ANITA FERNANDA DOS SANTOS TEIXEIRA

RELATIONSHIPS AMONG SOIL MICROBIOLOGICAL ATTRIBUTES AND ABIOTIC FACTORS IN PHYTOPHYSIOGNOMIES INFLUENCED BY IRON MINING

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Aos meus amores: meus pais, Maria Dalva e Luiz, meus irmãos, Aline e Anderson, meu afilhado Miguel, e meu namorado Sérgio, por me fazerem acreditar que eu sou capaz.

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A Deus,
por todas as bênçãos que recebi e por ter me dado forças para chegar até aqui.

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O Cio da Terra

“Debulhar o trigo

Recolher cada bago do trigo

Forjar no trigo o milagre do pão

E se fartar de pão

Decepar a cana

Recolher a garapa da cana

Roubar da cana a doçura do mel

Se lambuzar de mel

Afagar a terra

Conhecer os desejos da terra

Cio da terra, a propícia estação

E fecundar o chão”

(Milton Nascimento e Chico Buarque)

RESUMO

O Quadrilátero Ferrífero é uma área conhecida mundialmente por seus depósitos de minério de ferro. Nessa região se encontram Cerrado e Mata Atlântica, dois grandes biomas do Brasil, além de diversas áreas modificadas pela mineração e áreas de bancadas lateríticas, conhecidas como canga. Assim, além da importância econômica, a área é um *hot spot* de diversidade. O solo é um sistema onde ocorrem diversos serviços ecossistêmicos que garantem a manutenção da vida no planeta. Desta forma, a manutenção da qualidade do solo é importante para a manutenção desses serviços. Este trabalho objetivou avaliar a influência de fatores abióticos em atributos microbiológicos do solo e espacializar alguns desses em fitofisionomias do Quadrilátero Ferrífero. Solo foi amostrado em fitofisionomias de Mata Atlântica, Cerrado, Canga, Eucalipto e em áreas em reabilitação alteradas pela mineração de ferro. Neste trabalho foram avaliadas a diversidade de fungos micorrízicos arbusculares (FMA) em culturas armadilha e o potencial de inóculo micorrízico. Também foram realizadas predições e espacializações dos indicadores microbiológicos de qualidade do solo carbono da biomassa microbiana, respiração basal do solo, quociente metabólico (qCO_2), quociente microbiano (qMic), atividade de hidrólise do diacetato de fluoresceína (FDA), urease, fosfatase ácida, fosfatase alcalina e β-glicosidase. Para predição desses últimos indicadores citados foram utilizados dados de fertilidade e textura do solo, teores de elementos obtidos por equipamento portátil de fluorescência de raios-X (pXRF) e dados de atributos de terreno. O maior potencial do inóculo micorrízico foi encontrado em canga. O uso de culturas armadilhas aumenta a diversidade de espécies de FMA capturadas. Considerar fitofisionomia e estação no modelo melhora a predição de indicadores microbiológicos de qualidade do solo. A fertilidade do solo e textura podem predizer carbono da biomassa microbiana, respiração microbiana, qCO_2 e qMic. Atributos do terreno são os melhores preditores de respiração basal do solo. Elementos obtidos por pXRF, propriedades físico-químicas do solo e atributos do terreno fornecem bons modelos preditivos de atividade de enzimas do solo. A espacialização da atividade das enzimas e dos atributos microbiológicos permite uma melhor visão geral da variabilidade desses em cada fitofisionomia e estação.

Palavras-chave: Indicadores microbiológicos de qualidade do solo. Fungos micorrízicos arbusculares. Modelos de predição. Espacialização de dados microbiológicos.

ABSTRACT

The *Quadrilátero Ferrífero* is an area known worldwide for its iron ore deposits. In this region two large biomes of Brazil meet, Cerrado and Atlantic Forest, in addition to being found several areas modified by mining and areas of ironstone outcrops, known as canga. Thus, in addition to the economic importance, the area is a hot spot of diversity. Soil is a system where there are many ecosystem services that guarantee the maintenance of life on the planet. In this way, the maintenance of soil quality is important for the maintenance of these services. This work aimed to evaluate the influence of abiotic factors on soil microbiological attributes and to spatialize some of these in phytophysiognomies of the *Quadrilátero Ferrífero*. Soil was sampled in phytophysiognomies of Atlantic Forest, Cerrado, Canga, Eucalyptus, and in areas in rehabilitation altered by iron mining. In this work the diversity of arbuscular mycorrhizal fungi (AMF) in trap cultures and the potential of mycorrhizal inoculum were evaluated. Soil microbiological indicators biomass microbial carbon, basal soil respiration, metabolic quotient (qCO_2), microbial quotient (qMic), hydrolysis activity of fluorescein diacetate (FDA), urease, acid phosphatase, alkaline phosphatase and β-glycosidase were also predicted and spatialized. In order to predict these last mentioned indicators, soil fertility and texture data, element contents obtained by portable X-ray fluorescence equipment (pXRF) and terrain attribute data were used. The highest potential of the mycorrhizal inoculum was found in canga. The use of trap cultures increases the diversity of AMF species captured. To Consider phytophysiognomy and season in the model improves the prediction of microbiological indicators of soil quality. Soil fertility and texture can predict biomass microbial carbon, basal soil respiration, qCO_2 and qMic. Terrain attributes are the best predictors of basal soil respiration. Elements obtained by pXRF, soil physicochemical properties and terrain attributes provide good predictive models for soil enzymes activity. The spatialization of the enzymes activity and of the other microbiological attributes allows a better overview of the variability of these in each phytophysiognomy and season.

Keywords: Microbiological indicators of soil quality. Arbuscular mycorrhizal fungi. Predictive models. Spatialization of microbiological data.

SUMÁRIO

1. INTRODUÇÃO

O Brasil se destaca no cenário mundial pela mineração e exportação de Ferro. Apesar da importância econômica, a mineração causa mudanças que impactam o ambiente. No processo de mineração, a abertura da cava consiste em remover a camada que recobre o minério. Essa camada é depositada em forma de pilhas (taludes) que também podem ser formadas por rejeitos da mineração. Desta forma, diversas perturbações são causadas em áreas sub influência de mineração, principalmente no solo, sendo necessárias intervenções para revegetação e reabilitação desses locais.

O solo é um sistema complexo onde a fase sólida forma agregados, e as fases líquida e gasosa se encontram constantemente em dinâmica nos poros presentes na estrutura do solo. A heterogeneidade do solo faz com que ele apresente diversos tipos de habitats, que podem abrigar diferentes organismos. Desta forma, o solo apresenta variedade de condições que permite que organismos metabolicamente distintos possam conviver lado a lado (CARDOSO; ANDREOTE, 2016; MOREIRA; SIQUEIRA, 2006; RESENDE et al., 2014). Entre os diversos organismos que habitam o solo, os microrganismos desempenham papel fundamental para a formação do solo e a manutenção de processos biológicos. Eles são responsáveis pela excreção de compostos diversos que auxiliam no intemperismo do material de origem, além de terem papel na agregação das partículas do solo (SCHULZ et al., 2013). Entre os diversos processos biológicos intermediados pelos microrganismos do solo, podemos destacar a formação da matéria orgânica do solo, o processo de ciclagem de nutrientes, como carbono, nitrogênio, fósforo e enxofre, além de outros processos que garantem a manutenção da vida no planeta (BENDER; WAGG; VAN DER HEIJDEN, 2016).

Apesar de os microrganismos do solo influenciarem diretamente no funcionamento do ecossistema solo, eles também são influenciados por fatores bióticos e abióticos do solo. Diversos fatores abióticos como pH, teores de nutrientes, temperatura, água, mineralogia/estrutura, entre outros, refletem na composição e atividade da microbiota de diferentes formas, provocando diferentes respostas da microbiota. Essa resposta é geralmente refletida rapidamente pelos microrganismos do solo, que são componentes bastante sensíveis no sistema solo.

Nesta complexa rede de interações, há necessidade de compreensão de como os fatores abióticos podem afetar os microrganismos do solo sob uma mesma fitofisionomia. Diversos estudos apontam que alguns fatores abióticos são mais determinantes que outros na atividade e

composição da comunidade microbiana e, em grande parte dos estudos, apenas fatores relacionados à fertilidade do solo são considerados (DE CARVALHO et al., 2016; TEIXEIRA et al., 2017; SILVA et al., 2018a), ficando os outros fatores, como relevo e composição química do solo em segundo plano. O entendimento das diferentes relações dos fatores abióticos com os atributos microbiológicos do solo é um fator chave para melhor compreensão do comportamento desses que, por responderem rapidamente a variações no ambiente, são bons indicadores de qualidade do solo.

A qualidade do solo é a capacidade do solo de funcionar nos limites do ecossistema sustentando plantas, animais e seres humanos (DORAN; PARKIN, 1994). Existem diversos indicadores de qualidade do solo, estando esses relacionados com a física, química ou a biologia do mesmo. Entre esses, os indicadores microbiológicos apresentam vantagens em relação aos demais pela rapidez com que respondem a mudanças no ambiente (DORAN; PARKIN, 1994). Como a diversidade e a atividade dos microrganismos do solo é influenciada por diversos fatores, mudanças que ocorrem no meio levam o ecossistema solo a se reajustar, reflexo da resiliência do mesmo. Os microrganismos do solo, por sua grande sensibilidade às variações no meio em que estão inseridos, são os primeiros a reagirem às novas condições em seu habitat. Essa readaptação faz com que eles variem sua diversidade, reprodução, taxa de respiração, e até mesmo a quantidade de substâncias excretadas.

A diversidade de microrganismos encontrada em um solo também é um indicador de sua qualidade. Em ecossistemas estáveis, os organismos que ali habitam se encontram em condição de relativo equilíbrio. Os organismos do solo apresentam grande diversidade funcional, podendo um mesmo organismo executar diferentes funções em um mesmo sistema. Diferentes microrganismos podem desempenhar a mesma função, garantindo a redundância funcional necessária à resiliência do ecossistema (ZHANG et al., 2016). A resiliência do ecossistema é determinada por um conjunto de fatores físicos e químicos do solo e do seu efeito na comunidade microbiana (GRIFFITHS; PHILIPPOT, 2013).

Entre os microrganismos que apresentam grande diversidade funcional, os fungos micorrízicos arbusculares (FMA) se destacam. Esses fungos apresentam capacidade de auxiliar a agregação do solo pela produção de enzimas ou efeitos físicos, além de serem simbiontes da maioria das espécies de plantas, auxiliando-as na obtenção de água e nutrientes, além de facilitar o desenvolvimento dessas em ambientes sob estresse (JEFFRIES et al., 2003; KLIRONOMOS et al., 2000; VILELA et al., 2014). Os FMA são biotróficos obrigatórios que só completam seu ciclo de vida na presença de uma planta hospedeira. Os esporos desses fungos, além de serem

sua estrutura de propagação e resistência, são muito úteis para identificação das espécies de FMA. Apesar de não haver especificidade entre planta e FMA, existe preferencialidade, podendo uma mesma espécie de FMA beneficiar ou não diferentes espécies de plantas (BEVER et al., 2009; DE SOUZA et al., 2008). Desta forma, a diversidade desses fungos também é relacionada à qualidade do solo, por sua diversidade ser importante para garantir o equilíbrio do ecossistema.

Entre as substâncias excretadas, não apenas por FMA, como por diferentes microrganismos do solo, as enzimas apresentam papel funcional nos ecossistemas. As enzimas do solo aceleram reações em diversos ciclos como do carbono, nitrogênio e fósforo. A depender das condições encontradas no habitat, os microrganismos podem excretar diferentes enzimas e as mesmas podem estar mais ou menos ativas (BURNS et al., 2013; GIANFREDA et al., 2002).

O Quadrilátero Ferrífero é reconhecido mundialmente por seus grandes depósitos de Ferro. Além disso, essa região é considerada "hot spot" de diversidade, por sua grande diversidade de ambientes diferentes em curtas distâncias. Por sua heterogeneidade de habitats, essa é uma região bastante atrativa para estudos relacionados à ecologia. Lado a lado com áreas de preservação, há diversas áreas alteradas pela mineração que estão em fase de recuperação ou que precisam ou precisarão ser recuperadas, sendo conhecidos dois grandes desastres humanos e ambientais que foram o rompimento das barragens de rejeitos da mina Fundão (Mariana) e da mina Córrego do Feijão (Brumadinho), aumentando o apelo ecológico e ambiental para estudos nessas áreas.

O ecossistema solo encontra-se, portanto, em equilíbrio dinâmico com as condições do ambiente, e os indicadores microbiológicos de qualidade do solo, por refletirem rapidamente mudanças externas que rompam este equilíbrio, são de extrema importância para avaliações de impacto e degradação do solo, como ocorre em áreas de mineração. Desta forma, estudos de fatores abióticos que variam ao longo de ambientes interferindo nesses indicadores podem auxiliar no manejo adequado para retorno de sua resiliência. Este trabalho objetivou, portanto, avaliar as relações entre atributos microbiológicos do solo e fatores abióticos em áreas sob influência da mineração de ferro.

2. PRIMEIRA PARTE – REFERENCIAL TEÓRICO

2.1 O Quadrilátero Ferrífero

O Quadrilátero Ferrífero é uma região reconhecida mundialmente por seu grande depósito de minério de ferro. Localizada no estado de Minas Gerais, no sudeste do Brasil, a região abrange 25 municípios (Barão de Cocais, Belo Horizonte, Belo Vale, Bonfim, Brumadinho, Caeté, Catas Altas, Congonhas, Contagem, Esmeraldas, Ibirité, Igarapé, Itabira, Itabirito, Mariana, Moeda, Nova Lima, Ouro Branco, Ouro Preto, Raposos, Rio Acima, Rio Piracicaba, Sabará, Santa Bárbara e Santa Luzia) correspondendo a uma área de aproximadamente 7200 km² (DO CARMO; JACOBI, 2016).

Localizado no Cráton São Francisco, a região passou por um processo de soerguimento, e o nome de quadrilátero provém da forma relativamente quadrangular que a região apresenta. O termo ferrífero é devido à alta concentração de ferro nas formações geológicas da região, composta por itabiritos, dolomitos ferruginosos e rochas básicas a ultrabásicas (CARVALHO FILHO; CURI; SHINZATO, 2010; FARINA et al., 2016; VARAJÃO; SALGADO; VARAJÃO; BRAUCHER; COLIN; NALINI JUNIOR et al., 2009)

Os solos da região apresentam diversas características que refletem a composição de seus materiais de origem. A depender do relevo e material de origem, os solos da região variam desde ilhas de solo em Plintossolo Pétricos a Latossolos profundos. Assim, as fitofisionomias da região refletem as características do solo em que as plantas estão se desenvolvendo. Por essa grande diversidade de ambientes, o Quadrilátero Ferrífero é reconhecido como um *hot spot* de diversidade (CARVALHO FILHO; CURI; SHINZATO, 2010; CASTRO et al., 2017; TEIXEIRA et al., 2017; VARAJÃO et al., 2009).

Mata Atlântica e Cerrado, dois grandes biomas do Brasil, se encontram no Quadrilátero Ferrífero. Também são observadas áreas de canga, com bancadas lateríticas que apresentam ilhas de solo com grande concentração de diversidade. Além de fitofisionomias naturais, diversas localidades apresentam paisagens alteradas pela mineração de ferro, apresentando pilhas de estéril e / ou taludes de estabilização de solo que tenha sofrido alguma alteração e barragens de rejeitos.

Dois grandes acidentes com barragens de rejeitos conhecidos mundialmente ocorreram no Quadrilátero Ferrífero. O primeiro ocorreu em 15 de novembro de 2015, no município de Mariana. A barragem de rejeitos Fundão se rompeu, derramando aproximadamente 62 milhões de metros cúbicos de lama que destruiu o subdistrito de Bento Rodrigues, afetou cerca de 1.400 hectares e matou 19 pessoas (IBAMA, 2015). O segundo acidente ocorreu em 25 de janeiro de

2019, em Brumadinho. A barragem 1 da mina Córrego do Feijão se rompeu e derramou cerca de 12 milhões de metros cúbicos de rejeito no ambiente. Dados preliminares estimam 315 vítimas (mortos e desaparecidos) e que cerca de 200 hectares tenham sido afetados pela lama.

A mineração provoca mudanças drásticas na paisagem e no solo, podendo gerar graves consequências à sua sustentabilidade. Desta forma, o Quadrilátero Ferrífero apresenta grande diversidade de fitofisionomias com características particulares, sendo uma importante área para estudos de diversidade, ecossistemas e de grande apelo ecológico e ambiental.

2.2 O ecossistema solo

O solo é a parte superficial da crosta terrestre que resulta da ação dos fatores de formação como clima e organismos agindo sobre um material de origem em um dado relevo ao longo do tempo. Esse sistema heterogêneo, complexo e dinâmico apresenta uma ampla gama de habitats, proporcionando variabilidade de ambientes, proporcionando condições para que organismos metabolicamente distintos possam conviver lado a lado em equilíbrio dinâmico (KER et al., 2012; MOREIRA et al., 2013; RESENDE et al., 2014).

A estrutura do ecossistema solo compreende a comunidade biológica em riqueza, distribuição e densidade de espécies que nele habitam, além da quantidade e distribuição de componentes abióticos. Já as funções do ecossistema solo, envolvem os processos que nele ocorrem, como fluxo de energia, ciclos biogeoquímicos, formação da matéria orgânica, e a regulação mútua entre organismos e ambientes (TOWNSEND; BEGON; HARPER, 2010). Para compreensão do ecossistema solo, primeiramente deve-se entender a gama de habitats que o compõem. O solo é trifásico, contendo partículas sólidas além das fases líquida e gasosa. A parte sólida é composta por partículas minerais, matéria orgânica, organismos e raízes nele presente. Os principais constituintes da fase sólida do solo são, geralmente, partículas minerais de tamanho areia, silte ou argila. Juntamente com os outros constituintes sólidos, essas partículas se arranjam formando os agregados, e os espaços vazios entre as partículas e agregados formam os poros. A fase sólida do solo geralmente representa, aproximadamente 50 % do volume do solo (~45% de partículas minerais e ~5% de matéria orgânica). Os outros 50% do volume do solo encontram-se em equilíbrio dinâmico entre água e ar (CARDOSO; ANDREOTE, 2016; FERREIRA, 2010; MOREIRA; SIQUEIRA, 2006).

Os microrganismos do solo influenciam e são influenciados pelo seu habitat. Apesar de representarem uma pequena parcela do ecossistema que habitam, os microrganismos atuam

colonizando os materiais de origem e sedimentos nos primórdios da formação do solo; na sua agregação e nos ciclos biogeoquímicos que nele ocorrem (PAUL, 2007; SCHULZ et al., 2013).

A composição do material de origem do solo, as cargas das argilas (predominantemente negativas), a capacidade de troca de cátions, a superfície específica das partículas entre outras, são características que influenciam os microrganismos. Os microrganismos têm suas propriedades superficiais específicas, como carga, característica dos compostos excretados, entre outras e, essas propriedades são determinantes para a sua sobrevivência, sucessão, interações e atividade. Uma vez aderidos às partículas, os microrganismos ficam menos sujeitos a serem removidos do sistema e mais aptos a explorar os nutrientes e substâncias no seu micro-habitat. Por sua vez, as cargas positivas de células microbianas e hifas de fungos do solo aumentam a estabilidade do agregado ou do complexo argila-bactéria em argilas que apresentam carga líquida negativa (CARDOSO; ANDREOTE, 2016; MOREIRA; SIQUEIRA, 2006; ŠTURSOVÁ et al., 2016).

Os fatores que influenciam os microrganismos do solo são diversos. Entre esses, podemos citar características da fase sólida do solo, presença de substrato, fatores de crescimento, nutrientes, gases, temperatura, radiação solar (nos primeiros centímetros) e fatores relacionados à umidade e solução do solo como composição, pH, potencial redox, força iônica, entre outros (AGUILERA et al., 2016; SANTOYO et al., 2017). Mudanças em algum fator ou perturbações externas, refletem na atividade e até mesmo na diversidade de organismos do ecossistema, os quais buscarão um novo estado de equilíbrio para a comunidade. A rapidez dessa resposta faz com que os atributos microbiológicos do solo sejam considerados os bons indicadores de qualidade do solo.

2.3 Indicadores microbiológicos de qualidade do solo

Sustentar a qualidade do solo é essencial para a manutenção da vida na terra. O solo é responsável pela produção de alimentos para sustentar os mais de sete bilhões de habitantes do planeta Terra. Além desse serviço de provimento, outros serviços ecossistêmicos de regulação e de suporte ocorrem no solo e, sem os quais, a vida no planeta estaria ameaçada (PULLEMAN et al., 2012).

A “qualidade do solo” é "a capacidade de um solo funcionar dentro dos limites de um ecossistema natural ou manejado, para sustentar a produtividade de plantas e animais, manter ou aumentar a qualidade do ar e da água e promover a saúde das plantas, dos animais e dos

homens"(DORAN, 1997; DORAN; PARKIN, 1994). Os indicadores de qualidade do solo são parâmetros químicos, físicos e biológicos que refletem a condição de sustentabilidade do ecossistema. Características importantes para um atributo ser considerado um bom indicador de qualidade do solo são sua relação com processos ecossistêmicos, capacidade de refletir características físicas, químicas e biológicas e sensibilidade a variações (ARSHAD; MARTIN, 2002). Desta forma, atributos microbiológicos do solo são bons indicadores de qualidade pois são sensíveis a variações no ecossistema, apresentam estreita correlação com funções benéficas do solo, podem elucidar processos ecossistêmicos, são úteis para interpretação por gestores e relativamente fáceis de serem mensurados (SCHLOTER; DILLY; MUNCH, 2003).

A diversidade de microrganismos no solo é responsável por garantir serviços e funções ecossistêmicos. Entre os grupos de microrganismos que desempenham funções no ecossistema estão os FMA. Os FMA são simbiontes biotróficos obrigatórios que interagem com mais de 80% das espécies vegetais (SMITH; SMITH; JAKOBSEN, 2003). As hifas desses fungos penetram nas células do córtex tanto inter como intracelularmente formando arbúsculos e desenvolvendo hifas extra-radiculares que apresentam importância significativa para a captação de água e nutrientes em solos, principalmente os nutrientes de baixa mobilidade como o fósforo. Uma vez estabelecida a simbiose, suas hifas funcionam como um prolongamento das raízes do vegetal, podendo também melhorar os aspectos físicos do solo em que se encontram, além de melhorarem a absorção de água e nutrientes pelas plantas (MOREIRA; SIQUEIRA, 2006).

