (i.e. Se, Cr, Fe, and Ni) in tailings and the shifts on traits related to ecophysiology and activity of the microbial community. Except for bulk density, pH, extractable Se, Cr, Fe, and silt percentage, which presented the highest negative loadings on the first axis, all the other environmental variables presented positive loadings and were positively associated with undisturbed reference soils (Table 4). Extractable Ni and C:N ratio presented high negative loadings for the second axis.

4. Discussion

4.1 Impact of tailings on microbial ecophysiology

Our study provides evidence that the mine tailings spilled from the Fundão dam caused significant shifts in the microbial traits. The negative relationships between microbial biomass and extractable Ni, Cu, Mn, Fe, Se, and Cr contents confirm the sensitivity of microbes to heavy metal presence, even in low concentrations (Fig. 5).

This adverse effect was unexpected in our study as concentrations of these elements in mine tailings of the Fundão dam are considered low (Guerra et al., 2017; Queiroz et al., 2018; Silva et al., 2016). Despite those negative relationships, db-RDA analysis revealed that decreases on microbial biomass were more importantly related to pH value ($R^2 = 0.75$, $p < 0.001$) and silt percentage ($R^2 = 0.72$, $p < 0.001$) that had, in turn, relatively higher negative loadings on the first axis (−0.87 and −0.84, respectively) than extractable heavy metals contents (Fig. 7, Tables 4 and S4). Our results also indicate an associated effect of other environmental unfavorable factors that might affect microbes in mine tailings. For instance, organic matter acts as a source of nutrients and energy for soil microbial communities (Schimel and Schaeffer, 2012). Consequently, decreases in organic matter content reduce the availability of organic substrates and nutrients, which results in stoichiometric and energetic limitations for the growth and activity of microbial communities (Silva et al., 2018; Sinsabaugh et al., 2009; Waring et al., 2014). Such limitations, in turn, are critical for the carbon storage into the mine tailings in disturbed areas of our study since microbial-derived necromass was shown to be an important precursor of organic matter formation and stabilization in the soil (Kallenbach et al., 2016; Liang et al., 2017; Miltner et al., 2012). Clay content also had an important positive effect on microbial biomass in Und over the different studied sites through their ability to retain organic matter and supply of air, moisture, and nutrients for microbes, since for these soils the correlations between C_{org}, Nt or moisture and clay content were positive and associated to undisturbed reference soils for all sites (Figs. 5 and 7). This was also observed by previous studies (Jiang et al., 2013; Rui et al., 2016; Wardle, 1992).

Microbial respiration rates are determined by the biomass and activity of the soil microbes (Delgado-Baquerizo et al., 2016; Schimel and Schaeffer, 2012).

Consequently, factors that influence microbial biomass might exert substantial influence on other microbial traits (Wardle, 1992). Similar to biomass, microbial respiration decreased in the tailings at sites B and C in response to the same environmental factors, which suggest a reduction in the activity of the heterotrophic community in these sites (Figs. 2b and 5). This result suggests that under relatively low bioavailable heavy metal contents and resource limitation (e.g. C_{org}, Nt, Table 2), a proportionally lower microbial activity allow limited net mineralization of organic substrates in mine tailings, resulting in lower microbial biomass (Delgado-Baquerizo et al., 2016; Malik et al., 2018). Consequently, reductions in microbial biomass and activity directly affect microbial contributions to the restoration process in an ecosystem of mine tailings, as microbes influence soil carbon storage and turnover, nutrient (re)cycling, and primary productivity (Bardgett and Van Der Putten, 2014; Delgado-Baquerizo et al., 2016; Schimel and Schaeffer, 2012). Furthermore, pH negatively influenced microbial respiration rates in our study (Fig. 5), which highlights an important role of this environmental factor on microbial ecophysiology (Malik et al., 2018). Moreover, silt percentage had higher importance in determining the microbial respiration rates ($R^2 = 0.72$, $p < 0.01$) than bulk density ($R^2 = 0.32$, $p < 0.01$, Fig. 5, Table S4). Indeed, several studies have shown the influence of physical structure and texture of soils on respiratory flow by heterotrophic microbes (Jiang et al., 2013; Rui et al., 2016), which might explain the lower microbial respiration associated to mine tailings with high slit percentage, particularly at sites B and C (Figs. 5, 7, and S1).

On the other hand, microbial respiration increased in the tailings at site A, resulting in higher losses of microbial C, as suggested by the increase in metabolic quotient – $q\mathrm{CO}_2$ (Fig. 2c). The $q\mathrm{CO}_2$ is often used as a proxy of carbon use efficiency by microbes, as well as an indicator of ecosystem stress, and is believed to be increased under stressful conditions (Anderson and Domsch, 1993; Insam and Haselwandter, 1989). The negative correlations between $q\mathrm{CO_2}$ and microbial biomass, C_{org}, and Nt contents support the evidence that microbial C losses associated with resource limitation reduces microbial biomass and suggests the interdependence of resource availability and microbial growth efficiency (Malik et al., 2018). Such microbial losses can reduce the pool of carbon in ecosystems under disturbance since microbial growth efficiency controls the proportion of carbon stabilized in the soil versus that released as CO_2 to the atmosphere (Delgado-Baquerizo et al., 2016; Liang et al., 2017; Malik et al., 2018). In contrast, positive correlations of $q\mathrm{CO_2}$ with pH and extractable Cr, Se, Cu, and Ni contents reveal a stressful environment for microbes, responsible for significant differences in microbial traits among mine tailings and Und (Figs. 5 and 6, Table 3). It is plausible that under such a scenario of resource limitation, coupled with near-neutral pH value and the presence of heavy metals, even at low concentrations, microbes have invested in physiological strategies that allow them to survive in a stressful environment by shifting their growth metabolism to maintenance respiration (Malik et al., 2018, 2017; Parsons and Smith, 1989).

