UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Agronomia Eliseu Maciel
Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água

DISSERTAÇÃO

β-glucosidase and arylsulfatase enzymes and POxC content as indicators of minesoil restoration across a three-decade chronosequence

Gabriel Henrique Silva das Dôres

Pelotas, 2026

Gabriel Henrique Silva das Dôres

β-glucosidase and arylsulfatase enzymes and POxC content as indicators of minesoil restoration across a three-decade chronosequence

Dissertação apresentada ao Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências.

Orientador(a): Drª. Jakeline Rosa de Oliveira

Coorientadores: Drª. Lizete Stumpf
Dr. Ezequiel Cesar Carvalho Miola
Dr. Pablo Miguel

Pelotas, 2026

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação da Publicação

D695b Dores, Gabriel Henrique Silva das

β-glucosidase and arylsulfatase enzymes and POxC content as indicators of minesoil restoration across a three-decade chronosequence [recurso eletrônico] / Gabriel Henrique Silva das Dores ; Jakeline Rosa de Oliveira, orientadora ; Lizete Stumpf, Ezequiel Cesar Carvalho Miola, Pablo Miguel, coorientadores. — Pelotas, 2026.
61 f. : il.

Dissertação (Mestrado) — Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2026.

1. Chronosequence. 2. Soil acidity. 3. Soil health. 4. Soil fertility. 5. Biological activity. I. Oliveira, Jakeline Rosa de, orient. II. Stumpf, Lizete, coorient. III. Miola, Ezequiel Cesar Carvalho, coorient. IV. Miguel, Pablo, coorient. V. Título.

CDD 631.42

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

Gabriel Henrique Silva das Dôres

β-glucosidase and arylsulfatase enzymes and POxC content as indicators of minesoil restoration across a three-decade chronosequence

Dissertação aprovada, como requisito parcial para obtenção do grau de Mestre em Ciências, Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu, Universidade Federal de Pelotas.

Data da defesa: 04/03/2026

Banca examinadora:

Profa. Dra. Jakeline Rosa de Oliveira (Orientadora)

Doutora em Ciência do Solo pela Universidade Federal de Lavras

Profa. Dra. Ieda de Carvalho Mendes

Doutora em Ciência do Solo pela Oregon State University

Prof. Dr. Luiz Fernando Spinelli Pinto

Doutor em Ciência do Solo pela Universidade Federal do Rio Grande do Sul

Resumo

DORES, Gabriel Henrique Silva das. **Enzimas β-glicosidase e arilsulfatase e o teor de carbono oxidável por permanganato (POxC) como indicadores da restauração de minesoils em uma cronossequência de três décadas.** Orientadora: Jakeline Rosa de Oliveira. 2026. 61f. Dissertação (Mestrado em Manejo e Conservação do Solo e da Água) - Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2026.

Este estudo avaliou a restauração de minesoils com camada superficial argilosa ao longo de uma cronossequência de até 30 anos em Candiota, sul do Brasil, sob revegetação natural dominada por espécies do Bioma Pampa. A saúde do solo foi avaliada por meio da quantificação das atividades das enzimas arilsulfatase e β-glicosidase, dos teores de carbono oxidável por permanganato (POxC) e de carbono orgânico total (COT), em relação aos atributos de acidez e fertilidade do solo ao longo do tempo de restauração. Um Rhodic Lixisol não minerado adjacente foi amostrado como referência (solo não minerado). A atividade da arilsulfatase aumentou significativamente com o tempo de restauração ($R^2 = 0,74$; $p = 0,028$), enquanto a β-glicosidase não apresentou tendência temporal significativa ($R^2 = 0,352$; $p = 0,215$), com atividades variando de 26 a 132 e de 19 a 85 μg de p-nitrofenol g de $solo^{-1}$ h^{-1}, respectivamente. Ainda assim, ambas as enzimas permaneceram em níveis inferiores nos minesoils em comparação ao solo não minerado ao longo de toda a cronossequência. O POxC e o COT também aumentaram com a idade de restauração, indicando melhoria progressiva na dinâmica da matéria orgânica do solo e no acúmulo de carbono. De acordo com o modelo dos quatro quadrantes, no entanto, os minesoils avaliados permaneceram distintos do solo de referência não minerado após 30 anos, sugerindo recuperação incompleta das funções biológicas e químicas. Entre os indicadores avaliados, a arilsulfatase foi a mais sensível ao tempo de restauração, enquanto o POxC mostrou-se útil para acompanhar as trajetórias de restauração quando interpretado em conjunto com a atividade enzimática, o COT e os atributos de fertilidade. Os resultados indicam que a revegetação natural promove melhorias graduais na qualidade dos minesoils, mas a restauração completa pode requerer períodos mais longos e monitoramento contínuo. A calagem parece ser importante para mitigar o baixo pH e a saturação por alumínio, fatores que podem limitar a atividade biológica e retardar o progresso da restauração.

Palavras-chave: cronossequência, saúde do solo, atividade biológica, TOC, acidez do solo, fertilidade do solo

Abstract

DORES, Gabriel Henrique Silva das. **β-glucosidase and arylsulfatase enzymes and POxC content as indicators of minesoil restoration across a three-decade chronosequence.** Orientadora: Jakeline Rosa de Oliveira. 2026. 61f. Dissertação (Mestrado em Manejo e Conservação do Solo e da Água) - Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2026.

This study evaluated the restoration of clayey-topsoil minesoils along a chronosequence of up to 30 years in Candiota, southern Brazil, under natural revegetation dominated by Pampa Biome species. Soil health was assessed by quantifying arylsulfatase and β-glucosidase enzyme activities, permanganate-oxidizable carbon (POxC), and total organic carbon (TOC) contents in relation to soil acidity and fertility attributes over restoration time. An adjacent unmined Rhodic Lixisol was sampled as a reference (unmined soil). Arylsulfatase activity increased significantly with restoration time ($R2 = 0.74$; $p = 0.028$), whereas β-glucosidase showed no significant temporal trend ($R2 = 0.352$; $p = 0.215$), with activities ranging from 26 to 132 and from 19 to 85 µg p-nitrophenol g $soil^{-1}$ h^{-1}, respectively. Even so, both enzymes remained lower in minesoils than in the unmined soil throughout the chronosequence. POxC and TOC also increased with restoration age, indicating progressive improvement in soil organic matter dynamics and carbon accumulation. According to the four-quadrant model, however, the evaluated minesoils remained distinct from the unmined reference soil after 30 years, suggesting incomplete recovery of biological and chemical functions. Among the evaluated indicators, arylsulfatase was the most sensitive to restoration time, while POxC was useful for tracking restoration trajectories when interpreted together with enzymatic activity, TOC, and fertility attributes. The results indicate that natural revegetation promotes gradual improvements in minesoil quality, but full restoration may require longer periods and continued monitoring. Liming appears to be important for mitigating low pH and aluminum saturation, which can constrain biological activity and slow restoration progress.

Key-words: chronosequence, soil health, biological activity, TOC, soil acidity, soil fertility

Sumário

1. Introdução

A mineração desempenha papel relevante na economia, contudo, gera impactos ambientais significativos. O carvão mineral, segunda principal fonte global de energia primária, é extensivamente utilizado nas indústrias e na geração de energia elétrica (Williams, 2023). Em 2023, a demanda global cresceu 2,5%, atingindo 8.687 Mt, principalmente impulsionada por países como China (4.883 Mt) e Índia (1.245 Mt) (IEA, 2023).

A extração de carvão pode ser realizada em minas subterrâneas ou a céu aberto, sendo essa última predominante devido à maior eficiência e menores custos quando aplicada a jazidas superficiais (Ben-Awuah *et al.*, 2016). Após a extração, o carvão passa por beneficiamento visando aumentar seu poder calorífico, remoção de impurezas e adequação ao mercado consumidor. Os resíduos oriundos desse processo, compostos por partículas finas de carvão, ganga mineral e água, constituem rejeitos (*tailings*) (Firpo *et al.*, 2021). Stumpf, Pauletto e Pinto (2016) descrevem que, na mineração de carvão a céu aberto em Candiota, Rio grande do Sul – Brasil, as etapas envolvem remoção dos horizontes superficiais, transporte do material, escavação das rochas sedimentares, extração do carvão e recomposição topográfica utilizando estéreis e camadas de solo ou argila, resultando em minesoils subsequentemente revegetados.

A revegetação ocorre após a recomposição topográfica e pode ser potencializada por técnicas que promovam melhorias físicas, químicas (Ye *et al.*, 1999) e biológicas (Valentín-Vargas *et al.*, 2018). Existem duas abordagens: revegetação natural, geralmente lenta, e revegetação ativa, conduzida com espécies selecionadas para fins específicos, como tolerância a metais pesados (Zhang *et al.*, 2018). Estudos relatam o uso de gramíneas (Stumpf; Pauletto; Pinto, 2016; Oliveira, 2023; Miguel *et al.*, 2023) e espécies arbóreas (Mukhopadhyay; Maiti; Masto, 2013; Šnajdr *et al.*, 2013; Dias, 2024), entre outras.

Perfis de minesoils estudados na área de Candiota apontam que minesoils de minas de carvão com topsoil apresentam drenagem ácida em profundidade devido à oxidação da pirita (Bitencourt *et al.*, 2015), compactação superficial que dificulta o crescimento de raízes (Stumpf; Pauletto; Pinto, 2016) e contaminação por metais pesados, proveniente de estéreis contaminados com pirita ou deposição superficial, afetando a restauração (Dias, 2024). A restauração de minesoils pode ser avaliada por componentes biológicos, como a microbiota, que serve como bioindicador sensível da saúde do solo (Mendes *et al.* 2018; Wang *et al.*, 2012). A atividade enzimática do solo, medida por enzimas como arilsulfatase (envolvida no ciclo do enxofre) e β-glicosidase (degrada celulose para liberar glicose), pode ser usada para avaliar a microbiota do solo (Mcgill; Cole, 1981; Carneiro *et al.* 2024). Mendes *et al.* (2021), criaram um método inovador para avaliar a saúde dos solos agrícolas no Brasil, utilizando as atividades das enzimas β-glicosidase e arilsulfatase como base, relacionando-as aos teores de carbono orgânico total. Além disso, Mendes *et al.* (2024) destacam que, quando essas enzimas são preservadas pelo solo, isso indica condições propícias para o acúmulo de matéria orgânica.

Um outro indicador de saúde de solos agrícolas que vem sendo amplamente utilizado é o Carbono Oxidável por Permanganato (POxC), o qual indica o teor de carbono orgânico lábil ou ativo no solo. Culman *et al.* (2012) apontam que o POxC é sensível a alterações de manejo e à decomposição microbiana. Diversos estudos relacionam a atividade de enzimas como a β-glicosidase com a formação das frações de POxC, destacando-as como indicadores biológicos eficientes das transformações do carbono orgânico lábil no solo (Xu *et al.* 2021; Bharadwaj *et al.* 2025).

Diante deste contexto, o objetivo do trabalho foi testar a sensibilidade das enzimas β-glicosidase e Arilsulfatase e o teor de POxC como indicadores da restauração de minesoils em uma cronossequência de três décadas, atrelando-os aos atributos químicos ligados à acidez e fertilidade. Os objetivos específicos foram: a) Avaliar se os teores de POxC aumentam no decorrer dos anos de restauração dos minesoils e se está relacionado com a atividade enzimática; b) Avaliar se os teores de COT aumentaram com o tempo de restauração, e se a maior presença de COT está diretamente correlacionada com maior atividade das enzimas β-glicosidase e Arilsulfatase em uma cronossequência de 30 anos; C) Avaliar se as condições químicas como o pH e fertilidade do solo, influenciam a restauração dos minesoils, relacionando-os ao retorno da microbiota do solo expressa pela atividade das enzimas β-glicosidase e Arilsulfatase.

A hipótese é que a saúde de um minesoil esteja restaurado a partir de duas décadas de revegetação natural, mostrando elevada atividade das enzimas β-glicosidase e

Arilsulfatase, e elevado teor de POxC, reflexo da cobertura total da área com a vegetação característica do Bioma Pampa

2. Revisão de literatura

2.1. Impactos da Mineração de Carvão e a Recuperação dos Solos

A atividade mineradora está intrinsecamente vinculada ao desenvolvimento econômico, entretanto a mineração está atrelada a uma série de impactos ambientais negativos. Dentre os minérios energéticos, o carvão mineral destaca-se por ser a segunda maior fonte global de energia primária (Williams, 2023). Esta substância é amplamente empregada em processos industriais, especialmente em siderúrgicas, e na geração de eletricidade em usinas termoelétricas. De acordo com a Agência Internacional de Energia, no ano de 2023 a demanda global por carvão mineral apresentou um acréscimo de 2,5%, em comparação com o ano anterior, atingindo 8.687 milhões de toneladas (Mt). Este aumento foi impulsionado pela demanda proveniente de países em desenvolvimento, como a China (4.883 Mt) e a Índia (1.245Mt).

A mineração de carvão ocorre por meio de lavra subterrânea ou a céu aberto, a depender das características geológicas do depósito, como a profundidade da jazida e espessura e inclinação da camada de carvão (Ben-Awuah *et al*., 2016). A mineração a céu aberto é predominantemente empregada, em razão de seus custos operacionais relativamente inferiores e de sua maior eficiência na extração de depósitos superficiais, o que lhe confere maior viabilidade econômica (Ben-Awuah *et al.*, 2016). Porém, este método de lavra é cercado por problemáticas ambientais e sociais, que abrangem desde a definição da área de instalação do empreendimento até a gestão do passivo ambiental gerado. Os danos ambientais incluem a supressão do solo e das rochas que antecedem os bancos de carvão (Albert *et al.*, 2022), a perda de biodiversidade (Feng *et al*., 2019; Hu *et al*., 2020; Fabbri *et al*., 2021) e a alta geração de resíduos (Weiler *et al*., 2020).

Subsequente à mineração, o carvão passa pelo processo de beneficiamento para o aumento do poder calorífico, remoção de impurezas e a adequação às especificações do mercado consumidor. No caso da mina de Candiota esse processo não é realizado. O material residual deste processo, composto por partículas finas de carvão, ganga mineral e água, é considerado um resíduo da mineração (Firpo *et al*., 2021). A disposição incorreta desses resíduos é um determinante para a geração de drenagem ácida de mina (DAM), a qual é uma das principais problemáticas relacionadas a mineração de carvão, pois persiste por décadas após o fechamento do empreendimento (Jiao *et al*., 2023).

A DAM ocorre pela exposição de minerais sulfetados, como pirita, galena, arsenopirita, calcopirita e esfarelita a condições oxidantes (Guerrero *et al*., 2023). Durante a extração do carvão, esses minerais são expostos ao oxigênio atmosférico, à água e à ação de microrganismos acidófilos, desencadeando o processo de sulfurização. Esse processo químico gera ácido sulfúrico e libera íons sulfato (Bitencourt *et al*., 2015). Como consequência, forma-se um meio com forte acidez que favorece a mobilização de metais pesados e a precipitação de minerais secundários, como hidróxidos e óxidos de ferro e alumínio, resultando na contaminação de solos e corpos hídricos superficiais e subterrâneos (Silva *et al.*, 2011).

Neste contexto, a destinação adequada dos resíduos gerados pela mineração de carvão é essencial para uma gestão ambiental eficaz e sustentável. Uma alternativa promissora é a construção de minesoils os quais permitem a reutilização desses materiais na recuperação da área degradada. Os minesoils são solos resultantes da atividade humana, característicos do que é chamado por alguns de Antropoceno (Era geológica atual), em maioria, estes são construídos visando suprir uma necessidade específica, como o gerenciamento de resíduos e a restauração de ecossistemas degradados por atividades industriais, como a mineração (Leguédois *et al*., 2016).

2.2. Revegetação e seu impacto na restauração do solo

Após a recomposição topográfica o solo sofre correção (calagem e adubação) e então é feita a revegetação, que são técnicas que visam melhorias físicas, químicas (Ye *et al.*, 1999) e biológicas (Valentín-Vargas *et al.*, 2018). O processo de revegetação pode ocorrer por sucessão natural, de forma passiva, ou pode ser intervenção humana através de manejo, chamada de revegetação ativa ou artificial. Alguns estudos apontam que a revegetação por sucessão natural é um processo muito lento (Bradshaw, 2000). Já na revegetação ativa ou artificial, são escolhidas espécies vegetais de interesse de acordo com suas características, nesse momento pode-se pensar em espécies para o tratamento de metais pesados (Zhang *et al.*, 2018).

Há na literatura relatos de revegetação com espécies de gramíneas (Stumpf; Pauletto; Pinto, 2016; Oliveira, 2023; Miguel *et al.*, 2023) e espécies arbóreas (Mukhopadhyay; Maiti; Masto, 2013; Šnajdr *et al.*, 2013, Dias, 2024), dentre outros. Shen *et al.*, (2014) identificaram o efeito da sucessão vegetal na melhoria da qualidade do solo, principalmente no que tange aos teores de MOS. Porém ressaltam que devido as condições físicas e biológicas (baixa diversidade e quantidade de microrganismos) a velocidade de humificação estava baixa, necessitando de manejo. Liu *et al.* (2012) demonstram o efeito da revegetação no aporte de nitrogênio em solos reconstruídos em mina de cobre na China. Zhan e Sun (2012) apontam que a diversidade de fixadores de nitrogênio está ligada as mudanças nos teores de nutrientes e metais pesados causadas pela colonização da comunidade vegetal.

Sabendo-se da importância da revegetação na restauração de solos degradados pela mineração e com base nas informações acima expostas, entende-se a importância do estabelecimento da vegetação nesses minesoils. Bem como a importância da relação microrganismos/plantas, fundamental nos processos de formação do solo e no restabelecimento de funções do solo como a ciclagem de nutrientes (Rosario *et al.*, 2007; Mendez; Glenn; Maier, 2007).

