UNIVERSIDADE FEDERAL DE LAVRAS

ÉDER RODRIGUES BATISTA

ECOPHYSIOLOGY, ACTIVITY, AND DIVERSITY OF MICROBIAL COMMUNITIES IN AREAS DISTURBED BY A MINING DAM COLLAPSE

LAVRAS – MG
2021

ÉDER RODRIGUES BATISTA

ECOPHYSIOLOGY, ACTIVITY, AND DIVERSITY OF MICROBIAL COMMUNITIES IN AREAS DISTURBED BY A MINING DAM COLLAPSE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração: Biologia, Microbiologia e Processos Biológicos do Solo, para a obtenção do título de Doutor.

Prof. Dr. Marco Aurélio Carbone Carneiro
Orientador

LAVRAS – MG
2021

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Batista, Éder Rodrigues.
Ecophysiology, activity, and diversity of microbial communities in areas disturbed by a mining dam collapse / Éder Rodrigues Batista. - 2021.
130 p.

Orientador(a): Marco Aurélio Carbone Carneiro.

Tese (doutorado) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. Soil microbial communities. 2. Mining tailings. 3. Ecosystem functioning. I. Carneiro, Marco Aurélio Carbone. II. Título.

ÉDER RODRIGUES BATISTA

ECOPHYSIOLOGY, ACTIVITY, AND DIVERSITY OF MICROBIAL COMMUNITIES IN AREAS DISTURBED BY A MINING DAM COLLAPSE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração: Biologia, Microbiologia e Processos Biológicos do Solo, para a obtenção do título de Doutor.

APROVADA em 16 de dezembro de 2021.

Dr. José Oswaldo Siqueira	UFLA
Dr. Cláudio Roberto Fonsêca Sousa Soares	UFSC
Dr. Orivaldo José Saggin Junior	EMBRAPA
Dr. Cecílio Frois Caldeira Junior	ITV

Marco

Prof. Dr. Marco Aurélio Carbone Carneiro
Orientador

LAVRAS – MG
2021

A minha família, por todo amor, apoio e incentivo incondicionais

Dedico

Fazemos a ciência que lemos

Gilson Volpato

AGRADECIMENTOS

A Deus por esta realização.

A minha família, pelo apoio e amor incondicionais.

À sociedade, como provedora dos recursos empenhados nesta pesquisa.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) por todo o suporte financeiro às análises desta pesquisa.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela concessão de bolsa de doutorado.

À Universidade Federal de Lavras (UFLA) e ao Departamento de Ciência do solo, pela oportunidade de formação acadêmico-profissional.

Ao Instituto Tecnológico Vale, nas figuras dos Drs. Guilherme Oliveira e Eder Gonçalves, pela valiosa colaboração nesta pesquisa.

A toda equipe da Fundação Renova, pelo apoio logístico durante as campanhas de campo.

Ao professor Dr. Victor Salter Pylro, do Departamento de Ciências Biológicas (UFLA), e a sua orientada, Julie, pelo suporte nas rotinas moleculares.

Ao professor Dr. Marco Aurélio Carbone Carneiro, meu querido orientador, pela amizade e cuidadosa orientação.

Aos demais docentes do Departamento de Ciência do Solo pela dedicação em compartilhar seus conhecimentos durante o curso.

Aos funcionários da UFLA e aos técnicos do Departamento de Ciência do Solo, sempre solícitos em atender nossas demandas.

Aos estudantes de iniciação científica, pelo valioso suporte em diversos momentos durante desta pesquisa.

A todos os colegas de departamento, que direta ou indiretamente fizeram parte desta realização.

Muito obrigado!

RESUMO GERAL

O rompimento da barragem de Fundão, em Mariana, Minas Gerais, modificou irreversivelmente a paisagem, impactando ecossistemas e, sobretudo, as comunidades bióticas. Dentre tais comunidades, as microbianas se destacam como importantes reguladoras do funcionamento dos ecossistemas e, dada sua elevada sensibilidade e rápida resposta a perturbações ambientais, são ferramentas úteis para avaliação da qualidade do solo. Esta tese avaliou a resposta microbiana a alterações nas propriedades edáficas de áreas impactadas pela deposição de rejeitos da barragem de Fundão. O artigo 1 avaliou mudanças na ecofisiologia e na atividade microbiana associadas ao impacto da deposição de rejeitos da barragem de Fundão ao longo do rio Gualaxo do Norte. No geral, as amostras de rejeito apresentaram elevados valores de pH, silte e densidade, porém baixos valores de argila, carbono orgânico (C_{org}), nitrogênio total (Nt), metais pesados biodisponíveis e umidade. A biomassa microbiana, as atividades enzimáticas e o potencial de atividade microbiana total foram geralmente baixos no rejeito em relação aos solos de referência. A integração da atividade enzimática usando diferentes índices de qualidade do solo indicou redução significativa na eficácia degradativa das comunidades microbianas no rejeito em todos os locais estudados. O artigo 2 investigou mudanças na dinâmica do carbono controlada pelos microrganismos em resposta à disponibilidade de substrato orgânico e à atividade enzimática em duas profundidades do horizonte superior (A1: 0-3 cm e A2: 10-13 cm) em áreas sob influência do rejeito da barragem de Fundão. Observou-se que o menor crescimento microbiano (biomassa microbiana) e as maiores perdas de C microbiano (alto qCO_2) foram associadas a diminuições tanto na disponibilidade de substrato orgânico como na atividade enzimática (índice GMean). A respiração basal aumentou com o aumento na atividade enzimática apenas em A2. Já a redução na atividade enzimática foi associada ao menor conteúdo de matéria orgânica no rejeito, sugerindo importantes mudanças na dinâmica microbiana do C em áreas impactadas. No artigo 3 empregou-se abordagem metagenômica para investigar a diversidade taxonômica e funcional de comunidades microbianas em amostras coletadas durante o período chuvoso de 2017 e 2019. Apenas a diversidade alfa das funções da comunidade microbiana sofreu influência do impacto da deposição de rejeito. A composição taxonômica e funcional, entretanto, foi consistentemente alterada com o impacto e, em alguns casos, entre os períodos de amostragem. A análise de valor indicador identificou um total de 792 gêneros distribuídos em 108 classes microbianas associados tanto às áreas impactadas como às de referência. Do total de 8162 funções anotadas, 1433 foram identificadas como indicadores funcionais, sendo 779 delas características das áreas impactadas e 654 de áreas de referência. No geral, a ecofisiologia das comunidades microbianas foi sensivelmente alterada por mudanças nas propriedades físico-químicas do rejeito, com destaque para o pH, disponibilidade de recursos (i.e., C_{org}, Nt), porcentagem de silte e densidade. A atividade enzimática, por sua vez, foi principalmente inibida pela presença de Se, Cr, Fe e Ni, mesmo em baixas concentrações. Além disso, mudanças na disponibilidade de substrato orgânico e na atividade enzimática promovem compensações na alocação de recursos em nível de comunidade, com consequências para a dinâmica microbiana do C nos rejeitos de mineração. Além disso, as mudanças nas características edáficas observadas nas áreas impactadas alterou a diversidade alfa funcional, bem como as composições taxonômica e funcional das comunidades microbianas, com implicações para processos microbianos fundamentais para o restabelecimento do ecossistema nas áreas impactadas.

Palavras-chave: Biomassa microbiana. Atividade enzimática. Índice de qualidade do solo. Diversidade taxonômica. Diversidade funcional. Funcionamento do ecossistema. Rejeito de mineração.

GENERAL ABSTRACT

Fundão dam collapse, in Mariana, Minas Gerais, irreversibly modified the landscape, impacting ecosystems and, mostly, biotic communities. Microbial communities are important regulators of ecosystems functioning and given their high sensitivity and rapid responses to environmental disturbances, these communities are useful tools for assessing environmental quality. This thesis assessed the microbial response to changes in edaphic properties in areas disturbed by Fundão dam tailings deposition. The Paper 1 evaluated changes in microbial ecophysiology and activity associated with tailings deposition impacts across Gualaxo do Norte River. Overall, tailings samples exhibited high values of pH, silt, and bulk density, but low values of clay, organic carbon (C_{org}), total nitrogen (Nt), bioavailable heavy metals, and moisture. Microbial biomass, enzymatic activities, and total microbial activity potential were generally low in the tailings compared to undisturbed reference soils. Enzymatic activity integration using different soil quality indices indicated a significant reduction in the degradative efficacy of the microbial communities in the tailings in all the studied sites. Paper 2 has investigated the changes in microbial-controlled carbon dynamics in response to organic substrate availability and enzymatic activity at two upper horizon depths (A1: 0-3 cm and A2: 10-13 cm) in areas under the influence of tailings of the Fundão dam. It was observed the lower microbial growth (microbial biomass) and higher losses of microbial C (high $q\mathrm{CO}_2$) were associated with decreases in both the availability of organic substrate and in enzymatic activity (GMean index). Basal respiration has increased with increasing enzyme activity only at A2. Reduction in enzyme activity was associated with a lower content of organic matter in tailings, suggesting important changes in the microbial C dynamics in impacted areas. In paper 3, a metagenomic approach was used to investigate the taxonomic and functional diversity of microbial communities in samples collected during the rainy season of 2017 and 2019. Only the alpha diversity of microbial community functions was influenced by the impact of tailings deposition. The taxonomic and functional compositions, however, were consistently altered with impact and between sampling periods in some cases. Indicator value analysis has identified a total of 792 genera distributed in 108 microbial classes associated with both impacted and reference areas. Of the 8162 functions annotated, 1433 were identified as functional indicators, 779 of which were characteristic of impacted areas and 654 of reference areas. Overall, the ecophysiology of microbial communities was sensitively altered by changes in physicochemical properties of tailings, with emphasis on pH, resource availability (i.e., C_{org}, Nt), silt percentage, and bulk density. Enzyme activity, in turn, was mainly inhibited by the presence of Se, Cr, Fe, and Ni, even at low concentrations. Also, changes in organic substrate availability and enzyme activity promote trade-offs at the community-level resource allocation with consequences for microbial C dynamics in mine tailings. Additionally, the changes observed in taxonomic diversity, even small, as well as in the abundance and functional diversity of microbial communities, have implications for microbial processes fundamental to the ecosystem restoration in the impacted areas.

Keywords: Microbial biomass. Enzymatic activity. Soil quality index. Taxonomic Diversity. Functional Diversity. Ecosystem functioning. Mining tailings.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A atividade de mineração é um importante setor para a economia de diversos países. No entanto, as grandes quantidades de rejeito geradas após o beneficiamento do minério bruto trazem consigo grandes desafios ambientais, sobretudo em termos de armazenamento. Existem, no Brasil, cerca de 883 barragens destinadas à contenção de rejeitos e resíduos resultantes da exploração de pelo menos 58 tipos de minério (ANM, 2021). Em muitos casos, tais estruturas oferecem riscos ao meio ambiente por serem susceptíveis a rompimentos, causando impactos ambientais e socioeconômicos que vão além daqueles observados durante a abertura e operação da mina.

O rompimento da barragem de Fundão, ocorrido em novembro de 2015, próximo ao município de Mariana, MG, marcou a história do Brasil como sendo o maior desastre ambiental já ocorrido no país e um dos maiores do mundo envolvendo barragens de mineração (ESCOBAR, 2015). A onda de lama e rejeito proveniente da mineração de ferro percorreu mais de 600 km causando impactos nos rios Gualaxo do Norte, do Carmo e Doce, alcançando a foz do oceano Atlântico dias depois. Os impactos desse evento foram catastróficos e se caracterizaram pela destruição do distrito de Bento Rodrigues, localizado logo jusante da barragem, perda de grandes áreas de vegetação nativa, mortandade de comunidades bióticas aquáticas e terrestres, e por uma crise de abastecimento que afetou comunidades ribeirinhas locais e cidades inteiras devido ao aumento na turbidez e à poluição da água (IBAMA, 2015).

Além disso, grande parte do material vazado da barragem de Fundão ficou depositada sobre os solos adjacentes e nas margens dos rios impactados (IBAMA, 2015). O solo constitui uma das maiores fontes de biodiversidade do planeta, cujo funcionamento depende de uma gama altamente diversificada de processos realizados pelas comunidades bióticas que o habitam, e que estão, por sua vez, intrinsecamente ligadas a diferentes aspectos da multifuncionalidade dos ecossistemas e da sustentabilidade ambiental (HECTOR; BAGCHI, 2007).

Devido às intersecções formadas entre o solo e outras esferas ambientais como a atmosfera, a litosfera, a hidrosfera e a biosfera, ele pode indicar, por meio de suas propriedades químicas, físicas e biológicas, variações na qualidade ambiental (LEHMANN et al., 2020). Características relacionadas à microbiota são consideradas ferramentas sensíveis e eficazes para avaliar como intervenções antrópicas determinam mudanças que afetam o solo e o ecossistema. O estudo de características como a biomassa e as atividades enzimáticas são importantes para determinar o tamanho do compartimento microbiano do solo e o controle da ciclagem biogeoquímica de nutrientes pela microbiota. O uso de uma abordagem metagenômica, nas perspectivas taxonômica e funcional, permite monitorar alterações na diversidade, riqueza, abundância e composição das comunidades microbianas, possibilitando a compreensão dos mecanismos ecológicos que determinam funções e processos modulados pelas comunidades microbianas do solo e a sustentabilidade do ecossistema (MALIK et al., 2017; OSBURN et al., 2019).

Nesse sentido, os objetivos das pesquisas compreendidas no âmbito desta tese foram: (1) investigar os principais fatores ambientais modulando mudanças na ecofisiologia e na atividade microbiana em áreas impactadas pelo rompimento da barragem de Fundão (Artigo 1); (2) avaliar mudanças nas principais características ecofisiológicas microbianas em resposta à disponibilidade de substrato orgânico e atividade enzimática, e como mudanças nessas características afetam a dinâmica microbiana do carbono nas áreas impactadas (Artigo 2); e (3) determinar como o impacto gerado pela deposição de rejeitos afeta a abundância e as diversidades taxonômica e funcional das comunidades microbianas usando abordagem metagenômica de alto rendimento, bem como identificar táxons e funções características da microbiota edáfica das áreas impactadas (Artigo 3).

2 REFERENCIAL TEÓRICO

2.1 Mineração de ferro: importância e impactos em potencial

O ferro (Fe) constitui 98% da matéria-prima usada na fabricação do aço e suas reservas globais são estimadas em cerca de 170 bilhões de toneladas, das quais quase 81 bilhões correspondem a Fe metálico contido (HAQUE; NORGATE, 2015; USGS, 2020). O Brasil é o terceiro colocado em disponibilidade de minério de Fe, com reservas que somam 23 bilhões de toneladas e representam cerca de 13,5% das reservas globais (MTPA, 2018). Em 2019, o Fe representou 72,8% do valor total da produção e 69,1% das exportações dentre as substâncias metálicas exploradas no país, com uma produção beneficiada de 396,8 Gt e teor médio de 64,3%. O estado de Minas Gerais, um dos principais produtores brasileiros de Fe, alcançou uma produção beneficiada de 203,6 Gt nesse mesmo período, respondendo por 61% da produção nacional (ANM, 2020).

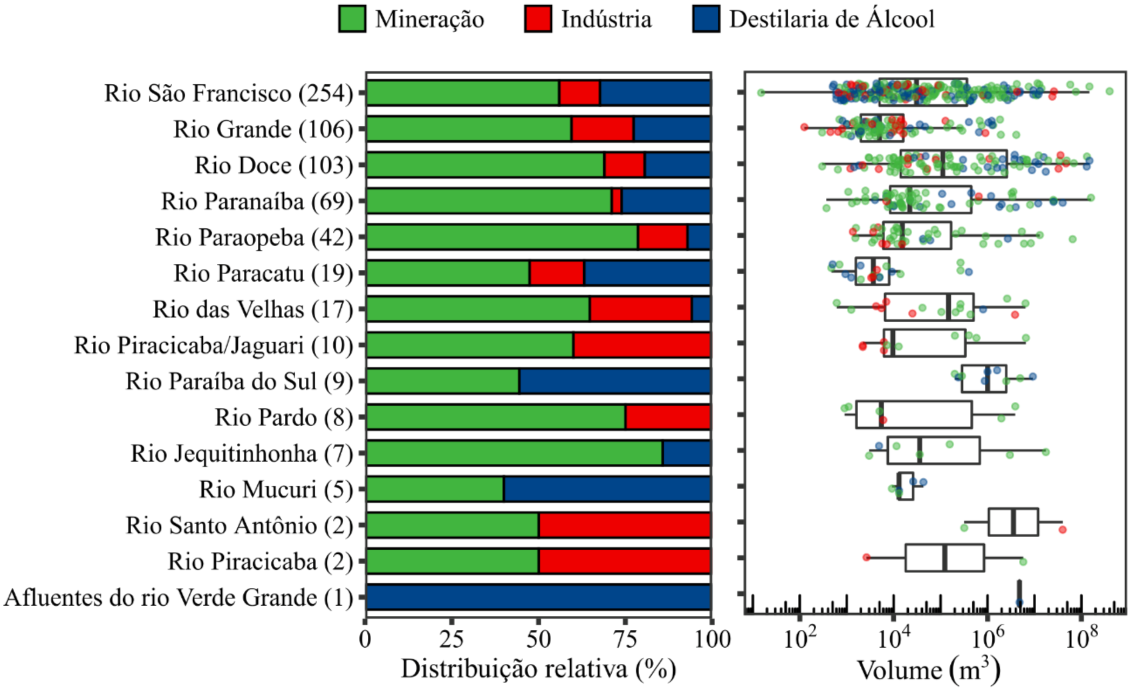
Apesar da importância econômica e metalúrgica do Fe para o mundo, a maior parte de sua extração é feita por meio de operações a céu aberto (FERREIRA; LEITE, 2015; HAQUE; NORGATE, 2015). Embora seja um meio eficiente e de custo relativamente baixo para a exploração de recursos minerais, esse tipo de operação está entre as intervenções antrópicas que mais causam impactos ambientais, incluindo, entre outros, a perda de vegetação nativa, a fragmentação de ecossistemas, a degradação e contaminação do solo e da água, a diminuição da diversidade de comunidades bióticas acima e abaixo do solo e alterações na paisagem em escalas local, regional e global (PALMER et al., 2010; SONTER; ALI; WATSON, 2018; SONTER; BARRETT; SOARES-FILHO, 2014; WAHSHA et al., 2017).

Além dos impactos inerentes à abertura e operação da mina, atividades de mineração produzem grandes quantidades de rejeito, o subproduto resultante do beneficiamento de minérios que inclui uma mistura de rocha britada, pequenas quantidades residuais do mineral de interesse econômico, resíduos químicos de processamento, água e metais pesados (ADIANSYAH et al., 2015). Em uma escala global, as quantidades de rejeito armazenadas em barragens de contenção são estimadas em mais de 100 bilhões de toneladas (TAYEBI-KHORAMI et al., 2019). No Brasil, essa quantidade ultrapassa 3,84 bilhões de m^3 entre rejeitos ou resíduos oriundos da exploração de pelo menos 58 tipos de minério que incluem metais, gemas, argilas, materiais arenosos, sais entre outros (ANM, 2020).

Barragens de rejeito representam o passivo ambiental mais significativo associado a operações de mineração (HUDSON-EDWARDS; JAMIESON; LOTTERMOSER, 2011; WISE, 2021). Estima-se que existam aproximadamente 3.500 barragens de rejeito de mineração ativas em todo o mundo (TAYEBI-KHORAMI et al., 2019). No Brasil, o Sistema Nacional de Informações sobre Segurança de Barragens (SNISB), mantido pela Agência Nacional de Mineração (ANM), registrava, até setembro de 2021, um total de 883 barragens, sendo Minas Gerais um dos estados com o maior número dessas estruturas (ANM, 2021).

De acordo com o inventário de barragens da Fundação Estadual de Meio Ambiente de Minas Gerais (FEAM), o estado possui atualmente 654 estruturas registradas distribuídas ao longo de quinze bacias hidrográficas, das quais 85 (13%) são destinadas ao armazenamento de resíduos industriais, 165 (25,2%) para resíduos de destilaria de álcool e 404 (61,8%) para rejeitos de mineração (FEAM, 2021). As bacias dos rios São Francisco, Grande e Doce são as que apresentam o maior número de barragens, com 254, 106 e 103 estruturas, respectivamente (Figura 1).

Figura 1 – Resumo do inventário de barragens da Fundação Estadual de Meio Ambiente de Minas Gerais (FEAM).



Distribuição relativa de barragens de contenção estratificadas por tipologia situadas em bacias hidrográficas do estado de Minas Gerais (à esquerda) e distribuição do volume de rejeitos por bacia (à direita). Os valores entre parênteses representam o número total de barragens em cada bacia hidrográfica. Cada ponto no painel à direita representa uma barragem.

Apesar do menor número de barragens na bacia do rio Doce em relação a outras bacias, 68,9% delas, isto é, 71 estruturas, são destinadas à contenção de rejeitos de mineração, o que gera grande preocupação, dado o risco inerente de rompimentos. Tais eventos podem ter proporções catastróficas, causando perdas humanas e grandes impactos ambientais e socioeconômicos, sobretudo no caso de estruturas contendo grandes volumes de rejeito (KOSSOFF et al., 2014). Estima-se que entre os anos de 1962 e 2020, cerca de 1.907 pessoas perderam a vida em desastres envolvendo barragens de rejeito em todo o mundo, sendo o Brasil o terceiro colocado, respondendo por 9% dessas fatalidades registradas entre 1986 e 2020 (ICMM, 2021; WISE, 2021). No total, o Brasil registrou dez acidentes nesse período, e um de seus casos recentes foi o do rompimento da barragem de Fundão, em Mariana, Minas Gerais (WISE, 2021).

2.2 O rompimento da barragem de Fundão

A barragem de Fundão pertencia ao complexo minerário de Germano e estava localizada no distrito de Bento Rodrigues, em Mariana, Minas Gerais. O rompimento da estrutura ocorreu em 5 de novembro de 2015 e foi considerado uma das maiores tragédias ambientais relacionadas à mineração no país e uma das maiores do mundo envolvendo barragens de rejeito (ESCOBAR, 2015). O acidente liberou no ambiente uma quantidade equivalente a 34 milhões de m^3 de rejeitos de mineração de Fe, em uma onda de lama que galgou a barragem de Santarém, logo abaixo, e que seguiu o curso dos rios Gualaxo do Norte e Carmo até atingir a barragem de Candonga, na usina hidrelétrica Risoleta Neves. Embora boa parte da lama tenha ficado represada nesse ponto, uma parcela considerável seguiu por cerca de 668 km pelo rio Doce até o município de Linhares, no Espírito Santo, onde alcançou a foz do Oceano Atlântico (IBAMA, 2015; SCHAEFER et al., 2016).

Dos inúmeros impactos imediatos do acidente, houve a destruição completa do distrito de Bento Rodrigues, localizado imediatamente a jusante da barragem, resultando em 19 vítimas fatais (MARTA-ALMEIDA et al., 2016). No trecho de aproximadamente 110 km entre as barragens de Fundão e Candonga, áreas nativas de diversos cursos d’água foram impactadas pelo fluxo e subsequente deposição do material sobre o solo, o que destruiu parcial ou totalmente cerca de 1.469 ha de vegetação nativa (IBAMA, 2016), sendo boa parte áreas de proteção permanente (APP), isto é, áreas protegidas que são voltadas à preservação dos recursos hídricos, da paisagem, da estabilidade geológica e da biodiversidade com a finalidade de garantir o fluxo gênico da fauna e da flora, proteger o solo e assegurar o bem-estar humano (BRASIL, 2012).

A onda de rejeito também provocou o assoreamento de áreas de várzea, mudanças nos terraços, elevação do leito dos rios impactados e supressão de áreas ribeirinhas tradicionalmente usadas para cultivo e pastagem (IBAMA, 2016; SCHAEFER et al., 2016). O derramamento do rejeito aumentou consideravelmente a quantidade de material sólido suspenso nos cursos d'água, o que também aumentou sua turbidez e provocou a mortandade de milhares de espécies de peixes e crustáceos, muitas delas endêmicas (HATJE et al., 2017; IBAMA, 2015). Além disso, houve a liberação de elementos e compostos químicos oriundos do processo de beneficiamento, tais como como eteramina e sódio (Na), além de alguns metais pesados como cromo (Cr), chumbo (Pb), mercúrio (Hg) e arsênio (As), o que causou uma crise de abastecimento que afetou milhares de famílias nas regiões impactadas (QUEIROZ et al., 2018; SEGURA et al., 2016).

O acidente também provocou impactos na microbiota do rio Doce a curto prazo, que incluíram um aumento nas abundâncias de classes bacterianas como Actinobacteria e Bacteroidetes e de genes relacionados à virulência, motilidade, respiração, transporte de membrana e metabolismo de ferro e nitrogênio, sugerindo mudanças nos perfis taxonômico e metabólico das comunidades microbianas aquáticas (CORDEIRO et al., 2019). Nos solos impactados pela deposição de rejeito, foram observadas mudanças na biomassa microbiana (SANTOS et al., 2019) e alterações drásticas na diversidade microbiana procariótica e eucariótica (GIONGO et al., 2020).

A Samarco Mineração S/A, uma *joint venture* societária entre a Vale S/A e a BHP Biliton Brasil Ltda, e uma das responsáveis diretas pela reparação dos danos, criou a Fundação Renova, uma empresa destinada a monitorar, avaliar e reparar os danos decorrentes do acidente por meio de projetos para recuperação das áreas degradadas. Das ações imediatas adotadas, pelo menos 170 mil m^3 de rejeito foram removidos do município de Barra Longa, enquanto cerca de vinte tributários dos rios Carmo e Gualaxo do Norte foram recuperados por meio da limpeza dos locais impactados, retirada dos sedimentos depositados dentro e nas margens dos riachos e a revegetação das áreas com gramíneas e leguminosas para o controle da erosão (SAMARCO, 2016).

Uma das principais ações adotadas pelo governo de Minas Gerais foi proibir a concessão de licença ambiental para operação ou ampliação de barragens destinadas à disposição de rejeitos de mineração ou de resíduos industriais que utilizassem o método de alteamento a montante (ALMG, 2019). Em setembro de 2020, o governo federal sancionou a Lei nº 14.066, que estendeu a proibição para o território nacional e estabeleceu prazos para a descaracterização (desconstrução) de barragens que empregavam esse mesmo tipo de alteamento (BRASIL, 2020).

2.3 Comunidades microbianas como indicadoras de qualidade do solo

A qualidade ambiental é definida como uma medida da condição de um ambiente em relação aos requisitos de uma ou mais espécies, bem como quaisquer necessidades ou propósitos humanos, e é composta pelas qualidades do ar, da água e do solo (JOHNSON et al., 1997). Ao nos referirmos ao ar ou à água, o termo qualidade baseia-se geralmente no grau de poluição e envolve a análise de contaminantes específicos, com valores-limites conhecidos, podendo incluir, no caso da água, propriedades biológicas como a presença de espécies-alvo de peixes, invertebrados, macrófitas ou algas para a definição de um estado ideal de qualidade (BASTIDA et al., 2008; PANDER; GEIST, 2013).

O solo, por sua vez, é um ecossistema complexo, multifuncional e dinâmico e forma uma importante intersecção entre a atmosfera, a litosfera, a hidrosfera e a biosfera, cuja função é crítica para a manutenção da qualidade ambiental (LEHMANN et al., 2020). Isso torna o termo qualidade mais complexo nesse caso, pois engloba aspectos cuja importância é primária para o funcionamento e a sustentabilidade dos ecossistemas, extrapola os limites da poluição e leva em conta o uso de propriedades químicas, físicas, biológicas e ecológicas para a definição de um estado ideal de qualidade (KARLEN et al., 1997). Além disso, a inter-relação entre essas propriedades possibilita uma avaliação mais acurada de impactos ambientais, com potencial de também orientar práticas de manejo agrícola e de reabilitação de áreas degradadas após atividades de mineração (KARLEN et al., 1997; MCDONALD; JONSON; DIXON, 2016).

O termo qualidade do solo (QS) pode ser definido como a capacidade do solo de funcionar, dentro dos limites do ecossistema e do uso da terra, como um sistema capaz de sustentar a produtividade biológica, promover a qualidade ambiental e manter a saúde de plantas e animais, incluíndo a humana (DORAN; PARKIN, 1994; WALL; NIELSEN; SIX, 2015). A avaliação da QS é feita com base em propriedades mensuráveis que influenciam a capacidade de um determinado solo de desempenhar uma determinada função ou processo, convencionalmente chamadas de indicadores de qualidade do solo (ACTON; PADBURY, 1993). Dentre as propriedades utilizadas para avaliação da QS, as biológicas se destacam das demais por sua maior sensibilidade e rápida resposta a perturbações ambientais, sobretudo aquelas relacionadas às comunidades microbianas (SILVA et al., 2018).