Os benefícios dos FMA e a importância da diversidade desses microrganismos não se limita apenas a melhorias dos aspectos nutricionais das plantas e à melhor agregação do solo. Diversos estudos com esses microrganismos interagindo com plantas tem relatado a minimização da toxicidade às plantas micorrizadas em solos com altos teores de elementos tóxicos (KHAN et al., 2000). Desta forma, como há preferencialidade entre as plantas e os FMA, quanto maior a diversidade presente no solo, maior a chance de a planta estabelecer associação com espécie de FMA mais benéfica. Assim, a diversidade desses microrganismos é especialmente importante como indicadora da qualidade do solo, principalmente pela sua aplicação na recuperação de solos (MATIAS et al., 2009). Uma vez que os FMA possibilitam o estabelecimento de plantas em locais em reabilitação, eles facilitam o reestabelecimento do equilíbrio ambiental pela retomada dos ciclos biogeoquímicos pela adição de carbono orgânico pela planta no sistema.

Além da diversidade de microrganismos, toda a comunidade microbiana, sua atividade e alguns metabólitos são também indicadores de qualidade essenciais para a avaliação da

qualidade do solo. A biomassa microbiana do solo, além de apresentar funções-chave já mencionadas, também é funciona como reserva de nutrientes no solo (SCHLOTER; DILLY; MUNCH, 2003). Desta forma, a biomassa microbiana, além de ser responsável por ciclar os nutrientes, também é fonte desses para o ecossistema. Sendo assim, solos com maior biomassa microbiana estocam e ciclam mais nutrientes no solo.

A respiração do solo é a medida da atividade da biomassa microbiana. O CO_2 é proveniente da oxidação da matéria orgânica do solo pelos microrganismos, sendo a respiração uma função chave no ciclo do carbono. A respiração do solo reflete diversas variações no solo que vão desde os ciclos de umedecimento, adição de substrato até a condições de estresse causadas por fatores externos como contaminantes no solo (CARDOSO; ANDREOTE, 2016; MOREIRA; SIQUEIRA, 2006).

As enzimas do solo são essenciais para o ciclo dos elementos. Os microrganismos do solo são os principais produtores de enzimas no solo. Assim, a atividade enzimática varia de acordo com a atividade microbiana. Por serem fundamentais para os ciclos dos elementos, a atividade das enzimas revela informações sobre funções chave no solo, sendo assim, elas também são boas indicadoras da qualidade do solo (BURNS et al., 2013; GIANFREDA et al., 2002). Entre as principais enzimas indicadoras de qualidade do solo, temos a β-glicosidase, que participa do ciclo do carbono (celobiose), fosfatases ácida e alcalina que atuam no ciclo do fósforo, urease que atua na quebra da ureia no clico do nitrogênio além da atividade do diacetato de fluoresceína (FDA) que quantifica a atividade de hidrólise, refletindo a atividade no geral.

Cabe ressaltar que nenhum indicador de qualidade do solo deve ser usado sozinho. A escolha dos indicadores a serem usados deve levar em consideração variações locais, heterogeneidade de habitats e diferentes micro-sítios de atividade (ocasionados por exemplo, por diferenças de fitofisionomias). Desta forma, o quociente metabólico (razão entre respiração basal do solo e carbono da biomassa microbiana) (ANDERSON; DOMSCH, 1993), e o quociente microbiano (razão entre carbono da biomassa microbiana e carbono orgânico) (SPARLING, 1992), em conjunto com outros indicadores biológicos, físicos e químicos, auxiliam na correta interpretação da qualidade do solo.

2.4 Modelagem e espacialização de dados na Ciência do Solo

A descoberta de novas tecnologias tem levado a avanços nos estudos relacionados a solos. Atualmente se busca não só o aumento da produtividade pelo uso eficiente dos insumos

agrícolas que possibilitam melhorar o manejo (LOPES; GUILHERME, 2016) como também da manutenção do equilíbrio ambiental nessas áreas e em áreas em reabilitação e de preservação ambiental. Desta forma, essas novas tecnologias proporcionam não somente o desenvolvimento da agricultura, como também a sustentabilidade da produção agrícola e a segurança ambiental.

Entre as diversas tecnologias que surgiram nos últimos anos, diversos sensores, que são equipamentos que respondem a um estímulo físico ou químico, podem ter seus dados usados para fins de medição de variáveis relacionadas a outras de mais difícil acesso, possibilitando assim, sua aplicação na agricultura (MANCINI et al., 2019; SILVA et al., 2018b). Sensores que auxiliam na obtenção de dados de solos são de grande importância em estudos ambientais, uma vez que o desenvolvimento de plantas se dá diretamente no solo estando relacionado com a posição na paisagem, localização geográfica e com as características físicas, químicas e biológicas do solo.

Para facilitar a interpretação dos dados, sejam eles de sensores próximos, remotos ou de outros tipos de análises, e diminuir os custos com grande quantidade de amostras para análises, ferramentas de geoprocessamento podem ser utilizadas. As ferramentas de geoprocessamento permitem que os dados coletados em pontos georreferenciados, ou seja, com coordenadas geográficas conhecidas, nas áreas sejam correlacionados com dados obtidos por sensores remotos no local de coleta de amostras de solos e, utilizando as correlações entre informações de dados de sensores espacializados e os dados pontuais, esses últimos podem ser preditos e espacializados para toda a área de estudo. A geoestatística é uma ferramenta aliada do geoprocessamento, utilizada para determinação da distribuição espacial de atributos do solo e outros fatores em função da distância entre os locais de amostras coletadas, permitindo melhor compreensão dos atributos de solo de forma espacial (SILVA et al., 2003).

Para viabilizar o uso das informações obtidas por sensores, os softwares de análises de dados topográficos e geoprocessamento são essenciais. Esses softwares apresentam diversas ferramentas essenciais para a predição e espacialização de dados de solos. Entre eles, temos softwares gratuitos como SAGA GIS (CONRAD et al., 2015), QGIS (www.qgis.org), e pagos como ArcGIS (ESRI). O uso de ferramentas de geoprocessamento facilita a análise de informações obtidas por sensores. Essas ferramentas utilizam modelos matemáticos, estatísticos e geoestatísticos para espacializar informações pontuais obtidas do solo, tendo como base informações já espacializadas obtidas dos sensores. Entre as diversas técnicas utilizadas para espacialização pode-se citar a krigagem, lógica fuzzy, árvores de decisão, random forest, support vector machine, entre outras.

O uso de ferramentas de geoprocessamento e geoestatísticas, além de auxiliar a predição de classes de solo, também pode levar a um ganho significativo de área mapeada (SILVA et al., 2016b), diminuindo os custos com as análises necessárias para o mapeamento e auxiliando a interpretação sobre a aptidão de determinada classe de solo em área não previamente amostrada. O geoprocessamento de dados também facilita a pesquisa em diversas áreas do conhecimento, desde o estudo de bacias hidrográficas e hidropedologia (PINTO et al., 2017) a estudos de florestas (DOS REIS et al., 2018).

Entre os sensores mais utilizados em solos, temos os receptores dos sensores remotos do sistema de posicionamento global (GPS). Os dados obtidos de satélites são de grande utilidade na predição de atributos de solos (SILVA et al., 2016a). A correlação da heterogeneidade do solo com os atributos topográficos obtidos por sensores remotos (satélites) tem sido explorada para auxiliar de diversas formas estudos relacionados a solos. A partir de imagens de satélites, podemos obter uma gama de informações muito úteis e frequentemente utilizadas para mapeamento digital de solos, como geomorfologia, sombreamento, altitude, altitude acima da rede de drenagem, índice de umidade, entre outras (SILVA et al., 2014; MENEZES et al., 2014; SILVA et al., 2016b)

Além dos sensores remotos, diversos sensores próximos têm auxiliado estudos sobre solos. Dentre os sensores próximos utilizados atualmente, o aparelho portátil de fluorescência de raios-X (pXRF) detecta teores totais de elementos em segundos e sem produção de resíduos (WEINDORF; BAKR; ZHU, 2014), sendo capaz de detectar teores totais dos elementos entre o Mg e o U da Tabela Periódica em amostras de solo (RIBEIRO et al., 2017).

Alguns trabalhos têm mostrado bons resultados para predição de atributos químicos do solo, como pH (SHARMA et al., 2014) e capacidade de troca de cátions (SHARMA et al., 2015), mas poucos tentaram associar esses dados a dados de Sistemas de Informação Geográfica (SIG) para geoprocessamento e espacialização dos dados (DUDA et al., 2017), em especial quando se trata do uso de dados de pXRF junto a dados microbiológicos.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 – Arbuscular mycorrhizal fungal community in an iron mining area and its surroundings: inoculation potential and diversity of spores in trap culture related to soil properties

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ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY IN AN IRON MINING AREA AND ITS SURROUNDINGS: INOCULATION POTENTIAL AND DIVERSITY OF SPORES IN TRAP CULTURE

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ABSTRACT: Arbuscular mycorrhizal fungi (AMF) interact symbiotically with most plant species, facilitating revegetation of areas under rehabilitation. The aim of this study was to evaluate the inoculum potential and diversity of AMF spores obtained by trap culture from five phytophysiognomies. Soil samples were collected in a mining area and its surroundings in the *Quadrilátero Ferrífero*, Minas Gerais (Brazil): tailings piles in rehabilitation with grass, canga, Cerrado, native forest, and eucalyptus plantation. Spores were directly extracted from trap cultures (TCs) established in two locations in the Southeast and South regions of Brazil for taxonomic identification of the species. Mycorrhizal inoculum potential was determined thirty days after inoculation. A total of 49 species were captured. Among them, 28 were not captured in field samples. Canga showed higher inoculum potential. The development of TCs in two locations allowed a wider diversity of AMF species to be captured.

Keywords: tailings piles; glomeromycota; ironstone; rehabilitation.

RESUMO: Fungos micorrízicos arbusculares (AMF) interagem simbioticamente com a maioria das espécies de plantas, facilitando a revegetação de áreas sob reabilitação. O objetivo deste trabalho foi de avaliar o potencial de inóculo e a diversidade de esporos de AMF obtidos por cultura armadilha em cinco ambientes. Amostras de solo foram coletadas em cinco ambientes em área de mineração e seu entorno no Quadrilátero Ferrífero, Minas Gerais (Brasil): pilha de rejeitos em reabilitação com capim, canga, Cerrado, mata nativa e plantação de eucalipto. A extração de esporos de culturas armadilha (TCs), estabelecidas em dois locais nas regiões Sul e Sudeste do Brasil, foi feita para identificação taxonômica das espécies. Foi determinada a riqueza de espécies. Potencial de inóculo micorrízico foi determinado 30 dias após inoculação. O total de 49 espécies foi capturada. Entre estas, 28 foram capturadas em

amostras de campo. Canga apresentou o maior potencial de inóculo. O desenvolvimento de TCs em dois locais faz com que maior diversidade de espécies seja capturada.

Palavras-chave: pilhas de rejeitos; glomeromycota; canga; reabilitação.

INTRODUCTION

Brazil is the third largest producer of iron ore worldwide, and approximately 70% of this ore is extracted in the state of Minas Gerais (Brasil, 2016), which has areas with high concentrations of iron (Fe). One of these areas in the central part of Minas Gerais is known as the *Quadrilátero Ferrífero,* which is of great historical-cultural and economic importance due to iron ore extraction (Carvalho Filho; Curi; Shinzato, 2010). However, although the extraction of Fe is economically important, iron mining brings about changes in the landscape, with impacts on plant cover, on soil biodiversity, and, consequently, on the biogeochemical cycles of the elements (Siqueira et al., 2007; Xing et al., 2015) since the process of opening mining pits involves removing soil over the ore (which is sterile) and depositing it in other locations, forming piles.

Surrounding these mining areas in the *Quadrilátero Ferrífero*, there are diverse floristic domains with vestiges of secondary forest that are characteristic of the Atlantic Forest and *Cerrado* (Brazilian tropical savanna) biomes, both considered as worldwide hotspots of diversity (Hopper; Silveira; Fiedler, 2016). The Cerrado, particularly, is recognized as one of the most ecologically important savannas of the world due to its high diversity of habitats, home to an estimated 11,627 species of native plants (Brasil, 2015). Among the habitats of the Cerrado, the *canga* is prominent through its association with ferruginous outcroppings and underground hardpans, its scarcity of soil volume, and the intense daily variation in

temperature, which hinders establishment of plants and makes it one of the most endangered ecosystems of Brazil (Matias et al., 2009; Skirycz et al., 2014).

The stability and resilience of the environment depends on the biodiversity that ensures ecological services (Mori; Furukawa; Sasaki, 2013). Revegetation can provide the biodiversity necessary for recovery of areas impacted by mining. In this context, arbuscular mycorrhizal fungi (AMF – Glomeromycota phylum) represent important components of the soil biota that promote diverse services in the ecosystem (Pellegrino; Bedini, 2014), such as better plant nutrition and growth (Thirkell; Cameron; Hodge, 2016), soil aggregation (Leifheit; Verbruggen; Rillig, 2015), and an increase in plant tolerance to biotic stresses (e.g., pathogens) (Liang et al., 2015) and abiotic stresses (e.g., potentially toxic elements in the soil) (Cabral et al., 2015), and assume an important role in the rehabilitation process of areas affected by mining. However, since AMF are necessarily biotrophic, they are also affected by removal of original vegetation and interventions in the soil of an area. Removal of the surface layer of the soil for ore extraction and creation of tailings piles has a negative influence on soil structure and can reduce its mycorrhizal inoculation potential since this breaks down the network of infective hyphae in the soil (Siqueira et al., 2007; Soares; Carneiro, 2010).

Among the propagules of AMF able to begin mycorrhizal colonization, such as hyphae in the soil and colonized roots, spores are the most resistant fungal structures and hold the important morphological characteristics for determination of the species (Pagano et al., 2016). Analysis of the diversity of AMF species and of inoculum potential in areas affected by mining and their surroundings represents one step in identifying the fungal species with potential for use in revegetation processes for the purpose of recovering areas affected by mining. However, few studies have evaluated the diversity of AMF in Fe mining and deposition areas.

Rehabilitation of mining areas is normally performed through planting of grasses, which assist in stabilization of the tailings piles. In dealing with soil with human intervention, studies

comparing the soil biota of these areas to less altered surrounding areas can indicate the effectiveness of the recovery process adopted. Thus, the aim of this study was to evaluate the potential of mycorrhizal inoculum, and diversity of AMF spores obtained by trap culture in an area in rehabilitation after Fe mining and its surroundings, consisting of five phytophysiognomies: tailings piles in rehabilitation, canga, Cerrado, forest, and planted eucalyptus forest.

MATERIAL AND METHODS

Study area

The study was carried out at the Córrego do Meio mine (19°51'41.23"S, 43°48'11.13'W), totally deactivated in 2006, in the municipality of Sabará, MG, Brazil, in five different phytophysiognomies: tailings piles in rehabilitation (TP), canga (CN), Cerrado (CE), forest (FT), and planted eucalyptus (PE) (Table 1, Figure 1). The five phytophysiognomies evaluated are located within the morphostructural unit of the *Quadrilátero Ferrífero*. Climate in the region is highland tropical, Cwa according to Köppen, with warm and humid summers and cold and dry winters. Mean annual rainfall is 1700 mm, with a short dry period in the winter, and mean annual temperature is 22°C.

In each sampling phytophysiognomy, four soil samples were collected. Each sample was composed of 12 subsamples collected at the depth of zero to 20 cm, with four of them taken at three meters and eight taken at six meters from the georeferenced center point (Figure 2), according to the sampling arrangement proposed by Huising et al. (2008). Samples were subjected to analysis of chemical and physical properties (personal information from Patrícia de Freitas Costa)

Table 1 - Description and range of elevation of the phytophysiognomies studied at the Córrego do Meio mine, state of Minas Gerais, Brazil.

Phytophysiognomy	Description	Range of Elevation (m)	Soil Class[1]
Tailings piles	Area in process of environmental recovery after iron mining, replanted with molasses grass (*Melinis minutiflora* P. Beauv.).	1041 to 1060	Anthrosol
Canga	Rocky environment well preserved over rocky outcroppings.	1196 to 1201	Litholic Neosol
Cerrado	Typical Cerrado vegetation with a low degree of anthropic influence.	992 to 1006	Haplic Cambisol
Planted eucalyptus forest	Reforested area planted predominantly with *Eucalyptus* spp.	926 to 971	Haplic Cambisol
Forest	Secondary vegetation at different stages of natural regeneration, originally belonging to the Atlantic Forest biome.	880 to 966	Haplic Cambisol

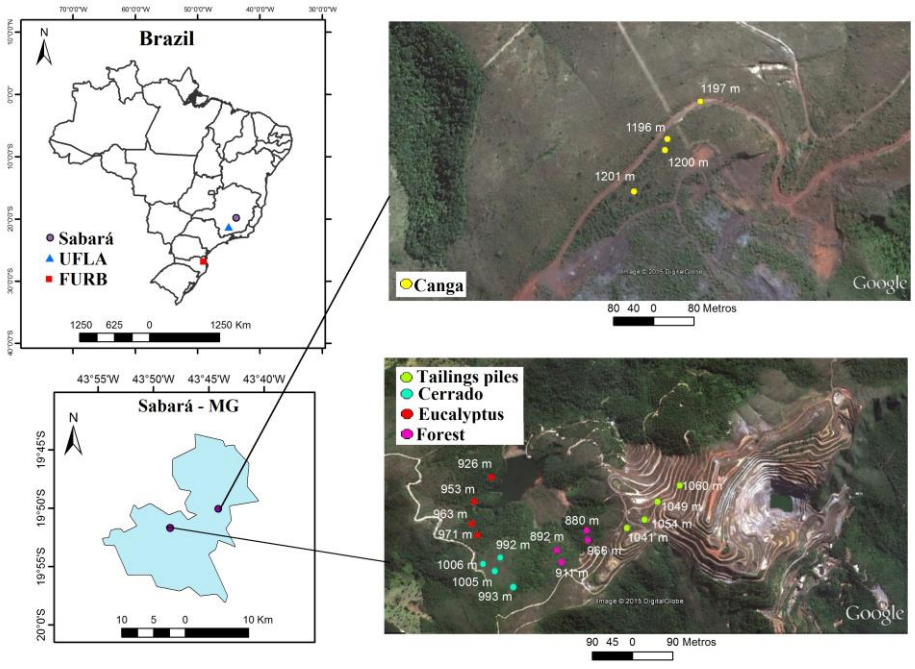

Figure 1- Location of study area.

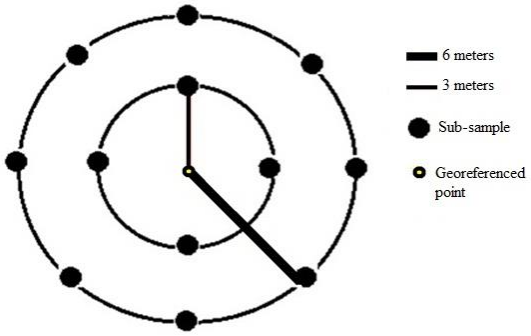

Figure 2- Soil sampling system

Composite soil samples that were used for microbiological analyses were homogenized and arranged for transport to the Soil Biology, Microbiology, and Biological Processes Laboratory of the Universidade Federal de Lavras (UFLA), where they were placed in cold storage at 4°C until evaluation.

Trap cultures

Trap cultures (TCs), used to try to capture species that were not found sporulating on field soil, were set up in the Soil Science Department of the Universidade Federal de Lavras (UFLA) in Lavras, Minas Gerais, in the Southeast region of Brazil, and in the Natural Sciences Department of the Universidade Regional de Blumenau (FURB) in Blumenau, Santa Catarina, in the South region of Brazil. The climate of Lavras, according to the Köppen climate classification, is Cwa, rainy temperate (mesothermal), with a dry winter and rainy summer, subtropical; and the mean temperature of the hottest month is greater than 22°C. Blumenau has a Cfa climate, constantly humid, subtropical, without a dry season and with hot summers (mean temperature of the hottest month is greater than 22°C). Mean annual temperature ranges from 19.1 to 20.0 °C; monthly maximum temperatures range from 26.0 to 27.6 °C and monthly minimum temperatures range from 15.4 to 16.8 °C (Thomé et al., 1999). The greenhouse at FURB is made of alveolar polycarbonate and is shaded during part of the morning, whereas the

greenhouse at UFLA is made of glass and is not shaded.

To establish the trap cultures, 500 mL of inoculum soil of each sample were mixed with 500 mL of sterile sand. This mixture was placed between two layers (200 mL each one) of sterile sand in 1.5 kg pots. Eighty seeds of palisade grass (*Urochloa brizantha* (Stapf) R.D.Webster) were sown and the plants were kept in a greenhouse for five months with application of Hoagland solution, with zero or 50% of the standard phosphorus (P) concentration, according to the nutritional needs of the plants in the initial stage. After the period of cultivation, the soil of each pot was homogenized and placed in cold storage at 4ºC until evaluation.

The AMF spores were extracted from 100 mL of each soil sample following the wet sieving and decanting technique (Gerdemann; Nicolson, 1963), combined with the water and 50% sucrose centrifuge technique (Jenkins, 1964). Spores were observed in a stereo microscope and separated into morphotypes according to color, size, and shape and mounted on slides with polyvinyl lactoglycerol (PVLG) and Melzer reagent for observation on a microscope and taxonomic identification through spore morphology. AMF species were identified considering the size, shape, color, presence of subcellular structures of the spores, and comparison with the descriptions contained in the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (Morton, 2016) and in Błaszkowski (2012). In addition, the presence of sporulating species was registered.

Inoculum potential

For evaluation of mycorrhizal inoculum potential, 20 mL of inoculum soil from each sample was placed in 90 mL of a mixture (1:1) of sterile soil and sand in plastic containers in duplicate for each composite field sample. Thirty seeds of *Urochloa brizantha* were added, maintaining from 5 to 10 plants. Thirty days after establishment, the roots were separated from

the substrate and washed, and 1 g of them was placed in capsules to be clarified and stained with trypan blue (0.05%), according to the method of Koske and Gemma (1989). Determination of the mycorrhizal colonization percentage was estimated by the intersection method in a square laboratory dish (Giovannetti; Mosse, 1980) and used as an estimate of the mycorrhizal inoculum potential.

Analysis of the AMF Community and Statistical Analyses

Species richness (R) was calculated as the number of species present in each phytophysiognomies. In regard to capture in different locations, species found in trap cultures at FURB and at UFLA were classified as present (1) or absent (0) in each phytophysiognomy.

