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ÁGHATA CRISTIE REWA CHARNOBAY

**ATRIBUTOS MICROBIOLÓGICOS DE SAÚDE DO SOLO EM  
SISTEMAS DIVERSIFICADOS DE PRODUÇÃO DE SOJA**

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Londrina  
2024

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutora em Agronomia.

Orientador: Prof. Dr. Marco Antonio Nogueira

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Londrina, 27 de fevereiro de 2024.

Aos meus pais, Darcio e Lourdes,  
e aos meus irmãos André e Yuri

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“Em algum lugar, alguma coisa incrível está esperando para ser descoberta”

*Carl Sagan*

"A natureza, em seus caprichos e mistérios, condensa em pequenas coisas o poder de dirigir as grandes; nas sutis, a potência de dominar as mais grosseiras; nas coisas simples, a capacidade de reger as complexas”

*Ana Maria Primavesi*

CHARNOBAY, Ághata Cristie Rewa. **Atributos microbiológicos de saúde do solo em sistemas diversificados de produção de soja** 2024. 101 folhas. Tese (Doutorado em Agronomia) – Universidade Estadual de Londrina, Londrina, 2024.

## RESUMO

O manejo sustentável do solo é essencial para a manutenção da sua saúde e da sustentabilidade da produção agropecuária. A diversificação dos sistemas de produção, utilizando diferentes espécies no plano de rotação, é uma prática de manejo conservacionista preconizada e que pode contribuir para a melhoria da saúde do solo (SS). A avaliação da SS é realizada por indicadores físicos, químicos e biológicos, que juntos determinam a qualidade do sistema de produção. Entender a influência da diversificação de culturas sobre esses indicadores, bem como a sua interrelação, é importante para dar suporte a recomendações de manejo visando sistemas de produção mais sustentáveis. O objetivo deste trabalho foi avaliar o efeito da diversificação de culturas em sistemas de produção de soja sobre a saúde do solo, utilizando indicadores microbiológicos. Um experimento foi desenvolvido no Centro de Pesquisa Agrícola da Copacol, em Cafelandia – PR, para avaliar o efeito da diversificação da sucessão soja/milho com diferentes culturas após a soja (trigo, aveia, milho consorciado com braquiária), bem como a introdução de uma terceira safra em sucessão (trigo ou aveia preta) ou em rotação (soja/milho/aveia branca; soja/trigo; soja/milho/aveia preta), em atributos microbiológicos de um Latossolo no curto prazo (1 a 3 anos). Outro ensaio foi conduzido na Embrapa Soja, Londrina – PR, para verificar como o cultivo de culturas de interesse comercial (milho e trigo) e de cobertura (crotalária e braquiária) em sucessão à soja afetam atributos microbiológicos de um Latossolo e a produtividade da soja no médio prazo (5 a 7 anos), comparado ao pousio. Os atributos microbiológicos avaliados foram o carbono e o nitrogênio da biomassa microbiana, a respiração basal do solo, quociente microbiano e metabólico, e atividade das enzimas  $\beta$ -glucosidase, arilsulfatase, fosfatase ácida e L-glutaminase, bem como as taxas de amonificação e nitrificação. Foram também avaliados os atributos químicos pH, carbono orgânico, nitrogênio total, e na forma de amônio e nitrato, e macronutrientes. Em ambos estudos, a inclusão de culturas de cobertura em sucessão (aveia preta e braquiária) à soja ou como terceira safra (aveia preta), entre a colheita do milho e a semeadura da soja, promoveu os atributos microbiológicos, principalmente ligados à atividade microbiana, comparado ao sistema soja/milho, com efeitos mais proeminentes com o aumento do tempo de adoção (3 a 7 anos). No curto prazo, atributos microbiológicos relacionados ao ciclo do carbono se mostraram mais sensíveis em discriminar as modificações nos agroecossistemas resultantes da diversificação de culturas. No médio prazo, a melhoria dos atributos microbiológicos foi acompanhada por aumento da produtividade da soja. Este trabalho reforça a importância da utilização de indicadores biológicos no monitoramento da saúde do solo e apresenta sistemas de diversificação alternativos que promovem a biologia do solo em comparação à sucessão soja/milho e o pousio.

**Palavras-chave:** Manejo do solo; Qualidade do solo; Sistema plantio direto; Bioindicadores.

CHARNOBAY, Ághata Cristie Rewa. **Microbial attributes of soil health in diversified soybean production systems**. 2024. 101 pages. Thesis (Postgraduate Program of Agronomy) – State University of Londrina, Londrina, 2024.

## ABSTRACT

Sustainable soil management is essential for maintaining soil health (SH) and the sustainability of agroecosystems. The diversification of production systems, using different species in the rotation plan, can improve SH. The evaluation of SH is carried out by physical, chemical and biological indicators, which together determine the quality of the production system. Understanding the influence of crop diversification on these indicators and their interrelation is important for supporting recommendations of management towards more sustainable production systems. This study aimed to evaluate the effect of crop diversification on soil health in Oxisols under soybean production systems, using microbiological indicators. An experiment was conducted at the Copacol Agricultural Research Center in Cafelandia, PR, to assess the effect of soybean/maize succession diversification with alternative crops after soybean (wheat, oats, maize intercropped with brachiaria), as well as the introduction of a third crop in succession (wheat and black oats) or rotation (soybean/maize/white oats; soybean/wheat; soybean/maize/black oats), on short-term changes (1 to 3 years) in microbial attributes. Another trial was conducted at Embrapa Soja, Londrina, PR, to access the effects of off-season cash crops (maize and wheat) and cover crops (crotalaria and ruzigrass) on microbial attributes and soybean yield in the medium-term (5 to 7 years), compared with fallow. The assessed microbial attributes included microbial biomass carbon and nitrogen, soil basal respiration, microbial and metabolic quotients, the activities of enzymes  $\beta$ -glucosidase, arylsulfatase, acid phosphatase, and L-glutaminase, as well as ammonification and nitrification rates. Chemical attributes such as pH, organic carbon, total nitrogen, ammonium and nitrate, and macronutrients were also evaluated. In both studies, including cover crops in succession (black oats and ruzigrass) after soybean or as a third crop (black oats) between maize harvesting and soybean sowing, promoted microbial attributes, mainly related to microbial activity, in comparison with the soybean/maize system and fallow, with more prominent effects with increasing adoption time (3 to 7 years). In the short term, microbial attributes related to the carbon cycle proved more sensitive in discriminating modifications in the agroecosystems resulting from crop diversification. In the medium-term, improvement in microbiological attributes was followed by increased soybean productivity. This study reinforces the importance of using microbial indicators for monitoring soil health and presents alternative diversification systems that promote soil biology compared with the soybean/maize succession and fallow.

**Keywords:** Soil management; Soil quality; No till system; Bioindicators.

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## 1. INTRODUÇÃO

O solo é a base para a produção agrícola e desempenha diversos serviços ecossistêmicos, sendo a manutenção da saúde do solo (SS), essencial para a sustentabilidade dos agroecossistemas. A SS é resultado de interações complexas entre seus atributos físicos, químicos e biológicos, e pode ser avaliada por meio de indicadores, medidas quantitativas ou semi-quantitativas, de fácil mensuração que possam expressar os efeitos das práticas de uso e manejo na qualidade física, química e biológica do solo.

Atributos microbiológicos e bioquímicos do solo respondem rapidamente às mudanças no seu uso e manejo. Os microrganismos do solo desempenham importantes funções relacionadas à mineralização e síntese da matéria orgânica, ciclagem de nutrientes e degradação de poluentes. O monitoramento da biomassa e da atividade respiratória microbiana e da atividade de enzimas participantes dos ciclos biogeoquímicos, bem como a avaliação da estrutura e funcionalidade da comunidade microbiana, são importantes e promissores indicadores da SS.

Sistemas de produção que priorizem práticas conservacionistas favorecem a manutenção ou recuperação da SS e devem ser estimulados. Entre as práticas conservacionistas com potencial de melhoria da SS, destaca-se a diversificação de culturas. Sistemas de produção diversificados auxiliam no controle de pragas e doenças e proporcionam melhorias físico-químico-biológicas na SS, quando comparados a sistemas de monocultura ou sucessão, comumente utilizados em áreas de produção de grãos no Brasil.

O cultivo de entressafra com culturas de interesse comercial como o trigo (*Triticum aestivum*) e a aveia branca (*Avena sativa*) em substituição ou rotação com milho (*Zea mays*), bem como a inclusão de espécies de leguminosas (e.g. crotalárias – *Crotalaria* spp.) e gramíneas (e.g. braquiárias – *Urochloa* spp, aveia preta – *Avena strigosa*) como culturas de cobertura são alternativas que podem ser adotadas para aumentar a diversificação das culturas na sucessão soja/milho e soja/pousio. Além disso, utilizar a janela entre a colheita do milho e a semeadura da soja para produção de grãos (como terceira safra) ou cobertura é uma opção para aumentar o aporte de resíduos culturais ao solo e o rendimento por unidade de área cultivada.

O objetivo deste trabalho foi avaliar o efeito da diversificação de culturas em sistemas de produção de soja sobre a saúde do solo, utilizando indicadores microbiológicos. No capítulo 1, atributos microbiológicos foram avaliados quanto aos efeitos da adoção de sistemas de diversificação (com culturas alternativas para sucessão ou inclusão na terceira safra) após o uso contínuo de soja/milho em curto prazo. No capítulo 2, objetivou-se compreender os efeitos

de diferentes culturas de interesse comercial e de cobertura cultivadas na entressafra sobre atributos microbiológicos do solo e na produtividade da soja.

## 2. REVISÃO BIBLIOGRÁFICA

### 2.1 SAÚDE DO SOLO: HISTÓRICO, CONCEITO E INDICADORES

Solos saudáveis são recursos essenciais e valiosos pois fornecem múltiplos serviços ecossistêmicos ligados à regulação dos ciclos hidrológicos e biogeoquímicos, produção agrícola e segurança alimentar, mitigação da mudança climática e saúde dos seres vivos. No entanto, como em outros habitats, os solos estão sob crescente pressão devido às atividades antrópicas (FIERER; WOOD; BUENO DE MESQUITA, 2021). Um relatório sobre os recursos terrestres do planeta mostra que 33% dos solos agrícolas são considerados degradados (FAO, 2015), impactando negativamente o bem-estar de pelo menos 3,2 bilhões de pessoas em todo o mundo (IPBES, 2018). A degradação dos solos está principalmente relacionada às mudanças e intensificação do uso da terra pela atividade agrícola e a pressão para o aumento da produtividade, com intensificação do uso de fertilizantes, pesticidas, irrigação e mecanização (FOLEY *et al.*, 2011), além de baixa diversificação de culturas e frequente revolvimento do solo (TELLES *et al.*, 2022).

A promoção de serviços ecossistêmicos está ligada à saúde do solo (SS), definida como a capacidade do solo funcionar dentro dos limites dos ecossistemas naturais ou manejados, para sustentar a produtividade de plantas e animais, e manter ou aumentar a qualidade do ar e da água e promover a saúde dos seres vivos (DORAN; ZEISS, 2000). O conceito, apesar de ter se tornado familiar entre pesquisadores e produtores nos últimos anos, provém de diversas contribuições e terminologias empregadas ao longo do tempo e ainda em evolução (Tabela 1) (BÜNEMANN *et al.*, 2018; KARLEN *et al.*, 2019; 2021). Há autores que utilizam os termos qualidade e saúde do solo como sinônimos (CREAMER *et al.*, 2022), enquanto outros argumentam que saúde e qualidade do solo se diferenciam pelo fato de o primeiro se estender além da saúde humana para objetivos de sustentabilidade mais amplos, que incluem a saúde planetária (FIERER; WOOD; BUENO DE MESQUITA, 2021; LEHMANN *et al.*, 2020).

Existe consenso, no entanto, quanto à importância de monitorar a SS a fim de identificar formas de uso e manejo do solo mais apropriadas e estimular a adoção de práticas para mitigar problemas relacionados (BAI *et al.*, 2018). Por ser resultante de interações complexas entre os atributos físicos, químicos e biológicos, a SS não pode ser mensurada diretamente. Para tanto, são utilizados indicadores, medidas quantitativas ou qualitativas

selecionadas de acordo com as funções do solo e relacionadas aos atributos que são importantes para medir a sustentabilidade de sistemas cultivados e naturais (KARLEN *et al.*, 1997).

Os indicadores de SS devem ser sensíveis às variações de manejo, de fácil mensuração e acesso tanto para cientistas, como para agricultores, extensionistas, além de ter baixo custo e explicar processos importantes para a manutenção das funções do ecossistema em estudo (RINOT *et al.*, 2019). Além disso, um indicador é útil somente quando seu valor pode ser corretamente interpretado e comparado com valores ou áreas de referência (BÜNEMANN *et al.*, 2018), ou ainda comparados com curvas de calibração (LOPES *et al.*, 2013), sendo estes dependentes do contexto local - características do solo e do manejo (FIERER; WOOD; BUENO DE MESQUITA, 2021).

Recomenda-se o uso de um conjunto de indicadores, ao invés de um único atributo, a fim de melhor compreender os efeitos do uso e manejo do solo, já que os atributos são interconectados e geram interferências mútuas, embora tenham diferentes níveis de resposta a mudanças (CARDOSO *et al.*, 2013). É importante, ainda, a seleção e utilização de indicadores de diferentes naturezas (física, química e biológica), visando abrangência holística da SS (ZORNOZA *et al.*, 2015). Entretanto, estudos recentes indicam que a avaliação da atividade de duas enzimas, a arilsulfatase e a beta-glucosidase, é capaz de sumarizar a condição geral da saúde do solo (LOPES *et al.*, 2013). Atributos do solo que respondam rapidamente às ações naturais ou antrópicas são considerados bons indicadores da SS. A textura e a densidade de partículas são, por exemplo, características físicas inerentes do solo e não são influenciadas pelas práticas de uso e manejo (STEFANOSKI *et al.*, 2013). A estrutura, no entanto, é sensível às mudanças antrópicas, sendo importante indicador da SS por governar diversos processos como a retenção e infiltração de água, as trocas gasosas, a dinâmica da matéria orgânica e dos nutrientes, a facilidade de crescimento de raízes e a suscetibilidade à erosão (RABOT *et al.*, 2018). A estrutura do solo pode ser avaliada pela sua densidade, pelo volume e tamanho de poros e agregação. Estes indicadores fornecem medidas do grau de compactação, do volume passível de ser ocupado por água e ar, da ação das raízes e da fauna edáfica e da dinâmica de infiltração e armazenamento de água do solo (CARDOSO *et al.*, 2013).

A saúde química do solo está relacionada com a capacidade deste em fornecer nutrientes para as plantas e reter elementos químicos ou compostos prejudiciais ao ambiente e ao crescimento das plantas. Os indicadores químicos de SS mais utilizados são a acidez (pH), teor de matéria orgânica, de nutrientes e de elementos tóxicos, como o alumínio (SCHOENHOLTZ; MIEGROET; BURGER, 2000).

**Tabela 1:** Definições e terminologias utilizadas ao longo da evolução do conceito de saúde do solo.

Conceito	Definição	Referência
Aptidão do solo ( <i>soil tilth</i> )	Condição física do solo ligada à sua facilidade de preparo, aptidão como berço de sementes e promoção da emergência de plântulas e penetração radicular	Karlen <i>et al.</i> (1990)
Fertilidade do solo	A capacidade de um solo fornecer nutrientes em quantidades e proporções para o crescimento de plantas específicas	Doula; Sarris (2016)
Qualidade do solo	A capacidade dos solos de produzir milho, soja e trigo sob condições de manejo de alto nível	Mausel (1971)
Qualidade do solo	Capacidade do solo funcionar para sustentar a produtividade biológica, manter a qualidade ambiental e promover a saúde de plantas e animais	Doran; Parkin (1994)
Saúde do solo	Capacidade do solo funcionar como um sistema vital para sustentar a produtividade de plantas e animais, manter ou aumentar a qualidade do ar e da água e promover a saúde dos seres vivos, dentro dos limites dos ecossistemas naturais ou manejados	Doran; Zeiss (2000)
Fertilidade sistêmica	Abordagem integrativa do conceito de fertilidade com base em propriedades físicas, químicas e biológicas do solo associadas ao sistema de manejo	Anghinoni; Vezzani (2021)
Segurança do solo	Capacidade do solo de sustentar funções para fornecer serviços planetários, bem-estar humano e proteção geral dos ecossistemas. O conceito reconhece cinco dimensões do solo (1) capacidade, (2) condição, (3) capital, (4) conectividade e (5) codificação, que abrangem as ciências sociais, econômicas e biofísicas, bem como aspectos públicos e legais	McBratney; Field; Koch (2014)
Saúde única	Abordagem que considera a saúde das pessoas, dos animais e do meio ambiente interconectada, propondo solução colaborativa de problemas em escala local, nacional e global	FAO (2007)

Além dos atributos físicos e químicos, atributos microbiológicos para estimativa da saúde do solo têm sido explorados devido à importância dos microrganismos na provisão de inúmeros serviços ecossistêmicos. Entre os indicadores microbiológicos do solo mais utilizados estão a biomassa microbiana (BM), a respiração basal (RB), o quociente microbiano ( $q_{Mic}$ ) e o quociente metabólico ( $q_{CO_2}$ ) (BASTIDA *et al.*, 2008), bem como a atividade de enzimas envolvidas nos ciclos biogeoquímicos (MENDES *et al.*, 2019).

## 2.2 DIVERSIFICAÇÃO DE CULTURAS

As práticas de manejo podem impactar positivamente ou negativamente a saúde do solo, afetando a sustentabilidade da produção agrícola. O sistema plantio direto (SPD) é caracterizado pela semeadura direta sobre os resíduos das culturas anteriores e é uma prática conservacionista reconhecida para manutenção ou aumento da conservação do solo (SILVA *et al.*, 2014). Ao reduzir os impactos negativos na estrutura do solo, promovendo cobertura permanente, revolvimento limitado à linha de semeadura e diversificação de culturas, o SPD resulta em melhoria dos atributos físicos (MORAES *et al.*, 2016), elevação dos teores de água e matéria orgânica do solo (LAL, 2016) e maior biomassa e atividade microbiana, uma vez que a maior intensidade dos processos microbiológicos ocorre na camada superficial do solo (BABUJIA *et al.*, 2010).

O Brasil é referência mundial na adoção do SPD com mais de 36 Mha cultivados sob esse sistema (FEDRAPDP, 2018). Entre as principais *commodities* cultivadas em SPD, a soja [*Glycine max* (L.) Merrill] se destaca. Na safra 2022/2023, 154,6 milhões de toneladas da oleaginosa foram colhidas em 44,1 Mha cultivados (CONAB, 2023). O Paraná é o segundo maior produtor de soja brasileiro, com 22,5 milhões de toneladas naquela safra (CONAB, 2023), possuindo também a segunda maior área cultivada sob SPD do país (FEDRAPDP, 2018).

Um dos pilares do SPD é a diversificação de culturas, entendida como o arranjo temporal e espacial das espécies que compõem um sistema agrícola (DEBIASI *et al.*, 2015). Sistemas diversificados favorecem a saúde física, química e biológica do solo pelo aumento da matéria orgânica e da ciclagem de nutrientes, pela supressão de plantas daninhas e o controle de pragas e doenças, levando ao aumento de produtividade e redução de custos de produção (ADETUNJI *et al.*, 2020). No entanto, os sistemas de produção de soja predominantes na agricultura brasileira envolvem a sucessão da oleaginosa na primavera-verão e milho (*Zea mays* L.) no outono-inverno (GARBELINI *et al.*, 2020). Como exemplo, durante a safra

2021/2022, cerca de 37% da área de produção de soja (~16,4 milhões de hectares) foram cultivados com milho em sucessão (CONAB, 2023). Nas regiões oeste e centro-oeste do estado do Paraná, importantes produtoras de soja, cerca de 65% da área de soja é cultivada com milho no outono-inverno (DERAL, 2023). Um questionário realizado com 234 agricultores do oeste paranaense a respeito de práticas de manejo adotadas para agricultura conservacionista evidenciou o problema, mostrando que 67% dos agricultores não estavam familiarizados com todos os pilares do SPD, em especial a rotação de culturas (49%) (TELLES *et al.*, 2022). Em adição, em muitas regiões do Brasil, sobretudo em áreas com baixa pluviosidade durante o outono-inverno, há dificuldade em selecionar culturas adaptadas às condições climáticas para o cultivo entre duas safras de soja. Essas áreas são geralmente deixadas em pousio no período outono-inverno, o que reduz a sustentabilidade do SPD (BALBINOT JUNIOR *et al.*, 2017). A simplificação dos sistemas de cultivo facilita a rotina operacional nas fazendas, mas, por outro lado, intensifica os problemas agronômicos, principalmente relacionados à redução da saúde do solo e ao aumento de problemas fitossanitários (DEBIASI *et al.*, 2015). Além disso, estudos recentes mostram maior retorno econômico e estabilidade de produção quando a rotação de culturas e o consórcio milho + braquiária foi utilizado, comparado à sucessão soja/milho (BALBINOT JUNIOR *et al.*, 2024; GARBELINI *et al.*, 2020).