Os microrganismos se destacam em relação aos demais grupos edáficos por ser o mais abundante, diverso e funcionalmente importante da Terra (SHOEMAKER; LOCEY; LENNON, 2017). Coletivamente, essas comunidades regulam o funcionamento dos ecossistemas por meio de interações entre si e com o ambiente, e estão intrinsecamente ligadas ao fornecimento de serviços ecossistêmicos, isto é, as contribuições que os ecossistemas fornecem, direta ou indiretamente, para o bem-estar humano (MARTINY et al., 2015; PERALTA et al., 2014). Tais serviços incluem a decomposição de resíduos orgânicos, himificação, agregação, mineralização e ciclagem de nutrientes, produtividade primária, regulação do clima, armazenamento de carbono, transformação de poluentes e controle de pragas e doenças (ADHIKARI; HARTEMINK, 2016; ESCALAS et al., 2019).

Apesar da reconhecida importância das comunidades microbianas para o ecossistema, seu uso ainda constitui menos de 20% em estudos de avaliação da QS, o que demonstra que na última década não houve a inclusão de uma proporção maior de indicadores biológicos quando comparados a outras classes de indicadores (LEHMANN et al., 2020). Nesse sentido, o estudo de características que determinam a aptidão microbiana em resposta a mudanças nas condições ambientais (ecofisiologia), bem como de sua atividade e diversidade, é fundamental para a caracterização, o monitoramento e a avaliação da QS em resposta a mudanças no uso da terra, bem como do processo de reabilitação de áreas pós mineradas, devendo ser consideradas em estudos sobre a resposta dos ecossistemas a perturbações ambientais (WANG et al., 2019).

2.3.1 Biomassa microbiana

A biomassa microbiana (BM) está compreendida entre 1% e 5% da fração ativa da matéria orgânica do solo (MOS) e inclui bactérias, arqueias, actinobactérias, fungos, vírus, protistas e outros eucariotos microbianos (JENKINSON, 1976; JENKINSON; LADD, 1981). É a principal fonte de enzimas no solo e sua atividade decompositora é uma importante característica do ecossistema que garante a reciclagem e a estabilização de nutrientes essenciais que sustentam a produtividade primária (MOREIRA; SIQUEIRA, 2006). Também modula o acúmulo de carbono orgânico estável no solo, cujas contribuições podem variar de 50% a 80% via reciclagem de biomassa e acúmulo de necromassa em relação à decomposição e transformação da serapilheira (LIANG; BALSER, 2011; LIANG; SCHIMEL; JASTROW, 2017; MILTNER et al., 2012).

Do ponto de vista da fertilidade do solo, a BM representa uma propriedade biológica fundamental devido à rápida taxa de reciclagem dos microrganismos (LIANG; SCHIMEL; JASTROW, 2017). Isso a torna um importante reservatório lábil de nutrientes, com uma quantidade imobilizada que varia de 2% a 5% para o nitrogênio (N), 2% a 20% para o fósforo (P) e 2% a 3% para o enxofre (S) (KAISER, 1994; SINGH et al., 1989; SRIVASTAVA; SINGH, 1991). A atuação da BM se estende ainda a processos como a degradação de xenobióticos, regulação do clima, estruturação e agregação do solo e ao fornecimento de recursos genéticos (MOREIRA; SIQUEIRA, 2006; SACCÁ et al., 2017).

A BM é considerada um sensível indicador de alterações nas qualidades do solo e ambiental e, portanto, pode ser utilizada como ferramenta de monitoramento da integridade de um ecossistema sob a influência de potenciais estressores ambientais, bem como de práticas de manejo e reabilitação de áreas degradadas por atividades de mineração (SILVA et al., 2018). Sua avaliação permite medir variações no tamanho do compartimento microbiano edáfico, bem como relacionar tais variações com flutuações em propriedades químicas e físicas do solo, que incluem a textura, o teores de matéria orgânica e nutrientes, o pH, a umidade e concentrações de elementos tóxicos (AZARBAD et al., 2013; CHODAK; NIKLIŃSKA, 2010; SANTOS et al., 2016).

Foram demonstradas, em áreas pós mineradas, reduções na BM relacionadas à presença de elevadas concentrações de metais como Zn, Cu, Pb e Cd (SANTOS et al., 2016). Por outro lado, a BM foi sensível em indicar o sucesso do processo de reabilitação, em que foi possível observar um aumento da mesma em resposta ao estímulo da vegetação por meio das entradas de C via rizodeposição e decomposição de serapilheira (MUÑOZ-ROJAS et al., 2016). Efeito similar foi observado com relação à idade da parcela estudada, demonstrando a contribuição positiva do desenvolvimento da vegetação e do aumento da complexidade do ecossistema com o processo de reabilitação (CHODAK; NIKLIŃSKA, 2010; HELINGEROVÁ; FROUZ; ŠANTRŮČKOVÁ, 2010).

2.3.2 Respiração basal

A respiração basal do solo (RBS) representa todas as funções metabólicas onde o CO_2 é produzido, principalmente durante o processo de decomposição da matéria orgânica (ALEF, 1995). Ocorre em maior grau nas camadas superficiais do solo, onde se concentra a maior parte dos resíduos orgânicos, e suas taxas também são determinadas pela biomassa e atividade dos microrganismos edáficos (DELGADO-BAQUERIZO et al., 2016). Taxas mais elevadas de RBS podem indicar alta atividade microbiana, uma vez que aumentos nas taxas de decomposição aumentam rapidamente a incorporação de C biomassa microbiana, elevando o fluxo de CO_2, que pode ser traduzido como um aumento nas taxas metabólicas da comunidade (SCHIMEL; SCHAEFFER, 2012).

Entretanto, como as células microbianas são encontradas em diferentes estados fisiológicos no solo, essas elevadas taxas de RBS também podem estar relacionadas a algum estresse ambiental (JOERGENSEN; WICHERN, 2018). Em geral, observa-se um aumento na RBS em áreas de mineração, caracterizando uma mudança do metabolismo de crescimento para o de respiração de manutenção, como estratégia fisiológica para sobreviver em um ambiente estressante, sobretudo sob a influência de metais pesados (SANTOS et al., 2013, 2016; SILVA et al., 2018). Por outro lado, após 19 anos, a revegetação de áreas de exploração de carvão demonstrou ter efeito positivo nas taxas de RBS devido à recuperação dos teores de matéria orgânica e ao estímulo propiciado pelas entradas de C sobre a atividade microbiana (QUADROS et al., 2016).

2.3.3 Atividade enzimática extracelular

Os microrganismos transformam os resíduos orgânicos por meio da atividade degradativa de enzimas extracelulares (SINSABAUGH; HILL; FOLLSTAD SHAH, 2009). Essas proteínas ativas despolimerizam macromoléculas orgânicas, produzindo substratos orgânicos simples (monômeros) e nutrientes solúveis para assimilação pelas plantas e uso pelos microrganismos na síntese de novas biomassa e enzimas (KALLENBACH; FREY; GRANDY, 2016; SINSABAUGH; HILL; FOLLSTAD SHAH, 2009). A atividade de enzimas extracelulares nos solos tem sido estudada por mais de um século com o objetivo de entender a influência de mudanças globais e outras perturbações sobre a biogeoquímica e a ciclagem de nutrientes como o carbono (C), o nitrogênio (N), o fósforo (P) e o enxofre (S) (SINSABAUGH; FOLLSTAD SHAH, 2012).

A atividade enzimática controla a dinâmica dos fluxos de matéria e energia dos ecossistemas terrestres, e uma série de enzimas têm sido sugeridas como indicadores adequados de QS, pois: (1) estão relacionadas à ciclagem e transformação de nutrientes e, portanto, apresentam elevado potencial de indicar variações na atividade degradativa das comunidades microbianas; (2) apresentam elevada sensibilidade às mudanças causadas por fatores naturais e antropogênicos; e (3) podem ser medidas de forma fácil e relativamente econômica (DICK, 1994; DICK; BREAKWELL; TURCO, 1996; GIANFREDA et al., 2005; HINOJOSA et al., 2004).

As enzimas são de longe os indicadores bioquímicos mais estudados do solo (DICK, 1994). Hidrolases do ciclo do C, tais como a α- e β-1,4-glicosidase e a β-galactosidase, são amplamente distribuídas na natureza e participam da ciclagem global de C por meio da hidrólise de componentes complexos da matéria orgânica (MO), que incluem celulose e outros β-1,4 glicanos, para formar monômeros de açúcar que são uma importante fonte de C para plantas e microrganismos (EIVAZI; TABATABAI, 1988; LJUNGDAHL; ERIKSSON, 1985).

As enzimas urease e a N-acetil-β-glicosaminidase são importantes hidrolases que atuam na ciclagem biogeoquímica de N. A urease está envolvida na hidrólise a ureia, com liberação de CO_2 e mineralização de N na forma de amônia (TABATABAI; BREMNER, 1972). A N-acetil-β-glicosaminidase desempenha importante papel na degradação tanto de quitina, que consiste de resíduos de N-acetil-β-glicosamina unidos por ligações β-1,4, como de outros polímeros com ligações similares, em um papel comparável ao da β-1,4-glicosidase, uma vez que também pode participar do ciclo do C (PARHAM; DENG, 2000).

As fosfatases são enzimas amplamente estudadas nos solos que atuam na ciclagem biogeoquímica de P. A Comissão sobre Enzimas da União Internacional de Bioquímica as classificou em pelo menos cinco grupos divididos em fosfomonoesterases, fosfodiesterases, fosfotriesterases, enzimas que atuam sobre compostos contendo fosforiol-anidridos e aquelas que atuam em compostos contendo ligações P-N, tais como as fosfoamidases (EIVAZI; TABATABAI, 1977). As principais fosfatases estudadas no solo são as fosfomonoesterases, que hidrolisam compostos de fosfomonoester e incluem as fosfatases ácida e alcalina, ambas responsáveis pela liberação do P na forma iônica ($H_2PO_4^-$) (DICK; BREAKWELL; TURCO, 1996).

As sulfatases formam um importante grupo de enzimas que controla a ciclagem biogeoquímica do S no solo e, assim como as fosfatases, também são agrupadas a depender do tipo de éster de sulfato que hidrolisam em arilsulfatase, alquilsulfatase, sulfatase esteroide, glicosulfatase, condrosulfatase e mirosulfatase (ROY, 1960). Dessas, a arilsulfatase é a principal enzima estudada nos solos, pois hidrolisa ésteres de sulfato orgânico, mineralizando o S na forma de sulfato inorgânico (SO_4^{2-}) (TABATABAI; BREMNER, 1970). O diacetato de fluoresceína (DAF) mede o potencial de atividade microbiana total no solo, sobretudo por sua relação com a respiração basal do solo, e é um substrato hidrolisado por diversas enzimas que incluem as proteases, lipases e esterases (SCHNÜRER; ROSSWALL, 1982).

A atividade enzimática é influenciada por diferentes propriedades do solo, tais como o pH, seu estado iônico, a temperatura e a presença íons metálicos ou metaloides (BURNS; DICK, 2002). Dentre os inibidores mais comuns estão os metais pesados, que promovem reduções drásticas na atividade de muitas enzimas, sobretudo em áreas sob a influência de atividades de mineração e também pós mineradas, afetando as atividades das principais enzimas do solo como arilsulfatase, β-1,4-glicosidase, urease e fosfatase ácida (HINOJOSA et al., 2004; MAJER et al., 2002; MIKANOVA, 2006; PAZ-FERREIRO et al., 2007). Embora em áreas impactadas pela mineração de bauxita as atividades enzimáticas tenham sido reduzidas, o DAF nos solos impactados apresentou rápida recuperação, atingindo valores similares aos dos solos de referências ainda no primeiro ano de reabilitação (CARNEIRO et al., 2008).

2.4 Índices biológicos de qualidade do solo: agregando indicadores

Diversos autores têm ressaltado que indicadores microbiológicos e bioquímicos individuais podem apresentar diferenças em sua sensibilidade e, por isso, tem sido sugerido o uso agregado desses indicadores em um único índice capaz refletir adequadamente mudanças na QS (LEHMANN et al., 2020; PAZ-FERREIRO; FU, 2016). Um índice de qualidade do solo pode ser definido como um conjunto mínimo de parâmetros que, quando inter-relacionados, fornecem dados numéricos sobre a capacidade de um solo desempenhar uma ou mais funções (ACTON; PADBURY, 1993).

Esses constituem uma abordagem integrativa pela qual propriedades relacionadas podem ser combinadas, e funcionam como ferramentas de decisão capazes de orientar práticas de manejo e reabilitação de ecossistemas degradados (PAZ-FERREIRO; FU, 2016). O uso de índices para avaliar a QS tanto em sistemas agrícolas como em áreas impactadas pela mineração ainda é limitado (BASTIDA et al., 2008). A compilação de dados de uma série de mais de 500 estudos sobre a avaliação de indicadores e índices de QS revelou que apenas 1% deles usaram pelo menos um índice (JIAN; DU; STEWART, 2020).

Esses índices podem ser simples, envolvendo apenas dois parâmetros, ou multiparamétricos, em que se considera mais que dois parâmetros (BASTIDA et al., 2008). Um dos índices mais simples e comuns da literatura é o quociente metabólico ($q\text{CO}_2$), que expressa a relação entre a respiração basal e a biomassa microbiana (INSAM; HASELWANDTER, 1989). Do ponto de vista ecofisiológico, o qCO$_2$ descreve a eficiência microbiana de uso de carbono, bem como o grau de estresse da comunidade, refletindo maior investimento em biossíntese (produção de biomassa) quando reduzido e maiores perdas de C-CO$_2$ microbiano quando elevado (JOERGENSEN; WICHERN, 2018). O qCO$_2$ está conceitualmente baseado na teoria de desenvolvimento do ecossistema proposta pelo ecólogo Eugene Odum, que sugere que atributos e processos ecossistêmicos fundamentais mudam em vários níveis de organização, do indivíduo ao sistema, à medida que os ecossistemas se desenvolvem em direção ao equilíbrio (ODUM, 1969). O qCO$_2$ segue um padrão similar, tendendo a ser elevado em comunidades microbianas de ecossistemas em estágios iniciais de desenvolvimento e reduzido naqueles em estágios mais avançados, agindo também como um indicador de maturidade do ecossistema (BASTIDA et al., 2008).

Outro índice simples comumente utilizado na literatura é o quociente microbiano (qMIC), que corresponde à relação entre a biomassa microbiana e o C orgânico total do solo (ANDERSON; DOMSCH, 1989). O qMIC representa tanto a capacidade da comunidade microbiana de converter substratos orgânicos em biomassa como uma medida da contribuição microbiana para a formação da matéria orgânica em ecossistemas terrestres (LIANG et al., 2019). Isso se deve ao fato de os microrganismos serem centrais em dois mecanismos críticos contrastantes: o primeiro diz respeito à redução dos estoques de C do solo via mineralização para CO$_2$; e o segundo corresponde ao aumento desses estoques por meio da formação de biomassa microbiana e estabilização de seus resíduos (necromassa), seja por associação a minerais dentro da estrutura do solo, ou por incrustação com, por exemplo, precipitados de Fe ou Si (KÄSTNER; MILTNER, 2018; LIANG; SCHIMEL; JASTROW, 2017).

Índices que se baseiam na atividade enzimática extracelular são bons exemplos de índices multiparamétricos e têm sido propostos como ferramentas para avaliação da QS. A média geométrica (GMean) e a média ponderada (WMean) da atividade enzimática são consideradas índices adequados para avaliação da QS por condensar todo o conjunto de valores de atividade enzimática estudado em um único valor numérico (HINOJOSA et al., 2004; LESSARD; SAUVÉ; DESCHÊNES, 2014). Ambas as médias servem como preditores da eficácia degradativa a nível de comunidade e funcionam como medidas de diversidade funcional pelo fato de, no solo, as enzimas serem produzidas por diversos membros da comunidade microbiana nativa (BURNS et al., 2013; LESSARD; SAUVÉ; DESCHÊNES, 2014).

O índice de resposta biológica integrada (IBRv2) também combina múltiplas enzimas e foi proposto para avaliar a saúde do ecossistema e identificar o impacto de estressores ambientais (SANCHEZ; BURGEOT; PORCHER, 2013). Esse índice é uma versão aprimorada do índice de resposta biológica proposto por (BELIAEFF; BURGEOT, 2002), e se baseia em diversos valores de atividade enzimática, que são padronizadas em relação a valores de referência centrados em zero. Assim, pode-se representar a variação na atividade enzimática, de modo que valores acima do limiar de referência apontam para um aumento na atividade decompositora da comunidade microbiana, enquanto valores abaixo desse limiar denotam reduções nessa atividade (SANCHEZ; BURGEOT; PORCHER, 2013).

2.5 Metagenômica de comunidades microbianas do solo

A genômica ambiental ou metagenômica é definida como uma abordagem molecular independente de cultivo que se baseia no isolamento e estudo de sequências de DNA obtidas diretamente de amostras ambientais, e que permite examinar e explorar tanto a diversidade taxonômica das comunidades microbianas como as funções codificadas pelos metagenomas dessas comunidades (DANIEL, 2005). Essa abordagem pode ser aplicada ao estudo das comunidades microbianas de duas formas: a primeira faz uso do sequenciamento de metagenomas inteiros, englobando todos os microrganismos em um nicho ambiental específico; a segunda se baseia no sequenciamento de amplicons de DNA, a partir da reação em cadeia da polimerase, e é direcionada a genes marcadores específicos como o 16S rRNA para procariotos e o 18S rRNA para eucariotos (XU, 2006).

A metagenômica revolucionou o estudo das comunidades microbianas do solo, onde os microrganismos não cultiváveis representam a maioria, oferecendo aos cientistas a possibilidade de superar as limitações dos métodos tradicionais de cultivo em laboratório (DANIEL, 2005). Essa abordagem também trouxe como vantagens a possibilidade de acesso a novos biocatalisadores para processos biossintéticos, biodegradação de poluentes e síntese de biocombustíveis (VOGEL et al., 2009), a construção de perfis funcionais das comunidades microbianas (LANGILLE et al., 2013), a descoberta de novos organismos, genes ou vias metabólicas (CULLIGAN et al., 2014) e também possibilitou investigar tanto genes diferencialmente expressos (MUNK et al., 2018) como táxons diferencialmente abundantes (VIEIRA et al., 2020).

Os solos constituem uma das maiores fontes de biodiversidade da Terra e são considerados os mais desafiadores entre os ambientes naturais, sobretudo devido à complexidade e ao tamanho dos metagenomas, sua dinâmica temporal e espacial e à enorme diversidade das comunidades microbianas que o habitam (BAHRAM et al., 2018). Estima-se que cada grama de solo contenha entre 3.000 e 11.000 genomas microbianos, com uma quantidade de sequências genômicas que pode chegar a 1.000 giga pares de bases (Gpb) (SCHMEISSER; STEELE; STREIT, 2007; VOGEL et al., 2009). Essa enorme quantidade de informação se reflete tanto nas diversidades taxonômica e filogenética, como na complexidade funcional dessas comunidades, determinando sua capacidade de tolerar condições ambientais adversas, obter recursos, interagir com outros membros da comunidade e modular a multifuncionalidade dos ecossistemas (HECTOR; BAGCHI, 2007; JING et al., 2015).

A metagenômica foi introduzida na ciência do solo dezessete anos atrás e representou um importante avanço para o estudo das comunidades microbianas edáficas (DANIEL, 2005; VESTERGAARD et al., 2017). Informações como a abundância, riqueza, estrutura e composição de espécies microbianas e de funções codificadas pelo metagenoma dessas comunidades podem constituir promissores indicadores ecológicos da qualidade e dos serviços ecossistêmicos fornecidos pelos solos (MARON et al., 2018). Com ela foi possível investigar os principais impulsionadores da biodiversidade nas perspectivas taxonômica, filogenética e funcional em resposta a mudanças nas propriedades edáficas causadas pela atividade de mineração (ESCOBAR et al., 2015; LEWIS et al., 2010) e identificar os mecanismos ecológicos que determinam alterações nos serviços ecossistêmicos modulados pelas comunidades microbianas do solo (MALIK et al., 2017; OSBURN et al., 2019).

REFERÊNCIAS

ACTON, D. F.; PADBURY, G. A. A conceptual framework for soil quality assessment and monitoring. In: ACTON, D. F. (Ed.). . **A program to assess and monitor soil quality in Canada: Soil quality evaluation summary**. Ottawa: [s.n.]. p. 201.

ADHIKARI, K.; HARTEMINK, A. E. Linking soils to ecosystem services — A global review. **Geoderma**, v. 262, p. 101–111, jan. 2016.

ADIANSYAH, J. S. et al. A framework for a sustainable approach to mine tailings management: Disposal strategies. **Journal of Cleaner Production**, v. 108, p. 1050–1062, 2015.

ALEF, K. Field methods. In: ALEF, K.; NANNIPIERI, P. (Ed.). . **Methods in Applied Soil Microbiology and Biochemistry**. [s.l.] Elsevier, 1995. p. 463–490.

ALMG. **Lei 23.291, de 25 de fevereiro de 2019**Belo Horizonte Assembleia Legislativa de Minas Gerais (ALMG), , 2019. Disponível em: <https://www.almg.gov.br/consulte/legislacao/completa/completa.html?tipo=LEI&num=23291&ano=2019>

ANDERSON, T.-H.; DOMSCH, K. H. Ratios of microbial biomass carbon to total organic carbon in arable soils. **Soil Biology and Biochemistry**, v. 21, n. 4, p. 471–479, 1989.

ANM. **Anuário Mineral Brasileiro: principais substâncias metálicasAgência Nacional de Mineração (ANM)**. Brasília: Agência Nacional de Mineração (ANM), 2020.

ANM. **Classificação Nacional de Barragens de Mineração**. Disponível em: <https://app.anm.gov.br/SIGBM/Publico/ClassificacaoNacionalDaBarragem>.

AZARBAD, H. et al. Microbial community structure and functioning along metal pollution gradients. **Environmental Toxicology and Chemistry**, v. 32, n. 9, p. 1992–2002, 2013.

BAHRAM, M. et al. Structure and function of the global topsoil microbiome. **Nature**, v. 560, n. 7717, p. 233–237, 2018.

BASTIDA, F. et al. Past, present and future of soil quality indices: A biological perspective. **Geoderma**, v. 147, n. 3–4, p. 159–171, 2008.

BELIAEFF, B.; BURGEOT, T. Integrated biomarker response: A useful tool for ecological risk assessment. **Environmental Toxicology and Chemistry**, v. 21, n. 6, p. 1316–1322, 2002.

BRASIL. **LEI Nº 12.651, DE 25 DE MAIO DE 2012**. Brasília: [s.n.].

BRASIL. **LEI Nº 14.066, DE 30 DE SETEMBRO DE 2020**. Brasília: [s.n.].

BURNS, R. G. et al. Soil enzymes in a changing environment: Current knowledge and future directions. **Soil Biology and Biochemistry**, v. 58, p. 216–234, 2013.

BURNS, R. G.; DICK, R. P. **Enzymes in the Environment: Activity, Ecology, and Applications**. New York: Marcel Dekker, Inc., 2002.

CARNEIRO, M. A. C. et al. Carbono orgânico, nitrogênio total, biomassa e atividade microbiana do solo em duas cronosseqüências de reabilitaç ão após a mineração de bauxita. **Revista Brasileira de Ciencia do Solo**, v. 32, n. 2, p. 621–632, 2008.

CHODAK, M.; NIKLIŃSKA, M. Effect of texture and tree species on microbial properties of mine soils. **Applied Soil Ecology**, v. 46, n. 2, p. 268–275, 2010.

CORDEIRO, M. C. et al. Insights on the freshwater microbiomes metabolic changes associated with the world's largest mining disaster. **Science of the Total Environment**, v. 654, p. 1209–1217, 2019.

CULLIGAN, E. P. et al. Metagenomics and novel gene discovery. **Virulence**, v. 5, n. 3, p. 399–412, abr. 2014.

DANIEL, R. The metagenomics of soil. **Nature Reviews Microbiology**, v. 3, n. 6, p. 470–478, jun. 2005.

DELGADO-BAQUERIZO, M. et al. Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. **Functional Ecology**, v. 30, n. 11, p. 1862–1873, 2016.

DICK, R. P. Soil Enzyme Activities as Indicators of Soil Quality. In: DORAN, J. W. et al. (Eds.). . **Defining Soil Quality for a Sustainable Environment**. Madson: Soil Science Society of America, 1994. p. 107–124.

DICK, R. P.; BREAKWELL, D. P.; TURCO, R. F. Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators. In: DORAN, J. W.; JONES, A. J. (Eds.). . **Methods for Assessing Soil Quality**. Madson: Soil Science Society of America, 1996. p. 247–271.

DORAN, J. W.; PARKIN, T. B. Defining and Assessing Soil Quality. In: DORAN, J. W. et al. (Eds.). . **Defining Soil Quality for a Sustainable Environment**. [s.l.] Soil Science Society of America, Inc., 1994. v. 35p. 3–21.

EIVAZI, F.; TABATABAI, M. A. Phosphatases in soils. **Soil Biology and Biochemistry**, v. 9, n. 3, p. 167–172, jan. 1977.

EIVAZI, F.; TABATABAI, M. A. Glucosidases and galactosidases in soils. **Soil Biology and Biochemistry**, v. 20, n. 5, p. 601–606, jan. 1988.

ESCALAS, A. et al. Microbial functional diversity: From concepts to applications. **Ecology and Evolution**, v. 9, n. 20, p. 12000–12016, 2019.

ESCOBAR, H. Mud tsunami wreaks ecological havoc in Brazil. **Science**, v. 350, n. 6265, p. 1138–1139, 2015.

ESCOBAR, I. E. C. et al. Changes in Microbial Community Structure and Soil Biological Properties in Mined Dune Areas During Re-vegetation. **Environmental Management**, v. 55, n. 6, p. 1433–1445, 2015.

FEAM. **Inventário de Barragens**. Disponível em: <http://www.feam.br/gestao-de-barragens/inventario-de-barragens>. Acesso em: 19 fev. 2021.

FERREIRA, H.; LEITE, M. G. P. A Life Cycle Assessment study of iron ore mining. **Journal of Cleaner Production**, v. 108, p. 1081–1091, 2015.

GIANFREDA, L. et al. Soil enzyme activities as affected by anthropogenic alterations: Intensive agricultural practices and organic pollution. **Science of the Total Environment**, v. 341, n. 1–3, p. 265–279, 2005.

GIONGO, A. et al. Adaption of microbial communities to the hostile environment in the Doce River after the collapse of two iron ore tailing dams. **Heliyon**, v. 6, n. 8, 2020.

HAQUE, N.; NORGATE, T. Life cycle assessment of iron ore mining and processing. In: **Iron Ore**. [s.l.] Elsevier, 2015. p. 615–630.

HATJE, V. et al. The environmental impacts of one of the largest tailing dam failures worldwide. **Scientific Reports**, v. 7, n. 1, p. 10706, 6 dez. 2017.

HECTOR, A.; BAGCHI, R. Biodiversity and ecosystem multifunctionality. **Nature**, v. 448, n. 7150, p. 188–190, 2007.

HELINGEROVÁ, M.; FROUZ, J.; ŠANTRŮČKOVÁ, H. Microbial activity in reclaimed and unreclaimed post-mining sites near Sokolov (Czech Republic). **Ecological Engineering**, v. 36, n. 6, p. 768–776, jun. 2010.

HINOJOSA, M. B. et al. Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. **Soil Biology and Biochemistry**, v. 36, n. 10, p. 1637–1644, 2004.

HUDSON-EDWARDS, K. A.; JAMIESON, H. E.; LOTTERMOSER, B. G. Mine Wastes: Past, Present, Future. **Elements**, v. 7, n. 6, p. 375–380, 1 dez. 2011.

IBAMA. **Laudo Técnico Preliminar: Impactos ambientais decorrentes do desastre envolvendo o rompimento da barragem de Fundão, em Mariana, Minas Gerais**Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, , 2015.

IBAMA. **Relatório Geral de Vistoria: Operação Áugias – Fase Hélios**. Brasília: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, 2016.