The inoculum potential was evaluated through Analysis of Variance (ANOVA), followed by the Scott-Knott test at the level of 5% significance. Analyses were carried out using the Assistat statistical software (Silva; Azevedo, 2016). Principal component analysis was performed using soil fertility, spore count and inoculum potential data in the R software (R Development Core Team, 2017) with vegan package (Oksanen et al., 2017).

RESULTS AND DISCUSSION

Diversity of Spores in Trap Cultures

The data obtained in field extraction of spores can be observed in the original article (TEIXEIRA et al., 2017) and, although it has been used to assist in the discussion of this chapter, will not be mentioned here because they are part of the master's thesis. Considering both locations (UFLA and FURB) used for carrying out TCs, a total of 49 species were captured (Table 2). Among them, 28 were not captured in field samples, thus leading to an increase of more than 90% in the total number of species in relation to direct extraction from the soil in the field. The only species found both in the field and in the TCs considering all the

174 phytophysiognomies was *Acaulospora morrowiae*. Fourteen species were captured in the
175 phytophysiognomies in addition to those found by direct extraction from the field.

176 The capture of species only in TCs and not from direct extractions in the field shows the
177 importance of this technique for studies of AMF diversity since some species may exhibit low
178 or no sporulation in the field (Stutz; Morton, 1996). Some species for which spores had not
179 previously been found in the field sporulated in TCs, showing that there were viable propagules
180 able to establish association with the trap species (*U. brizantha*). In contrast, some species
181 whose spores were found in the field were not captured in TCs. This may be explained by the
182 low density of spores of some AMF species found in the field, and may also be due to the
183 preferences of the trap species itself since the host plant is able to show preference for AMF
184 species that are more efficient in the association (Bever et al., 2009).

185

186 Table 2 - Arbuscular mycorrhizal fungi (AMF) species found in Trap Cultures
187 (TCs), their occurrence at the Universidade Federal de Lavras (UFLA) and the
188 Universidade Regional de Blumenau (FURB) (1 = presence; 0 = absence of
189 species), and total species richness (R) in tailings piles (TP), Cerrado (CE), canga
190 (CN), planted eucalyptus (PE), and Forest (FT), or species not found in direct
191 extraction in the field (N).

AMF species in TCs	UFLA					FURB					Site of direct extraction
	TP	CE	CN	PE	FT	TP	CE	CN	PE	FT	
Family Archaeosporaceae											
Archaeospora trappei	0	0	0	0	1	1	1	1	0	0	CE
Family Ambisporaceae											
Ambispora leptoticha	0	1	1	1	1	1	1	0	1	1	CE PE FT
Family Acaulosporaceae											
Acaulospora alpina	0	0	0	0	1	1	0	0	0	1	TP
Acaulospora colombiana	0	1	0	0	0	1	1	0	0	1	TP CE PE
Acaulospora delicata	0	0	0	0	0	1	1	0	1	0	N
Acaulospora foveata	0	0	0	0	0	0	1	1	1	0	N
Acaulospora lacunosa	0	0	0	0	1	0	1	1	0	1	CN FT
Acaulospora mellea	1	1	0	1	1	1	1	1	1	1	TP CN PE FT
Acaulospora morrowiae	1	1	1	1	1	1	1	1	1	1	TP CN PE FT

192 Continue...

193 Table 2 continuation...

AMF species in TCs	UFLA					FURB					Site of direct extraction
	TP	CE	CN	PE	FT	TP	CE	CN	PE	FT	
Acaulospora rehmii	1	0	0	0	1	1	0	0	0	1	N
Acaulospora scrobiculata	1	0	1	0	1	1	0	0	0	1	TP
Acaulospora sp1	0	0	0	0	0	1	0	0	0	1	N
Acaulospora sp2	0	0	0	0	0	0	1	0	1	1	N
Acaulospora spinosa	1	0	0	0	0	1	0	0	0	1	TP FT
Acaulospora spinosissima	0	0	0	0	0	1	0	0	0	1	N
Family Diversisporaceae											
Corymbiglomus tortuosum	0	0	0	0	0	0	1	0	0	0	N
Diversispora sp.	1	0	1	1	0	1	0	1	1	1	TP CN PE FT
Family Gigasporaceae											
Cetraspora pellucida	1	0	0	0	0	0	0	1	0	0	TP FT
Dentiscutata biornata	0	0	1	0	0	0	1	1	0	0	CN
Dentiscutata heterogama	0	0	1	1	0	1	0	0	0	0	CE
Dentiscutata cf. *scutata*	1	0	0	0	0	1	1	1	0	1	N
Gigaspora albida	0	0	0	0	0	1	0	0	1	1	N
Gigaspora gigantea	0	0	0	0	0	0	0	1	0	0	N
Gigaspora sp.	1	0	0	0	0	0	0	0	0	0	TP CN
Scutellospora pernambucana	0	0	0	0	0	0	1	0	0	1	N
Family Claroideoglomeraceae[1]											
Claroideoglomus etunicatum	0	0	0	0	0	1	0	0	0	0	N
Family Glomeraceae											
Dominikia sp.	1	1	1	1	0	1	1	0	1	1	TP PE
Glomus cf. *aggregatum*	1	0	0	0	0	0	0	0	0	1	N
Glomus glomerulatum.	0	0	0	1	1	1	1	1	1	1	N
Glomus cf. *invermaium*	1	0	0	0	0	0	0	0	0	0	N
Glomus microaggregatum	0	0	0	0	0	1	0	1	1	1	TP ,CN, FT
Glomus microcarpum	0	0	0	0	0	0	1	1	0	1	N
Glomus sp2	1	1	1	1	1	0	0	0	0	0	T
Glomus sp3	0	1	0	1	1	0	0	0	0	0	N
Glomus sp4	1	0	1	0	0	0	0	0	0	0	N
Glomus sp5	0	0	1	0	0	0	0	0	0	0	N
Glomus sp6	0	0	0	0	0	1	0	0	1	1	N
Glomus sp7	0	0	0	0	0	0	0	0	0	1	N
Glomus sp8	0	0	0	0	0	1	0	0	0	1	N
Glomus spinuliferum	0	0	0	0	0	1	1	0	0	0	N
Rhizophagus clarus	1	0	0	1	0	1	1	0	0	1	TP CE PE FT
Rhizophagus fasciculatus	0	0	0	0	0	1	1	0	1	1	CE, PE
Rhizophagus diaphanus	1	1	1	1	1	0	0	0	0	0	FT
Sclerocystis coremioides	0	0	0	0	0	1	1	1	0	1	N
Sclerocystis taiwanensis	0	0	0	0	0	0	0	0	0	1	N
Sclerocystis sinuosa	0	0	0	0	0	0	0	0	0	1	N
Septoglomus sp1	0	0	0	0	0	1	0	0	0	0	N
Septoglomus viscosum	0	1	1	1	1	1	1	1	1	1	TP
Incertae sedis											
Entrophospora infrequens	1	0	0	0	0	1	1	0	0	1	N
R	17	9	12	12	13	29	22	15	14	31	

194

195 It is believed that the species *Glomus* sp2 has greater capability of establishing
196 association with plants and sporulating under the climate conditions of the TCs at UFLA, since

it did not sporulate in TCs at FURB. Since this species was found in a higher number of spores in all field areas, it may also have preferentially colonized the roots of the trap species in detriment to other species present, as reported by Bever et al. (2009). Thus, the TCs at UFLA captured a much lower number of species. However, the greater richness of species captured at FURB may have been determined by the fact that the greenhouse was shaded part of the day and that environmental conditions at this site are quite different from those of the *Quadrilátero Ferrífero* compared to UFLA. As the AMF species respond in different manners and have different tolerances depending on the disturbance (Van der Heyde et al., 2017), the sporulation of a greater number of species at FURB may be due to the response of the AMF species in overcoming adversity and surviving under environmental conditions different from their conditions of origin. Lower insolation at FURB may have resulted in milder temperatures, allowing better development of the plants and, consequently, greater investment in mycorrhization on the part of the host plant.

The TCs at UFLA were able to capture 28 AMF species, whereas at FURB, 43 were captured; a considerable increase in species richness could be observed with the use of TCs under different environmental conditions (Table 3). The species *Rhizophagus diaphanus* and *Glomus* sp2 sporulated in all the TCs at UFLA and did not sporulate at FURB.

Canga, when evaluated only by field spores, exhibited lower R and H' (TEIXEIRA et al., 2017); however, when evaluated in TCs (Table 3), species richness equal to that of the PE phytophysiognomy was observed. The TP phytophysiognomy, when only field spores were evaluated, was the phytophysiognomy that exhibited the highest R, followed by FT and CE, and the highest H'. A total of 59 species were found in all the phytophysiognomies considering TCs and direct extraction from the field and, among them, 15 could not be identified at the species level.

Table 3 - Species richness of arbuscular mycorrhizal fungi and increases in species richness by the use of trap cultures (TCs) at the Universidade Federal de Lavras (UFLA) and the Universidade Regional de Blumenau (FURB) in soils from tailings piles replanted to grass (TP), Cerrado (CE), canga (CN), planted eucalyptus (PE), and forest (FT).

	TP	CE	CN	PE	FT
Direct extraction from soil	20	14	9	14	15
Richness increases TCs UFLA	7	3	8	5	7
Richness increases TCs FURB	18	15	9	8	23
Total richness increases TCs	22	17	16	11	25
Total richness	42	31	25	25	40

The lower diversity of AMF spores found in CN may be related with the small islands of soil and other factors that hinder establishment of plants in these environments (Skirycz et al., 2014). In a similar way, in PE, the lower diversity of plant species may have influenced the lower diversity of AMF spores. Thus, as these fungi are necessarily biotrophic, the stability of the ecosystems in regard to the constant presence of hosts can ensure the survival of AMF species (Siqueira; Colozzi-Filho; Oliveira, 1989). Even though lower in relation to CP, CE, and FT, the diversity of AMF spores found in CN is the greatest yet reported so far in studies of these areas (Matias et al., 2009) and may be contributing to conservation of this environment.

Although disturbances affect soil properties and may diminish the diversity of AMF (Lins et al., 2006), higher R and H' found in TP in the field, as well as greater total richness of species, indicate that AMF diversity was not only recovered, but also increased in the phytophysiognomy in rehabilitation. Since there is preferentiality between macro- and microsymbionts in mycorrhizas (Bever et al., 2009; Kiers et al., 2011), this greater diversity of AMF species can facilitate the entrance of mycotrophic plant species in the phytophysiognomy, thus assisting the continuity of its rehabilitation process. Considering that there are currently only 289 species of AMF described worldwide (Goto, 2017), the species described in this study

represent 15% of all species, showing that these phytophysiognomies are hotspots of diversity of AMF.

Inoculum Potential

Inoculum potential differed among the phytophysiognomies evaluated (Figure 3), with the CN phytophysiognomy showing the highest potential, with 39.9% of the roots with colonization. The CE phytophysiognomy exhibited the second highest inoculum potential, with 22.7% of the roots colonized, whereas the other phytophysiognomies did not differ among themselves and exhibited inoculum with lower potential for colonization, with fewer than 13% of roots colonized by AMF.

The viability of AMF propagules in the soil can decrease in accordance with the disturbance to which they are subjected, thus reducing their infective capacity (Trejo; Barois; Sangabriel-Conde, 2016). However, the infective capacity of the AMF species is not related to propagule density (Caproni et al., 2003; Santos et al., 2000), since it infective capacity was not correlated with spore density in the present study. In contrast, dormancy of the spores varies considerably among species (Juge et al., 2002). Greater infectivity of the inocula of CN shows that although it exhibits the lowest species diversity and has spore density similar to the other phytophysiognomies, the inocula quickly colonize the host and can assist in the resilience of this environment subject to stress factors.

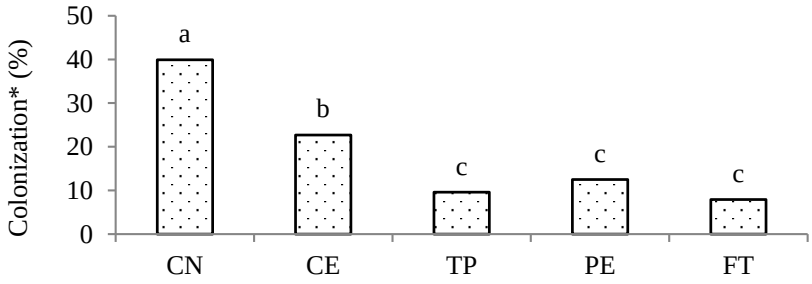

Figure 3 - Mycorrhizal inoculum potential given by the percentage of mycorrhizal colonization of arbuscular mycorrhizal fungi in *Urochloa brizantha* inoculated with soil from the

265 phytophysiognomies of tailings piles replanted to grass (TP), Cerrado (CE), canga (CN),
266 planted eucalyptus (PE), and forest (FT). * Mean values followed by the same lowercase letters
267 do not differ statistically by the Scott-Knott test at the level of 5% significance.
268
269 The inoculum potential and number of spores in the trap culture experiment was
270 influenced by organic matter content, Fe, cation exchange capacity at pH 7 and effective and
271 potential acidity (Figure 4). It was also observed that higher values of pH, Mn and silt inversely
272 influence inoculum potential and sporulation.

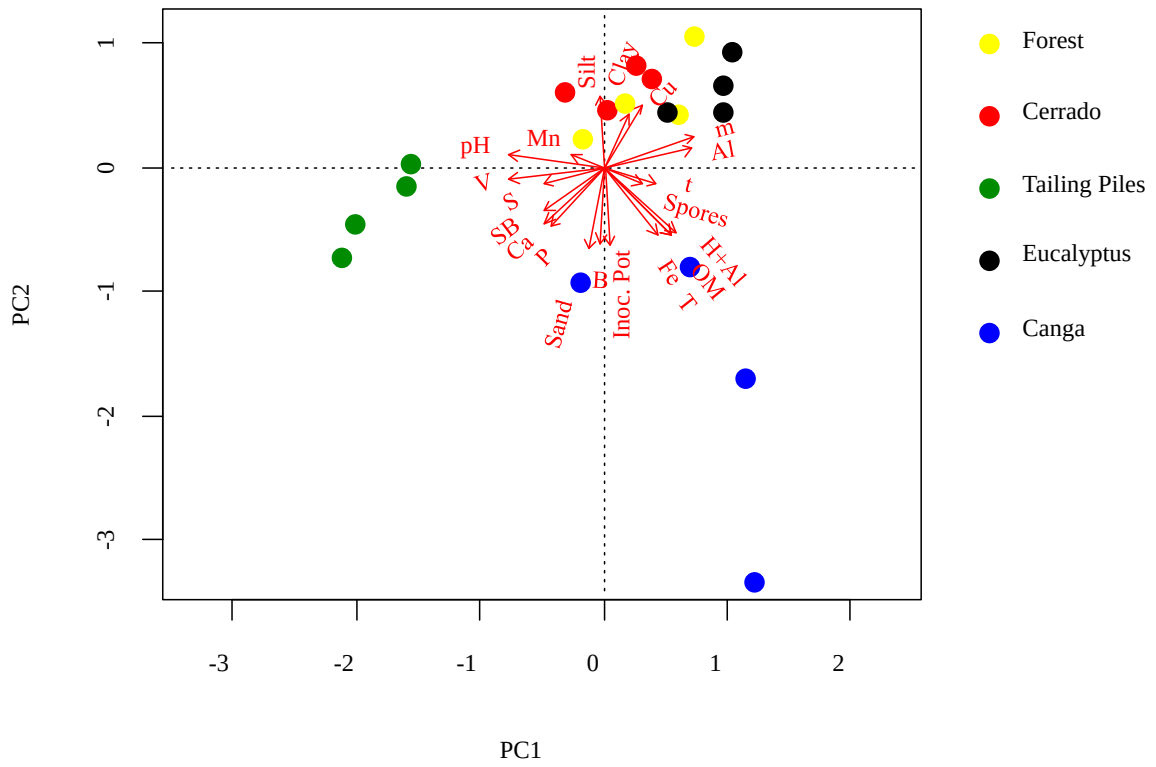

Figure 4 – Ordination diagram of Principal Component Analysis of soil properties, inoculum potential (Inoc. Pot.), and sporulation (Spores) related to the ordination axes for the five phytophysiognomies studied: forest, canga, Cerrado, tailings piles replanted to grass, and planted eucalyptus.

CONCLUSIONS

The greater potential of mycorrhizal inoculum in the canga phytophysiognomies can contribute to resilience in this phytophysiognomy.

The use of Trap Cultures in both locations of Brazil increases the diversity of AMF species captured insofar as different species tend to develop better under distinct environmental conditions.

Abiotic factors related to soil texture and fertility influence the inoculum potential of arbuscular mycorrhizal fungi.

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ARTIGO 2 – Prediction of microbiological indicators of soil quality: A proximal and remotely sensed data approach

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Abstract

Microbiological indicators of soil quality respond to changes in the environment before other indicators. For large-scale determination of soil quality, many sampling points are needed. The search for techniques that allow for the reduction of the quantity of samples evaluated and the accurate prediction of values at non-sampled places within an area are vital in advancing soil science. This work sought to predict soil microbiological attributes based on soil fertility and soil physical data, elemental concentrations determined by portable X-ray fluorescence (pXRF), and terrain attribute data. Soil was collected in dry and rainy seasons in four phytophysiognomies of the *Quadrilátero Ferrífero* in Minas Gerais, Brazil. Biomass microbial carbon (BMC), basal soil respiration (BSR), metabolic quotient (qCO_2), and microbial quotient (qMic) were used as microbiological indicators of soil quality. Values of terrain attributes, soil fertility, and texture attributes, and elemental concentrations obtained by pXRF, as well as phytophysiognomy and season of collect (dry or rainy) were used (separately and together) to predict each of the microbiological indicators. Predictions were performed using conditional random forest modeling and leave one out cross-validation. These methods are not intended to replace conventional analyses of microbiological indicators, but to reduce the cost, time, and number of samples collected and laboratory analyses needed to obtain microbiological information. The best predictions were obtained when phytophysiognomy and season where included as predictors. BSR was better predicted when using only terrain attributes as predictors ($R^2 = 0.91$). The qCO_2 was best predicted by the model using fertility and texture data together with terrain data ($R^2 = 0.79$). The qMic was best predicted when using only soil fertility and texture data. BMC and qMic presented lower coefficient of determination values ($R^2 \geq 0.65$) while the highest value was found for BSR ($R^2 = 0.91$). Therefore, it is possible to predict the microbiological indicators BMC, BSR, qCO_2, and qMic from soil fertility, physical data, and terrain attributes. Terrain attributes can be used to predict microbiological indicators of soil quality, and may be useful for spatial analysis of these attributes across an area of interest.

Keywords: Soil microbiology, cforest, soil basal respiration, microbial biomass carbon, prediction models

1 Introduction

Soil is a complex and dynamic environment responsible for several ecosystem processes that support life. Microbiological indicators of soil quality, such as basal soil respiration and microbial biomass carbon, are important attributes for detecting soil disturbances, as they are sensitive to variations allowing rapid assessment of soil quality (Krüger et al., 2017; Santos et al., 2016). The microorganisms, their metabolism, and metabolites in the soil, respond to natural or anthropic variations such as temperature, precipitation, soil revolving, deposition of residues, and changes of land use and cultivation systems commonly reflect changes in the environment (Bonilla-Bedoya et al., 2017; Lopes et al., 2018; dos Santos et al., 2013; Muñoz et al., 2017; Silva et al., 2018a). For an accurate interpretation of these indicators, it is necessary to collect a high number of samples throughout large areas, which can be an obstacle to more frequent use of these important attributes in decision making on land use and management.

Soil physical properties (e.g., texture) and chemical attributes related to fertility, such as pH, CEC, and nutrient availability influence soil metabolism and microbial processes (Ragot et al., 2016; Souza et al., 2016). These attributes have been commonly studied alone or together with the microbiological indicators to verify if these and any other factors provokes a response of the microbiota (Santos et al., 2013; Muñoz et al., 2017). These soil attributes are known to influence soil microbiota and consequently, soil biological quality (Silva et al., 2018a). For instance, pH, moisture and distribution of organic fractions in the soil are key drivers of microbial communities (Burt and Butcher, 1985; de Carvalho et al., 2016; Fierer and Jackson, 2006; Florinsky et al., 2004; Jesus et al., 2009; Teixeira et al., 2017; Yu et al., 2017; Zhu et al.,

2017). Soil attributes related to fertility and physical properties have been widely studied and they are commonly used to assist decision making on soil management. However, many different chemical reagents, specialized manpower and time are needed to evaluate each of these attributes. The same is true for soil microbiological indicators, which are frequently less used than soil fertility indicators (Joergensen and Brookes, 1990 McLean et al., 1958; Shoemaker et al., 1961).

With the development of remote sensing technology and data processing software, obtaining information on terrain features has become easy. Increasingly, better satellite images are being used to extract information and create digital elevation models that show information about topography and terrain continuously over an area of interest (Gupta, 2018). This information is now easily obtained, at low costs, and has been efficiently used mostly for prediction of several soil physical and chemical properties (Arrouays et al., 2014; Florinsky et al., 2002; Lecours et al., 2017; McKenzie and Ryan, 1999; Menezes et al., 2018; Silva et al., 2016a; Zhu et al., 2010). The relationship between terrain attributes and specific groups of organisms, such as denitrifiers, has already been shown (Florinsky et al., 2004) and slope has also been reported as a topographic factor influencing soil quality (Nabiollahi et al., 2018). Although studies have already verified the influence of topographic attributes on specific groups of organisms, works using terrain information to predict and spatially render microbiological attributes related to soil quality indicators are less common (Berryman et al., 2018; Kaleita et al., 2017; Zhang et al., 2018; Zhong et al., 2018).

The spatial analysis of microbiological indicators of soil quality allows observation of how each indicator varies throughout each area. Spatial analysis techniques have been widely used for making maps of soil classes and properties (Abd-Elmabod et al., 2017; Silva et al., 2016a). These techniques are useful because they allow inferences about attribute values at non-sampled points across the evaluated area (Mancini et al., 2019), and can improve the cost-benefit of

attribute mapping due to the smaller number of sampling points required when remote sensing data is used as predictor variables.