2.3. Mineração de Carvão em Candiota/ RS e os minesoils gerados.

No Brasil, o estado do Rio Grande do Sul (RS) detém aproximadamente 80% das reservas de carvão mineral do país, no município de Candiota (RS), localiza-se a maior jazida de carvão mineral do Brasil, a Mina de Candiota, sob concessão da Companhia Riograndense de Mineração (CRM). Nesta localidade, as reservas de carvão são cerca de 1 bilhão de toneladas, com viabilidade a serem minerados por apresentarem profundidades inferiores a 50 metros (Pinto *et al.*, 2020). A atividade de mineração em Candiota teve início na década de 60 e atualmente 750,68 hectares já foram impactados (Albert *et al.*, 2022). Na Mina Candiota, a recuperação ambiental ocorre de forma concomitante às operações de mineração. Após a recomposição topográfica, as áreas recuperadas parte foram devolvidas para os proprietários, utilizando para cultivar eucalipto, e parte não receberam nova destinação funcional devido às limitações edáficas, como baixos teores de macronutrientes, elevado grau de compactação e baixo pH, características que inviabilizam a implantação de atividades agropecuárias.

Stumpf, Pauletto e Pinto (2016) listam as etapas da mineração de carvão a céu aberto, desde a lavra à reposição topográfica, que ocorre no município de Candiota. A primeira etapa está ligada à retirada dos horizontes A, B e/ou C do solo original, esse material é imediatamente transportado a uma área topograficamente aplainada. Seguindo para a remoção das rochas sedimentares sobrejacentes utilizando escavadeira de alta capacidade (dragline), com posterior detonação e remoção dos bancos de carvão. Após a lavra é feita a recomposição topográfica da área, começando com a reposição dos estéreis (mistura de rochas e carvão não aproveitados), que são aplainados por tratores de esteira formando a camada de cobertura (*overburden)* das áreas pós-mineração (Stumpf *et al.*, 2023). Após essa etapa é acrescentado outra camada de solo (*topsoil*), que originalmente era o horizonte A e/ou B removidos durante a primeira etapa, e/ou uma camada de argila selante, finalizando a recomposição topográfica. Esse processo dá origem aos minesoils que são posteriormente revegetados (Stumpf; Pauletto; Pinto, 2016).

Na mina de Candiota, pesquisadores da UFPel estudaram minesoils, onde foram cultivadas plantas como *Hemarthria altissima*, *Paspalum notatum cv. Pensacola*, *Cynodon dactylon cv. Tifton* e *Urochloa brizantha* em um experimento de campo. Essas áreas passaram por calagem, receberam adubação e foram avaliadas periodicamente por diferentes equipes de pesquisa. Anteriormente, Nunes (2002) constatou que minesoils com 1 e 5 anos apresentaram maior densidade, menor macroporosidade, resistência mecânica elevada e pH baixo (~2,4) na camada superficial em alguns pontos do solo com 1 ano de idade. Em comparação ao solo natural, esses minesoils apresentaram maior densidade e menor porosidade total.

Para o monitoramento da dinâmica de restauração dos solos construídos em Candiota, o Departamento de Solos da Universidade Federal de Pelotas (UFPel) instalou uma área experimental no ano de 2003, entretanto esta apresentou limitações no estabelecimento da vegetação devido a condições climáticas adversas. Por esse motivo, no ano de 2007 foi implantada uma nova área experimental. A área de 2003 foi reduzida e mantida para fins comparativos, com o objetivo de avaliar a evolução natural dos solos construídos submetidos a diferentes estratégias de revegetação.

Quando as espécies estavam implantadas há 28 meses, avaliou-se a agregação dos minesoils, porém não foi observado diferença significativa entre as espécies de gramíneas nas camadas de 0,00-0,10m e de 0,10-0,20m. E o teor médio de carbono variou pouco em profundidade, na camada de 0,00-0,10m foi de 5,34 g kg^{-1} e na camada de 0,10-0,20 m de 5,18 g kg^{-1} (Franco, 2006). Aos 41 meses de restauração desses minesoils, também não foi observado diferença entre as espécies em relação à agregação do solo, porém verificou-se aumento na concentração de agregados na classe de 1,00-0,25mm, tanto na camada de 0,00-0,10m (40,13%) como na camada de 0,10-0,20m (35,73%). Constatou-se o aumento do teor médio de carbono orgânico na camada de 0,00-0,10m (7,38 g kg^{-1}) e na camada de 0,10- 0,20m (6,20 g kg^{-1}) (Gonçalves, 2008)

Holanda Neto (2011) aos 48 meses de implantação do experimento com minesoils sob revegetação com Urochloa brizantha, Cynodon dactylon cv. capim vaquero, Panicum maximum, Urochloa humidicola, Hemarthria altissima e Cynodon dactylon cv. Tifton constatou um aumento nos teores de COT e respiração basal se comparado a minesoils não revegetado. Também identificou espécies de fungos, a saber: Acaulospora mellea, Entrophospora infrequens, Glomus clarum, Glomus macrocarpum, Glomus tortuosum, Glomus sp., Glomus clavisporum, Glomus dominikii, Glomus etunicatum, Glomus.

Com 72 meses de revegetação desses minesoil com espécies de gramíneas, foi avaliado a pressão de pré-consolidação, com menor valor relacionado a Hemarthria altissima (71 kPa) e os maiores valores proporcionados pelo Paspalum notatum cv. Pensacola (120 KPa), Cynodon dactylon cv. Tifton (120 kPa) e a Urochloa brizantha (118 kPa) (Miola, 2010). Em outro estudo que realizou o fracionamento da matéria orgânica observou-se que os minesoils sob revegetação com Hemarthria altissima e a Urochloa brizantha apresentaram maiores estoques de carbono na fração leve livre (1,22 Mg ha-1 e 1,27 Mg ha^{-1} respectivamente) em relação aos minesoils revegetados com Paspalum notatum (0,86 Mg ha-1) e Cynodon dactylon (0,83 Mg ha-1) na camada de 0,00-0,03m. Em relação ao estoque de carbono da fração leve oclusa o minesoils sob revegetação com Hemarthria altissima e o Cynodon dactylon apresentaram os maiores teores (1,09 Mg ha-1 e 1,02 Mg ha-1 respectivamente) em relação ao revegetado com Paspalum notatum (0,61 Mg ha-1)(Leal, 2011).

Com 3 anos de restauração, os minesoils sob revegetação com as espécies de gramíneas (Cynodon dactylon cv. capim vaquero, Panicum maximum, Urochloa humidicola e Urochloa brizantha), Reis *et al.* (2014) observaram que a resistência tênsil de agregados esteve negativamente associada ao teor de COT na camada superficial do minesoil (0,00-0,15m). E que os valores de resistência tênsil variaram de 82,41 kPa a 112,28 kPa na camada 0,00-0,05m e de 91,40 kPa a 191,41 kPa na camada 0,10-0,15m. Após 78 meses de restauração, constatou-se que a concentração de macroagregados foi maior na camada de 0,10-0,20 m (87,56%) em comparação com a camada de 0,00-0,10 m (81,15%). O teor médio de carbono orgânico na camada superficial (0,00-0,10 m) foi de 8,46 g kg^{-1}, enquanto na camada de 0,10-0,20 m esse valor foi de 6,39 g kg^{-1} (Castro, 2012).

Bitencourt *et al.*, 2015 avaliaram o potencial de acidificação por pirita e contaminação por metais pesados em solos construídos após mineração de carvão, comparando áreas antigas (I e II, sem recuperação ambiental) e atuais (IV e VII, com topsoil e argila). Nas áreas antigas, observou-se acidificação extrema ao longo de todo o perfil, com pH abaixo de 3,2 e altas concentrações de alumínio. A adição de topsoil e argila nas áreas atuais melhorou as condições químicas superficiais, mas não impediu a formação de drenagem ácida em profundidade, embora a acidificação tenha sido menos intensa. A análise multivariada evidenciou que a liberação de metais se relaciona principalmente à geração de drenagem ácida, pois os materiais geológicos da camada de estéril não possuem teores elevados de metais pesados naturalmente.

Na área experimental de 2003, após 103 meses Stumpf, Pauletto e Pinto, (2016) avaliaram a agregação e o crescimento vegetal considerando as espécies de gramíneas perenes *Hemarthria altissima*, *Paspalum notatum* cv. *Pensacola, Cynodon dactylon* cv. *Tifton* e *Urochloa brizantha*. Os autores concluíram que a espécie *Urochloa brizantha* apresenta um maior potencial para recuperação dos atributos físicos das áreas degradadas por apresentar maior densidade, volume, comprimento e área radicular. As raízes de todas as espécies de gramíneas perenes analisadas promoveram uma recuperação da condição física do solo na camada superficial (0,00–0,10 m), com a formação de agregados, aumento da macroporosidade e diminuição da densidade do solo. Entretanto, observou-se uma significativa diminuição do desenvolvimento radicular de todas as espécies abaixo da camada superficial, o que reflete uma condição física restritiva do solo devido à presença de agregados compressivos associados baixa macroporosidade e alta densidade do solo.

A elevada compactação do solo é uma limitação recorrente observada nos solos construídos na Mina Candiota, de acordo com Barboza *et al.* (2021) a umidade da camada de topsoil deve ser um fator considerado durante a reconstrução topográfica para minimizar os impactos do tráfego de máquinas pesadas. O solo apresentou umidade equivalente a 216,31 g kg^{-1} e, após o primeiro evento de tráfego de máquinas, observou-se um aumento na densidade do solo, bem como uma redução de 76% na macroporosidade na profundidade de 0,20 m. A formação de agregados compressivos e o aumento na retenção de água no solo foram registrados após o décimo segundo evento de tráfego de máquinas. Isso também acaba por limitar o desenvolvimento da vegetação (Stumpf; Pauletto; Pinto, 2016).

Fernandez (2022) analisou os efeitos da revegetação com gramíneas perenes de verão sob diferentes manejos de roçada, ao longo de 18 e 11,6 anos, na qualidade biológica e química do solo de uma área degradada pela mineração de carvão em Candiota/RS. A pesquisa utilizou funis de Berlese-Tüllgren modificados para identificar organismos edáficos, análises microbiológicas para CBM, RB e atividade da urease, e quantificou frações da matéria orgânica para avaliar humificação. Houve melhora no CBM e na RB em relação ao solo de referência, indicando efeito positivo das gramíneas; porém, a roçada com permanência da biomassa ainda não é considerada o manejo mais eficaz para recuperação ecológica. Um outro estudo avaliou a fauna edáfica e os atributos químicos e físicos de um minesoil, em Candiota, após 14,6 anos de revegetação com gramíneas (Cynodon *dactylon*, *Panicum maximum*, *Urochloa humidicola* e *Urochloa brizantha*). Amostras coletadas em 2022 mostraram que os atributos físicos do solo estão consolidados, mas a relação entre fauna, biomassa microbiana e carbono orgânico ainda está em fase inicial, possivelmente devido ao tempo relativamente curto de recuperação da área minerada (Oliveira, 2023).

Após 10,6 anos da implantação do experimento de 2003 e 4,6 anos do experimento de 2007, Miguel *et al*. (2023) analisaram as propriedades físicas dos minesoils e constataram melhorias nas camadas superficiais (0-0,10m), como a quebra dos agregados compressivos e aumento da macroporsidade, as quais foram promovidas pela expansão do sistema radicular das gramíneas perenes. No entanto, na camada de 0,10–0,20 m a compactação persiste.

Fernandez *et al.* (2023) indicaram que, após 18 anos de revegetação com gramíneas perenes, o minesoil ainda se encontra em estágio inicial de recuperação quando considerada sua função ecossistêmica como habitat para organismos do solo. A deposição contínua de biomassa aérea pelas gramíneas, ao longo de mais de uma década, não foi suficiente para promover avanços significativos na formação de matéria orgânica humificada. Esse resultado demonstra que o minesoil ainda carece de atributos ecológicos que favoreçam a colonização e atuação efetiva dos organismos decompositores da matéria orgânica. Stumpf *et al.* (2018) destacam a importância de considerar os minesoils como um novo sistema, no qual o monitoramento de atributos a longo prazo é essencial, uma vez que as relações positivas entre o carbono orgânico total e a agregação levam tempo para serem restauradas, devido à intensa compactação das áreas mineradas.

Em 2024, minesoils com 8,6 e 20 anos de restauração por gramíneas perenes (*Hemarthria altissima*, *Cynodon dactylon* e *Urochloa brizantha*) foram avaliados quanto a elementos potencialmente poluidores (EPTs). O fator de contaminação e o índice de carga poluente apontaram poluição moderada. Constataram-se que os teores de Cobre, Vanádio, Bário e Cromo ultrapassaram os valores de referência. Já o Manganês, apresentou maior disponibilidade para as plantas nos minesoil. *Urochloa brizantha* resultou em menor acúmulo de EPTs em minesoils, demonstrando sua eficiência na redução da mobilidade desses elementos. Não foi detectado Zinco ou Vanádio nas plantas, somente Bário, sem diferenças significativas entre espécies. Conclui-se que idade da restauração e escolha das gramíneas impactam a dinâmica dos EPTs (Brisolara, 2024).

A restauração das funções ecológicas dos solos em áreas mineradas permanece um desafio complexo, embora a revegetação com *Urochloa brizantha* tenha aumentado a abundância da fauna edáfica (2.397 indivíduos vs. 483 no controle) (Oliveira *et al.*, 2025). Os parâmetros biológicos essenciais ainda não atingiram níveis comparáveis a ecossistemas não perturbados. A atividade enzimática e a diversidade da mesofauna mantêm-se abaixo dos valores de referência, indicando uma recuperação incompleta das interações biológicas do solo (Fernandez *et al.*, 2023).

2.4. Acidez do solo, saturação por alumínio e impacto no retorno da biota

Os minesoils provenientes da extração de carvão de Candiota, compostos por uma camada de estéreis (*overburden*) sob uma camada superficial (*topsoil*), apresentam condições físico-químicas desfavoráveis ao desenvolvimento vegetal, como alta acidez, compactação e presença de metais pesados (Moradi *et al.*, 2020; Fernandez, 2022). Como citado nos itens acima, os minesoils provenientes de mineração de carvão são, na camada estéril, ricos em $S\text{-}FeS_2$ (pirita), $S\text{-}SO_4$ (sulfato) e S-orgânico que ao oxidar, inicia um processo pedogenético chamado de sulfurização, produção de ácido sulfúrico na solução do solo, que resulta em um solo com pH baixo (Bitencourt *et al.*, 2015; Pinto e Kämpf, 2002). Esse processo resulta no intemperismo e dissolução de minerais no solo com a consequente elevação dos teores de metais pesados no solo (Barnhisel *et al*. 2015) O intemperismo em solos de baixo pH libera alumínio na solução, promove a lixiviação de cátions alcalinos e mantém os grupos funcionais dos colóides protonados. Isso resulta em solos com alta acidez ativa e potencial, elevada saturação da CTC por Al e baixa saturação por bases. O risco de toxidez nas raízes e para a microbiota do solo aumenta com a profundidade do solo, pois há menos complexação de alumínio pela matéria orgânica e menores concentrações de cátions básicos, como cálcio, magnésio e potássio (Poschenrieder *et al.* 2008; CQFS, 2016; Mulazzani *et al.* 2024)

A alta concentração de metais pesados, torna o ambiente inóspito a muitas formas de vida no solo. A fauna edáfica, macrofauna (>2mm) e mesofauna (2mm) (Lavelle; Spain, 2001) sofre efeito negativo em sua população e diversidade quando em solos com elevados teores de metais pesados (Lu *et al.*, 2021). A contaminação por metais pesados afeta negativamente a matéria orgânica e a atividade biológica no solo. Há estudos que demonstram o potencial desses xenobióticos em induzir danos ao DNA de microrganismos (Souza *et al.*, 2021). Além disso, esses elementos reagem com a matéria orgânica do solo, o que resulta na complexação dos metais e da MOS, a tornando indisponível para a microbiota do solo e para a vegetação (Zamulina *et al.*, 2021).

Esses elementos xenobióticos inibem a atividade de enzimas como dehidrogenases e urease, que são essenciais para o metabolismo microbiológico e que fazem parte da ciclagem de nutrientes no solo (mineralização da MOS), bem como reduz a população microbiana (Uzarowicz *et al.*, 2020). Ou seja, a inibição da atividade microbiológica afeta as transformações da MOS, que por sua vez afeta seus teores e disponibilidade no solo (Wolinska *et al.*, 2020). Também impacta a produção de polissacarídeos (substâncias cimentantes) ou hifas fúngicas (substância ligantes), alterando a agregação do solo (Rigotti, 2002).

Zamulina *et al.* (2021) destacam que há microrganismos que podem se adaptar, com o tempo, à presença desses metais, auxiliando no processo de precipitação desses elementos em carbonatos, o que reduz sua mobilidade e toxicidade. Há na literatura estudos indicando a atividade de bactéria do gênero Bacillus relacionados à sobrevivência em condições ambientais adversas como minesoil contaminados com metais pesados (Uzarowicz *et al.*, 2020).

Por mais que a presença dos metais pesados prejudique o desenvolvimento da microbiota do solo, há fatores ainda mais limitantes à sobrevivência desses organismos. Os parâmetros físico-químicos do solo, como o pH e salinidade, apresentam forte influência no desenvolvimento da microbiota. Portanto, solos ácidos e com altos teores de sais apresentam menor atividade microbiológica. Enzimas como desidrogenase, urease e β-glicosidase são sensíveis a esses parâmetros. Ou seja, solos que com pH mais elevado e altos teores de MO apresentaram maior atividade enzimática. Para a recuperação desses minesoils, com pH muito baixo, é necessário, primeiramente, a neutralização da acidez e para isso é só aplicar calcário (Iakovleva *et al.*, 2015).