Estratégias para aumentar a diversidade e a sustentabilidade em sistemas de produção são inúmeras e envolvem práticas como a diversificação de culturas, com pelo menos três espécies no sistema de rotação (HIRAKURI *et al.*, 2012), o consórcio de plantas (BAPTISTELLA *et al.*, 2020), e o uso de plantas de cobertura (KIM *et al.*, 2020). Pensando na diversificação dos sistemas de produção de soja, a rotação ou substituição do milho por outras culturas de interesse comercial ou como cobertura (BALBINOT JUNIOR *et al.*, 2024; YOKOYAMA *et al.*, 2022), bem como a intensificação da sucessão soja/milho com o cultivo de terceira safra (entre a colheita do milho e a semeadura da soja) são opções.

As culturas comerciais de entressafra são cultivadas entre duas safras de verão com intuito de substituir o pousio e obter retorno econômico. O cultivo do trigo (*Triticum aestivum* L.) na entressafra da soja é uma alternativa ao milho, principalmente na região sul do Brasil (BALBINOT JUNIOR *et al.*, 2020). Além do retorno econômico, o cultivo do trigo pode auxiliar na manutenção da cobertura do solo no período outono/inverno, fornecendo de 3 a 5 Mg ha<sup>-1</sup> de massa seca com alta relação C:N (BALBINOT JUNIOR *et al.*, 2024; YOKOYAMA *et al.*, 2022). A aveia branca (*Avena sativa* L.) é o segundo cereal de inverno mais produzido no Brasil, superado apenas pelo trigo (CONAB, 2023). A gramínea pode ser utilizada na alimentação humana e animal (silagem, feno, rações) devido a sua composição nutricional (62%

carboidratos e 11% proteínas), bem como inserida na rotação de culturas visando a manutenção da qualidade do solo pela adição de resíduos (COELHO *et al.*, 2019).

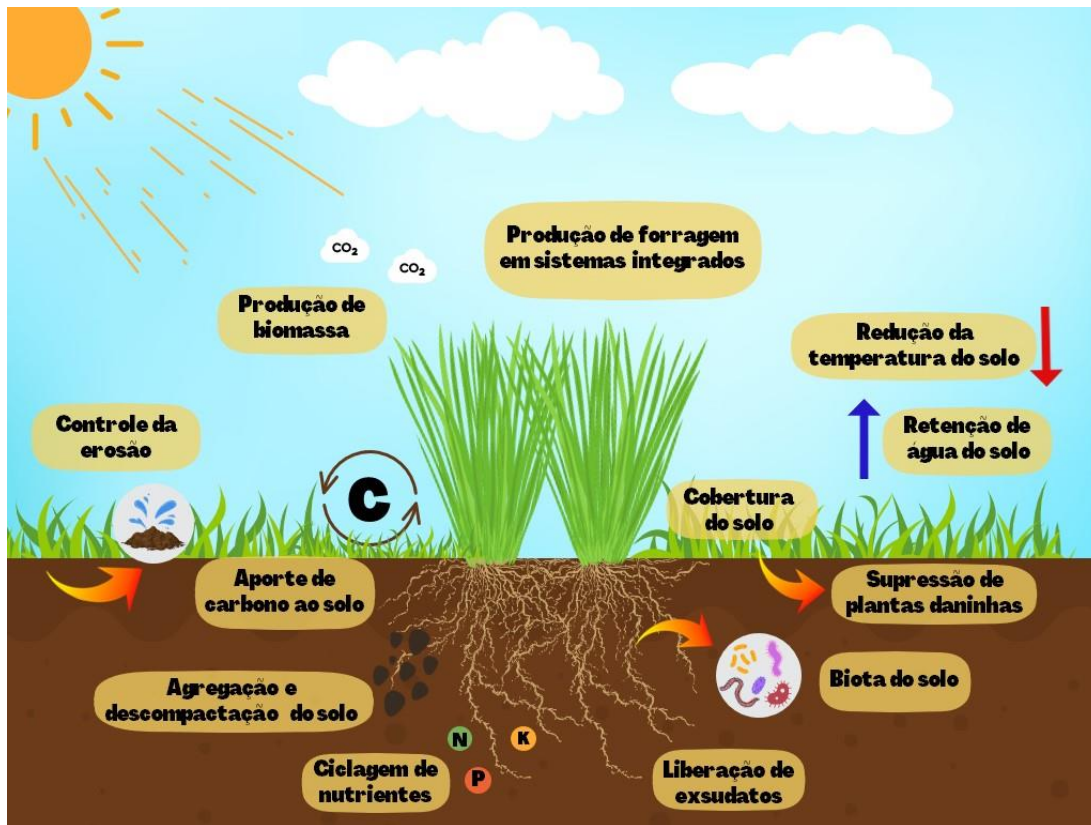
Culturas de cobertura (CC) são utilizadas para promover a proteção e melhoria dos solos, geralmente no outono-inverno, entre o cultivo de culturas de interesse econômico. Entre as culturas mais comumente utilizadas como CCs pode-se citar leguminosas como a ervilhaca (*Vicia sativa* L.), ervilha (*Pisum sativum* L.), e crotalárias (*Crotalaria* spp.), bem como as gramíneas aveia branca e preta (*Avena strigosa* Schreb.) (ADETUNJI *et al.*, 2020) e, em agroecossistemas tropicais, forrageiras como as braquiárias (*Urochloa* spp. syn. *Brachiaria*) (MOMESSO *et al.*, 2022b). Dadas as diferenças na qualidade e quantidade de biomassa produzidas (Tabela 2), a utilização de leguminosas como CC propicia rápida ciclagem de nutrientes e processos como a fixação biológica de nitrogênio, enquanto que a utilização de gramíneas promove maior crescimento de raízes e aporte de biomassa e consequente cobertura, proteção do solo e liberação mais gradual de nutrientes (SOUZA *et al.*, 2024).

Algumas características das plantas forrageiras como a adaptação às condições ambientais tropicais e solos pouco férteis, elevada produção de biomassa, sistema radicular agressivo e produção de exsudatos radiculares, estão ligadas à prestação de importantes serviços ecossistêmicos e as tornam excelentes alternativas como CC (BAPTISTELLA *et al.*, 2020). As braquiárias são usualmente semeadas isoladas ou consorciadas com o milho de segunda safra, e os benefícios da adoção de espécies desse gênero no sistema de rotação (Figura 1) incluem alta produção de matéria seca para a formação de cobertura do solo (FRANCHINI *et al.*, 2014), ciclagem de nutrientes (ALMEIDA *et al.*, 2019; SOUZA *et al.*, 2024) e supressão de plantas daninhas (SÃO MIGUEL *et al.*, 2018). Estas gramíneas também são benéficas para a conservação do solo, uma vez que propiciam maior cobertura (BALBINOT JUNIOR *et al.*, 2024) e melhoram seus atributos físico-químico-biológicos (CRUSCIOL *et al.*, 2015; SHIMIZU *et al.*, 2022; TONON-DEBIASI *et al.*, 2024), melhorando o rendimento de outras culturas em sucessão, como a soja (BALBINOT JUNIOR *et al.*, 2017). Nos últimos anos, estudos relacionados a estratégias de uso das forragens (MACHADO *et al.*, 2017; VOLF *et al.*, 2021), adubação (BOSSOLANI *et al.*, 2021; MOMESSO *et al.*, 2022a) e resposta a inoculantes (GUIMARÃES *et al.*, 2022, 2023), bem como promoção de saúde física, química e biológica (MOMESSO *et al.*, 2022a, 2022b; SILVA *et al.*, 2021, 2022) do solo têm sido desenvolvidos, buscando melhores recomendações de uso e manejo destas culturas. De maneira geral, observam-se melhorias em atributos da saúde do solo e componentes de produção das culturas de interesse, sem afetar negativamente a produtividade da cultura principal quando consorciada com a forragem.

Tabela 2: Características de culturas de interesse comercial e cobertura de solo com possibilidade de utilização na diversificação de sistemas de produção de soja.

Nome comum	Nome científico (Família)	Massa seca (Mg ha <sup>-1</sup> ) <sup>(1)</sup>	Relação C: N
<i>Culturas de interesse comercial</i>			
Milho	<i>Zea mays</i> L. (Poaceae)	5 - 20	71:1 <sup>(2)</sup> 105:1 <sup>(3)</sup>
Trigo	<i>Triticum aestivum</i> L. (Poaceae)	4 - 6	89:1 <sup>(3)</sup>
Aveia Branca	<i>Avena sativa</i> L. (Poaceae)	7 - 15	39:1 <sup>(4)</sup>
<i>Culturas de cobertura</i>			
Aveia preta	<i>Avena strigosa</i> Schreb (Poaceae)	5 - 10	37:1 <sup>(5)</sup> 37:1 <sup>(3)</sup>
Braquiária	<i>Urochloa</i> spp. (Poaceae)	10 - 16	45:1 <sup>(6)</sup>
Crotalária	<i>Crotalaria spectabilis</i> Roth. (Fabaceae)	4 - 6	20:1 <sup>(3)</sup>

<sup>(1)</sup> Dados obtidos de Carvalho et al. (2022); <sup>(2)</sup> Silva et al., (2009); <sup>(3)</sup> Chaves et al. (2021); <sup>(4)</sup> Redin et al. (2018); <sup>(5)</sup> Giacomini et al. (2003); <sup>(6)</sup> Rosolem, Werle e Garcia (2012).



**Figura 1.** Benefícios à saúde do solo favorecidos pela inclusão de *Urochloa* spp. como cultura de cobertura na diversificação dos sistemas de produção de grãos.

### 2.3 ATRIBUTOS MICROBIOLÓGICOS COMO INDICADORES DA SAÚDE DO SOLO

Uma das conquistas mais importantes ao longo da evolução do conceito de SS foi a adição do componente biológico para avaliação dos efeitos do manejo do solo (LEHMANN *et al.*, 2020). Durante meados do século XIX, estudos de microbiologistas como Louis Pasteur, Selman Waksman e Sergei Winogradsky formaram a base para o desenvolvimento da microbiologia do solo moderna (WALL *et al.*, 2019). Importantes descobertas em relação à microbiologia agrícola se seguiram, e hoje tem-se clareza da importância dos microrganismos do solo na decomposição de resíduos orgânicos, mineralização e solubilização de nutrientes, degradação de compostos e poluentes xenobiontes, bem como, na promoção do crescimento e saúde das plantas a partir de interações na rizosfera, tanto em ecossistemas naturais como manejados (FIERER, 2017; KASCHUK; ALBERTON; HUNGRIA, 2010).

Apesar dos avanços alcançados ao longo do desenvolvimento como ramo da ciência, a microbiologia do solo ainda é uma das fronteiras mais inexploradas associadas à

saúde do solo (BÜNEMANN *et al.*, 2018; SIMON *et al.*, 2022; VALANI *et al.*, 2021). Os acontecimentos pós-guerras mundiais que influenciaram a agricultura moderna colocaram maior peso nos atributos físicos e químicos do solo (LEHMAN *et al.*, 2015), sendo a inclusão das propriedades biológicas nas avaliações da saúde do solo iniciada no final da década de 1980/início da década de 1990 (DORAN; ZEISS, 2000). Atualmente, dada à sensibilidade dos microrganismos às mudanças nos agroecossistemas em curto prazo, indicadores microbiológicos são considerados promissores como ferramentas para avaliação da saúde do solo (BÜNEMANN *et al.*, 2018).

### 2.3.1 Biomassa Microbiana

A biomassa microbiana (BM) é a fração viva da matéria orgânica do solo constituída pelos organismos menores que  $5 \times 10^3 \mu\text{m}^3$  (BROOKES, 2001). A BM representa de 1 a 5% da matéria orgânica do solo (MOREIRA; SIQUEIRA, 2006) e a maior parte da biomassa total (PULLEMAN *et al.*, 2012), estando envolvida em processos e interações ecológicas que influenciam as funções do solo desde a microescala até a escala da paisagem (NANNIPIERI *et al.*, 2020).

A comunidade microbiana representada pela BM do solo é responsável pela decomposição de resíduos orgânicos e síntese de matéria orgânica, mineralização e solubilização de nutrientes, degradação de compostos e poluentes xenobiontes, bem como a promoção do crescimento e saúde das plantas a partir de interações na rizosfera (KASCHUK; ALBERTON; HUNGRIA, 2010; SCHLOTTER *et al.*, 2018). Além disso, a BM é o destino inicial dos nutrientes do solo e, devido à sua rápida ciclagem com a morte dos microrganismos, é um importante reservatório lábil de nutrientes como nitrogênio, fósforo e enxofre, protegendo-os contra perdas por lixiviação ou fixação e tornando-os disponíveis para as plantas (MOREIRA; SIQUEIRA, 2006).

Alterações nos atributos físicos e químicos do solo influenciadas pelo manejo podem afetar fatores que influenciam diretamente a biomassa e a atividade microbiana, como o teor de água e matéria orgânica, aeração e temperatura do solo, bem como, a interação entre a matéria orgânica e a fração mineral (TORABIAN; FARHANGI-ABRIZ; DENTON, 2019). As mudanças na BM do solo podem ser avaliadas pela quantificação do carbono (CBM) e do nitrogênio (NBM) presentes nas células microbianas (BROOKES, 2001).

### 2.3.2 Respiração Basal do Solo

A atividade metabólica dos microrganismos do solo pode ser quantificada pela respiração basal (RB), medida que representa o CO<sub>2</sub> produzido por microrganismos aeróbicos ao atuar na oxidação da matéria orgânica (ALEF, 1995). Juntamente com a BM, a RB é frequentemente empregada como indicador de saúde do solo em ambientes naturais e agrícolas (BASTIDA *et al.*, 2008).

A RB é influenciada pelo manejo e é regulada pela quantidade e qualidade dos resíduos orgânicos presentes no solo (HUNGRIA *et al.*, 2009; LOPES *et al.*, 2013). A conversão de áreas de florestas nativas em áreas de cultivo reduz a respiração microbiana, consequência da diminuição das entradas de carbono (BINI *et al.*, 2013). Por sua vez, a presença de resíduos frescos e de fácil decomposição (FRANCHINI *et al.*, 2007), assim como a construção de um ambiente favorável aos microrganismos, com condições adequadas de temperatura, água e oxigênio, e manutenção da estrutura do solo, favorecem a atividade biológica (BABUJIA *et al.*, 2010; BINI *et al.*, 2014; HUNGRIA *et al.*, 2009).

Valores de RB elevados podem resultar do aporte de grande quantidade de substrato lábil, mas podem também ser decorrentes da rápida oxidação de carbono proveniente, por exemplo, da desestruturação do solo promovida por técnicas inadequadas de preparo, que facilitam o acesso da comunidade microbiana ao reservatório de matéria orgânica, antes protegida no interior dos agregados (FRANCHINI *et al.*, 2007; HUNGRIA *et al.*, 2009). Assim, apesar de bom indicador da SS, a RB deve ser interpretada com cuidado, sendo recomendada sua associação a outros indicadores e conhecimento sobre o ambiente estudado, já que valores elevados podem indicar tanto alta produtividade do sistema, quanto situação de estresse para a comunidade microbiana (ISLAM; WEIL, 2000).

### 2.3.3 Quociente Microbiano e Metabólico

Índices importantes utilizados como indicadores da QS são derivados do CBM, como a razão CBM/COT (carbono orgânico total do solo), conhecida como quociente microbiano ( $q_{Mic}$ ) (INSAM; DOMSCH, 1988). Já a razão entre a respiração basal do solo e o CBM, razão RB/CBM, resulta no quociente metabólico ( $q-CO_2$ ) (INSAM; HASELWANDTER, 1989).

O  $q_{Mic}$  é um índice que fornece informações sobre a capacidade do solo em favorecer o crescimento microbiano, pois indica quão propício é o ambiente para a BM, aumentando a proporção do C nas células microbianas em relação ao COT do solo (INSAM; DOMSCH, 1988). Os valores de  $q_{Mic}$  têm relação com a adição, transformação e qualidade da

matéria orgânica e são influenciados pela intensidade de manejo do solo. Em geral, solos florestais e agrícolas possuem  $q_{\text{Mic}}$  em variando entre 2,0 e 4,4, sendo valores abaixo de 2,0 considerados limitantes para o funcionamento biológico do solo (ANDERSON, 2003).

Espera-se que solos saudáveis apresentem maiores valores de  $q_{\text{Mic}}$  (KASCHUK; ALBERTON; HUNGRIA, 2010). Trabalhos em ambiente tropical têm verificado que sistemas conservacionistas de manejo do solo, como o SPD, apresentam maiores valores de  $q_{\text{Mic}}$  quando comparados ao cultivo convencional (BABUJIA *et al.*, 2010; FRANCHINI *et al.*, 2007; HUNGRIA *et al.*, 2009). Estes resultados destacam a importância dos microrganismos no ciclo do carbono em ambiente tropical, além de validarem o  $q_{\text{Mic}}$  como indicador para detectar o impacto do uso e manejo do solo na microbiota do solo.

O  $q\text{-CO}_2$  permite avaliar o estado metabólico dos microrganismos do solo (INSAM; HASELWANDTER, 1989). Em geral, menores valores de  $q\text{-CO}_2$  estão associados a sistemas mais estáveis, sugerindo uso mais eficiente de C pelos microrganismos (ANDERSON; DOMSCH, 2010). Sistemas de manejo conservacionista proporcionam maiores valores de CBM e menores valores de RB, resultando em menores valores de  $q\text{-CO}_2$  (BINI *et al.*, 2014; FRANCHINI *et al.*, 2007). Por outro lado, valores elevados de  $q\text{-CO}_2$  representam menor eficiência metabólica, indicando atividade biológica (RB) mais intensa para a manutenção da biomassa microbiana, decorrente de uma condição estressante (ANDERSON; DOMSCH, 2010). Em trabalho avaliando sistemas de preparo de solo e rotação de culturas, Franchini *et al.* (2007) encontraram valores de  $q\text{-CO}_2$  55% menores em SPD comparado ao plantio convencional (PC). Resultado semelhante foi encontrado por Hungria *et al.* (2009), que verificaram  $q\text{-CO}_2$  cinco vezes maior em PC comparado ao SPD, indicando uma comunidade microbiana de menor eficiência metabólica e um ambiente desfavorável.

Assim como a RB, o  $q\text{-CO}_2$  deve ser interpretado com cuidado, pois valores elevados, além de indicar condições de estresse à comunidade microbiana, podem indicar aportes de C orgânico facilmente degradável, que promovem prontamente a atividade microbiana (BABUJIA *et al.*, 2010).

#### 2.3.4 Atividade de enzimas do solo

Entre as funções essenciais da BM do solo estão a decomposição, ciclagem de resíduos orgânicos e a degradação de compostos poluentes (KASCHUK; ALBERTON; HUNGRIA, 2010). Em geral, estas ações são mediadas por enzimas, catalisadores em diferentes

reações dos ciclos biogeoquímicos, que processam compostos orgânicos complexos em subunidades assimiláveis, como açúcares e aminoácidos (CALDWELL, 2005).

Os microrganismos são a fonte primária de enzimas no solo, embora plantas e animais também possam produzi-las (TABATABAI, 1994). As enzimas do solo podem ser encontradas no interior das células microbianas (endoenzimas), ligadas à membrana externa ou excretadas no ambiente (exoenzimas) (BALOTA *et al.*, 2013). Exoenzimas estabilizadas, também chamadas de abiônticas, podem permanecer ativas no solo por períodos variáveis, adsorvidas em superfícies orgânicas e inorgânicas, ou complexadas às substâncias húmicas (BURNS *et al.*, 2013). Nessa condição, as exoenzimas apresentam redução de sua capacidade catalítica, mas se mantêm por longos períodos intermediando reações bioquímicas (NOGUEIRA; HUNGRIA, 2013). Assim, a atividade enzimática total de um solo é o somatório da atividade enzimática dos organismos vivos e das enzimas abiônticas (MENDES *et al.*, 2018).

A atividade enzimática do solo fornece informações sobre o seu potencial de realizar reações biogeoquímicas dos ciclos do carbono (C), nitrogênio (N), fósforo (P) e enxofre (S) (Tabela 3), sendo sensível na detecção dos impactos do uso e manejo na saúde do solo (NANNIPIERI; TRASAR-CEPEDA; DICK, 2018). Os métodos de avaliação são simples, precisos, e geralmente, baseados em curtos períodos de incubação em laboratório (NANNIPIERI *et al.*, 2012), o que faz das enzimas bons indicadores da SS.

Diversos trabalhos demonstram o potencial da atividade de enzimas do solo como indicadores sensíveis dos efeitos dos sistemas de manejos agrícolas (BALOTA *et al.*, 2004; BINI *et al.*, 2014; LOPES *et al.*, 2018; MENDES *et al.*, 2019). Além disso, a atividade enzimática geralmente tem correlação positiva com teores de matéria orgânica e produtividade das culturas (BARBOSA *et al.*, 2023; LOPES *et al.*, 2018; MENDES *et al.*, 2019). Maior atividade das enzimas amilase, celulase e arilsulfatase (BALOTA *et al.*, 2004), glutaminase e fosfatase ácida (BINI *et al.*, 2014) e  $\beta$ -glucosidase (LOPES *et al.*, 2018) foram encontradas em áreas de SPD, quando comparadas com áreas sob cultivo convencional. Os autores atribuem os resultados à melhor qualidade estrutural e aporte de resíduos do SPD, que propiciam um ambiente mais adequado para a atividade da comunidade microbiana.