In addition to remote sensors, proximal sensors have also been successfully used for better soil characterization and prediction of its attributes (O'Rourke et al., 2016; Peluco et al., 2015; Silva et al., 2016b; Weindorf et al., 2018). Among these sensors, the portable X-ray fluorescence spectrometer (pXRF) has been increasingly used in recent years for analysis of soil, water, and vegetation (Cardelli et al., 2017; McGladdery et al., 2018; Pearson et al., 2017; Peinado et al., 2010; Weindorf et al., 2012a). The pXRF is able to determine the concentration of elements from Mg to U in the soil and other materials, quickly and without generation of chemical waste (Ribeiro et al., 2017; Weindorf et al., 2014). The contents of the elements obtained by pXRF have also been efficiently used for the prediction of soil physical and chemical attributes (Aldabaa et al., 2015; Chakraborty et al., 2016; Duda et al., 2017; Silva et al., 2017; Teixeira et al., 2018; Wang et al., 2015; Weindorf et al., 2012b; Zhu et al., 2011). Weindorf et al. (2018) successfully applied visible near infrared diffuse reflectance spectroscopy for modeling of soil biological properties. However, there are still no studies on the use of elemental data produced by pXRF in predicting microbiological attributes of soil quality.

Mathematical models are used for data prediction; regression and machine learning algorithms such as random forest are common approaches for application development of proximally sensed data (Sharma et al., 2014; Towett et al., 2015; Wang et al., 2015; Zhu et al., 2011). Models based on random forests have achieved quality results for predicting soil attributes (Cardelli et al., 2017; Pelegrino et al., 2018; Silva et al., 2017). Importantly, modeling and spatial prediction are location specific. Further, these approaches are not meant to replace laboratory analyses of microbiological indicators of soil quality. Rather, they can be an

alternative to reduce the number of samples to be collected and analyzed, as well as promote predictions at non-sampled locals within a given area.

Thus, this work aimed to predict microbiological attributes of soil quality based on soil fertility and texture data, chemical elemental contents obtained by pXRF, and terrain attribute data. The hypotheses tested were a) it is possible to predict microbiological attributes of soil quality from data of soil fertility, texture, pXRF elemental data, and terrain attributes, and b) while modeling microbiological parameters, terrain attributes and pXRF reported soil elemental data can produce comparable prediction accuracy to those produced by soil fertility and texture data.

2 Methods

2.1 Soil sampling

Soil samples were collected in four phytophysiognomies, in the municipalities of Brumadinho and Nova Lima, located in the *Quadrilátero Ferrífero*, in the state of Minas Gerais, Brazil. The climate of the region features cold, dry winters and warm, rainy summers; this corresponds to the Cwa classification per the Köppen system (Alvares et al., 2013), with mean annual temperature of 21° C and precipitation of 1390 mm.

The area contains four vegetation types with contrasting characteristics (phytophysiognomies) described by Castro et al. (2017) and Silva et al. (2018) as follows: Ironstone Outcrops (IO) (known as “canga”) on Petric Plinthosol (Typic Plinthaquox), Neotropical Savanna (NS) (known as “Brazilian Cerrado”) on Petric Plinthosol (Typic Plinthaquox), Atlantic Forest (AF) on a toposequence of Red Latosol (Rhodic Haplustox), Red-Yellow Latosol (Typic Haplustox), and Haplic Cambisol (Typic Dystrustept); rehabilitated area revegetated with grass (RA) is found on anthropic soil (Figure 1) (Coelho et al., 2017) [soils classified per Brazilian Soil Classification System (Embrapa, 2013) and per Keys to Soil

126 Taxonomy (Soil Survey Staff, 2014), the latter in parenthesis]. The IO and NS
127 phytophysiognomies are located at the Ferrous Technology Center (Miguelão) (Nova Lima –
128 MG), while the phytophysiognomies under AF and RA are located in the Córrego do Feijão
129 Mine (Brumadinho – MG).

130 Soil sampling was conducted in both dry (August, 2015) and rainy (January, 2016) seasons
131 in two transects in each phytophysiognomy with 50 m of distance between each other. Ten
132 samples composed of five sub-samples were collected at depth of 0 to 20 cm in each
133 phytophysiognomy. Subsamples were collected as follows: from a central position within the
134 transect, the first subsample was collected, followed by the collection of four other subsamples
135 at 5 and 10 m to the west and east of the central subsample, comprising five subsamples (Silva
136 et al., 2018a).

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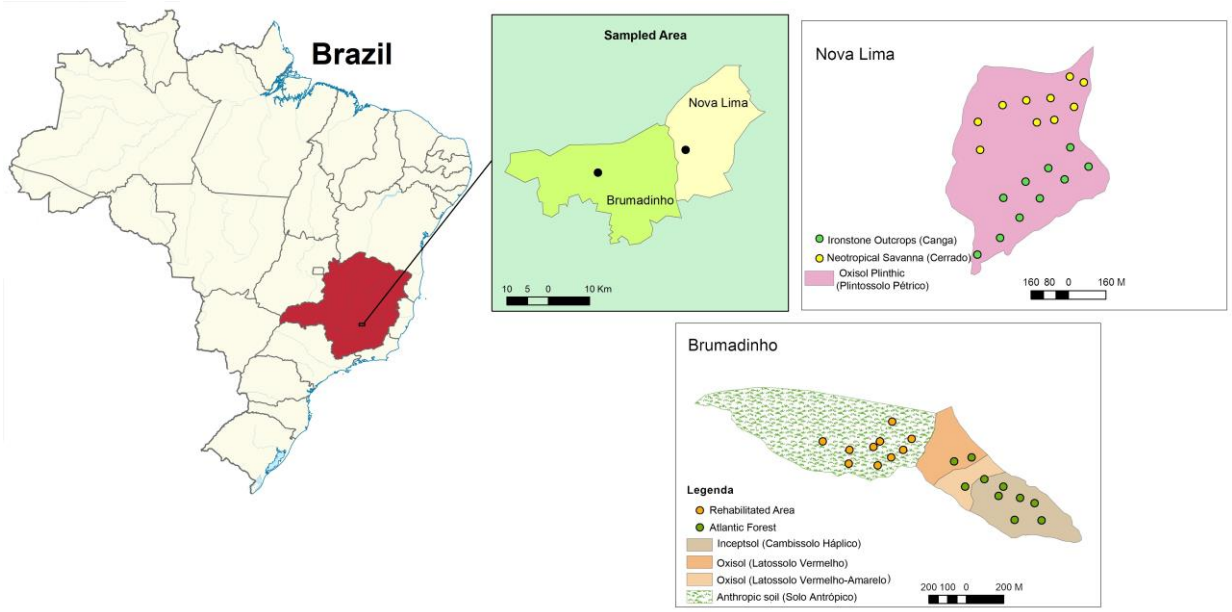


138

139 Figure 1: Location of the soil sampling sites at the Ferrous Technology Center (Miguelão
140 - Nova Lima) and the Córrego do Feijão Mine (Brumadinho) in Minas Gerais State, Brazil.

2.2 Soil analysis

The microbiological indicators of soil quality used in this study were determined by Silva et al. (2018), including organic carbon (C_{org}) (Walkley and Black, 1934), biomass microbial carbon (BMC) (Vance et al., 1987; Islam and Weil, 1998), and basal soil respiration (BSR) (Anderson and Domsch, 1993). The metabolic quotient (qCO_2 = BSR / BMC) (Anderson and Domsch, 1993) and the microbial quotient (qMic = BMC/C_{org}) (Sparling, 1992) were also calculated.

The analyses of soil fertility and textural parameters (displayed with "$_f$" added to their symbol to avoid confusion with some pXRF attributes) were performed on samples collected during the dry and rainy seasons, and were presented by Castro et al. (2017) and Silva et al. (2018). The attributes evaluated were pH in water (1:2.5) ($_f$pH); available P ($_f$P), K ($_f$K), Fe ($_f$Fe), Zn ($_f$Zn), Mn ($_f$Mn) and Cu ($_f$Cu) (Mehlich, 1953); remaining P (P_{rem}) (Alvarez and Fonseca, 1990); exchangeable Ca^{2+} ($_f$Ca), Mg^{2+} ($_f$Mg), and Al^{3+} ($_f$Al) (Mclean et al., 1958); potential acidity ($_f$H+Al) (Shoemaker et al., 1961); available S ($_f$S) (Hoeft et al., 1973) and B ($_f$B) (Raij et al., 2001); total soil N ($_f$Ntotal) (Joergensen and Brookes, 1990); effective cation exchange capacity (CEC), CEC at pH 7 ($_f$T), Al^{3+} saturation ($_f$m), bases saturation ($_f$V) (Alvarez et al., 1999), soil organic matter ($_f$OM) (Walkley and Black, 1934), and sand ($_f$sand), silt ($_f$silt) and clay ($_f$clay) contents (Bouyoucos, 1951).

To obtain the values of terrain attributes, a digital elevation model (Alos Palsar) with spatial resolution of 12.5 m obtained in the digital platform of the Alaska Satellite Facility (https://vertex.daac.asf.alaska.edu/) was used. Using the software System for Automated Geoscientific Analysis (SAGA) GIS v 2.1.4 (Conrad et al., 2015), the following terrain attributes were generated: aspect (aspect), channel network base level (channbl), cross-sectional curvature (csc), hillshade (hillsh), longitudinal curvature (longcurv), multi-resolution ridge top flatness (mrrtf), multi-resolution index of valley bottom flatness (mrvbf), relative slope

(relslop), valley depth (valleydep), vertical distance to channel network (vertdis), topographic wetness index (twi) (Beven and Kirkby, 1979) and slope (slope). These terrain attributes have been commonly used in modeling and predicting soil attributes (Adhikari et al., 2014; Arrouays et al., 2014; Jafari et al., 2014; Silva et al., 2016a; Taghizadeh-Mehrjardi et al., 2015). The values of these terrain attributes were extracted at the central sampling places using the software ArcGis 10.3 (ESRI, The Redlands, CA, USA).

An S1 Titan LE pXRF (Bruker® Nano Analytics, Kennewick, WA, USA) (50 kV, e100 μA, Rh X-ray tube) was used to determine soil elemental contents. Air-dried samples were sieved at 2 mm and analyzed by pXRF using the GeoChem software in Trace mode (dual soil) for 60 seconds, in triplicate. The accuracy of the equipment was evaluated by analyzing the 2710a and 2711a reference materials certified by the National Institute of Standards and Technology (NIST) as well as a standard sample provided by the equipment manufacturer (check sample - CS). The percentage of recovery for the elements used in this work was calculated for the certified elements in the samples (% of recovery = 100 x obtained content / total certified content). The elements used in the present study were those that were detected in all the repetitions of at least one phytophysiognomy under study ($_{px}$ was added before the symbol to avoid confusion with some fertility attributes): Al_2O_3($_{px}Al_2O_3$), As ($_{px}$As), Bi ($_{px}$Bi), CaO ($_{px}$CaO), Ce ($_{px}$Ce), Cl ($_{px}$Cl), Cr ($_{px}$Cr), Cu ($_{px}$Cu), Fe ($_{px}$Fe), K_2O ($_{px}K_2O$), Mn ($_{px}$Mn), Nb ($_{px}$Nb), Ni ($_{px}$Ni), P_2O_5 ($_{px}P_2O_5$), Pb ($_{px}$Pb), Rb ($_{px}$Rb), SiO_2 ($_{px}SiO_2$), Ta ($_{px}$Ta), Ti ($_{px}$Ti), V ($_{px}$V), Y ($_{px}$Y), Zn ($_{px}$Zn) and Zr ($_{px}$Zr). Their recovery values, in the sequence 2710a/2711a/CS (%), were: Al (78.9/69.4/88.4), As (0/0/0), Bi (0/0/0), Ca (36.1/43.2/0), Ce (0/0/0), Cl (0/0/0), Cr (0/125.1/0), Cu (83.8/77.0/94.4), Fe (76.1/67.8/89.2), K (56.7/46.1/84.2), Mn (69.9/61.8/81.3), Nb (0/0/0), Ni (0/114.9/90.1), P (412.0/577.9/0), Pb (109.4/106.4/105.6), Rb (88.1/90.5/0), Si (57.9/49.9/90.2), Ta (0/0/0), Ti (78.2/71.5/0), V (0/0/0), Y (0/0/0), Zn

(95.5/78.1/0) and Zr (104.6/0/0). Zero values indicate that the element was not present in the certified material or that it was non-detectable via pXRF.

2.3 Data analysis

For the modeling and validation of BMC, BSR, qCO_2, and qMic, soil organic matter, soil fertility and texture (F), terrain (T), and pXRF (pX) attributes were used separately and together, comprising a total of seven distinct combinations of predictor datasets for model creation: pX data only; F data only; T data only; F + pX data; F + T data; T + pX data; and F + pX + T data. Moreover, each of the seven models was also calibrated with and without addition of phytophysiognomy and season (dry or rainy) as predictor variables, separately or together (both phytophysiognomy and season; only phytophysiognomies; only season; neither phytophysiognomy or season), totaling 28 models per microbiological attribute (7 predictors datasets x 4 combinations of phytophysiognomy and season). In this study, the 'partykit' package with 'cforest' function was used in R (R Development Core Team, 2017) to fit a conditional random forest model (Hothorn et al., 2006; Strobl et al., 2007). The models were validated by the leave-one-out cross validation (LOOCV) via the 'caret' package (Kuhn et al., 2018), where the following parameters were obtained: coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). For the models with better results, the most and least important variables were analyzed.

For models which produced good prediction results using only terrain attribute data, with or without the influence of phytophysiognomy and season, the predicted microbiological attribute was spatially rendered across the study phytophysiognomies. The values of these terrain attributes were extracted at the central sampling places using ArcGis 10.3 software. R software ('raster' and 'rgdal' packages) was used to apply the model generated with the terrain for prediction along each phytophysiognomy in the two seasons.

3 Results

Quality prediction models of soil microbiological attributes were obtained. The models containing phytophysiognomy and season as predictors produced generally better results than those in which those variables were omitted, as indicated by higher R^2 values and lower values of RMSE and MAE (Figure 2). However, for BMC the decrease in model performance when the phytophysiognomy, season or both were omitted was smaller than those models which used only F, pX, or T as predictors, with losses in R^2 ranging from 0.04 when only fertility data were used and 0.12 when only pXRF data were used. Therefore, it can be concluded that F, pX, and T were able to correctly capture variations in BMC across different phytophysiognomies and seasons. For instance, the R^2 of the model including F+pX+T was 0.65 when phytophysiognomy and season were included, and 0.62 when they were omitted. Lower R^2 and higher RMSE and MAE were observed when using only pX (A- S-: R^2= 0.48, RMSE= 174 mg g^{-1}, MAE= 137; A+ S+: R^2= 0.59, RMSE= 154 mg g^{-1}, MAE= 122) or T (A- S-: R^2= 0.5, RMSE= 178 mg g^{-1}, MAE= 148; A+ S+: R^2= 0.61, RMSE= 152 mg g^{-1}, MAE= 122) as the predictor and a substantial reduction in predictive capacity for all models was apparent when phytophysiognomy and season were both omitted. Although the models using soil fertility and texture data showed better results, incorporation of phytophysiognomy and/or season did not produce substantial changes in R^2 values. While the lowest R^2 (0.59) was produced when using pXRF, adding phytophysiognomy and season as predictors exhibited an R^2 of 0.65 using fertility and texture parameters data as predictors.

235

236 Figure 2. Root mean square error (RMSE), coefficient of determination (R^2), and mean absolute
237 error (MAE) for prediction of soil microbiological attributes using soil fertility and texture data
238 (F), pXRF (pX) and terrain (T), separately or together as predictors, in Minas Gerais, Brazil.
239 P+ S+ Both phytophysiognomy and season included as predictors; P + S- only
240 phytophysiognomy included as predictors; P- S+ only season included as predictors; P- S- not
241 including phytophysiognomy or season as predictors.

242

The accuracy of the model predicting BSR was highly dependent on both phytophysiognomy and season, and there were large losses of predictive power for the models when one or both of them were removed from the list of predictors. When phytophysiognomy and season were used, the predictions always yielded $R^2 > 0.85$ while the highest value was obtained when using only terrain attributes as predictors ($R^2 = 0.91$). When data from phytophysiognomy and season were not included, the model using only pXRF data presented the worst R^2 values among all models and all predicted microbiological attributes ($R^2 = 0.01$).

Comparing the R^2 values of the models obtained only with terrain attributes for BMC and qCO_2 with the models obtained using fertility and texture data, when data from phytophysiognomy and season were included, the reduction in R^2 values was very small (BMC ~3%, qCO_2 ~5%). In contrast, to predict *q*Mic, the loss in R^2 value was higher (~20%).

The qCO_2 was best predicted by the model using fertility and texture data together with terrain data, with an R^2 of 0.79. The worst adjustments for the qCO_2 prediction models were obtained when physiognomy and season were not among the predictors. However, when phytophysiognomy and season were used, the R^2 was always > 0.67 even with only pXRF data as the predictor, showing the importance of phytophysiognomy and season to predict the qCO_2.

The *q*Mic was best predicted when using only fertility and texture data along with season and phytophysiognomy. As observed for BMC, the modeling of *q*Mic was not sensitive to omission of phytophysiognomy or season, with small variations in RMSE, R^2, and MAE values. The BMC and *q*Mic predictions presented lower values of R^2 although they were still > 0.65 when only fertility data were used (BMC: $R^2 = 0.65$; *q*Mic: $R^2 = 0.66$), showing reasonable accuracy (Figure 3).

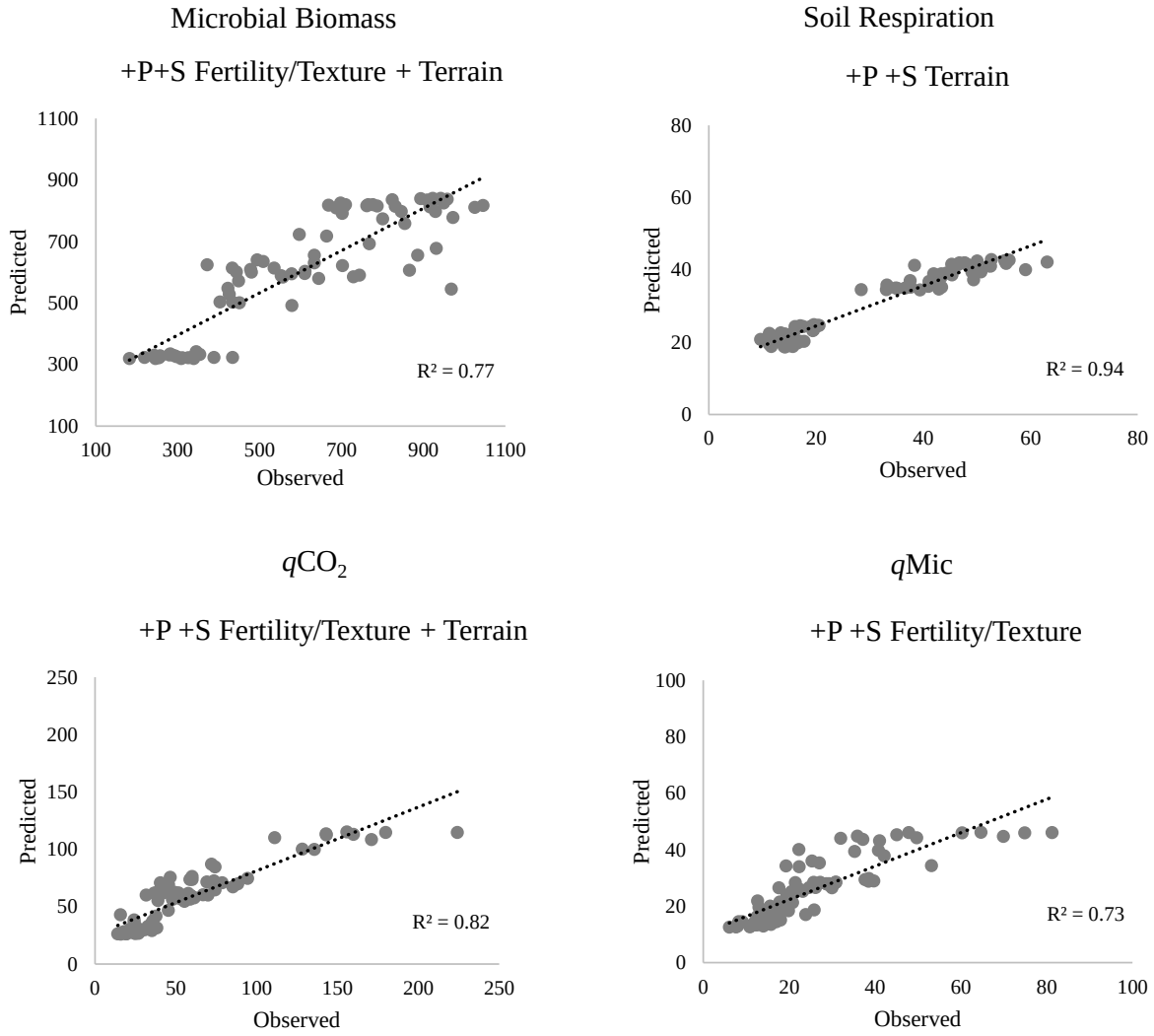


Figure 3. Observed and predicted values from the best models for predicting Microbial Biomass, Basal Soil Respiration, metabolic quotient (qCO_2), and metabolic quotient (qMic), in Minas Gerais, Brazil. +P: with phytophysiognomy; +S: with season

With respect to variable importance, season was most important for the determination of BSR and qCO_2 (Figure 4). However, this variable was not among the five most important for predicting BMC and was among the four variables with negative values of importance for prediction of the qMic. Phytophysiognomy was among the most important predictors for BMC and qCO_2.