ICMM. **Safety Performance: Benchmarking progress of ICMM members in 2020**. London, UK: [s.n.].

INSAM, H.; HASELWANDTER, K. Metabolic quotient of the soil microflora in relation to plant succession. **Oecologia**, v. 79, n. 2, p. 174–178, 1989.

JENKINSON, D. S. The effects of biocidal treatments on metabolism in soil—IV. The decomposition of fumigated organisms in soil. **Soil Biology and Biochemistry**, v. 8, n. 3, p. 203–208, jan. 1976.

JENKINSON, D. S.; LADD, J. N. Microbial biomass in soil: measurement and turnover. In: PAUL, E. A.; LADD, J. N. (Eds.). . **Soil biochemistry**. 5. ed. New York: Marcel Dekker, Inc., 1981. p. 415–471.

JIAN, J.; DU, X.; STEWART, R. D. A database for global soil health assessment. **Scientific Data**, v. 7, n. 1, p. 3–10, 2020.

JING, X. et al. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. **Nature Communications**, v. 6, n. 1, p. 8159, 2 nov. 2015.

JOERGENSEN, R. G.; WICHERN, F. Alive and kicking: Why dormant soil microorganisms matter. **Soil Biology and Biochemistry**, v. 116, n. October 2017, p. 419–430, 2018.

JOHNSON, D. L. et al. Meanings of Environmental Terms. **Journal of Environmental Quality**, v. 26, n. 3, p. 581–589, 1997.

KAISER, E. A. Significance of Microbial Biomass for Carbon and Nitrogen Mineralization in Soil. **Zeitschrift Fur Pflanzenernahrung Und Bodenkunde**, v. 157, n. 4, p. 271–278, 1994.

KALLENBACH, C. M.; FREY, S. D.; GRANDY, A. S. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. **Nature Communications**, v. 7, p. 13630, 28 nov. 2016.

KARLEN, D. L. et al. Soil Quality: A Concept, Definition, and Framework for Evaluation (A Guest Editorial). **Soil Science Society of America Journal**, v. 61, n. 1, p. 4, 1997.

KÄSTNER, M.; MILTNER, A. SOM and Microbes—What Is Left From Microbial Life. In: GARCIA, C.; NANNIPIERI, P.; HERNANDEZ, T. B. T.-T. F. OF S. C. (Eds.). . **The Future of Soil Carbon**. [s.l.] Elsevier, 2018. p. 125–163.

KOSSOFF, D. et al. Mine tailings dams: Characteristics, failure, environmental impacts, and remediation. **Applied Geochemistry**, v. 51, p. 229–245, 2014.

LANGILLE, M. G. I. et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. **Nature Biotechnology**, v. 31, n. 9, p. 814–821, 2013.

LEHMANN, J. et al. The concept and future prospects of soil health. **Nature Reviews Earth & Environment**, v. 1, n. 10, p. 544–553, 2020.

LESSARD, I.; SAUVÉ, S.; DESCHÊNES, L. Toxicity response of a new enzyme-based functional diversity methodology for Zn-contaminated field-collected soils. **Soil Biology and Biochemistry**, v. 71, p. 87–94, 2014.

LEWIS, D. E. et al. Soil functional diversity analysis of a bauxite-mined restoration chronosequence. **Microbial Ecology**, v. 59, n. 4, p. 710–723, 2010.

LIANG, C. et al. Quantitative assessment of microbial necromass contribution to soil organic matter. **Global Change Biology**, v. 25, n. 11, p. 3578–3590, 2019.

LIANG, C.; BALSER, T. C. Microbial production of recalcitrant organic matter in global soils: Implications for productivity and climate policy. **Nature Reviews Microbiology**, v. 9, n. 1, p. 75, 2011.

LIANG, C.; SCHIMEL, J. P.; JASTROW, J. D. The importance of anabolism in microbial control over soil carbon storage. **Nature Microbiology**, v. 2, n. 8, p. 1–6, 2017.

LJUNGDAHL, L. G.; ERIKSSON, K. Ecology of Microbial Cellulose Degradation. In: MARSHALL K.C. (Ed.). . **Advances in Microbial Ecology**. Boston, MA: Springer, 1985. p. 237–299.

MAJER, B. J. et al. Effects of heavy metal contamination of soils on micronucleus induction in Tradescantia and on microbial enzyme activities: A comparative investigation. **Mutation Research - Genetic Toxicology and Environmental Mutagenesis**, v. 515, n. 1–2, p. 111–124, 2002.

MALIK, A. A. et al. Bacterial Physiological Adaptations to Contrasting Edaphic Conditions Identified Using Landscape Scale Metagenomics. **mBio**, v. 8, n. 4, p. 1–13, 6 set. 2017.

MARON, P.-A. et al. High Microbial Diversity Promotes Soil Ecosystem Functioning. **Applied and Environmental Microbiology**, v. 84, n. 9, p. 1–13, 16 maio 2018.

MARTA-ALMEIDA, M. et al. Fundão Dam collapse: Oceanic dispersion of River Doce after the greatest Brazilian environmental accident. **Marine Pollution Bulletin**, 2016.

MARTINY, J. B. H. et al. Microbiomes in light of traits: A phylogenetic perspective. **Science**, v. 350, n. 6261, p. aac9323–aac9323, 6 nov. 2015.

MCDONALD, T.; JONSON, J.; DIXON, K. W. National standards for the practice of ecological restoration in Australia. **Restoration Ecology**, v. 24, p. S4–S32, 2016.

MIKANOVA, O. Effects of heavy metals on some soil biological parameters. **Journal of Geochemical Exploration**, v. 88, n. 1- 3 SPEC. ISS., p. 220–223, 2006.

MILTNER, A. et al. SOM genesis: Microbial biomass as a significant source. **Biogeochemistry**, v. 111, n. 1–3, p. 41–55, 2012.

MOREIRA, F. M. S.; SIQUEIRA, J. O. Microbiologia e Bioquímica do Solo. **Editora UFLA**, 2006.

MTPA. **Corredores Logísticos Estratégicos: Complexo de Minério de Ferro**. Brasília: Ministério dos Transportes, Portos e Aviação Civil, 2018.

MUNK, P. et al. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. **Nature Microbiology**, v. 3, n. 8, p. 898–908, 23 ago. 2018.

MUÑOZ-ROJAS, M. et al. Soil quality indicators to assess functionality of restored soils in degraded semiarid ecosystems. **Restoration Ecology**, v. 24, n. April, p. S43–S52, ago. 2016.

ODUM, E. P. The Strategy of Ecosystem Development. **Science**, v. 164, n. 3877, p. 262–270, 18 abr. 1969.

OSBURN, E. D. et al. Soil Bacterial and Fungal Communities Exhibit Distinct Long-Term Responses to Disturbance in Temperate Forests. **Frontiers in Microbiology**, v. 10, n. December, 2019.

PALMER, M. A. et al. Mountaintop Mining Consequences. **Science**, v. 327, n. 5962, p. 148–149, 8 jan. 2010.

PANDER, J.; GEIST, J. Ecological indicators for stream restoration success. **Ecological Indicators**, v. 30, p. 106–118, jul. 2013.

PARHAM, J. A.; DENG, S. P. Detection, quantification and characterization of β-glucosaminidase activity in soil. **Soil Biology and Biochemistry**, v. 32, n. 8–9, p. 1183–1190, ago. 2000.

PAZ-FERREIRO, J. et al. Biochemical properties of acid soils under native grassland in a temperate humid zone. **New Zealand Journal of Agricultural Research**, v. 50, n. 4, p. 537–548, 22 dez. 2007.

PAZ-FERREIRO, J.; FU, S. Biological Indices for Soil Quality Evaluation: Perspectives and Limitations. **Land Degradation & Development Development**, v. 27, n. 1, p. 14–25, 2016.

PERALTA, A. L. et al. A social-ecological framework for “micromanaging” microbial services. **Frontiers in Ecology and the Environment**, v. 12, n. 9, p. 524–531, 2014.

QUADROS, P. D. DE et al. Coal mining practices reduce the microbial biomass, richness and diversity of soil. **Applied Soil Ecology**, v. 98, p. 195–203, fev. 2016.

QUEIROZ, H. M. et al. The Samarco mine tailing disaster: A possible time-bomb for heavy metals contamination? **Science of the Total Environment**, v. 637–638, p. 498–506, 2018.

ROY, A. B. The Synthesis and Hydrolysis of Sulfate Esters. In: F. F. NORD (Ed.). . **Advances in Enzymology and Related Areas of Molecular Biology**. New York, NY: John Wiley & Sons, Ltd, 1960. p. 205–235.

SACCÁ, M. L. et al. Ecosystem Services Provided By Soil Microorganisms. In: **Soil Biological Communities and Ecosystem Resilience**. Cham: Springer International Publishing, 2017. p. 9–24.

SAMARCO. **Um ano do rompimento de Fundão**. [s.l.] Gerência Geral de Comunicação e Relações Institucionais, 2016.

SANCHEZ, W.; BURGEOT, T.; PORCHER, J.-M. A novel "Integrated Biomarker Response" calculation based on reference deviation concept. **Environmental Science and Pollution Research**, v. 20, n. 5, p. 2721–2725, 4 maio 2013.

SANTOS, J. V. DOS et al. Soil biological attributes in arsenic-contaminated gold mining sites after revegetation. **Ecotoxicology**, v. 22, n. 10, p. 1526–1537, 2013.

SANTOS, O. S. H. et al. Understanding the Environmental Impact of a Mine Dam Rupture in Brazil: Prospects for Remediation. **Journal of Environment Quality**, v. 48, n. 2, p. 439, 2019.

SANTOS, J. V. et al. Biological attributes of rehabilitated soils contaminated with heavy metals. **Environmental Science and Pollution Research**, v. 23, n. 7, p. 6735–6748, 10 abr. 2016.

SCHAEFER, C. E. G. R. et al. Paisagens de lama: os tecnossolos para recuperação ambiental de áreas afetadas pelo desastre da barragem do Fundão, em Mariana. In: **Boletim informativo Sociedade Brasileira de Ciência do Solo**. [s.l.] Sociedade Brasileira de Ciência do Solo, 2016. p. 18–23.

SCHIMEL, J. P.; SCHAEFFER, S. M. Microbial control over carbon cycling in soil. **Frontiers in Microbiology**, v. 3, n. SEP, p. 1–11, 2012.

SCHMEISSER, C.; STEELE, H.; STREIT, W. R. Metagenomics, biotechnology with non-culturable microbes. **Applied Microbiology and Biotechnology**, v. 75, n. 5, p. 955–962, 30 jul. 2007.

SCHNÜRER, J.; ROSSWALL, T. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. **Applied and Environmental Microbiology**, v. 43, n. 6, p. 1256–1261, 1982.

SEGURA, F. R. et al. Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). **Environmental Pollution**, v. 218, p. 813–825, nov. 2016.

SHOEMAKER, W. R.; LOCEY, K. J.; LENNON, J. T. A macroecological theory of microbial biodiversity. **Nature Ecology & Evolution**, v. 1, n. 5, p. 0107, 3 maio 2017.

SILVA, A. O. et al. Soil microbiological attributes indicate recovery of an iron mining area and of the biological quality of adjacent phytophysiognomies. **Ecological Indicators**, v. 93, n. December 2017, p. 142–151, 2018.

SINGH et al. Microbial biomass acts as a source of plant nutrient in dry tropical forest and savanna. **Nature**, n. JANUARY, p. 21–23, 1989.

SINSABAUGH, R. L.; FOLLSTAD SHAH, J. J. Ecoenzymatic Stoichiometry and Ecological Theory. **Annual Review of Ecology, Evolution, and Systematics**, v. 43, n. 1, p. 313–343, 2012.

SINSABAUGH, R. L.; HILL, B. H.; FOLLSTAD SHAH, J. J. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. **Nature**, v. 462, n. 7274, p. 795–798, 2009.

SONTER, L. J.; ALI, S. H.; WATSON, J. E. M. Mining and biodiversity: Key issues and research needs in conservation science. **Proceedings of the Royal Society B: Biological Sciences**, v. 285, n. 1892, 2018.

SONTER, L. J.; BARRETT, D. J.; SOARES-FILHO, B. S. Offsetting the impacts of mining to achieve no net loss of native vegetation. **Conservation Biology**, v. 28, n. 4, p. 1068–1076, 2014.

SRIVASTAVA, S. C.; SINGH, J. S. Microbial C, N and P in dry tropical forest soils: Effects of alternate land-uses and nutrient flux. **Soil Biology and Biochemistry**, v. 23, n. 2, p. 117–124, 1991.

TABATABAI, M. A.; BREMNER, J. M. Arylsulfatase Activity of Soils. **Soil Science Society of America Journal**, v. 34, n. 2, p. 225–229, mar. 1970.

TABATABAI, M. A.; BREMNER, J. M. Assay of urease activity in soils. **Soil Biology and Biochemistry**, v. 4, n. 4, p. 479–487, 1972.

TAYEBI-KHORAMI, M. et al. Re-Thinking Mining Waste through an Integrative. **Minerals**, v. 9(5), n. 2019, p. 286, 2019.

USGS. **Mineral Commodity Summaries**. Disponível em: <https://www.usgs.gov/centers/nmic/iron-ore-statistics-and-information>.

VESTERGAARD, G. et al. Making big data smart—how to use metagenomics to understand soil quality. **Biology and Fertility of Soils**, v. 53, n. 5, p. 479–484, 16 jul. 2017.

VIEIRA, S. et al. Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. **ISME Journal**, v. 14, n. 2, p. 463–475, 2020.

VOGEL, T. M. et al. TerraGenome: a consortium for the sequencing of a soil metagenome. **Nature Reviews Microbiology**, v. 7, n. 4, p. 252–252, abr. 2009.

WAHSHA, M. et al. Microbial enzymes as an early warning management tool for monitoring mining site soils. **Catena**, v. 148, p. 40–45, 2017.

WALL, D. H.; NIELSEN, U. N.; SIX, J. Soil biodiversity and human health. **Nature**, v. 528, n. 7580, p. 69–76, 2015.

WANG, J. T. et al. Linking microbial diversity with ecosystem functioning through a trait framework. **Journal of biosciences**, v. 44, n. 5, p. 2–4, 2019.

WISE. **WISE - World information service on energy uranium project**. Disponível em: <http://www.wise-uranium.org/mdaf.html>. Acesso em: 18 set. 2021.

XU, J. Microbial ecology in the age of genomics and metagenomics: concepts, tools, and recent advances. **Molecular Ecology**, v. 15, n. 7, p. 1713–1731, 8 mar. 2006.

SEGUNDA PARTE – ARTIGOS

3 ARTIGO 1 – Environmental drivers of shifts on microbial traits in sites disturbed by a large-scale tailing dam collapse

Standards of the journal – *Science of The Total Environment* (ISSN: 0048-9697)
(Final published version)

DOI: https://doi.org/10.1016/j.scitotenv.2020.139453

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

Éder Rodrigues Batista, Joana Junqueira Carneiro, Flávio Araújo Pinto, Jessé Valentim dos Santos, Marco Aurélio Carbone Carneiro*

Setor of Biology, Microbiology and Soil Biological Processes, Department of Soil Science, Federal University of Lavras (UFLA), Lavras - MG, Brazil.

*Corresponding author.
Address correspondence to
marcocarbone@ufla.br
Tel. *+55 35 2142-2051*

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 (Gomes 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 (*q*CO_2) and microbial (*q*MIC) 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 kEC factor of 2.64 (Vance et al., 1987). Microbial basal respiration (BR, µg C–CO2 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 (*q*MIC, %) was calculated from the MBC: Corg 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:

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

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

WMean index calculation was performed as follows:

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

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:

$$w_i = \frac{\mho_i}{\sum_{i=1}^{n} \mho_i}$$

where $\mho_i$ 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:

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

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

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

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):

$$\mathrm{A_i} = \mathrm{Z_i} - \mathrm{Z_0}$$

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:

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

where $\mathrm{A_i}$ is the deviation of ith enzymatic activity and n is the total number of studied enzymes.

2.6. 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 ≥ 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 qCO_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 – $q\text{CO}_2$ (Fig. 2c). The $q\text{CO}_2$ 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 qCO_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 qCO_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 Corg, 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.

References

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

Alef, K., 1995. Field methods, in: Alef, K.; Nannipieri, P. (Ed.), Methods in Applied Soil Microbiology and Biochemistry. Elsevier, pp. 463–490. https://doi.org/10.1016/B978-012513840-6/50025-2

Allison, S.D., Vitousek, P.M., 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol. Biochem. 37, 937–944. https://doi.org/10.1016/j.soilbio.2004.09.014

Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen’s climate classification map for Brazil. Meteorol. Zeitschrift. https://doi.org/10.1127/0941-2948/2013/0507

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1046/j.1442-9993.2001.01070.x

Anderson, T.-H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem. 21, 471–479. https://doi.org/10.1016/0038-0717(89)90117-X

Anderson, T.H., 2003. Microbial eco-physiological indicators to asses soil quality. Agric. Ecosyst. Environ. 98, 285–293. https://doi.org/10.1016/S0167-8809(03)00088-4

Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO2(qCO2) as a specific activity parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass of forest soils. Soil Biol. Biochem. 25, 393–395. https://doi.org/10.1016/0038-0717(93)90140-7

Bardgett, R.D., Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. https://doi.org/10.1038/nature13855

Barton, K., 2019. MuMIn: Multi-Model Inference.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J. Vis. Exp. 1–16. https://doi.org/10.3791/50961

Bonan, G.B., Hartman, M.D., Parton, W.J., Wieder, W.R., 2013. Evaluating litter decomposition in earth system models with long-term litterbag experiments: An example using the Community Land Model version 4 (CLM4). Glob. Chang. Biol. 19, 957–974. https://doi.org/10.1111/gcb.12031

Bouyoucos, G.J., 1962. Hydrometer Method Improved for Making Particle Size Analyses of Soils 1. Agron. J. 54, 464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Bremner, J.M., 1960. Determination of nitrogen in soil by the Kjeldahl method. J. Agric. Sci. 55, 11–33. https://doi.org/10.1017/S0021859600021572

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58, 216–234. https://doi.org/10.1016/j.soilbio.2012.11.009

Carmo, Flávio Fonseca do, Kamino, L.H.Y., Junior, R.T., Campos, I.C. de, Carmo, Felipe Fonseca do, Silvino, G., Castro, K.J. da S.X. de, Mauro, M.L., Rodrigues, N.U.A., Miranda, M.P. de S., Pinto, C.E.F., 2017. Fundão tailings dam failures: the environment tragedy of the largest technological disaster of Brazilian mining in global context. Perspect. Ecol. Conserv. 15, 145–151. https://doi.org/10.1016/j.pecon.2017.06.002

Chodak, M., Gołebiewski, M., Morawska-Płoskonka, J., Kuduk, K., Niklińska, M., 2013. Diversity of microorganisms from forest soils differently polluted with heavy metals. Appl. Soil Ecol. 64, 7–14. https://doi.org/10.1016/j.apsoil.2012.11.004

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

Delgado-Baquerizo, M., Grinyer, J., Reich, P.B., Singh, B.K., 2016. Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. Funct. Ecol. 30, 1862–1873. https://doi.org/10.1111/1365-2435.12674

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172. https://doi.org/10.1016/0038-0717(77)90070-0

Empresa Brasileira de Pesquisa Agropecuária, 2011. Manual de métodos de análise de solo, 3°. ed, Manual de métodos de análise de solos. Embrapa Solos, Brasília.

Fließbach, A., Martens, R., Reber, H.H., 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem. 26, 1201–1205. https://doi.org/10.1016/0038-0717(94)90144-9

Frankenberger, W.T., Johanson, J.B., 1982. Effect of pH on enzyme stability in soils. Soil Biol. Biochem. 14, 433–437. https://doi.org/10.1016/0038-0717(82)90101-8

Gao, Y., Wang, J., Xu, J., Kong, X., Zhao, L., Zeng, D., 2013. Assessing the quality of oil-contaminated saline soil using two composite indices. Ecol. Indic. 24, 105–112. https://doi.org/10.1016/j.ecolind.2012.06.005

Gomes, L.E. de O., Correa, L.B., Sá, F., Neto, R.R., Bernardino, A.F., 2017. The impacts of the Samarco mine tailing spill on the Rio Doce estuary, Eastern Brazil. Mar. Pollut. Bull. 120, 28–36. https://doi.org/10.1016/j.marpolbul.2017.04.056

Grossman, R.B., Reinsch, T.G., 2002. The Solid Phase, in: Dane, J.H., Topp, C.G. (Eds.), Methods of Soil Analysis. Part 4. Physical Methods. Soil Science Society of America, Madison, pp. 201–228. https://doi.org/10.2136/sssabookser5.4.c9

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. 228. 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, 10706. https://doi.org/10.1038/s41598-017-11143-x

Hinojosa, M.B., García-Ruíz, R., Viñegla, B., Carreira, J.A., 2004. Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. Soil Biol. Biochem. 36, 1637–1644. https://doi.org/10.1016/j.soilbio.2004.07.006

Insam, H., Haselwandter, K., 1989. Metabolic quotient of the soil microflora in relation to plant succession. Oecologia 79, 174–178. https://doi.org/10.1007/BF00388474

Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O’Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs, H.W., 2019. vegan: Community Ecology Package.

Jiang, Y., Sun, B., Jin, C., Wang, F., 2013. Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil. Soil Biol. Biochem. 60, 1–9. https://doi.org/10.1016/j.soilbio.2013.01.006

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630. https://doi.org/10.1038/ncomms13630

Kozak, M., Piepho, H.-P., 2018. What’s normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J. Agron. Crop Sci. 204, 86–98. https://doi.org/10.1111/jac.12220

Legendre, P., Anderson, M.J., 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecol. Monogr. 69, 1. https://doi.org/10.2307/2657192

Lenth, R., 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Leprince, F., Quiquampoix, H., 1996. Extracellular enzyme activity in soil: Effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus Hebeloma cylindrosporum. Eur. J. Soil Sci. 47, 511–522. https://doi.org/10.1111/j.1365-2389.1996.tb01851.x

Lessard, I., Sauvé, S., Deschênes, L., 2014. Toxicity response of a new enzyme-based functional diversity methodology for Zn-contaminated field-collected soils. Soil Biol. Biochem. 71, 87–94. https://doi.org/10.1016/j.soilbio.2014.01.002

Li, Y., Jia, Z., Sun, Q., Zhan, J., Yang, Y., Wang, D., 2016. Ecological restoration alters microbial communities in mine tailings profiles. Sci. Rep. 6, 25193. https://doi.org/10.1038/srep25193

Li, Y.T., Rouland, C., Benedetti, M., Li, F. bai, Pando, A., Lavelle, P., Dai, J., 2009. Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. Soil Biol. Biochem. 41, 969–977. https://doi.org/10.1016/j.soilbio.2009.01.021

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 1–6. https://doi.org/10.1038/nmicrobiol.2017.105

Lu, H., Li, Z., Fu, S., Méndez, A., Gascó, G., Paz-Ferreiro, J., 2015. Combining phytoextraction and biochar addition improves soil biochemical properties in a soil contaminated with Cd. Chemosphere 119, 209–216. https://doi.org/10.1016/j.chemosphere.2014.06.024

Malik, A.A., Puissant, J., Buckeridge, K.M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H.S., Peyton, J.M., Mason, K.E., van Agtmaal, M., Blaud, A., Clark, I.M., Whitaker, J., Pywell, R.F., Ostle, N., Gleixner, G., Griffiths, R.I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. Nat. Commun. 9, 3591. https://doi.org/10.1038/s41467-018-05980-1

Malik, A.A., Thomson, B.C., Whiteley, A.S., Bailey, M., Griffiths, R.I., 2017. Bacterial Physiological Adaptations to Contrasting Edaphic Conditions Identified Using Landscape Scale Metagenomics. MBio 8, 1–13. https://doi.org/10.1128/mBio.00799-17

Marigómez, I., Garmendia, L., Soto, M., Orbea, A., Izagirre, U., Cajaraville, M.P., 2013. Marine ecosystem health status assessment through integrative biomarker indices: A comparative study after the Prestige oil spill “mussel Watch.” Ecotoxicology 22, 486–505. https://doi.org/10.1007/s10646-013-1042-4

Mendez, M.O., Neilson, J.W., Maier, R.M., 2008. Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailing site. Appl. Environ. Microbiol. 74, 3899–3907. https://doi.org/10.1128/AEM.02883-07

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: Microbial biomass as a significant source. Biogeochemistry 111, 41–55. https://doi.org/10.1007/s10533-011-9658-z

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 5, 1–10. https://doi.org/10.3389/fmicb.2014.00022

Nielsen, U.N., Wall, D.H., Six, J., 2015. Soil Biodiversity and the Environment. Annu. Rev. Environ. Resour. 40, 63–90. https://doi.org/10.1146/annurev-environ-102014-021257

Parsons, L.L., Smith, M.S., 1989. Microbial Utilization of Carbon-14-Glucose in Aerobic vs. Anaerobic Denitrifying Soils. Soil Sci. Soc. Am. J. 53, 1082. https://doi.org/10.2136/sssaj1989.03615995005300040016x

Paz-Ferreiro, J., Fu, S., 2016. Biological Indices for Soil Quality Evaluation: Perspectives and

Limitations. L. Degrad. Dev. Dev. 27, 14–25. https://doi.org/10.1002/ldr.2262

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2019. nlme: Linear and Nonlinear Mixed Effects Models.

Prado, I.G. de O., da Silva, M. de C.S., Prado, D.G. de O., Kemmelmeier, K., Pedrosa, B.G., Silva, C.C. da, 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

Queiroz, H.M., Nóbrega, G.N., Ferreira, T.O., Almeida, L.S., Romero, T.B., Santaella, S.T., Bernardino, A.F., Otero, X.L., 2018. The Samarco mine tailing disaster: A possible time-bomb for heavy metals contamination? Sci. Total Environ. 637–638, 498–506. https://doi.org/10.1016/j.scitotenv.2018.04.370

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Rui, Y., Murphy, D. V., Wang, X., Hoyle, F.C., 2016. Microbial respiration, but not biomass, responded linearly to increasing light fraction organic matter input: Consequences for carbon sequestration. Sci. Rep. 6, 35496. https://doi.org/10.1038/srep35496

Sanchez-Hernandez, J.C., Sandoval, M., Pierart, A., 2017. Short-term response of soil enzyme activities in a chlorpyrifos-treated mesocosm: Use of enzyme-based indexes. Ecol. Indic. 73, 525–535. https://doi.org/10.1016/j.ecolind.2016.10.022

Sanchez, W., Burgeot, T., Porcher, J.-M., 2013. A novel “Integrated Biomarker Response” calculation based on reference deviation concept. Environ. Sci. Pollut. Res. 20, 2721–2725. https://doi.org/10.1007/s11356-012-1359-1

Santos, J. V., Varón-López, M., Fonsêca Sousa Soares, C.R., Lopes Leal, P., Siqueira, J.O., de Souza Moreira, F.M., 2016. Biological attributes of rehabilitated soils contaminated with heavy metals. Environ. Sci. Pollut. Res. 23, 6735–6748. https://doi.org/10.1007/s11356-015-5904-6

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 1–11. https://doi.org/10.3389/fmicb.2012.00348

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Environ. Microbiol. 43, 1256–1261. https://doi.org/0099-2240/82/061256-06$02.00/0

Serafim, A., Company, R., Lopes, B., Fonseca, V.F., Frana, S., Vasconcelos, R.P., Bebianno, M.J., Cabral, H.N., 2012. Application of an integrated biomarker response index (IBR) to assess temporal variation of environmental quality in two Portuguese aquatic systems. Ecol. Indic. 19, 215–225. https://doi.org/10.1016/j.ecolind.2011.08.009

Silva, A.C., Cavalcante, L.C.D., Fabris, J.D., Júnior, R.F., Barral, U.M., Farnezi, M.M. de M., Viana, A.J.S., Ardisson, J.D., Fernandez-Outon, L.E., Lara, L.R.S., Stumpf, H.O., Barbosa, J.B.S., Silva, L.C. da, 2016. Chemical, mineralogical and physical characteristics of a material accumulated on the river margin from mud flowing from the collapse of the iron ore tailings dam in Bento Rodrigues, Minas Gerais, Brazil. Rev. Espinhaço 5, 44–53.