The microbial quotient (*q*MIC) refers to the potential of the microbial community to convert organic substrates into biomass (Anderson and Domsch, 1989). Values below 2.0% could be considered as critical for soils with a neutral soil pH (Anderson, 2003). In our study, *q*MIC had values below 2%, except for Di (3.2%) and Pi (2.4%) at site B and Pi (3.4%) at site C (Fig. 2d). However, even in values higher than Und, microbial conversion of organic substrates is limited in mine tailings by unfavorable factors. For instance, this microbial trait was negatively correlated with C_{org}, Nt, C:N ratio, β-1,4-glucosidase and acid phosphatase activities, and FDA hydrolysis (Fig. 5). Such evidence demonstrates that resource limitation in the mine tailings contributes to lower incorporation of organic substrates into microbial biomass. Similar to previous studies, our results demonstrate that microbial communities rely strongly on enzymes for organic substrates acquisition and subsequent synthesis of their biomass (Kallenbach et al., 2016; Sinsabaugh et al., 2009).

4.2 Impact of tailings on enzyme activities

Overall, enzyme activity presented variable sensibility to disturbance of mine tailings (Fig. 3). This shows that enzyme activities generally have different responses to fluctuations in environmental conditions, particularly to different heavy metals (Gao et al., 2013; Li et al., 2009). Furthermore, once released into the environment, the microbes have little control over the functions and fates of enzymes (Burns et al., 2013; Sinsabaugh et al., 2009, 2008). Enzymatic activities are affected by the soil pH, presence of heavy metals, and the physical structure and texture of soils and sediments (Allison and Vitousek, 2005; Santos et al., 2016; Sinsabaugh et al., 2008). After conducting correlation and db-RDA analyses, we found that physicochemical properties (e.g. pH, silt, BD) and extractable heavy metals contents, except for the Zn and Pb, were the main drivers of inhibition in enzyme activities and total microbial activity potential in the mine tailings than resource limitation (Figs. 5 and 7). These findings apply to all the enzymes measured in our study and suggest that the biogeochemical processes by microbes are widely affected in disturbed areas by the Fundão dam collapse. Heavy metals are the main responsible to inhibit enzyme activities by complexing the substrate, or by reacting with the enzyme–substrate complex (Fließbach et al., 1994; Li et al., 2009). As a result, the inhibition of enzymatic activities can impair plant development and restoration of disturbed ecosystems in the long-term since (re)cycling and release of carbon and nutrients by microbes become proportionally reduced (Delgado-Baquerizo et al., 2016; Sinsabaugh et al., 2009, 2008). On the other hand, although the hydrolases activities (e.g. β-1,4-glucosidase, acid/alkaline phosphatases) were found to be more closely related to the availability of organic substrates (Sinsabaugh et al., 2008), our study also reveals that pH of mine tailings was an important environmental predictor modulating enzymatic responses (Figs. 5 and 7). This shows that the decline in enzyme activities is also related to the optimum pH range, which may affect the substrate availability, enzyme-substrate complex stability, and promoting partial or irreversible inactivation of the enzyme or a combination of these effects (Bell et al., 2013; Frankenberger and Johanson, 1982; Sinsabaugh et al., 2008). This evidence comes from negative correlations between all enzyme activities and pH (Fig. 5) as well as the NMDS and db-RDA analyses, that showed that shifts in enzyme activities were associated to changes in the pH of the mine tailings relative to undisturbed reference soils (Figs. 6 and 7, Table 2). Furthermore, the lower clay and organic matter contents in the mine tailings were problematic due to the risk of pH denaturation. This is compatible with the inhibition pattern observed in our study and agrees with classical evidence which suggests that the adherence of the enzymes to the humic-clay fractions would allow some resistance to pH denaturation (Frankenberger and Johanson, 1982; Leprince and Quiquampoix, 1996).

4.3 Enzyme-based indexes for environmental quality assessment

Our study extends the use of the enzyme-based indexes beyond the soil pollution with their application as a robust tool for assessment of environmental quality based on microbial degradative efficacy in mine tailings spilled from Fundão dam. Many studies have shown the effectiveness of the GMea and WMean indexes as indicators of soil pollution (Hinojosa et al., 2004; Lessard et al., 2014), soil contamination by oil spilling (Gao et al., 2013), heavy metals (Lu et al., 2015), and chlorpyrifos-treated soils (Sanchez-Hernandez et al., 2017). In our study, these indexes provided equivalent results, highlighting the significantly negative impact of the mine tailings on the biogeochemical processes mediated by microbes (R^2_m GMea = 0.949, R^2_m WMean = 0.951, $p \leq 0.001$, Table S2). In general, mine tailings reduced global enzymatic activity in a range varying between 44–75% compared to undisturbed reference soils at all studied sites (Fig. 4a). This resulted in decreased microbial biomass ($R^2 = 0.98$, $p < 0.0001$) which reveals, in turn, a reduced ability of these communities to break down organic substrates at mine tailings (Fig. S4). In other words, these findings suggest low degradative efficacy of microbial communities in mine tailings, where the influence of adverse environmental conditions might lead to the lowest biomass and activity of bacteria and fungi, modulated by the alterations in the physicochemical environment for microbes (Lessard et al., 2014; Santos et al., 2016; Silva et al., 2018).