2.5. Atividade biológica e POxC como indicadores de qualidade de minesoil em recuperação.

A matéria orgânica do solo, pode ser subdividida de acordo com sua função e taxa de decomposição em: lábil (ativa), lenta (intermediário) e estável (inerte, resistente) (Lützow *et al*., 2007; Kopecký *et al*., 2021). Sendo que as frações lábeis da MOS servem como fonte de energia e substrato para os microrganismos, que por sua vez contribuem na ciclagem de nutrientes no solo. Já a parte mais estável da matéria orgânica do solo está ligado aos serviços ecossistêmicos de armazenamento de carbono no solo (Haynes, 2010).

De acordo com a literatura, a proteção contra a biodegradação da MOS é dada pela inacessibilidade espacial (protegida em agregados) e a interação com superfícies minerais e íons metálicos (formando complexos organominerais) (Lützow *et al*., 2006; Llorente; Glaser; Turrión, 2010; Kopecký *et al*., 2021). Portanto a formação dos complexos organometálicos se dá pela ligação desses elementos com grupos funcionais da matéria orgânica como os grupos carboxílicos, fenólicos, hidroxílicos, principalmente em ácidos húmicos e fúlvicos. Assim, resultando em mais estabilidade a MO e como consequência reduzindo a biodisponibilidade da MO à microbiota (Karpukhin e Bushuev, 2007; Zamulina *et al.*, 2021).

A microbiota do solo é essencial para decompor e utilizar o carbono lábil do solo, atuando por meio de enzimas secretadas como a β-glicosidase, que regula a quebra de polissacarídeos no solo (Wang *et al*. 2022; Bharadwaj *et al*. 2025). A degradação do carbono consiste na decomposição de compostos orgânicos como amido, celulose, hemicelulose, quitina e pectina. Já a estabilização do carbono no solo ocorre principalmente pelo ciclo Calvin-Benson-Bassham, importante para a estabilização do carbono em solos ricos em nutrientes (Yuan *et al*. 2025). O carbono lábil do solo pode ser avaliado pelas frações: carbono orgânico dissolvido, carbono oxidável com permanganato (POXC) e carbono orgânico de fração leve (Wang *et al*. 2022). O POxC é amplamente empregado como indicador de saúde do solo pela sua praticidade e baixo custo, e se mostra sensível a alterações no manejo ou ambiente. Ele se relaciona principalmente com frações menores (53–250 μm) e mais densas (>1,7 g cm−3) do carbono orgânico particulado, indicando um carbono lábil relativamente mais processado (Culman *et al*. 2012).

Componente biológicos do solo também se mostram como indicadores sensíveis a saúde de solo (Mendes *et al*., 2018; Mendes *et al.*, 2018). Lu *et al.* (2021) demonstram que a fauna edáfica pode ser utilizada como um indicador de poluição do solo e Wang *et al.* (2012) apontam que a microbiota pode fornecer informações complementares sobre a contaminação do solo, podendo ser considerados também como um bioindicador.

Há na literatura algumas metodologias criadas para mensurar a atividade microbiana do solo, como a respiração basal, Carbono da BioMassa-CBM e a atividade enzimática. Na respiração basal é medida a atividade metabólica dos microrganismos no solo a partir da captura de CO_2 liberado pela respiração desses microrganismos (Mendes *et al.*, 2019). A CBM corresponde a quantidade de carbono da biomassa encontrado em determinado volume de solo, refletindo a parte viva e metabolicamente ativa da MOS (Mendes *et al.*, 2019). Já a leitura da atividade enzimática do solo fornece dados sobre a atividade de enzimas como a arilsulfatase e a β-glicosidase que fazem parte do metabolismo da microfauna (Mendes *et al.*, 2019). A β-glicosidase é uma exocelulase que participa da transformação (quebra) da celulose em glicose, ou seja, na decomposição da matéria orgânica no solo. Catalisando a quebra, principalmente, da celobiose em glicose, que por sua vez será utilizada como fonte de energia pela microbiota. A arilsulfatase é da classe das esterases e que participa do ciclo do enxofre. Proveniente da excreção de bactérias, agem na hidrólise de sulfatos aromáticos resultando na liberação de SO_4^{2-} (Mcgill; Cole, 1981; Carneiro *et al.*, 2024).

Dentre essas metodologias, analisando os teores dessas enzimas, consegue-se ter um melhor entendimento sobre as reações bioquímicas que ocorrem no solo, relacionadas à mineralização de nutrientes (Veres *et al.*, 2015). Esse parâmetro biológico pode ser usado para estimar, por meio de índices, o impacto da ação humana nos solos e a poluição resultante (Nannipieri; Trasar-Cepeda; Dick, 2018; Siwik-Ziomek *et al.*, 2018) Essas enzimas por serem muito sensíveis às mudanças no ambiente, como pH baixo, alta salinidade e presença de Elementos Potencialmente Tóxicos (EPTs) com os metais pesados, podem ser utilizadas como bioindicadores da restauração ecológica de minesoils (De Varennes *et al.*, 2010; Pająk *et al.*, 2018; Uzarowicz *et al.*, 2020).

Mendes *et al*. (2021), criaram um método inovador para avaliar a saúde dos solos agrícolas no Brasil (BioAs), utilizando as atividades das enzimas β-glicosidase e arilsulfatase como base, relacionando-as aos teores de carbono orgânico total. A partir da BioAs, Chaer *et al*. (2023) propôs o modelo dos quatro quadrantes, usando a atividade específica das enzimas β-glicosidase e arilsulfatase (ASEA), calculada pela média enzimática em relação ao teor de TOC e expressa em µg PNP g–1 TOC h–1. Neste modelo a saúde do solo é classificada em quatro quadrantes: Estado de equilíbrio com alta qualidade do solo (*High soil quality*), apresentando alta atividade biológica e alto teor de TOC; Estado transitório com perda de qualidade do solo (*Soil losing quality*), apresentando baixa atividade biológica e tendência de perda de TOC; Estado de equilíbrio com baixa qualidade do solo (*Low soil quality*), apresentando baixa atividade biológica e baixo teor de TOC; Estado transitório, com ganho de qualidade do solo (*Improving soil quality*), apresentando alta atividade biológica e tendência de aumento do TOC (Mendes *et al*., 2023). Além disso, Mendes *et al*. (2024) destacam que, quando essas enzimas são preservadas pelo solo, isso indica condições propícias para o acúmulo de matéria orgânica.

Capítulo 1

β-glucosidase and arylsulfatase enzymes and permanganate-oxidizable carbon content as indicators of minesoil restoration across a three-decade chronosequence

1. Chapter 1: β-glucosidase and arylsulfatase enzymes and permanganate-oxidizable carbon content as indicators of minesoil restoration across a three-decade chronosequence (Submetido no número especial da revista "Journal of Soil Science e Plant Nutrition")

1.1. Introduction

Coal remains as the largest source for electricity generation worldwide, highlighting its continued relevance for global economy (IEA, 2024). However, coal combustion in thermoelectric power plants continues to face substantial criticism due to the magnitude of its polluting effects, releasing large amounts of CO_2 into the atmosphere (IPCC, 2023). In addition, surface coal mining causes significant environmental impacts, including the complete removal of soil layers and vegetation (Ruiz et al., 2023).

The adoption of topographic reconstruction of mined areas minimizes the visual impact on the post-mining landscape degradation. Even so, despite the revegetation of reconstructed landscapes, the resulting minesoils exhibit, over the long term, low edaphic biodiversity (Fabbri et al., 2021; Feng et al., 2019; Hu et al., 2020; Olivera et al., 2025), persistent soil compaction (Fernandez et al., 2025; Leal et al., 2025a), and slow accumulation of soil organic matter (SOM) (Leal et al., 2025b; Stumpf et al., 2018).

In minesoils, time is a key factor influencing the accumulation of SOM, which is associated with the recovery of edaphic and microbial diversity (Mukhopadhyay and Masto, 2022). In turn, these processes are linked to the reestablishment of essential ecosystem services, such as nutrient cycling, as reflected by enzymatic activity (Ezeokoli et al. 2020). In Brazil, the enzymatic activity of arylsulfatase and β-glucosidase is used as a sensitive indicator of agricultural soil health, as it enables a better understanding of biochemical reactions associated with nutrient mineralization and indicates conditions favorable for SOM accumulation (Carneiro et al., 2024; Mendes et al., 2024). The activity of these two enzymes also serves as an indicator of soil structural status. In biogenic aggregation, higher arylsulfatase activity is observed compared to β-glucosidase, suggesting that fungi play a substantial role in soil functioning within natural and agricultural settings in Brazil (Coelho-Junior et al., 2026).

Arylsulfatase and β-glucosidase are also used, in minesoils, as bioindicators of ecological restoration due to their high sensitivity to changes in pH, salinity, and the presence of heavy metals (Ezeokoli et al., 2020; Pająk et al., 2018; Uzarowicz et al., 2020). In South Africa, an increase in β-glucosidase activity was observed along a minesoil chronosequence of up to 18 years of restoration (66, 176, and 530 μg p-nitrophenol g^{-1} h^{-1} for <1, 1.5, and 18 years of restoration, respectively), which was associated with the recovery of bacterial diversity in these areas (Ezeokoli et al., 2020).

Another soil health indicator that has been widely used in agricultural soils is permanganate-oxidizable carbon (POxC), which represents the labile or active organic carbon fraction in the soil (Pulleman et al. 2021). POxC is a rapid and cost-effective method for quantifying soil labile carbon and is highly sensitive to changes in soil management and environmental conditions (Culman et al., 2012). This labile organic carbon fraction is more closely associated with smaller (53–250 μm) and denser (>1.7 g cm^{-3}) particulate organic carbon fractions, reflecting a relatively more microbiologically processed carbon pool (Culman et al., 2012). Hence, POxC is considered a reliable indicator of soil carbon sequestration, as it reflects management practices that promote the accumulation or stabilization of soil organic matter (SOM) (Hurisso et al., 2016).

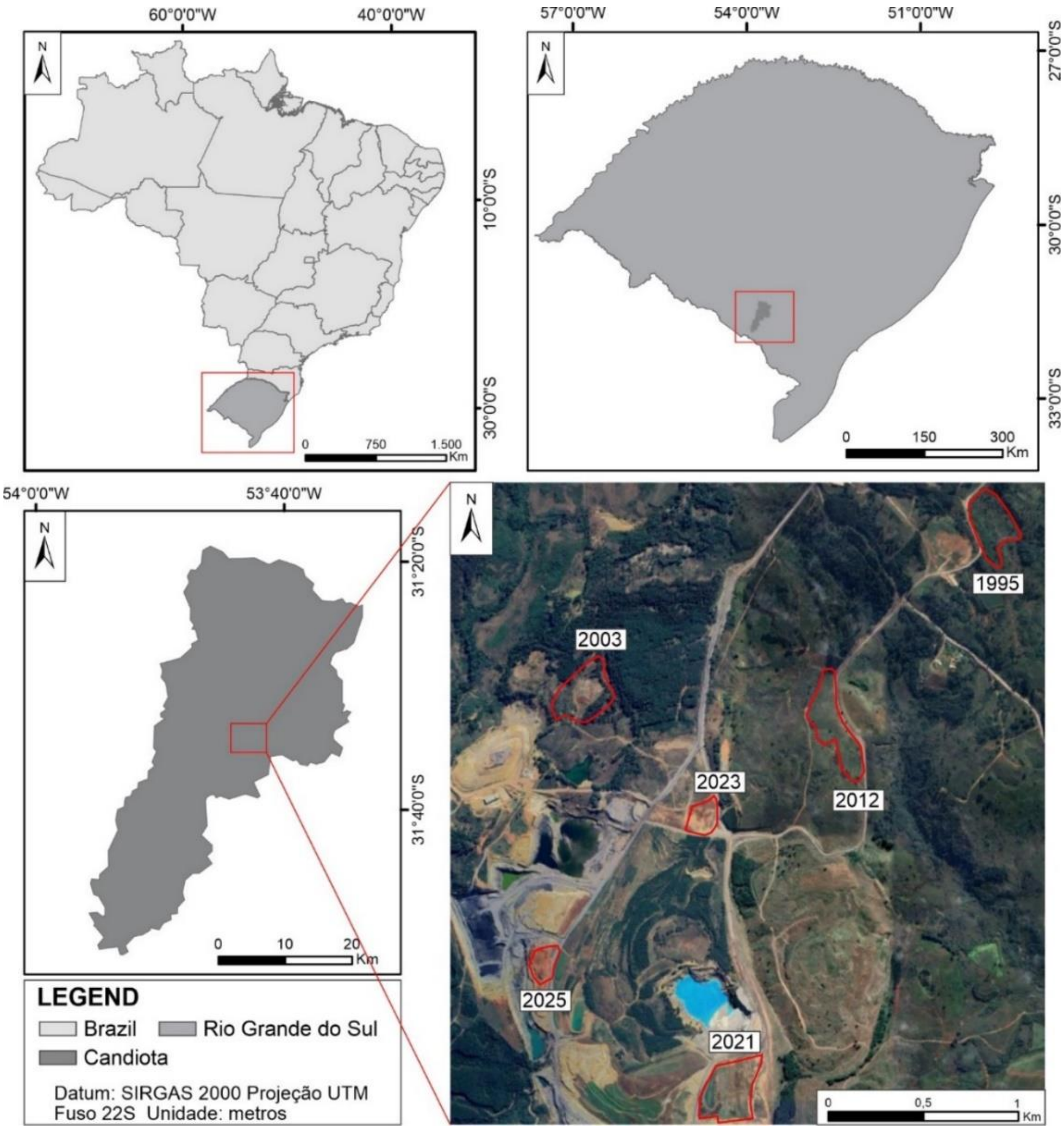
Within this context, the objective of this study was to test the sensitivity of β-glucosidase and arylsulfatase enzymes, as well as POxC content, as indicators of restoration evolution along a chronosequence of minesoil revegetated for three-decades, linking them to soil acidity and fertility attributes. We hypothesize that minesoil health improves with restoration time under natural revegetation indicated by increase in β-glucosidase and arylsulfatase activities and POxC content.

1.2. **Materials e Methods**

1.2.1. Study area characterization

The study was conducted at the Candiota coal mine, located in southern Brazil, along a minesoil chronosequence with up to 30 years of restoration (Figure 1). The climate is classified as humid subtropical (Cfa), with a mean annual air temperature of 17 °C and a mean annual precipitation of 1,400 mm, characterized by cold winters and hot summers (Alvares et al., 2013).

Figure 1. Location of minesoils created in 2025, 2023, 2021, 2012, 2003, 1995 at the Candiota coal mine, southern Brazil forming a chronosequence of minesoils with <1, 2, 4, 13, 22 and 30 years, respectively.



Source: Created by the author (2025)

Restoration at the Candiota mine involves topographic reconstruction of the landscape by covering the mining pit with a thick layer of rock fragments and waste materials generated during coal extraction, referred to as the overburden layer (Albert et al., 2022). Thereafter, the overburden layer is covered by a soil layer derived from the soil horizons removed from the mining front, known as topsoil (Stumpf et al., 2016). This is followed by the establishment of vegetation on the minesoils. The topsoil used in topographic reconstruction is derived from the B horizon of a Rhodic Lixisol (IUSS

Working Group WRB, 2015), the predominant soil type at the Candiota Coal Mine (Stumpf et al., 2016; Fernandez et al., 2025).