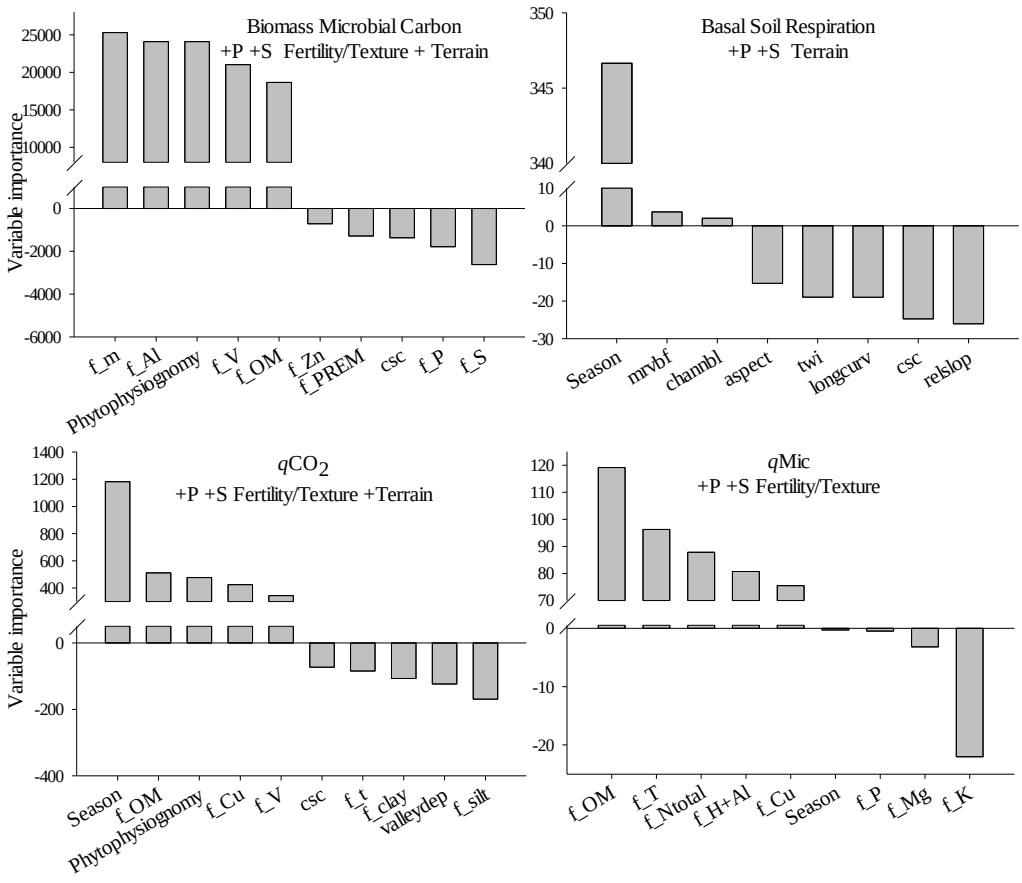
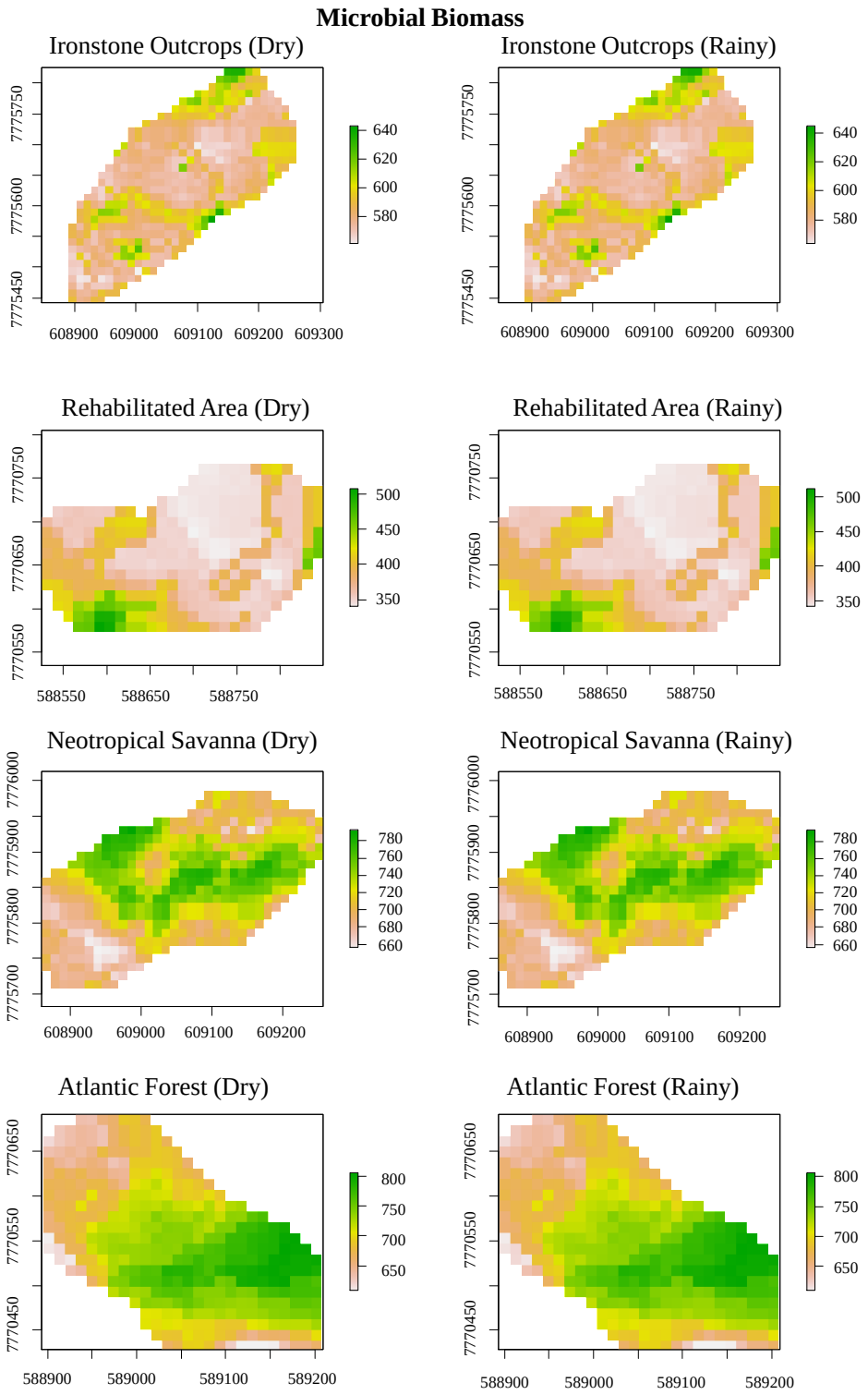


Figure 4. Importance of the variables of the best prediction models for Microbial Biomass, Basal Soil Respiration, metabolic quotient (qCO_2), and metabolic quotient (qMic), in Minas Gerais, Brazil. +P: with phytophysiognomy; +S: with season

Among the five most important variables for the prediction of BMC, besides season and phytophysiognomy, two are related to the presence of exchangeable Al^{3+} in the soil ($_f$Al and $_f$m) and one to the organic matter of the soil ($_f$OM). For predicting BSR, in addition to the season, only two other variables presented positive values of impact: multi-resolution index of valley bottom flatness and channel network base level.

To predict the qCO_2, soil organic matter, base saturation, and available $_F$Cu appeared among the five most important variables. The most important variables for determination of the qMic were soil organic matter, CEC at pH 7, soil pH, potential acidity, and $_f$Cu available.

Since the terrain attributes presented significant results for prediction of microbial attributes (Figure 2), and these data are spatially available, the generated model was used for the production of spatial variability maps of these attributes across different phytophysiognomies and seasons of the year (Figures 5, 6, 7 and 8). These maps show the potential of using these tools to visualize the biological parameters in the phytophysiognomies. The variation between seasons observed in BMC and *q*Mic were very small (Figures 5 and 8). Contrariwise, for BSR and qCO_2 (Figures 6 and 7) differences between phytophysiognomies and seasons were clearly observed. In addition to these expected differences, the spots with different colors observed on the map indicated the variation of BSR and qCO_2 according to terrain attributes.



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299 Figure 5. Spatial variability maps of microbial biomass according to the model generated using
300 phytophysiognomy, season, and terrain attributes as predictor variables, in Minas Gerais,
301 Brazil.

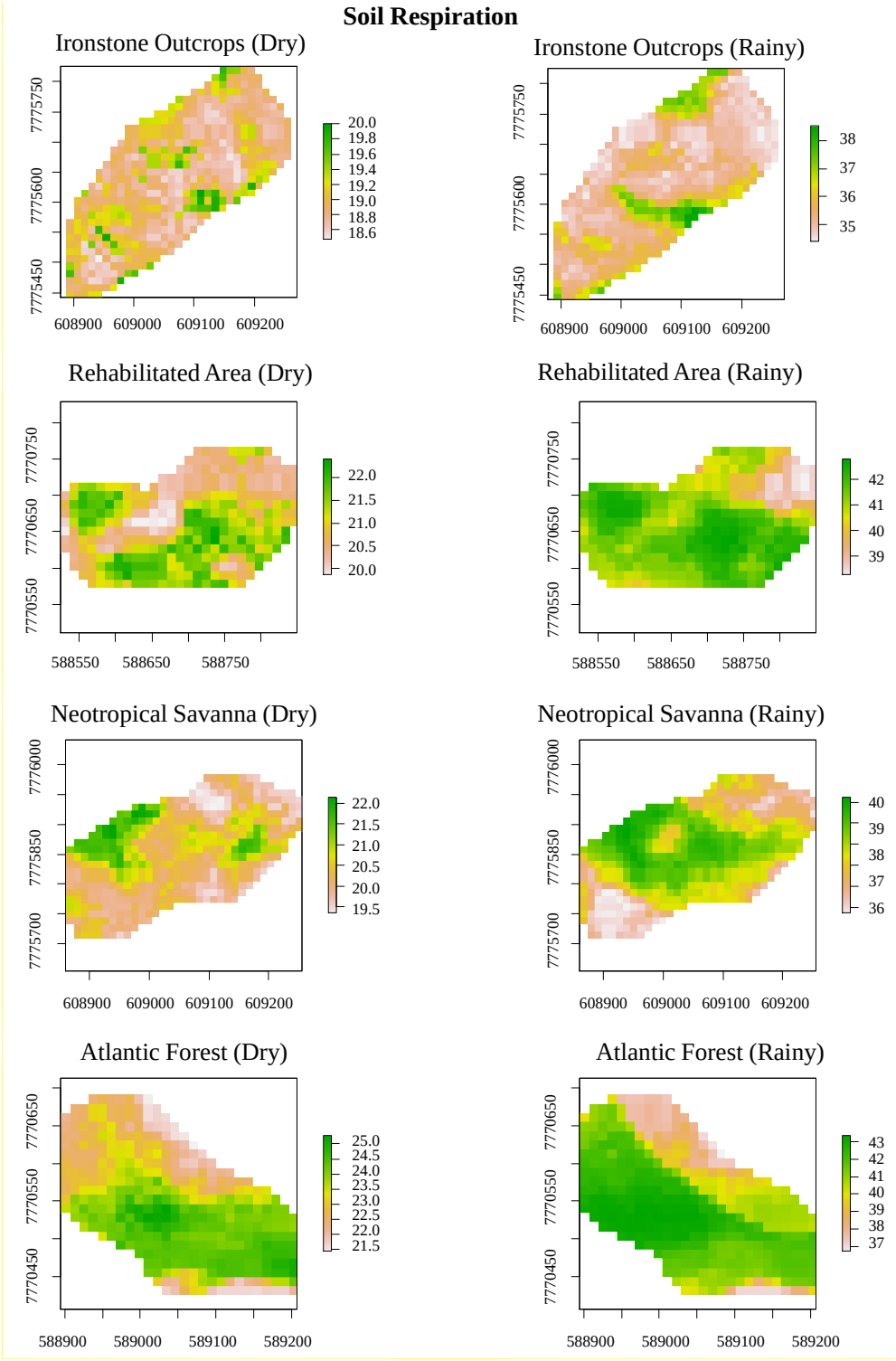
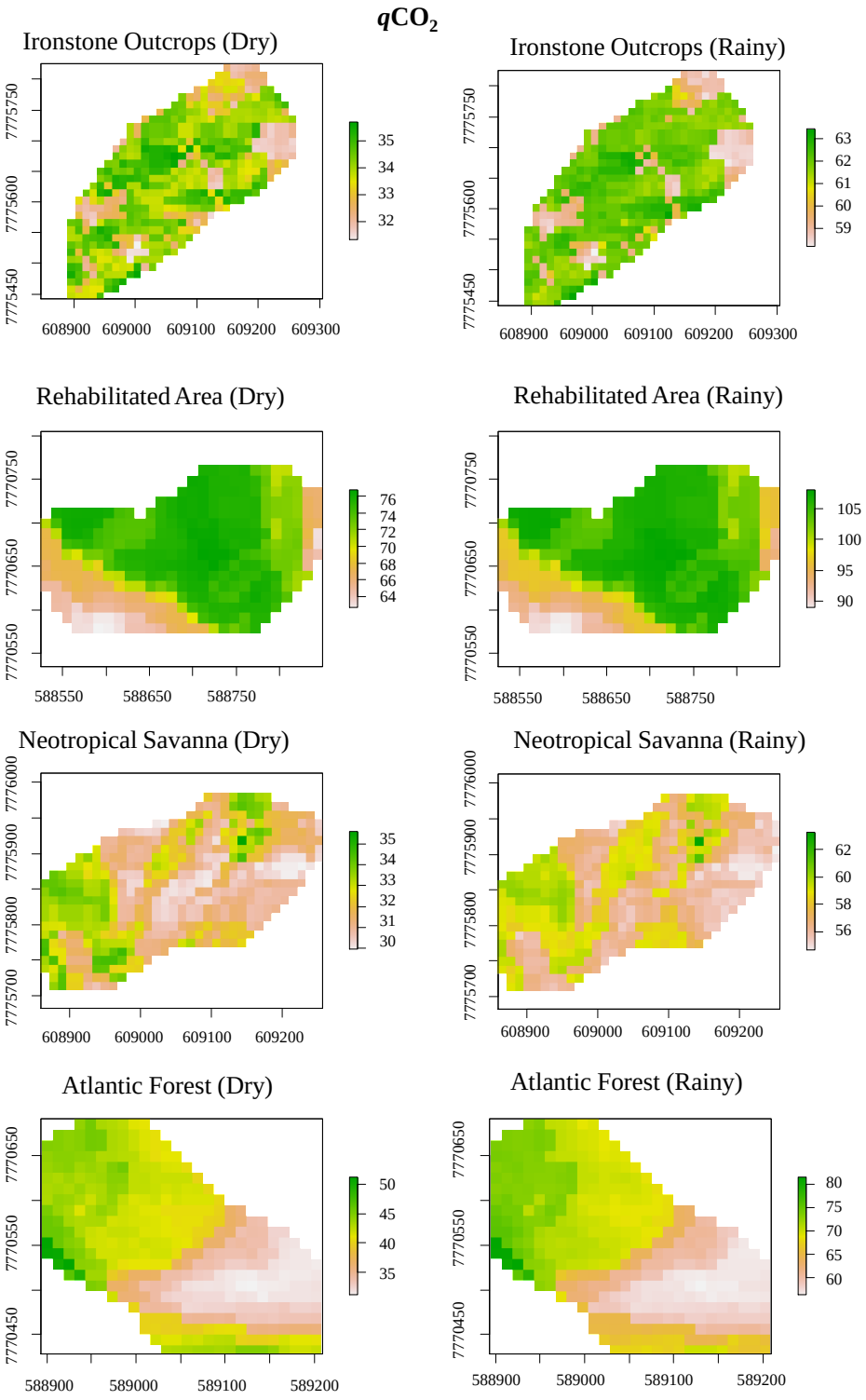


Figure 6. Spatial variability maps of soil respiration according to the model generated using phytophysiognomy, season, and terrain attributes as predictor variables, in Minas Gerais, Brazil.



306

307 Figure 7. Spatial variability maps of metabolic quotient according to the model generated using

308 phytophysiognomy, season, and terrain attributes as predictor variables, in Minas Gerais,

309 Brazil.

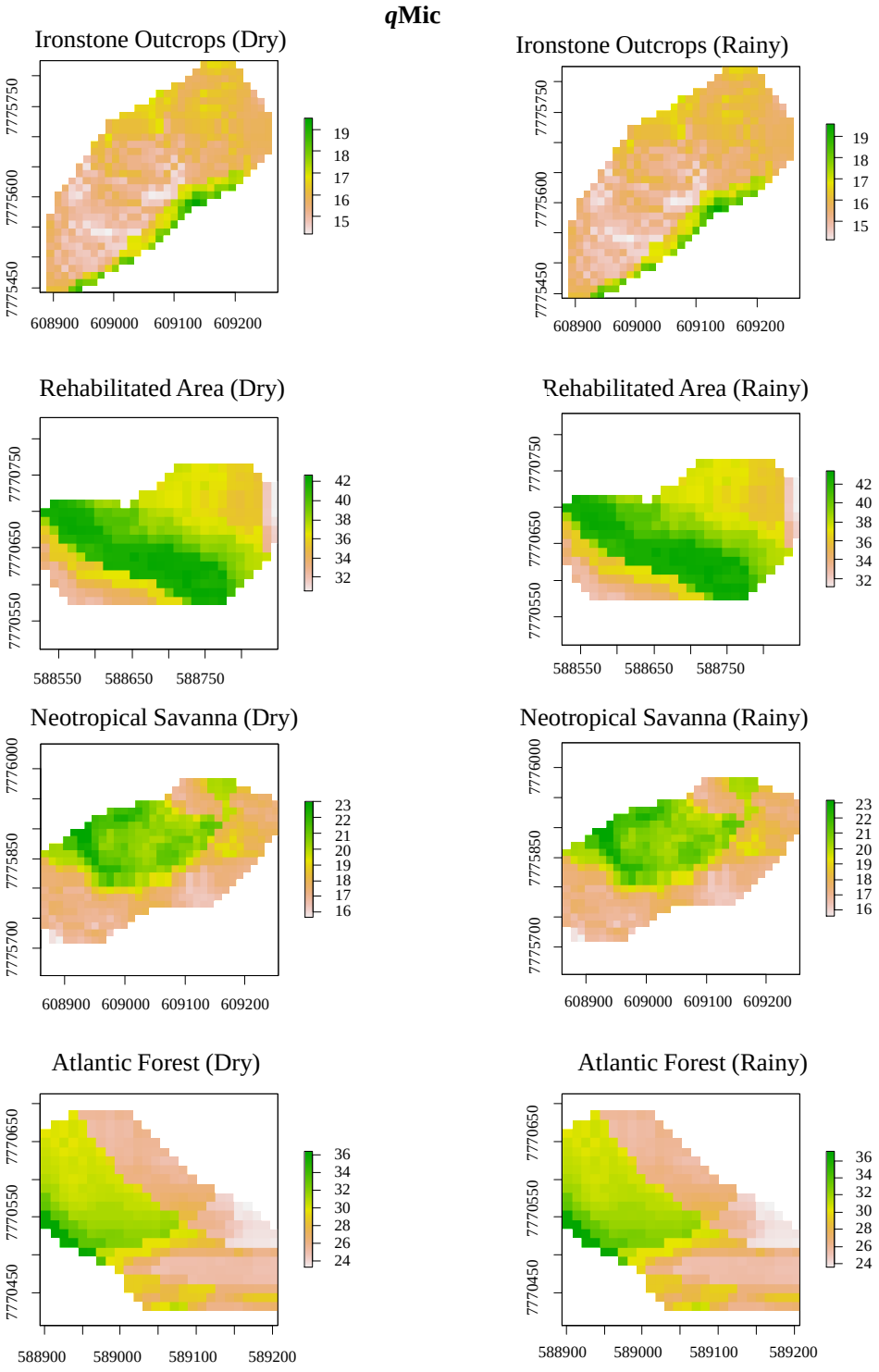


Figure 8. Spatial variability maps of microbial quotient spatialized according to the model generated using phytophysiognomy, season and terrain attributes as predictor variables, in Minas Gerais, Brazil.

4 Discussion

315 The microbial activity is influenced by humidity and temperature, rapidly responding to
316 disturbances in the environment, variations in the season of soil sampling, the type of land use
317 or vegetation cover (Silva et al., 2018a). The influence of both season and phytophysiognomy
318 was indicated by the best models for prediction of BMC, BSR, qCO_2, and *q*Mic. Significant
319 reduction in model fit was observed by the omission of phytophysiognomy and/or season for
320 BSR and qCO_2, indicating that these were the sensitive parameters to respond to stresses and
321 variations caused by land use and climate.

322 Conversely, when season and/or phytophysiognomy were not used, small reductions in
323 accuracy were observed for BMC and *q*Mic prediction models. The BMC itself is related to soil
324 organic carbon and its decomposition, which further depend on temperature and humidity
325 (Sierra et al., 2015). Thus, the low importance of season and phytophysiognomy was probably
326 related to the covariation of these variables with other variables considered important in the
327 model, which reflect the variations of season and phytophysiognomy.

328 This work confirms the combined influence of the soil fertility, texture, and terrain for the
329 prediction of BMC, BSR, and qCO_2, producing R^2 values close or even higher than the models
330 using only soil fertility parameters and texture. The BSR was best predicted with terrain
331 attributes, while the predictions of BMC and qCO_2 showed only small losses in prediction
332 capacity with only these attributes. This indicated the potential of terrain attributes to
333 complement or even replace fertility and textural attributes of the soil, reducing the related
334 expenses. The ease of obtaining terrain data makes this an important alternative to complement
335 studies related to soil microorganisms and soil microbiological attributes, which depend on
336 several factors such as humidity, temperature (Salazar-Villegas et al., 2016), and shading which
337 are correlated with terrain attributes. Moreover, it makes it possible to spatially render this
338 information.

339 This is the first study assessing the suitability of modeling microbiological indicators based
340 on total elemental content obtained through pXRF. For prediction of BMC, when
341 phytophysiognomy and/or season were used in conjunction with pXRF data as predictors,
342 values of R^2 close to those of the models using soil fertility and texture and terrain attributes
343 were found. Elemental contents obtained by pXRF have already shown good correlation with
344 available nutrient contents and other soil fertility attributes such as pH, CEC, and base
345 saturation percentage (Aldabaa et al., 2015; Pelegrino et al., 2018; Ribeiro et al., 2017; Sharma
346 et al., 2015, 2014; Rawal et al., 2019), in addition to soil mineralogy and parent material (Silva
347 et al., 2018b).

348 However, terrain attributes, soil fertility and texture were more efficient in the prediction
349 of the microbiological indicators evaluated here. As soil total elemental content under natural
350 conditions tends to take longer periods of time to undergo changes in relation to managed areas
351 or other types of anthropogenic influence, it is expected that the microbial soil quality indicators
352 evaluated here also show few alterations due to changes in the content of elements obtained by
353 pXRF under conditions of low or no anthropic influence. Nevertheless, Mancini et al. (2018)
354 were able to verify the anthropic influence by the variation of soil elemental contents obtained
355 by pXRF between managed and unmanaged areas. Therefore, further studies on alterations of
356 microbial attributes as a function of pXRF reported elements under the influence of fertilizer
357 applications or other types of anthropic influence are required.

358 The *q*Mic was the only microbiological attribute that showed little sensitivity to the
359 prediction by season or phytophysiognomy, probably because it reflects the relationship
360 between two indicators: BMC and soil organic carbon. The best prediction of *q*Mic was
361 obtained using the predictors of soil fertility and texture, and the greatest importance of organic
362 matter and total nitrogen in its prediction was obtained, since the soil organic carbon is a fraction

of soil organic matter. In turn, the soil organic matter is an important reservoir of nitrogen in the soil ecosystem (Moreira et al., 2006).