The IBRv2 index provides both a graphical illustration of the responses for different enzymes as well as a value that integrates all these responses and distinguish between increase, inhibition, or no change relative to reference values (Sanchez et al., 2013). The possibility of plotting these scores in a sunray plot enables a quick visual inspection of the type of response of each enzyme (Sanchez-Hernandez et al., 2017). In our study, the negative A_i–scores of this index indicated a strong inhibition of all enzymes in the mine tailings (A_i–scores below the zero line) at site A, whereas β-1,4-glucosidase was less severely inhibited in Di condition at site B and both β-1,4-glucosidase and alkaline phosphatase in Pi condition at site C (Fig. 4b). These findings highlight the integrated biological response as a robust indicator of environmental disturbances assessment such as the study of temporal variation of environmental quality in aquatic systems (Serafim et al., 2012), oil-contaminated marine ecosystems (Marigómez et al., 2013), and chlorpyrifos-treated soils (Sanchez-Hernandez et al., 2017).

5. Conclusions

This study provides an insight into the importance of microbial communities in restoring a productive and functional ecosystem in areas disturbed by the Fundão dam collapse. Our findings indicate that the reduced capacity of microbial communities to store C (biomass synthesis) in the tailings is linked to the low resource availability existing that, in turn, is responsible for restrictions in the substrate availability for microbial growth and activity. Moreover, the high pH value, bulk density, and predominance of small-sized particles (especially the silt) of mine tailings, also has high importance in modulates the incorporation of organic C into the microbial biomass and act as the important environmental drivers of changes in microbial traits. An important finding to emerge from this study is that microbial traits sensitively respond to heavy metals at relatively low concentrations in mine tailings. Consequently, all these environmental factors decrease the biomass and activity of these communities, leading to reduced rates of important processes (e.g. litter decomposition, nutrient cycling) and sustainability of the ecosystem in the long-term.

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Table 1. Description of the conditions for the different study sites

Sites	Condition	Description
Site A	Di	Vegetation with poorly overgrowing in the tailings
	Pi	Without understorey, some grasses, low litter content
	Und	*Eucalyptus* plantation (*Eucalyptus grandis*)
Site B	Di	Voluntary growth of grass and shrub species and presenting process of natural regeneration
	Pi	Vegetation is underdeveloped, with some shrub species and few kinds of grasses and influence of vegetation in their Und
	Und	Native forest with typical regional species
Site C	Di	Area under a revegetation process with seed mix (Table S1). Present grasses in different degrees of development and some shrubs
	Pi	Vegetation consists of undeveloped understory with low litter content and influence of vegetation in their Und.
	Und	Native forest with typical regional species

Di, directly impacted; Pi, partially impacted; Und, undisturbed reference soils

Table 2. Average values of physicochemical properties and extractable content of heavy metals for the surface layer (0-10 cm) from different study sites

Properties	Site A			Site B			Site C		
	Di	Pi	Und	Di	Pi	Und	Di	Pi	Und
pH (H_2O)	6.72	7.18	5.32	8.32	6.60	4.84	8.04	6.00	3.82
S.E.	0.18	0.13	0.11	0.12	0.25	0.12	0.28	0.22	0.04
C_{org} (g kg^{-1})	3.06	2.6	8.48	1.73	5.01	19.4	7.94	4.14	19.2
S.E.	0.33	0.56	1.26	0.24	0.68	1.19	4.96	1.0	0.83
Nt (g kg^{-1})	0.34	0.32	0.76	0.53	0.54	2.69	0.34	0.34	2.41
S.E.	0.04	0.02	0.02	0.07	0.07	0.12	0.03	0.04	0.04
C:N	9	8	11	3	9	7	21	13	8
S.E.	1.8	1.7	1.6	0.64	0.89	0.3	12.1	3.3	0.23
Moisture (%)	12.70	8.21	19.97	9.21	11.83	17.72	11.35	12.10	15.74

S.E.	0.64	1.97	0.88	1.44	0.78	0.64	1.22	2.13	0.35
Fe (mg kg^{-1})	170.52	134.35	111.86	140.71	190.91	70.86	198.82	263.75	120.37
S.E.	9.42	3.96	5.39	6.12	13.32	12.17	22.93	38.85	21.84
Mn (mg kg^{-1})	40.97	95.08	47.19	102.20	93.95	127.22	122.32	145.81	31.06
S.E.	3.39	4.49	3.63	3.18	2.86	35.19	13.83	14.14	10.25
Zn (mg kg^{-1})	0.61	0.12	2.05	0.13	0.36	1.11	0.33	0.44	1.20
S.E.	0.07	0.02	0.51	0.03	0.01	0.20	0.05	0.13	0.46
Cu (mg kg^{-1})	1.98	2.04	0.76	0.39	5.12	0.58	0.99	0.94	0.52
S.E.	0.97	0.01	0.13	0.11	1.86	0.08	0.09	0.06	0.03
Se (mg kg^{-1})	77.69	113.70	22.18	145.93	184.83	16.08	122.48	82.60	15.28
S.E.	10.49	4.72	3.18	18.53	7.40	5.82	17.37	10.31	1.57
Cr (mg kg^{-1})	0.07	0.28	0.05	0.27	0.33	0.00	0.23	0.09	0.00
S.E.	0.01	0.01	0.01	0.06	0.01	0.00	0.07	0.02	0.00
Pb (mg kg^{-1})	0.67	0.63	1.24	0.46	0.75	2.10	0.62	0.79	3.37
S.E.	0.03	0.06	0.07	0.09	0.14	0.10	0.08	0.32	0.33
Ni (mg kg^{-1})	0.69	1.12	1.65	0.52	0.55	0.38	0.53	0.59	0.30
S.E.	0.11	0.05	0.16	0.04	0.01	0.01	0.03	0.02	0.02
BD (g cm^{-3})	2.1	2.0	1.8	1.9	2.0	1.4	1.6	1.3	1.1
S.E.	0.06	0.10	0.04	0.16	0.25	0.07	0.01	0.09	0.01