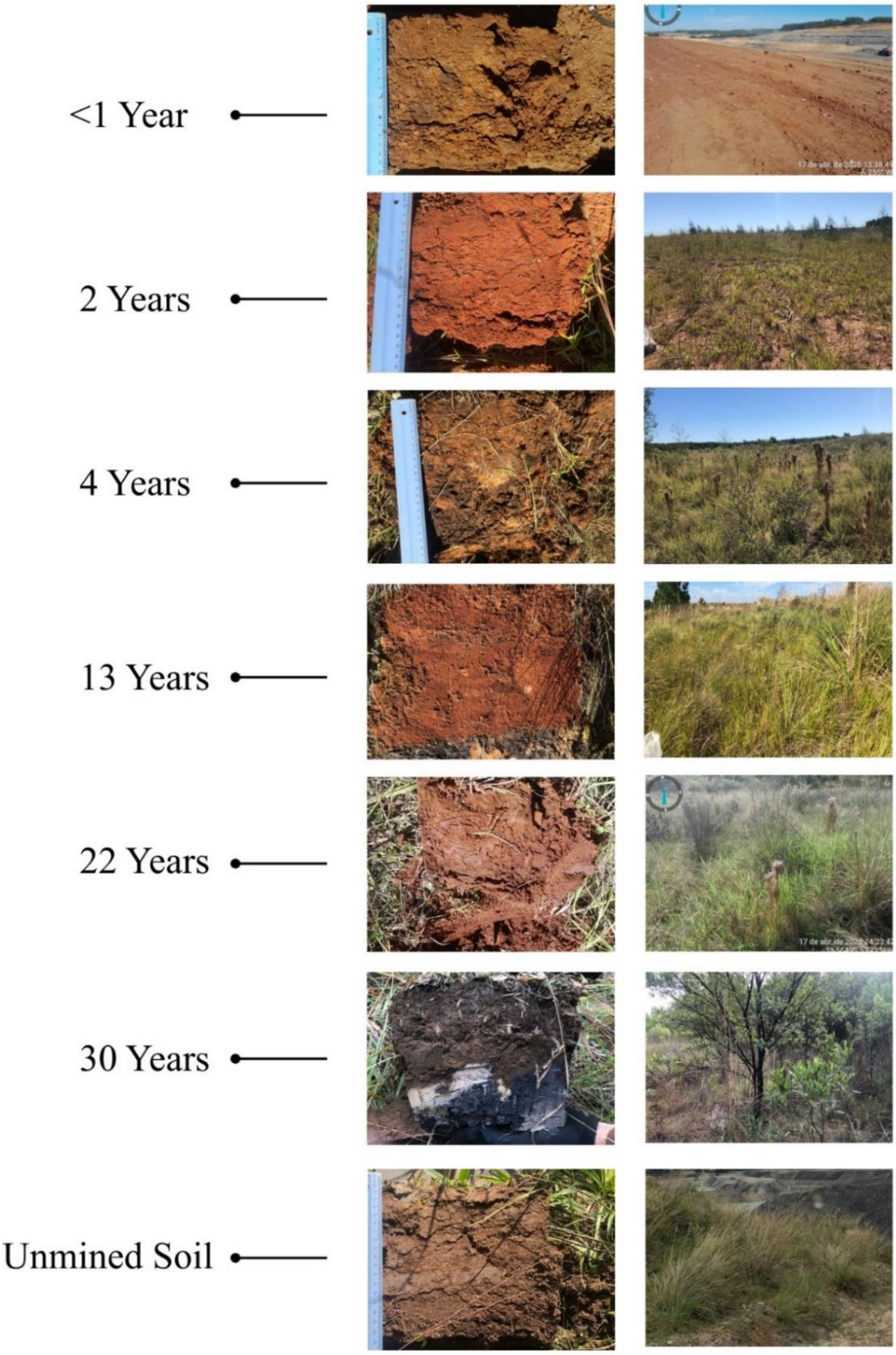
The areas selected for this study did not receive agronomic management (liming, fertilization, or subsoiling) by the mining company and have been subjected to natural revegetation since 2025, 2023, 2021, 2012, 2003 and 1995, with a predominance of grass species characteristic of the Pampa Biome originating from the topsoil seed bank. Exceptionally, part of the area restored in 2003 served as a temporary limestone stockpile in mid-2002. Therefore, the minesoils form a restoration chronosequence with different stages of vegetation cover, comprising <1, 2, 4, 13, 22 and 30 year(s) since the establishment of natural vegetation in the area (Figure 2).

Unmined soil under native vegetation, located near the mining pit, was selected as the reference area (Figure 2). Vegetation cover intensity and the botanical description of the predominant species in each minesoil and in the unmined soil, based on vegetative characteristics at the family or functional group level (Gonçalves and Lorenzi, 2011; Silva Junior et al., 2023), are presented below. Species identification was limited by the absence of plant reproductive structures due to the sampling period (December 2025):

a) Minesoil with 30 years of restoration (Figure 2): Vegetation covered the entire sampled area and was dominated by pioneer species, including *Asteraceae* (*Baccharis* sp.), tufted *Poaceae*, and terrestrial *Bromeliaceae*, as well as *pteridophytes* (*Pteridium* sp.) and juvenile woody individuals (*Pinus* sp., *Myrsine* sp., and *Dodonaea* sp.). The floristic assemblage exhibited herbaceous to subshrub growth forms, associated with fasciculate root systems.

b) Minesoil with 22 years of restoration (Figure 2): Vegetation covered the entire sampled area and consisted of perennial tufted grasses (*Poaceae*), terrestrial bromeliads (*Bromeliaceae*), and pioneer *Asteraceae* species (*Baccharis* sp. and herbaceous *Asteraceae*), in addition to juvenile woody individuals (*Myrsine* sp.). During this period, vegetation was predominantly herbaceous to subshrubby, with fasciculate root system architecture.

Figure 2. Vegetation cover and minesoil profile along a 30-year natural restoration chronosequence.



Source: Created by the author (2025)

c) Minesoil with 13 years of restoration (Figure 2): Vegetation covered the entire sampled area and was composed of perennial herbaceous and subshrub species, mainly represented by *Asteraceae* (*Baccharis* sp.), perennial tufted grasses (*Poaceae*), and terrestrial bromeliads (*Bromeliaceae*). Vegetation structure remained dominated by herbaceous to subshrub growth forms, associated with fasciculate root systems.

d) Minesoil with 4 years of restoration: It was not possible to collect predominant species due to adverse weather conditions. Nevertheless, vegetation covered the entire area and visually resembled the dominant species observed in the minesoil with 2 years of restoration.

e) Minesoil with 2 years of restoration (Figure 2): Vegetation did not uniformly cover the area and was dominated by perennial tufted grasses (*Poaceae*), associated with herbaceous to subshrub *Asteraceae* and prostrate subshrub dicotyledonous species. At this stage, herbaceous subshrub growth forms predominant, with root systems concentrated in the surface soil layer and a high density of fine roots.

f) Minesoil with less than 1 year of restoration (Figure 2): Vegetation cover was incipient. However, the few species present in the sampled area included pioneer and opportunistic species, represented by *Asteraceae* (*Baccharis* sp.), Poaceae with prostrate to decumbent growth forms (likely *Paspalum* sp.), a spiny *Solanaceae* (*Solanum sisymbriifolium*), and a juvenile shrub individual of *Primulaceae* (*Myrsine* sp.). The floristic assemblage consisted mainly of herbaceous to subshrub species, with occasional young shrubs, whose root systems exhibited clearly identifiable fasciculate or taproot architectures and were still in the establishment phase.

g) Unmined soil (Figure 2): Vegetation composition was dominated by herbaceous *Asteraceae* (e.g., *Conyza* sp. and other basal rosette forms), associated with perennial tufted *Poaceae* and *Cyperaceae*. Plants exhibited predominantly herbaceous growth forms, with root systems of defined architecture, characterized by taproots in Asteraceae and fasciculate roots in *Poaceae* and *Cyperaceae.*

1.2.2. Sample collection and analysis

In April 2025, a grid of five georeferenced sampling points was established in each area of the chronosequence and in one unmined area, with a mean distance of 75.16 m between points. At each point, soil samples were collected from the 0.00–0.10 m layer using a shovel. The samples were air-dried at room temperature after gentle disaggregation and subsequently sieved through a 2 mm mesh for further analyses. Soil texture across the minesoil chronosequence ranged from 34.7 to 51.7% clay, 37.8 to 48.1% sand, and 7.9 to 17.8% silt (Table 1).

Table 1**.** Particle-size distribution in the 0.00–0.10 m soil layer of the 30-year minesoil restoration chronosequence and the unmined soil under native vegetation.

Time restoration	Clay	Sand	Silt
	----------------------------------g kg^{-1}	--	
< 1 yr	51.7 ± 4.9	40.4 ± 4.8	7.9 ± 0.9
2 yrs	43.0 ± 4.7	48.0 ± 6.3	8.9 ± 2.9
4 yrs	41.4 ± 9.8	38.5 ± 2.7	20.1 ± 5.2
13 yrs	44.4 ± 7.4	37.8 ± 7.0	17.8 ± 4.7
22 yrs	43.1 ± 5.3	39.1 ± 1.9	17.8 ± 10.9
30 yrs	34.7 ± 6.2	48.1 ± 9.6	17.2 ± 10.6
Unmined Soil	29.3 ± 3.4	48.6 ± 6.6	22.1 ± 3.3

Source: Created by the author (2025)

β-glucosidase and arylsulfatase enzymatic activity

Soil samples for β-glucosidase and arylsulfatase determination were prepared according to the FERTBIO soil sample concept, following the protocol established by Mendes et al. (2019). Soil samples were air-dried, gently disaggregated, and sieved through a 2-mm mesh. This preparation facilitates routine analyses, reduces variability among subsamples, and minimizes the need for refrigeration during storage of moist soils. Although this procedure may reduce enzyme contents, the literature indicates that treatment ranking and the relationships between SOC and microbial indicators remain consistent compared with samples analyzed under field-moist conditions (Mendes et al., 2019). The samples were stored at room temperature, and the analyses were completed within one year after air-drying. β-glucosidase and arylsulfatase activities were determined in duplicate, with one control included for each assay (Tabatabai, 1994). Because the incubation period was short (1 h), toluene was not used in the assays.

β-glucosidase activity was determined according to the methodology proposed by Tabatabai (1994), using *p-nitrophenyl-D-glucopyranoside* (PNG) as the substrate. Arylsulfatase activity was determined following the method proposed by Tabatabai and Bremner (1970), using *p-nitrophenyl sulfate* (PNS) as the substrate. Absorbance readings were obtained using a *Metash* spectrophotometer (UV-5100 UV-VIS Spectrophotometer, no. AE2302076) at wavelengths of 410 and 420 nm for *arylsulfatase* and *β-glucosidase*, respectively. For the calculation of enzymatic activity, a standard curve was constructed for each enzyme, resulting in the following regression equation 1 for β-glucosidase, and equation 2 for arylsulfatase.

$$y = 103.85x - 1.1018 \quad (1)$$

$$y = 82.907x + 0.176 \quad (2)$$

In these equations, x corresponds to the absorbance value measured by the spectrophotometer. Based on the sample mass (approximately 1 g of dry soil), enzyme activity was calculated as the concentration of p-nitrophenol (μg p-nitrophenol per gram of dry soil), after subtracting the control values from each replicate. Mean values, standard deviations, and coefficients of variation (CV) were calculated using the replicate values corrected for the controls. The CV was calculated as equation 3. Enzymatic activity is expressed as μg of p-nitrophenol produced per hour per gram of soil (μg p-nitrophenol h^{-1} g^{-1} soil).

$$CV = \left(\frac{standard\ deviation}{mean} \right) \times 100 \cdot \quad (3)$$

1.2.3. POxC, TOC and soil chemical attributes

The POxC content was determined according to the method described by Weil et al. (2003) and the protocol adapted by Culman et al. (2014). Briefly, an equivalent of 2.5 g of oven-dry soil (< 2 mm) from each sample was placed in a 50 mL Falcon tube and shaken at 240 rpm for 2 minutes with 18.0 mL of deionized water and 2.0 mL of 0.2 M potassium permanganate ($KMnO_4$), resulting in a soil:solution ratio of 1:8 (w/v). After shaking, the soil suspension was allowed to settle in the dark for 10 minutes. Subsequently, 0.5 mL of the supernatant was diluted in 49.5 mL of deionized water in Falcon tubes. The absorbance of the diluted solution was measured at 550 nm using a spectrophotometer. The absorbance of four $KMnO_4$ standard solutions was measured to generate a standard curve, and sample absorbance was converted to POxC using the equation proposed by Weil et al. (2003).

Total organic carbon (TOC) contents were determined by method (Walkley-Black), following the procedures described by Mendonça and Matos (2017). The TOC values were multiplied by 1.724 to estimate soil organic matter (SOM) content assuming that SOM contains 58% of carbon on average (Stockmann et al., 2013). The SOM levels were classified as low (≤ 2.5%), intermediate (2.6–5.0%), or high (> 5.0%), according to the official regional guidelines for soil fertilization and liming (CQFS, 2016).

Soil acidity and fertility attributes were determined following the methodology described by Teixeira et al. (2017). Soil pH was measured in water at a 1:1 ratio. Available soil phosphorus (P) was determined using the *Mehlich*$^{-1}$ method, with an extracting solution composed of 0.05 mol L^{-1} HCl and 0.0125 mol L^{-1} H_2SO_4 and quantified by *Metash UV–Vis spectrophotometry* at 660 nm using a standard curve prepared in the extracting solution. Results were expressed as mg kg^{-1} of available P. Exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with 1 mol L^{-1} KCl. Ca^{2+} and Mg^{2+} were analyzed by atomic absorption spectrophotometry, and Al^{3+} by titration with NaOH. Available K^+ was determined using the *Mehlich*$^{-1}$ method and analyzed by flame photometry. Potential acidity ($H^+ + Al^{3+}$) was extracted with calcium acetate and determined by titration with NaOH. The sum of bases (SB) was calculated as equation 4 and used to calculate cation exchange capacity (CEC). CEC at pH 7.0 was calculated as equation 5 Effective CEC (CECe) was calculated as equation 6 and used to calculate aluminum saturation (M%). Base saturation (BS%) was calculated as equation 7. And aluminum saturation was calculated as equation 9.

$$SB = Ca^{2+} + Mg^{2+} + K^{+} + Na^{+} \qquad (4)$$

$$CEC = SB + \left(Al^{3+} + H^{+} \right) \qquad (5)$$

$$CEC_e = SB + Al^{3+} \qquad (6)$$

$$BS(\%) = \frac{100 \times SB}{CEC} \qquad (7)$$

$$M\% = \frac{100 \times Al^{3+}}{(CEC_e)} \qquad (8)$$

1.2.4. Assessment of minesoil health based on the four-quadrant model

Chaer et al. (2023) proposed the assessment of agricultural soil health using a four-quadrant model that considers the mean enzymatic activity of two enzymes in relation to TOC content, generating the average specific enzymatic activity of β-glucosidase and arylsulfatase (ASEA), expressed as µg PNP g-1 TOC h-1. In this study, the four-quadrant model adopted TOC and ASEA threshold values of 15.5 g kg-1 and 4.3 µg PNP g-1 TOC h-1, respectively, considering the high clay contents observed in the minesoils. Within this framework, soil health is classified into four quadrants: (i) an equilibrium state with High soil quality, characterized by high biological activity and high TOC content; (ii) a transitional state with Soil losing quality, characterized by low biological activity and a tendency toward TOC loss; (iii) an equilibrium state with Low soil quality, characterized by low biological activity and low TOC content; and (iv) a transitional state with Improving soil quality, characterized by high biological activity and a tendency toward increasing TOC (Chaer et al., 2023).

1.2.5. Statistical analysis

To evaluate the effects of soil texture (clay content percentage) on TOC, arylsulfatase, and β-glucosidase contents, linear regression analysis was performed. Correlation coefficients, coefficients of determination (R^2), regression equations, and model significance were obtained using RStudio software.

Temporal trends were evaluated using simple linear regression, modeling each response variable as a function of restoration time (Years) using *lm()* function in *RStudio 2025.09.2* (R version 4.4.1), following the equation 9.

$$Y=\beta_0+\left(\beta_1\times X\right)+\varepsilon \quad (9)$$

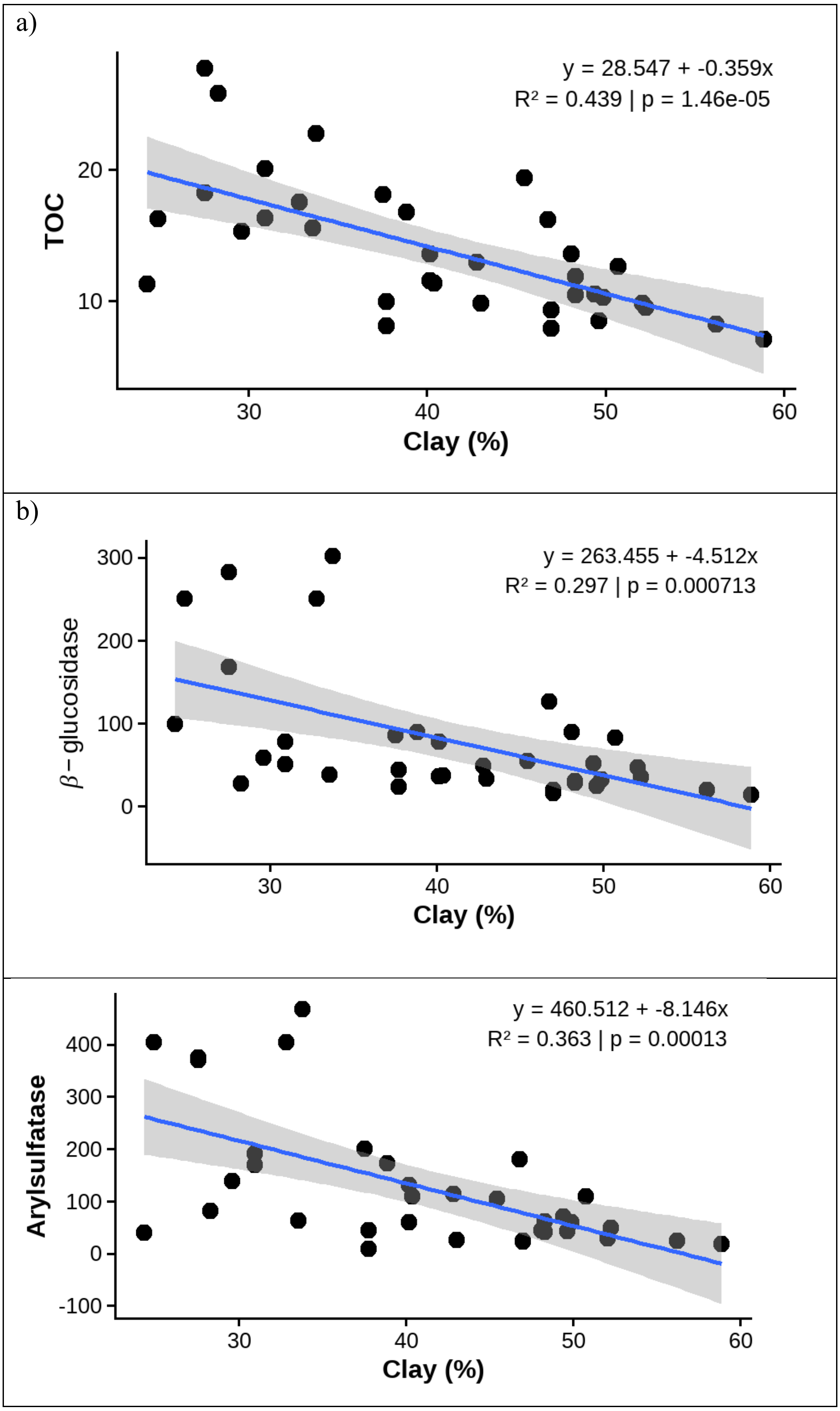
Where Y represents the response variable (arylsulfatase, β-glucosidase, permanganate-oxidizable carbon (POxC), total organic carbon (TOC), pH (H_2O), phosphuros, aluminium saturation (M%) and base saturarion (BS%), X is the restoration time in years, β_0 the intercept, β_1 the slope coefficient, and ε the random error. Model fit quality was assessed using the coefficient (R^2) and the significance of the slope coefficient (t-test, $\alpha = 0.05$). To contextualize the values observed along the chronosequence, the mean and standard deviation of the unmined soil were calculated and graphically represented as a ±1 standard deviation band overlaid on the regression plots. Graphs were generated using the ggplot2 package.