Basal soil respiration was the only indicator that presented the best model without the use of soil fertility attributes. The most important predictors of terrain were the multi-resolution index of valley bottom flatness and channel network base level. The former identifies valley bottoms using a slope classification was restricted to convergent areas (Gallant and Dowling, 2003), while the latter represents the vertical distance to the level of the base channel of a localized network. Both are related to soil moisture and sediment deposition areas, important factors that influence the respiratory activity of soil microorganisms (Orchard and Cook, 1983).

The spatial analysis of soil attributes has been much sought after in recent years; more commonly pedological attributes have been spatially rendered (Corassa et al., 2016; Demattê et al., 2016; Silva et al., 2017). BSR's solid prediction from terrain data made it possible to verify the variation of this attribute over the different phytophysiognomies in the two evaluated seasons. Associated microbiological parameters should be studied to determine their feasability for spatial rendering. Visualization of microbiological attributes can be an important strategy for ecological studies, contributing to a better sampling of the biodiversity and assisting in optimizing soil quality assessment.

5 Conclusions

Consideration of phytophysiognomy and season improved the prediction of microbiological indicators of soil quality. Soil fertility and physical attributes can predict the microbiological indicators of soil quality of BMC, BSR, *q*Mic, and qCO_2. Contents of chemical elements obtained by pXRF failed to predict these microbiological indicators of soil quality. Terrain attributes are the best predictors of BSR, allowing for rapid and easy prediction as well as supporting the spatial analysis of attributes throughout the phytophysiognomy. These

methods do not totally replace the conventional analyses of microbiological indicators, but reduce the cost, time, and the number of samples and laboratory analyses needed to obtain such data.

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ARTIGO 3 – Soil physicochemical properties and terrain information predict soil enzymes activity

Artigo formatado nas normas da revista Remote Sensing of Environment

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Abstract

Soil enzymes act in biogeochemical cycles and are indicators of soil quality since they rapidly reflect changes in the environment. Moreover, enzymes are related to soil physical-chemical properties, but their spatial distribution has been rarely evaluated. The hypothesis of this work is that soil attributes related to fertility and texture (F), contents of chemical elements obtained by portable X-ray fluorescence (pX) spectrometry and terrain attributes (T) can be used as predictors to soil enzyme activity. The objective of this work was to predict soil enzymes activity and assess the spatial distribution of these enzymes in phytophysiognomies of the *Quadrilátero Ferrífero* mineral province, in Brazil. Soil samples were collected in five phytophysiognomies in dry and humid seasons, and activity of β-glycosidase, acid phosphatase, alkaline phosphatase, urease, and hydrolysis of fluorescein diacetate (FDA) were determined. Phytophysiognomy, season, F, T, and pX, were used together or separately, to predict the enzymes through conditional random forest and leave-one-out cross validation. The importance of the variables was determined. Model generated using T was used for the spatialization of the enzymes. The generated models presented good accuracy, with coefficient of determination (R^2) varying from 0.63 (FDA by pX) to 0.82 (β-glycosidase by F and T). Spatialization generated maps showing the variation of the enzymes along the phytophysiognomies. PX variables were more important for predicting acid phosphatase and urease, while F variables were more important for predicting β-glucosidase. Prediction of soil enzymes is possible through pXRF, terrain, fertility and texture data.

Keywords: Portable X-ray fluorescence; β-glucosidase; Urease; Phosphatase; Relief, Soil Quality; prediction models

1 Introduction

The *Quadrilátero Ferrífero* is one of the largest mineral provinces in the world. In this region, there is a great variation in the soil composition besides a great biological diversity, being considered a hot spot of the diversity (Carvalho Filho et al., 2010; Castro et al., 2017; Silva et al., 2018; Skirycz et al., 2014; Teixeira et al., 2017).

Soil are in the interface of the spheres of the planet, being in contact with atmosphere, lithosphere, and hydrosphere, besides containing a great diversity of organisms and presenting important functions for the maintenance of the biosphere. In soil, besides macro, meso, and microfauna, as well as microorganisms are present, which perform functions of great environmental importance, despite their small size (Singh et al., 2018). The microorganisms are responsible directly and indirectly for the decomposition of residues, cycling of the elements and maintenance of the biogeochemical cycles that guarantee the continuity of life on the planet (Plante, 2007).

Soil enzymes are largely excreted by the inhabiting microorganisms. These enzymes play key roles in accelerating processes related to the nutrient cycling and catalysis of reactions that would take several years to occur without their action. Enzymes involved in cycles of soil elements, such as in the carbon, nitrogen and phosphorus, have been considered indicators of soil quality, since their activity rapidly reflects changes in the environment, as a consequence of their relationship with soil physicochemical properties and with the structure of the microbial community (Paz-Ferreiro and Fu, 2016).

The activity of the soil enzymes is influenced by several factors, from attributes related to fertility, soil moisture, texture, and to the chemical composition of the soil. On the other hand, some chemical elements can be toxic to the microorganisms producing these enzymes (Gianfreda et al., 2002; Mounissamy et al., 2017; Wang et al., 2007). Few studies evaluated the influence of several chemical elements meansured by conventional methods in the soil enzymes

activity, being more common the evaluation of these elements in contaminated areas (dos Santos et al., 2016; Wang et al., 2007). Modern tools, such as the portable X-ray fluorescence (pXRF), spectrometry have proved successful for predicting various soil attributes and making the quantification of elemental contents easier, more rapid and less costly (Mancini et al., 2019; Ribeiro et al., 2017; Silva et al., 2017; Teixeira et al., 2018; Weindorf et al., 2012; Zhu et al., 2011).

Soil enzymes are good microbiological indicators of soil quality (dos Santos et al., 2013; Nadimi-Goki et al., 2018; Paz-Ferreiro and Fu, 2016; Silva et al., 2018). However, for a throughout characterization, soil sampling to determine the activity of these enzymes should be performed in several points on the area of interest, increasing the number of samples, which may constraint the work. Moreover, in between the sampling sites there are gaps where information on enzyme activity is not contemplated by the sampling scheme. Currently, several works have sought to represent in a spatial way the variability of soil physical, chemical, and biological attributes and, for that end, several techniques can be used (Duda et al., 2017; Forkuor et al., 2017; Hengl et al., 2017; Liu et al., 2012; Malone et al., 2017; Pelegrino et al., 2018; Qu et al., 2018; Spohn et al., 2013a; Vasu et al., 2017). Among these techniques, terrain attributes, i.e., representation of topography features in a raster type (pixel-based) format, such as slope, curvature and topographic wetness index, that correlate with the variation of the data of interest (e.g., soil enzymes activity) along the areas have been used for prediction of these attributes of interest, generating a map with continuous information of these attributes variability across the study area.

The hypothesis of this work is that contents of chemical elements obtained by portable X-ray fluorescence (pX) spectrometry and terrain attributes (T) can be used as predictors to soil enzyme activity. The objective of this work was to predict soil enzymes activity and to assess

the spatial distribution of these enzymes in phytophysiognomies of the *Quadrilátero Ferrífero* mineral provice, in Brazil.

2 Material and Methods

2.1 Soil sampling

This study was conducted in Brumadinho and Nova Lima counties, located in the *Quadrilátero Ferrífero* region, in the state of Minas Gerais, Brazil. The climate of the region is Cwa according to Köppen-Geiger classification, with dry winters and hot summers (Alvares et al., 2013).

The evaluated areas contains four vegetation types with contrasting characteristics (phytophysiognomies) described by Castro et al. (2017) and Silva et al. (2018) as follows: Ironstone Outcrops (IO) (known as "Canga") on Petric Plinthosol (Typic Plinthaquox), Neotropical Savanna (NS) (known as "Brazilian Cerrado") on Petric Plinthosol (Typic Plinthaquox), Atlantic Forest (AF) on a toposequence of Red Latosol (Rhodic Haplustox), Red-Yellow Latosol (Typic Haplustox), and Haplic Cambisol (Typic Dystrustept); rehabilitated area revegetated with grass (RA) is found on anthropic soil (Coelho et al., 2017) (Figure 1) [soils classified per Brazilian Soil Classification System (Embrapa, 2013) and per Keys to Soil Taxonomy (Soil Survey Staff, 2014), the latter in parenthesis] (Figure 1) (Castro et al., 2017; Silva et al., 2018). Soil sampling was carried out by collecting 10 samples per phytophysiognomy, each of them composed of five sub-samples collected at 0 to 20 cm depth at five and ten meters to the east and west of a central georeferenced sampling point (Silva et al., 2018).

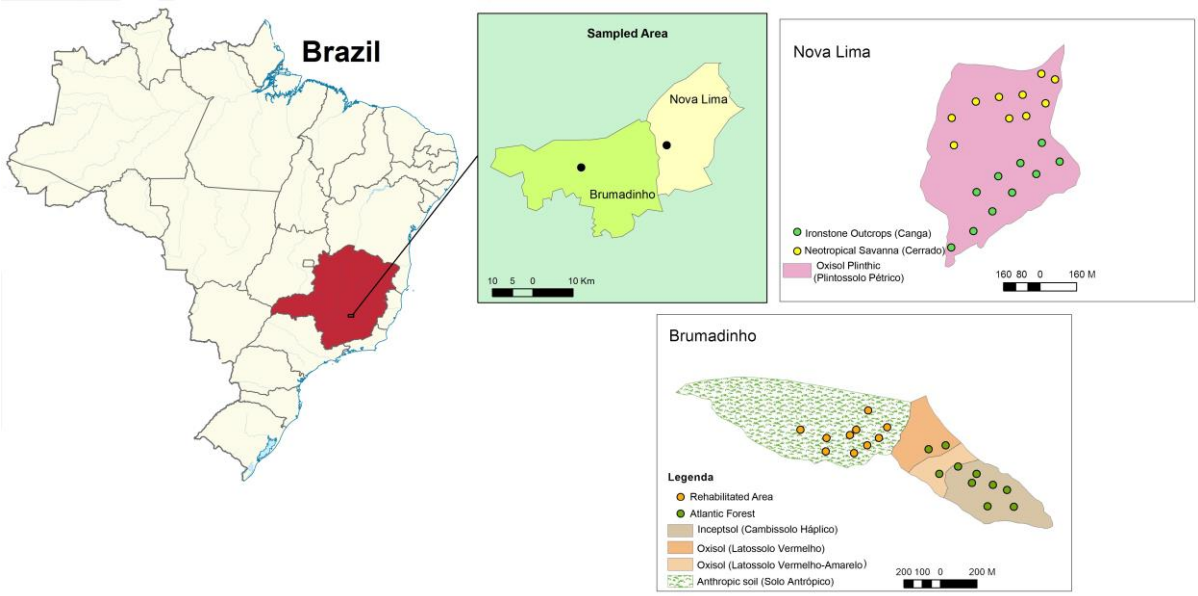


Figure 1: Location of the soil sampling sites at the Ferrous Technology Center (Miguelão - Nova Lima) and the Córrego do Feijão Mine (Brumadinho) in Minas Gerais State, Brazil.

2.2 Soil analysis

The enzymes evaluated were β-glucosidase (Eivazi and Tabatabai, 1988), acid phosphatase, alkaline phosphatase (Eivazi and Tabatabai, 1977), and urease (Keeney and Nelson, 1982; Tabatabai and Bremner, 1970), in addition to total enzyme activity by hydrolysis of fluorescein diacetate (FDA) (Dick et al., 1996). The activity values of the soil enzymes used in this study were determined by Silva et al. (2018).

Mean values of soil fertility and texture were presented by Castro et al. (2017) and Silva et al. (2018). The following soil properties were determined: total N contents ($_f$Ntotal) (Joergensen and Brookes, 1990), exchangeable contents of Ca^{2+} ($_f$Ca), Mg^{2+} ($_f$Mg), and Al^{3+} ($_f$Al), (Mclean et al., 1958); pH in water (1:2.5) ($_f$pH); available contents of P ($_f$P), K ($_f$K), Fe ($_f$Fe), Zn ($_f$Zn), Mn ($_f$Mn) and Cu ($_f$Cu) (Mehlich, 1953); remaining P (Prem) (Alvarez V. and Fonseca, 1990); potential acidity ($_f$H+Al) (Shoemaker et al., 1961); available S ($_f$S) (Hoeft et al., 1973) and B ($_f$B) (Raij et al., 2001); effective cation exchange capacity (CEC) ($_f$t), potential CEC at pH 7 ($_f$T), aluminum saturation ($_f$m), base saturation ($_f$V) (Alvarez V. et al., 1999), and soil organic

matter ($_{f}$OM) obtained by oxidation with potassium dichromate in acidic medium (Walkley and Black, 1934). The sand ($_{f}$sand), silt ($_{f}$silt), and clay ($_{f}$clay) contents were obtained through soil texture determination by the pippete method (Bouyoucos, 1951).

The terrain attributes were derived from an Alos Palsar digital elevation model (spatial resolution of 12.5m) obtained on the digital platform of the Alaska Satellite Facility (https://vertex.daac.asf.alaska.edu). The terrain attributes Slope, Topographic Wetness Index (twi) (Beven and Kirkby, 1979), Aspect (aspect), Hillshade (hillsh), Channel Network Base Level (channbl), Cross-sectional Curvature (csc), Longitudinal Curvature (longcurv), Multi-resolution Ridge Top Flatness (mrrtf), Relative Slope (relslop), Valley Depth (valleydep) and Vertical Distance to Channel Network (vertdis) were obtained using the software System for Automated Geoscientific Analysis (SAGA) GIS v 2.1.4 (Conrad et al., 2015). The values of these terrain attributes were extracted at the sampling sites.

Soil chemical elemental contents were obtained through a pXRF Bruker® model S1 Titan LE (50 kV e100 μA X-ray tubes). For such analyses, the samples were air dried, sieved to 2 mm, and scanned in triplicate with the equipment, for 60 seconds, at Trace (dual soil) mode, using the GeoChem software. The accuracy of the equipment was assessed by scanning reference materials certified by the National Institute of Standards and Technology (NIST), 2710a, 2711a, and by equipment manufacturer (check sample - CS). The recovery percentage (% of recovery = 100 x Obtained content / Total certified content) for the elements used in this study was calculated. The elements used in this study were Al_2O_3($_{px}$Al2O3), As ($_{px}$As), Bi ($_{px}$Bi), CaO ($_{px}$CaO), Ce ($_{px}$Ce), Cl ($_{px}$Cl), Cr ($_{px}$Cr), Cu ($_{px}$Cu), Fe ($_{px}$Fe), K_2O ($_{px}$K2O), Mn ($_{px}$Mn), Nb ($_{px}$Nb), Ni ($_{px}$Ni), P_2O_5 ($_{px}$P2O5), Pb ($_{px}$Pb), Rb ($_{px}$Rb), SiO_2 ($_{px}$SiO2), Ta ($_{px}$Ta), Ti ($_{px}$Ti), V ($_{px}$V), Y ($_{px}$Y), Zn ($_{px}$Zn) and Zr ($_{px}$Zr). These elements were used since they presented results in all the repetitions of at least one phytophysiognomy under study. The recovery values of samples 2710a / 2711a / CS are, respectively: Al (78.8/68.9/88.3 %), As (0/0/0), Bi (0/0/0),

Ca (36.2/42.9/0), Ce (0/0/0), Cl (0/0/0), Cr (0/124.7/0), Cu (84.0/77.2/94.3), Fe (75.8/67.6/89.3), K (55.6/45.9/84.3), Mn (69.9/61.2/80.5), Nb (0/0/0), Ni (0/115.0/90.2), P (411.8/577.9/0), Pb (108.2/106.3/105.6), Rb (88.3/91.1/0), Si (57.4/49.3/90.5), Ta (0/0/0), Ti (77.7/72.0/0), V (0/0/0), Y (0/0/0), Zn (94.2/77.8/0) and Zr (105.0/0/0). Zero values indicate either that the element was not present in the certified material or that pXRF could not detect it.

2.3 Data analysis

The software R (R Development Core Team, 2017) was used for fitting the models using the partykit package (Zeileis and Hothorn, 2014) and the cforest algorithm, which creates random forests from unbiased classification trees (Hothorn et al., 2006; Strobl et al., 2007). For modeling and validation of β-glucosidase, acid phosphatase, alkaline phosphatase, urease and FDA, the following variables were used as predictors: Phytophysiognomy; season; soil organic matter, soil fertility and texture (F); terrain attributes (T); and pXRF data (pX). The sets of predictor variables F, T and pX were used separately or in combination, encompassing seven conditions: a) (pX); b) (F); c) (T); d) (F + pX); e) (F + T); f) (T + pX); and g) (F + pX + T). In addition to the seven combinations of variables, an eighth model was developed with the 15 most important variables for predicting each enzyme (15+) as well as a ninth model with all variables that presented positive importance values (All +), totaling nine models.

The importance of the variables was determined for the F + pX + T models. All models were validated by the Leave One Out Cross-Validation (LOOCV) method of the caret package (Kuhn et al., 2018). The values of coefficient of determination (R^2), root mean square error (RMSE), and absolute mean error (MAE) were obtained, comparing the values estimated by the model with the observed (real) values. The predicted and observed values of the two best models (largest R^2 and smallest RMSE and MAE) were also plotted in 1:1 graphs for

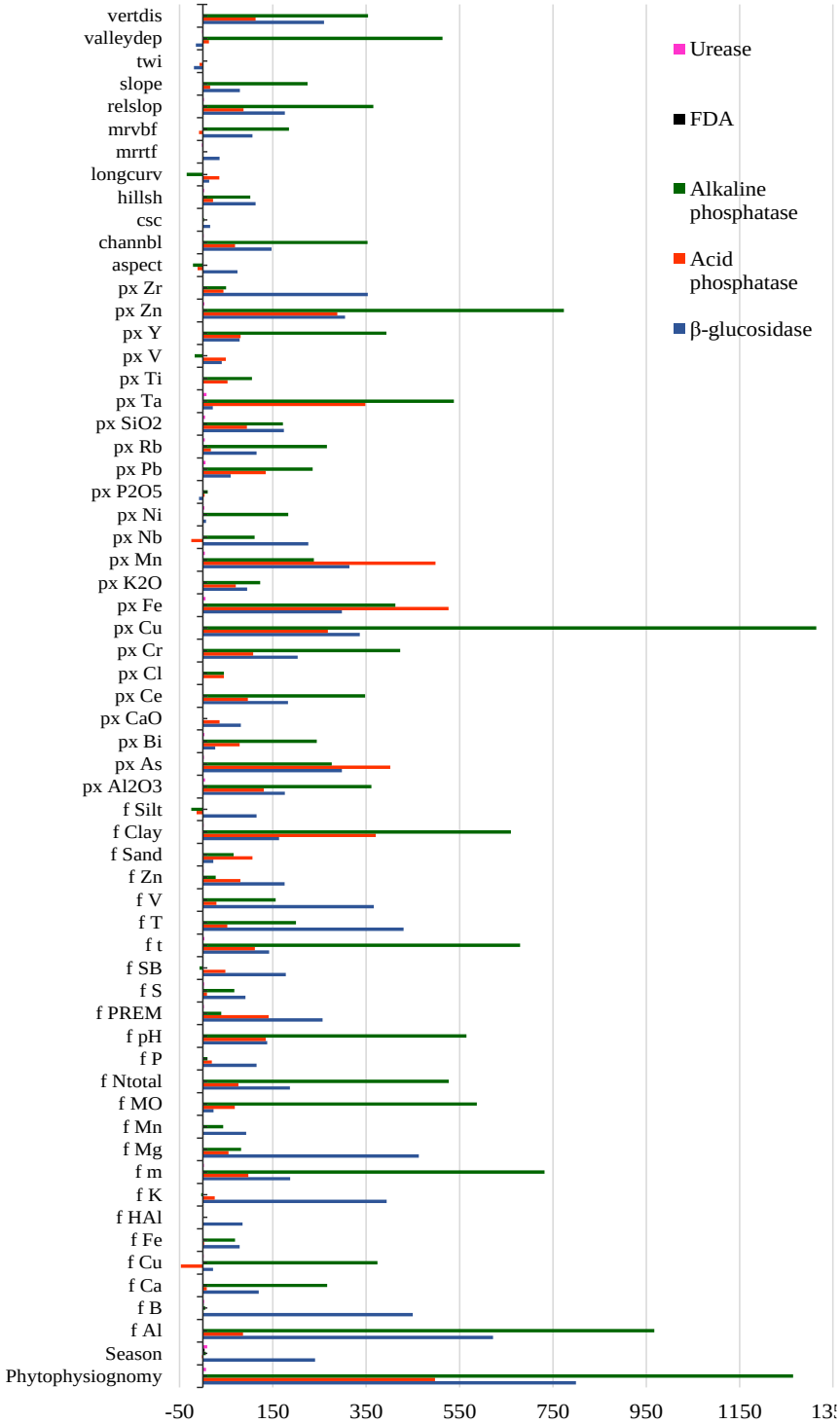
comparison. Moreover, the predicted enzymes activity were spatialized to the phytophysiognomies using the models created based on T data. For that, the values of the terrain attributes were extracted at the central sampled point. Then, the raster and rgdal packages of the R software were applied to the generated models for spatial prediction along each phytophysiognomy in each season, providing a spatial overview of the soil enzymes activities within the study phytophysiognomies.

3 Results

3.1 Variables importance

The most important variables for the β-glucosidase prediction were phytophysiognomy, $_{f}$Al, $_{f}$Mg, $_{f}$B and $_{f}$T (Figure 2). Besides the phytophysiognomy, only variables related to soil fertility were among the five most important ones to predict this enzyme activity. For the Acid Phosphatase prediction, the most important variables were $_{px}$Fe, $_{px}$Mn, phytophysiognomy, $_{px}$As and $_{f}$Clay (Figure 2), which means that among the five most important variables, only one is related to soil texture, and three ($_{px}$Fe, $_{px}$Mn and $_{px}$As) are related to the results of elemental contents obtained by pXRF.

Variables obtained by pXRF appear among the five most important also for Alkaline Phosphatase and FDA prediction (Figure 2). The most important variables for the Alkaline Phosphatase prediction were $_{px}$Cu, phytophysiognomy, $_{f}$Al, $_{px}$Zn and $_{f}$m. For the FDA prediction, the most important variables were Season, $_{f}$B, $_{f}$S, $_{px}$Ti and $_{px}$As. Similarly to the FDA prediction, Season was also the most important variable for the Urease prediction, followed by $_{px}$Ta, phytophysiognomy, $_{px}$Pb and $_{px}$Fe (Figure 2). Twi was the only variable that negatively influenced the modeling of all enzymes (Figure 2). The terrain variables did not appear among the most important for the models probably because they are constant throughout the year, as opposed to the enzymes activity.