S.E., standard error of the mean (n = 5, total n = 45). Di, directly impacted; Pi, partially impacted; Und, undisturbed reference soils. C_{org}, organic carbon; Nt, total nitrogen; C:N, C_{org}-to-Nt ratio; BD, bulk density

Table 3. PerMANOVA based on pairwise comparisons of all microbial traits between the disturbed conditions and undisturbed reference soils within each site ($p < 0.05$, permutations = 9,999)

Tested pair	Site A			Site B			Site C		
	F	r^2	*p-value*	F	r^2	*p-value*	F	r^2	*p-value*
Di × Und	10.2	0.56	0.01	16.4	0.67	0.02	16.2	0.67	0.02
Pi × Und	10.6	0.57	0.02	8.0	0.50	0.02	16.1	0.67	0.02

Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soils. The *p*-values are Bonferroni corrected in all cases.

Table 4. Results of distance-based redundancy analysis for the environmental drivers of shifts on microbial traits

Measurements/variables	db-RDA1	db-RDA2
Eigenvalues	0.79	0.26
Variance explained (%)	39	13
Loadings		
pH	–0.87	0.005
Nt	0.87	0.24
Clay	0.85	0.02
Silt	–0.84	–0.10
Pb	0.80	0.18
C_{org}	0.79	0.04
Se	–0.73	0.06
Cr	–0.66	0.05
Moisture	0.66	–0.13
Zn	0.57	–0.12
BD	–0.52	0.20
Fe	–0.46	–0.29
Sand	0.44	0.14
Mn	–0.20	0.06
Ni	–0.18	–0.36
Cu	–0.14	0.03
C:N	–0.07	–0.35

C_{org}, organic carbon; Nt, total N; C:N, C_{org} to total N ratio

Fig. 1. Sampling sites along Gualaxo do Norte river, Minas Gerais, Brazil

Fig. 2. Ecophysiological traits of microbial community. (a) microbial biomass carbon (MBC), (b) basal respiration (BR), (c) metabolic quotient (qCO_2), and (d) microbial quotient (*q*MIC) in different sites disturbed after the Fundão dam collapse. Bars show the mean for each studied condition (n = 5) and the error bars indicate the standard error of the mean. Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soil. Different lowercase letters indicate significant differences ($p < 0.05$) among conditions within the same site; Different uppercase letters indicate significant differences ($p < 0.05$) for the same condition among sites.

Fig. 3. Extracellular enzymatic activities. (a) arylsulfatase (Aryl), (b) β-1,4-glucosidase (BG), (c) acid phosphatase (AcP), (d) alkaline phosphatase (AlkP), and (e) fluorescein diacetate hydrolysis (FDA) at different sites disturbed after the Fundão dam collapse. Bars show the mean for each studied condition (n = 5) and the error bars indicate the standard error of the mean. Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soil. Different lowercase letters indicate significant differences ($p < 0.05$) among conditions within the same site; Different uppercase letters indicate significant differences ($p < 0.05$) for the same condition among sites.

Fig. 4. Enzyme-based indexes. (a) average values of the GMea and WMean indexes from disturbed conditions (tailings) compared to undisturbed reference soils within each studied site and (b) sunray plots for distribution of A_i–scores for the IBRv2 index as calculated for each enzyme activity measured in tailings at each site across Gualaxo do Norte river. Error bars indicate the standard error of the mean. Aryl, arylsulfatase; BG, β-1,4-glucosidase; AcP, acid phosphatase; AlkP, alkaline phosphatase; FDA, fluorescein diacetate hydrolysis. Di, directly impacted, Pi, partially impacted, Und,

undisturbed reference soil. Different letters indicate significant differences (Tukey *post-hoc* test, $p < 0.05$) among conditions within each site.

Fig. 5. Correlogram between microbial traits and environmental drivers. The color intensity and inclination degree of ellipses indicate the magnitude of correlation for a specific pair of variables. The ellipses of positive correlations are shown in blue and the negative correlations in red. The crosses represent absence of significance ($p > 0.05$). *q*MIC, microbial quotient (Microbial biomass C: C_{org} ratio), qCO_2, metabolic quotient (Basal respiration: Microbial biomass C ratio).

Fig. 6. Non-metric multidimensional scaling (NMDS) plot for the differences in microbial traits among disturbed conditions and undisturbed reference soils for each site after the Fundão dam collapse. Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soils.

Fig. 7. Distance-based redundancy analysis (db-RDA) of different microbial traits with forward selection of predictor variables followed by Monte Carlo permutation tests (9,999 permutations). Solid arrows represent predictor (physicochemical variables and heavy metals) significantly associated with the variation in traits related to the microbial ecophysiology and activity. C_{org}, organic carbon; Nt, total nitrogen; MC, moisture content; BD, bulk density. Di, directly impacted; Pi, partially impacted; Und, undisturbed reference soils.