In addition, a principal component analysis (PCA) was performed to identify the main correlations between enzymatic activity and soil chemical attributes related to acidity and fertility in the minesoils, as well as their similarities with the unmined soil after 30 years of restoration. Principal component analysis (PCA) was performed in RStudio 2025.09.2 (R version 4.4.1) using a correlation matrix with automatic standardization. Standardization included mean-centering by subtracting the variable mean and scaling by dividing by the standard deviation, resulting in variables with mean 0 and variance 1. The variables included in the analysis were β-glucosidase, arylsulfatase, BS%, M%, soil pH, phosphorus, organic matter, and POxC.

1.3. **Results**

Linear regression was used to assess the effect of soil texture (clay content) on TOC and the activities of both enzymes (Figure 3). Because the observed relationships were weak and negative, soil texture does not appear to be a major driver of the dynamics of these variables in the evaluated minesoils.

Figure 3 Relationship between clay and total organic carbon (TOC) (a) *β-glucosidase* (b) and *Arylsulfatase* (c) activity along a 30-year minesoil restoration chronosequence under natural revegetation and in an unmined soil (red dashed line average value and highlighted area standard deviation).



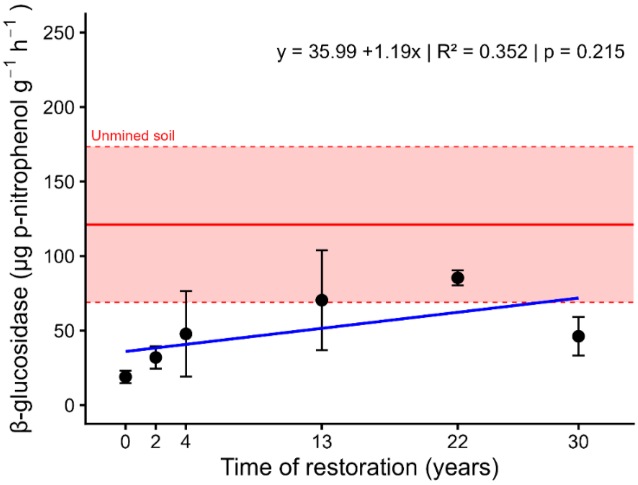
Source: Created by the author (2025)

1.3.1. β-glucosidase and arylsulfatase activity, and POxC and TOC contents

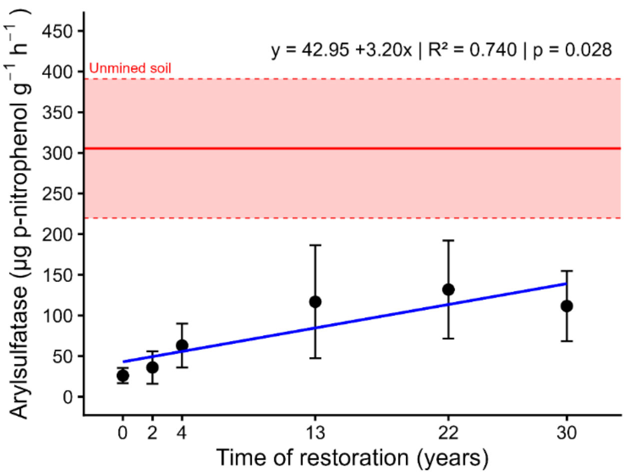
β-glucosidase activity ranged, on average, from 19 to 85 μg *p*-nitrophenol g^{-1} soil h^{-1} across the minesoil chronosequence, but it did not show a statistically significant relationship with restoration age (R^2 = 0.352; p = 0.215) (Figure 4a). Mean β-glucosidase activity remained below 100 μg *p*-nitrophenol g^{-1} soil h^{-1} in all minesoils, whereas the unmined soil reached 121 μg *p*-nitrophenol g^{-1} soil h^{-1} (Figure 4a). Among the restored areas, the 22-year minesoil showed the closest value to the unmined soil, with 85.4 μg *p*-nitrophenol g^{-1} soil h^{-1}, and also exhibited the largest increase (349 %) relative to the youngest minesoil (<1 year). With respect to arylsulfatase activity in minesoils, it ranged, on average, from 26 to 132 μg *p*-nitrophenol g^{-1} soil h^{-1}, showing a strong and significant linear relationship with restoration age (R^2 = 0.74; p = 0.028) (Figure 4b) contrasting with β-glucosidase (Figure 4a). Notably, arylsulfatase activity exceeded 100 μg *p*-nitrophenol g^{-1} soil h^{-1} in minesoils with 4, 13, 22, and 30 years of restoration, whereas younger minesoils exhibited activities ranging from 24 to 61 μg *p*-nitrophenol g^{-1} soil h^{-1} (Figure 4b). Nevertheless, all minesoils showed arylsulfatase activity markedly lower than those observed in the unmined soil (305.4 μg *p*-nitrophenol g^{-1} soil h^{-1}) (Figure 4b). Minesoils with 2 years and <1 year of restoration exhibited activities that were 88.2% and 91.5% lower, respectively, than that of the unmined soil (Figure 4b). The minesoil under 22 years of restoration also exhibited the greatest increase in arylsulfatase activity (407 %) compared with the youngest minesoil (<1 year of restoration).

Figure 4. Relationship between restoration time and the activity of *β-glucosidase* (a) and *arylsulfatase* (b) along a 30-year minesoil restoration chronosequence and in an unmined soil under natural revegetation (red dashed line average value and highlighted area standard deviation).

a)



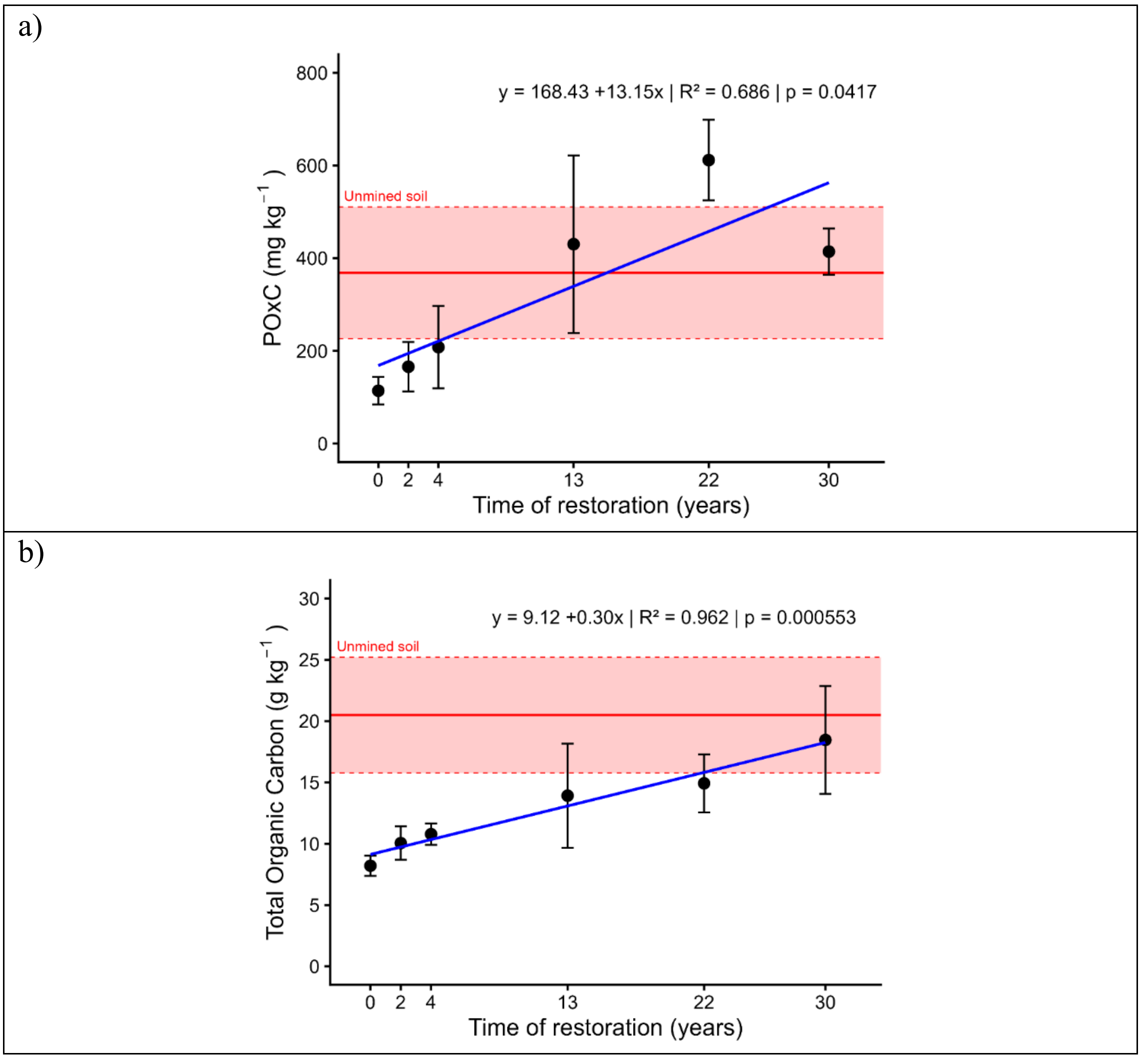
b)



Source: Created by the author (2025)

POxC contents ranged, on average, from 114 to 612 mg kg^{-1} along the minesoil chronosequence, with higher values strongly associated with restoration age (R^2 = 0.686; p = 0,0417) (Figure 5a). Notably, minesoils with 13, 22, and 30 years of restoration (430, 612, and 414 mg kg^{-1}, respectively) exceeded the mean POxC content observed in the unmined soil (369 mg kg^{-1}). In contrast, minesoils with 4, 2, and <1 year(s) of restoration exhibited mean POxC contents of 208, 166, and 114 mg kg^{-1}. Minesoils with 13, 22, and 30 years of restoration exhibited increases of 277.3%, 436.4%, and 263.3%, respectively, in POxC contents relative to the youngest minesoil (<1 year) (Figure 5a). The TOC content of minesoils significantly and linearly increased with restoration age (R^2 = 0.962; p = 0.000553) (Figure 5b). This temporal effect is evident when comparing TOC contents in the longer-restored minesoils. Between <1 and 4 years of restoration, the TOC increased by 31.5%. Between 13 and 22 years of restoration, the TOC increased by 18.5%, and between 22 and 30 years, it increased by 17.8%. Between <1 and 30 years of restoration the TOC increased by 114.3%. However, all minesoil TOC contents were lower than those observed in the unmined soil (20.5 g kg^{-1}) (Figure 5b).

Figure 5. Relationship between restoration time and permanganate-oxidizable carbon (POxC) (a) and total organic carbon (TOC) (b) contents along a 30-year minesoil restoration chronosequence under natural revegetation and in an unmined soil (red dashed line average value and highlighted area standard deviation).

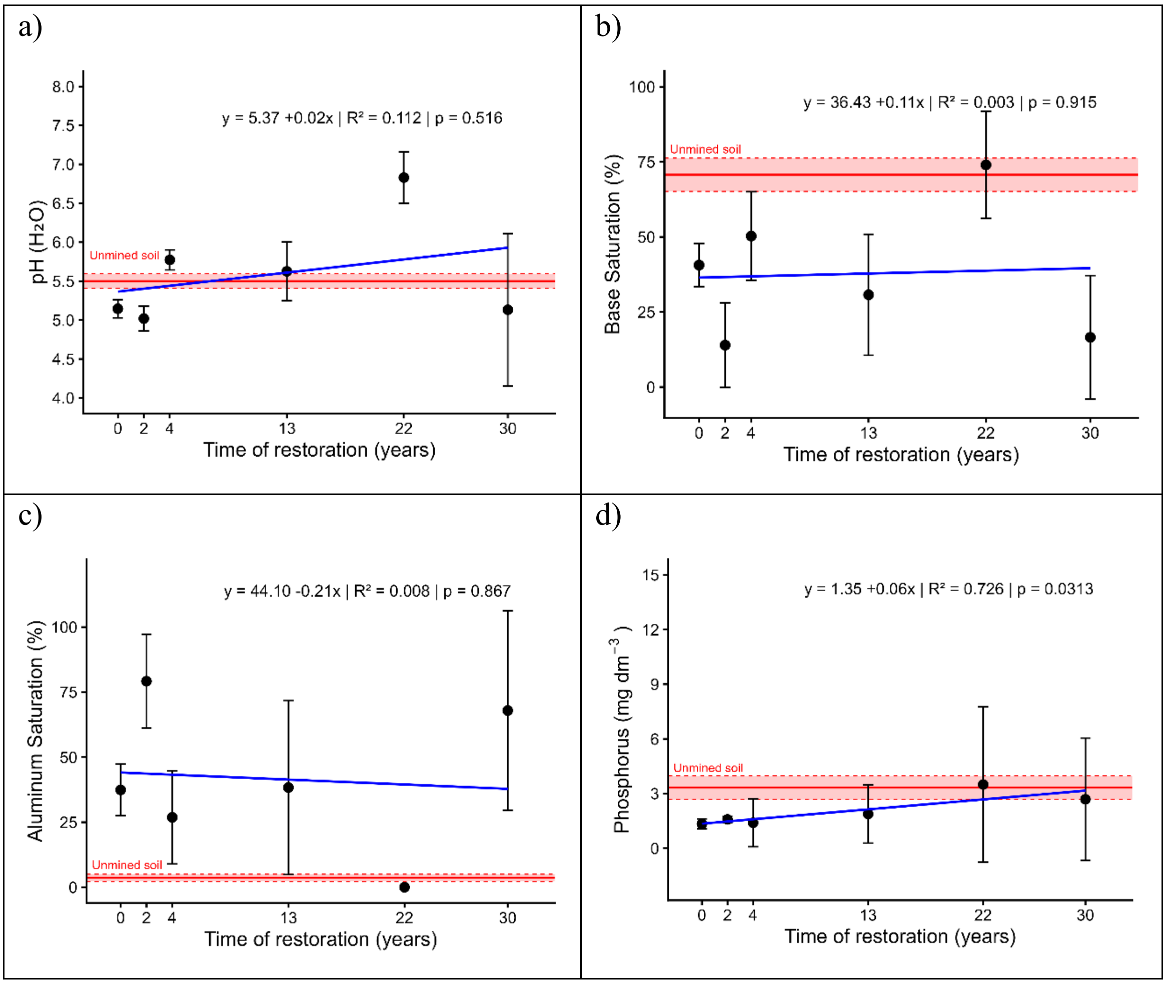


Source: Created by the author (2025)

1.3.2. Soil acidity and fertility

Soil pH (R^2 = 0.112; p = 0.516; Figure 6a), base saturation (R^2 = 0.003; p = 0.915; Figure 6b), and aluminum saturation (R^2 = 0.008; p = 0.867; Figure 6c) did not show significant relationships with restoration time. In contrast, phosphorus content was significantly related to restoration time (R^2 = 0.726; p = 0.0313; Figure 6d).

Figure 6: Relationship between restauration time and soil pH (a), base saturation (b) and aluminium saturation (c), and phosphorus content (d) along a 30-year minesoil restoration chronosequence under natural revegetation and in an unmined soil (red dashed line average value and highlighted area standard deviation).



Source: Created by the author (2026)

Compared with the unmined soil, the 22-year minesoil showed markedly higher mean soil pH, reaching values close to 7.0 (Figure 6a). Consequently, it was the only area in the chronosequence with base saturation above 70% (Figure 6b), while aluminum saturation remained below 10% (Figure 6c), both values being close to the mean observed in the unmined soil.

According to Comissão de Química e Fertilidade do Solo - RS/SC (CQFS, 2016), no reference pH value is established for soils under natural grassland, which is the predominant vegetation cover in both the unmined soil and the minesoils. Therefore, decision-making for the assessment of soil acidity and the consequent risk of Al^{3+} toxicity is based on base saturation (BS%), with critical conditions considered when BS% ≤ 40%. In this context, the unmined soil and the minesoils with 22, 4, and <1 year(s) of restoration exhibited adequate acidity conditions (BS% > 40%). In contrast, minesoils with 30, 13, and 2 years of restoration showed BS values of 16, 31, and 14%, respectively, along with critical M% values of 68, 38, and 79%, respectively (Table 2).

Table 2. Soil acidity and fertility attributes in the 0.00–0.10 m layer of a 30-year minesoil restoration chronosequence under natural revegetation and in an unmined soil under native vegetation.