Tabela 3: Enzimas utilizadas como indicadores da saúde do solo e suas funções nos ciclos biogeoquímicos.

Enzima	Ciclo	Função	Método de análise
$\beta$ -glicosidase	C	Degradação da celobiose em glicose	Tabatabai (1994)
Arilsulfatase	S	Mineralização do S orgânico a $\text{SO}_4^{2-}$	Tabatabai; Bremner (1970)
Fosfatase ácida	P	Hidrólise de ésteres e anidros de fosfato em $\text{H}_2\text{PO}_4^-$	Tabatabai; Bremner (1969)
L-glutaminase	N	Hidrólise da L-glutamina em amônia e ácido L-glutâmico	Frankenberger; Tabatabai (1991)

Fonte: Adaptado de Cardoso et al. (2013)

A interpretação dos valores das atividades enzimáticas é, em geral, realizada com base em comparações com áreas de referência (MENDES *et al.*, 2019). Contudo, estudos têm sido desenvolvidos para determinação de valores críticos de atividade de enzimas do solo em condições brasileiras (LOPES *et al.*, 2018; LOPES *et al.*, 2013; MENDES *et al.*, 2019), que culminaram no lançamento da tecnologia BioAS (Bioanálise do Solo), pela Embrapa Cerrados (MENDES *et al.*, 2018). A BioAS consiste na determinação de indicadores biológicos da saúde do solo e na interpretação dos seus valores, utilizando os princípios das curvas de calibração de nutrientes (LOPES *et al.*, 2013). O método disponibiliza tabelas de interpretação para duas enzimas do solo,  $\beta$ -glicosidase e arilsulfatase, no bioma Cerrado (MENDES *et al.*, 2018) e, com auxílio de redes de pesquisas, já foram desenvolvidas tabelas para cultivos anuais para o estado do Paraná. A BioAS engloba ainda um novo método de amostragem de solo para análises microbiológicas, o método FertBio, no qual as análises microbiológicas podem ser realizadas nas mesmas amostras empregadas para análises químicas, o que facilita as rotinas de amostragem e análise de solo (MENDES *et al.*, 2019).

Em conjunto com o desenvolvimento da BioAS também foi desenvolvido o índice de qualidade do solo (IQS) FertBio, que combina atributos biológicos e químicos em três funções do solo, mensuradas por seus respectivos indicadores: (F1) ciclagem de nutrientes (baseada nas atividades das enzimas do solo  $\beta$ -glucosidase e arilsulfatase); (F2) armazenamento de nutrientes (carbono orgânico do solo ou do acrônimo em inglês, SOC e capacidade de troca catiônica, CTC); e (F3) fornecimento de nutrientes (teores de Ca, Mg, K, P, pH, H + Al; Al, soma de bases e saturação por bases) (MENDES *et al.*, 2021). Além disso, recentemente Chaer

et al. (2023) propuseram um modelo de quatro quadrantes para interpretação da saúde do solo (Tabela 4) baseado nas funções de ciclagem e armazenamento de nutrientes em Latossolos argilosos do Cerrado. O modelo leva em consideração as atividades médias de arilsulfatase e  $\beta$ -glicosidase por unidade de SOC (atividade enzimática específica média, ASEA) e o SOC, assumindo que áreas sob práticas de manejo do solo de longo prazo alcançam equilíbrio entre ASEA e SOC. Porém, como as enzimas do solo são indicadores mais sensíveis às mudanças no curto prazo, esse equilíbrio é temporariamente alterado após a adoção de práticas de manejo agrícola que melhorem ou degradem a saúde do solo (CHAER et al., 2023).

Tabela 4. Classes para interpretação da saúde do solo de acordo com o modelo de quatro quadrantes proposto por Chaer et al. (2023).

Classes para interpretação	Enzimas - ASEA (ciclagem)	Matéria orgânica - SOC (armazenamento)
Solo saudável	Alta	Alta
Solo adoecendo	Baixa	Alta
Solo doente	Baixa	Baixa
Solo em recuperação	Alta	Baixa

É importante ressaltar que os modelos propostos tanto para a BioAS, quanto para o IQS FertBio e o modelo de quatro quadrantes são resultado de calibrações estabelecidas entre os indicadores químico-biológicos tanto com o SOC quanto com a produtividade das culturas, possibilitando a avaliação da SS levando em consideração aspectos ambientais e econômicos que colocam o Brasil na vanguarda do desenvolvimento e uso de tecnologias para a avaliação da biologia e da saúde do solo em larga escala (MENDES et al., 2019; MENDES et al., 2021; CHAER et al., 2023).

#### 2.4 EFEITO DA DIVERSIFICAÇÃO DE CULTURAS EM ATRIBUTOS BIOLÓGICOS DO SOLO

Solos saudáveis têm a capacidade de manter os processos biológicos funcionando de maneira sustentável continuamente (CARDOSO *et al.*, 2013). Sistemas de manejo que priorizam a não mobilização do solo e a diversidade de culturas promovem o aumento do CBM e do NBM, comparados a sistemas que envolvem o revolvimento do solo (BINI *et al.*, 2014; FRANCHINI *et al.*, 2007; KASCHUK; ALBERTON; HUNGRIA, 2010; SILVA *et al.*, 2010; SILVA *et al.*, 2014). O aporte de resíduos orgânicos ao solo é

correlacionado positivamente com o CBM (BASTOS *et al.*, 2023), com maiores valores de carbono da biomassa microbiana sendo encontrados em sistemas diversificados em comparação ao pousio (PIRES, M. *et al.*, 2020) e a sucessão soja/trigo, (PIRES, C. *et al.*, 2020; SILVA *et al.*, 2010), especialmente pela adoção de CCs. Avaliando a adoção de culturas de cobertura (milheto – *Pennisetum glaucum*, sorgo - *Sorghum bicolor* e crotalária) como alternativa ao monocultivo de soja, Leite *et al.* (2021) encontraram valores de CBM e NBM reduzidos pela metade no solo sob pousio (132 e 8,44 mg kg<sup>-1</sup>, respectivamente) quando comparados ao uso de CCs; o CBM não diferiu entre as culturas de cobertura, no entanto, menores valores de NBM foram encontrados para crotalaria (*Crotalaria juncea*) comparados aos encontrados nos solos cultivados com gramíneas. Em metanálise avaliando o efeito da inclusão de culturas de cobertura em atributos biológicos do solo, Kim *et al.* (2020) observaram aumento de 25 e 26% para CBM e NBM, respectivamente, em comparação a solos sob pousio. O consórcio milho/braquiária (*Urochloa ruziziensis*) aumentou o carbono e o nitrogênio da BM comparado ao cultivo de milho solteiro em sistemas de sucessão com soja (SANTOS *et al.*, 2021). Entre os principais efeitos dos sistemas conservacionistas que influenciam positivamente a BM citados nos artigos estão a proteção do solo, aumento nos teores de matéria orgânica e substrato para o crescimento microbiano, bem como melhoria das condições físico-químicas com maior retenção de água e diminuição da oscilação de temperatura do solo.

A diversificação dos sistemas de produção também influencia a respiração e a eficiência metabólica da comunidade microbiana. Por exemplo, o uso de CCs aumentou a RB em 35% comparado a solos sob pousio (Kim *et al.*, 2020), provavelmente devido à maior disponibilidade de substrato para a atividade da BM. O uso de aveia preta e braquiária *ruziziensis* na entressafra da soja aumentaram a RB e o  $qCO_2$  comparados ao milho, sendo estes resultados atribuídos ao estímulo da comunidade microbiana, principalmente pelo maior aporte de material orgânico de fácil degradação ao solo (TONON-DEBIASI *et al.*, 2024). O trabalho de Leite *et al.* (2021) não encontrou diferenças para RB entre o pousio e o uso de CCs (milheto, sorgo, crotalária) na entressafra da soja, porém menores valores de  $qMic$  foram associados ao sistema soja/pousio, denotando menor proporção de carbono microbiano no carbono orgânico total do solo. Em outro estudo, a adoção de braquiária *ruziziensis* consorciada com milho na sucessão soja/milho não alterou a RB, no entanto, menores valores de  $qCO_2$  foram encontrados no consórcio, demonstrando que a inclusão da forrageira tropical no sistema favoreceu a eficiência de uso do C pelos microrganismos quando comparado ao milho solteiro (SANTOS *et al.*, 2021). Em trabalho visando entender a interação entre culturas de verão (milho, soja, ou milho em sucessão à soja) e culturas de entressafra (milho, sorgo, milheto, crotalária e guandu)

em atributos biológicos do solo, Xavier *et al.* (2019) não encontraram diferenças significativas entre as culturas de entressafra cultivadas após o milho e a rotação soja/milho; no entanto, quando a soja foi cultivada no verão, os maiores valores de fluxo de CO<sub>2</sub> (FCO<sub>2</sub>) foram encontrados no solo cultivado com o sorgo, crotalária e guandu em comparação ao milho; os valores de FCO<sub>2</sub> foram correlacionados com o CBM e os teores de matéria orgânica do solo.

A presença de substratos de diferentes culturas provenientes da diversificação dos sistemas de produção têm sido associada ao aumento da atividade de enzimas do solo, em especial aquelas ligadas aos ciclo biogeoquímicos do C, P, K e N. A metanálise de Kim *et al.* (2020) revelou aumento na atividade das enzimas  $\beta$ -glucosidase e fosfatase ácida da ordem de 14 e 18%, respectivamente, com o uso de CCs comparado ao pousio. Em condições de Cerrado, Anghinoni *et al.* (2021) observaram maior atividade da enzima  $\beta$ -glucosidase na sucessão soja/braquiária ruziziensis e na rotação (ano 1) soja/milho+braquiária, (ano 2) braquiária, (ano 3) soja/crotalária (*Crotalaria ochroleuca*) em comparação com a sucessão soja/milho e soja/pousio. Em experimento de longo prazo (32 anos) conduzido no sul do Brasil, testando o efeito da sucessão soja/trigo (R0) e dois modelos de rotação de cultura [ (R1: soja/trigo;soja/aveia); (R2: soja/aveia; milho/nabo/aveia+ervilhaca; soja/trigo)], Pires C. *et al.* (2019) encontraram maiores valores de  $\beta$ -glucosidase, fosfatase ácida e N-acetil-glucosaminidase em R1 e R2 comparado a R0, atribuindo os resultados ao incremento do aporte de resíduos ao solo, a maior demanda por P em sistemas diversificados e aumento da biomassa microbiana. Os resultados de 73 publicações avaliando a atividade enzimática do solo em seis biomas brasileiros e oito ordens de solos foram sumarizados por Barbosa *et al.* (2023), sendo encontrados maiores valores de atividade de enzimas (principalmente  $\beta$ -glucosidase e arilsulfatase) em sistema plantio direto e integração lavoura-pecuária.

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### 3 ARTIGO A – SHORT-TERM EFFECTS OF CROP DIVERSIFICATION ON SOIL MICROBIAL BIOMASS AND ACTIVITY IN TROPICAL SOYBEAN SYSTEMS

**Abstract** – Designing soybean production systems for maximizing economic returns while preserving soil health in alternative to the traditional maize off-season/soybean double cropping system is crucial for sustainability. Soil microorganisms are important components of soil health and quickly respond to soil and crop managements. We assessed the short-term effects of off-season crop diversification on soil microbial attributes in cropping systems cultivated with maize off-season/soybean for 5 years, in Southern Brazil. Treatments included the successions maize/soybean (CS1), wheat/soybean (CS2), oats/soybean (CS3), and maize intercropped with ruzigrass/soybean (CS4), as well as systems with a third crop after maize, in succession [wheat (CD1) or black oats (CD2)], or in rotation [white/black oats (CD3)]. Soil microbial attributes were evaluated after off-season crops termination after one and three years of diversification. Soil under treatments CS1 and CD1 exhibited the highest microbial biomass (MB) carbon and microbial metabolic efficiency (higher  $q_{\text{Mic}}$ ; lower  $q_{\text{CO}_2}$ ) in both sampling years, while the other diversifications increased the labile-C, measured as salt extractable organic carbon, microbial respiration, and  $q_{\text{CO}_2}$ . CD2 enhanced soil nitrogen (N) processes after 3 years, showing higher total N, nitrate, and MB-N. The activity of  $\beta$ -glucosidase, arylsulfatase, acid phosphatase, and glutaminase were mostly affected after three years, with general increases in CS3 and CD treatments. Soil C-cycling microbial attributes were the most effective to discriminate among the diversification strategies. Overall, our results show that off-season crop diversification influences soil microbial attributes in the short-term and highlight the capacity of bioindicators to monitor soil processes aiming at soil health.

**Keywords** – Soil quality; Crop rotation; No-till system; Soil microbiological indicators; Soil enzymes; Oxisols.

#### 1. Introduction

Soils play crucial roles in provisioning various environmental services, including the regulation of hydrological and biogeochemical cycles, atmospheric carbon sequestration, and the conservation of global biodiversity. However, like other habitats, soils have been under increasing pressure due to anthropogenic activities (Derpsch et al., 2024). The driver to increase crop yields to support the global population growth has relied on the

intensification of agricultural practices such as the use of irrigation, fertilizers, pesticides, and mechanization (Foley et al., 2011), as well as low diversified crop systems (Tamburini et al., 2020), which can all together impair the capacity of soils to work as vital living ecosystems that sustain plants, animals and humans, a concept named as soil health (Doran and Parkin, 1994; Lehmann et al., 2020).

Healthy soils result from the balanced interaction between physicochemical and biological attributes. Microorganisms are fundamental drivers of biological processes that support ecological services and agricultural yield, such as the formation and decomposition of soil organic matter, nutrient cycling, and plant growth promotion (Philippot et al., 2023). As the living component of the soil, the microbial community is highly responsive to agricultural practices that may affect the diversity and function of microorganisms by changing soil conditions such as water and organic matter contents, aeration and temperature (Hashimi et al., 2023). For these reasons, soil microbial attributes have been used as indicators for guiding the adoption of conservation practices that maintain or improve soil health (Chaer et al., 2023; Mendes et al., 2021; Mendes et al., 2019).

Increasing the diversity in agroecosystems has been suggested as a tool to promote environmental sustainability without compromising crop yields (Tamburini et al., 2020; Volsi et al., 2022). Agricultural diversification ranges from the inclusion of a single crop into rotation schemes to the implementation of more complex management strategies arranged in space and time, which includes the use of cover crops, intercropping, and mix of crops (Adetunji et al., 2020; Brannan et al., 2023; Cordeiro et al., 2021). Crop diversification contributes to weed suppression (Naeem et al., 2021), improvement of soil physical attributes (Bertollo et al., 2021), nutrient cycling (Souza et al., 2024; Tiecher et al., 2017), and higher and more stable grain yields in production systems (Balbinot Junior et al., 2024; Garbelini et al., 2020). The inputs of organic residues from different crop species also influence the soil microbial community (Leite et al., 2021). A recent study conducted in soybean (*Glycine max.* (L.) Merr.) cropping systems in Southern Brazil found that, after three years, the replacement of maize (*Zea mays* L.) with off-season black oats (*Avena strigosa* Schreb) or ruzigrass (*Urochloa ruziziensis* R. Germ. & C. M. Evrard) has resulted in higher soil basal respiration and cellulase activity (Tonon-Debiasi et al., 2024). The activity of  $\beta$ -glucosidase was also enhanced by off-season ruzigrass and crop rotation compared with soybean monocrop or soybean/maize succession in an 8-year study in central Brazil (Anghinoni et al., 2021). Besides carbon-related attributes, previous studies have also found positive effects of the inclusion of off-season crops such as black oats on microbial biomass nitrogen (N) and the activity of the

N-cycling enzyme glutaminase, when compared with fallow (Bini et al., 2014). The increases in microbial biomass and activity attributes in cropping systems have been correlated mainly to the amount of crop residues added to the system (Bastos et al., 2023; Santos et al., 2022).

Despite the several benefits associated with crop diversification and its status as pillar of the no-till system, adopted across 33 million hectares in Brazil (Fuentes-Llanillo et al., 2021), farmers in the country often prioritize simpler cropping systems. This choice is influenced by factors such as short-term profit maximization (Chaplin-Kramer et al., 2023), facilitation of farm operational routine (Debiasi et al., 2015), the absence of off-season crops adapted to regions with dry fall/winter season (Balbinot Junior et al., 2017), and a lack of awareness regarding the importance of crop diversification (Telles et al., 2022). Although Brazil has recently become the world's biggest soybean producer, many soybean-cultivated areas exhibit low crop diversity due to the predominance of the soybean/maize succession (Garbelini et al., 2020). Even though this double crop system is well established in various parts of the country, the economic return of off-season maize is highly variable due to weather conditions, primarily related to water stress (Andrea et al., 2018). Additionally, there is low soil coverage during the window between maize harvest and soybean sowing (June to October) (Balbinot Junior et al., 2023), which potentially impacts soil physical, chemical, and biological attributes.

In this context, the development of more diversified systems aiming at increasing yields while reducing costs, financial risks and environmental impacts becomes imperative for the sustainability of the cropping system. Cropping wheat (*Triticum aestivum* L.) or oats in replacement or in rotation with maize (Balbinot Junior et al., 2020), and intercropping maize with forage grasses such as ruzigrass (Balbinot Junior et al., 2023) are alternatives that can be adopted for increasing crop diversification in soybean/maize successions. In addition, using the period between the second season maize and soybean sowing for grain production (as a third crop) or cover crop is a possible option to enhance the input of crop residues into the soil and yield per unit of cultivated area.

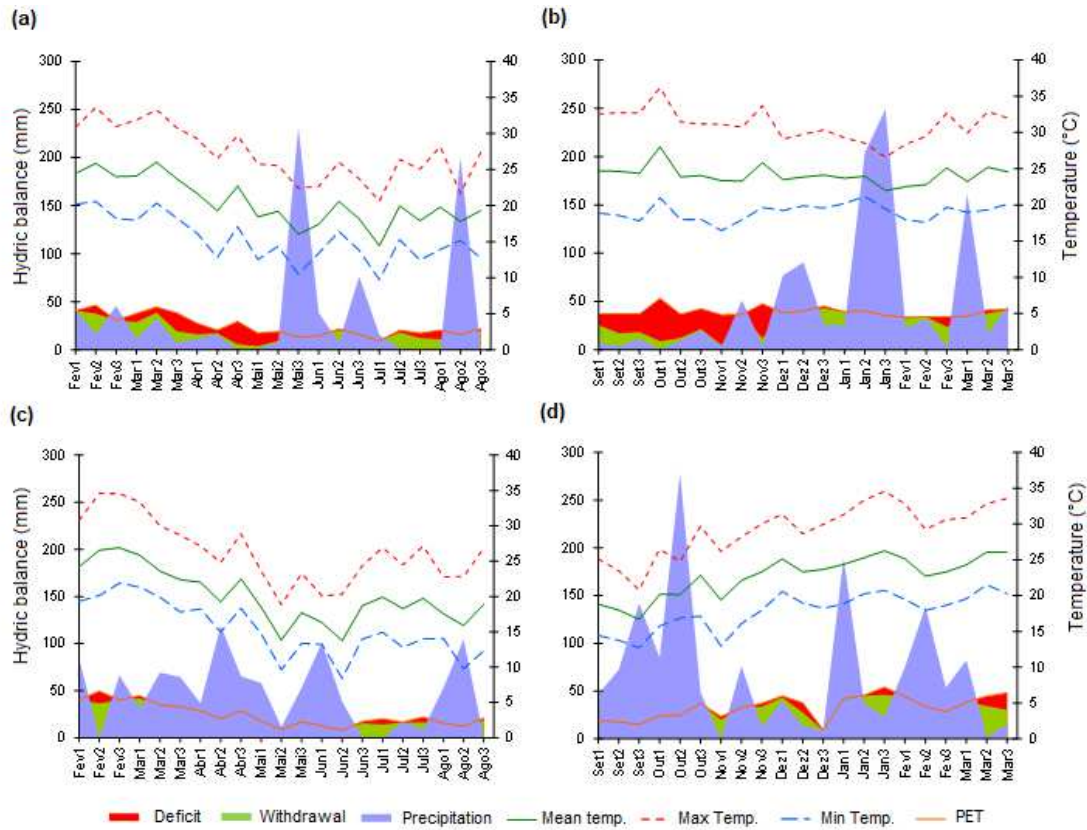
As soil microbial attributes are potential fast sensors for monitoring soil health, we aimed to evaluate the short-term effects of alternative off-season crop diversification on the soil microbial biomass and activity in soybean cropping systems. We hypothesized that (i) diversifying the soybean off-season with different succession crops and (ii) intensifying the off-season period with the inclusion of a third crop enhance the soil microbial attributes compared with the commonly used soybean/maize succession.