Declaration of competing interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

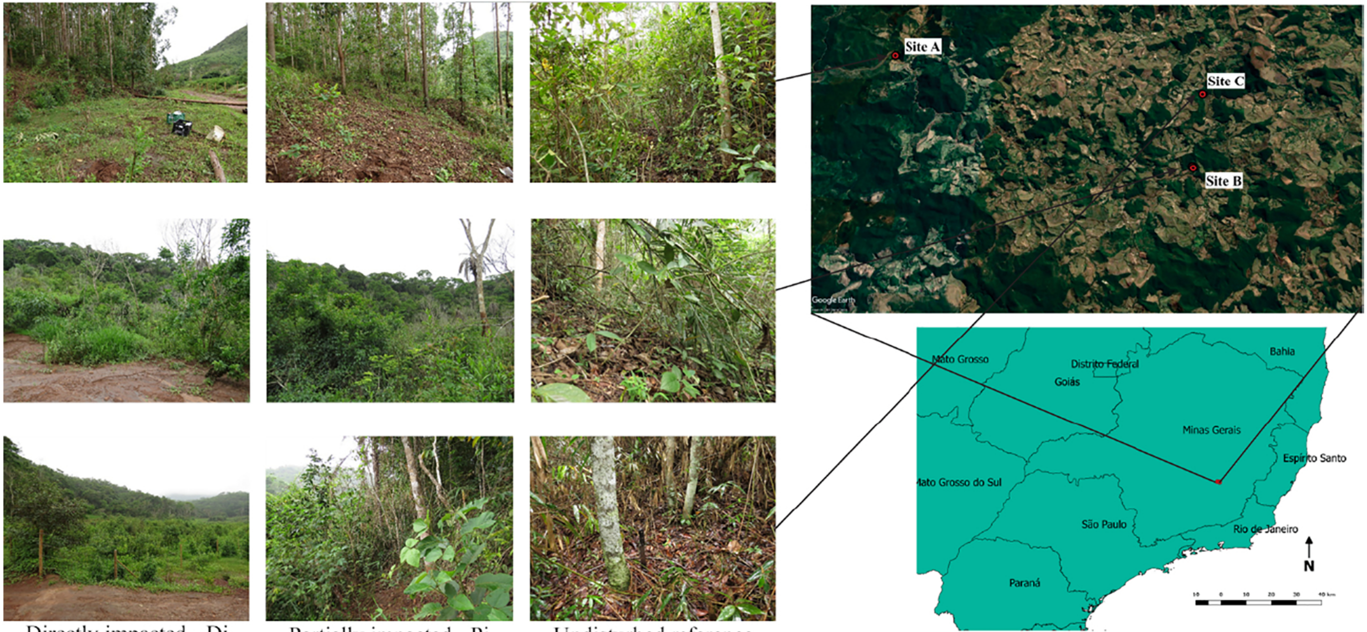
Éder Rodrigues Batista: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft, Writing - review & editing. **Joana Junqueira Carneiro:** Conceptualization, Investigation, Validation, Writing - review & editing. **Flávio Araújo Pinto:** Conceptualization, Investigation, Validation, Writing - review & editing. **Jessé Valentim dos Santos:** Conceptualization, Investigation, Validation, Writing - review & editing. **Marco Aurélio Carbone Carneiro:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - review & editing, Funding acquisition, Project administration, Supervision.

Graphical abstract

Conceptual diagram of differences in microbial traits among disturbed conditions and undisturbed reference soils after the large-scale Fundão dam collapse across Gualaxo do Norte river, Brazil. AMF, arbuscular mycorrhizal fungi.

Highlights

- Effects of Fundão dam tailings on microbial traits of disturbed areas were assessed
- Microbial biomass and activity at tailings were generally low
- Enzyme-based indexes were successfully used to measure environmental quality
- Physicochemical properties had high importance in shifting microbial traits at tailings