Time restoration	pH	Ca	Mg	K	P	SOM[1]	BS[2]	M[3]
	H_2O	----$cmol_c$ dm^{-3}-----		-------mg dm^{-3}-------		-----------%------------		
< 1 yr	5.1±0.1	4.1±1.2	0.0±0.0	37.6±16.5	1.3±0.3	1.4±0.1	41±7	37±10
2 yrs	5.0±0.2	0.5±0.9	0.1±0.3	68.4±8.0	1.6±0.2	1.7±0.2	14±14	79±18
4 yrs	5.8±0.1	2.4±1.3	1.0±0.8	129.9±80.7	1.4±1.3	1.9±0.1	50±15	27±18
13 yrs	5.6±0.4	1.2±0.9	0.1±0.2	74.2±12.2	1.9±1.6	2.4±0.7	31±20	38±33
22 yrs	6.8±0.3	3.1±0.7	1.1±0.4	89.5±17.2	3.5±4.2	2.6±0.4	74±18	0±0
30 yrs	5.1±1.0	1.0±1.6	0.0±0.0	78.5 ±22.3	2.7±3.3	3.2±0.8	16±20	68±38
Unmined soil	5.5±0.1	14.5±6.9	0.1±0.1	103.8± 22.5	3.3±0.6	3.5±0.8	71±6	3±1

[1] soil organic matter; [2] base saturation; [3] aluminum saturation. Source: Created by the author (2026)

The exchangeable Ca^{2+} content ranged from 0.5 to 4.1 cmolc dm^{-3} among the minesoils, whereas the unmined soil exhibited 14.5 cmolc dm^{-3}. According to CQFS (2016), minesoils with 2, 13, and 30 years of restoration show low Ca availability (<2.0 cmolc dm^{-3}). In contrast, minesoils restored for 22, 2, and <1 year exhibit medium Ca^{2+}

availability (between 2 and 4 cmolc dm^{-3}). Only the unmined soil showed high Ca^{2+} availability, with concentrations well above the threshold of 4 cmolc dm^{-3} (Table 2).

The Mg^{2+} contents presented in Table 2 indicate that minesoils restored for 22 years (1.09 cmolc dm^{-3}) and 4 years (1.04 cmolc dm^{-3}) have high availability (>1.0 cmolc dm^{-3}), whereas the remaining minesoils and the unmined soil show low availability (<0.5 cmolc dm^{-3}), according to CQFS (2016).

Regarding K^+ contents, the unmined soil (104 mg dm^{-3}) and minesoils with 30 (78 mg dm^{-3}), 22 (89 mg dm^{-3}), 13 (74 mg dm^{-3}), and 2 years of restoration (68 mg dm^{-3}) were classified as having high availability (46–90 mg dm^{-3}), according to CQFS (2016). In contrast, minesoils restored for 4 years (129 mg dm^{-3}) and <1 year (38 mg dm^{-3}) were classified as having very high and low K^+ availability, respectively (Table 2).

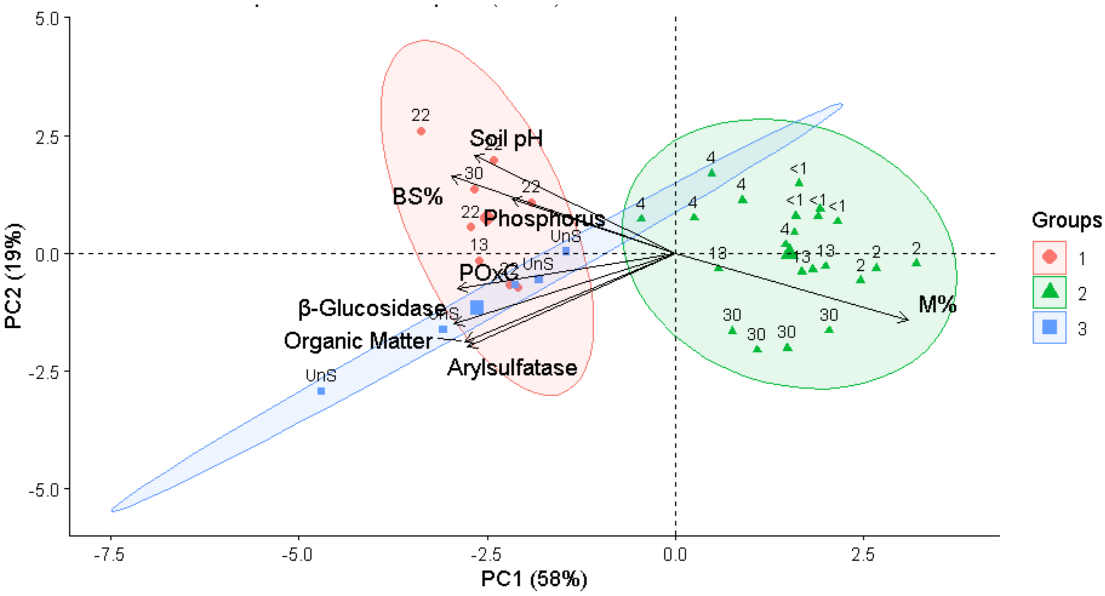
Phosphorus contents in the unmined soil (3.3 mg dm^{-3}) and in the minesoil with 22 years of restoration (3.5 mg dm^{-3}) were classified as low. In turn, minesoils with 30 years (2.7 mg dm^{-3}), 13 years (1.9 mg dm^{-3}), 4 years (1.4 mg dm^{-3}), 2 years (1.6 mg dm^{-3}), and <1 year (1.3 mg dm^{-3}) of restoration were classified as having very low P availability (CQFS, 2016).

Table 2 also shows that soil organic matter (SOM) contents in minesoils restored for 30 and 22 years and in the unmined soil ranged from 2.6 to 3.5%, being classified as medium SOM availability (2.6–5.0%). In contrast, the minesoil with 13 years of restoration and the younger minesoils (<1 to 4 years of restoration) exhibited low SOM contents (<2.5%), according to CQFS (2016).

1.3.3. Correlation between enzymatic activity, soil acidity, and fertility

The PCA components 1 and 2 explained 58% and 19% of the total variance of the dataset, respectively (Figure 7). The PCA highlighted clear differences between the evaluated minesoils and the unmined soil according to their biological and chemical attributes.

Figure 7. Principal component analysis of arylsulfatase and β-glucosidase enzymatic activities, soil organic matter, permanganate-oxidizable carbon (POxC), Phosphuros, base saturation (BS%), and aluminum saturation (M%) in a chronosequence of minesoils and an unmined soil under natural revegetation.



Source: Created by the author (2026)

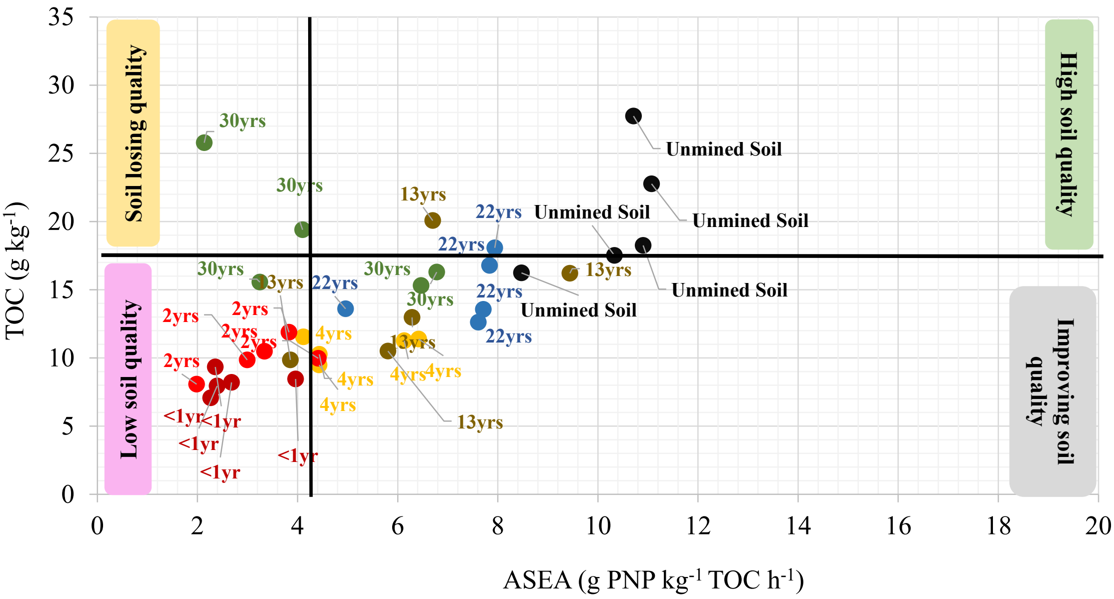
The unmined soil (Group 3) was predominantly associated with β-glucosidase, arylsulfatase, POxC, and soil organic matter, indicating greater biological activity, higher labile carbon content, and increased organic matter accumulation in these areas. The close association among these variables suggests strong interactions between soil organic matter dynamics and microbial functioning under more established vegetation conditions. Group 1, composed mainly of the minesoil with 22 years of restoration, occupied an intermediate position in the ordination space and was more closely associated with phosphorus, soil pH, and base saturation (BS%), reflecting the importance of soil fertility attributes in the recovery process of these minesoils. In contrast, Group 2, which included the oldest minesoil (30 years of restoration) together with the youngest minesoils, was more strongly associated with aluminum saturation (M%), indicating a greater influence of soil acidity-related constraints on the distribution of these samples. The clustering of the 30-year-old minesoil with the younger restored areas suggests that, despite the longer restoration period, some chemical limitations associated with soil acidity persist. Furthermore, the opposite orientation of M% relative to the biological variables suggests a negative association between M% and soil biological quality (Figure 7).

1.3.4. Four-quadrant model for assessing the health of minesoils and the unmined soil under natural revegetation

All replicates of the minesoils restored for 2 and <1 year were plotted in the “low soil quality” quadrant (TOC < 15.5 g kg^{-1} and ASEA < 4.3 g PNP kg^{-1} TOC h^{-1}) (Figure 8). In contrast, minesoils restored for 4, 13, and 22 years had most of their replicates ordinated in the quadrant indicating “improving soil quality”, with TOC < 15.5 g kg^{-1} but ASEA > 4.3 g PNP kg^{-1} TOC h^{-1}.Interestingly, the minesoil restored for 30 years was the only one that did not show a consistent pattern, with its replicates displayed across quadrants indicating soil loosing quality, low quality and improving quality (Figure 8).

Finally, the unmined soil was clearly classified with the status of healthy soil, i.e., with TOC > 15.5 g kg^{-1} and ASEA > 4.3 g PNP kg^{-1} TOC h^{-1} (Figure 8).

Figure 8. Four-quadrant model relating total organic carbon (TOC) and the mean specific activity of the enzymes β-glucosidase and arylsulfatase in a chronosequence of minesoils and an unmined soil under natural revegetation.



Source: Created by the author (2026)

1.4. Discussion

Mostly arylsulfatase activity was sensitive in capturing the effect of restoration time in the minesoils (Figure 4b), suggesting that recovery of the sulfur cycle enzyme was more strongly associated than the carbon cycle (β-glucosidase) along the 30-year chronosequence. Although β-glucosidase did not show a positive relationship with restoration time (Figure 4a), the PCA indicated that this enzyme was more closely associated with organic matter and the unmined soil (Figure 7), suggesting greater dependence on carbon quality and a slower recovery process. Nevertheless, both arylsulfatase and β-glucosidase activities remained much lower than those observed in the unmined soil (Figure 4a–b), indicating limited biota recovery in soils strongly disturbed by mining even after revegetation has been established in the oldest areas (Figure 2). The microbial community is primarily responsible for producing these enzymes in soil, although vegetation also contributes through root exudation and biomass decomposition (Bharadwaj et al., 2025; Chen et al., 2022).

From a vegetation perspective, the minesoils restored for 13, 22, and 30 years showed large ground cover, mainly by species of the families *Asteraceae*, *Bromeliaceae*, and *Poaceae*. In the 30-year-old minesoil, juvenile woody individuals (*Pinus sp*., *Myrsine sp*., and *Dodonaea sp*.) were also present and dominated the area (see Materials and Methods). However, despite the well-established vegetation cover, the mean β-glucosidase and arylsulfatase activities in the 30-year-old minesoil were lower (46.2 and 112 µg p-nitrophenol g $soil^{-1}$ h^{-1}, respectively). This result does not corroborate the findings of Pajak et al. (2018), who reported a strong positive correlation between soil enzymatic activity (*dehydrogenase*, *urease*, and *β-glucosidase*) and vegetation biodiversity in a minesoil in Poland. This pattern suggests that vegetation establishment alone may not be sufficient to explain variations in enzymatic activity among restored minesoils.

The negative correlations observed for the minesoil with 30 years of restoration in the PCA, relative to enzymatic activity (Figure 7), may be associated, at least in part, with the high Al^{3+} content observed in this area (Table 2). This may be related to chemical conditions less favorable to the survival and activity of the microorganisms responsible for producing the investigated enzymes. Elevated Al^{3+} activity in soils is well known to exert toxic effects on microorganisms and plant roots (Mulazzani et al., 2024; Poschenrieder et al., 2008). In addition, the proximity of a thermoelectric power plant to the analyzed area may also be associated with potential heavy metal contamination, which may also be related to reduced microbial enzymatic activity in this soil type (Pajak et al., 2018). Pollution in the Candiota Mine is corroborated by Oliveira et al. (2026), who reported high concentrations of vanadium, barium, and chromium in a minesoil with 20 years of restoration, which were also associated with coal extraction and combustion activities typical of the mining sector, generating dust potentially associated with contamination in surrounding areas. Likewise, Uzarowicz et al. (2020) reported lower *arylsulfatase* and *β-glucosidase* activities in the A horizon of a 45-year-old minesoil under complete vegetation cover, which the authors associated with contamination by trace elements. Assessments of heavy metal contents in this chronosequence will be conducted in future studies to further evaluate their possible association with enzymatic activity results.

PCA also indicated that minesoil with 22 years of restoration was associated with positive correlations among both enzymes, POxC, organic matter, BS%, and soil pH, like the unmined soil (Figure 7). In agricultural soils, the positive relationship between enzymatic activity and soil fertility is well established and is commonly associated with the interaction of chemical and biochemical processes related to microbial activity and extracellular enzyme functioning in soil (Daunoras et al., 2024; Mól et al., 2023). *β-glucosidase* is produced predominantly by soil microorganisms and, to a lesser extent, by plant roots and decomposing plant residues; its primary function is to catalyze the final step of cellulose, cellobiose, and other β-glycoside degradation, releasing glucose, which is a major component of the POxC fraction and an important energy source for the soil microbiota (Daunoras et al., 2024; Bharadwaj et al., 2025). *Arylsulfatase* is also produced mainly by soil microorganisms and catalyzes the hydrolysis of sulfate esters, releasing sulfate anions that are essential for both the microbial community and vegetation (Chen et al., 2022; Klose et al., 2011; Slezack-Deschaumes et al., 2012).

The outstanding performance of the minesoil with 22 years of restoration in terms of enzymatic activity and fertility (Figure 7) suggest that restoration time alone may not be the main factor associated with the recovery of biological activity in highly anthropized mining areas, considering that the minesoil with the longest restoration period (30 years) showed a contrasting pattern relative to these correlations . This contrasting pattern may be associated with differences in the chemical characteristics of these minesoils: the former exhibited high pH (6.8) and BS (74%), and absence of M%, whereas the latter exhibited a pH close to 5.0, low BS (16%), and M% of nearly 70% (Table 2). These differences in chemical attributes may be related to the history of the minesoil constructed in 2003 (22 years minesoil), which, according to the mining company (*Companhia Riograndense de Mineração*), was used as a dolomitic limestone disposal site. Enzymatic activity, and consequently SOM decomposition, are commonly associated with less acidic pH conditions, in which enzyme structural stability may be better maintained and microbial diversity tends to be higher (Ezeokoli et al., 2020; Li et al., 2016; Xue et al., 2025; Wolińska and Stępniewski, 2012; Wu et al., 2017). Fernandez et al. (2026) reported that liming has a positive and long-term effect on minesoils under revegetation with perennial grasses, being associated with improved soil chemical conditions, higher biomass production, and greater root expansion down to 0.20 m, which coincided with more than a twofold increase in TOC in the surface layer between 8.6 and 20 years of restoration. Therefore, the less acidic pH and higher BS may be associated with more favorable conditions for bacterial communities and enzymatic activity in the minesoil with 22 years of restoration (Figure 7). Similar findings were reported by Uzarowicz et al. (2020), who observed positive correlations between soil pH and BS with arylsulfatase ($r = 0.43$ and $r = 0.41$, respectively), β-glucosidase ($r = 0.47$ and $r = 0.43$, respectively), and the microbial population dominated by *Bacillus* ($r = 0.61$ and $r = 0.47$, respectively) in a minesoil in Poland.

In addition to pH effects on microorganism activity, higher POxC contents may be associated with greater enzymatic activity in minesoils (Kowalska et al.2023), reflecting increased substrate availability for microorganisms, which may stimulate enzyme production and, consequently, SOM degradation, thereby increasing carbon levels in the soil (Bharadwaj et al., 2025). In this regard, the minesoils with 13, 22, and 30 years of restoration exhibited surprisingly higher mean POxC contents than the unmined soil (Figure 5a), suggesting a possible overcompensation of labile carbon pools. The positive correlation among POxC, TOC, and enzymatic activity, which occurred mainly in the minesoil with 22 years of restoration (Figure 7), was also associated with more favorable environmental conditions, such as lower acidity and higher fertility, which are commonly related to a larger fraction of active carbon and are associated with increased microbial and enzymatic activity in soils (Margenot et al., 2017; Tirol-Padre and Ladha, 2004; Xu et al., 2021b Weil et al., 2003). Greater availability of biologically active carbon may favor the production of hydrolytic enzymes such as *β-glucosidase* and *arylsulfatase*, as the decomposition of plant polysaccharides and organic sulfur compounds becomes energetically advantageous for microorganisms (Bharadwaj et al., 2025). In parallel, SOM itself contributes to enzyme protection and persistence in soil by favoring the formation of complexes among enzymes, humic compounds, and clay minerals (Deng and Tabatabai, 1997; Mendes et al., 2003, 2024), which may have been particularly facilitated by the considerable clay content of the minesoils (Table 1). The TOC content in the minesoils was about half of that of the reference area (Figure 5b) only partial recovery of long-term carbon pools. Together with POxC data, this suggests that in minesoils, labile carbon pools may recover more rapidly, whereas more stable pools require longer periods to be restored, even if clay content is considerable, and that enzymatic functions respond differently to the restoration process and time. Although carbon accumulation in minesoils is evident over the course of restoration (Figure 5b), the results suggest that full replenishment may require periods longer than 30 years.