## 2. Material and methods

### 2.1 Site description and treatments

The study was carried out at the Copacol Center for Agricultural Research, located in Cafelândia, Paraná state, Southern Brazil (24°37'13" S; 53°18'14" W; 581 m a. s. l.) over three cropping seasons (2020/21, 2021/22, and 2022/23) in a site managed under no-tillage with soybean/maize succession since 2015. The climate of the region is humid subtropical (Cfa) according to Köppen. The soil at the experimental site is classified as an Oxisol [“Latossolo Vermelho distroférico”, in the Brazilian classification (Santos, 2018); or Rhodic Eutrudox, in the USA classification (Soil Survey Staff, 2014)], with very clayey texture (770 g kg<sup>-1</sup> of clay, 99 g kg<sup>-1</sup> of sand, and 131 g kg<sup>-1</sup> of silt). Soil chemical properties (0-0.20 m) before the experiment set up were: soil organic carbon (Walkley Black), 18.6 g dm<sup>-3</sup>; pH in CaCl<sub>2</sub>, 5.1; Al, 0.1 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al (SMP) 4.16 cmol<sub>c</sub> dm<sup>-3</sup>; Ca (KCl), 3.91 cmol<sub>c</sub> dm<sup>-3</sup>; Mg (KCl), 1.56 cmol<sub>c</sub> dm<sup>-3</sup>; K (Mehlich-1), 0.46 cmol<sub>c</sub> dm<sup>-3</sup>; P (Mehlich-1), 19 mg dm<sup>-3</sup>; cation-exchange capacity, 10.08 cmol<sub>c</sub> dm<sup>-3</sup>; and base saturation, 58 %. Meteorological data for the cropping seasons when soil was sampled (2020/2021 and 2022/2023) are shown in Figure 1.

The experiment was carried out in a complete randomized blocks design with four replicates. Treatments included eight cropping systems designed for off-season diversification in soybean systems, established in the fall/winter of 2020 (Table 1) in plots measuring 150 m<sup>2</sup> (5 × 30 m). Systems comprised the usual maize/soybean succession (CS1), the crop succession alternatives wheat/soybean (CS2), oat/soybean (CS3), and maize intercropped with ruzigrass/soybean (CS4) as well as systems with wheat (CD1), black oats (CD2), and white/black oats (CD3) cropped between the second season maize harvest and the soybean sowing, as a third crop in the same agricultural year. White oats were cultivated as cash crop (CS3 and CD3) and black oats as a cover crop (CS3, CD2, and CD3). More details can be seen in Supplementary Figure S1.



**Figure 1:** Average temperatures, average maximum temperatures, average minimum temperatures, and sequential water balance measured at 10-day intervals according to the method described by Thornthwaite and Mather (1955) over 2020/2021 (a and b) and 2022/2023 (c and d) cropping seasons.

**Table 1:** Sequence of crops grown in the diversification systems for soybean production during the seasons 2020/2021, 2021/2022, and 2022/2023.

Cropping system	Fall/Winter 2020		Spring/Summer 2020/2021		Fall/Winter 2021		Spring/Summer 2021/2022		Fall/Winter 2022		Spring/Summer 2022/2023	
	2nd crop	3rd crop <sup>†</sup>	1st crop	2nd crop	1st crop	2nd crop	3rd crop <sup>‡</sup>	1st crop				
CS1	Maize	Fallow	Soybean	Maize	Soybean	Maize	Fallow	Soybean				
CS2	Wheat	Fallow	Soybean	Wheat	Soybean	Wheat	Fallow	Soybean				
CS3	White Oats	Fallow	Soybean	Black Oats	Soybean	Black Oats	Fallow	Soybean				
CS4	Maize + Ruzigrass	Fallow	Soybean	Maize + Ruzigrass	Soybean	Maize + Ruzigrass	Fallow	Soybean				
CD1	Maize	Wheat	Soybean	Maize	Soybean	Maize	Wheat	Soybean				
CD2	Maize	Black Oats	Soybean	Maize	Soybean	Maize	Black Oats	Soybean				
CD3	Maize	White Oats	Soybean	Wheat	Soybean	Maize	Black Oats	Soybean				

<sup>†</sup>First soil sampling: December 21, 2020, <sup>‡</sup>Second soil sampling: October 27, 2022.

## 2.2 Crop sowing and management

Information regarding crop genotypes, sowing and harvesting dates, and base and topdressing fertilization for each crop in all cropping seasons are presented in Supplementary Table S1. Soybean and maize off-season were sown with row spacing of 0.50 m. For soybeans, seeding rate was adjusted to achieve a final population of 240,000 plants (2020/21) and 360,000 plants per hectare (2021/22 and 2022/23). Soybean seeds were pre-treated with pyraclostrobin (2.5 g a.i. 100 kg<sup>-1</sup> of seeds), thiophanate-methyl (22.5 g a.i. 100 kg<sup>-1</sup>), fipronil (25 g a.i. 100 kg<sup>-1</sup>), and a liquid inoculant containing *Bradyrhizobium elkanii*. For the second crop maize, the seed drill was adjusted to deliver a population of around 60,000 plants per hectare in all seasons. In the maize + ruzigrass intercropping, the establishment, fertilization, and cultural practices were the same used for the sole second crop maize cultivation, except the post-emergence weed control. The forage grass was broadcasted immediately before maize sowing, on the same day. Coated ruzigrass seeds, with a cultural value close to 80%, were used at seeding rates of 10 kg ha<sup>-1</sup>, 8 kg ha<sup>-1</sup>, and 12 kg ha<sup>-1</sup> in 2020, 2021, and 2022, respectively.

Wheat, black oats, and white oats were sown with rows spaced 17 cm apart. The seeder was adjusted to achieve a population of 300 plants per m<sup>2</sup> for wheat, and for white and black oats sown in the normal period (CS 2, 3, 6, and 8). When the winter cereals were sown in the window between the second-season maize harvest and soybean sowing (CD 1 and 2), the population was adjusted to establish 350 plants per m<sup>2</sup> due to the difficulty of seed deposition at the appropriate depth, due to the amount of maize residues left on the soil surface by the time of sowing. Nitrogen fertilizer topdressing was applied at the rate of 200 kg ha<sup>-1</sup> of urea approximately 28 days after the emergence of wheat (2020 and 2021) or white oats (2020). Nitrogen topdressing was not applied to black oats used as a cover crop in all seasons, as well as for wheat in the 2022 season. More details can be seen in Supplementary Table S1.

Weed control during the crops pre and post-emergence varied according to the rate of infestation and the crops present in each CS. Black oat termination was performed using glyphosate (1.25 kg a.i. ha<sup>-1</sup>). Other cultural practices, including weed, pest, and disease control, were carried out according to the regional technical recommendations for each crop.

## 2.3 Soil sampling

Soil samples for microbial and chemical analyses were taken at the 0.0–0.10 m depth at two different times: (i) in December 2020, after the first fall/winter season; (ii) in October 2022, after three fall/winter seasons and two summer seasons. Soil cores were collected

5 times (one at the center of the planting row and other two on each side of the inter-row spacing) at three points within each plot using a soil auger, following the FERTBIO soil sampling concept (Mendes et al., 2019). The 15 subsamples were homogenized in a bucket and an 800-g sample stored in plastic bags and transported to the laboratory. An aliquot of each composite soil sample was air-dried at room temperature, sieved (2 mm), and stored at room temperature until analysis. Results from chemical analysis can be seen in Supplementary Table S2. The remaining soil at field moisture was stored at 4 °C after sieving for the analysis of inorganic nitrogen, net ammonification, and potential nitrification rate.

#### *2.4 Soil microbial biomass and chemical attributes*

Microbial biomass carbon (MB-C) and nitrogen (MB-N) were accessed by the fumigation-extraction method (Vance et al., 1987) after soil rewetting for adjusting moisture to 60% of the water holding capacity (WHC) in a pre-incubation in the dark (7 days at 28 °C). Fumigated (F) aliquots underwent fumigation with chloroform for 24 h whereas the non-fumigated (NF) ones were not exposed to chloroform and were used for soil C and N backgrounds. Soluble organic C and N from F and NF samples were extracted using  $K_2SO_4$  0.5 mol L<sup>-1</sup>. Quantification of C was performed by wet oxidation with  $K_2Cr_2O_7$  followed by titration with  $Fe(NH_4)_2(SO_4).6H_2O$  0.033 mol L<sup>-1</sup> in  $H_2SO_4$  using phenanthroline as indicator. Quantification of N was performed by the indophenol blue method after digestion with sulfuric acid (Feijl and Anger, 1972). MB-C and MB-N were estimated as the difference between the F and NF samples, using  $K_C = 0.33$  and  $K_N = 0.68$  as extraction coefficients for C and N, respectively (Brookes et al., 1985). The soluble carbon from NF aliquots was considered a labile soil carbon fraction, defined as salt-extractable organic carbon (Baldwin-Kordick et al., 2022; Potter et al., 2023). Soil basal respiration (BR) was assessed based on the  $CO_2$  released from a soil sample with moisture adjusted to 60% of WHC and incubated in hermetically sealed vials for 7 days (28 °C in the dark) using 20 mL of 0.5 mol L<sup>-1</sup> NaOH as a  $CO_2$ -trap (Alef, 1995). The microbial quotient ( $qMic$ ) was calculated as the ratio between MB-C and soil organic carbon (SOC) (Insam and Domsch, 1988). The metabolic quotient ( $qCO_2$ ) was calculated as the ratio between BR and MB-C (Insam and Haselwandter, 1989).

The influence of CS treatments on nitrogen mineralization and microbial oxidation of ammonium ( $NH_4^+$ ) over nitrate ( $NO_3^-$ ) was assessed in a laboratory incubation experiment with soil samples taken in 2022. Ammonification and potential net nitrification rates were determined considering the concentration of  $NH_4^+$  and  $NO_3^-$  before and after the incubation

of field-moist soil with moisture adjusted to 60% WHC, in the dark, for 21 days at 28 °C, with and without the addition of 125  $\mu\text{g g}^{-1}$  of  $\text{NH}_4^+$  as ammonium sulfate (Schuster and Schröder, 1990).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were extracted using  $\text{KCl } 2 \text{ mol L}^{-1}$  and determined by the salicylate (Searle, 1984) and delta-absorbance (Apha, 2005) methods, respectively. Ammonification rate was computed as the difference between the final (without N) and initial  $\text{NH}_4^+$  (prior incubation) divided by the number days of incubation. Potential nitrification rate was calculated by the difference between the final (with added  $\text{NH}_4^+$ ) and initial  $\text{NO}_3^-$  divided by the number of days of incubation.

The activities of  $\beta$ -glucosidase (BG; EC 3.2.1.21), arylsulfatase (ARYL; EC 3.1.6.1), and acid phosphatase (PHOS; EC 3.1.3.2) were determined in air-dried samples (Mendes et al., 2019) following Tabatabai (1994). Glutaminase (GLUT; EC 3.5.1.2) was measured as established by Frankenberger and Tabatabai, (1991), following extraction with  $\text{KCl } 2.5 \text{ mol L}^{-1}$  and  $\text{AgSO}_4 100 \text{ mg L}^{-1}$ , steam distillation, and quantification by titration with  $\text{H}_2\text{SO}_4 0.005 \text{ mol L}^{-1}$ .

The assessed soil chemical attributes were pH in  $\text{CaCl}_2 0.01 \text{ mol L}^{-1}$ , soil organic carbon (SOC) (Walkley-Black), P and K (Mehlich-1), Ca, Mg, and Al ( $\text{KCl } 1 \text{ mol L}^{-1}$ ), H+Al (SMP), cation-exchange capacity (CEC), and base saturation (V%). In 2022, soil ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) were extracted and quantified as described above. Total inorganic nitrogen was computed as the sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .

## 2.5 Data analysis

Data from each soil sampling were analyzed separately. Normality and homoscedasticity of data were assessed using Shapiro-Wilk and Bartlett tests, respectively. The data were analyzed for variance using one-way ANOVA and means were compared by the Tukey's test ( $P \leq 0.05$ ). A canonical discriminant analysis (CDA) was performed to find the microbial attributes that best described the separation among the diversification systems. Hierarchical clustering analysis (HCA) was employed to verify the formation of clusters of diversification systems with similar microbial attributes using the Euclidean distance and the average linkage aggregation method. All analyses were performed using the software R (Core Team, 2023).

## 3. Results

### 3.1 Soil organic carbon and carbon-related microbial attributes

Cropping systems designed for diversification of the soybean/maize succession affected the soil organic carbon (SOC) and microbial attributes related to C cycling (Table 2). After the first year, in 2020, the succession maize/soybean (CS1) presented the highest SOC and the succession wheat/soybean (CS2) the lowest; the other systems showed intermediate SOC values. In 2022, after 3 years of off-season diversification, the highest SOC was observed in CD3 (maize or wheat/white or black oats/soybean), CD2 (maize/black oats/soybean), and CS2 (wheat/fallow/soybean) when compared with CS3 (white or black oats/fallow/soybean), which presented the lowest SOC. The soil extractable organic carbon (SEOC) was higher in all cropping systems compared with CS1 in 2020; in 2022, CS2, CS3, and CD3 presented the highest SEOC compared to CS1 and CD1 (maize/wheat/soybean). The highest microbial biomass carbon (MB-C) was observed in CS1 in both years, not differing from CD1 in 2020, and CD1 and CD3 in 2022. Soil basal respiration (BR) was higher in CD2 and differed from CS2 in 2020; in 2022, CD2 had the highest BR compared with the successions CS1 and CS2, and CD3. The soil under CS1 along with CD1 exhibited the highest  $q_{\text{Mic}}$  in both sampling seasons. The systems CD2 and CD3 promoted the highest metabolic quotient ( $q_{\text{CO}_2}$ ) in 2020, whereas CS1 had the lowest; in 2022, CD2 also exhibited higher  $q_{\text{CO}_2}$  compared with CS1 and CD3, not differing from the other cropping systems.

### *3.2 Soil inorganic N and N-related microbial attributes*

The highest amount of total soil inorganic N was found in CD2 while CS3 presented the lowest (Table 3). Soil ammonium ( $\text{N-NH}_4^+$ ) and the net ammonification rate (AR) did not differ among the treatments. Soil nitrate ( $\text{N-NO}_3^-$ ) was higher in CD2 compared with CS1 and CS3, but no differences among treatments were found for potential net nitrification rate (NR).

Soil microbial biomass N (MB-N) was affected in both samplings (Table 3). In 2020, the highest MB-N was observed in the soil under CS1 and CS4 compared with CS2. In 2022, the soil under CD2 showed higher MB-N, while CS1 and CS4 resulted in lower MB-N values.

**Table 2:** Soil organic carbon and carbon-related microbial indicators at the topsoil (0-0.1 m) of an Oxisol as affected by one (2020) and three (2022) years of diversification of off-season crops after soybean.

Cropping Systems † (year)	SOC (g dm <sup>-3</sup> )	SEOC (mg kg <sup>-1</sup> )	MB-C	BR (mg C-CO <sub>2</sub> kg <sup>-1</sup> h <sup>-1</sup> )	qMic %	qCO <sub>2</sub> (mg C-CO <sub>2</sub> g <sup>-1</sup> MB-C h <sup>-1</sup> )
<b>(2020)</b>						
CS1	23.1 a	358 b	515 a	27.4 ab	2.32 a	2.20 b
CS2	19.8 b	544 a	303 c	25.5 b	1.55 b	3.22 ab
CS3	20.3 ab	593 a	285 c	28.1 ab	1.40 b	4.11 ab
CS4	21.4 ab	593 a	343 bc	27.9 ab	1.57 b	3.36 ab
CD1	21.2 ab	537 a	436 ab	28.1 ab	2.08 a	2.91 ab
CD2	21.7 ab	601 a	323 c	32.2 a	1.44 b	4.31 a
CD3	21.8 ab	598 a	322 c	31.6 ab	1.48 b	4.16 a
P-value	0.035	< 0.001	< 0.001	0.040	< 0.001	< 0.005
CV (%)	5.82	9.1	11.9	9.9	11.7	19.3
<b>(2022)</b>						
CS1	18.5 bc	359 c	714 a	14.9 b	3.90 a	0.87 c
CS2	19.2 ab	609 a	506 b	16.9 b	2.63 b	1.52 ab
CS3	17.7 c	675 a	466 b	17.8 ab	2.63 b	1.58 ab
CS4	18.3 bc	516 ab	506 b	19.4 ab	2.39 b	1.48 ab
CD1	18.4 bc	441 bc	615 ab	19.2 ab	3.24 ab	1.39 abc
CD2	19.3 ab	595 ab	503 b	22.3 a	2.6 b	1.87 a
CD3	20.0 a	618 a	542 ab	17.0 b	2.7 b	1.18 bc
P-value	< 0.05	< 0.001	< 0.05	0.030	< 0.001	< 0.001
CV (%)	3.46	12.9	14.0	11.6	13.8	17.4

Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). SOC – soil organic carbon; SEOC – salt extractable organic carbon; MB-C – microbial biomass carbon; BR – basal respiration; qMic – microbial quotient; qCO<sub>2</sub> – metabolic quotient; CV - coefficient of variation. † For details on the cropping systems, please refer to Table 1.

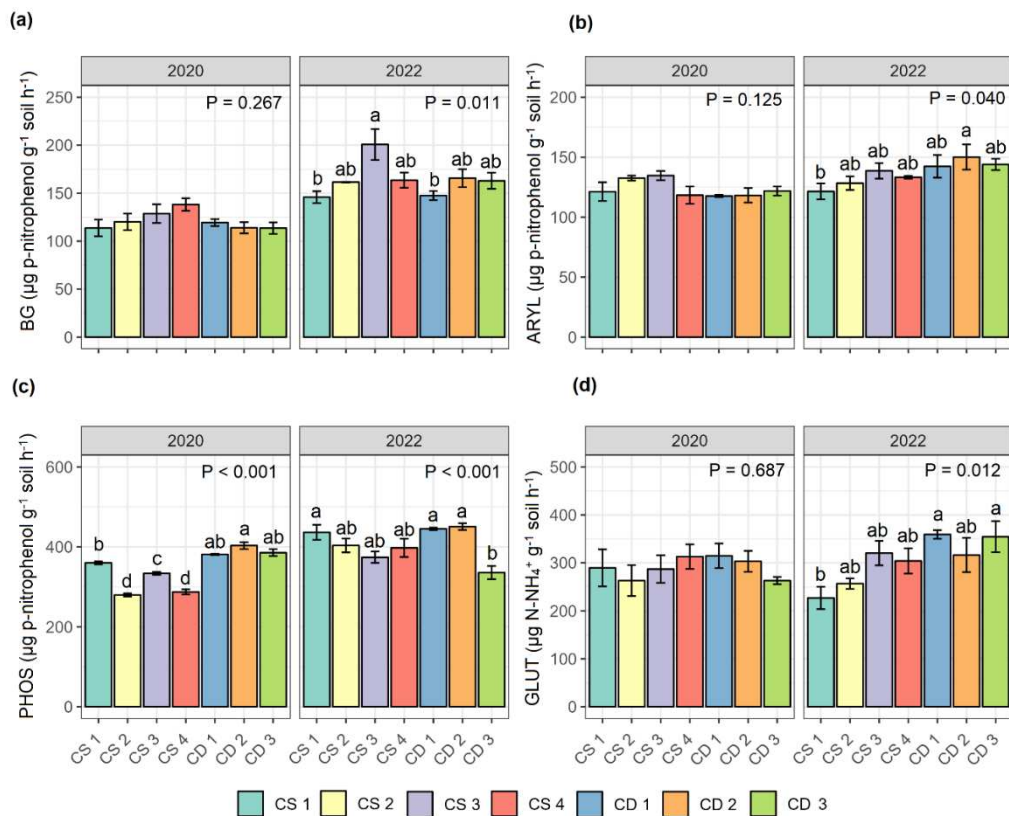
**Table 3:** Soil inorganic N and N-related microbial indicators at the topsoil (0-0.1 m) of an Oxisol as affected by one (2020) and three (2022) years of diversification of off-season crops after soybean.

Cropping Systems †	Total N	N-NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	N-NO <sub>3</sub> <sup>-</sup>	2022		MB-N (mg kg <sup>-1</sup> )	
				AR (mg N-NH <sub>4</sub> <sup>+</sup> kg <sup>-1</sup> d <sup>-1</sup> )	NR (mg N-NO <sub>3</sub> <sup>-</sup> kg <sup>-1</sup> d <sup>-1</sup> )	2020	2022
CS1	14.7 ab	4.69	10.0 b	0.52	7.87	79.3 a	36.9 b
CS2	15.4 ab	4.80	11.0 ab	0.62	8.03	49.3 b	51.8 ab
CS3	14.4 b	4.84	9.60 b	0.68	7.46	65.4 ab	44.0 ab
CS4	15.7 ab	4.36	11.4 ab	0.59	7.67	79.1 a	37.4 b
CD1	18.4 ab	6.17	12.2 ab	0.48	8.47	63.6 ab	44.7 ab
CD2	18.8 a	4.78	13.0 a	0.60	7.53	59.4 ab	52.4 a
CD3	15.4 ab	3.86	11.6 ab	0.71	7.76	63.4 ab	41.2 ab
P-value	0.012	0.104	< 0.05	0.175	0.214	< 0.05	0.041
CV (%)	11.1	20.3	10.4	21.3	7.03	18.2	16.8

Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). Total N- Total inorganic nitrogen; N-NH<sub>4</sub><sup>+</sup> ammonium; N-NO<sub>3</sub><sup>-</sup> - nitrate; AR – net ammonification rate; NR- potential net nitrification rate; MB-N – microbial biomass nitrogen; CV – coefficient of variation. † For details on the cropping systems, please refer to Table 1.