Silva, A.O., da Costa, A.M., dos Santos Teixeira, A.F., Azarias Guimarães, A., Valentim dos Santos, J., de Souza Moreira, F.M., 2018. Soil microbiological attributes indicate recovery of an iron mining area and of the biological quality of adjacent phytophysiognomies. Ecol. Indic. 93, 142–151. https://doi.org/10.1016/j.ecolind.2018.04.073

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798. https://doi.org/10.1038/nature08632

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Nelson, D.W., Sommers, L.E., 1996. Total Carbon, Organic Carbon, and Organic Matter, in: Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America and American Society of Agronomy, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Stefanowicz, A.M., Kapusta, P., Szarek-Łukaszewska, G., Grodzińska, K., Niklińska, M., Vogt, R.D., 2012. Soil fertility and plant diversity enhance microbial performance in metal-polluted soils. Sci. Total Environ. 439, 211–219. https://doi.org/10.1016/j.scitotenv.2012.09.030

Tabatabai, M.A., Bremner, J.M., 1970. Arylsulfatase Activity of Soils. Soil Sci. Soc. Am. J. 34, 225. https://doi.org/10.2136/sssaj1970.03615995003400020016x

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. Camb. Philos. Soc. 67, 321–358. https://doi.org/10.1111/j.1469-185X.1992.tb00728.x

Waring, B.G., Weintraub, S.R., Sinsabaugh, R.L., 2014. Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117, 101–113. https://doi.org/10.1007/s10533-013-9849-x

Wei, T., Simko, V., 2017. R package “corrplot”: Visualization of a Correlation Matrix.

Woch, M.W., Radwańska, M., Stanek, M., Łopata, B., Stefanowicz, A.M., 2018. Relationships between waste physicochemical properties, microbial activity and vegetation at coal ash and sludge disposal sites. Sci. Total Environ. 642, 264–275. https://doi.org/10.1016/j.scitotenv.2018.06.038

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)

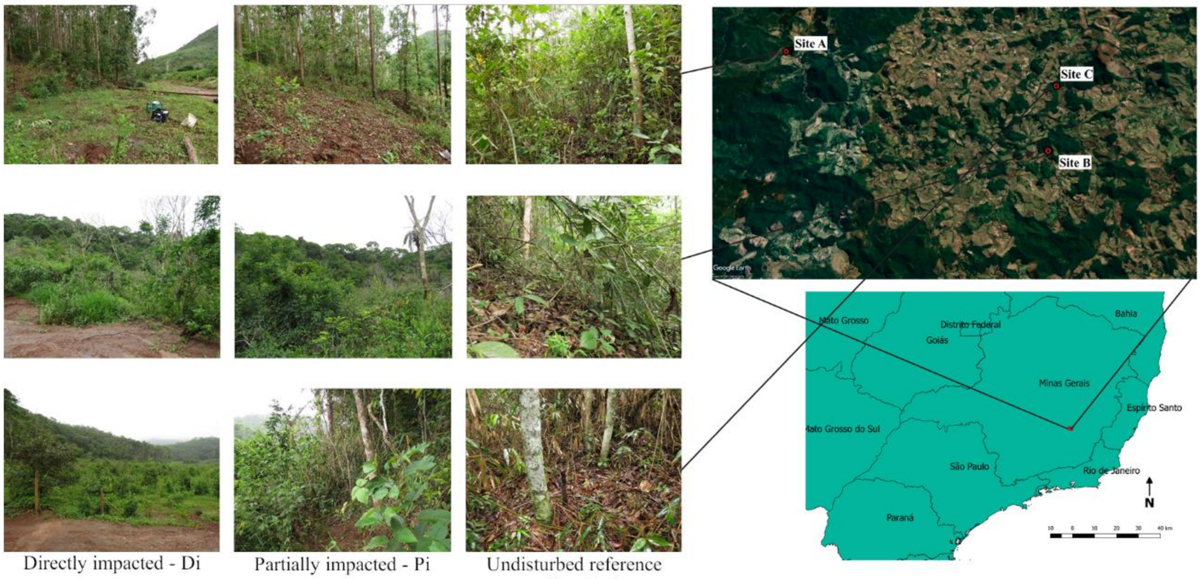
Tested pair	Site A			Site B			Site C		
	F	r^2	*p-Value*	F	r^2	*p-Value*	F	r^2	*p-Value*
Di × Und	10.2	0.56	0.01	16.4	0.67	0.02	16.2	0.67	0.02
Pi × Und	10.6	0.57	0.02	8.0	0.50	0.02	16.1	0.67	0.02

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



Directly impacted - Di

Partially impacted - Pi

Undisturbed reference soils - Und

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

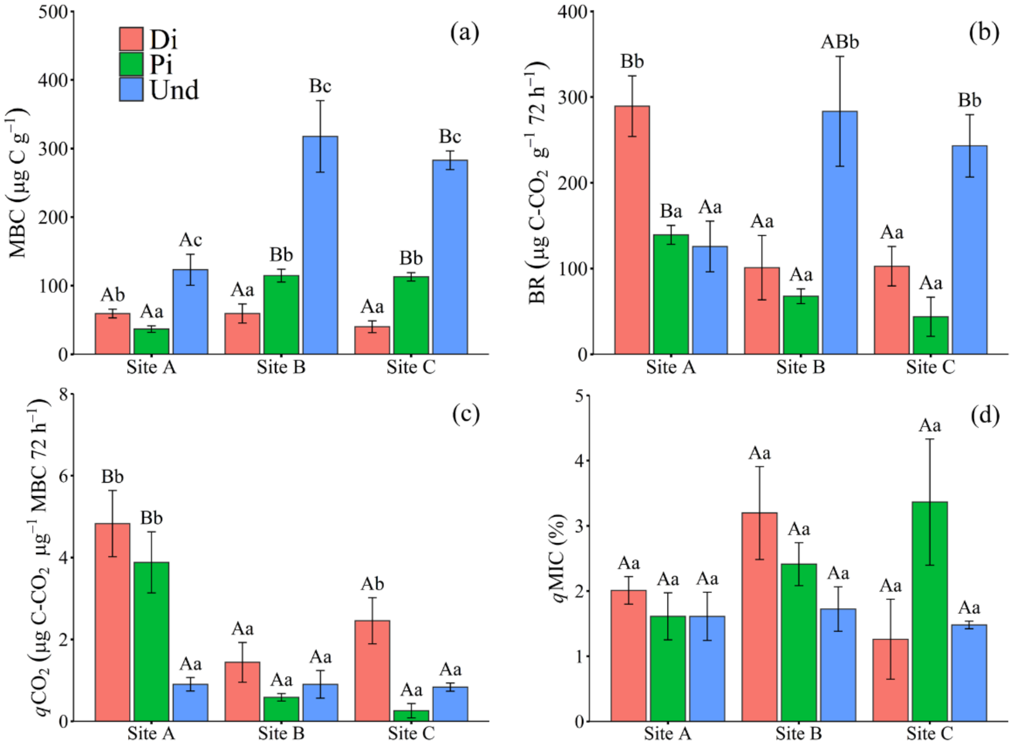


Fig. 2. Ecophysiological traits of microbial community. (a) microbial biomass carbon (MBC), (b) basal respiration (BR), (c) metabolic quotient (qCO$_2$), and (d) microbial quotient (qMIC) in different sites disturbed after the Fundão dam collapse. Bars show the mean for each studied condition (n = 5) 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.

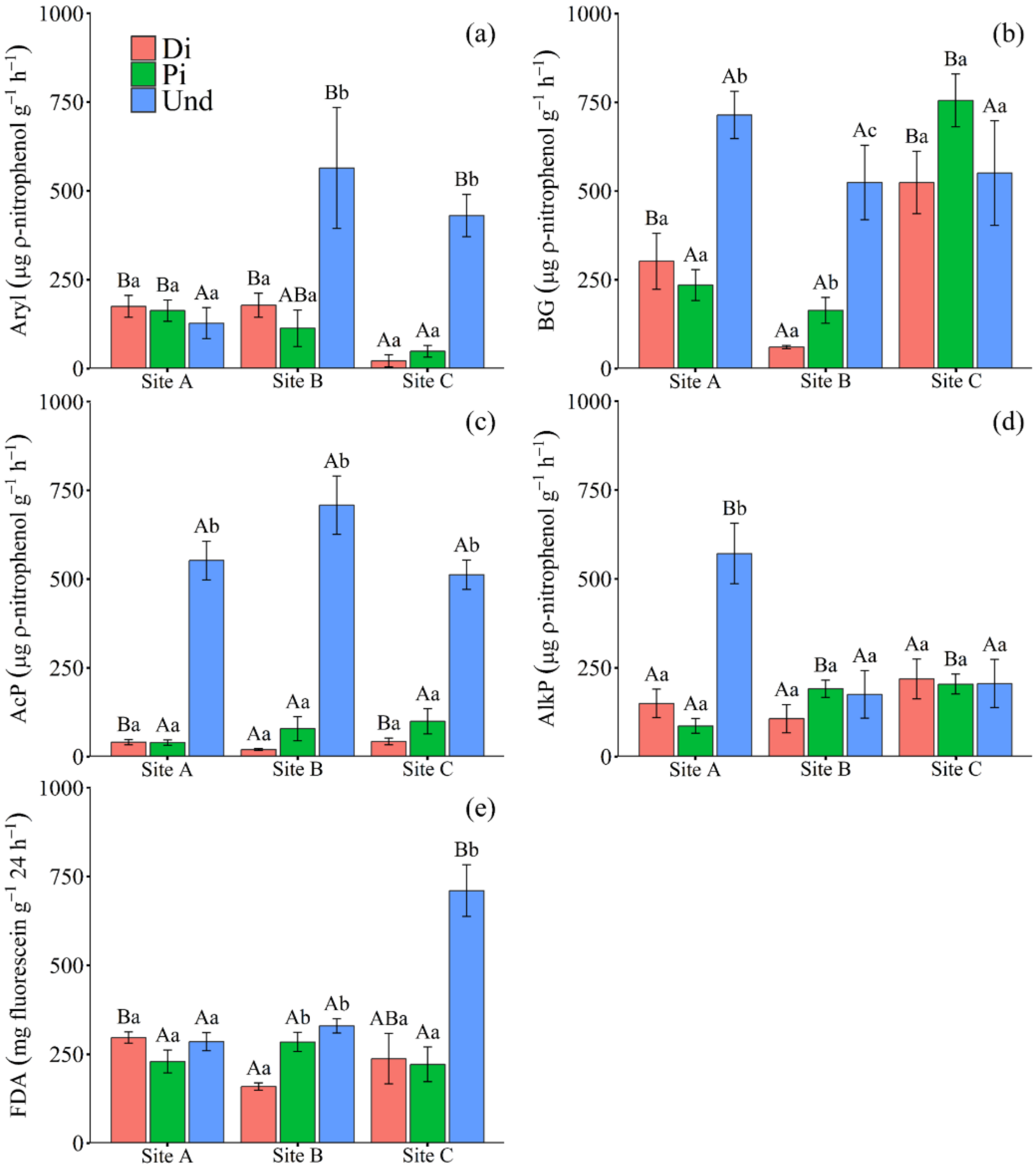


Fig. 3. Extracellular enzymatic activities. (a) arylsulfatase (Aryl), (b) β-1,4-glucosidase (BG), (c) acid phosphatase (AcP), (d) alkaline phosphatase (AlkP), and (e) fluorescein diacetate hydrolysis (FDA) at different sites disturbed after the Fundão dam collapse. Bars show the mean for each studied 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.

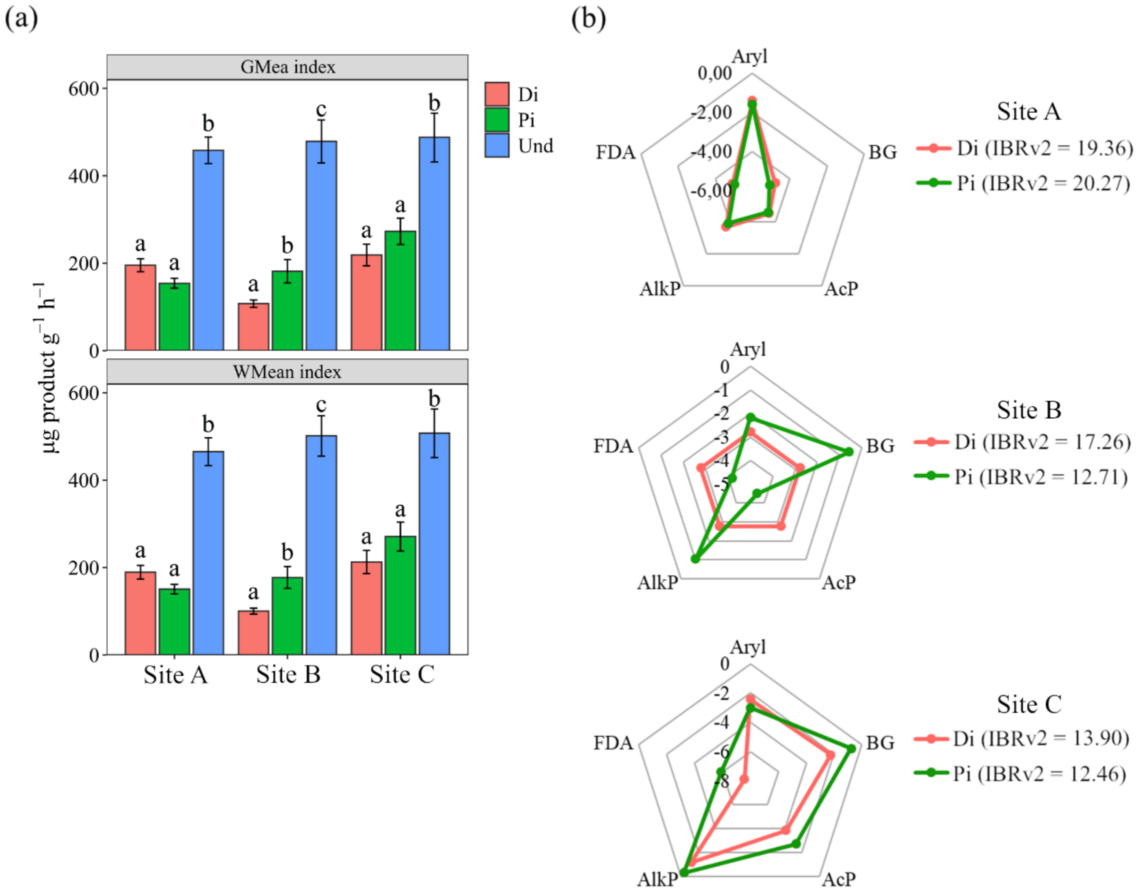


Fig. 4. Enzyme-based indexes. (a) average values of the GMea and WMean indexes from disturbed conditions (tailings) compared to undisturbed reference soils within each studied site and (b) sunray plots for distribution of A_i–scores for the IBRv2 index as calculated for each enzyme activity measured 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.

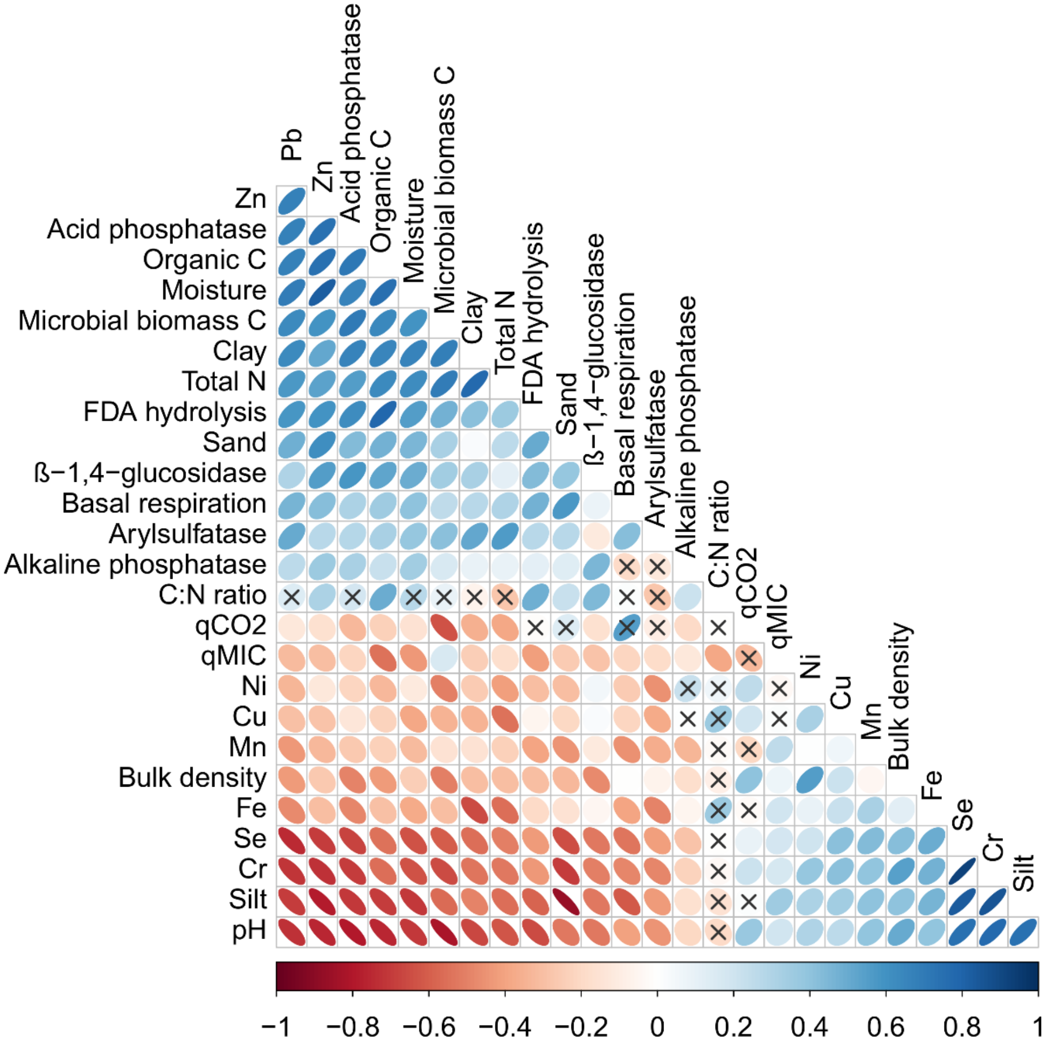


Fig. 5. Correlogram between microbial traits and environmental drivers. The color intensity and inclination degree of ellipses indicate the magnitude of correlation for a specific pair of variables. The ellipses of positive correlations are shown in blue and the negative correlations in red. The crosses represent absence of significance ($p > 0.05$). *q*MIC, microbial quotient (Microbial biomass C: C_{org} ratio), *q*CO_2, metabolic quotient (Basal respiration: Microbial biomass C ratio).

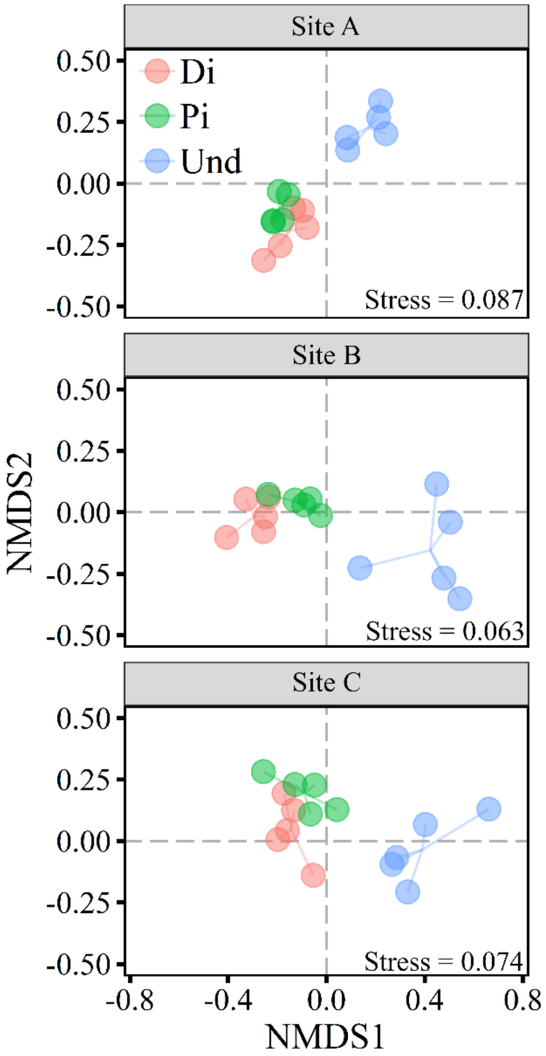


Fig. 6. Non-metric multidimensional scaling (NMDS) plot for the differences in microbial traits among disturbed conditions and undisturbed reference soils for each site after the Fundão dam collapse. Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soils.

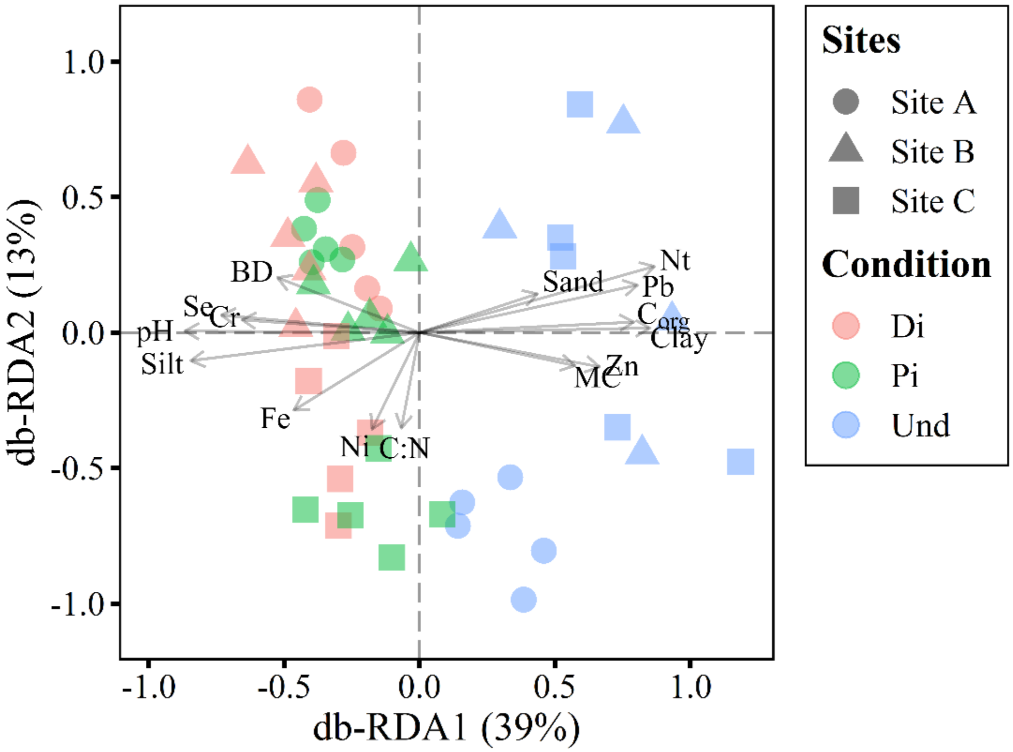


Fig. 7. Distance-based redundancy analysis (db-RDA) of different microbial traits with forward selection of predictor variables followed by Monte Carlo permutation tests (9,999 permutations). Solid arrows represent predictor (physicochemical variables and heavy metals) significantly associated with the 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.

4 ARTIGO 2 – Organic substrate availability and enzyme activity affect microbial-controlled carbon dynamics in areas disturbed by a mining dam failure

Standards of the journal – *Applied Soil Ecology* (ISSN: 0929-1393)
(Final published version)

DOI: https://doi.org/10.1016/j.apsoil.2021.104169

Organic substrate availability and enzyme activity affect microbial-controlled carbon dynamics in areas disturbed by a mining dam failure

Éder Rodrigues Batista, Aline de Jesus Franco, Ana Paula Valadares da Silva, Jessyca Adriana Gomes Florêncio da Silva, Davi Santos Tavares, Josemara Karina de Souza, Aline Oliveira Silva, Marisangela Barbosa, Jessé Valentim dos Santos, Marco Aurélio Carbone Carneiro*

Federal University of Lavras, Department of Soil Science, 37200-900 Lavras, Minas Gerais, Brazil

*Corresponding author.
Address correspondence to
marcocarbone@ufla.br
Tel. *+55 35 2142-2051*

ABSTRACT: We investigated key ecophysiological traits of microbial communities across different disturbance levels for two upper horizon depths (A1: 0–3 cm and A2: 10–13 cm) in areas disturbed by Fundão dam failure across Gualaxo do Norte river. We found that lower microbial growth (microbial biomass) and higher microbial-C losses (high $q\mathrm{CO}_2$) were linked to both decreases in organic substrate availability and enzyme activity suggesting important feedbacks on C dynamics in areas disturbed by Fundão dam failure. Basal respiration was not affected by organic substrate availability but increased with enzyme activity at A2 suggesting an enzymatic compensation in response to a lower plant-derived input. Lower degradative activity of extracellular enzymes (expressed as GMean index) was linked to lower organic matter content in mine tailings. Our results suggest that changes in organic substrate availability reflect changes in the microbial-controlled C dynamics in mine tailings. Thus, the design of restorative strategies based on microbial traits has the potential to accelerate the rehabilitation in areas disturbed by Fundão dam failure.

Keywords: Microbial Activity, Microbial Growth, Enzyme Activity, Carbon Cycle, Ecosystem Functioning, Mine Tailings

1. Introducion

Fundão dam failure, in November 2015, released approximately 43 million m^3 of iron ore tailings in one of the largest mining disasters known to date, contaminating soils and water resources and suppressing thousands of hectares of native vegetation (Carmo et al., 2017). Recent studies investigating Fundão dam failure impacts on microbial communities have provided evidence on the shifts in their ecophysiology and enzyme activities (Batista et al., 2020) as well as in the diversity of prokaryotic and eukaryotic groups in areas disturbed by the accident (Giongo et al., 2020; Prado et al., 2019). However, information on how microbial ecophysiological traits respond to organic substrate availability and enzyme activity and how shifts in these traits affect the microbial-controlled carbon dynamics in disturbed areas are lacking. It is recognized that microbes have the potential to control the turnover, accumulation, and losses of C from terrestrial ecosystems (Liang et al., 2017; Schimel and Schaeffer, 2012). For instance, microbial biomass (MBC) – a small, highly labile, and active fraction of organic matter (OM) – promotes the accumulation and stabilization of C into the soils through microbial-OM formation stabilized as microbial necromass (i.e. dead cell residues), and thus soils with greater MBC has the potential to have increased C pools (Bradford et al., 2013; Kallenbach et al., 2016; Liang et al., 2017). Indeed, recent studies have provided evidence that microbial necromass makes up between 50 and 80% of soil C pools (Liang and Balser, 2011; Sokol and Bradford, 2019). Furthermore, microbial community size and activity, characterized by the release of CO2 from soils (also referred to as basal respiration), drives decomposition rates and also determines the microbial regulation of soil C (Schimel, 2013). At the same time, microbes are reliant on organic substrate availability for building up their own biomass and enzymes (Allison et al., 2010; Kalbitz et al., 2000; Sokol and Bradford, 2019). For instance, dissolved organic carbon (DOC) derived from rhizodeposition (e.g. root exudates) or that available from the degradative activity of extracellular enzymes represents a high-energy value labile substrate for microbes and can stimulate microbial growth, thus increasing the potential of microbial-derived C accumulation through increases in biomass and necromass of these communities (Kalbitz et al., 2000; Sokol and Bradford, 2019; Soong et al., 2020). However, recent studies have revealed that Fundão dam tailings are not only OM- and nutrient-limited (Batista et al., 2020; Silva et al., 2016) as also present restrict plant growth, which might limit plant-derived DOC inputs (Cruz et al., 2020). Consequently, such resource-limited conditions can alter the microbial community resource allocation as a metabolic trade-off to survive in a stressful environment, leading to changes from growth to maintenance respiration (Malik et al., 2018; Schimel et al., 2007). Thus, if more resource is devoted to cell maintenance instead of growth, an increase in biomass-specific respiration ($q\mathrm{CO}_2$) is expected. The $q\mathrm{CO}_2$ (basal respiration-to-biomass ratio) represents a simplified indicator of energy demands for cell maintenance and microbial C use efficiency (Anderson and Domsch, 1993; Joergensen and Wichern, 2018). Consequently, higher $q\mathrm{CO}_2$ rates indicate potential increases in microbial-derived C losses and, along with decreases in MBC, can be linked to greater C loss from soils, with implications for ecosystem functioning and soil-atmosphere feedbacks (Malik et al., 2018; Schimel, 2013; Schimel et al., 2007). Likewise, labile C limitation (e.g. DOC) also leads microbial communities to depend strongly on the degradative activity of extracellular enzymes for resource acquisition (Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2012). Such agents of decomposition depolymerize polymeric OM and microbial debris to produce labile substrates for microbial assimilation and, therefore, play an important role in controlling microbial ecophysiology (Allison et al., 2010; Moorhead et al., 2013). In this study, we investigated the responses of microbial communities from two upper horizon depths to altered substrate availability and enzyme activity in areas disturbed by the Fundão dam failure. We hypothesized that changes in organic substrate availability and enzyme activity would affect the microbial metabolism, leading to community-level trade-offs and shifts in microbial-controlled carbon dynamics. Further, we hypothesized that changes in both factors would determine depth-dependent effects at the upper horizon on microbial community traits in mine tailings. To test these hypotheses, we (1) investigated empirical relationships of labile C content and enzyme activity with key microbial ecophysiological traits and (2) assessed the microbial ecophysiological control on C pool.