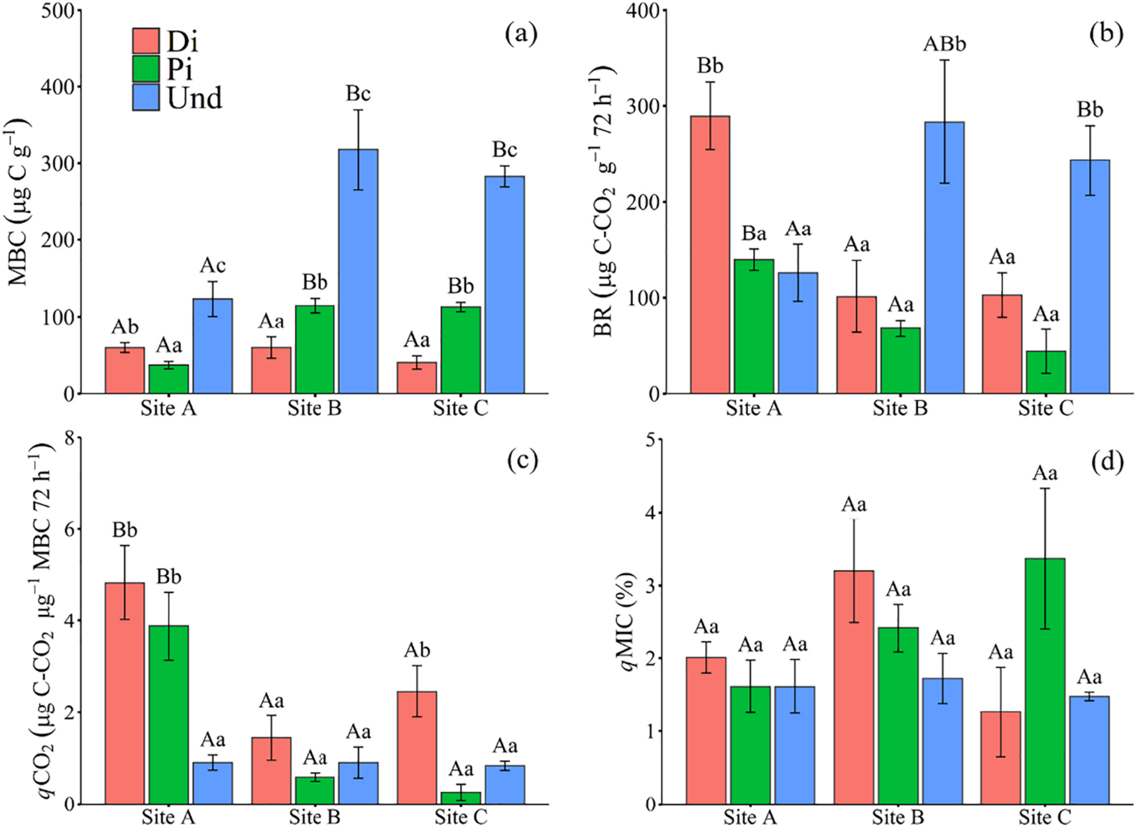


Directly impacted - Di

Partially impacted - Pi

Undisturbed reference soils - Und

Figure 1



(a)
MBC (μg C g^{-1})
Di
Pi
Und
Ab
Aa
Ac
Aa
Bb
Bc
Aa
Bb
Bc
Site A
Site B
Site C
(b)
BR (μg C-CO$_2$ g^{-1} 72 h^{-1})
Bb
Ba
Aa
Aa
Aa
ABb
Aa
Aa
Bb
(c)
qCO$_2$ (μg C-CO$_2$ μg^{-1} MBC 72 h^{-1})
Bb
Bb
Aa
Aa
Aa
Aa
Ab
Aa
Aa
(d)
qMIC (%)
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa

Figure 2

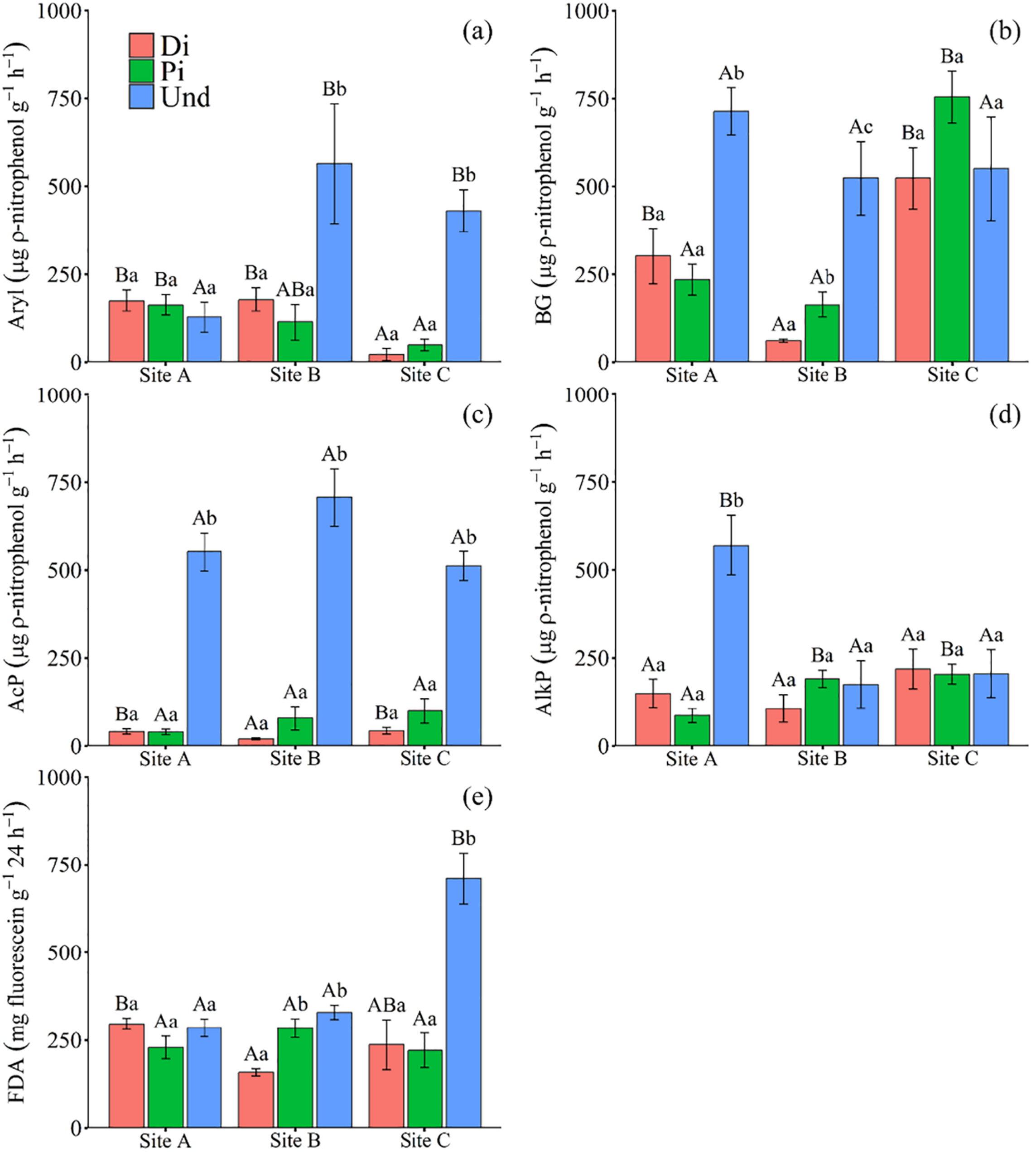

(a)
Aryl (μg ρ-nitrophenol g^{-1} h^{-1})
Di
Pi
Und
Ba Ba Aa Ba ABa Bb Aa Aa Bb
(b)
BG (μg ρ-nitrophenol g^{-1} h^{-1})
Ba Aa Ab Aa Ab Ac Ba Ba Aa
(c)
AcP (μg ρ-nitrophenol g^{-1} h^{-1})
Ba Aa Ab Aa Aa Ab Ba Aa Ab
(d)
AlkP (μg ρ-nitrophenol g^{-1} h^{-1})
Aa Aa Bb Aa Ba Aa Aa Ba Aa
(e)
FDA (mg fluorescein g^{-1} 24 h^{-1})
Ba Aa Aa Aa Ab Ab ABa Aa Bb
0
250
500
750
1000
Site A
Site B
Site C