### 3.3 Activities of soil enzymes

In the first sampling year 2020, only the activity of acid phosphatase (PHOS) was affected by the cropping systems (Figure 2) with the highest PHOS observed in CD2 compared with the other systems, not differing from CD1 and CD3; CS2 and CS4 (maize + ruzigrass/soybean) had the lowest PHOS in 2020. In the second sampling year 2022, off-season systems influenced the activity of all assessed enzymes.  $\beta$ -glucosidase (BG) was higher in the soil under CS3 compared with CS1 and CD1. Higher arylsulfatase (ARYL) activity was observed in the soil under CD2 compared with CS1, while the other systems resulted in intermediate ARYL activity values in the soil. PHOS was higher in the soil under CS1, CD1, and CD2 when compared with CD3. Glutaminase (GLUT) activity was the highest in the soil under CD1 and CD3 when compared with CS1.



**Figure 2:** Activity of the soil enzymes  $\beta$ -glucosidase – BG (a), arylsulfatase – ARYL (b), acid phosphatase – PHOS (c), and glutaminase – GLUT (D) at the topsoil (0-0.1 m) of an Oxisol as affected by one (2020) and three (2022) years of diversification of off-season crops after soybean. Error bars represent the standard error of means. Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). For details on the cropping systems, please refer to Table 1.

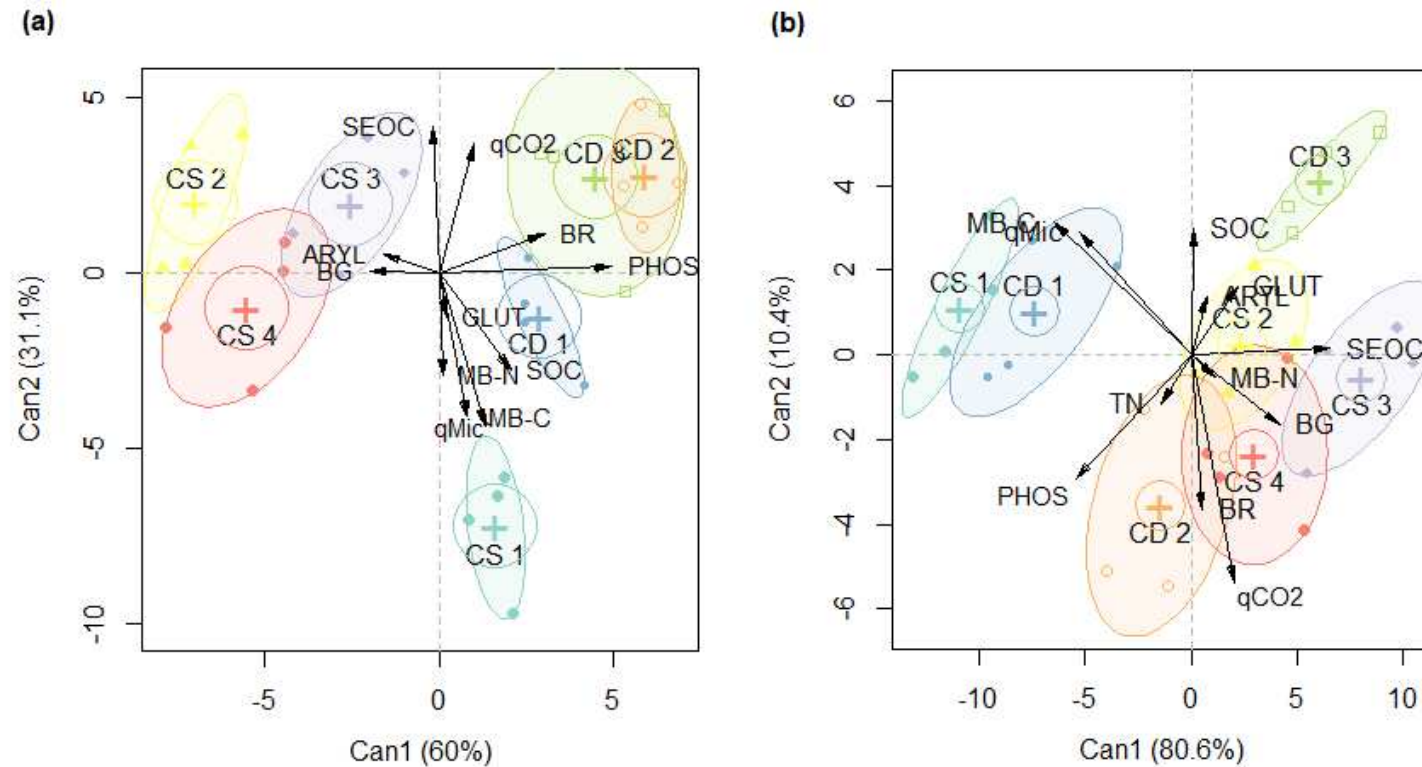
### 3.4 Global data analysis

The canonical discriminant analysis (CDA) showed high canonical correlations with the first and second discriminant functions (Can1 and Can2) in both sampling years (Figure 3). In 2020, Can1 accounted for 60% of the discriminating power whereas Can2 accounted for 31% (Figure 3a). The CDA plot showed the formation of three groups (1- CS1; 2- CS2, CS3, and CS4; and 3- CD1, CD2, and CD3) based on differences in microbial attributes, SOC, and TN. The activity of PHOS and BR were important to discriminate diversification treatments for Can1, while MB-C, SEOC,  $q_{\text{Mic}}$ , and  $q_{\text{CO}_2}$  were relevant in discriminating treatments for Can2 (Table 4). In 2022, Can1 and Can2 accounted for 80.6 and 10.4% of the discriminating power, respectively (Figure 3b). From the CDA plot, the formation of 2 groups was observed: 1- CS1 and CD1; and 2- CS2, CS3, CS4, CD2 and CD3. The most relevant attributes for Can1 discriminating function were PHOS, MB-C, SEOC, and  $q_{\text{Mic}}$ , while  $q_{\text{CO}_2}$  was highly associated to Can2 (Table 4).

The hierarchical clustering analysis (HCA) was performed to confirm the formation groups based on similar soil microbial attributes among the diversification treatments (Figure 4). The dendrogram from 2020 showed a separation between CS1 and the other treatments, forming other two groups, one encompassing the more diversified systems (CD1, CD2 and CD3), and other with the remaining succession systems (CS2, CS3, and CS4) (Figure 4a), in a similar fashion as in the CDA (Figure 3a); in 2022, CS1 and CD1 formed a group, differentiating from the other crop successions (Figure 4b), corroborating again the findings of the CDA (Figure 3b).

## 4. Discussion

Crop diversification is a promising conservative practice known to enhance soil health, primarily due to soil protection and the introduction of crop residues in varying quantity and quality (Balbinot Junior et al., 2024; Pires et al., 2020). A range of studies have linked the variety and supply of nutrients provided by the use of different crop sequences to alterations on soil microbial community abundance, diversity, and functionality over time (Araujo et al., 2023; Hungria et al., 2009; Kim et al., 2020; Leite et al., 2021; Silva et al., 2010). Here, we have found that the diversification of the soybean/maize succession with different crops after soybean (CS2, CS3, and CS4), as well as introducing a third crop either in succession (CD1 and CD2) or in rotation (CD3), significantly influenced the soil microbial biomass and activity after just only one year.

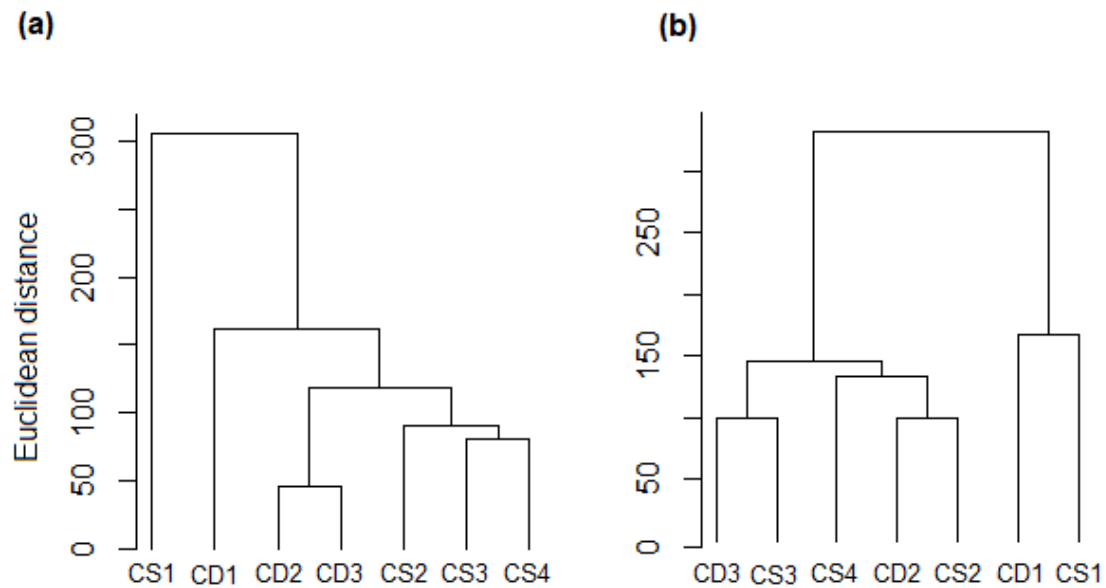


**Figure 3:** Canonical discriminant analysis (CDA) considering soil microbial attributes, soil organic carbon, and total inorganic nitrogen assessed after one (a) and three (b) years of diversification of off-season crops after soybean. MB-C – microbial biomass carbon; BR – basal respiration; SOC – soil organic carbon; SEOC – salt extractable organic carbon; qMic – microbial quotient; qCO<sub>2</sub> – metabolic quotient; BG –  $\beta$ -glucosidase; ARYL: arylsulfatase; PHOS; acid phosphatase; GLUT: glutaminase; MB-N – microbial biomass nitrogen; TN – total inorganic nitrogen. For details on the cropping systems, please refer to Table 1.

**Table 4:** Loading values for the canonical discriminating function 1 (Can1) and 2 (Can2) based on soil microbial attributes, organic carbon and inorganic nitrogen after one (2020) and three (2022) years of diversification of off-season crops after soybean.

Attribute	2020		2022	
	Can1	Can2	Can1	Can2
BG	-0.395	0.013	0.522	-0.205
ARYL	-0.320	0.109	0.090	0.173
PHOS	<b>0.987</b>	0.036	<b>-0.675</b>	-0.364
GLUT	0.042	-0.250	0.274	0.202
MB-C	0.264	<b>-0.871</b>	<b>-0.813</b>	0.391
MB-N	0.021	-0.582	0.143	-0.060
BR	<b>0.603</b>	0.228	0.063	-0.455
SOC	0.408	-0.549	0.015	0.371
SEOC	-0.037	<b>0.837</b>	<b>0.819</b>	0.019
TN	-	-	-0.178	-0.143
<i>q</i> Mic	0.164	<b>-0.816</b>	<b>-0.660</b>	0.361
<i>q</i> CO <sub>2</sub>	0.201	<b>0.740</b>	0.252	<b>-0.670</b>
R <sup>2</sup>	0.97	0.95	0.98	0.89
P-value	<0.001	<0.001	<0.001	<0.001

Correlation coefficients in bold (> 0.6) represent strong discriminant potential with a given discriminant function in the CDA. BG –  $\beta$ -glucosidase; ARYL: arylsulfatase; PHOS; acid phosphatase; GLUT: glutaminase; MB-C – microbial biomass carbon; MB-N – microbial biomass nitrogen; BR – basal respiration; SOC – soil organic carbon; SEOC – salt extractable organic carbon; TN – total inorganic nitrogen; *q*Mic – microbial quotient; *q*CO<sub>2</sub> – metabolic quotient.



**Figure 4:** Dendrogram of similarity based on the hierarchical clustering analysis showing the formation of groups characterized by similar soil microbial attributes following one (a) or three years (b) of diversification of off-season crops after soybean. For details on the cropping systems, please refer to Table 1.

Soil organic matter (SOM) significantly influences the soil physical, chemical, and biological properties and changes in soil management can disrupt the balance between the formation and mineralization of SOM, consequently impacting the C-related attributes (Bini et al., 2013). The results for microbial C-cycling attributes suggest that the introduction of new crops following five years of soybean/maize cultivation (after 2020) has stimulated the soil microbial community, probably due to increase in the labile carbon fractions (SEOC), which is an easily-use C for microbial growth. The greater diversity and amount of soluble C compounds in the soil under diversified cropping systems can increase the decomposition of the more soluble C fractions (Spohn et al., 2023), increasing the  $q\text{CO}_2$  for most cropping systems compared with CS1, mainly in CD2 and CD3. The presence of oats, which present lower C:N ratio (37 and 39:1, respectively) (Redin et al., 2018), speeds-up the decomposition rate of decomposition of residues compared with wheat and ruzigrass residues (Redin et al., 2018; Rosolem et al., 2010). Previous study in similar soil and climate conditions have found higher  $q\text{CO}_2$  along with higher BR rates and labile-C under crop diversification strategies (Tonon-Debiasi et al., 2024), corroborating our findings. Schmidt et al. (2018) also demonstrated that diversification of cover crops tends to promote a more generalist microbial

community formed by moderately to fast-growing microorganisms that present a wider metabolic capacity and are responsive for a more diversified unfolding of organic substrates, resulting in higher concentrations of SEOC in the soil.

The cultivation of maize/soybean (CS1) in the previous 6 years without diversification resulted in higher SOC, MB-C, and  $q_{\text{Mic}}$ , and lower  $q_{\text{CO}_2}$  compared with the other cropping systems, except for CD1, which was grown with wheat between maize and soybean. Higher  $q_{\text{Mic}}$  and lower  $q_{\text{CO}_2}$  are typically observed under agricultural conservationist management practices, such as no-till (Bini et al., 2014; Franchini et al., 2007; Hungria et al., 2009), suggesting higher amounts of living C in the SOC and lower energy requirements to maintain the soil microbial community. These results found for CS1 might indicate that the continuous use of the maize after soybean has resulted in a stable environment in terms of input of residues (Zhang et al., 2018), which ended up selecting for slow-growers and more specialized microbial community in the soil (Schmidt et al., 2018). Araujo et al. (2023), exploring the effects of different maize/soybean systems on microbial community diversity and composition, found that soybean/maize succession increased the relative abundance of bacterial genera with high potential to degrade cellulose, a major component of maize biomass (Chaves et al., 2021). Interestingly, the inclusion of wheat as a third crop following maize (CD1) has resulted in MB-C,  $q_{\text{Mic}}$  and  $q_{\text{CO}_2}$  similar to CS1, indicating that, probably due to the high C:N ratio of wheat compared with oats and ruzigrass (Redin et al., 2018; Rosolem et al., 2010), CD1 did not promote deep alterations in the composition of the existing soil microbial community.

After the third year of crop diversification, CS1 and CD1 kept lower  $q_{\text{CO}_2}$ , showing higher microbial metabolic efficiency, and presented higher MB-C, getting closer to each other regarding the similarity of microbial attributes in the systems, as demonstrated the CDA and hierarchical clustering analyses. For the other diversification systems, a more complex picture appeared, probably due to the cumulative effects of residues added to the soil (Silva et al., 2010). For instance, the inclusion of wheat in rotation and oats in succession to maize (CD3) has resulted in higher SOC compared with CS1, while MB-C and  $q_{\text{CO}_2}$  did not differ from maize/soybean, indicating a possible ongoing adaptation of the microbial community to the increase of crop diversity with time. In fact, studies have shown that the effects of crop diversification on soil microbial attributes are more evident in the long-term (Wood and Bowman, 2021).

As the three years of diversification did not affect the net ammonification and potential nitrification rates, differences in total N and nitrate might have been a result of stimulation of microbial activity and their effects on the decomposition of crop residues. The

MB-N represents the pool of N immobilized organically in the microbial cells, which is protected from losses by leaching or denitrification (Nogueira et al., 2014). Furthermore, MB-N acts as a reservoir of readily available N, since it is easily mineralized after microbial death (Cardoso et al., 2013). Notably, the inclusion of black oats as a third crop have resulted in the highest total N,  $\text{NO}_3^-$ , and MB-N, indicating that the closure of the window between maize harvest and soybean sowing could be beneficial to increase soil N availability and cycling.

Extracellular enzymes act in the decomposition of organic compounds (Mendes et al., 2019; Sobucki et al., 2021) and their activity tend to increase with the addition of crop residues to the soil (Kim et al., 2020; Pires et al., 2020). Our results demonstrated that, with the exception of PHOS, the activity of BG, ARYL, and GLUT responded to crop diversification only by the third year, suggesting that enzymes required some time to respond following continuous double cropping (maize/soybean). However, we must also consider that the first year was drier than the first one (Figure 1), what may have impaired the enzyme activity. In fact, the effects of plant diversification on the soil carbon depends on the climate (Spohn et al., 2023), which mechanisms of transformation depend on the soil enzymes.  $\beta$ -glucosidase is one of the most studied soil enzymes acting in the last steps of decomposition of cellulose within the carbon biogeochemical cycle (Tabatabai, 1994). Given its positive correlation with plant residues (Anghinoni et al., 2021; Pires et al., 2020), it is likely that the straw from black oats used as cover crop and the proportion of labile carbon fractions in the soil under CS3 contributed to stimulate the BG activity compared with CS1 and CD1. As for ARYL, the presence of organic sulfur can stimulate the production of arylsulfatase, since this enzyme mediates the mineralization of sulfur from organic sources (Sobucki et al., 2021). Moreover, there is a strong correlation between ARYL and SOC (Mendes et al., 2019). The introduction of black oats as third crop in the window between maize harvest and soybean sowing (CD2) increased the ARYL activity in the soil compared with CS1, probably due to the increased addition of organic residues to the system. In agreement with this finding, Crespo et al. (2021) observed higher organic sulfur after the introduction of black oats as soybean off-season compared with fallow when studying ways to improve sulfur supply in soybean-based cropping systems.

Acid phosphatase exhibited early sensitivity to diversification strategies in the short-term, demonstrating high discriminatory power to differentiate among the diversification treatments in the first year, according to the CDA that showed high loading values of PHOS with the discriminating function 1. Bini et al. (2014), evaluating soil microbial attributes following the inclusion of cover crops (black oats) in a 5-year no-till soybean/fallow

system, also elected acid phosphatase activity as a reliable discriminator among diversification systems. Finally, the glutaminase activity, which catalyzes the hydrolysis of glutamine into glutamate and ammonia, plays crucial role in the transformation of organic nitrogen compounds into mineral N accessible to microorganisms and plants (Frankenberger and Tabatabai, 1991). Our results showed that the introduction of new crop species in succession and, specially, the addition of a third crop in succession (CD1) or in rotation (CD3) contributed to increase the GLUT activity in the soil compared with the soybean/maize system on the third year, contributing to speed up the soil N-cycling.

Contrary to previous findings regarding the higher sensitivity of soil microbial nitrogen indicators to differentiate between soil management and off-season use (Bini et al., 2014; Silva et al., 2014), we found that, at both sampling years, the most significant microbial attributes were related to the carbon cycle (MB-C, BR, SEOC,  $q_{\text{Mic}}$ , and  $q_{\text{CO}_2}$ ). Our results corroborate other studies such as Strickland et al. (2019) who found significant changes in the soil microbial C attributes after one year of adoption of cover crops. When evaluating the response of soil microbial attributes to changes from native forest to reforested sites (with native and exotic species) and croplands, Bini et al. (2013) observed that MB-C, BR, and cellulase activity were the best indicators to discriminate the variation in the vegetation composition. In another study on the inclusion of cover crops after 36 years of continuous maize/soybean succession, Kim et al. (2022) found that two years of cover crops were not enough to influence the N-cycling microbial communities. On the other hand, our findings indicate that soil microbial attributes related to C-cycling can be good indicators of soil health to evaluate changes resulting from crop diversification in the short-term.

## 5. Conclusions

Three years of intensification of plant residues inputs to an agroecosystem formerly based on soybean/maize succession by adopting diversification of crops in the window between maize and soybean increased the soil labile organic carbon and microbial activity but did not increase the soil microbial biomass carbon and metabolic efficiency compared with the continuous soybean/maize system. Including black oats as diversifying cover crop in the off-season interval between maize and soybean or in rotation with soybean replacing maize increased the soil microbial biomass nitrogen and soil enzyme activities after three years. Microbial attributes related to carbon cycle (microbial biomass carbon, salt extractable organic carbon, basal respiration, microbial quotient and metabolic quotient) proved to be the most

effective discriminators of crop diversification effects on attributes related to soil health in the short-term.

Despite the detected changes in the short-term, overall trends should be clearer in the long-term to confirm our hypothesis on the improvement of microbial attributes following the adoption of different off-season crops and a third season crop compared with the commonly soybean/maize succession.

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**Supplementary table S1:** Details on crop genotypes, sowing and harvesting dates, and fertilization practices for crops cultivated during the three cropping seasons during the experiment.