188

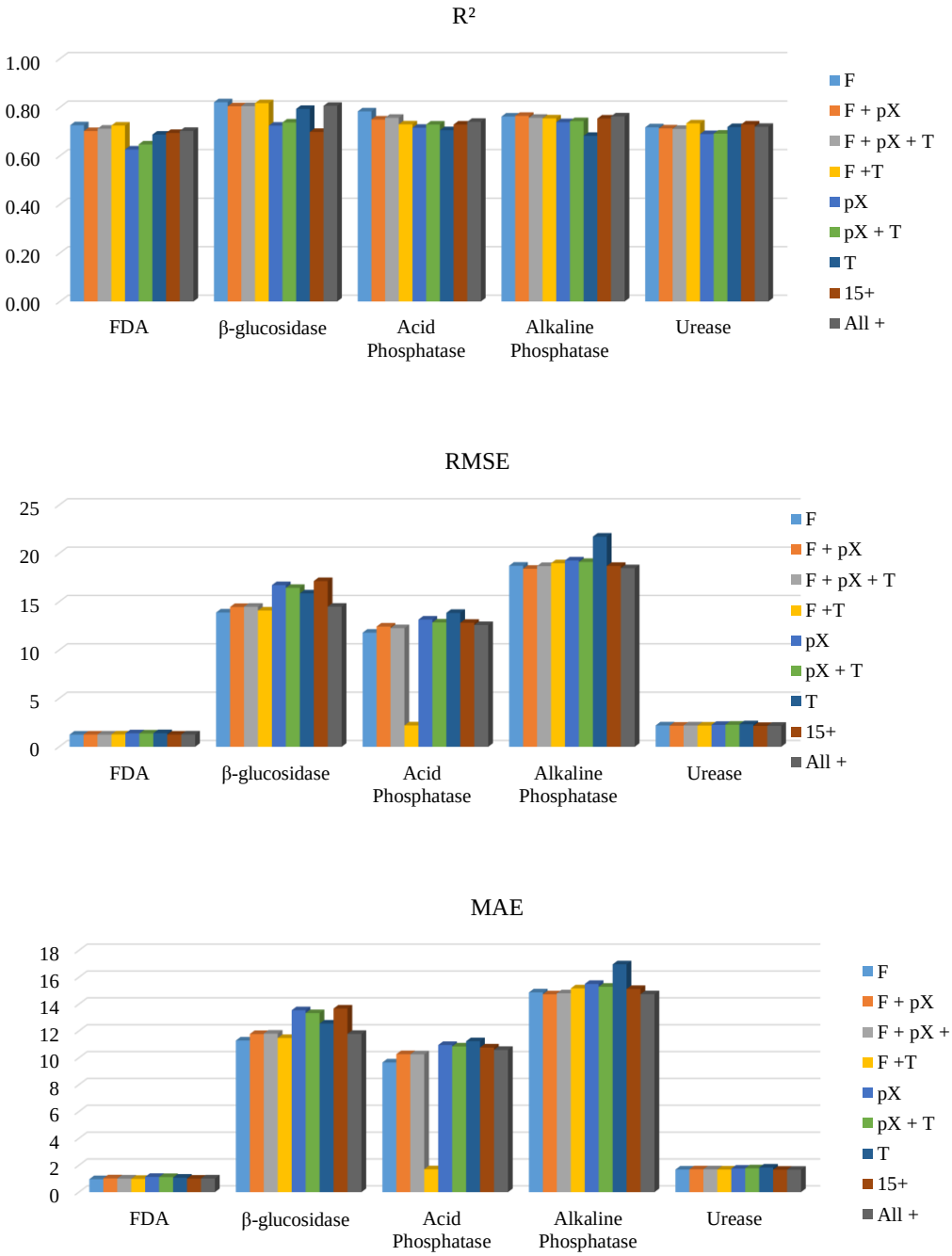
189 Figure 2: Importance of the variables for the prediction of the enzymes β-glucosidase, acid
190 phosphatase, alkaline phosphatase, urease and fluorescence diacetate hydrolysis activity
191 (FDA).

192 **3.2 Prediction of soil enzymes**

193 Better values of R^2 are observed in the prediction of Urease, β-glucosidase and FDA as
194 well as considerable decreases in RMSE and MAE values for Acid Phosphatase prediction
195 when the soil variables obtained by pXRF and variables related to terrain attributes are used in
196 addition to those of soil fertility (Figure 3). The R^2 values obtained for the 9 models evaluated
197 were higher than 0.60, reaching 0.82 in the β-glucosidase prediction (F and F + T models)
198 (Figure 3). The lowest R^2 was obtained in the FDA model using only pX data ($R^2 = 0.63$). When
199 only terrain attributes were used, observed R^2 values were close to those obtained using other
200 predictor variables.

201 The RMSE and MAE values behaved similarly (Figure 3). The highest values of these
202 parameters in relation to the other values for prediction of the same enzyme activity were
203 observed in the models for the Alkaline Phosphatase prediction. To predict this enzyme activity,
204 the model that used only the T data was 2 to 4 units higher than the values of the other models.
205 The lowest values of RMSE and MAE for the prediction of the same enzyme were observed in
206 the Acid Phosphatase prediction. In this case, the F+T model had at least 8 units lower than the
207 other models used to predict this enzyme.

208 The selection of variables for enzymes prediction did not present significant results. None
209 of the models for enzyme prediction with selection of variables by importance (All + and 15+)
210 presented higher results than the models in which all the data were used.

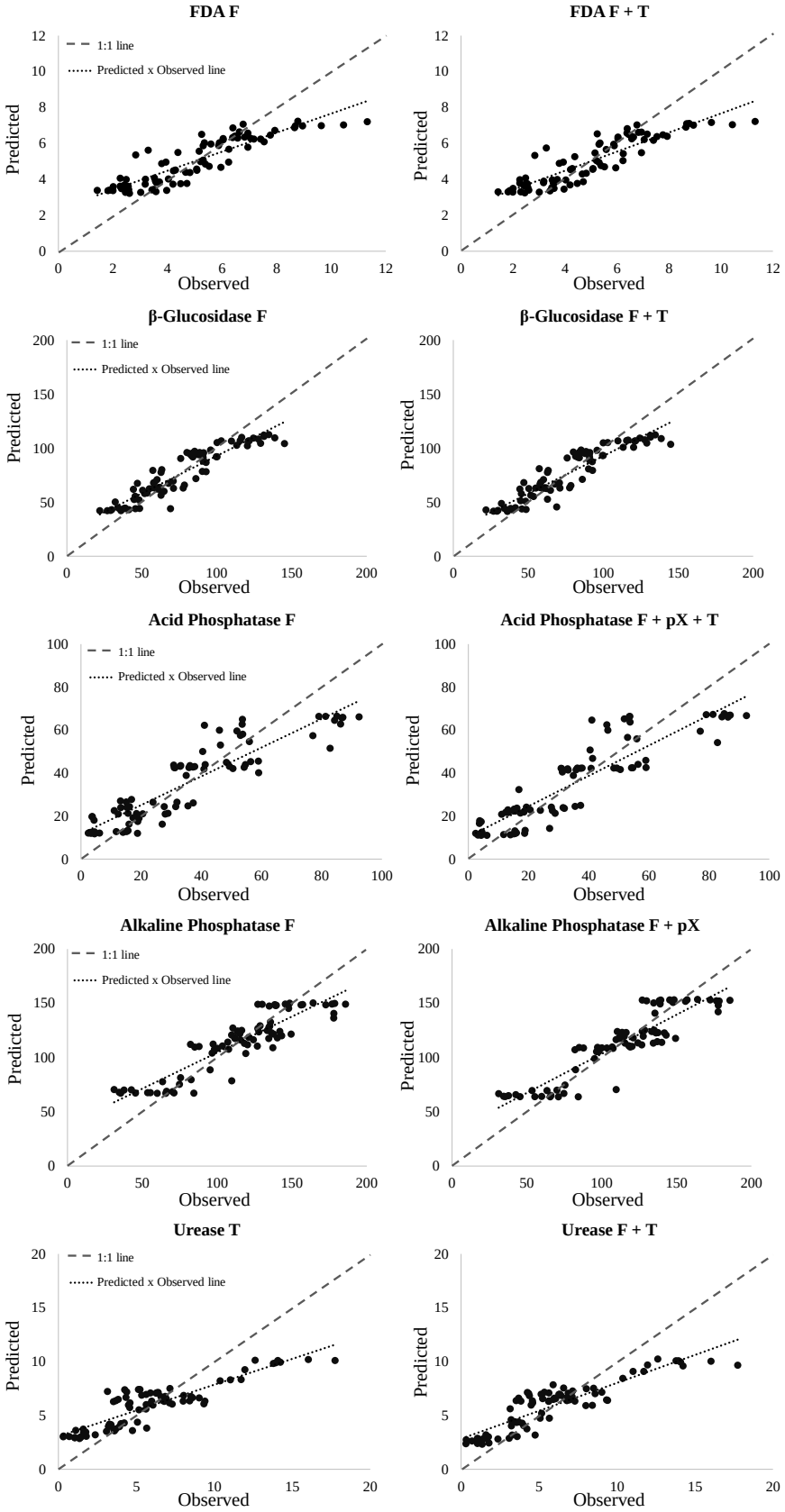


211

212 Figure 3: Coefficient of determination (R^2), root mean square error (RMSE) and absolute mean
213 error (MAE) for prediction of hydrolysis of fluorescein diacetate and of the enzymes β-
214 glucosidase, Acid Phosphatase, Alkaline Phosphatase and Urease through models with varying
215 predictor variables: soil fertility and texture (F), portable X-ray fluorescence (pX) data, terrain
216 attributes (T), the 15 most important variables (15+), and all variables with positive importance
217 (All+).

In the two best models for FDA prediction (F and F+T) and the enzymes β-glucosidase (F and F + T) and Urease (T and F + T) the points were close to the trend line, showing the good accuracy of both models (Figure 4). When comparing these graphs with the 1:1 graphs generated by the predicted vs. observed values of Acid Phosphatase (F and F+pX+T) and Alkaline Phosphatase (F and F+pX), it is noted that they are a little more scattered trend line.

The best model for Urease enzyme prediction was obtained only using terrain attribute data (Figure 3). Although the models generated only with terrain attributes to predict the other enzymes and the FDA were not the best, the lowest R^2 value observed was 0.68 for Alkaline Phosphatase, a small variation when it is considered that the highest R^2 value obtained for prediction of this enzyme was 0.76.



236 Figure 4: Values predicted two best prediction models and observed values of the FDA and the
237 enzymes β-glucosidase, Acid Phosphatase, Alkaline Phosphatase and Urease. F = attributes of

238 soil fertility and texture; T = terrain attributes; pX = values obtained by portable fluorescence
239 X-ray spectrometer.
240
241 **3.3 Spatialization of soil enzymes**
242 The models generated only with terrain attribute data were used for enzyme and FDA
243 spatialization in the phytophysiognomies evaluated in the two seasons (Figures 5, 6, 7, 8 and
244 9). As the terrain attributes do not vary from one season to another, enzyme and FDA responses
245 were also similar in their distribution on maps, with variations mainly on the scales. The
246 enzymes β-glucosidase (Figure 5), Urease (Figure 8), and FDA (Figure 9) were the enzymes
247 that varied most in relation to the collection season, while the Acid Phosphatase enzymes
248 (Figure 6) and Alkaline Phosphatase (Figure 7) showed very small variations.

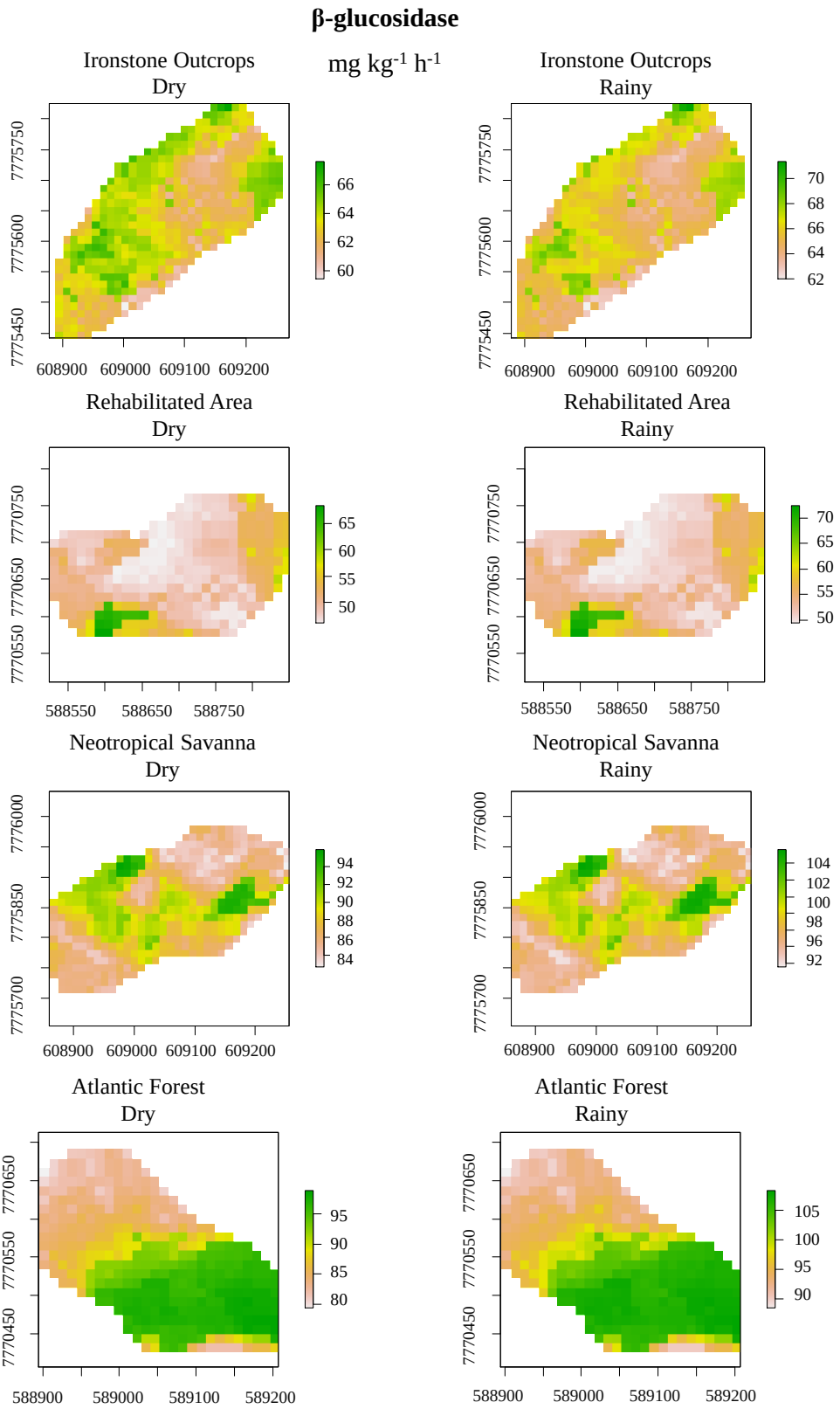


Figure 5: Spatialization of the predicted values of the β-glucosidase activity based on the model generated with terrain attributes (T) for the Ironstone Outcrops, Rehabilitated Area, Neotropical Savanna and Atlantic Forest phytophysiognomies in the Dry and Rainy seasons.

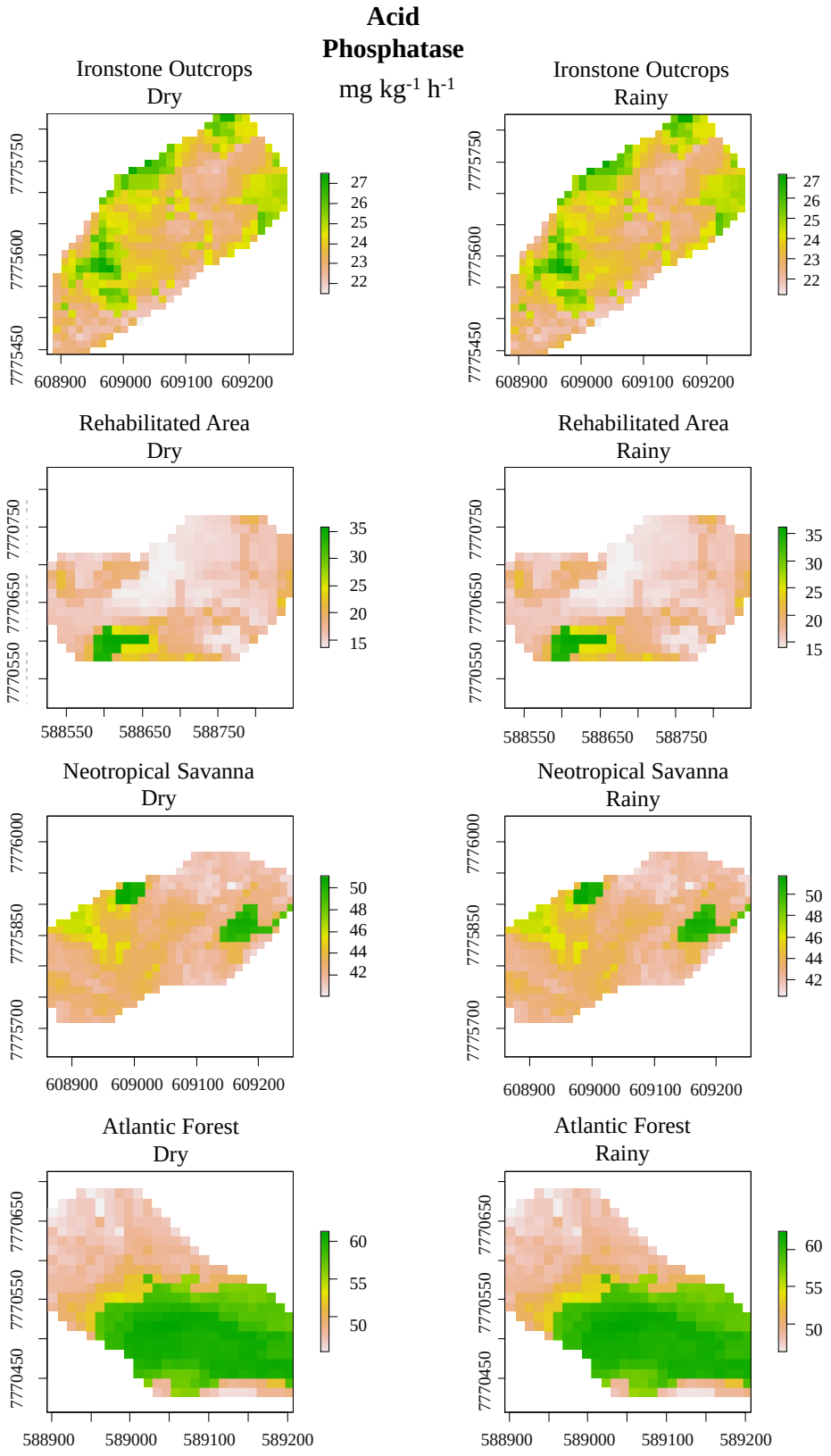
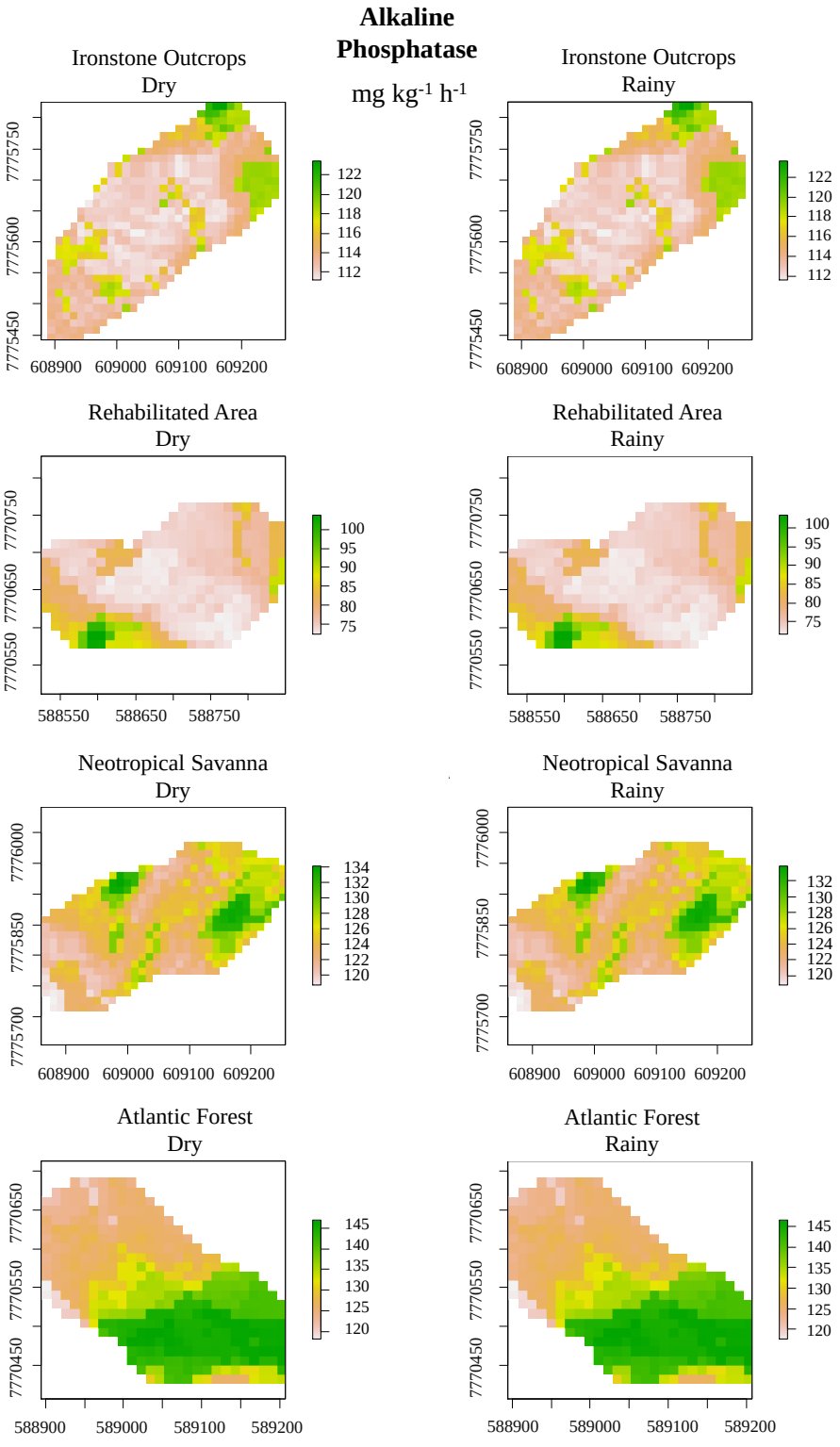


Figure 6: Spatialization of predicted values of Acid Phosphatase activity based on the model generated with terrain attributes (T) for the Ironstone Outcrops, Rehabilitated Area, Neotropical Savanna and Atlantic Forest phytophysiognomies in the Dry and Rainy seasons.