2. Material and Methods

2.1. Field sites and sampling

Samples were collected across three sites disturbed by Fundão dam failure [(site A: 20°16′0.034″ S; 43°18′ 14.177″ W), (site B: 20°17′47.916″ S; 43°12′18.584″ W), and (site C: 20°16′22.272″ S; 43°12′4.293″ W)] at September 2019, four years after the accident. The field sites and disturbance levels (i.e. Di, direct impact, Pi, intermediate impacted, Und, undisturbed soil) studied here are described in detail at Batista et al. (2020). From five equally spaced points (5 m from each other) along a transect established within each disturbance level, five soil and tailings cores (4 cm inner diameter) were randomly taken from two depths representing the top (0–3 cm, A1) and approximately middle (10–13 cm, A2) of the upper horizon and pooled to obtain one composite sample per point. This produced 30 composite samples per site, totalizing 90 samples. We carefully removed the litter layer before sampling. Cored samples stratification at specific layers (A1 and A2, each 3 cm thickness) was made to assessing the influence of changes in organic substrate availability and enzyme activity on the microbial community traits on a compact depth scale. In the laboratory, all samples were then freshly sieved to 2 mm mesh size after removing coarse debris and roots and stored at 4 °C in sterile, hermetically sealed plastic bags until analyses.

2.2. Measurements of biotic and abiotic variables

Total organic carbon (TOC, g kg^{-1}) – a common proxy for soil organic matter – was determined in the soil and tailings samples using a standard protocol, based on the potassium dichromate oxidation method (Nelson and Sommers, 1996). Samples pH (tailings and soil) was determined using a glass electrode in deionized water solution (1: 2.5 v/v). Extractable calcium (Ca^{2+}) and magnesium (Mg^{2+}) were extracted with 1M of KCl extractant, whereas phosphorus (P), potassium (K^{+}), iron (Fe), manganese (Mn), zinc (Zn^{2+}), and copper (Cu^{2+}) were extracted from samples by Mehlich-1 extractant. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry. P and K^{+} were determined by colorimetry and atomic emission spectroscopy, respectively. Fe, Mn, Zn^{2+}, and Cu^{2+} were determined in an ICP-OES analyzer. Base sum (BS) was calculated as the sum of Ca^{2+}, Mg^{2+}, and K^{+}. Effective cation exchange capacity (CECe) was calculated as the sum of Ca^{2+}, Mg^{2+}, K^{+}, and Al^{3+}, whereas potential cation exchange capacity (CECp) as the sum of Ca^{2+}, Mg^{2+}, K^{+}, Al^{3+}, and H^{+}. Textural characterization was performed by based on Bouyoucos method (Bouyoucos, 1962).

Microbial biomass carbon (MBC, µg C g^{-1}) and dissolved organic carbon (DOC, µg C g^{-1}) were determined through the chloroform fumigation-extraction method (Vance et al., 1987). Fumigated and unfumigated (controls) soil and tailings samples (20 g) were horizontally shaken on a shaker table with 100 mL of 0.5 M K_2SO_4 for 30 min. Extractable organic C in the samples was quantified by titration of potassium dichromate excess with 33.3 mM ammonium ferrous sulfate. We calculated the MBC as the difference between the C content in fumigated and unfumigated samples, which was then multiplied by a k_{EC} factor of 2.64 (Vance et al., 1987), whereas the extractable C derived from unfumigated samples was reported as DOC (Bolan et al., 1996). Microbial basal respiration (respired C, μg CO_2 g^{-1} 72 h^{-1}) was used as a proxy for the active microbial community and measured following incubation of soil and tailings samples (20 g) in glass jars of 1 L completely sealed in the presence of 0.05 M NaOH for 72 h in the dark at room temperature (25 °C) (Alef, 1995). Afterward, cumulative CO_2 efflux was estimated by titration of NaOH with a 0.05 M HCl solution. Controls were performed by incubating completely sealed jars only containing NaOH solution following the same conditions as described above. Microbial basal respiration was then divided by the microbial biomass to obtain the biomass-specific respiration or microbial metabolic quotient (qCO_2, μg C $μg^{-1}$ MBC 72 h^{-1}), which was used as a proxy for microbial C use efficiency (Anderson and Domsch, 1993; Joergensen and Wichern, 2018).

The potential activities of β-1,4-glucosidase (BG, EC 3.2.1.21) and acid phosphomonoesterase (orthophosphoric monoester phosphohydrolase, AcP, EC 3.1.3.2) were measured following a common colorimetric assay protocol (Dick, 2011). These enzymes are among the most relevant exocellulase in the soil and are important agents of organic matter cycling: BG was chosen as it is involved in the hydrolysis of glucose from the nonreducing end of cellobiose, as well as the cellulose and other β-1,4 glucans and hence can be used as a proxy for C acquisition by microbial communities, whereas AcP was chosen as it is involved in the hydrolysis of phosphate from phosphoesters and represents a proxy for P acquisition (Dick, 2011). Briefly, 1 g of soil and tailings samples (three technical replicates) was mixed with 4 mL of modified universal buffer (MUB, pH 6.0) and 1 mL of 25 mM MUB-substrate solution (ρ-nitrophenyl-β-D-glucopyranoside for BG and ρ-nitrophenyl phosphate tetrahydrate for AcP, Sigma Chemical Co., St. Louis, MO). Reactions were performed for 1 h at 37 °C. Following incubation, 1 mL of 0.5 M CaCl2 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 the supernatant of AcP received the same amount of $CaCl_2$ with 4 mL of NaOH. After samples filtering, the ρ-nitrophenol (ρNP) release was quantified through absorbance readings on a spectrophotometer at 410 nm. Controls were performed as described above, but the substrate addition was made only after the reactions' interruption. The potential extracellular enzyme activities were expressed in the unit of μg ρNP g^{-1} h^{-1}. We then applied the geometric mean of enzyme activity (GMean index) to investigate the integrated responses of microbial community enzyme activity, calculated following Hinojosa et al. (2004) as:

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

where y_i is the enzyme activity and n is the total number of studied enzymes. GMean index was expressed as µg g^{-1} h^{-1}.

2.5. Statistical analyses

Regression analyses were performed for each depth to assessing the ecophysiological response of microbial communities to organic substrate availability (i.e. DOC content) and extracellular enzyme activity (i.e. GMean index) across different disturbance levels. We also established relationships of TOC – a proxy for soil organic matter – with MBC, $q\text{CO}_2$, and GMean index to assess, respectively, the influence of microbial growth (i.e. biomass synthesis) and microbial C use efficiency on C sequestration dynamics, and substrate availability (i.e. organic matter) on degradative enzyme activity across disturbance levels. Regression analyses were performed with *nlme* package v3.1.144 (Pinheiro et al., 2020) and visualizations were generated using the *ggplot2* package v3.3.1 (Wickham, 2016) in R statistical environment v.3.6.3 (R Core Team, 2020).

3. Results and discussion

Our study reveals significant relationships of microbial biomass with organic substrate availability (DOC content) and enzyme activity (GMean index) in both studied depths, suggesting interdependence between the microbial growth and organic substrate availability (Fig. 1a and b, supplementary table S1). These results show that organic substrate derived from rhizodeposition (e.g. root exudates) or from the extracellular enzyme activity represents a high-energy value source for soil microbes (Kalbitz et al., 2000; Sokol and Bradford, 2019). At the same time, the lower contents of Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, as well as lower values of base sum (BS), effective cation exchange capacity (CECe), potential cation exchange capacity (CECp), and clay in both depths suggest lower capability of mine tailings in supplying nutrients to plants and microbes (low fertility) relative to undisturbed soils (Table 1). Indeed, low fertility of mine tailings was shown to be a constrained factor to plant growth (Cruz et al., 2020), which might result in lower plant-derived inputs (Sokol and Bradford, 2019). Consequently, decreases in microbial growth driven by organic substrate limitation alter microbial-derived C accumulation in areas disturbed by Fundão dam failure since microbial necromass was shown to be both a prerequisite to and the main component of the long-lasting organic matter, rather than decomposed plant debris (Bradford et al., 2013; Kallenbach et al., 2016). Evidence for decreased microbial contributions to the C pool in mine tailings comes from the TOC pool depletion with corresponding reductions in microbial biomass at both depths (Fig. 2a, supplementary table S2), which suggests a lower potential for C sequestration resultant from living microbial biomass and ultimately from dead cell residues (i.e. necromass) in disturbed areas, contributing to imbalances in microbial carbon cycling processes and the ecosystem sustainability (Joergensen and Wichern, 2018; Liang et al., 2017; Soong et al., 2020).

Although the labile C availability is expected to stimulate microbial basal respiration, a proxy for the soil active microbial community (Moorhead et al., 2013; Schimel and Schaeffer, 2012), we did not observe the influence of DOC on microbial CO_2 efflux independently of studied depth (Fig. 1c, supplementary table S1). However, such an efflux increased with increasing enzyme activity at A2 (Fig. 1d, supplementary table S1). This result suggests that due to the lower organic substrate availability at the subsurface layer in mine tailings, microbial breakdown of organic matter assumes a crucial role on substrate availability at this layer (Moorhead et al., 2013; Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2012). On the other hand, microbial communities have presented increased biomass-specific respiration (qCO_2) with decreasing DOC and GMean index in disturbed areas, suggesting community-level metabolic trade-offs in response to the organic substrate-limited environment of mine tailings (Fig. 1e and f, supplementary table S1). This shows that lower organic substrate availability in mine tailings leads to increases in the qCO_2 due to more energy demands for maintenance functions, which results in more microbial-C being respired instead of being converted into microbial biomass (Joergensen and Wichern, 2018; Malik et al., 2018). Also, our results show that organic substrate availability is an important factor regulating microbial-C partitioning and, therefore, can be considered as an environmental predictor of constraints on microbial growth efficiency, as well as C accumulation and losses from soils (Kallenbach et al., 2016; Schimel, 2013). Evidence for such microbial-derived C–CO_2 losses at higher biomass-specific respiration rates comes from the observation that the TOC pool in the environment of mine tailings was lower with increasing qCO_2 (Fig. 2b, supplementary table S2). Such an observation is in line with previous studies which showed that microbial acclimatization to an organic substrate-limited environment leads to shifts from a growth metabolism to maintenance respiration and might contributes to C losses from soil (Malik et al., 2018; Schimel, 2013; Schimel et al., 2007).

Extracellular enzyme activity reflects the response of the heterotrophic microbial communities to supply its metabolic demands in response to resource availability in the environment (Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2012). According to our findings, decreases in microbial biomass and increases in biomass-specific respiration with corresponding reductions in the GMean index reveals the influence of organic matter depolymerization rates on microbial ecophysiology and suggests that in mine tailings with lower organic substrate, resource limitation leads microbial communities to depend strongly on enzyme activity to convert polymeric organic matter to labile, assimilable forms (displayed as points size of TOC, Fig. 1b, d and f). Decreases in GMean index, in turn, were associated with the lower TOC pool in mine tailings when compared to the undisturbed soils for both depths (Fig. 3, supplementary table S3). This result shows that the presence of organic matter is crucial for both extracellular enzyme activity and the resultant control from this activity (e.g. organic substrate availability) on the ecophysiology of microbial communities (Allison et al., 2010; Sinsabaugh and Follstad Shah, 2012). Also, our findings suggest dynamic relationships between microbial growth, microbial C use efficiency, and extracellular enzyme activity (Fig. 4) since the organic substrates availability is an important prerequisite not only to biomass synthesis but also the enzyme production and activity (Moorhead et al., 2013; Sinsabaugh et al., 2009; Sokol and Bradford, 2019).

In conclusion, our study provides evidence that changes in organic substrate availability and enzyme activity lead to trade-offs in community-level resource allocation as a tolerance strategy to stressful environmental conditions. These trade-offs have consequences for microbial-controlled carbon dynamics in disturbed areas since increases in microbial-C losses reduce the biomass synthesis (i.e. growth) and, therefore, the potential to C accumulation and stabilization in mine tailings through the microbial path. Also, a relevant question to emerge from this study lies in the importance of designing restorative strategies (e.g. afforestation, organic amendments) microbial community-oriented aimed at mitigating the environmental disturbances caused by the Fundão dam failure. Such strategies, in turn, can improve microbial processes related to carbon cycling (i.e. accumulation, stabilization, decomposition, losses), accelerate the rehabilitation of disturbed areas, and enhance the ecosystem functioning over the long-term.

References

Alef, K., 1995. Field methods, in: Alef, K.; Nannipieri, P. (Ed.), Methods in Applied Soil Microbiology and Biochemistry. Elsevier, pp. 463–490. https://doi.org/10.1016/B978-012513840-6/50025-2

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nat. Geosci. 3, 336–340. https://doi.org/10.1038/ngeo846

Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO2(qCO2) as a specific activity parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass of forest soils. Soil Biol. Biochem. 25, 393–395. https://doi.org/10.1016/0038-0717(93)90140-7

Batista, É.R., Carneiro, J.J., Araújo Pinto, F., dos Santos, J.V., Carneiro, M.A.C., 2020. Environmental drivers of shifts on microbial traits in sites disturbed by a large-scale tailing dam collapse. Sci. Total Environ. 738, 139453. https://doi.org/10.1016/j.scitotenv.2020.139453

Bolan, N.S., Baskaran, S., Thiagarajan, S., 1996. An evaluation of the methods of measurement of dissolved organic carbon in soils, manures, sludges, and stream water. Commun. Soil Sci. Plant Anal. 27, 2723–2737. https://doi.org/10.1080/00103629609369735

Bouyoucos, G.J., 1962. Hydrometer Method Improved for Making Particle Size Analyses of Soils 1. Agron. J. 54, 464–465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Bradford, M.A., Keiser, A.D., Davies, C.A., Mersmann, C.A., Strickland, M.S., 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry 113, 271–281. https://doi.org/10.1007/s10533-012-9822-0

Carmo, F.F., Kamino, L.H.Y., Tobias Junior, R., Campos, I.C., Carmo, F.F., Silvino, G., Castro, K.J.S.X., Mauro, M.L., Rodrigues, N.U.A., Miranda, M.P.S., Pinto, C.E.F., Fonseca Do Carmo, F.F., Hiromi, L., Kamino, Y., Tobias Junior, R., Christina De Campos, I., Fonseca Do Carmo, F.F., Silvino, G., Junio, K., Xavier De Castro, S., Mauro, M.L., Uchoa, N., Rodrigues, A., Paulo De Souza Miranda, M., Ferreira Pinto, C.E., 2017. Fundão tailings dam failures : the environment tragedy of the largest technological disaster of Brazilian mining in global context. Perspect. Ecol. Conserv. 15, 145–151. https://doi.org/http://dx.doi.org/10.1016/j.pecon.2017.06.002

Cruz, F.V. da S., Gomes, M.P., Bicalho, E.M., Della Torre, F., Garcia, Q.S., 2020. Does Samarco's spilled mud impair the growth of native trees of the Atlantic Rainforest? Ecotoxicol. Environ. Saf. 189, 110021. https://doi.org/10.1016/j.ecoenv.2019.110021

Dick, R.P., 2011. Methods of Soil Enzymology, Methods of Soil Enzymology, SSSA Book Series. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, USA. https://doi.org/10.2136/sssabookser9

Giongo, A., dos Anjos Borges, L.G., Marconatto, L., de Lara Palhano, P., Serbent, M.P., Moreira-Silva, E., de Abreu Siqueira, T., Martinho, C.T., Barili, R., Paz, L.V., Moser, L.I., De Marco Veríssimo, C., Ketzer, J.M.M., Medina-Silva, R., 2020. Adaption of microbial communities to the hostile environment in the Doce River after the collapse of two iron ore tailing dams. Heliyon 6. https://doi.org/10.1016/j.heliyon.2020.e04778

Hinojosa, M.B., García-Ruíz, R., Viñegla, B., Carreira, J.A., 2004. Microbiological rates and enzyme activities as indicators of functionality in soils affected by the Aznalcóllar toxic spill. Soil Biol. Biochem. 36, 1637–1644. https://doi.org/10.1016/j.soilbio.2004.07.006

Joergensen, R.G., Wichern, F., 2018. Alive and kicking: Why dormant soil microorganisms matter. Soil Biol. Biochem. 116, 419–430. https://doi.org/10.1016/j.soilbio.2017.10.022

Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: A review. Soil Sci. 165, 277–304. https://doi.org/10.1097/00010694-200004000-00001

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630. https://doi.org/10.1038/ncomms13630

Liang, C., Balser, T.C., 2011. Microbial production of recalcitrant organic matter in global soils: Implications for productivity and climate policy. Nat. Rev. Microbiol. 9, 75. https://doi.org/10.1038/nrmicro2386-c1

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 1–6. https://doi.org/10.1038/nmicrobiol.2017.105

Malik, A.A., Puissant, J., Buckeridge, K.M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H.S., Peyton, J.M., Mason, K.E., van Agtmaal, M., Blaud, A., Clark, I.M., Whitaker, J., Pywell, R.F., Ostle, N., Gleixner, G., Griffiths, R.I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. Nat. Commun. 9, 3591.

https://doi.org/10.1038/s41467-018-05980-1

Moorhead, D.L., Rinkes, Z.L., Sinsabaugh, R.L., Weintraub, M.N., 2013. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: Informing enzyme-based decomposition models. Front. Microbiol. 4, 1–12. https://doi.org/10.3389/fmicb.2013.00223

Nelson, D.W., Sommers, L.E., 1996. Total Carbon, Organic Carbon, and Organic Matter, in: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, M.E.S. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America and American Society of Agronomy, Madison, WI, USA, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2020. nlme: Linear and Nonlinear Mixed Effects Models.

Prado, I.G. de O., da Silva, M. de C.S., Prado, D.G. de O., Kemmelmeier, K., Pedrosa, B.G., Silva, C.C. da, 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, 2020. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Schimel, J., 2013. Microbes and global carbon. Nat. Clim. Chang. 3, 867–868. https://doi.org/10.1038/nclimate2015

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394. https://doi.org/10.1890/06-0219

Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 1–11. https://doi.org/10.3389/fmicb.2012.00348

Silva, A.C., Cavalcante, L.C.D., Fabris, J.D., Júnior, R.F., Barral, U.M., Farnezi, M.M. de M., Viana, A.J.S., Ardisson, J.D., Fernandez-Outon, L.E., Lara, L.R.S., Stumpf, H.O., Barbosa, J.B.S., Silva, L.C. da, 2016. Chemical, mineralogical and physical characteristics of a material accumulated on the river margin from mud flowing from the collapse of the iron ore tailings dam in Bento Rodrigues, Minas Gerais, Brazil. Rev. Espinhaço 5, 44–53.

Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory.

Annu. Rev. Ecol. Evol. Syst. 43, 313–343. https://doi.org/10.1146/annurev-ecolsys-071112-124414

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798. https://doi.org/10.1038/nature08632

Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nat. Geosci. 12, 46–53. https://doi.org/10.1038/s41561-018-0258-6

Soong, J.L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., Richter, A., 2020. Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. Glob. Chang. Biol. 26, 1953–1961. https://doi.org/10.1111/gcb.14962

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, Use R! Springer International Publishing, New York. https://doi.org/10.1007/978-3-319-24277-4

Table 1. Average values (±SE) of chemical and textural properties of samples for two depths of upper horizon: A1 (0–3 cm) and A2 (10–13 cm) across different disturbance levels.

Variable	A1			A2		
	Di	Pi	Und	Di	Pi	Und
pH (H_2O)	7 ± 0.3	6.1 ± 0.1	5.1 ± 0.2	6.8 ± 0.2	6.3 ± 0.2	4.7 ± 0.1
P (mg kg^{-1})	11.7 ± 3	4.9 ± 0.8	1.4 ± 0.2	6.4 ± 1.1	4.7 ± 0.7	0.7 ± 0.1
K^{+} (mg kg^{-1})	131 ± 59	35.5 ± 6.8	107 ± 17	35.8 ± 24	7.8 ± 3	47.2 ± 6
Ca^{2+} (cmol$_c$ dm^{-3})	1.6 ± 0.2	1.7 ± 0.4	3.4 ± 0.7	1.1 ± 0.2	0.9 ± 0.1	1.4 ± 0.4
Mg^{2+} (cmol$_c$ dm^{-3})	0.3 ± 0.1	0.4 ± 0.1	1.5 ± 0.2	0.1 ± 0.02	0.13 ± 0.03	0.74 ± 0.16
BS (cmol$_c$ dm^{-3})	2.3 ± 0.4	2.3 ± 0.5	5.3 ± 0.9	1.2 ± 0.3	1.1 ± 0.1	2.3 ± 0.5
CECe (cmol$_c$ dm^{-3})	2.3 ± 0.4	2.4 ± 0.5	5.6 ± 0.9	1.04 ± 0.2	1.1 ± 0.1	2.9 ± 0.4
CECp (cmol$_c$ dm^{-3})	3.2 ± 0.4	3.6 ± 0.6	9.9 ± 0.8	2.1 ± 0.3	2 ± 0.2	7.2 ± 0.5
Fe^{2+} (mg kg^{-1})	136 ± 11	98.1 ± 9	75.8 ± 11	229 ± 52	140 ± 11	83.4 ± 7.2
Zn^{2+} (mg kg^{-1})	1 ± 0.3	0.9 ± 0.1	3.03 ± 0.4	0.62 ± 0.1	0.52 ± 0.1	1.73 ± 0.2
Mn^{2+} (mg kg^{-1})	143 ± 15	163 ± 16	85.4 ± 9	151 ± 23	92.8 ± 11.4	45.7 ± 4.9
Cu^{2+} (mg kg^{-1})	0.74 ± 0.1	0.68 ± 0.1	0.75 ± 0.1	1.6 ± 0.8	0.9 ± 0.3	3.83 ± 2.9
Clay (%)	7.1 ± 0.1	8.7 ± 0.9	28.7 ± 0.6	7.3 ± 0.3	9 ± 0.3	29.9 ± 0.5
Silt (%)	46.3 ± 1.5	44.8 ± 0.7	20.2 ± 1	44.6 ± 0.8	44.7 ± 1.1	22.2 ± 0.9
Sand (%)	46.6 ± 1.5	46.5 ± 0.8	51.1 ± 1.3	48.2 ± 0.9	46.3 ± 1.2	47.8 ± 1.1

SE, standard error of the mean (n = 5, total n = 90). BS, base sum; CECe, effective cation exchange capacity; CECp, potential cation exchange capacity.

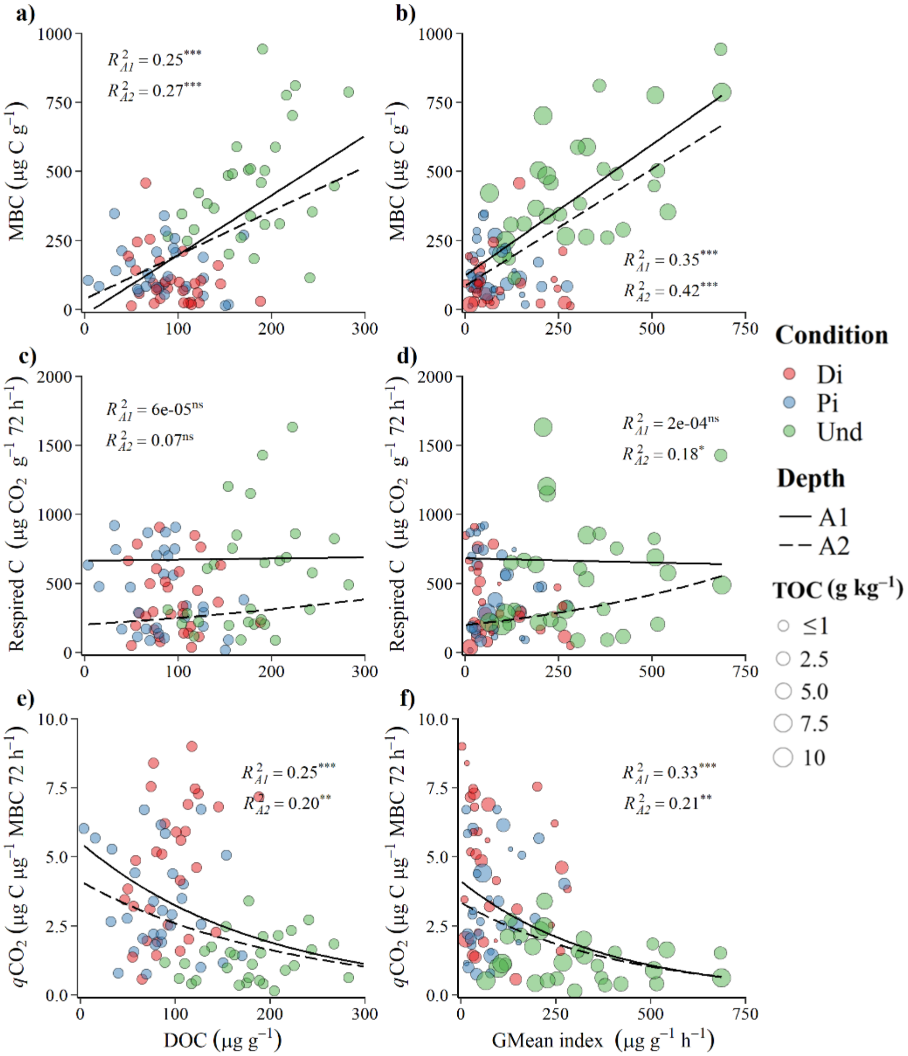


Fig. 1. Microbial ecophysiological response to labile organic carbon and extracellular enzyme activity. Regression trends of microbial biomass C (MBC), basal respiration (respired C), and biomass-specific respiration (qCO$_2$) with the dissolved organic C – DOC (**a**, **c**, **e**) and the geometric mean of enzyme activity – GMean index (**b**, **d**, **f**) across different disturbance levels for two upper horizon depths. Independent points here represent data from all soil and tailings samples (n = 90). Point sizes in **b**, **d**, and **f** represent organic matter content (measured as total organic carbon – TOC). Di, direct impact, Pi, intermediate impacted, Und, undisturbed soil. Solid and dashed trend lines represent the fitted regression models for the depths A1 (0–3 cm) and A2 (10–13 cm), respectively. Best-fitting regression models were: **a**) A1: $y = -17.7 + 2.2x$ and A2: $y = 37.9 + 1.6x$; **b**) A1: $y = 124.5 + 0.95x$ and A2: $y = 86.8 + 0.85x$; **c**) A1: $y = 664.9 + 0.002x$ and A2: $y = 202.5e^{0.002x}$; **d**) A1: $y = 682.3 - 0.001x$ and A2: $y = 198.2e^{0.002x}$; **e**) A1: $y = 5.5e^{-0.005x}$ and A2: $y =$

$4.1e^{-0.005x}$; **f**) A1: $y = 4.1e^{-0.003x}$ and A2: $y = 3.3e^{-0.002x}$. Significance levels are indicated by: $^{ns}\ p > 0.05$; $^{*}\ p < 0.05$; $^{**}\ p \leq 0.01$; $^{***}\ p \leq 0.001$.