Season	Crop	Genotype	Sowing (Month/Day)	Harvest/ Management	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O (kg ha <sup>-1</sup> )	Top dressing N (kg ha <sup>-1</sup> )
2020/2021	Soybean	M5705 IPRO	Oct/07	Mar/03	04-24-16 (310)	-
	Maize 2 <sup>nd</sup> season <sup>(1)</sup>	AG 9050 PRO3	Jan/21	May/25	10-15-15 (300)	Urea 45% N (200)
	Wheat	TBIO Sonic	May/10	Sep/05	10-15-15 (300)	Urea 45% N (200)
	White Oats	URS Altiva	May/10	Aug/08	10-15-15 (300)	Urea 45% N (200)
	Wheat 3 <sup>rd</sup> season	TBIO Sonic	Jun/10	Sep/25	10-15-15 (300)	Urea 45% N (200)
	White Oats 3 <sup>rd</sup> season	URS Altiva	May/29	Aug/15	10-15-15 (300)	Urea 45% N (200)
	Black Oats (CD2)	Embrapa 139	May/29	Sep/10 <sup>(2)</sup>	-	-
2021/2022	Soybean	BMX Lança	Oct/06	Jan/01	02-20-18 (300)	-
	Maize 2 <sup>nd</sup> season <sup>(1)</sup>	AG 9000 PRO3	Mar/10	Jun/20	10-15-15 (310)	Ammonium nitrate 27% N (150)
	Wheat	TBIO Audaz	May/05	- <sup>(3)</sup>	10-15-15 (300)	Urea 45% N (200)
	Black Oats (CS3)	Embrapa 139	May/13	Aug/15 <sup>(2)</sup>	-	-
2022/2023	Soybean	BMX Zeus	Oct/17	Mar/05	02-20-18 (300)	-
	Maize 2 <sup>nd</sup> season <sup>(1)</sup>	AG 9000 PRO3	Jan/24	May/31	10-15-15 (300)	Urea 45% N (150)
	Wheat	TBIO Ponteiro	Apr/29	Sep/10	10-15-15 (300)	Urea 45% N (200)
	Black Oats (CS3)	Embrapa 139	Apr/29	Aug/20 <sup>(2)</sup>	-	-
	Wheat 3 <sup>rd</sup> season	TBIO Audaz	Jun/15	Oct/10	10-15-15 (300)	Urea 45% N (200)
	Black Oats (CD2/CD3)	Embrapa 139	Jun/15	Sep/20 <sup>(2)</sup>	-	-

<sup>(1)</sup> Refers to 2<sup>nd</sup> season maize in single cultivation or intercropped with *Urochloa ruziziensis*; <sup>(2)</sup> Cover crop termination; <sup>(3)</sup> Harvest lost due to the occurrence of frost in the reproductive phase of the wheat.

**Supplementary table S2:** Soil chemical characteristics at the topsoil (0-0.1 m) of an Oxisol as affected by one (2020) and three (2022) years of off-season diversification in soybean cropping systems.

Cropping System (year)	pH (CaCl <sub>2</sub> )	P (mg kg <sup>-1</sup> )	K -----	Ca	Mg	H+Al	SB	CEC	V (%)
----- (cmolc dm <sup>-3</sup> ) -----									
<b>2020</b>									
CS1	5.51	42.3 b	0.73 c	6.29	3.07	4.67	10.1	14.7	68.3
CS2	5.47	43.6 b	0.80 bc	6.20	2.83	4.97	9.83	14.8	66.4
CS3	5.47	39.0 b	0.89 abc	6.03	2.90	4.80	9.81	14.6	67.2
CS4	5.33	58.4 a	0.94 ab	6.20	2.66	5.25	9.99	14.6	67.3
CD1	5.56	45.2 b	0.77 bc	6.36	3.18	4.65	10.3	15.0	68.8
CD2	5.37	47.7 ab	1.07 a	5.83	2.84	5.11	9.74	14.8	65.3
CD3	5.48	45.0 b	1.07 a	6.19	2.90	4.83	10.1	14.9	67.5
P-value	0.543	< 0.05	< 0.001	0.612	0.150	0.502	0.857	0.910	0.678
CV (%)	3.2	11.7	10.1	6.63	8.6	9.5	6.09	3.3	4.0
<b>2022</b>									
CS1	5.52	62.4 abc	0.49 c	5.18	2.44	4.67	8.15	12.4	65.8
CS2	5.68	72.6 ab	0.73 ab	5.48	2.53	4.97	8.72	12.7	68.3
CS3	5.57	73.1 a	0.88 a	5.02	2.27	4.80	8.16	12.4	65.7
CS4	5.76	51.6 c	0.77 ab	5.18	2.54	5.25	8.54	12.3	69.2
CD1	5.64	55.6 abc	0.68 bc	5.27	2.51	4.65	8.46	12.4	68.1
CD2	5.72	68.8 abc	0.73 ab	5.5	2.58	5.11	8.87	12.7	69.5
CD3	5.46	53.5 bc	0.84 ab	5.24	2.30	4.83	8.37	12.4	67.4
P-value	0.245	< 0.05	< 0.001	0.824	0.350	0.502	0.736	0.575	0.673
CV (%)	3.24	13.1	11.0	9.5	9.3	9.5	8.3	3.3	5.6

Means followed by different letters in the column differ from each other by the Tukey test ( $P < 0.05$ ). pH – soil pH; P: phosphorus; K - potassium; Ca – calcium; Mg – magnesium; H+Al – exchangeable acidity; SB - sum of bases; CEC – cation exchange capacity; V (%) – base saturation. For details on the cropping systems, please refer to Table 1.

#### 4 ARTIGO B – SOIL MICROBIAL ATTRIBUTES AND SOYBEAN YIELD RESPONSE TO OFF-SEASON CROP DIVERSIFICATION IN AN OXISOL

**Abstract** – Soybean cropping in Brazil is frequently characterized by the soybean/maize succession or soybean/fallow systems, with negative effects on the soil biological traits. The diversification with off-season crops can improve the soil biological health and soybean yield. The aim of this study was to evaluate the effects of off-season crops on soil microbial attributes and soybean yield over a seven-year experiment carried out in Londrina, Paraná, Brazil. The treatments comprised five cropping systems in the off-season: (i) cash crop maize, (ii) cash crop wheat, (iii) ruzigrass (*Urochloa ruziziensis*) as cover crop, (iv) showy rattlebox (*Crotalaria spectabilis*) as cover crop, and (v) fallow. Soil microbial attributes and soybean yield were assessed over 2020/2021 and 2022/2023 cropping seasons. Ruzigrass had the highest production of straw (8,347 kg ha<sup>-1</sup> year<sup>-1</sup>) while showy rattlebox produced the lowest amounts of straw among the off-season crops (509 kg ha<sup>-1</sup> year<sup>-1</sup>). Ruzigrass and maize improved the soil organic carbon levels compared with fallow, whereas maize promoted the lowest amount of labile-C in the soil compared with the other treatments. Maize, ruzigrass, and wheat also increased the soil microbial biomass carbon. Cropping ruzigrass as off-season cover crop also improved N-cycling traits (microbial biomass N, and total inorganic N), microbial respiration, and the activity of  $\beta$ -glucosidase, arylsulfatase, acid phosphatase, and glutaminase. A multivariate analysis with soil microbiological and chemical attributes revealed a separation among the off-season treatments, specially between ruzigrass from showy rattlebox and fallow. Ruzigrass provided the highest soybean yield in succession (4,199 kg ha<sup>-1</sup> year<sup>-1</sup>) compared with fallow (3,525 kg ha<sup>-1</sup> year<sup>-1</sup>). These results highlight ruzigrass as an option to diversify the soybean production system to improve soil microbial attributes and soybean yield. Our findings also add on the understanding of crop diversification as a sustainable agricultural practice for promoting soil health.

**Keywords** – Soil bioindicators; soil quality; soil microbiota; soil enzymes; no-till system.

### 1. Introduction

Soybean (*Glycine max.* (L.) Merr.) holds significant importance as a valuable source of vegetable protein and oil for human and animal consumption, in addition to ingredients and raw material for a variety of industrial applications (Singh and Krishnaswamy, 2022). Brazil produced 154.6 million metric tons of soybean grains in 44.1 million hectares in

the 2022/2023 growing season, making it the world's largest producer and exporter (Conab, 2023). The majority of soybean cropping areas in the country have been cultivated under no-tillage (NT) system, which is characterized by low soil disturbance, permanent soil cover, and crop diversification, practices that combine environmental and economic benefits (Fuentes-Llanillo et al., 2021).

Soybean/maize (*Zea mays* L.) off-season double cropping system has become the dominant grain production system in Brazil (Garbelini et al., 2020). During the 2021/2022 season, approximately 16.4 million hectares (~37% of the soybean production area) were cropped with maize after soybean (Conab, 2023). This trend is even more pronounced in some key soybean production regions, like the northern of the Paraná state (South Brazil), where over 65% of the soybean area is cropped with maize in the off-season (Seab, 2023). In addition, in the Brazilian agricultural regions where rainfall is limited during the autumn-winter, areas are left under fallow due to the lack of adapted crops for cultivation between soybean seasons (Balbinot Junior et al., 2017; Yokoyama et al., 2022). The ongoing agroecosystem simplification with monocropping or crop succession reduces the sustainability of the NT system and raises concerns on the long-term profitability of soybean cropping systems, as the lack of crop diversification is associated to phytosanitary issues such as increased incidence of weed, pests, and diseases (Volsi et al., 2022), low soil cover in the window between maize harvest and soybean sowing (June to October) (Balbinot Junior et al., 2023), and decline of the soil physical, chemical, and biological health (Wang et al., 2022).

Crop diversification involves temporal and spatial arrangement of species, including strategies such as crop rotation, intercropping, and cover crops (Brannan et al., 2023). The diversification of species is considered a sustainable management practice for ensuring environmental conservation and increasing yield stability (Volsi et al., 2022). Wheat (*Triticum aestivum* L.) stands as an alternative cash crop that can be grown during the soybean off-season, either as a replacement or in rotation with maize in several regions of Brazil (Balbinot Junior et al., 2023a). Moreover, the use of cover crops between cash crop seasons aiming at soil coverage and improvement of soil attributes provide further diversification (Adetunji et al., 2020). Cover crop species such as showy rattlebox (*Crotalaria spectabilis* Roth.) and ruzigrass (*Urochloa ruziziensis* R. Germ. & C. M. Evrard) are suitable for soybean off-season cultivation. Showy rattlebox is a legume characterized by its production of biomass easily decomposable (Yokoyama et al., 2022), ability to suppress phytonematodes (Silva et al., 2022), and capacity of biological nitrogen fixation (Mendonça et al., 2017). Ruzigrass is widely used as cover crop in tropical regions of Brazil, in rotation or intercropped with cash crops (Baptistella et al., 2020;

Volf et al., 2023). This grass produces large amounts of biomass with high persistence on the soil surface, and has a vigorous and deep root system, which are desirable traits for a cover crop (Rosolem et al., 2019).

Soil microorganisms are key components of soil health due their pivotal role in various soil processes, such as formation and mineralization of soil organic matter (Tao et al., 2023), nutrient cycling (Bender et al., 2023), soil aggregation (Hartmann and Six, 2022), and plant growth-promotion (Volpiano et al., 2022). Soil microbial attributes (e.g.: microbial biomass, basal respiration, and activity of soil enzymes) are useful indicators of soil health due to their rapid sensitivity to shifts in management (Bünemann et al., 2018; Mendes et al., 2019) and their relationship with soil physical properties (Tonon-Debiasi et al., 2024), chemical fertility, and crop yield (Mendes et al., 2021). The diversification of cropping systems can shape the soil microbial community, affecting its composition and functions. The influence of crop species on soil microbial attributes can occur during the plant growth, with recruitment of specific microbial groups (Molefe et al., 2023), and during the decomposition of surface and belowground plant residues (Almagro et al., 2021; Williams et al., 2023). The amount of crop biomass added to the soil has been positively correlated with soil microbial biomass and the activity of soil enzymes, including  $\beta$ -glucosidase and arylsulfatase (Bastos et al., 2023; Santos et al., 2022). Furthermore, crops with rapid biomass decomposition, such as legumes, can increase the soil microbial activity, activity of enzymes, and microbial biomass, when included in the diversification (Borase et al., 2021; Wang et al., 2020).

Soil microbial responsiveness to crop diversification, however, relies on various environmental factors, such as climate, soil type, and texture. It also depends on the management practices, like soil preparation, use of fertilizers, the frequency of crop diversification, and the crop species (Kim et al., 2020; Morugán-Coronado et al., 2022; Muhammad et al., 2021). The selection of off-season crops more favorable to the soil microbial community may positively affect the soybean yield and soil health. Despite extensive research on indicators of soil physical and chemical health under different soybean diversification strategies in Southern Brazil (Balbinot Junior et al., 2024; Bertollo et al., 2021; Yokoyama et al., 2022), there are limited information on the influence on soil microbial attributes. Therefore, this study aimed to assess the effects of off-season crops on soil microbial attributes and soybean yield over a seven-year experiment in South Brazil. Our hypotheses are (i) using ruzigrass and showy rattlebox as cover crops enhances soil microbial attributes and soybean yield, and (ii) the fallow causes a decline in the soil biological health and soybean yield.

## 2. Material and methods

### 2.1 Site description

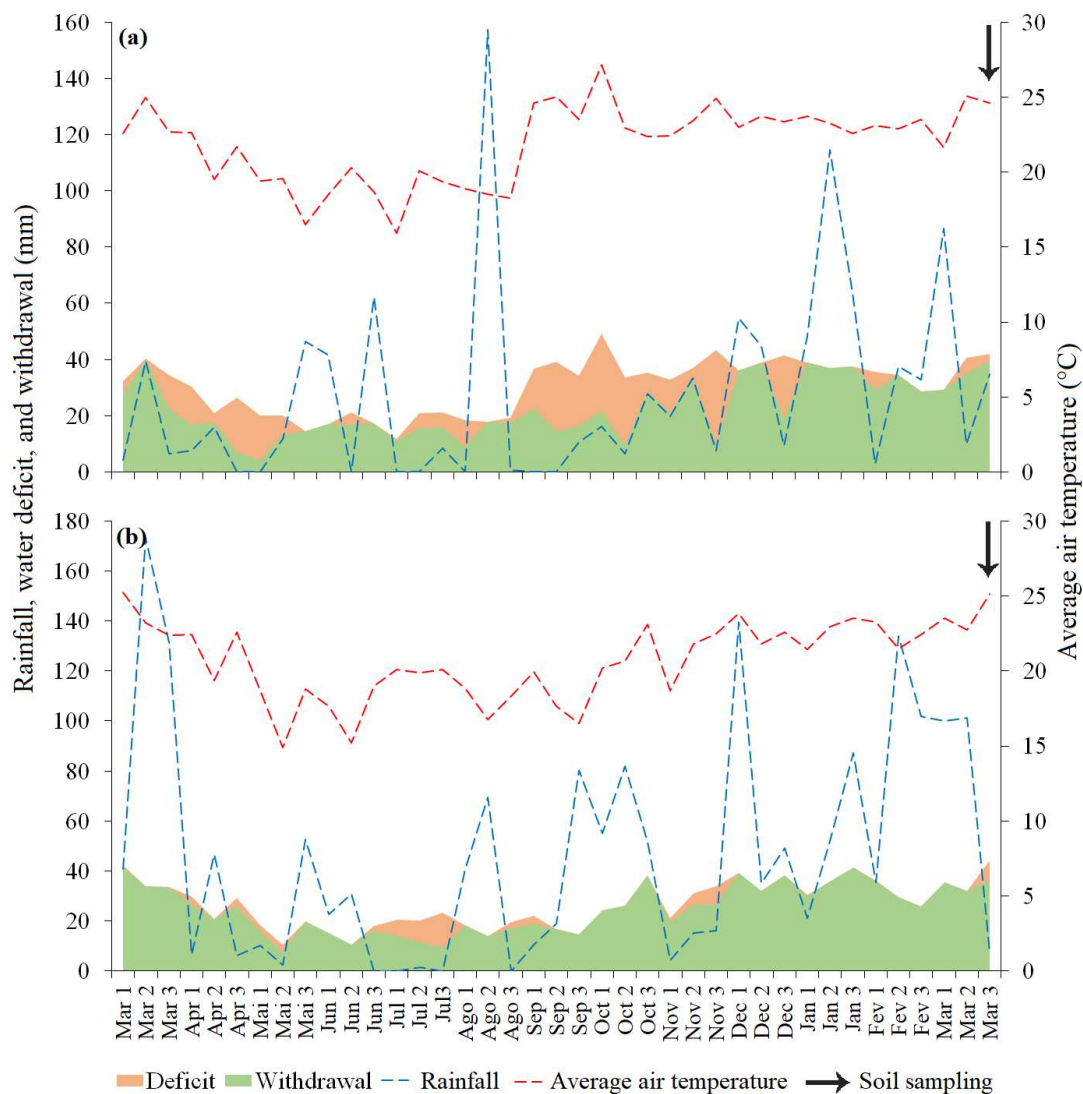
The study was carried out at the experimental station of Embrapa Soybean in Londrina, Paraná state, Brazil (24°36'40" S and 53°18'20" W; 630 m a.s.l.). According to Köppen, the climate of the region is humid subtropical (Cfa). The soil is an Oxisol [“Latossolo Vermelho distroférico”, according to the Brazilian classification (Silva, 2018); or Rhodic Eutrudox, according to the USA classification (Soil Survey Staff, 2014)], with clayey texture (710 g kg<sup>-1</sup> of clay, 82 g kg<sup>-1</sup> of sand, and 208 g kg<sup>-1</sup> of silt). The soil chemical properties (0-0.20 m) at the experiment set up (March 2016) were: soil organic carbon (Walkley Black), 17.8 g dm<sup>-3</sup>; pH in CaCl<sub>2</sub>, 5.1; Al, 0.0 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al (SMP) 5.2 cmol<sub>c</sub> dm<sup>-3</sup>; Ca, 4.41 cmol<sub>c</sub> dm<sup>-3</sup>; Mg, 1.52 cmol<sub>c</sub> dm<sup>-3</sup>; K, 0.85 cmol<sub>c</sub> dm<sup>-3</sup>; P (Mehlich-1), 37 mg dm<sup>-3</sup>; cation-exchange capacity, 12.0 cmol<sub>c</sub> dm<sup>-3</sup>; and base saturation, 57 %. Meteorological data during the cropping seasons (2020/2021 and 2022/2023) were recorded at the agroclimatological station at Embrapa Soybean and used for calculating the water balance according to Thornthwaite and Mather (1955) (Figure 1).

### 2.2 Experimental design, treatments, and crop management

The experiment was established in March 2016 to evaluate the effects of off-season crops on soil attributes and soybean yield. The experimental design was in randomized complete blocks with five treatments and five replications, totaling thirty plots (5.0 × 10.0 m). Treatments consisted of soybean production systems where soybean was cultivated as the spring/summer main crop, followed by different crops in the fall/winter succession: (i) Maize; (ii) Wheat; (iii) Ruzigrass; (iv) Showy rattlebox; and (v) Fallow after soybean and subjected to weed control. Maize and wheat were grown as cash crops and ruzigrass and showy rattlebox as cover crops.

The experimental area had been managed under no-tillage for 15 years, with soybean or maize in the spring/summer season and wheat or maize in the fall/winter. Before the beginning of the trial, black oats (*Avena strigosa* Schreb.) and millet (*Pennisetum glaucum* L.) were cultivated in total area, in the winter of 2015 and the summer of the 2015/2016 season, respectively. Liming (2.5 Mg ha<sup>-1</sup>) was applied in total area before the beginning of the trial. In all cropping seasons, maize, ruzigrass, and showy rattlebox were sown in mid-March, while wheat was sown at the end of April. The maize hybrid 'AG 9010 YG' was sown with 0.90 m between lines and a final plant density of 60,000 plants per hectare. For wheat (cv. 'BRS Gralha

Azul'), ruzigrass (cv. Kennedy), and showy rattlebox, seed rates of 350, 50, and 100 seeds m<sup>2</sup>, respectively, were used, with rows spaced 0.17 m apart. Both maize and wheat received N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O at rates of 24, 84, and 48 kg ha<sup>-1</sup>, respectively. Maize and wheat treatments did not receive topdressing fertilization, while ruzigrass, showy rattlebox, and fallow were not fertilized at either sowing or topdressing. Maize was harvested in August, whereas wheat harvest in September. In October, the area underwent desiccation with glyphosate (1.08 kg a.i. per hectare) in preparation for soybean sowing.



**Figure 1:** Sequential water balance measured at every 10-day intervals according to Thornthwaite and Mather (1955), rainfall, average air temperature, and soil sampling dates over the 2020/2021 (a) and 2022/2023 (b) cropping seasons.