Figure 3

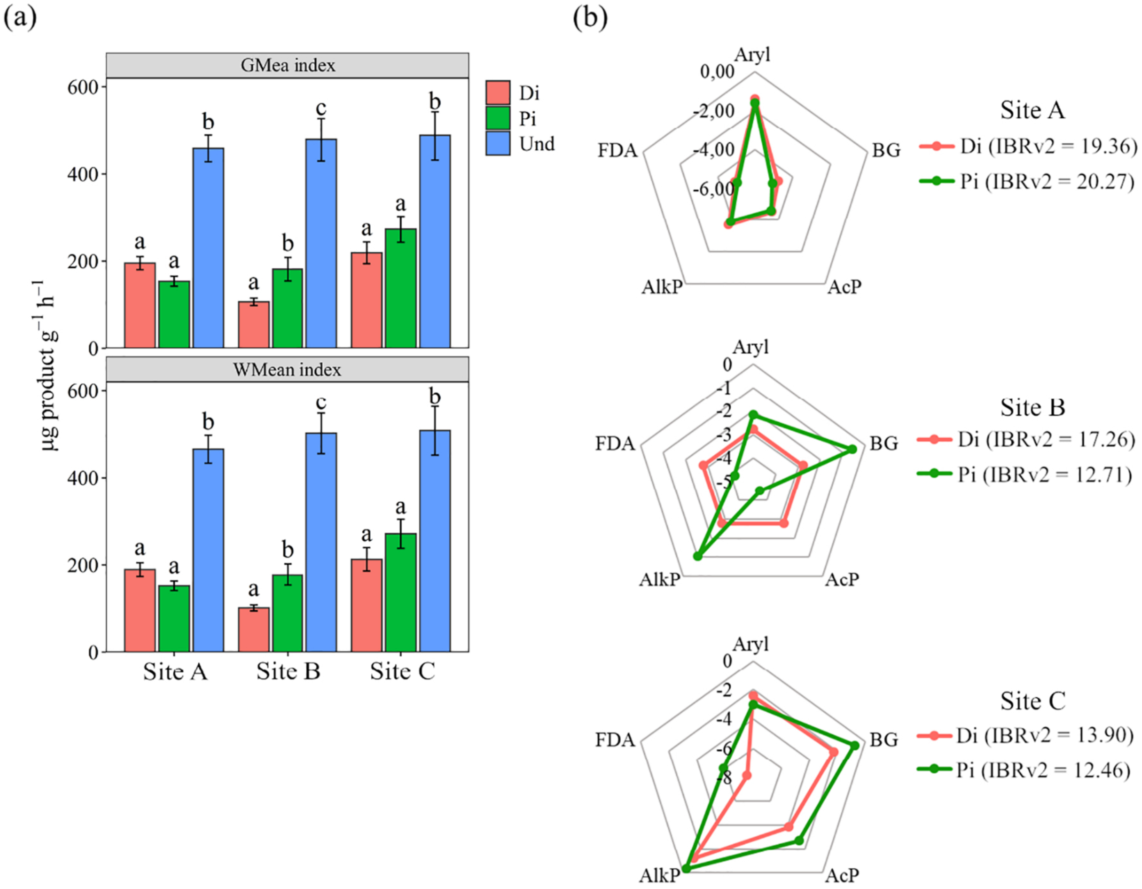

(a)
GMea index
WMean index
µg product g^{-1} h^{-1}
600
400
200
0
Di
Pi
Und
a
a
b
a
b
c
a
a
b
Site A
Site B
Site C
(b)
Aryl
BG
AcP
AlkP
FDA
0,00
-2,00
-4,00
-6,00
0
-1
-2
-3
-4
-5
-6
-8
Site A
Di (IBRv2 = 19.36)
Pi (IBRv2 = 20.27)
Site B
Di (IBRv2 = 17.26)
Pi (IBRv2 = 12.71)
Site C
Di (IBRv2 = 13.90)
Pi (IBRv2 = 12.46)