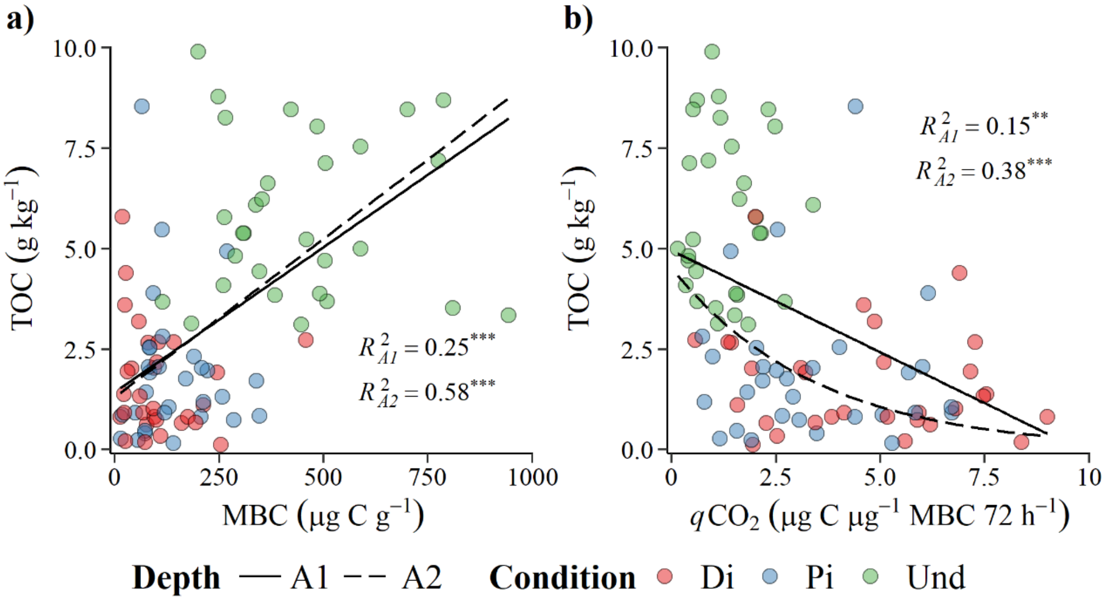


Fig. 2. Influence of microbial biomass and biomass-specific respiration on C pool (measured as total organic carbon – TOC). Regression trends of TOC with the microbial biomass C (a proxy for microbial growth – MBC, **a**) and biomass-specific respiration (a proxy for microbial C use – $q\mathrm{CO}_2$, **b**) across different disturbance levels for two upper horizon depths. Independent points here represent data from all soil and tailings samples (n = 90). Di, direct impact, Pi, intermediate impacted, Und, undisturbed soil. Solid and dashed trend lines represent the fitted regression models for the depths A1 (0–3 cm) and A2 (10–13 cm), respectively. Best-fitting regression models were: **a**) A1: $y = 1.4 + 0.007x$ and A2: $y = 1.3 + 0.008x$; **b**) A1: $y = 4.95 - 0.51x$ and A2: $y = 4.5e^{-0.29x}$. Significance levels are indicated by: ** $p \leq 0.01$; *** $p \leq 0.001$.

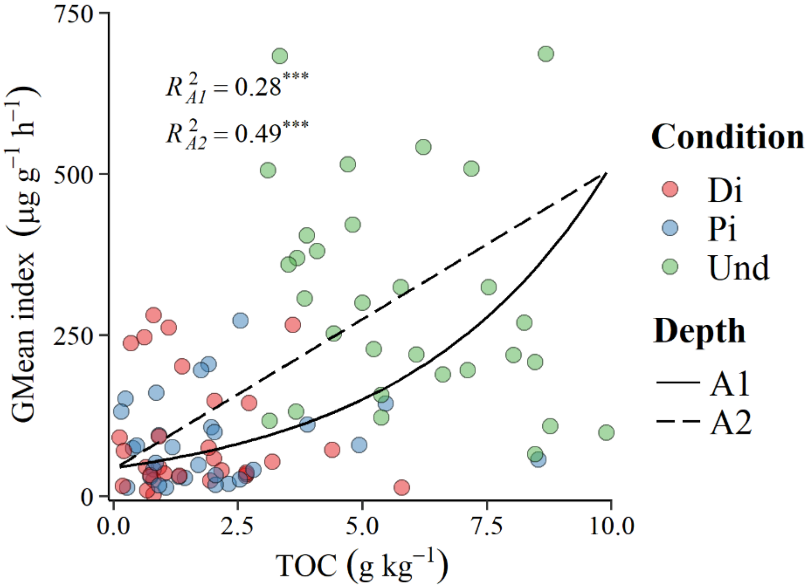


Fig. 3. Influence of resource availability on enzyme activity. Relationships of geometric mean of enzyme activity index (GMean index) with organic matter content – measured as total organic carbon (TOC) – across different disturbance levels for two upper horizon depths. Independent points here represent data from all soil and tailings samples (n = 90). Di, direct impact, Pi, intermediate impacted, Und, undisturbed soil. Solid and dashed trend lines represent the fitted regression models for the depths A1 (0–3 cm) and A2 (10–13 cm), respectively. Best-fitting regression models was: A1: $y = 43.8e^{0.25x}$ and A2: $y = 42.03 + 46.6x$. Significance level is indicated by: *** $p \leq 0.001$.

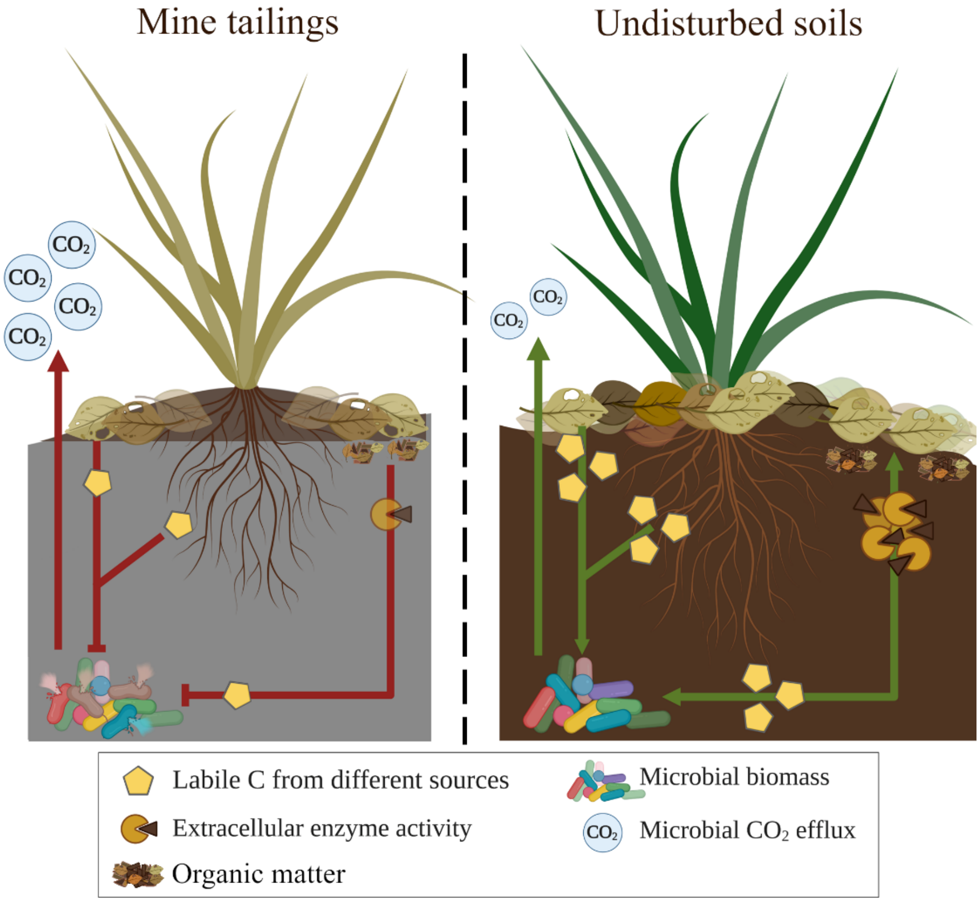


Fig. 4. Conceptual diagram for the influence of labile organic C from different sources on the ecophysiology of microbial communities in sites disturbed by Fundão dam failure (left) and undisturbed soils (right). Specifically, when labile C content and degradative activity decline, microbial biomass and C use efficiency also decline, increasing microbial-C losses as evidenced by higher qCO_2 in disturbed areas (mine tailings). On the other hand, when labile C content and degradative activity increase, the better C use by microbes reduces microbial-C losses and more substrate can be assimilated into biomass, improving C sequestration through the microbial path. Created with BioRender (https://biorender.com/).

5 ARTIGO 3 – Microbial taxonomical and functional diversity in areas disturbed by a mining dam collapse

Article written according to the standards of *The ISME Journal*
(Preliminar version)

Microbial taxonomical and functional diversity in areas disturbed by a mining dam collapse

Authors: Éder Rodrigues Batista[1], Julie Kenya[1], Aline Oliveira Silva[1], Marisangela Barbosa[1], Jessé Valentim dos Santos[1], Victor Salter Pylro[1], Guilherme Oliveira[2], Eder Soares[2], Marco Aurélio Carbone Carneiro[1*]

[1]Department of Soil Science, Federal University of Lavras. Av. Doutor Sylvio Menicucci, s/n, Aquenta Sol, 37200-000, Lavras, MG

[2]Instituto Tecnológico Vale, R. Boaventura da Silva, 955, CEP 66055-200, Bairro Nazaré Belém, Pará, Brazil

[*]corresponding author (marcocarbone@ufla.br)

Abstract: Changes in microbial community diversity and function were studied across areas disturbed by a mining dam collapse along two sampling period. We observed that taxonomic diversity was not influenced by disturbance, whereas functional diversity was increased in disturbed areas. Microbial community composition exhibited shifts in response to disturbance, but no influence was observed for sampling period. Our Indicator Analysis revealed several genera from Proteobacteria, including Alpha-, Beat-, Delta-, and Gamaproteobacteria classes, as well as Firmicutes and Actinobacteria in disturbed areas. We identified disturbance functional indicators related mostly to carbohydrates for both sampling periods. Our results show that although taxonomic diversity did not change in response to Fundão dam collapse, the accident lead to important changes in both taxonomic and functional community compositions. Furthermore, changes in microbial functional diversity revealed a high investment in resource acquisition by microbial communities, with consequences for ecosystem functioning.

Keywords: Microbial diversity; functional diversity; ecosystem functioning; mine tailings.

Introduction

In November 2015 occurred the Fundão dam failure, releasing approximately 34 million m3 of tailings related to iron ore mining, and was considered one of the most dangerous mining disasters in Brazil [1]. The accident resulted in diverse impacts on the environment, such as the presence of heavy metal in river banks [2], water pollution [3], loss of native vegetation [4], as well as terrestrial and aquatic species [4], and shifts in microbial community ecophysiology [5].

Mine tailings constitute an environment that differ from soils in several properties once they present small particle size, their structure has no aggregation, have low contents of organic substrates and nutrient, and generally present high levels of heavy metals [6, 7]. Fundão dam tailings have different chemical and mineralogical properties compared to undisturbed soils in, for instance, its mineralogy, that is predominantly quartz ($SiO2$) and hematite ($Fe2O3$) [8]. They have a granulometry basically sized as sand (42%) and silt (47.5%), which lead to a high particle density (2.94 g cm3), low organic matter and sorption capacity [9].

However, we still have a limited understanding on how taxonomic and functional diversity responds to impact in disturbed areas. Terrestrial ecosystems, such as the soil, harbor highly diverse microbial communities that are of crucial importance for soil functioning [10]. Microorganisms are the most abundant, diverse, and functionally important group in soils. These communities play several ecosystem processes such as organic matter decomposition, nutrient cycling, primary productivity and climate regulation [11–13].

Microbes have the capacity to quickly respond to environmental changes, which makes it possible to know changes in ecosystem functioning, particularly in areas under mining activities [14, 15]. Consequently, they act as indicators of environmental changes caused by anthropogenic activities, such as mining, as well as how these activities influence their diversity and function [16].

Changes in physical and chemical properties in tailings dam areas may impact microbial communities. For instance, the availability of the substrates is thought to be the basis for building up microbial biomass and enzymes [17, 18]. On the other hand, these properties may reduce the microbial diversity in the disturbed sites [19, 20]. Although there is no consensus on the effects of, for instance, heavy metals on microbial diversity, some studies have shown that both short-term and long-term exposure to metals results in the reductions of soil diversity [21]. Therefore, evaluating the diversity and functions of soil microbial communities in areas disturbed by mining activities can provides good response potential and easy interpretation.

We hypothesized that changes in edaphic conditions caused by Fundão dam collapse impose distinct responses on the taxonomic and functional diversities of microbial communities in areas disturbed. The main objective of the present study was to determine the effects of mine tailings deposition on bacterial, archaeal, and functional diversity using shotgun metagenomic approach.

Materials and methods

Study area and sampling

This study was carried out across three sites representative of Fundão dam tailings deposition on the river banks and background soils located near to Mariana municipality, Minas Gerais, Brazil [(site A: 20°16′0.034″ S; 43°18′ 14.177″ W), (site B: 20°17′47.916″ S; 43°12′18.584″ W), and (site C: 20°16′22.272″ S; 43°12′4.293″ W)]. The region's climate is classified as Cwa according to the Köppen-Geiger classification, with a dry season from April to September and a rainy season from October and March, an annual average temperature of 19 °C, and an annual average rainfall of 1375 mm [22]. Sampling was carried out during the rainy seasons of 2017 and 2019, representing two and four years after the accident. We considered two environmental conditions: the disturbed one was characterized by tailings deposition over background soils, with vegetation poorly overgrowing or voluntary growth of grass and shrub species, and low organic matter and litter contents; the reference one was characterized as undisturbed native soils, with native species of grass, shrub, and tree, and high organic matter and litter contents. At each sampling period, we randomly collected five soil and tailings subsamples at the 10 cm depth from three equally spaced points (5 m from each other) along a transect established within each condition. After the homogenization and removal of stones and roots, subsamples were pooled to obtain one composite sample per point. This produced 18 composite samples per sampling period (6 per site), totalizing 36 samples. At the field, 50 g of each sample were separated for molecular analyses and preserved at −80 °C until DNA extraction. In the laboratory, the samples for physicochemical characterization were air-dried, sieved at 4 mm mesh, and stored in hermetically sealed plastic bags until analyses.

Measurement of physicochemical variables

Organic matter content (SOM, %) was determined in the soil and tailings samples using a standard protocol, based on the potassium dichromate oxidation method [23]. Samples pH (tailings and soil) was determined using a glass electrode in deionized water solution (1: 2.5 v/v). Extractable calcium (Ca^{2+}) and magnesium (Mg^{2+}) were extracted with 1M of KCl extractant, whereas phosphorus (P), potassium (K^+), determined by colorimetry and atomic emission spectroscopy, respectively. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry. Extractable iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), lead (Pb), nickel (Ni), and selenium (Se) were extracted from samples by Mehlich-1 extractant and were determined in an ICP-OES analyzer. Se was determined using atomic absorption spectrometry. Effective cation exchange capacity (CECe) was calculated as the sum of Ca^{2+}, Mg^{2+}, K^+, and Al^{3+}, whereas potential cation exchange capacity (CECp) as the sum of Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, and H^+. Textural characterization was performed by based on Bouyoucos method [24].

DNA extraction, shotgun sequencing, and sequence processing

Total DNA was extracted from tailings and soil samples (0.1 g fresh weight) using the PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific Inc.), following the manufacturer's suggested protocol. We measured the DNA quality and concentration in each sample by using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Two samples were excluded from further analyses as we could not extract good-quality DNA. The remaining samples ($n = 34$) were sequenced on an Illumina NextSeq® 500 whole-genome sequencing platform (Illumina, San Diego, CA) using a High Output kit (2 × 300 cycles) at the Vale Institute of Technology – ITV (Belém, Pará, Brazil). Metagenomic sequencing data were annotated with the Metagenomics Rapid Annotation using Subsystems Technology (MG-RAST) server v4.0.3 [25]. Taxonomic assignments were carried out up to the genus level using the RefSeq database whereas functional classification was carried out using the SEED Subsystems database with a maximum E-value cutoff of $10{-}5$, minimum identity cutoff of 60%, and a minimum sequence alignment length of 15 nucleotides. Abundance tables derived from MG-RAST were then imported into R statistical environment v4.0.2 [26] for downstream analyses.

Diversity metrics and statistical analyses

We performed the rarefaction, diversity and ordination analyses using the vegan package v2.5-7 [27]. Alpha-diversity was then estimated as Hill numbers D [28]. This diversity measure, also referred to as actual diversity index, provides a general statistical framework sufficiently robust and flexible that makes it intuitively interpretable relative to the diversity concept [29] and is defined as:

$$ {}^{q}D = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)} \quad , q \neq 1 $$

where S is the number of species in the assemblage, pi is the relative abundance of ith species, and q is a parameter modulating the sensitivity of measure towards abundant and rare species.

We calculated Hill numbers with q from 0 to 2 as previously recommended [30]. For q = 0, D is simply the species richness; for q = 1, D is the exponential of Shannon's entropy; and for q = 2, D is equal to the inverse of Simpson's concentration index.

To visualize shifts in the taxonomic and functional composition of microbial communities, we used non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities calculated on square-root-transformed relative abundances. Effects of disturbance, sampling periods, and its interaction were determined through two-way PerMANOVA [31] with 10,000 permutations using the adonis() function in vegan package. We applied the Bonferroni correction to adjust P-values for multiple testing and considered significant only terms with a P smaller than 0.05 after correction. Euclidean matrices were used to perform a distance-based redundancy analysis (db-RDA) and identify the most important environmental drivers promoting shifts on community taxonomic and functional compositions based on Monte Carlo tests with 10,000 full permutations [32].

To identify genera and functions significantly associated with disturbed and reference conditions at each sampling period, we used Indicator Value analysis (IndVal) [33]. The IndVal score considers measures such as the relative frequency of occurrence (fidelity) and average relative abundance (specificity) to estimate an association value between a given genus or function and a given environmental condition [34]. We carried out this analysis using the relative abundances calculated on rarefied count data. We determined the IndVal scores and significance of associations with 10,000 permutations using the multipatt() function in the indicspecies package v1.7.9 [35]. Genera and functions above an IndVal score threshold of 0.50 and with a p-value smaller than 0.05 were considered significant.

We evaluated the effect of disturbance on alpha-diversity within and between sampling periods by fitting linear mixed-effects models (LMMs) with transect as the random effect using the nlme package v3.1.153 [36]. Models included the condition, sampling periods, and interaction terms as fixed effects. Two-way factorial ANOVA followed by Tukey post hoc tests were used to identify the statistical significance of model parameters using the emmeans() function in the emmeans package v1.7.0 [37]. Predicted values were obtained based on the fitted LMMs using the ggpredict() function in the ggeffects package v1.1.1.1 [38]. All models were checked for normality and homogeneity of variance through visual inspection of diagnostic plots as previously suggested [39]. Transformations were applied when necessary.

Results and discussion

Disturbance effects on functional diversity are not reflected in taxonomic diversity

To quantify the alpha-diversity of microbial communities, we estimated Hill numbers D, a robust and flexible measure that makes the diversity concept intuitively interpretable [29]. Overall, we observed that the sampling period did not significantly influence the community taxonomic and functional alpha-diversity (Fig. 1a, Table S1). Communities in disturbed areas exhibited higher functional alpha-diversity than reference communities (Fig. 1b, $P < 0.001$, Table S1). Functional richness ($q = 0$), Hill's Shannon ($q = 1$) and Hill's Simpson ($q = 2$) were higher in disturbed areas for both sampling periods, with an increase of 51%, 29%, and 20%, respectively. These responses cay indicate a higher investment in ecophysiological adaptations mechanisms to survive in a stressful environment [40, 41]. NMDS ordination analysis suggested that the taxonomic and functional compositions of communities exhibited a clear difference between disturbed and reference conditions (Fig. 1c, d). PerMANOVA results have shown that communities were taxonomically and functionally dissimilar in response to disturbance, but no influence was observed across the sampling periods (Fig. 2a, b, $P < 0.05$, Table S2). We also observed that community taxonomy exhibited a pattern less similar between samples than functions, that had higher similarities (Figs. S1 and S2).

Compositional patterns of microbial communities and their environmental drivers

We performed a db-RDA to investigate the environmental drivers modulating shifts in community composition. Our results demonstrated that disturbance modulated shifts in taxonomic (adj. $R^2 = 0.34$, $P = 0.0032$) and functional (adj. $R^2 = 0.15$, $P = 0.0003$) compositions irrespective to sampling period (Fig. 3a, b, Table S3 and S4). We observed that the pH significantly influenced the community compositions in 2017 ($R^2 = 0.66$, $P < 0.001$) and 2019 ($R^2 = 0.63$, $P < 0.001$). This reveals an important influence of changes in chemical and physical properties of tailings relative to reference soils in modulate microbial response [42]. We performed a correlation analysis based on Spearman's coefficients to investigate the specific response of functional alpha-diversity measures to environmental variables. We observed a significantly effect of the first axis of db-RDA on all Hill measures, characterized by an increase in disturbed areas (Fig. S3, $P < 0.0001$), showing a functional mechanism of adaptation by communities in disturbed areas in response to limited organic substrate and high pH value (Fig. S1), that modulates ecophysiological responses of microbial communities [43, 44].

Genera and functions unique to disturbance conditions

We used community-aggregated approach [45] as a proxy to assess the community taxonomy and function and its potential implications for ecosystem functioning. The overall proportions of identified genera were largely similar in bacterial and archaeal phyla over sampling periods, being 352 identified as disturbance indicators and 440 as reference indicators, totalizing 792 genera (Fig. 3a, b, Data S1). Our Indicator Value analysis identified genera related to phyla Proteobacteria, Firmicutes, Cyanobacteria, and Actinobacteria for both sampling periods. Proteobacteria presented the highest number of indicators, with a total of 345 genera, being 142 identified as disturbance indicators and 203 as reference indicators (Fig. 3c, Data S1). Firmicutes was the second most important phylum, with 176 indicators genera in total, with 90 as characteristic from disturbance areas and 86 from reference areas (Data S1). For instance, most of genera linked to Proteobacteria were belonged to Alphaproteobacteria class, such as *Bradyrhizobium*, *Mesorhizobium*, and *Azospirillum*, all important genera involved in the N_2 fixation by plants [16]. These findings agree with our ordination analysis results showing differences in taxonomic composition are linked to changes in edaphic conditions post-accident. These findings agree with our ordination analysis results showing differences in taxonomic composition are linked to changes in edaphic conditions post-accident [19, 20].

In total, 1,980 Subsystems functions were annotated, being 1,302 linked to disturbance areas, whereas 678 were related to reference areas (Fig. 4, Data S2). Of the 849 functional disturbance indicators in 2017, most belonged to the class carbohydrates (187 functions) and were linked to monosaccharides, di- and oligosaccharides, sugar alcohols, and central carbohydrate metabolism (Fig. 4a, Data S2). Similarly, we identified 639 functional disturbance indicators in 2019 (Fig 4b, Data S2). Of these, 101 were also related to the class carbohydrates and linked mostly to monosaccharides and di- and oligosaccharides (Data S2). In addition, in 2017, a total of 42 disturbance indicators were annotated to amino acids and derivatives, 69 to protein metabolism, and 40 to respiration. For 2019, disturbance indicators were distributed into 24 to amino acids and derivatives, 25 to protein metabolism, and 13 to respiration (Data S2). These results are in line with shifts in ecophysiological traits of microbial communities in areas disturbed by mining [46]. Also, the large number of functional indicators for carbohydrate and amino acid metabolism indicates increased investment in substrate degradation, uptake and assimilation in disturbed areas [41]. All these increases may also indicate an increased investment in these resource acquisition traits to degrade the organic substrates in disturbed areas. Indeed, disturbed areas have a depleted content of organic matter, which may lead to ecophysiological trade-offs by community [46].

Conclusion

In conclusion, our study shows that only functional alpha-diversity have been altered by disturbance in response to Fundão dam collapse. Furthermore, our results indicate that microbial community taxonomic and functional compositions were altered with dam depositions, indicating disturbance-specific drivers such as pH and resource availability modulating alpha- and beta-diversities. Our results also indicate that the metabolic and physiological costs of a stressful environment promote lead to trade-offs in acquisition resource by microbial communities, which constrain the microbial processes that make possible the ecosystem rehabilitation and functioning in disturbed areas.

References

1. Carmo FF, Kamino LHY, Tobias Junior R, Campos IC, Carmo FF, Silvino G, et al. Fundão tailings dam failures : the environment tragedy of the largest technological disaster of Brazilian mining in global context. Perspect Ecol Conserv 2017; 15: 145–151.

2. Queiroz HM, Nóbrega GN, Ferreira TO, Almeida LS, Romero TB, Santaella ST, et al. The Samarco mine tailing disaster: A possible time-bomb for heavy metals contamination? Sci Total Environ 2018; 637–638: 498–506.

3. Hatje V, Pedreira RMA, de Rezende CE, Schettini CAF, de Souza GC, Marin DC, et al. The environmental impacts of one of the largest tailing dam failures worldwide. Sci Rep 2017; 7: 10706.

4. IBAMA. Laudo Técnico Preliminar: Impactos ambientais decorrentes do desastre envolvendo o rompimento da barragem de Fundão, em Mariana, Minas Gerais. 2015. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA. , 38

5. Batista ÉR, Carneiro JJ, Araújo Pinto F, dos Santos JV, Carneiro MAC. Environmental drivers of shifts on microbial traits in sites disturbed by a large-scale tailing dam collapse. Sci Total Environ 2020; 738: 139453.

6. Santos J V., Varón-López M, Fonsêca Sousa Soares CR, Lopes Leal P, Siqueira JO, de Souza Moreira FM. Biological attributes of rehabilitated soils contaminated with heavy metals. Environ Sci Pollut Res 2016; 23: 6735–6748.

7. Mendez MO, Neilson JW, Maier RM. Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailing site. Appl Environ Microbiol 2008; 74: 3899–3907.

8. Couto FR, Ferreira A de M, Pontes PP, Marques AR. Physical, chemical and microbiological characterization of the soils contaminated by iron ore tailing mud after Fundão Dam disaster in Brazil. Appl Soil Ecol 2021; 158: 103811.

9. Silva AC, Cavalcante LCD, Fabris JD, Júnior RF, Barral UM, Farnezi MM de M, et al. Chemical, mineralogical and physical characteristics of a material accumulated on the river margin from mud flowing from the collapse of the iron ore tailings dam in Bento Rodrigues, Minas Gerais, Brazil. Rev Espinhaço 2016; 5: 44–53.

10. Escalas A, Hale L, Voordeckers JW, Yang Y, Firestone MK, Alvarez-Cohen L, et al. Microbial functional diversity: From concepts to applications. Ecol Evol 2019; 9: 12000–12016.

11. Schimel JP, Schaeffer SM. Microbial control over carbon cycling in soil. Front Microbiol 2012; 3: 1–11.

12. Sinsabaugh RL, Hill BH, Follstad Shah JJ. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 2009; 462: 795–798.

13. Bardgett RD, Van Der Putten WH. Belowground biodiversity and ecosystem functioning. Nature 2014; 515: 505–511.

14. Li Y, Jia Z, Sun Q, Zhan J, Yang Y, Wang D. Ecological restoration alters microbial communities in mine tailings profiles. Sci Rep 2016; 6: 25193.

15. Silva AO, da Costa AM, dos Santos Teixeira AF, Azarias Guimarães A, Valentim dos Santos J, de Souza Moreira FM. Soil microbiological attributes indicate recovery of an iron mining area and of the biological quality of adjacent phytophysiognomies. Ecol Indic 2018; 93: 142–151.

16. Oliveira Silva A, Azarias Guimarães A, Costa AM, Louzada Rodrigues T, Soares Carvalho T, Reis Sales F, et al. Plant growth-promoting rhizobacterial communities from an area under the influence of iron mining and from the adjacent phytophysiognomies which have high genetic diversity. L Degrad Dev 2020; 31: 2237–2254.

17. Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter A. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front Microbiol 2014; 5: 1–10.

18. Delgado-Baquerizo M, Grinyer J, Reich PB, Singh BK. Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. Funct Ecol 2016; 30: 1862–1873.

19. Prado IG de O, da Silva M de CS, Prado DG de O, Kemmelmeier K, Pedrosa BG, Silva CC da, et al. 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 2019; 141: 84–95.

20. Giongo A, dos Anjos Borges LG, Marconatto L, de Lara Palhano P, Serbent MP, Moreira-Silva E, et al. Adaption of microbial communities to the hostile environment in the Doce River after the collapse of two iron ore tailing dams. Heliyon 2020; 6.