Sowing of soybean was performed always in the first half of October with 0.45 m between rows and 320,000 plants ha<sup>-1</sup>. Soybean cultivars (all with indeterminate growth type and maturity group 6.1), varied across the cropping seasons: cv. BRS 1010IPRO in 2016/17, 2017/18, and 2018/19; cv. BRS 1003IPRO in 2019/20, 2020/21, and 2021/22; and cv. BRS 1061IPRO in 2022/23. Before sowing, seeds underwent chemical treatment with pyraclostrobin + thiophanate-methyl + fipronil (1 mL kg<sup>-1</sup>) and were inoculated with *Bradyrhizobium elkanii* strains SEMIA 587 and SEMIA 5019 (2 mL kg<sup>-1</sup>). Fertilization provided 70 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 70 kg ha<sup>-1</sup> of K<sub>2</sub>O in furrow simultaneously to the sowing.

## 2.2 Soil sampling

Soil samples for microbial and chemical analysis were collected at the 0.0–0.10 m depth after the soybean harvest (end of March) in two cropping seasons, 2020/2021 and 2022/2023, corresponding to the fifth and seventh year from the beginning of the experiment. Following the FERTBIO soil sample concept (Mendes et al., 2019), five soil cores were collected at three points in each plot using a soil probe, perpendicularly to the sowing row. In each of the three sampling points, one core was collected on the sowing row, while the other two were collected equidistantly on each side of the inter-row spacing. The composite soil samples were homogenized and transported to the laboratory. An aliquot of the soil sample was air-dried at room temperature, passed through a 2 mm sieve, and stored at room temperature until analysis, within two months. For analysis of soil inorganic nitrogen, net ammonification, and potential net nitrification rate, the soil was kept under field moisture and stored at 4 °C after sieving until analysis.

## 2.3 Soil analysis

### 2.3.1 Chemical attributes

Chemical attributes included soil pH in CaCl<sub>2</sub> 0.01 mol L<sup>-1</sup>, soil organic carbon (SOC) (Walkley-Black), available P (Mehlich-1), K (Mehlich-1), Ca, Mg, and Al (KCl 1 mol L<sup>-1</sup>), H+Al (SMP), cation-exchange capacity (CEC), and base saturation (V%). At the 2022/2023 cropping season, soil ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) were extracted from 20 g of field-moist soil with KCl 2 mol L<sup>-1</sup>. Quantifications of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were carried out based on the salicylate (Searle, 1984) and delta-absorbance (Apha, 2005) methods, respectively. The sum of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> was considered as total soil inorganic N. Results from chemical analysis are presented in Table S1.

### 2.3.2 Microbial attributes

#### 2.3.2.1 Microbial biomass, basal respiration, and metabolic indices

Microbial biomass carbon (MB-C) and nitrogen (MB-N) were determined by the chloroform fumigation–extraction method (Vance et al., 1987). Before analysis, soil aliquots were rewetted to achieve a moisture at 60% of the maximum soil water holding capacity (WHC), and pre-incubated for seven days in the dark at 28 °C. Two 20 g aliquots of moist soil were taken and one underwent incubation with chloroform in a desiccator connected to a vacuum pump for 24 h and named fumigated (F) whereas the other was not exposed to chloroform and named non-fumigated (NF). Soil organic carbon and nitrogen were extracted using 80 mL of  $\text{K}_2\text{SO}_4$  0.5 mol L<sup>-1</sup> and stirred at 150 rpm for 40 min. Carbon was quantified by wet oxidation with  $\text{K}_2\text{Cr}_2\text{O}_7$  at 100 °C for 30 min, followed by titration with  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4) \cdot 6\text{H}_2\text{O}$  0.033 mol L<sup>-1</sup> in  $\text{H}_2\text{SO}_4$  using phenanthroline as indicator. Nitrogen was determined after sulfuric digestion, followed by color development based on the indophenol blue method (Feijl and Anger, 1972) and absorbance measure at 630 nm. MB-C and MB-N were recorded based on the difference between the F and NF aliquots, using the extraction coefficients  $K_C = 0.33$  and  $K_N = 0.68$ , for C and N, respectively (Brookes et al., 1985). The C measured in NF samples (salt-extractable organic carbon - SEOC) was considered a labile soil carbon fraction (Baldwin-Kordick et al., 2022; Potter et al., 2023).

Soil basal respiration (BR) was determined by measuring the  $\text{CO}_2$  released from the soil (Alef, 1995) over a 7-day incubation period (28 °C in the dark) using 50 g of soil, with moisture adjusted to 60% of WHC. Incubation was conducted in hermetically sealed flasks containing the soil sample and a small vial containing 10 mL of 0.5 mol L<sup>-1</sup> NaOH. The released  $\text{CO}_2$  was captured by the addition of 1 mL of a saturated solution of  $\text{BaCl}_2$  and determined by titration with 0.5 mol L<sup>-1</sup> HCl using phenolphthalein (1%) as indicator. The control blank was a hermetically sealed flask without soil, containing a vial with 10 mL of 0.5 mol L<sup>-1</sup> NaOH. Derived indexes were computed as follows: (i) Microbial quotient ( $q_{\text{Mic}}$ ) was the ratio between microbial biomass carbon and total soil organic carbon (Insam and Domsch, 1988); (ii) Metabolic quotient ( $q_{\text{CO}_2}$ ) was calculated as the ratio between basal respiration and the microbial biomass carbon (Insam and Haselwandter, 1989).

#### 2.3.2.2 Soil enzyme activities

The activities of  $\beta$ -glucosidase (BG; EC 3.2.1.21), arylsulfatase (ARYL; EC 3.1.6.1), and acid phosphatase (PHOS; EC 3.1.3.2), related to carbon, sulfur, and phosphorus

cycling, respectively, were determined following Tabatabai (1994) and the air-dried sample protocol established by Mendes et al. (2019). The procedure is based on the colorimetric determination of p-nitrophenol formed after the hydrolysis of specific substrates for the respective enzyme (BG: p-nitrophenyl- $\beta$ -D-glycopyranoside; ARYL: p-nitrophenyl sulfate; and PHOS: disodium p-nitrophenyl phosphate tetrahydrate). Due to the short incubation period (1 h at 37 °C), toluene was omitted from the analyses. The activity of the N cycling-related enzyme glutaminase (GLUT; EC 3.5.1.2) was measured following Frankenberger and Tabatabai (1991) with L-glutamine as substrate. The  $\text{NH}_4^+$  produced from the hydrolysis of L-glutamine during a 2-h incubation at 37 °C was extracted with  $\text{KCl}$  2.5 mol  $\text{L}^{-1}$  and  $\text{AgSO}_4$  100 mg  $\text{L}^{-1}$ , steam distilled (Kjeldahl), and quantified by titration with standardized  $\text{H}_2\text{SO}_4$  0.005 mol  $\text{L}^{-1}$ .

### 2.3.2.3 Soil microbial N transformations

Soil  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and N-cycling related microbial attributes were evaluated only at the 2022/2023 season. Ammonification and potential nitrification rates (Schuster and Schröder, 1990) were determined considering the concentration of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  before and after the incubation of 20 g of soil (with moisture adjusted to 60% of WHC) for 21 days at 28 °C, in the dark, with and without the addition of 125  $\mu\text{g g}^{-1}$  soil of  $\text{NH}_4^+$  as ammonium sulfate. The extraction and determination of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were performed as described above. The daily ammonification rate was calculated by the difference between the final (without N) and initial  $\text{NH}_4^+$  (prior incubation) concentration divided by the number of days of incubation. The daily potential nitrification rate was computed by the difference between the final (with added  $\text{NH}_4^+$ ) and initial concentration of  $\text{NO}_3^-$  divided by the incubation days.

### 2.4 Average specific enzyme activity

The average specific activity of BG and ARYL per unit of SOC (average specific enzyme activity, ASEA) was determined according to Chaer et al. (2023). First, the activity of each enzyme was divided by the SOC to calculate the specific activity of BG (S-BG) and ARYL (S-ARYL). Then, ASEA was calculated as:

$$(i) \text{ ASEA} = (\text{S-BG} + \text{S-ARYL})/2$$

### 2.5 Plant analysis

The residual straw of off-season crops ( $\text{kg ha}^{-1}$ ) left on the soil surface was measured 20 days before soybean sowing. The amount of straw in  $1 \text{ m}^2$  per plot was collected, dried at  $65 \text{ }^\circ\text{C}$ , and weighted. Soybean grain yield was estimated after harvesting and weighing the grains of the central area in each plot. Grain yield was adjusted to 13 % moisture and expressed in  $\text{kg ha}^{-1}$ . Average straw dry matter production and average grain yield were computed as means of the results from 2020/2021, 2021/2022, and 2022/2023 seasons.

## 2.6 Statistical analysis

Data from each cropping season were analyzed separately. The normality of residues and the homogeneity of variances were assessed using the Shapiro-Wilk and Bartlett tests, respectively. Once the assumptions of the analysis of variance were met, data underwent one-way ANOVA, and mean comparisons were performed using the Tukey's test ( $P \leq 0.05$ ). A Pearson's correlation analysis was performed to evaluate the relationship among soil microbial attributes, residual straw, and soybean yield. A principal component analysis (PCA) was used to explore the ordination of samples according to the chemical and microbial attributes, as well as the association between the off-season crops with these indicators. PERMANOVA was used to test if the centroids of off-season crops were significantly different from each other. All analyses were performed using the software R (Core Team, 2023).

## 3. Results

### 3.1 Straw production by off-season crops

The off-season crops produced different amounts of straw across the two cropping seasons (Table 1). Ruzigrass yielded the highest amounts of straw in both seasons; in 2020/2021, maize produced more straw than wheat, while in 2022/2023 both off-season crops left similar amounts of straw on the soil surface. Showy rattlebox left the lowest amount of straw on the soil surface in both cropping seasons, as fallow was intentionally left without plants. Considering the mean of three years of the study, ruzigrass produced the highest amount of straw, averaging  $8,347 \text{ kg ha}^{-1} \text{ year}^{-1}$ , while showy rattlebox produced only  $509 \text{ kg ha}^{-1} \text{ year}^{-1}$ . Off-season maize and wheat presented intermediate amounts of straw ( $3,106$  and  $2,926 \text{ kg ha}^{-1} \text{ year}^{-1}$ , respectively), not differing statistically from each other (Table 1).

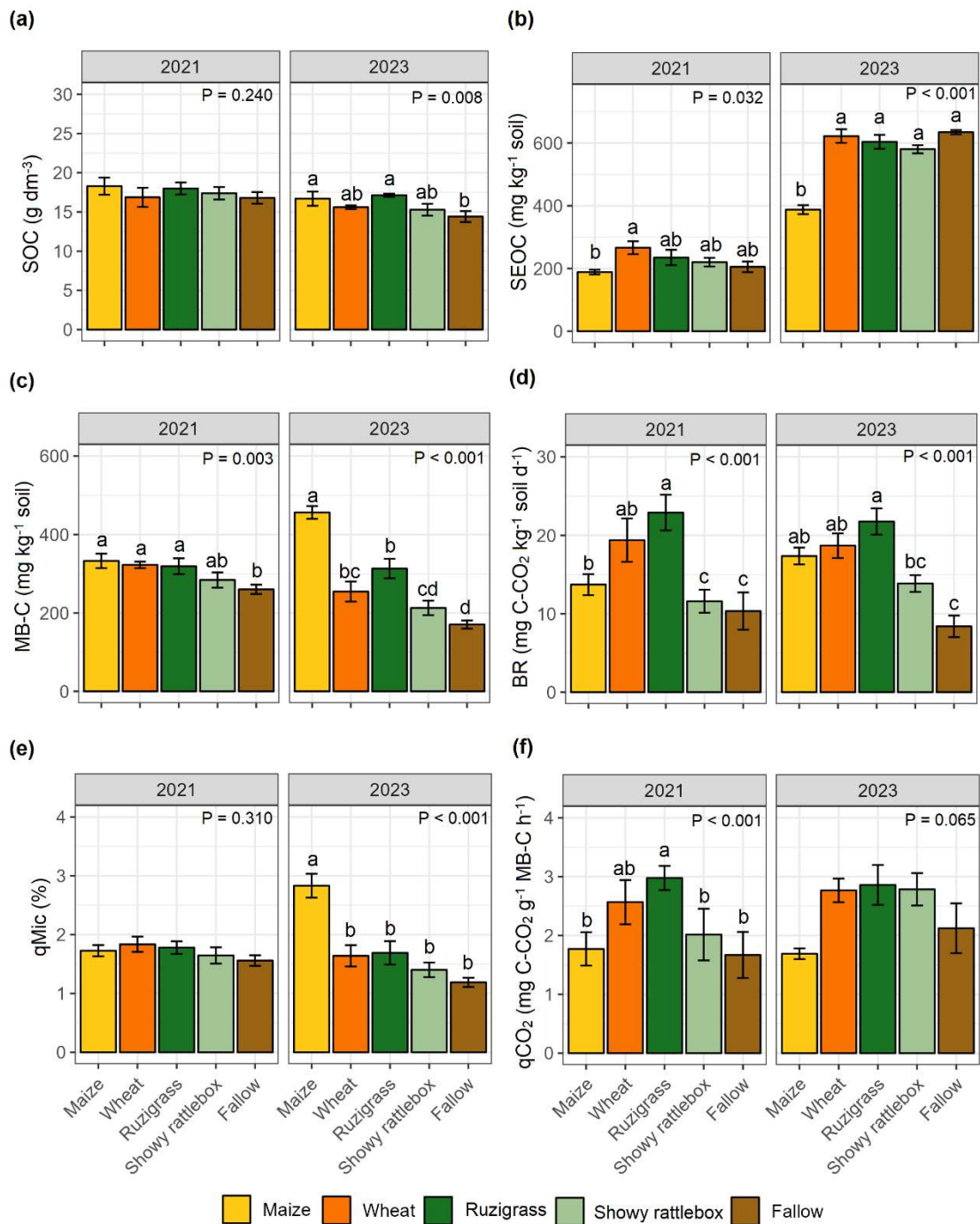
**Table 1:** Straw dry matter produced by off-season crops left on the soil surface before soybean sowing and soybean grain yield for the 2020/2021 and 2022/2023 cropping seasons, and average.

	Residual straw			Soybean yield		
	20/21 (kg ha <sup>-1</sup> )	22/23 (kg ha <sup>-1</sup> )	Average (20/23) (kg ha <sup>-1</sup> year <sup>-1</sup> )	20/21 (kg ha <sup>-1</sup> )	22/23 (kg ha <sup>-1</sup> )	Average (20/23) (kg ha <sup>-1</sup> year <sup>-1</sup> )
Maize	5586 b	2112 b	3106 b	3404 b	4061	3815 ab
Wheat	2894 c	3020 b	2926 b	3801 ab	4207	3958 ab
Ruzigrass	9100 a	8506 a	8347 a	4419 a	4118	4199 a
Showy rattlebox	0 d	628 c	509 c	3664 ab	3772	3744 ab
Fallow	-	-	-	3643 b	3734	3525 b
P-value	< 0.001	< 0.001	< 0.001	0.011	0.27	0.03
CV (%)	16.6	18.4	15.1	10.4	10.0	7.0

Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). CV: coefficient of variation.

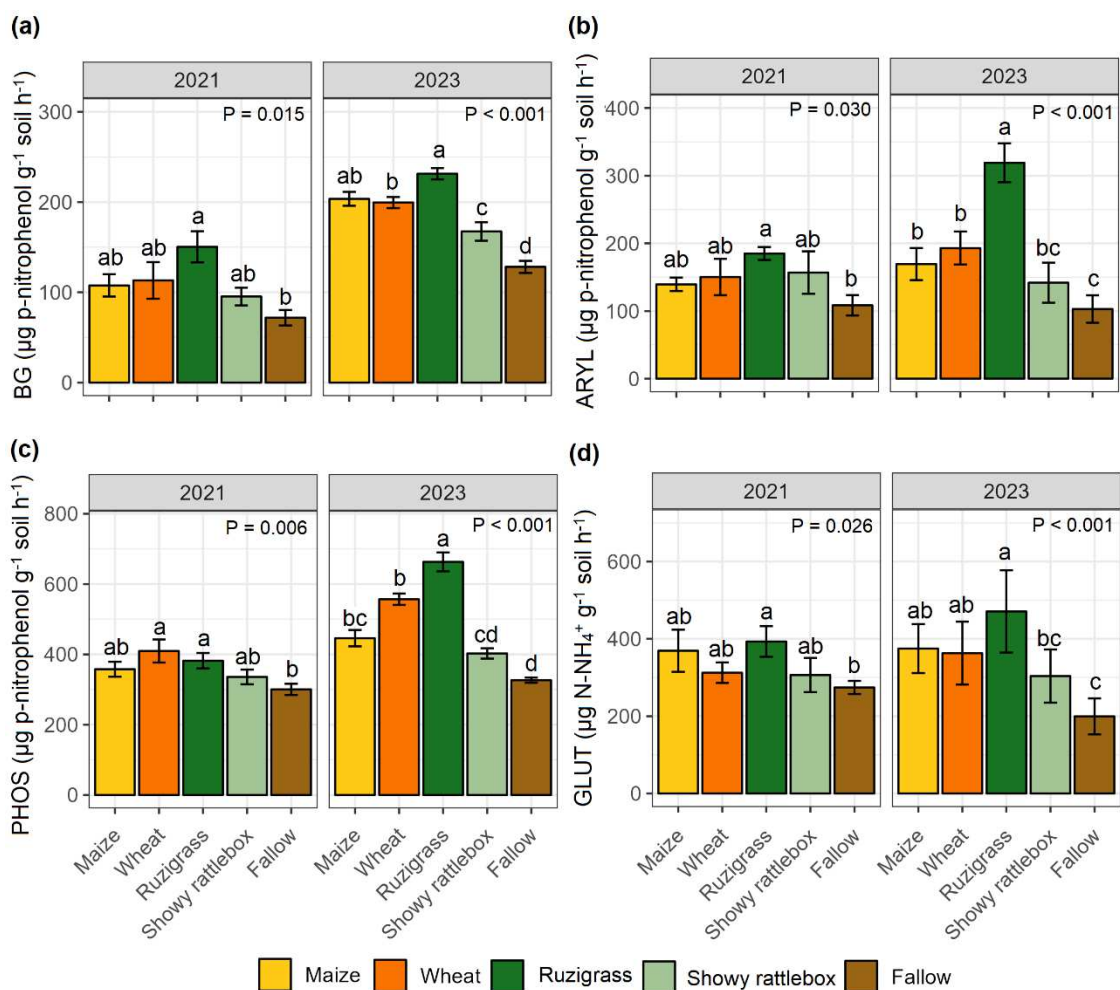
### 3.2 Soil microbial attributes, organic C and inorganic N

The off-season crops influenced the soil organic carbon (SOC) and carbon-related microbial attributes in the two cropping seasons, with variations between years (Figure 2). SOC was significantly affected only in 2022/2023, with ruzigrass and maize showing the highest SOC content compared with fallow (Figure 2a). Salt extractable organic carbon (SEOC) increased from 2020/2021 to 2022/2023 for all off-season crops and fallow, with maize showing the lowest SEOC in both sampling years (Figure 2b). In 2020/2021, maize, wheat, and ruzigrass led to the highest microbial biomass carbon (MB-C) compared with fallow; in 2022/2023, the soil cropped with maize had the highest MB-C while fallow had the lowest, not differing from showy rattlebox in both seasons (Figure 2c). Ruzigrass and wheat resulted in the highest soil basal respiration (BR) in both seasons, and also maize in 2022/2023, while showy rattlebox and fallow provided the lowest soil microbial activity in both sampling years. The off-season crops did not affect the microbial quotient ( $q_{Mic}$ ) in 2020/2021; however, in 2022/2023, maize exhibited the highest  $q_{Mic}$  compared with the other treatments (Figure 2e). Finally, the metabolic quotient ( $q_{CO_2}$ ) was the highest in the soil grown with ruzigrass and wheat in 2020/2021, whereas no significant differences were detected in 2022/2023 (Figure 2f).



**Figure 2:** Soil organic carbon – SOC (a), salt-extracted organic carbon – SEOC (b), microbial biomass carbon – MB-C (c), basal respiration – BR (d), microbial quotient –  $q_{\text{Mic}}$  (e), and metabolic quotient –  $q_{\text{CO}_2}$  (f) at the topsoil (0-0.1 m) of an Oxisol as affected by off-season crops after soybean harvest in 2020/2021 and 2022/2023 cropping seasons. Error bars represent the standard error of means. Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ).

The off-season crops affected the activity of the assessed soil enzymes (Figure 3). Overall, in the first sampling year 2020/2021 ruzigrass promoted the activities of BG, ARYL, PHOS, and GLUT compared with fallow, which showed the lowest enzyme activities; maize, wheat, and showy rattlebox presented intermediate activities, not differing statistically from fallow or ruzigrass. In 2022/2023, ruzigrass presented the highest activities of ARYL and PHOS compared with the other off-season crops and fallow. BG and GLUT activities were also higher in the ruzigrass soil, but did not differ from maize for BG, and maize and wheat for GLUT. Showy rattlebox and fallow had the lowest soil enzyme activities in 2022/2023.



**Figure 3:** Activities of the soil enzymes  $\beta$ -glucosidase – BG (a), arylsulphatase – ARYL (b), acid phosphatase – PHOS (c), and glutaminase – GLUT (D) at the topsoil (0-0.1 m) of an Oxisol as affected by off-season crops after soybean harvest in 2020/2021 and 2022/2023 cropping seasons. Error bars represent the standard error of means. Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ).