In the four-quadrant model, the classification of minesoils with 2 and <1 year of restoration as *low soil quality* (Figure 8) was associated with incipient or absent vegetation cover (Figure 2), together with less favorable chemical conditions in the topsoil, such as high M%, as indicated by the PCA (Figure 7). Conversely, minesoils restored for 4, 13, and 22 years were predominantly distributed within the *improving soil quality* quadrant. This is noteworthy given the large differences in vegetation among these areas (Figure 2) and the PCA indicating unsuitable chemical conditions (high M%) for the minesoils with 4 and 13 years of restoration, whereas the minesoil with 22 years of restoration exhibited positive correlations between enzymatic activity and fertility (Figure 7). Furthermore, the minesoil restored for 30 years showed no consistent pattern, with its samples scattered across quadrants reflecting declining, low, and improving quality (Figure 8), despite having better stablished vegetation cover (Figure 2). This variability may reflect long-term instability in restoration trajectories, indicating a need for extended monitoring. Once again, these results suggest that even in minesoils with many years of restoration and stablished revegetation, this does not necessarily translate to high soil quality in the newly formed soil profile, which may also be associated with persistent compaction (Fernandez et al., 2025) and/or high acidity and low base saturation (Albert et al., 2022). Tian et al. (2024) corroborated these findings in a study of a degraded soil in China under forest restoration for 10 to 40 years. The authors observed higher *β-glucosidase* activity, only between 30 and 40 years of restoration, associated with increases in SOC and the microbial community. However, all evaluated areas were under similar pH and BS% values.

In this context, the use of *arylsulfatase* and *β-glucosidase* activities in minesoils, as well as POxC and TOC, should also consider their associations with additional minesoil attributes, such as microbial community structure, faunal groups (especially that considered ecosystem engineers as earthworms), types of topsoil aggregates, and contamination by heavy metals. According to Coelho-Junior et al. (2026), soil quality and the impacts of management practices to which soils are subjected are best assessed through the combined analysis of physical, chemical, and biological indicators. The literature indicates that, in addition to the type and successional stage of vegetation cover, soil pH is associated with bacterial biodiversity patterns at the bacterial species level and influences heavy metal availability, factors that may also influence enzymatic activity (Uzarowicz et al., 2020). In turn, higher enzymatic activity may also be associated with biogenic aggregation and increased earthworm metabolic activity, which mix mineral and organic matter in their digestive tracts and release mucus that may stimulate soil microbial biomass (Coelho-Junior et al., 2026). In a 126-day mesocosm study conducted in China, Song et al. (2020) observed that in soils with surface plant residues, the presence of earthworms was associated with lower *β-glucosidase* activity and reduced fungal populations but increased the proportion of water-stable macroaggregates (>250 mm). According to the authors, in the absence of fungi, earthworms may assume a more relevant role in the cellulolytic decomposition of residues through their digestive processes, being associated with higher activities of protease, invertase, urease, and alkaline phosphatase enzymes.

1.5. **Conclusions**

This study demonstrated that minesoils constructed with clayey topsoil and subjected to natural revegetation with species of the Pampa biome exhibited progressive changes in their biological and chemical attributes along the restoration chronosequence. According to the four-quadrant model, the evaluated minesoils remained distinct from the unmined reference soil after 30 years of restoration, although this comparison should be interpreted cautiously due to the different textural conditions between the minesoils and the reference soil.

Among the enzymes analyzed, arylsulfatase was more sensitive in detecting the effects of restoration time on minesoil recovery. However, the activities of arylsulfatase and β-glucosidase remained lower than those observed in the unmined soil throughout the evaluated chronosequence. POxC contents were also sensitive to restoration time and, when associated with enzymatic activity, TOC, and fertility parameters, proved to be a useful indicator for evaluating soil health and restoration trajectories in the investigated minesoils.

The importance of implementing agronomic management practices in minesoils- especially liming- is emphasized to prevent low pH, and aluminum saturation from negatively affecting the progression of minesoil health. This appears to have occurred in the minesoil with 22 years of restoration, which served as a repository for dolomitic limestone, showed positive relations with enzymatic activity, POxC, pH, or fertility.

Future research is recommended to refine the use of arylsulfatase and β-glucosidase activities in minesoils. This includes further studies on the relationship between enzyme activity and SOM, and other restoration indicators as microbial population DNA profile, soil macro- and mesofauna diversity assessment as ecosystem engineers (such as earthworms), different compositional types of topsoil aggregates, and contamination by heavy metals.

2. Considerações finais

Este estudo constatou que minesoils argilosos no bioma Pampa precisam de mais de 30 anos para recuperar minimamente sua saúde, expressa pelos parâmetros biológicas e químicas analisados. A arilsulfatase foi o indicador mais sensível ao tempo da restauração, mas tanto as atividades das enzimas arilsulfatase quanto da β-glicosidase permaneceram muito abaixo das encontradas no unmined soil mesmo após três décadas. O POxC, combinado com atividade enzimática, TOC e fertilidade, indicou de forma eficaz o progresso da saúde do solo. A calagem é fundamental para melhorar o pH e reduzir a saturação por alumínio, como observado no minesoil com 22 anos de restauração, que serviu de depósito de calcário dolomítico e que apresentou maiores valores de atividade enzimática e fertilidade. Pesquisas futuras devem explorar as relações entre atividade enzimática e MOS, DNA microbiano, diversidade da fauna edáfica, tipos de agregados e contaminação por metais pesados.

Referências

ALBERT, R.P.; PINTO, L.F.S.; MIGUEL, P.; *et al.* Acidification potential of geological materials in a coal mining area in southern Brazil. **Journal of South American Earth Sciences**, v. 120, p. 104103, 2022.

ALVARES, Clayton Alcarde *et al.* Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 1 dez. 2013.

BARBOZA, Fabrício da Silva; STUMPF, Lizete; PAULETTO, Eloy Antonio; *et al.* Impact of machine traffic events on the physical quality of a minesoil after topographic reconstruction. **Soil and Tillage Research**, v. 210, p. 104981, 2021.

BARNHISEL, R. I. *et al*. Characteristics and Reclamation of "Acid Sulfate" Mine Spoils. *In*: KITTRICK, J.A.; FANNING, D.S.; HOSSNER, L.R. (Orgs.). **SSSA Special Publications**. Madison, WI, USA: Soil Science Society of America, 2015, p. 225–234. Disponível em: <http://doi.wiley.com/10.2136/sssaspecpub10.c13>. Acesso em: 24 out. 2024.

BEN-AWUAH, E. *et al.* Strategic mining options optimization: Open pit mining, underground mining or both. **International Journal of Mining Science and Technology**, v. 26, n. 6, p. 1065–1071, nov. 2016.

BITENCOURT, Dioni Glei Bonini; *et al*. Geração de drenagem ácida e de contaminação por metais pesados em perfis de solos construídos em área de mineração de carvão. **Revista Brasileira de Ciência do Solo**, v. 39, n. 6, p. 1821–1834, 2015.

BHARADWAJ, Anasuya *et al.* Unraveling labile carbon and soil enzyme dynamics across seasons and sediment layers in Deepor Beel: a degraded freshwater wetland in Assam, India. **Environmental Monitoring and Assessment**, v. 197, n. 8, p. 874, 8 jul. 2025.

BRADSHAW, Anthony. The use of natural processes in reclamation — advantages and difficulties. **Landscape and Urban Planning**, v. 51, n. 2–4, p. 89–100, 2000.

BRISOLARA, Bruna Lemons. **Elementos potencialmente poluidores em solo construído de mineração de carvão sob o cultivo de gramíneas perenes aos 8,6 e 20 anos**. Pelotas-RS. Dissertação (Mestrado) — Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2024.

CASTRO, R.C. **Avaliação temporal de atributos físicos de um solo construído em área de mineração de carvão recuperado com gramíneas perenes.** Pelotas RS. 99f. Dissertação (Mestrado em Solos). Programa de Pós-Graduação em Agronomia. Faculdade de Agronomia Eliseu Maciel - Universidade Federal de Pelotas 2012.

CARNEIRO, Roberto Guimarães *et al*, A soil health assessment tool for vegetable cropping systems in tropical soils based on beta-glucosidase, arylsulfatase, and soil organic carbon, **Applied Soil Ecology**, v. 198, p. 105394, 2024.

CHAER, Guilherme Montandon *et al.* Evaluating C trends in clayey Cerrado Oxisols using a four-quadrant model based on specific arylsulfatase and β-glucosidase activities. **Applied Soil Ecology**, v. 183, p. 104742, mar. 2023.

CHEN, Guozhu *et al.* Organic sulfur mineralization in surface soils from coastal wetlands with different flooding periods affected by the flow-sediment regulation in the Yellow River Delta, China. **CATENA**, v. 215, p. 106343, ago. 2022.

COELHO-JUNIOR, Marcondes G. *et al.* Soil health in land-use systems of a quilombola community in Brazil: Integrating soil aggregate analysis and enzymatic indicators. **Applied Soil Ecology**, v. 217, p. 106636, jan. 2026.

CQFS (2016) Manual de calagem e edubação para os estados do Rio Grande do Sul e de Santa Catarina. DR Publicidade

CRM - COMPANHIA RIOGRANDENSE DE MINERAÇÃO. **Balanço patrimonial do exercício 2020**. Disponível em: https://www.crm.rs.gov.br/upload/arquivos/202105/05114724-balanco-2020.pdf.

CULMAN, Steven W. *et al.* Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. **Soil Science Society of America Journal**, v. 76, n. 2, p. 494–504, mar. 2012.

DAUNORAS, Jokūbas; KAČERGIUS, Audrius; GUDIUKAITĖ, Renata. Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. **Biology**, v. 13, n. 2, p. 85, 29 jan. 2024.

DE VARENNES, Amerilis *et al.* Enzymatic Activity of a Mine Soil Varies According to Vegetation Cover and Level of Compost Applied. **International Journal of Phytoremediation**, v. 12, n. 4, p. 371–383, 26 abr. 2010.

DENG, S. P.; TABATABAI, M. A. Effect of tillage and residue management on enzyme activities in soils: III. Phosphatases and arylsulfatase. **Biology and Fertility of Soils**, v. 24, n. 2, p. 141–146, 17 fev. 1997.

DIAS, Sérgio da Costa. **Estudo pioneiro da restauração de solos minerados em uma cronosequência de 10,6 anos na mina de carvão de Moatize** – Moçambique— Pelotas, 2024. 111 f. Tese (Doutorado) — Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2024.

EZEOKOLI, Obinna T. *et al.* Structural and functional differentiation of bacterial communities in post-coal mining reclamation soils of South Africa: bioindicators of soil ecosystem restoration. **Scientific Reports**, v. 10, n. 1, p. 1759, 4 fev. 2020.

FABBRI, Debora; PIZZOL, Romeo; CALZA, Paola; *et al.* Constructed Technosols: A Strategy toward a Circular Economy. **Applied Sciences**, v. 11, n. 8, p. 3432, 2021.

FENG, Yu *et al*. Effects of surface coal mining and land reclamation on soil properties: A review. **Earth-Science Reviews**, v. 191, p. 12–25, 2019.

FERNANDEZ, Maria Bertaso de Garcia. **Avaliação de atributos químicos e biológicos na recuperação de solo minerado na região de Candiota-RS**. Pelotas-RS, 2022. 70 f. Dissertação (Mestrado) — Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2022.

FERNANDEZ, Maria Bertaso De Garcia *et al.* First assessment of soil mesofauna, microbiota, and humic substances associations in a minesoil revegetated with four grasses in Brazil: An 18-year field study. **European Journal of Soil Biology**, v. 118, p. 103533, set. 2023.

FERNANDEZ, Maria Bertaso De Garcia *et al.* Temporal dynamics of minesoil restoration in Southern Brazil: A two-decade monitoring of soil physical attributes and organic carbon content. **Soil and Tillage Research**, *253*, 106647, 2025.

FERNANDEZ, Maria Bertaso De Garcia *et al.* Changes in chemical properties of a coal minesoil over two decades of reclamation with perennial grasses in Southern Brazil. **Revista Brasileira de Ciência do Solo**, v. 50, n. spe1, p. e0250060, 14 jan. 2025.

FIRPO, Beatriz A.; WEILER, Jéssica; SCHNEIDER, Ivo A.H. Technosol made from coal waste as a strategy to plant growth and environmental control. **Energy Geoscience**, v. 2, n. 2, p. 160–166, 2021.

GONÇALVES, F.C. **Efeito de plantas de cobertura sobre os atributos físicos de um solo construído na área de mineração de carvão de Candiota-RS após três anos**. Pelotas-RS 2008. 92f. Dissertação (Mestrado em Solos). Programa de Pós Graduação em Agronomia. Faculdade de Agronomia Eliseu Maciel – Universidade Federal de Pelotas. 2008.

GONÇALVES, EG; Lorenzi, H. **Morfologia vegetal: organografia e dicionário ilustrado de morfologia das plantas vasculares**. 2. ed. Nova Odessa, SP: Instituto Plantarum de Estudos da Flora, 2011. 544 p.

GUERRERO, J.L.; SUÁREZ-VAZ, N.; PAZ-GÓMEZ, D.C.; *et al.* Spatiotemporal evolution of U and Th isotopes in a mine effluent highly polluted by Acid Mine Drainage (AMD). **Journal of Hazardous Materials**, v. 447, p. 130782, 2023.

HAYNES, R. J. Labile Organic Matter Fractions as Central Components of the Quality of Agricultural Soils: An Overview. Em: **Advances in Agronomy. [s.l.] Elsevier**, 2005. v. 85p. 221–268.

HOLANDA NETO, M. **Atributos microbiológicos de um solo construído vegetado com gramíneas após mineração de carvão em Candiota/RS**. Dissertação (Mestrado em Solos). 98f. Programa de Pós-Graduação em Agronomia. Faculdade de Agronomia Eliseu Maciel - Universidade Federal de Pelotas, 2011.

HU, Ruoyu *et al.* Weathering of Basalts by *Aspergillus sp.* FS-4 Strain: Glass Compositions are Prone to Weathering. **Geomicrobiology Journal**, v. 37, n. 2, p. 101–109, 7 fev. 2020.

HU, Zhenqi *et al.* Preparation of topsoil alternatives for open-pit coal mines in the Hulunbuir grassland area, China. **Applied Soil Ecology**, v. 147, p. 103431, mar. 2020.

HURISSO, Tunsisa T. *et al.* Comparison of Permanganate-Oxidizable Carbon and Mineralizable Carbon for Assessment of Organic Matter Stabilization and Mineralization. **Soil Science Society of America Journal**, v. 80, n. 5, p. 1352–1364, set. 2016.

IAKOVLEVA, Evgenia *et al*. Acid mine drainage (AMD) treatment: Neutralization and toxic elements removal with unmodified and modified limestone. **Ecological Engineering**, v. 81, p. 30–40, 2015.
IEA - INTERNATIONAL ENERGY AGENCY, Energy Statistics Data Browser. 2023. Disponível em: <https://www.iea.org/data-and-statistics/data-tools/energy-statistics-data-browser?country=WORLD&fuel=Energy%20supply&indicator=TESbySource>. Acessado em: 20/10/2024

IPCC (Painel Intergovernamental sobre Mudança do Clima). Mudança do Clima. Relatório Síntese. 2023. Disponível em < https://www.gov.br/mcti/pt-br/acompanhe-o-mcti/sirene/publicacoes/relatorios-do-ipcc/arquivos/pdf/copy_of_IPCC_Longer_Report_2023_Portugues.pdf>

JIAO, Yanan; ZHANG, Chunhui; SU, Peidong; *et al.* A review of acid mine drainage: Formation mechanism, treatment technology, typical engineering cases and resource utilization. **Process Safety and Environmental Protection**, v. 170, p. 1240–1260, 2023.

KLOSE, Susanne *et al.* Sulfur Cycle Enzymes. *In*: DICK, Richard P. (Org.). **SSSA Book Series**. 1. ed. *[S.l.]*: Wiley, 2011. v. 9 p. 125–159.

KOPECKÝ, M.; *et al.* Influence of selected maize cultivation technologies on changes in the labile fraction of soil organic matter sandy-loam cambisol soil structure. **Soil and Tillage Research**, v. 207, p. 104865, mar. 2021.

KOWALSKA, Aneta *et al.* Carbon Sequestration in Remediated Post-Mining Soils: A New Indicator for the Vertical Soil Organic Carbon Variability Evaluation in Remediated Post-Mining Soils. **Energies**, v. 16, n. 16, p. 5876, 8 ago. 2023.

LAVELLE, P., SPAIN, A.V.,. Soil Organisms. **Soil Ecology. Springer** . 2001. 201–356.