21. Narendrula-Kotha R, Nkongolo KK. Bacterial and fungal community structure and diversity in a mining region under long-term metal exposure revealed by metagenomics sequencing. Ecol Genet Genomics 2017; 2: 13–24.

22. Alvares CA, Stape JL, Sentelhas PC, De Moraes Gonçalves JL, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Zeitschrift 2013.

23. Nelson DW, Sommers LE. Total Carbon, Organic Carbon, and Organic Matter. In: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston

MES (ed). Methods of Soil Analysis. Part 3. Chemical Methods. 1996. Soil Science Society of America and American Society of Agronomy, Madison, WI, USA, pp 961–1010.

24. Bouyoucos GJ. Hydrometer Method Improved for Making Particle Size Analyses of Soils 1. Agron J 1962; 54: 464–465.

25. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, et al. The metagenomics RAST server - A public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 2008; 9: 1–8.

26. R Core Team. R: A Language and Environment for Statistical Computing. 2020. R Foundation for Statistical Computing, Vienna, Austria.

27. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: Community Ecology Package. 2020. , 298

28. Hill MO. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 1973; 54: 427–432.

29. Alberdi A, Gilbert MTP. A guide to the application of Hill numbers to DNA-based diversity analyses. Mol Ecol Resour 2019; 19: 804–817.

30. Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, et al. Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. Ecol Monogr 2014; 84: 45–67.

31. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol 2001; 26: 32–46.

32. Legendre P, Anderson MJ. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecol Monogr 1999; 69: 1.

33. Dufrene M, Legendre P. Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. Ecol Monogr 1997; 67: 345.

34. De Cáceres M, Legendre P, Wiser SK, Brotons L. Using species combinations in indicator value analyses. Methods Ecol Evol 2012; 3: 973–982.

35. De Cáceres M. How to use the indicspecies package (ver. 1.7.1). R Proj 2013; 29.

36. Pinheiro J, Bates D, DebRoy S, Sarkar D. nlme: Linear and Nonlinear Mixed Effects Models. 2020. , 337

37. Lenth R V., Buerkner P, Herve M, Love J, Riebl H, Singmann H. Estimated Marginal Means, aka Least-Squares Means. Am Stat . 2021. CRAN. , 85

38. Lüdecke D. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. J Open Source Softw 2018; 3: 772.

39. Kozak M, Piepho H-P. What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. J Agron Crop Sci 2018; 204: 86–98.

40. Malik AA, Thomson BC, Whiteley AS, Bailey M, Griffiths RI. Bacterial Physiological Adaptations to Contrasting Edaphic Conditions Identified Using Landscape Scale Metagenomics. MBio 2017; 8: 1–13.

41. Malik AA, Swenson T, Weihe C, Morrison EW, Martiny JBH, Brodie EL, et al. Drought and plant litter chemistry alter microbial gene expression and metabolite production. ISME J 2020; 14: 2236–2247.

42. Vieira S, Sikorski J, Dietz S, Herz K, Schrumpf M, Bruelheide H, et al. Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. ISME J 2020; 14: 463–475.

43. Malik AA, Puissant J, Buckeridge KM, Goodall T, Jehmlich N, Chowdhury S, et al. Land use driven change in soil pH affects microbial carbon cycling processes. Nat Commun 2018; 9: 3591.

44. Schimel J, Balser TC, Wallenstein M. Microbial stress-response physiology and its implications for ecosystem function. Ecology 2007; 88: 1386–1394.

45. Fierer N, BarberÃ¡n A, Laughlin DC. Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. Front Microbiol 2014; 5: 1–6.

46. Batista ÉR, Franco ADJ, Silva APV da, Silva JAGF da, Tavares DS, Souza JK De, et al. Organic substrate availability and enzyme activity affect microbial-controlled carbon dynamics in areas disturbed by a mining dam failure. Appl Soil Ecol 2022; 169: 104169.

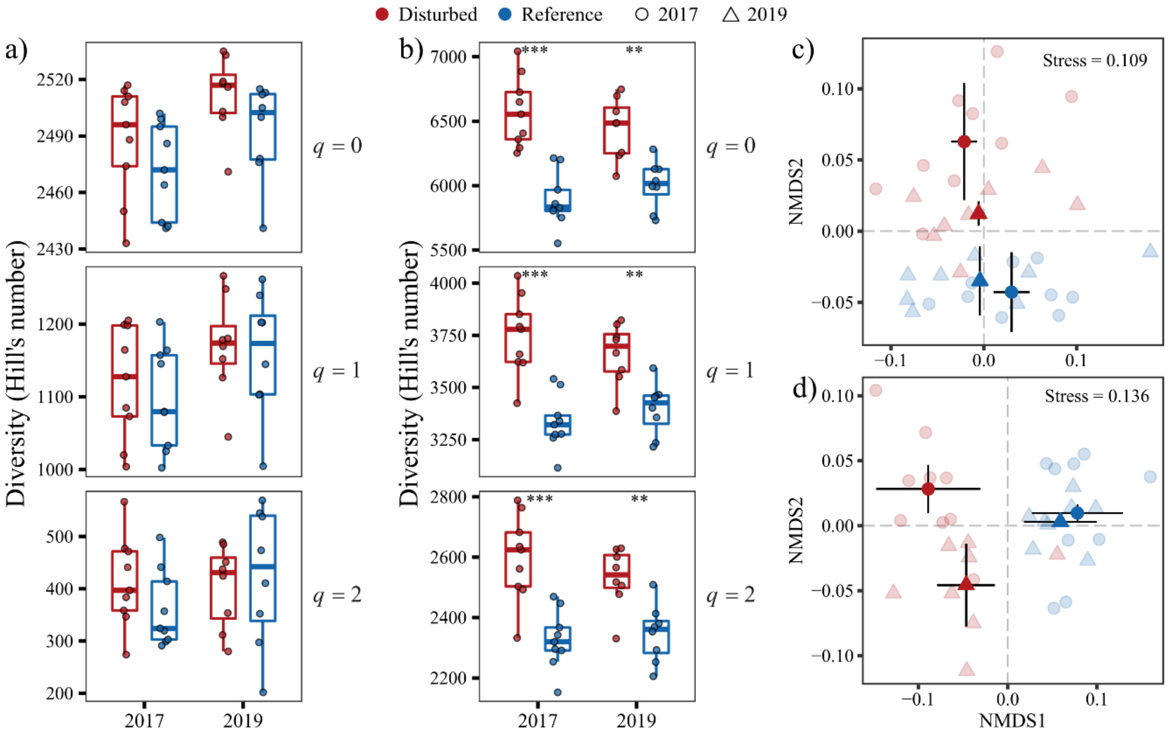


Fig. 1. Community taxonomic and functional diversity. (a) Alpha-diversity in Hill numbers for bacterial and archaeal communities at the genus level. (b) Alpha-diversity in Hill numbers for community functions at the function level. (c) NMDS ordination comparing the taxonomic compositions between disturbed and reference soils across sampling periods based on Bray–Curtis dissimilarity at the genus level. (d) NMDS ordination comparing the functional composition between disturbed and reference soils across sampling periods based on Bray–Curtis dissimilarity at the function level. From top to bottom: $q = 0$, richness; $q = 1$ exponential of Shannon entropy; $q = 2$, inverse of Simpson concentration index. The horizontal box lines represent the first quartile, the median and the third quartile. Whiskers denote the range of points within the first quartile − 1.5× the interquartile interval and the third quartile + 1.5× the interquartile interval, n = 34. Asterisks depict the significance levels between groups as analyzed by Tukey's post hoc test (*** $p < 0.001$; ** $p < 0.01$). Light and dark points in NMDS ordinations indicate original observations and mean ± 95% CI, respectively.

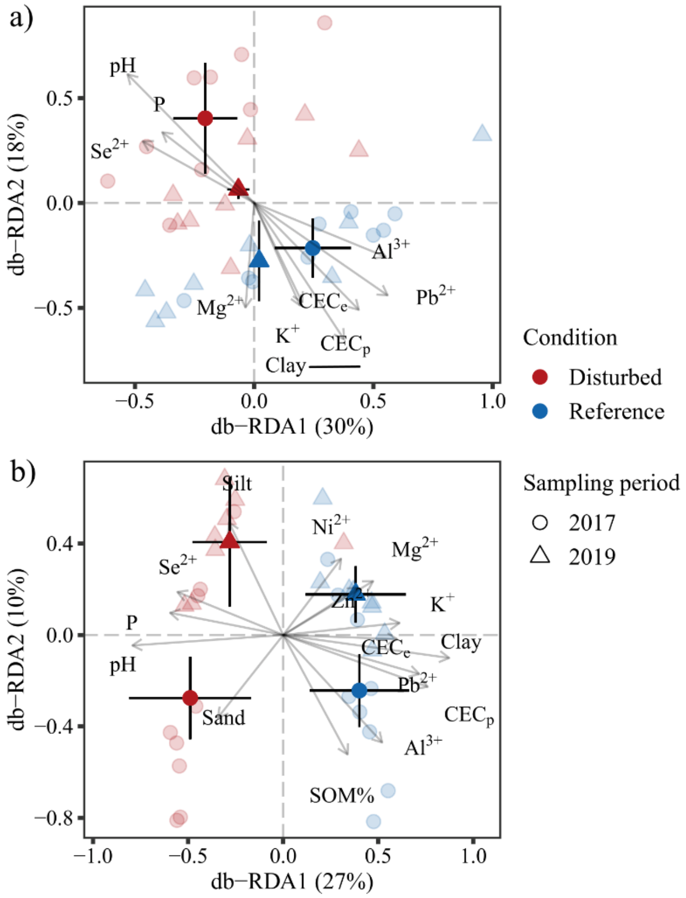


Fig. 2. Distance-based redundancy analysis (db-RDA) for the influence of ecological drivers on community taxonomical (a) and functional compositions (b) with forward selection of predictor variables followed by Monte Carlo permutation tests (10,000 permutations). Solid arrows represent environmental predictor significantly associated with the variation in community taxonomic and functional compositions. C_{org}, organic carbon; CECe, effective cation exchange capacity; CECp, potential cation exchange capacity; SOM%, soil organic matter. Light and dark points in ordinations indicate original observations and mean ± 95% CI, respectively.

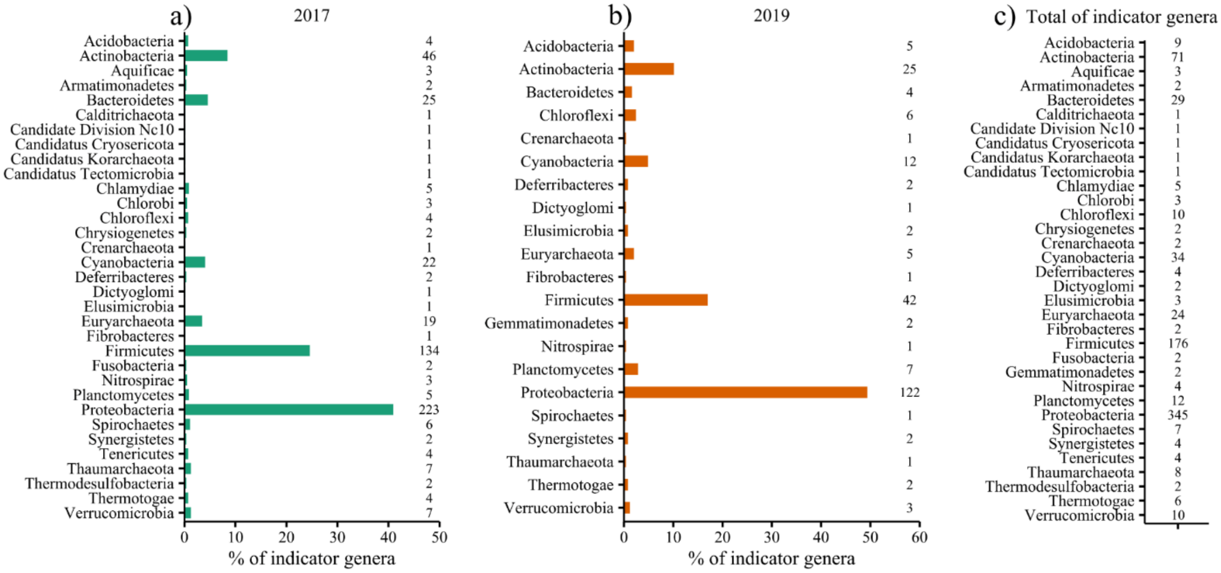


Fig. 3. Impact of disturbance on microbial community taxonomy in each sampling period. Percentage of bacterial and archaeal genera indicators for communities in 2017 (a) and 2019 (b), and (c) total number of indicators within each microbial group across both sampling periods (n = 34). Numbers indicate total number of indicators in each sampling period and the total number across both sampling periods.

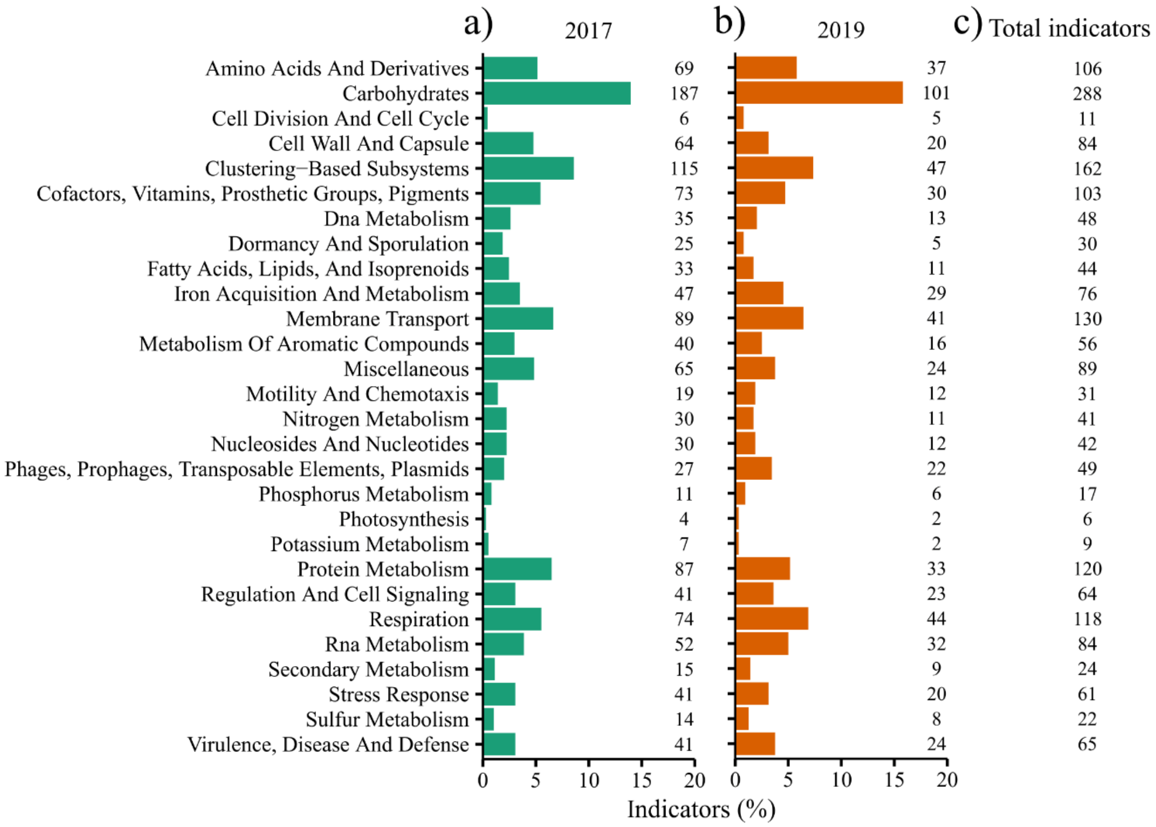
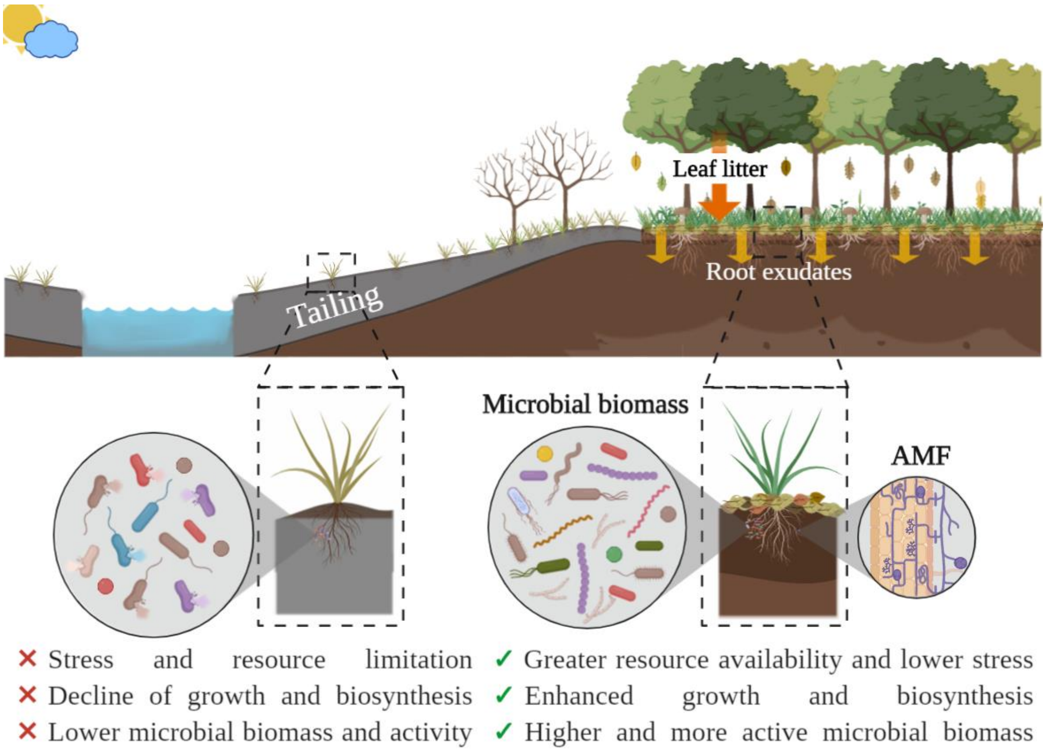


Fig. 4. Impact of disturbance on microbial community functions in each sampling period. Percentage of functional indicators within the level 1 of Subsystems classification for communities in 2017 (a) and 2019 (b), and (c) total number of indicators within each Subsystem level across both sampling periods (n = 34). Numbers indicate total number of indicators in each sampling period and the total number across both sampling periods.

ANEXOS

Anexo A1. Material suplementar referente ao artigo 1



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. Created with Biorender.com.

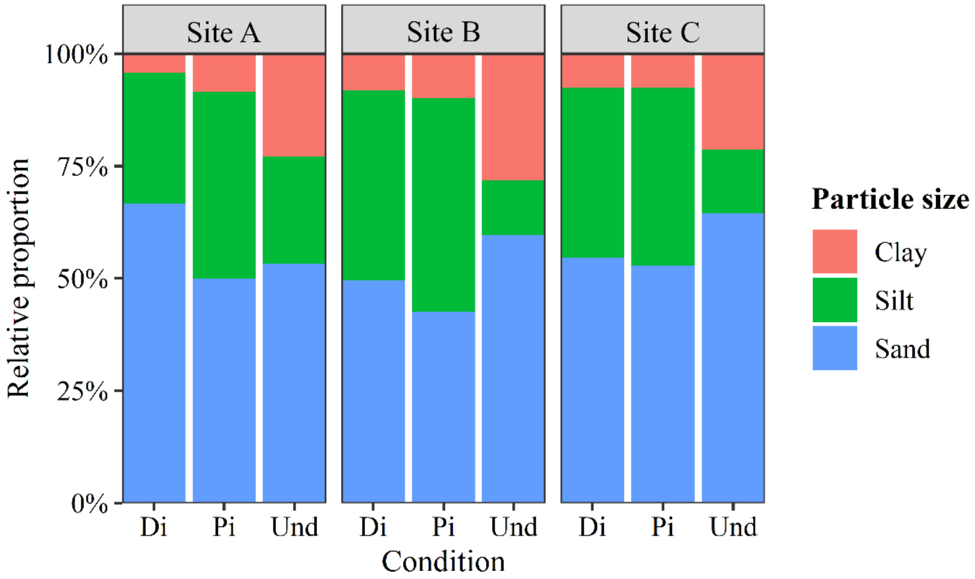
Supplementary Material

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

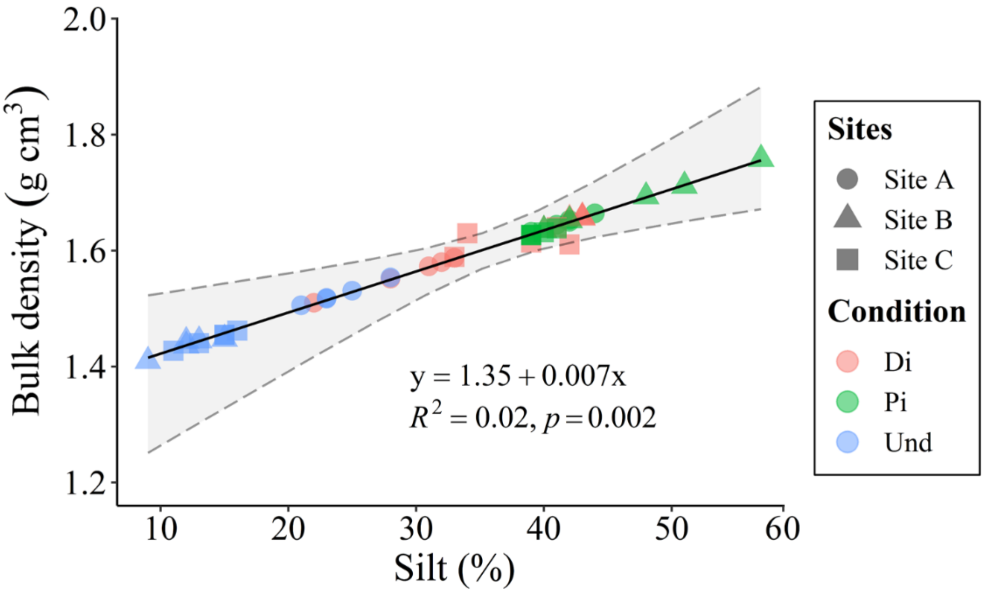
Authors: Éder Rodrigues Batista, Joana Junqueira Carneiro, Flávio Araújo Pinto, Jessé Valentim dos Santos, Marco Aurélio Carbone Carneiro[*]

[1]Department of Soil Science, Federal University of Lavras. Av. Doutor Sylvio Menicucci, s/n, Aquenta Sol, 37200-000, Lavras, MG

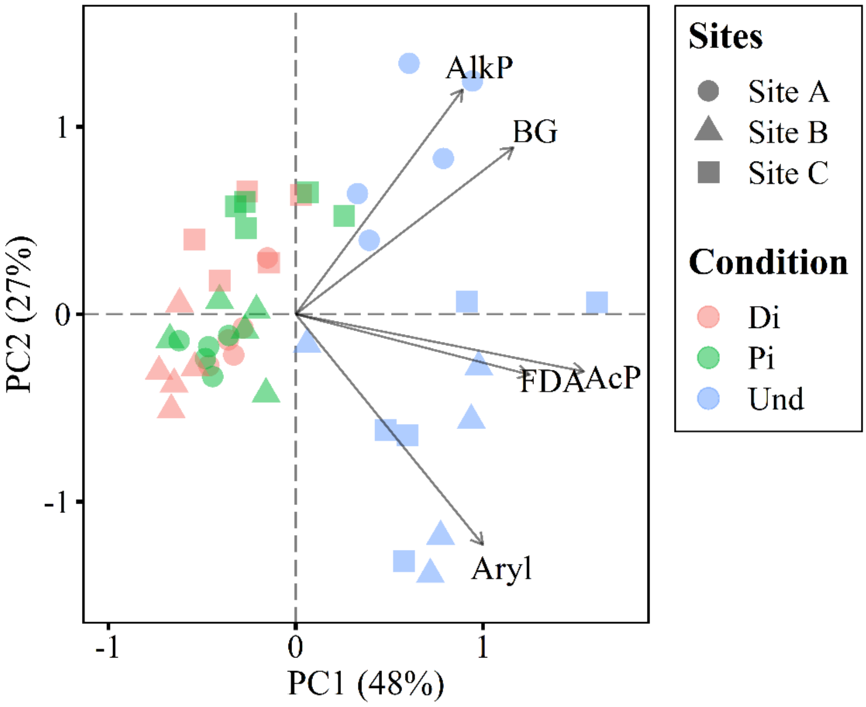
[*]corresponding author (marcocarbone@ufla.br)



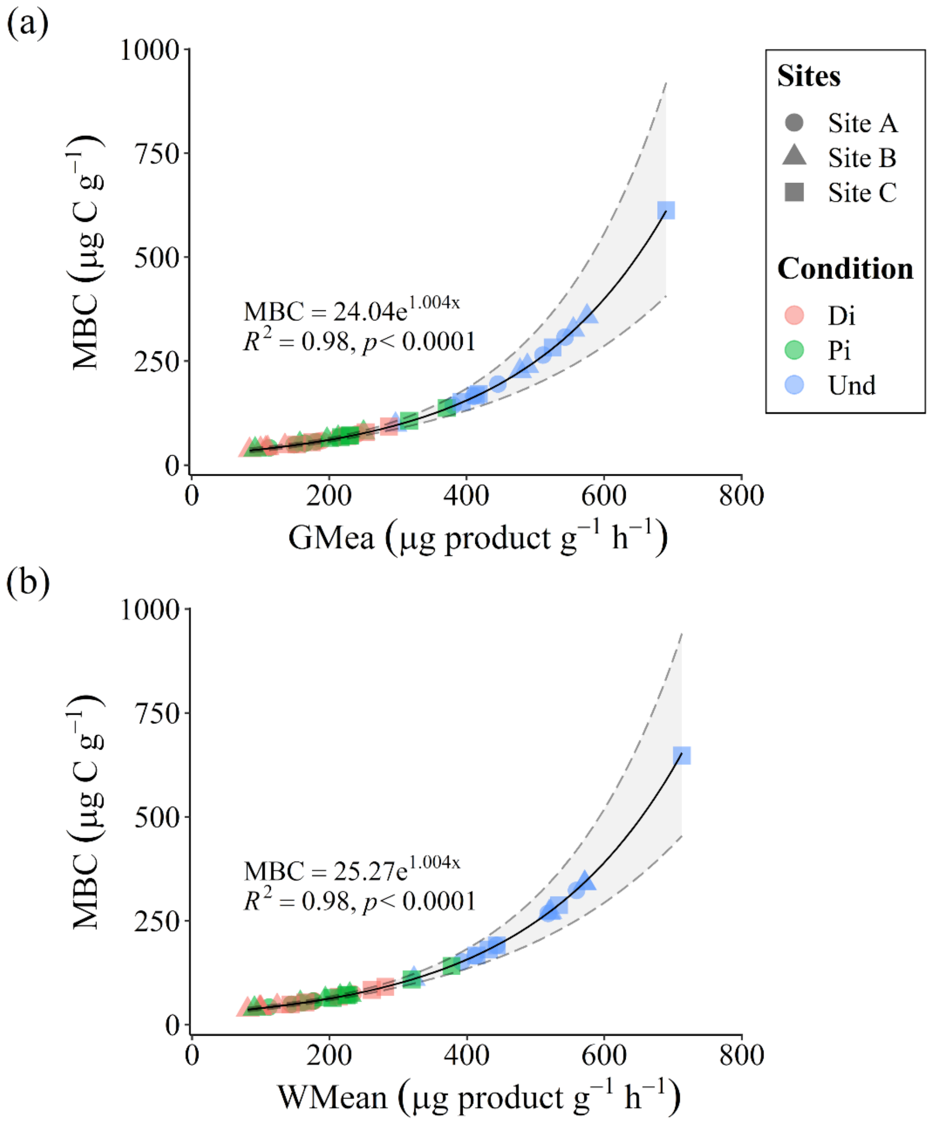
Supplementary figure S1. Samples texture and the particle size distribution for mine tailings and undisturbed reference soil from different study sites. Di, directly impacted; Pi, partially impacted; Und, undisturbed reference soils.