257

258 Figure 7: Spatialization of the predicted values of Alkaline Phosphatase activity based on the
259 model generated with terrain attributes (T) for the Ironstone Outcrops, Rehabilitated Area,
260 Neotropical Savanna and Atlantic Forest phytophysiognomies in the Dry and Rainy seasons.

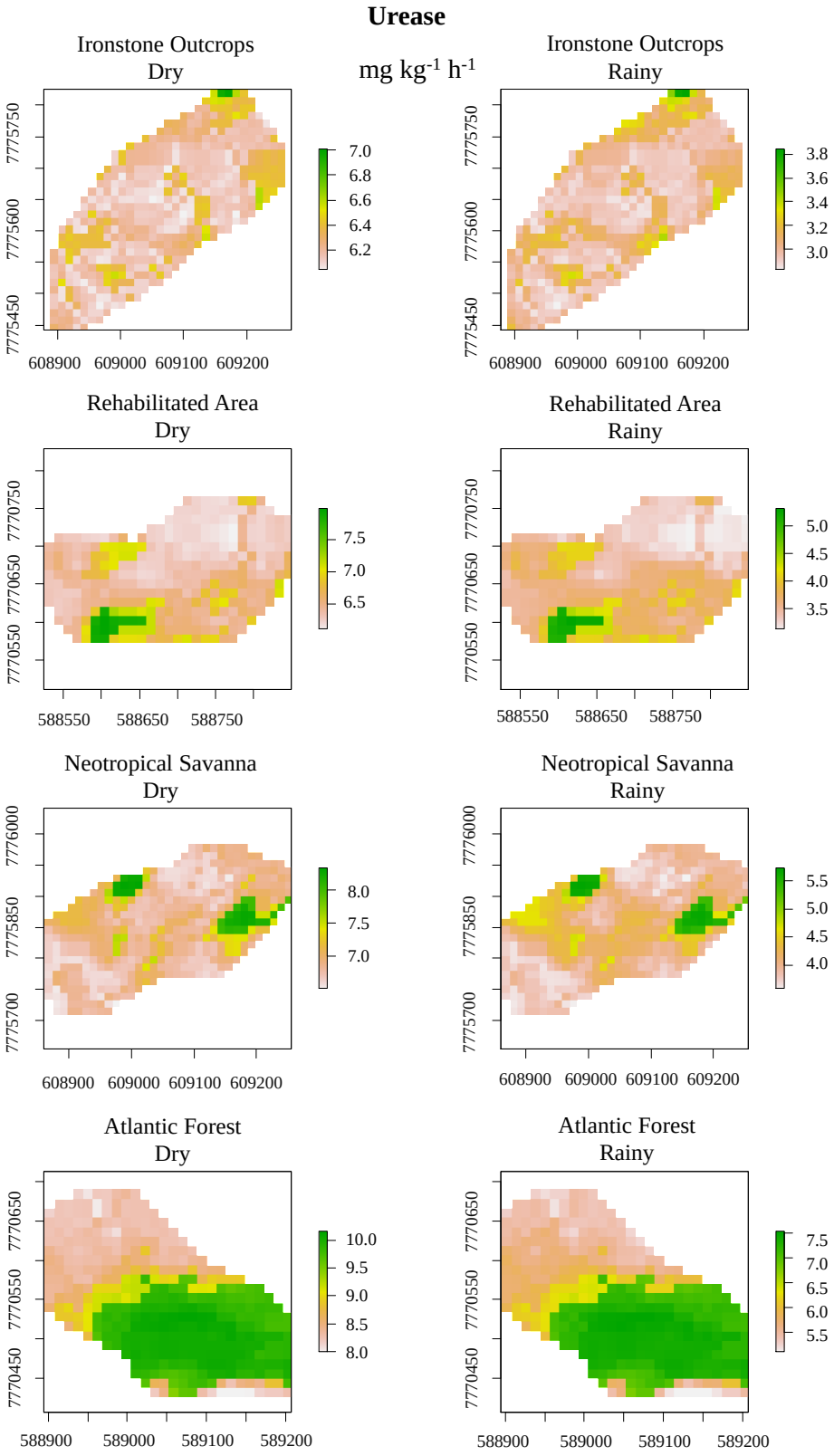


Figure 8: Spatialization of the predicted values of Urease enzyme based on the model generated with terrain attributes (T) for the Ironstone Outcrops, Rehabilitated Area, Neotropical Savanna and Atlantic Forest phytophysiognomies in the Dry and Rainy seasons.

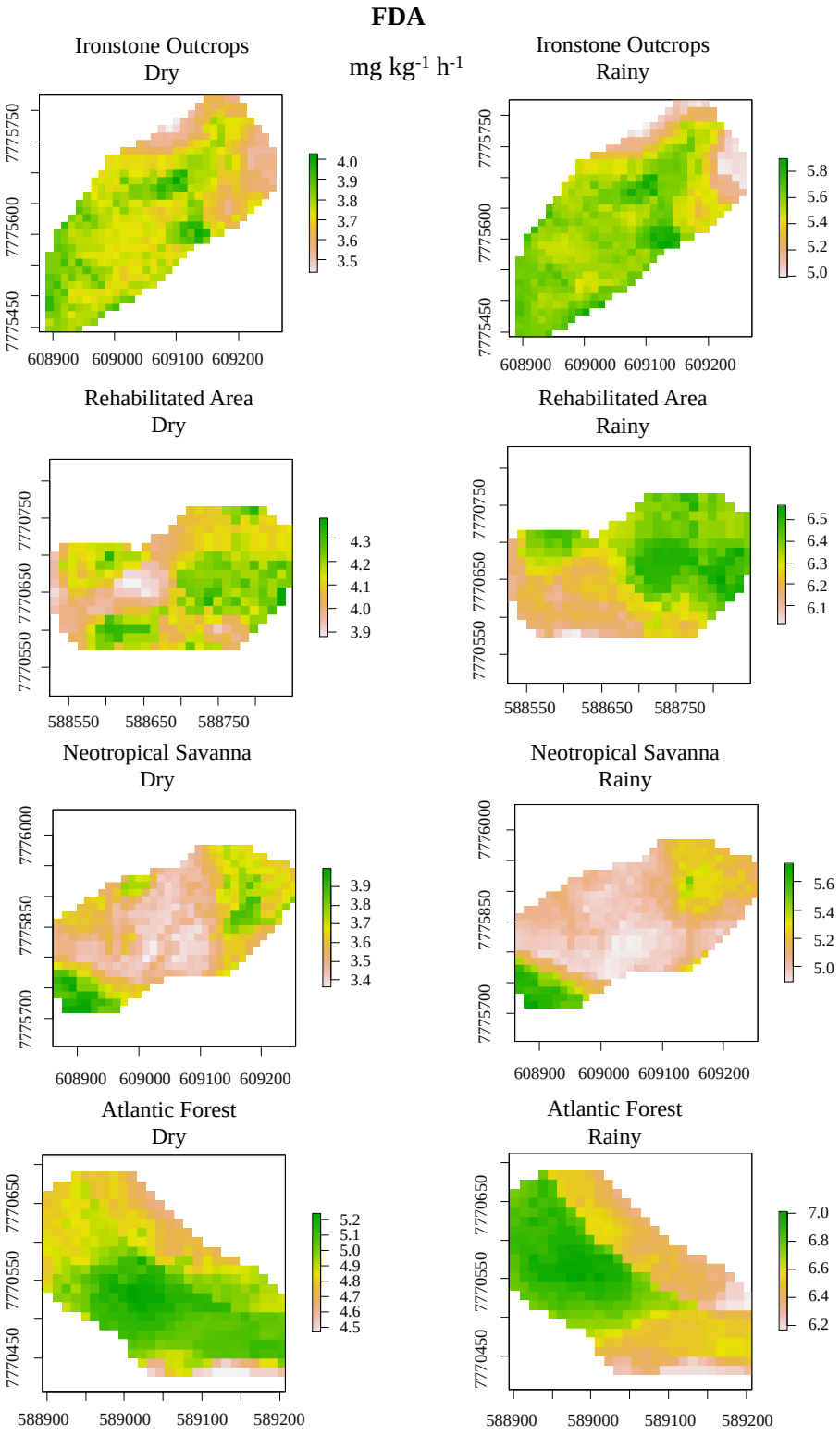


Figure 9: Spatialization of predicted values of FDA based on the model generated with terrain attributes (T) for the Ironstone Outcrops, Rehabilitated Area, Neotropical Savanna and Atlantic Forest phytophysiognomies in the Dry and Rainy seasons.

4 Discussion

4.1 Variables importance

Among the variables used in the models, phytophysiognomies, soil fertility and Season are variables already known to influence soil microbiology (Silva et al., 2018; Alkorta et al., 2017; Ravindran and Yang, 2015; Stone et al., 2015; Štursová et al., 2016). In this work it was demonstrated that the microbiological attributes studied are differently influenced by phytophysiognomy and season, depending on the microbiological parameter to be predicted. While phytophysiognomy appeared among the most important variables for the enzymes β-glucosidase, Acid Phosphatase, Alkaline Phosphatase and Urease, it did not appear among the most important for FDA prediction, which is used as a measure of general enzymatic activity. For FDA prediction, Season appears as the most important variable. The modeling of the importance of the variables was able to demonstrate that the enzymatic activities vary in different intensities when phytophysiognomy and/or season are different, as evidenced by Silva et al. (2018).

In addition to phytophysiognomy and season, variables related to soil fertility and texture commonly appeared among the most important ones in the models for predicting attributes related to soil microbiology. Variables related to soil physicochemical properties such as values of electrical conductivity, pH, organic carbon, soil texture and nutrient contents have already been cited as important for improving the accuracy of prediction models (Ebrahimi et al., 2017; Tavares et al., 2018).

4.2 Prediction of soil enzymes

For Acid Phosphatase prediction, the two most important variables were $_{px}$Fe and $_{px}$Mn. The influence of Fe and Mn oxides has been observed in the enzymes phosphatase and urease, promoting the abiotic polymerization of phenolic compounds, with the adsorbed enzyme

294 molecules exposed, presenting greater activities (Gianfreda et al., 2002). As the evaluated
295 phytophysiognomies are located in the *Quadrilátero Ferrífero*, region with highly weathered
296 soils derived from parent materials with high concentrations of Fe (Carvalho Filho et al., 2010),
297 it is expected that the mineralogy of these soils will present Fe oxides, known for their high P
298 fixing capacity (Kämpf et al., 2012; Resende et al., 2014), as well as Al and Mn oxides. We
299 believe that the importance of these elements obtained by pXRF is therefore related to the
300 mineralogy of the soils of the region. Mancini et al. (2019) demonstrated that pXRF is a tool
301 capable of detecting even slight chemical variations in soil parent materials, which would lead
302 to the formation of soils with different chemical compositions and, therefore, soil mineralogy.
303 This fact also explains why the importance of these elements obtained by pXRF was greater
304 than that of available contents of these elements for the same soil. Another explanation for the
305 influence of these elements on Acid Phosphatase is the P-fixing ability of these soils. As the
306 soils of the region are generally acidic and have a high P fixing capacity (Motta et al., 2002),
307 the higher Acid Phosphatase activity may be related to the lower P availability in these
308 environments, which is also a reflection of their mineralogy.

309 Conversely, for Alkaline Phosphatase prediction, $_{px}$Cu was the most important variable.
310 The importance of this variable may be related to the effect of Cu on the decrease of Alkaline
311 Phosphatase activity already demonstrated in studies in areas under influence of this element
312 (Mounissamy et al., 2017; Wang et al., 2007). The importance of $_{px}$Ta for Urease and $_{px}$As for
313 FDA predictions is probably related to their toxic effect to the microbial population, being able
314 to reduce the enzymatic activity (Li et al., 2016; Nadimi-Goki et al., 2018; Oladipo et al., 2014).

315 The R^2 values found for β-Glucosidase, Alkaline Phosphatase and Acid Phosphatase
316 prediction using phytophysiognomy, season and pXRF information by cForest were all higher
317 than those found by Comino et al. (2018) using infrared spectroscopy and Partial Least Squares
318 Regression model, indicating that different models and techniques can produce different results.

It demonstrates that pXRF is also an important tool for studies related to the soil microbiological quality of the soil. New technologies have been sought to aid in the study and understanding of soil microbiological parameters, such as enzyme activity. Technologies such as infrared spectroscopy have been able to partially predict individual soil enzymes and can be used to estimate the soil biological quality index (Comino et al., 2018). The models with selection of the variables of positive importance (All + and 15+) did not present better results compared to the other models, showing the importance of maintaining all variables for the prediction of enzyme activity.

4.3 Spatialization of soil enzymes

The stability of the enzyme Phosphatase, observed in the spatialization of the attributes for the phytophysiognomies at the different seasons (Figures 8 and 9) has been previously reported. Lopes et al. (2018), in evaluations over 5 years, observed that the enzyme Acid Phosphatase did not present significant variations, remaining stable. Urease was the only enzyme that had its activity reduced in the rainy season.

The use of microbiological attributes related to soil quality, such as enzymes, is a worldwide trend (Spohn et al., 2013b). The spatialization of soil microbiological attributes is an important alternative for assessing soil quality, since these attributes can vary, even in unchanged areas, under the same vegetation type and other similar conditions (Figures 7, 8, 9, 10 and 11). The influence of terrain along with the variability of soil physicochemical attributes at short distances on soil enzyme activities is still little studied and can be a great key factor for determining the reference values of microbiological attributes for each phytophysiognomy, making interpretation and extrapolation easier to similar areas.

5. Conclusions

Elements obtained by pXRF and soil physicochemical properties and terrain attributes provide the generation of predictive models for soil enzymes with great accuracy. The spatialization of the enzymes using models generated with terrain attributes data allows a better overview of the variability of the enzymatic activity across each study phytophysiognomy in a given season of the year.

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TERCEIRA PARTE – CONSIDERAÇÕES FINAIS

A região do Quadrilátero Ferrífero, além de ser uma área de depósitos de Fe, é considerada um "hot spot" de biodiversidade. A relação entre fatores químicos, físicos e biológicos do solo com as fitofisionomias, como em todos os ecossistemas naturais, pode ser afetada negativamente pela atividade antropogênica, comprometendo esse "hot spot". Os microrganismos do solo são responsáveis por processos que garantem a manutenção da vida no planeta e podem auxiliar de forma direta e indireta na recuperação de áreas degradadas. Eles são influenciados por diversos fatores, entre os quais, os fatores abióticos. Portanto, há necessidade de maior compreensão de como os fatores abióticos podem influenciar e determinar os atributos microbiológicos do solo sob uma mesma fitofisionomia. Assim, entender as relações entre fatores abióticos e atributos microbiológicos do solo foi o que motivou a condução deste trabalho em fitofisionomias sob influência de mineração nos municípios de Sabará (artigo 1), Brumadinho e Nova Lima (artigos 2 e 3), no Quadrilátero Ferrífero.

Este trabalho faz parte do projeto multidisciplinar "Diversidade de plantas e de organismos dos solos com potencial biotecnológico e indicadores de impacto ambiental, no Estado de Minas Gerais" [CRA- RDP- 00136- 10 (FAPEMIG/ FAPESP/ FAPESPA/ Vale SA)]. Este projeto buscou aumentar o conhecimento sobre biodiversidade e processos do solo, visando sua conservação e o aumento da produtividade e da qualidade ambiental por meio de uma abordagem holística daquele importante "hot spot".

Neste trabalho foram realizadas coletas de solo e os dados relativos à fertilidade e textura do solo foram determinados em todas as amostras compostas de solo. Esses fatores abióticos já são amplamente conhecidos por influenciarem a atividade dos organismos do solo. Nas áreas de Brumadinho – MG e Nova Lima – MG também foram determinados teores totais de elementos químicos por análise do solo em espectrômetro de fluorescência de raios-X portátil (pXRF), e atributos de terreno. Assim, os fatores abióticos relacionados a fertilidade e textura do solo, teores totais de elementos e atributos de terreno foram utilizados em conjunto e em separado para predição dos atributos microbiológicos. Os atributos de terreno foram utilizados ainda para espacialização dos atributos microbiológicos nas fitofisionomias avaliadas.

O pXRF é capaz de determinar o teor de diversos elementos químicos no solo em poucos segundos, sem geração de resíduos. Apesar de o pXRF já ser utilizado há algum tempo em algumas áreas da ciência, apenas há alguns anos esse equipamento começou a ser utilizado para estudos de solo e ainda não são encontrados estudos que relacionem seus dados a dados de atributos microbiológicos do solo.

Os atributos de terreno podem ser gerados a partir do modelo digital de elevação (MDE). O MDE apresenta pixels com valores de altitude ao longo das áreas e pode ser obtido com

grande facilidade e gratuitamente. Os diferentes atributos de terreno apresentam informações importantes como, índice de umidade, declividade, entre outros que podem influenciar os atributos microbiológicos do solo. Apesar da grande facilidade de obtenção desses dados, poucos estudos de atributos microbiológicos do solo utilizam atributos de terreno.

A predição de dados vem sendo cada vez mais utilizada em diversas áreas do conhecimento. Diversos modelos de *machine learning* têm surgido nos últimos anos. Esses modelos utilizam conjuntos de dados (de treinamento) para predição e tem se mostrado eficientes na predição de dados de diversos atributos de solos como, por exemplo, classes e mineralogia, mas poucos estudos sobre predição de atributos microbiológicos do solo são encontrados.

Portanto, nesse trabalho, buscamos integrar técnicas inovadoras de modelagem e de obtenção de dados (pXRF) para predizer atributos microbiológicos do solo a partir de diferentes grupos de fatores abióticos. Para isso, utilizamos a técnica de modelagem *Conditional Random Forests* (cforest) que tem se mostrado eficiente em predições relacionadas a atributos de solo.

No primeiro artigo o enfoque maior foi dado nos fungos micorrízicos arbusculares (FMA), que são microrganismos que podem auxiliar a reabilitação de áreas degradadas devido a sua simbiose mutualística com a maioria das espécies vegetais. A coleta de solo foi realizada em Sabará – MG em áreas de pilha de rejeitos revegetada, mata, eucalipto plantado, cerrado e canga. Foram montados experimentos de culturas armadilha para recuperação FMA, em Lavras – MG e Blumenau – SC, e experimento para determinação do potencial de inóculo de FMA. A influência dos fatores abióticos relacionados à fertilidade e textura do solo no potencial de inóculo de FMA foi avaliada. O maior potencial do inóculo micorrízico foi encontrado em ambiente de canga. Foi observado aumento da diversidade de espécies de FMA capturadas em culturas armadilha quando as mesmas são conduzidas em localidades diferentes. Além disso, foi observada a influência dos fatores abióticos relacionados à textura e fertilidade do solo no potencial inóculo FMA.

Os fatores abióticos análise de fertilidade e textura do solo, teores de elementos químicos obtidos por pXRF, e atributos de terreno obtidos por modelo digital de elevação do terreno foram utilizados como preditores de indicadores microbiológicos de qualidade do solo no segundo e terceiro artigos. Em ambos os artigos o solo foi coletado em duas estações (seca e chuvosa) em fitofisionomias de Cerrado (Nova Lima – MG), canga (Nova Lima – MG), Mata Atlântica (Brumadinho – MG) e pilhas de estabilização de área de armazenagem de minério pré-transporte (Brumadinho – MG).

No segundo artigo foram preditos carbono da biomassa microbiana, respiração basal do solo, quociente microbiano e quociente metabólico. O uso de fitofisionomia e estação de coleta do solo como preditores nos modelos melhorou a predição dos indicadores. Fertilidade do solo e textura foram importantes para a predição dados de. Os teores de elementos químicos obtidos pelo pXRF não foram capazes de predizer esses indicadores microbiológicos de qualidade do solo, enquanto os atributos do terreno foram os melhores preditores da respiração basal do solo.

No terceiro artigo, os indicadores de qualidade do solo preditos foram as enzimas do solo: urease, β-glicosidase, fosfatase ácida, fosfatase alcalina e atividade total de enzimas estimada pela hidrólise do diacetato de fluoresceína. Os teores de elementos obtidos pelo pXRF, as propriedades físico-químicas do solo e atributos do terreno conseguiram predizer as enzimas do solo com grande precisão, e os teores de elementos lidos por pXRF estiveram entre os mais importantes para a predição das enzimas.

A espacialização dos indicadores microbiológicos de qualidade do solo preditos foi feita, nos artigos 2 e 3, usando modelos gerados com dados de atributos do terreno. A espacialização permitiu melhor visão geral da variabilidade desses em cada área de estudo em uma determinada estação do ano. Neste trabalho, a espacialização foi feita com a finalidade de mostrar o potencial de uso da técnica para fins de estudos relacionados à microbiologia do solo, sendo necessários estudos com validação para comprovar sua eficácia e para auxiliar na interpretação e tomada de decisão sobre manejo a ser utilizado nas áreas avaliadas.

Mais estudos sobre influência de fatores abióticos nos atributos microbiológicos do solo e do uso desses fatores para a predição dos indicadores microbiológicos de qualidade do solo são necessários. Ressalta-se ainda que esses estudos não têm por objetivo a substituição das técnicas atualmente utilizadas para a determinação dos atributos microbiológicos pois os atributos microbiológicos determinados por técnicas clássicas são necessários para a modelagem dos dados. A vantagem da predição utilizando ferramentas de modelagem é a redução da quantidade de amostras necessárias para estudos em diferentes áreas.

Por fim, ressalta-se a importância dos estudos relacionados a biodiversidade e manutenção da qualidade do solo no Quadrilátero Ferrífero. Dois grandes desastres humanos e ambientais causaram perturbações antropogênicas drásticas nos últimos anos nesse importante "hot spot" de biodiversidade que foram o rompimento das barragens Fundão, em Mariana – MG, e Córrego do Feijão, em Brumadinho – MG. Esses desastres afetam direta e indiretamente a biodiversidade local e aumentaram ainda mais o apelo ecológico e ambiental para estudos nessas áreas. Esses estudos auxiliam no processo de busca por alternativas eficientes para a

recuperação de áreas impactadas direta ou indiretamente pela mineração, além de servirem como base de conhecimento para outras áreas.