Figure 4

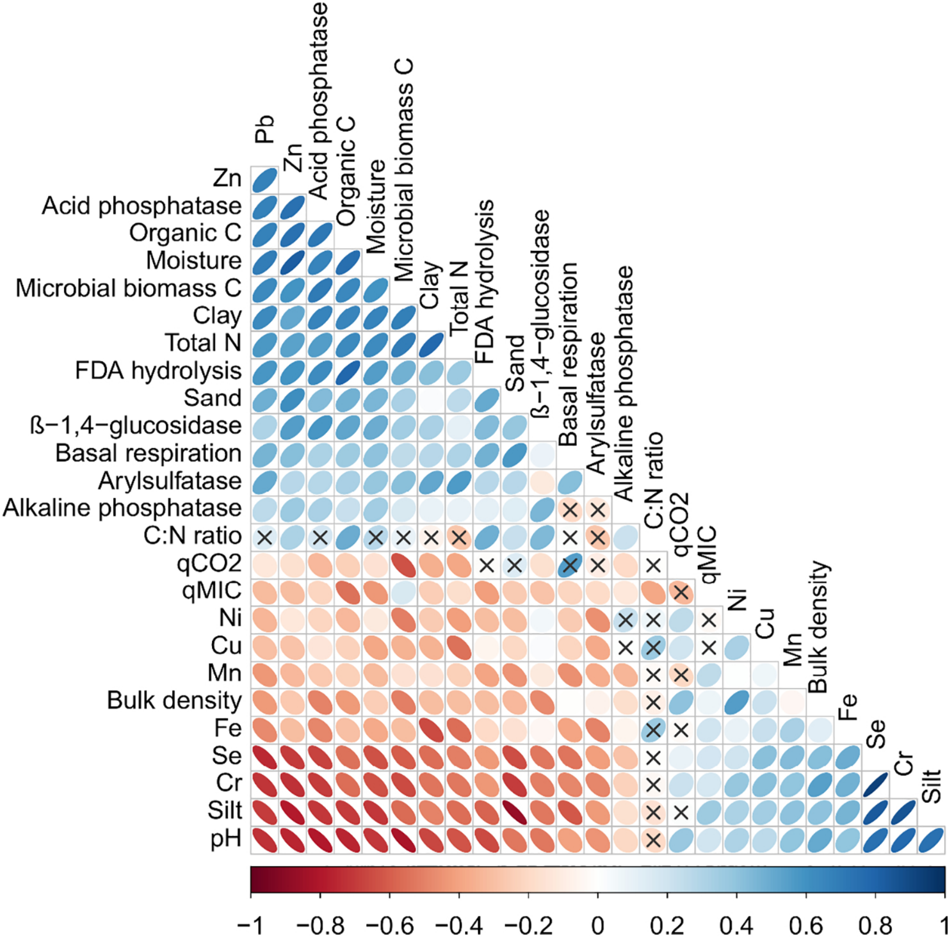

Pb
Zn
Acid phosphatase
Organic C
Moisture
Microbial biomass C
Clay
Total N
FDA hydrolysis
Sand
ß−1,4−glucosidase
Basal respiration
Arylsulfatase
Alkaline phosphatase
C:N ratio
qCO2
qMIC
Ni
Cu
Mn
Bulk density
Fe
Se
Cr
Silt
pH
−1
−0.8
−0.6
−0.4
−0.2
0
0.2
0.4
0.6
0.8
1

Figure 5

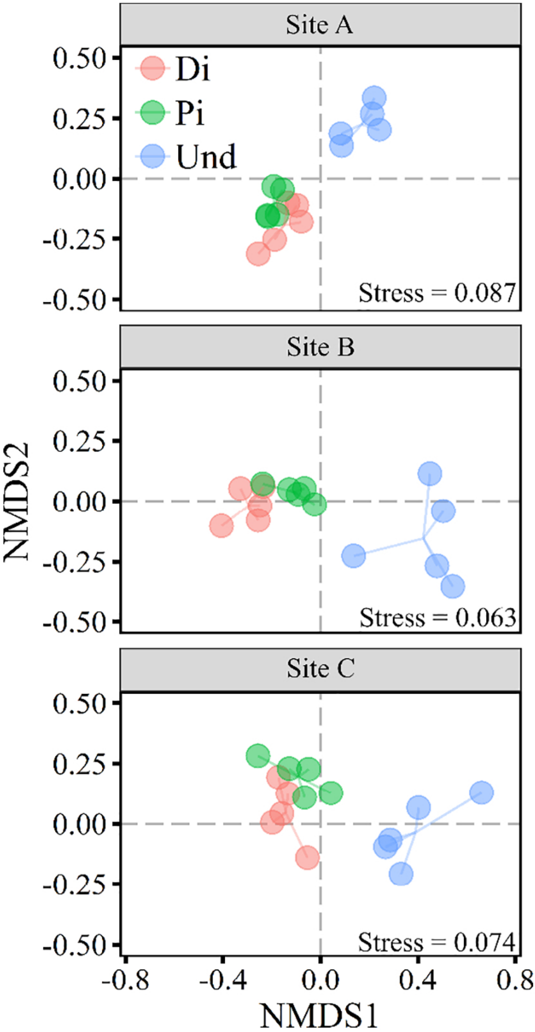

Site A
Di
Pi
Und
Stress = 0.087
Site B
Stress = 0.063
Site C
Stress = 0.074
0.50
0.25
0.00
-0.25
-0.50
NMDS2
-0.8
-0.4
0.0
0.4
0.8
NMDS1

Figure 6

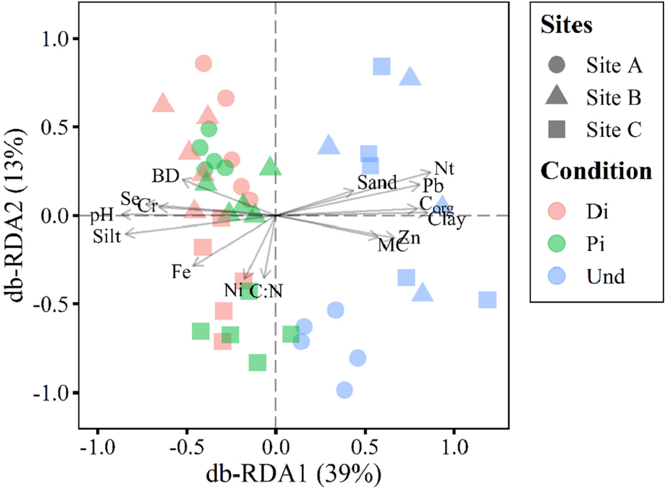

db-RDA2 (13%)
1.0
0.5
0.0
-0.5
-1.0
db-RDA1 (39%)
-1.0
-0.5
0.0
0.5
1.0
BD
Se
Cr
pH
Silt
Fe
Ni
C:N
Sand
Nt
Pb
Corg
Clay
Zn
MC
Sites
Site A
Site B
Site C
Condition
Di
Pi
Und

Figure 7