Supplementary figure S2. Relationship between silt percentage and bulk density. Model predictions for all conditions at each site are presented here as independent points. The shaded area represents 95% confidence interval around the trend line. Di, directly impacted, Pi, partially impacted, Und, undisturbed reference soil. Predicted values were obtained based on linear mixed-effect model fits extracted using the *ggeffects* package (LÜDECKE, 2018).



Supplementary figure S3. Principal component analysis of enzyme activities used to calculate the WMean index based on eigenvectors for each enzyme activity associated with the first axis. 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.



Supplementary figure S4. Influence of enzyme activity on microbial growth as assessed by enzyme-based numerical indexes GMea (a) and WMean (b) among tailings and undisturbed reference soils for each studied site along Gualaxo do Norte river. Model predictions from conditions for all sites are presented here as independent points. The shaded area represents 95% confidence interval around the trend line. Predicted values were obtained based on linear mixed-effect model fits extracted using the *ggeffects* package (LÜDECKE, 2018).

Supplementary tables

Supplementary table S1. Some of identified species in tailings of site C

Specific name	Common name
Triplaris americana	Pau formiga
Zeyheria digitalis	Bolsa de carneiro
Schinus terebinthifolius	Aroeirinha
Guapira sp.	Guapira
Cedrela fissilis	Cedro
Pterogyne nitens	Amendoim bravo
Cassia ferruginea	Chuva de ouro
Lonchocarpus cultratus	Embira de sapo
Tabernaemontana hystrix	Leiteira
Syagrus romanzoffiana	Jerivá

Source: personal communication from Dr. Soraya and collaborators, Department of Forest Sciences, Federal University of Lavras.

Supplementary table S2. Summary of the linear mixed-effects models for the effects of tested parameters on microbial ecophysiology and activity

Tested parameters	d.f.	MBC	BR	$q\mathrm{CO}_2$	*q*MIC	Aryl	BG	AcP	AlkP	FDA	GMea	WMean
Condition	2	195.67^{***}	12.08^{***}	24.79^{***}	9.08^{ns}	21.23^{***}	87.91^{***}	368.68^{***}	4.79^{**}	29.59^{***}	92.24^{***}	111.61^{***}
Site	2	27.51^{***}	15.56^{***}	10.56^{***}	1.82^{ns}	0.97^{ns}	37.71^{***}	3.71^{*}	3.54^{*}	19.18^{***}	–	–
Condition × Site	4	8.75^{***}	7.58^{***}	8.44^{***}	1.49^{ns}	6.95^{***}	4.01^{**}	4.31^{**}	6.77^{***}	14.99^{***}	–	–
Data transformation†		log	none	sqrt	sqrt	log	none	sqrt	none	sqrt	none	none
R^2_m		0.905	0.556	0.634	0.676	0.855	0.636	0.976	0.699	0.927	0.949	0.951
R^2_c		0.916	0.573	0.664	0.706	0.872	0.637	0.980	0.758	0.935	0.953	0.955

Numbers provide F-values and asterisks denote their significance: ^{ns} $p > 0.05$; * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. d.f., degrees of freedom. R^2_m, variance explained by the fixed effects; R^2_c, variance explained by the entire model, including fixed and random effects. MBC, microbial biomass C; BR, basal respiration; $q\mathrm{CO}_2$, metabolic quotient (BR:MBC ratio); *q*MIC, microbial quotient (MBC:C_{org} ratio); Aryl, arylsulfatase; BG, β-1,4-glucosidase; AcP, acid phosphatase; AlkP, alkaline phosphatase; FDA, fluorescein diacetate hydrolysis; GMea, geometric mean of enzyme activity; WMean, weighted mean index

†Transformations applied to data to meet model assumptions (log, logarithmic; sqrt, root square; none, no transformation necessary)

Supplementary table S3. Eigenvectors and weights used to calculate WMean index for each enzyme activity associated with the first axis (PC1) of the principal component analysis as shown in the Fig. S3.

Enzyme	Eigenvector ($Ʊ_i$)	Weight (w_i)
Acid phosphatase	1.5377	0.2635
FDA hydrolisis	1.2477	0.2138
β-1,4-glucosidase	1.1603	0.1989
Arylsulfatase	0.9987	0.1712
Alkaline phosphatase	0.8903	0.1526
Sum	5.8347	1.0000

Supplementary table S4. Importance and significance of physicochemical properties and extractable heavy metals content obtained from db-RDA analysis in the *vegan* package.

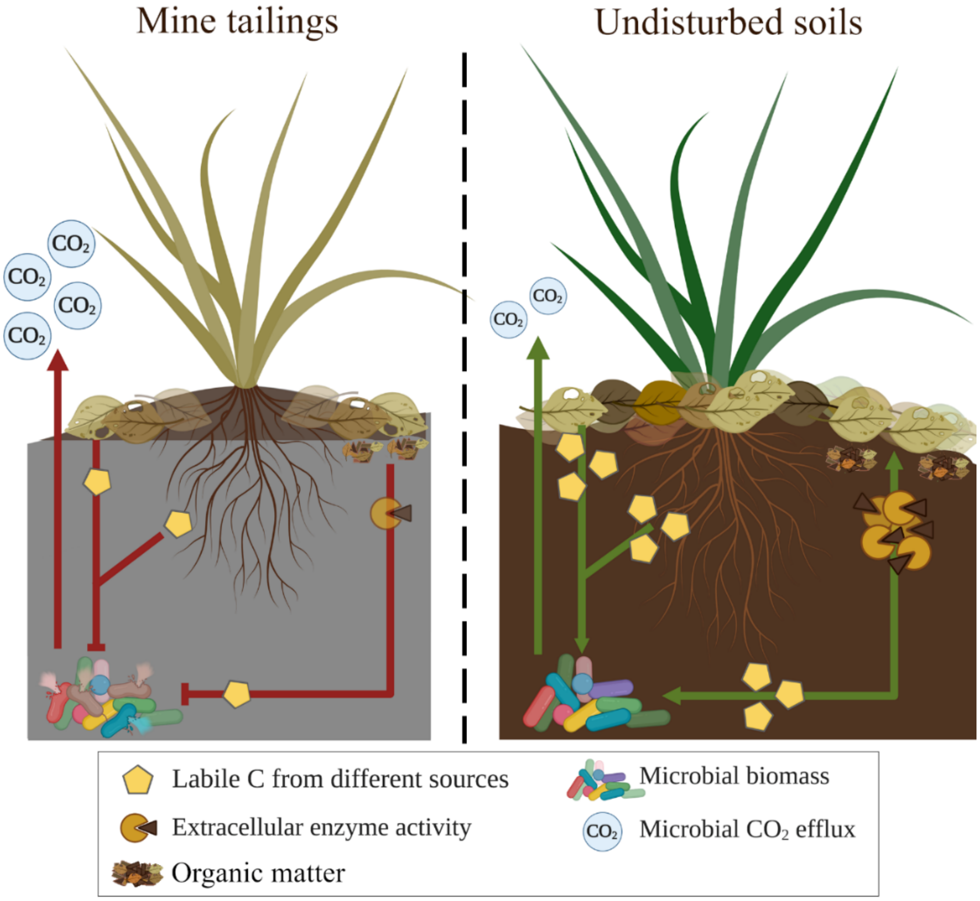
Physicochemical properties and heavy metals	R^2	*p*-value
Nt	0.81	0.0001
pH	**0.75**	**0.0001**
Silt	**0.72**	**0.0001**
Clay	0.72	0.0001
Pb	0.68	0.0001
C_{org}	0.63	0.0001
Se	**0.54**	**0.0001**
Moisture	0.46	0.0001
Cr	**0.43**	**0.0001**
Zn	0.34	0.0006
BD	**0.32**	**0.0006**
Fe	**0.30**	**0.0007**
Sand	0.21	0.006
Ni	**0.16**	**0.03**
C:N	**0.13**	**0.04**
Mn	0.04	0.41
Cu	0.02	0.67

Significant variables with high negative loadings are presented in bold

Supplementary references

Lüdecke, D., 2018. ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. J. Open Source Softw. 3, 772. https://doi.org/10.21105/joss.00772

Anexo A2. Material suplementar referente ao artigo 2



Supplementary Material

Organic substrate availability and enzyme activity affect microbial-controlled carbon dynamics in areas disturbed by a mining dam failure

Authors: Éder Rodrigues Batista, Aline de Jesus Franco, Ana Paula Valadares da Silva, Jéssyca Adriana Gomes Florêncio da Silva, Davi Santos Tavares, Josemara Karina de Souza, Aline Oliveira Silva, Marisangela Barbosa, Jessé Valentim dos Santos, Marco Aurélio Carbone Carneiro*

Department of Soil Science, Federal University of Lavras. Av. Doutor Sylvio Menicucci, s/n, Aquenta Sol, 37200-000, Lavras, MG

*corresponding author (marcocarbone@ufla.br)

Supplementary table S1. Results of best-fitting regression models used to assess microbial ecophysiological response relationship by analyzing the predictive power of dissolved organic carbon (DOC) content and extracellular enzyme activity (expressed as geometric mean of enzyme activity – GMean index) for each studied depth. Shown are model regression coefficients (Coef.), standard errors (SE), *t* and *p* values.

Microbial biomass × DOC content					**Microbial biomass × GMean index**				
Predictor variable	Coef.	SE	*t*-value	*p*-value	Predictor variable	Coef.	SE	*t*-value	*p*-value
A1					A1				
Intercept	–17.7	64.1	–0.3	0.78	Intercept	124.5	26.3	4.7	0.0000
Linear term	2.2	0.5	4.4	0.0000	Linear term	0.95	0.13	7.3	0.0000
A2					A2				
Intercept	37.9	52.7	14.0	0.48	Intercept	86.8	31.3	2.8	0.0085
Linear term	1.6	0.38	4.2	0.0001	Linear term	0.85	0.15	5.7	0.0000
Basal respiration × DOC content					**Basal respiration × GMean index**				
A1					A1				
Intercept	664.9	1.8	369.3	0.0000	Intercept	682.3	0.63	1083	0.0000
Linear term	0.002	0.01	0.2	0.89	Linear term	–0.001	0.004	–0.25	0.7727
A2					A2				
Intercept	202.5	1.2	168.8	0.0000	Intercept	198.2	1.2	165.2	0.0000
Linear term	0.002	0.001	2.0	0.12	Linear term	0.002	0.0006	3.3	0.0181
Biomass-specific respiration × DOC content					**Biomass-specific respiration × GMean index**				
A1					A1				
Intercept	5.5	1.2	4.6	0.0000	Intercept	4.1	1.1	3.7	0.0000
Linear term	–0.0053	0.0014	–3.8	0.0007	Linear term	–0.003	0.0005	–6.0	0.0000
A2					A2				
Intercept	4.1	1.3	3.2	0.0000	Intercept	3.3	1.2	2.8	0.0000
Linear term	–0.0046	0.0016	–2.9	0.0064	Linear term	–0.002	0.0008	–2.5	0.0051

Supplementary table S2. Results of best-fitting regression models used to assess total organic carbon (TOC) relationship by analyzing the predictive power of microbial biomass C (MBC) and biomass-specific respiration ($q\mathrm{CO}_2$) for each studied depth. Shown are model regression coefficients (Coef.), standard errors (SE), *t* and *p* values.

Microbial biomass C				
Predictor variable	Coef.	SE	*t*-value	*p*-value
A1				
Intercept	1.4	0.57	2.5	0.0169
Linear term	0.007	0.002	3.5	0.0001
A2				
Intercept	1.3	0.37	3.5	0.001
Linear term	0.008	0.001	8.0	0.0000
Specific respiration ($q\mathrm{CO}_2$)				
Predictor variable	Coef.	SE	*t*-value	*p*-value
A1				
Intercept	4.95	0.67	7.4	0.0000
Linear term	–0.51	0.17	–3.0	0.0053
A2				
Intercept	4.5	1.2	3.8	0.0001
Linear term	–0.29	0.05	–5.8	0.0001

Supplementary table S3. Results of best-fitting regression models used to assess the geometric mean of enzyme activity (GMean index) relationship by analyzing the predictive power of organic matter content – measured as total organic carbon (TOC) – for each studied depth. Shown are model regression coefficients (Coef.), standard errors (SE), *t* and *p* values.

Predictor variable	Coef.	SE	*t*-value	*p*-value
A1				
Intercept	43.8	1.5	29.2	0.0000
Linear term	0.25	0.05	5.0	0.0000
A2				
Intercept	42.03	40.9	1.03	0.3109
Linear term	46.6	5.8	8.03	0.0000

Anexo A3. Material suplementar referente ao artigo 3

Supplementary Material

Microbial taxonomical and functional diversity in areas disturbed by a mining dam collapse

Authors: Éder Rodrigues Batista[1], Julie Kenya[1], Aline Oliveira Silva1, Marisangela Barbosa1, Jessé Valentim dos Santos1, Victor Salter Pylro1, Guilherme Oliveira2, Eder Soares2, Marco Aurélio Carbone Carneiro1[*]

[1]Department of Soil Science, Federal University of Lavras. Av. Doutor Sylvio Menicucci, s/n, Aquenta Sol, 37200-000, Lavras, MG

[2]Instituto Tecnológico Vale, R. Boaventura da Silva, 955, CEP 66055-200, Bairro Nazaré Belém, Pará, Brazil

[*]corresponding author (marcocarbone@ufla.br)

Table S1. ANOVA results for the effects of disturbance and sampling period on the taxonomic and functional alpha-diversity of the microbial community.

				q = 0		*q* = 1		*q* = 2	
Group	Terms	numDF	denDF	F value	*P*	F value	*P*	F value	*P*
Taxonomy	Condition (C)	1	8	1.29	0.2881	0.0986	0.7616	0.0633	0.8077
	Sampling period (S)	1	8	3.24	0.1096	2.0191	0.1931	0.4165	0.5368
	C × S	1	8	0.00	0.9978	0.0079	0.9314	0.6137	0.4560
	Marginal R^2			0.20		0.13		0.06	
	Conditional R^2			0.64		0.72		0.60	
	Transf.			NA		NA		NA	
Function	Condition (C)	1	8	50.50	<0.001	51.95	<0.001	47.48	<0.001
	Sampling period (S)	1	8	0.10	0.78	0.001	0.98	0.230	0.64
	C × S	1	8	5.50	0.05	4.968	0.06	2.41	0.16
	Marginal R^2			0.66		0.67		0.65	
	Conditional R^2			0.67		0.67		0.65	
	Transf.			Log		NA		NA	

Alpha-diversity estimates are presented as Hill numbers under three *q* values: *q* = 0 corresponds to species richness; *q* = 1 corresponds to Shannon diversity; and *q* = 2 corresponds to Simpson diversity. Marginal R^2 accounts for the variance explained by the fixed effects; Conditional R^2 accounts for the variance explained by the entire model, including fixed and random effects.

Table S2. PerMANOVA results from adonis() function on Bray-Curtis dissimilarities for community taxonomy and function.

Taxonomy					
Terms	Df	*R^2*	Sum of Sqrts	Pseudo-F	*P*
Condition (C)	1	0.17	0.065	6.68	<0.001
Sampling period (S)	1	0.04	0.016	1.65	0.4422
Condition × Sampling period	1	0.04	0.017	1.70	0.4089
Function					
Terms	Df	*R^2*	Sum of Sqrts	Pseudo-F	*P*
Condition (C)	1	0.15	0.16	5.97	<0.001
Sampling period (S)	1	0.04	0.05	1.76	0.06
Condition × Sampling period	1	0.04	0.04	1.51	0.16

Table S3. Importance and significance of physicochemical properties and extractable heavy metals content modulating shifts in taxonomic composition obtained from db-RDA analysis in the vegan package.

Physicochemical properties and heavy metals	R^2	*p*-value
pH	0.659	9.9e-05
Clay	0.565	9.9e-05
Pb^{2+}	0.507	9.9e-05
CECp	0.452	0.0003
Al^{3+}	0.037	0.0006
Se^{2+}	0.305	0.0040
K^+	0.278	0.0057
CECe	0.265	0.0076
P	0.262	0.0042
Mg^{2+}	0.250	0.0111

Table S4. Importance and significance of physicochemical properties and extractable heavy metals content modulating shifts in functional composition obtained from db-RDA analysis in the vegan package.

Physicochemical properties and heavy metals	R^2	*p*-value
Clay	0.772	9.9e-05
pH	0.632	9.9e-05
CECp	0.629	9.9e-05
Pb^{2+}	0.541	9.9e-05
Al^{3+}	0.492	9.9e-05
SOM	0.388	0.0007
K^+	0.377	0.0011
P	0.364	0.0002
CECe	0.359	0.0009
Se^{2+}	0.345	0.0013
Silt	0.324	0.0028
Mg^{2+}	0.279	0.0054
Sand	0.249	0.0110
Ni^{2+}	0.204	0.0264
Zn^{2+}	0.197	0.0272

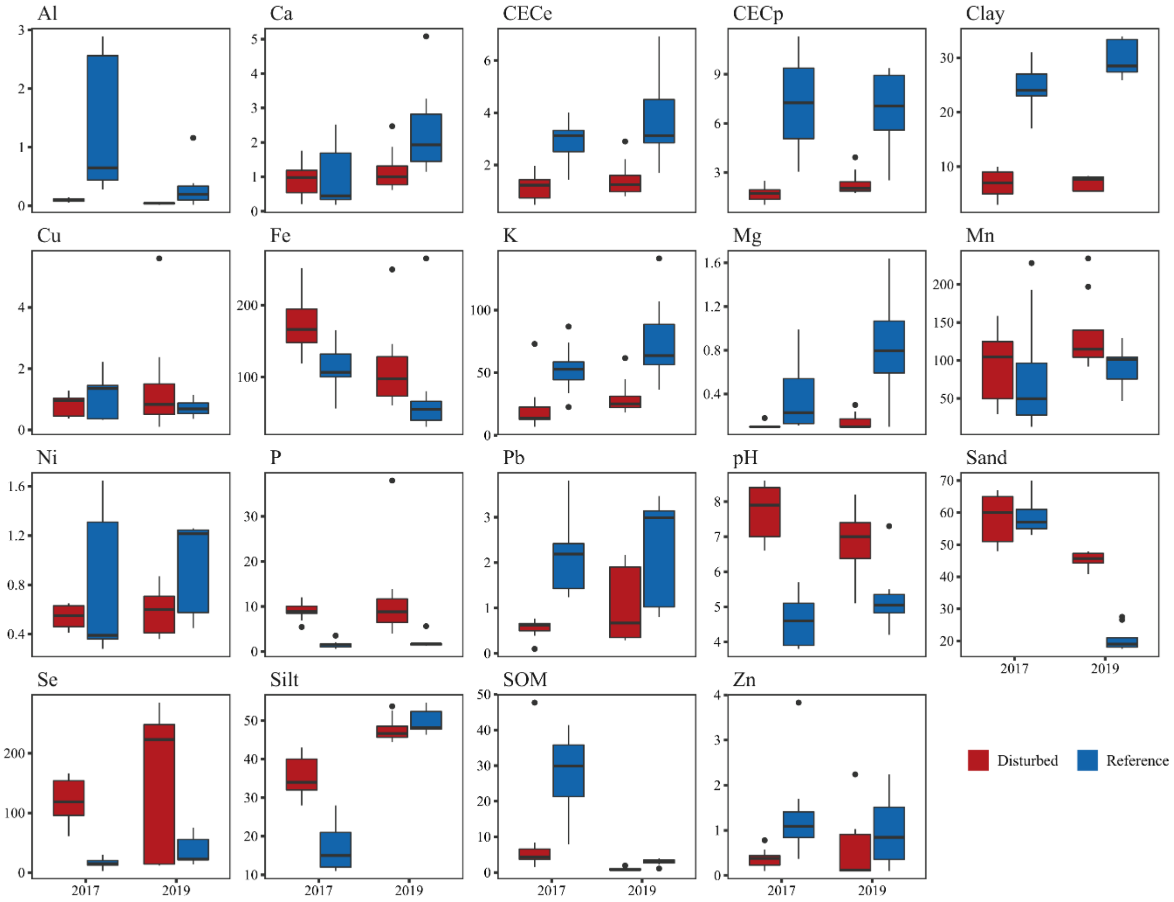


Fig. S1. Environmental variables from areas disturbed by a mining dam collapse.

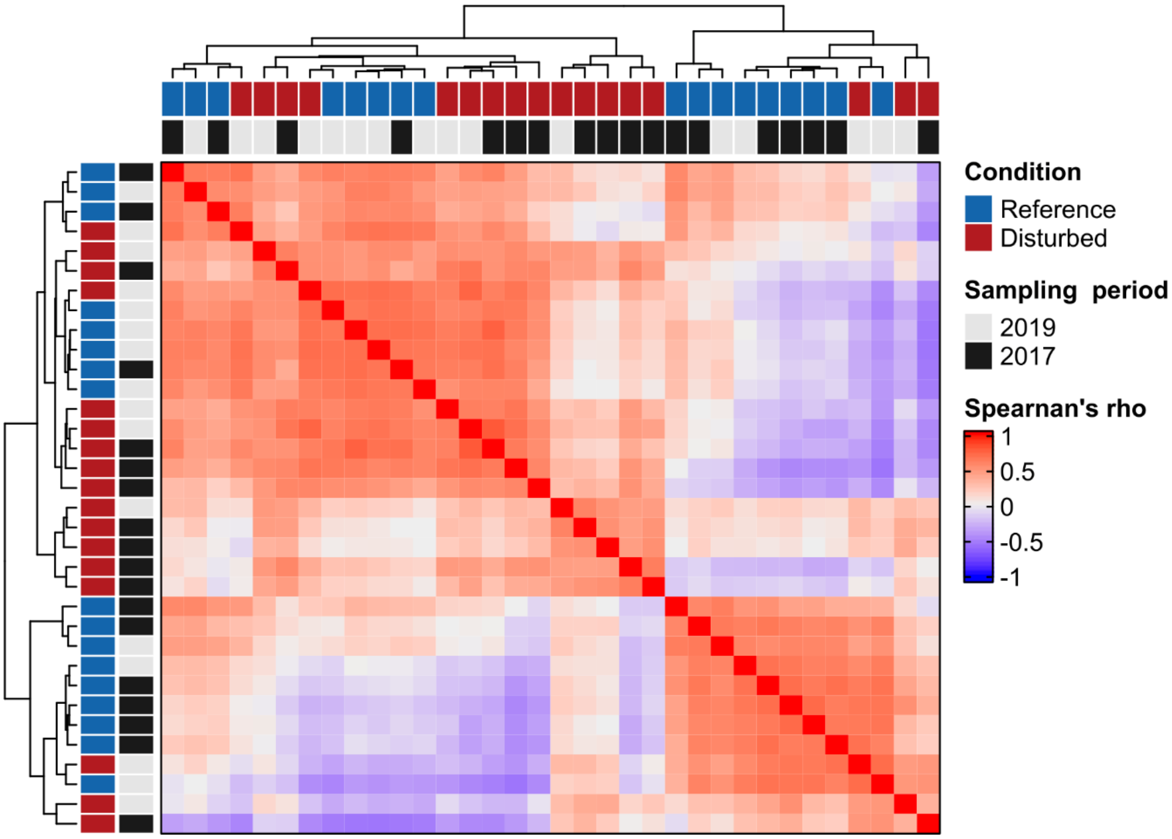


Fig. S2. Sample similarity heatmap for the composition of bacterial and archaeal phyla. Bray-Curtis dissimilarities were converted to similarities (%). Spearman correlation coefficients between samples were used for complete-linkage clustering of all samples. Sidebars color-annotate the condition and sampling period of each sample.

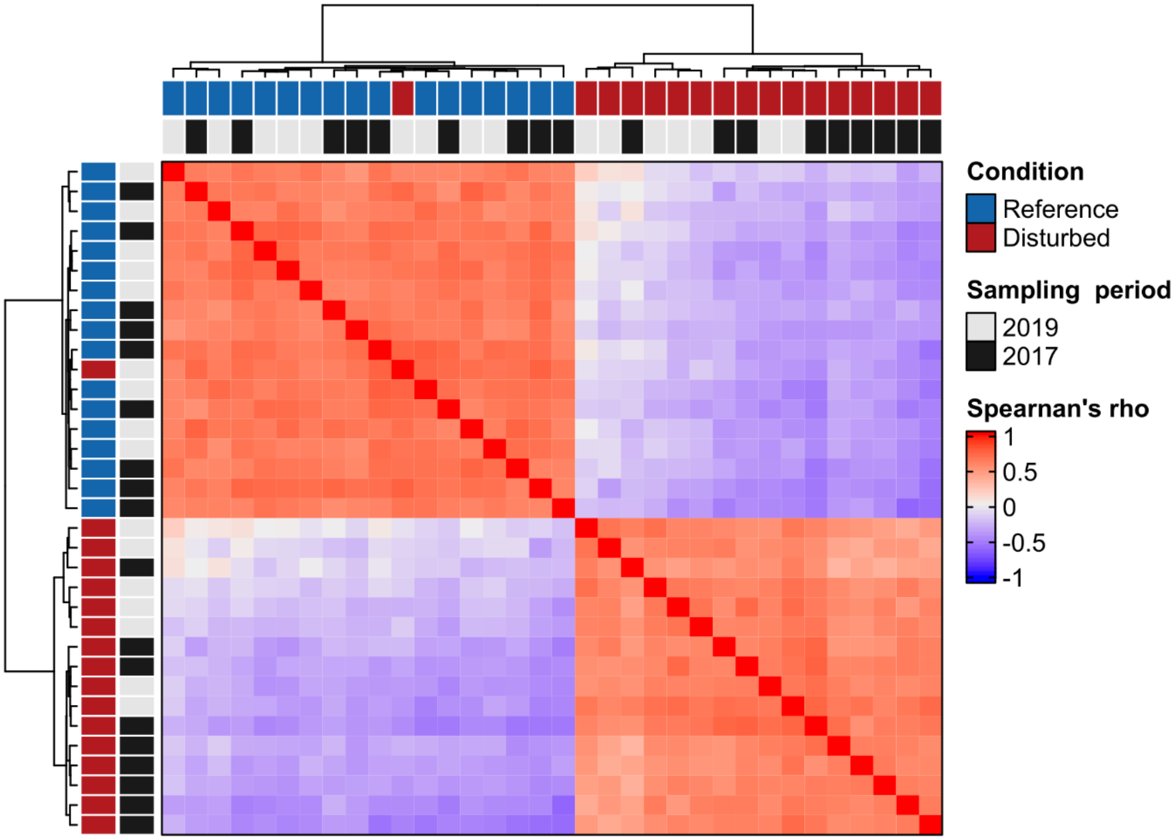


Fig. S3. Sample similarity heatmap for the community functional composition at the upper Subsystems level 1. Bray-Curtis dissimilarities were converted to similarities (%). Spearman correlation coefficients between samples were used for complete-linkage clustering of all samples. Sidebars color-annotate the condition and sampling period of each sample.

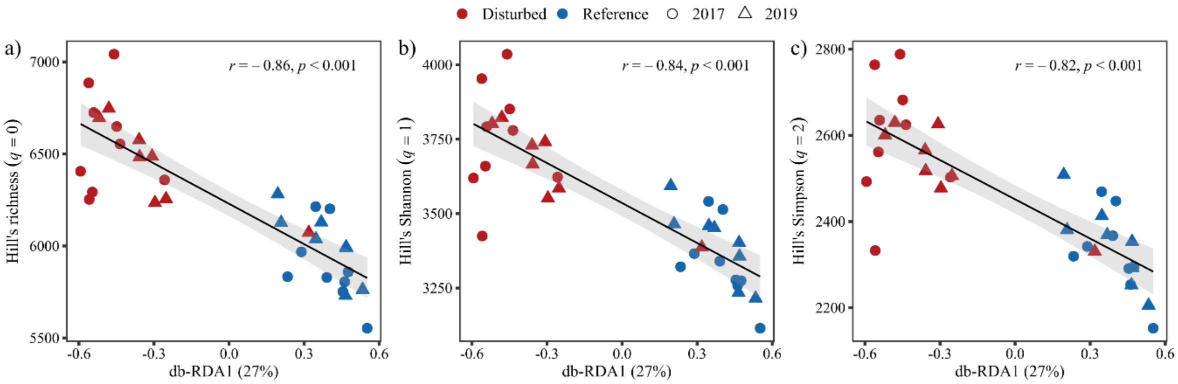


Fig. S4. Spearman's correlation coefficients for relationships between alpha-diversity measures and environmental variables. (a) relationship of first axis of db-RDA analysis with Hill's richness (a), Hill's Shannon (b), and Hill's Simpson (c).