LEAL, Otávio dos Anjos. **Frações e qualidade da matéria orgânica de um solo construído vegetado com gramíneas após a mineração de carvão**. Dissertação (Mestrado em Solos). 113f. Programa de Pós-Graduação em Agronomia. Faculdade de Agronomia Eliseu Maciel - Universidade Federal de Pelotas, 2011.

LEAL, Otávio dos Anjos *et al.*. Legacy of severe soil degradation hinders the buildup of mineral-associated soil organic carbon. ***Science of the Total Environment***, *978*, 179445, 2025a.
LEAL, Otávio Dos Anjos *et al.* First validation of the method Visual Evaluation of Soil Structure in coal mining area using a long-term field revegetation experiment as testbed. **Soil and Tillage Research**, v. 246, p. 106347, fev. 2025b.

LEAL, O.A, Reichel, R., Wissel, H., & Brüggemann, N. (2025a). Legacy of severe soil degradation hinders the buildup of mineral-associated soil organic carbon. ***Science of the Total Environment***, *978*, 179445.

LEGUÉDOIS, Sophie; SÉRÉ, Geoffroy; AUCLERC, Apolline; *et al.* Modelling pedogenesis of Technosols. **Geoderma**, v. 262, p. 199–212, 2016.

LI, Yang *et al.* Ecological restoration alters microbial communities in mine tailings profiles. **Scientific Reports**, v. 6, n. 1, p. 25193, 29 abr. 2016.

LIU, Wei-qiu *et al*. Nitrogen fixation in biotic crusts and vascular plant communities on a copper mine tailings. **European Journal of Soil Biology**, v. 50, p. 15–20, 2012.

LLORENTE, M.; GLASER, B.; TURRIÓN, M. B. Storage of organic carbon and Black carbon in density fractions of calcareous soils under different land uses. **Geoderma**, v. 159, n. 1–2, p. 31–38, out. 2010.

LU, Ping; *et al*. Composition and structure of soil fauna communities and their relationships with environmental factors in copper mine waste rock after re-vegetation. **Global Ecology and Conservation**, v. 32, p. e01889, 2021.

LÜTZOW, M. V.; *et al.* Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. **European Journal of Soil Science**, v. 57, n. 4, p. 426–445, ago. 2006.

LÜTZOW, M. V.; *et al.* SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. **Soil Biology and Biochemistry**, v. 39, n. 9, p. 2183–2207, set. 2007.

MCGILL, W.B.; COLE, C.V. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. **Geoderma**, v. 26, n. 4, p. 267–286, 1981.

MARGENOT, Andrew J. *et al.* Biochemical proxies indicate differences in soil C cycling induced by long-term tillage and residue management in a tropical agroecosystem. **Plant and Soil**, v. 420, n. 1–2, p. 315–329, nov. 2017.

MENDES, I. C. *et al.* Propriedades biológicas em agregados de um Latossolo Vermelho-Escuro sob plantio convencional e direto no Cerrado. **Revista Brasileira de Ciência do Solo**, v. 27, n. 3, p. 435–443, jun. 2003.

MENDES, Iêda Carvalho; SOUZA, DJMG; BUENO, dos RJF; *et al.* **Bioanálise de solo**: Aspectos teóricos e práticos. Tópicos em ciência do solo. Viçosa, MG: Sociedade Brasileira de Ciência do Solo, v. 10, p. 1–64, 2019.

MENDES, I.D.C. *et al.* Tecnologia BioAS: uma maneira simples e eficiente de avaliar a saúde do solo. Planaltina, DF: **Embrapa Cerrados**, 2021.

MENDES, Ieda Carvalho. **Fazendas de referência para a implementação do modelo de quatro quadrantes na avaliação de tendências do carbono do solo pela tecnologia BioAS**. Documentos Embrapa Cerrados 403:1–41, 2023.

MENDES, Ieda Carvalho *et al.* Soil Bioanalysis (SoilBio): A Sensitive, Calibrated, and Simple Assessment of Soil Health for B razil. *In*: MENDES, Ieda Carvalho; CHERUBIN, Maurício Roberto (Orgs.). **ASA, CSSA, and SSSA Books**. 1. ed. *[S.l.]*: Wiley, 2024. p. 292–326.

MENDEZ, Monica O.; GLENN, Edward P.; MAIER, Raina M. Phytostabilization Potential of Quailbush for Mine Tailings: Growth, Metal Accumulation, and Microbial Community Changes. **Journal of Environmental Quality**, v. 36, n. 1, p. 245–253, 2007.

MIGUEL, Pablo *et al*. Physical restoration of a minesoil after 10.6 years of revegetation. **Soil and Tillage Research**, v. 227, p. 105599, 2023.

MIOLA, E.C.C. **Qualidade física de um solo construído e cultivado com diferentes plantas de cobertura na área de mineração de Candiota – RS**. Pelotas-RS. 2010. 107f. Dissertação (Mestrado em Solos). Programa de Pós Graduação em Agronomia - Universidade Federal de Pelotas. 2010.

MÓL, Paula Chequer Gouveia *et al.* β-glucosidase: An overview on immobilization and some aspects of structure, function, applications and cost. **Process Biochemistry**, v. 130, p. 26–39, jul. 2023.

MORADI, Jabbar *et al*. Vertical distribution of soil fauna and microbial community under two contrasting post mining chronosequences: Sites reclaimed by alder plantation and unreclaimed regrowth. **Global Ecology and Conservation**, v. 23, p. e01165, 2020.

MUKHOPADHYAY, Sangeeta; MAITI, S.K.; MASTO, R.E. Use of Reclaimed Mine Soil Index (RMSI) for screening of tree species for reclamation of coal mine degraded land. **Ecological Engineering**, v. 57, p. 133–142, 2013.

MUKHOPADHYAY, Sangeeta; MASTO, Reginald E. Comparative evaluation of Cassia siamea and Albizia lebbeck for their potential to recover carbon and nutrient stocks in a chronosequence post-mining site. **CATENA**, v. 208, p. 105726, jan. 2022.

MULAZZANI, Rodrigo Pivoto *et al.* Chemical constraints are the major limiting factor of root deepening in southern Brazil soils. **Geoderma Regional**, v. 38, p. e00825, set. 2024.

NANNIPIERI, Paolo; TRASAR-CEPEDA, Carmen; DICK, Richard P. Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. **Biology and Fertility of Soils**, v. 54, n. 1, p. 11–19, 2018.

NUNES, M.C.D. **Condições físicas de solos construídos na área de mineração de carvão de Candiota-RS**. Pelotas-RS. 130f. Dissertação (Mestrado em Solos). Programa de Pós-Graduação em Agronomia. Faculdade de Agronomia Eliseu Maciel - Universidade Federal de Pelotas, 2002.

OLIVEIRA, Mauricio Silva de. **Fauna edáfica e sua relação com os atributos químicos e físicos de um solo construído, após 14,6 anos de revegetação**. Pelotas - RS. 48 f. Dissertação (Mestrado) Programa de Pós-Graduação em Manejo e Conservação do Solo e da Água, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2023.

OLIVEIRA, Mauricio silva *et al.* Abundance and diversity of mesofauna in relation to minesoil properties after 14.6 years of revegetation with grasses. **Ecological Engineering**, v. 214, p. 107578, abr. 2025.

OLIVEIRA, Jakeline Rosa *et al.* Environmental, ecological and human health risk assessment for Ba, Cr, Zn and V in minesoil after 20 years of restoration in southern Brazil. **Frontiers in Soil Science**, v. 6, p. 1754030, 2026. Disponível em: https://doi.org/10.3389/fsoil.2026.1754030

PAJĄK, Marek *et al*. Restoration of Vegetation in Relation to Soil Properties of Spoil Heap Heavily Contaminated with Heavy Metals. **Water, Air, & Soil Pollution**, v. 229, n. 12, p. 392, 2018.

PINTO L. F. S, KÄMPF, N. **Contaminação dos solos construídos**. In: Teixeira EC, Pires MJR, editores. Meio ambiente e carvão. Impactos da exploração e utilização. Porto Alegre: FINEP/CAPES/ PADCT/GTM/PUCRS/UFSC/FEPAM; 2002. p.69-92.

PINTO, Luiz Fernando Spinelli; STUMPF, Lizete; MIGUEL, Pablo; *et al.* Reclamation of soils degraded by surface coal mining. In: Mining techniques-past, present and future. [s.l.]: **IntechOpen**, 2020.

POSCHENRIEDER, Charlotte *et al.* A glance into aluminum toxicity and resistance in plants. **Science of The Total Environment**, v. 400, n. 1–3, p. 356–368, ago. 2008.

PULLEMAN, Mirjam *et al.* Soil mass and grind size used for sample homogenization strongly affect permanganate-oxidizable carbon (POXC) values, with implications for its use as a national soil health indicator. **Geoderma**, v. 383, p. 114742, fev. 2021.

REIS, D. A., DE LIMA, C. L. R., PAULETTO, E. A. Resistência tênsil de agregados e compressibilidade de um solo construído com plantas de cobertura em área de mineração de carvão em Candiota, RS. **Revista Brasileira de Ciência do Solo**, v.38, n.2, p. 669-678, 2014.

RIGOTTI, N. **Recuperação de áreas degradadas**: estudo de caso. Porto Alegre, FINEP/CAPES/PADCT/GTM/PUCRS/FEPAM, p. 99-113, 2002.

ROSARIO, Karyna *et al*. Bacterial Community Changes during Plant Establishment at the San Pedro River Mine Tailings Site. **Journal of Environmental Quality**, v. 36, n. 5, p. 1249–1259, 2007.

RUIZ, Francisco *et al.* Constructing soils for climate-smart mining. **Communications Earth & Environment**, v. 4, n. 1, p. 219, 19 jun. 2023.

SHEN, Zhang-Jum *et al*. Effect of Vegetation Succession on Organic Carbon, Carbon of Humus Acids and Dissolved Organic Carbon in Soils of Copper Mine Tailings Sites. **Pedosphere**, v. 24, n. 2, p. 271–279, 2014.

SILVA, Luis F. O.; WOLLENSCHLAGER, Marcus; OLIVEIRA, Marcos L. S. A preliminary study of coal mining drainage and environmental health in the Santa Catarina region, Brazil. **Environmental Geochemistry and Health**, v. 33, n. 1, p. 55–65, 2011.

Silva Junior VSE, *et al*.. Estudos em biologia vegetal: famílias botânicas. **Editora Conhecimento Livre**. V. 1. 2023.

SIWIK-ZIOMEK, Anetta *et al*. Biological parameters in technogenic soils of a former sulphur mine. **International Agrophysics**, v. 32, n. 2, p. 237–245, 2018.

SLEZACK-DESCHAUMES, Sophie *et al.* Dynamics of cultivable arylsulfatase-producing bacterial and fungal communities along the phenology of field-grown rape. **European Journal of Soil Biology**, v. 48, p. 66–72, jan. 2012.

ŠNAJDR, Jaroslav *et al*. Dominant trees affect microbial community composition and activity in post-mining afforested soils. **Soil Biology and Biochemistry**, v. 56, p. 105–115, 2013.

SONG, Ke *et al.* Earthworms accelerate rice straw decomposition and maintenance of soil organic carbon dynamics in rice agroecosystems. **PeerJ**, v. 8, p. e9870, 17 set. 2020.

SOUZA, Melissa Rosa de *et al*. Evaluation of soils under the influence of coal mining and a thermoelectric plant in the city of Candiota and vicinity, Brazil. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis**, v. 866, p. 503350, 2021.

STOCKMANN, Uta *et al.* The knowns, known unknowns and unknowns of sequestration of soil organic carbon. **Agriculture, Ecosystems & Environment**, v. 164, p. 80–99, jan. 2013.

STUMPF, Lizete. **Desenvolvimento radicular de gramíneas perenes e sua eficiência na recuperação de atributos físicos de um solo construído compactado em área de mineração de carvão.** Pelotas-RS. 122f. Tese (Doutorado) -Programa de Pós-Graduação em Agronomia/Solos. Universidade Federal de Pelotas, 2015.

STUMPF, Lizete; PAULETTO, Eloy Antonio; PINTO, Luiz Fernando Spinelli. Soil aggregation and root growth of perennial grasses in a constructed clay minesoil. **Soil and Tillage Research**, v. 161, p. 71–78, 2016.

STUMPF, Lizete *et al.* Tensile strength and organic matter fractions in aggregates of a grass-covered mined soil under early stage recovery. **Soil and Tillage Research**, v. 176, p. 69–76, mar. 2018.

STUMPF, Lizete *et al*. Impact of Revegetation on Ecological Restoration of a Constructed Soil in a Coal Mining in Southern Brazil. *In*: HUFNAGEL, Levente; A. EL-ESAWI, Mohamed (Orgs.). **Environmental Sciences**. [s.l.]: IntechOpen, 2023, v. 6. Disponível em: <https://www.intechopen.com/chapters/82754>. Acesso em: 24 out. 2024.

TABATABAI, M.A. Soil enzymes. In: WEAVER, R.W.; ANGLE,S. & BOTTOMLEY, P.J., eds. Methods of soil analysis: Microbiological and biochemical properties. 2. Madison, **SoilScience Society of America**, 1994. p.775-833.

TABATABAI, M. A.; BREMNER, J. M. Factors Affecting Soil Arylsulfatase Activity. **Soil Science Society of America Journal**, v. 34, n. 3, p. 427–429, maio 1970.

TEIXEIRA, Paulo César *et al.* Manual de métodos de análise de solo. 2017.

TIAN, Qian *et al.* Variation of soil organic carbon components and enzyme activities during the ecological restoration in a temperate forest. **Ecological Engineering**, v. 201, p. 107192, abr. 2024.

Tirol-Padre A, Ladha JK. Avaliação da confiabilidade do carbono oxidável por permanganato como índice de carbono lábil no solo. **Revista da Sociedade de Ciência do Solo da América**, *68*(3), 969–978, 2004.

UZAROWICZ, Łukasz *et al*. Technogenic soils (Technosols) developed from mine spoils containing Fe sulphides: Microbiological activity as an indicator of soil development following land reclamation. **Applied Soil Ecology**, v. 156, p. 103699, 2020.

VALENTÍN-VARGAS, Alexis *et al*. Treatment impacts on temporal microbial community dynamics during phytostabilization of acid-generating mine tailings in semiarid regions. **Science of The Total Environment**, v. 618, p. 357–368, 2018.

VERES, Zsuzsa *et al*. Soil extracellular enzyme activities are sensitive indicators of detrital inputs and carbon availability. **Applied Soil Ecology**, v. 92, p. 18–23, 2015.

XU, Hongwei *et al.* Responses of soil enzyme activity and soil organic carbon stability over time after cropland abandonment in different vegetation zones of the Loess Plateau of China. **CATENA**, v. 196, p. 104812, jan. 2021.

XU, Chen-yang *et al.* The interplay of labile organic carbon, enzyme activities and microbial communities of two forest soils across seasons. **Scientific Reports**, v. 11, n. 1, p. 5002, 2 mar. 2021.

XUE, Yuanyuan *et al.* The role of vegetation restoration in shaping the structure and stability of soil bacterial community of alpine mining regions. **Plant and Soil**, v. 513, n. 2, p. 2903–2924, ago. 2025.

WANG, Qiang *et al*. Using microbial community functioning as the complementary environmental condition indicator: A case study of an iron deposit tailing area. **European Journal of Soil Biology**, v. 51, p. 22–29, 2012.

WEIL, Ray R. *et al.* Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. **American Journal of Alternative Agriculture**, v. 18, n. 1, p. 3–17, mar. 2003.

WEILER, Jéssica; FIRPO, Beatriz A.; SCHNEIDER, Ivo A.H. Technosol as an integrated management tool for turning urban and coal mining waste into a resource. **Minerals Engineering**, v. 147, p. 106179, 2020.

WILLIAMS, G. Social and economic costs and benefits of coal. Em: The Coal Handbook. [s.l.] **Elsevier**, 2023. p. 3–29.

WOLINSKA, Agnieszka; STEPNIEWSK, Zofia. Dehydrogenase Activity in the Soil Environment. *In*: CANUTO, Rosa Angela (Org.). **Dehydrogenases**. *[S.l.]*: InTech, 2012.

WOLIŃSKA, Agnieszka *et al*. Soil Microbial Community Profiling and Bacterial Metabolic Activity of Technosols as an Effect of Soil Properties following Land

Reclamation: A Case Study from the Abandoned Iron Sulphide and Uranium Mine in Rudki (South-Central Poland). **Agronomy**, v. 10, n. 11, p. 1795, 2020.

WU, Yucheng *et al.* pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. **Scientific Reports**, v. 7, n. 1, p. 40093, 4 jan. 2017.

YE, Z.H *et al*. Lime and pig manure as ameliorants for revegetating lead/zinc mine tailings: a greenhouse study. **Bioresource Technology**, v. 69, n. 1, p. 35–43, 1999.

ZAMULINA, Inna V *et al*. The influence of long-term Zn and Cu contamination in Spolic Technosols on water-soluble organic matter and soil biological activity. **Ecotoxicology and Environmental Safety**, v. 208, p. 111471, 2021.

ZHAN, Jing; SUN, Qingye. Diversity of free-living nitrogen-fixing microorganisms in the rhizosphere and non-rhizosphere of pioneer plants growing on wastelands of copper mine tailings. **Microbiological Research**, v. 167, n. 3, p. 157–165, 2012.

ZHANG, Shaoliang; YANG, Yongjun; MI, Jiaxin. Research progress on mining ecological restoration: based on the report of three consecutive world ecological restoration conferences. **Acta Ecologica Sinica**, v. 38, n. 15, 2018. Disponível em: <http://www.ecologica.cn/stxb/ch/reader/view_abstract.aspx?doi=10.5846/stxb201711071992>. Acesso em: 25 out. 2024.