Ruzigrass cropped as off-season promoted the highest average specific enzyme activity (ASEA), differing statistically from the other treatments (except wheat in 2021/2022) (Table 2). Wheat, maize, and showy rattlebox cropping resulted in intermediate ASEA values, whereas fallow resulted in the lowest ASEA in both soil samplings.

**Table 2:** Average specific enzyme activity (ASEA) as affected by off-season crops after soybean harvest in 2020/2021 and 2022/2023 cropping seasons.

	ASEA (mg p-nitrophenol g <sup>-1</sup> SOC h <sup>-1</sup> )	
	2020/2021	2022/2023
Maize	6.42 bc	11.3 bc
Wheat	8.02 ab	12.6 b
Ruzigrass	9.33 a	16.1 a
Showy rattlebox	7.16 bc	10.1 bcd
Fallow	5.38 c	8.4 d
P-value	< 0.001	< 0.001
CV (%)	15.8	11.1

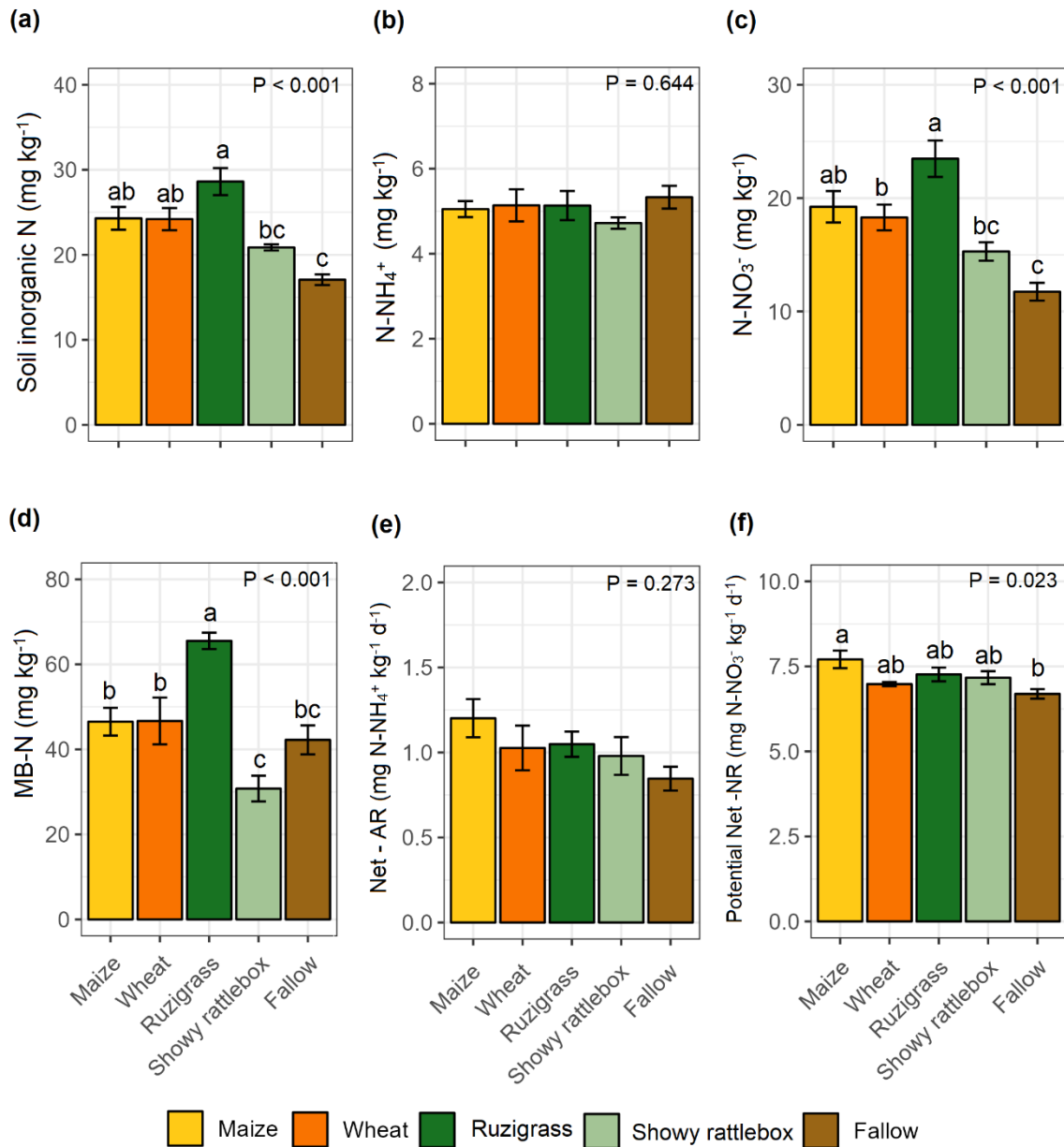
Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). CV: coefficient of variation.

The highest amount of total soil inorganic N was found in the soil with ruzigrass as off-season crop compared with showy rattlebox and fallow (Figure 4a). Soil ammonium and the net ammonification rate (AR) did not differ among the six off-season treatments (Figure 4b, 4e), while soil nitrate was higher in the soil with ruzigrass, which did not significantly differ from maize (Figure 4c). Maize promoted the highest potential net nitrification rate (NR), differing from fallow, which presented the lowest NR (Figure 4f). The amount of N in the microbial biomass (MB-N) was the highest in the soil with ruzigrass, followed by maize, wheat, and fallow, whereas showy rattlebox promoted the lowest MB-N (Figure 4d).

### 3.3 Soybean grain yield

Soybean yield varied among cropping years and the previous off-season crops (Table 1). In 2020/2021, higher soybean yield was observed after ruzigrass, whereas maize and fallow resulted in the lowest soybean yield. In the 2022/2023 season, off-season crops did not

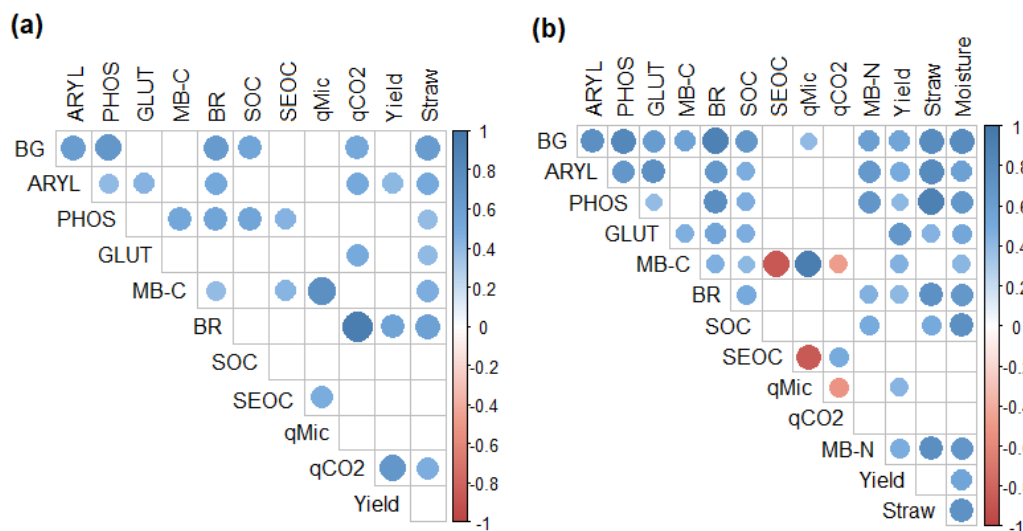
influence soybean yield (Table 1). Considering the mean yield from the three cropping seasons (2020 to 2023), soybean cropped after ruzigrass exhibited the highest yield compared with fallow, but not differing from maize, wheat, and showy rattlebox (Table 1).



**Figure 4:** Soil inorganic nitrogen (a), ammonium – N-NH<sub>4</sub><sup>+</sup> (b), nitrate – N-NO<sub>3</sub><sup>-</sup> (c), and N-related microbial indicators: microbial biomass N – MBN (d), and net ammonification (e) and potential nitrification rates – NR (e), at the topsoil (0-0.1 m) of an Oxisol as affected by off-season crops after soybean harvest in the 2022/2023 cropping season. Error bars represent the standard error of means. Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ).

### 3.4 Relationship between soil microbial attributes and off-season crops straw, soybean grain yield, and soil chemical fertility

In both cropping seasons, Pearson's correlation analysis revealed significant relationships between the amount of straw produced by off-season crops and microbial indicators. In 2020/2021, the amount of straw before soybean sowing positively correlated with MB-C, BR and the assessed soil enzymes (Figure 5a). In 2022/2023, besides these attributes, the amount of straw also correlated positively with SOC, MB-N, and soil moisture (Figure 5b). Soybean yield also significantly correlated with the biological indicators, especially in 2022/2023. In 2020/2021, only arylsulfatase, basal respiration, and the metabolic quotient exhibited positive correlations with soybean yield (Figure 5a). However, in 2022/2023, all the assessed soil enzymes, BR, microbial biomass C and N, and  $q_{Mic}$  positively correlated with soybean yield (Figure 5b).



**Figure 5:** Pearson's correlation between microbiological attributes at the topsoil (0-0.1 m) of an Oxisol, and soybean yield, as affected by off-season crops, in 2020/2021 (a) and 2022/2023 (b) cropping seasons. Only the significant correlations were plotted ( $P < 0.05$ ). BG –  $\beta$ -glucosidase; ARYL: arylsulfatase; PHOS; acid phosphatase; GLUT: glutaminase; MB-C – microbial biomass carbon; BR – basal respiration; SOC – soil organic carbon; SEOC – salt extractable organic carbon;  $q_{Mic}$  – microbial quotient;  $q_{CO_2}$  – metabolic quotient; MB-N – microbial biomass nitrogen; Yield - soybean grain yield; Straw – residues from off season crops left on the soil surface; Moisture – soil water content at the moment of sampling.

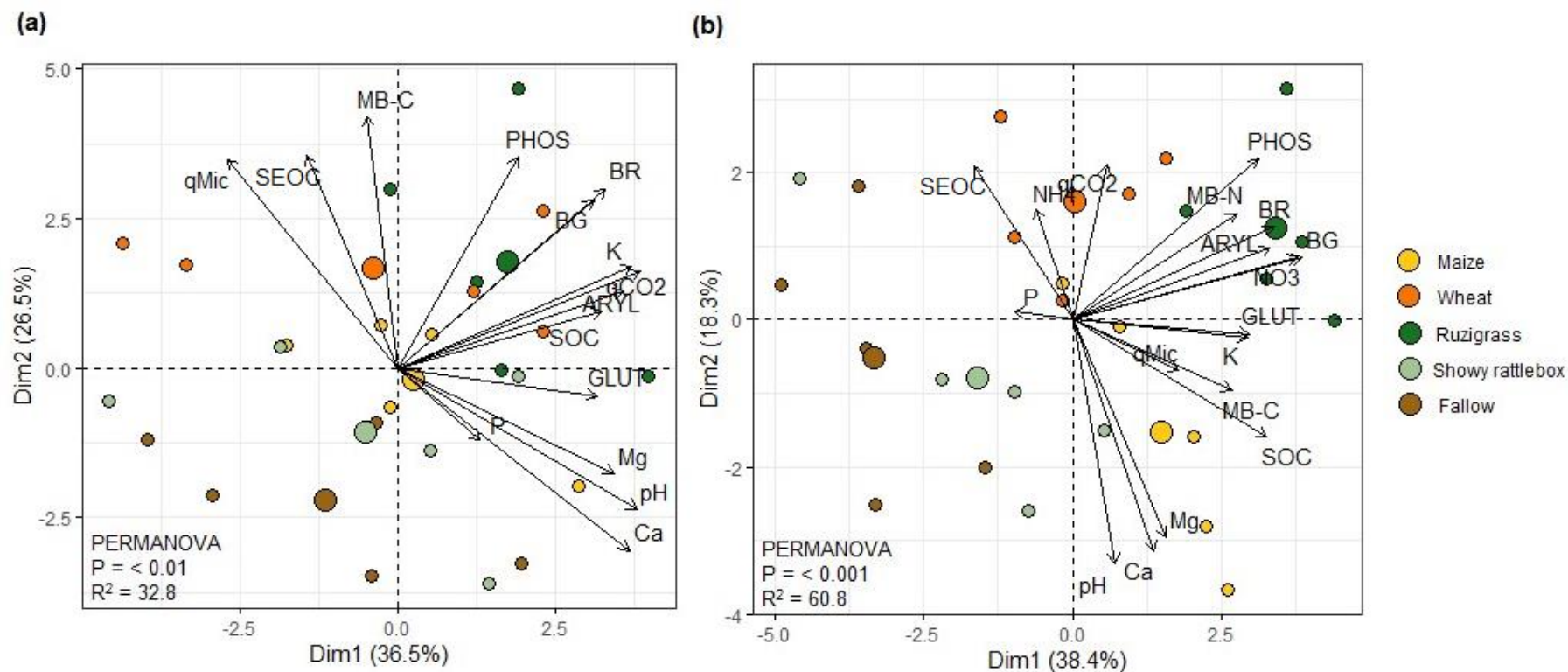
The principal component analysis (PCA) explored relationships among microbial and chemical variables and their association with the off-season treatments (Figure 6). For the 2020/2021 season, the first two components explained 63,0% of the total variance (Figure 6a). The attributes most associated with principal component 1 (Dim1), which explained 36.5% of the variance among the off-season crops were:  $q\text{CO}_2$ , pH, K, Ca, and ARYL. The variables MB-C, SEOC, and PHOS accounted for treatment dissimilarity along principal component 2 (Dim2), explaining 26.5% of the total variability (Figure 6a; Supplementary table S2). The off-season crops clustered according to the soil microbial and chemical attributes, and the PERMANOVA test statically validated that separation (Figure 6a). Ruzigrass stood on the right and upper side of Dim1 and Dim2, respectively, strongly associated with microbial activity indicators (BG, ARYL, PHOS, BR),  $q\text{CO}_2$ , K, and SOC; showy rattlebox and fallow, on the other hand, showed no association with any assessed soil attribute (Figure 6a).

In the 2022/2023 season, principal components 1 and 2 among microbial and chemical attributes explained 56.7% of the total variance (Figure 6b). The variables more associated with Dim1 (38.4% of the variance) were the activities of the enzymes BG, ARYL, PHOS, and GLUT, in addition to BR, SOC, K, and the N-related attributes MB-N and  $\text{NO}_3^-$ . pH, Ca, and Mg mostly correlated with Dim2, accounting for 18.3% of the variance (Figure 6b; Supplementary table S2). PERMANOVA test showed statistical significance, validating the separation between the off-season treatments ( $P < 0.01$ ). As in 2020/2021, ruzigrass and fallow were separated in opposite quadrants of the PCA plot. Besides K and the microbial activity indicators, ruzigrass was also associated with MB-N and nitrate, whereas showy rattlebox became more distant from the other treatments, getting closer to fallow (Figure 6b).

## 4. Discussion

### 4.1 Off-season crops and straw production

In addition to minimal soil moving, crop diversification and constant soil cover are interlinked pillars of the NT system (Fuentes-Llanillo et al., 2021). Plant species selected for crop rotation should provide continuous soil cover until the cultivation of the next crop (Balbinot Junior et al., 2024). Ruzigrass have shown to be a suitable off-season alternative to maintain the soil cover for the succeeding soybean, and improving the quality of the NT system. The high aboveground biomass production is a characteristic of grasses of the genus *Urochloa* that has stimulated its adoption as cover crop in tropical climates (Baptistella et al.,



**Figure 6:** Principal component analysis (PCA) of off-season crops considering soil microbial and chemical attributes assessed after soybean harvest in the 2020/2021 (a) and 2022/2023 (b) cropping seasons. Microbial attributes: MB-C – microbial biomass carbon; BR – basal respiration; SOC – soil organic carbon; SEOC – salt extractable organic carbon; qMic – microbial quotient; qCO<sub>2</sub> – metabolic quotient; BG –  $\beta$ -glucosidase; ARYL: arylsulfatase; PHOS; acid phosphatase; GLUT: glutaminase; MB-N – microbial biomass nitrogen. Chemical attributes: pH – soil pH; Ca – calcium; Mg – magnesium; K - potassium; NH<sub>4</sub> - ammonium; NO<sub>3</sub> – nitrate.

2020). In addition, ruzigrass straw presents a high C:N ratio (45:1) (Rosolem et al., 2010), which might decelerate the biomass mineralization, thereby extending the period of soil protection by its residues. Maize and wheat biomasses also undergo slower decomposition rates compared with legumes, due to the high C:N ratio of their residues, and lignification, especially the stem of mature plants. Although no differences on the straw amount were observed between maize and wheat in the average of the three seasons, studies have indicated higher percentage of soil cover of wheat compared with maize (Yokoyama et al., 2022) due to thinner residues left on the soil surface. Despite showy rattlebox provided only a small amount of straw at the pre-sowing of soybean, this species might add ecosystem services such as nematode control (Silva et al., 2022), biological nitrogen fixation (Mendonça et al., 2017), and faster nutrient cycling (Souza et al., 2024). However, the low biomass production in addition to low C:N ratio of their residues (Yokoyama et al., 2022) make showy rattlebox unsuitable as off-season crop for not providing adequate soil cover in NT systems in Southern Brazil.

#### *4.2 Soil organic carbon and mineral nitrogen*

The input of organic residues via plant biomass, either shoots or roots, can increase the fractions of soil organic carbon (SOC) (Li et al., 2024). Ruzigrass and maize increased the SOC compared with fallow, what can be attributed to higher amounts of residues these crops produced. Soil organic matter is a key component of soil health, affecting its physical, chemical, and biological properties. The lower plant biomass returning to the soil in the soybean/fallow may lead to a negative carbon balance, leading to a decrease in SOC and overall soil health over time. A meta-analysis study highlighted the benefits of adding cash crops and cover crops in rotation on SOC, with an increase of 3.6% and 8.5%, respectively compared with monocrop (McDaniel et al., 2014). Most of the studies included in the meta-analysis were conducted in North America, but similar results were found under tropical conditions, showing declines in SOC with soybean monocropping compared with diversified cropping systems, including cover crops (Tiecher et al., 2020). Crop diversification effects on SOC, however, has a typically long-term response (Poeplau and Don, 2015), what may explain the no effect of off-season crops at the first sampling, even after five years of the installation of the experiment. Bini et al. (2014) also did not find significant changes in SOC after black oats as off-season cover crop in conventional or NT systems in Northern Parana for five years.

The treatments, however, significantly affected the SEOC fraction, in both samplings. SEOC represents labile pools of carbon that can be easily used by the soil microbial

community, mainly low-molecular-weight, dissolved, and exchangeable organic C (Baldwin-Kordick et al., 2022). The composition of plant residues may affect the different pools of the SOC. As maize straw has almost 70% of cellulose and hemicellulose and less than 4% of lignin (Khatri et al., 2015), most part of the plant biomass is easily degradable by the action of the soil microbial community, what may explain the lower SEOC in the soil cropped with maize, especially in the second season. In addition, the lower concentration of SEOC in the soil agrees with hither MB-C (Figure 2c), suggesting that part of the SEOC might have been consumed by the soil microbial community, and converted into microbial biomass, causing an increase in the  $q_{\text{Mic}}$  (Figure 2e), which represents the living pool of SOC.

Plants and microorganisms uptake N mainly as inorganic forms ( $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$ ) that may come from N fertilizers, biological N fixation (BNF), and mineralization of soil organic matter (Robertson and Vitousek, 2009). The off-season crops did not affect the ammonification rate. Total soil inorganic N was predominantly  $\text{N-NO}_3^-$ , which is rapidly converted from  $\text{NH}_4^+$  in the soil (Coskun et al., 2017). Ruzigrass promoted the highest soil  $\text{N-NO}_3^-$  content, and consequently total soil inorganic N, probably linked to the amount of biomass produced by the forage, as shown by the positive correlation between  $\text{N-NO}_3^-$  and straw ( $r = 0.80$ ,  $P < 0.05$ ; data not shown) in 2022/2023. This trend was rather similar for MB-N, showing that the pool of organic N immobilized in the microbial biomass also reflected in the mineral N pool, and strengthen the importance of the microbial biomass as a source of N easily mineralizable in the soil. In addition, the cultivation of ruzigrass can increase the abundance of N-fixing microorganisms, potentially improving the free-living and associative biological N fixation and the availability of soil N (Merloti et al., 2023; Rocha et al., 2020). Some grasses belonging to the genus *Urochloa* (e.g. *Urochloa humidicola*) release root exudates that inhibit the microbial oxidation of ammonia in a process known as biological nitrification inhibition (BNI) (Subbarao et al., 2009). The inhibitory effects of ruzigrass on nitrification in soils have been investigated but are still not well understood with results varying from high (Merloti et al., 2023) to none BNI (Rocha et al., 2020) compared with other *Urochloa* spp. species. Our findings showed that ruzigrass did not decrease the potential nitrification rate in the soil, thus not confirming the BNI capacity in our study.

#### 4.3 Soil microbial biomass, microbial activity, and metabolic efficiency indices

Most of the soil microbial attributes were affected after five years of off-season crop diversification after soybean cropping, highlighting the sensitivity of bioindicators

to crop management in the short term and their importance for monitoring the soil health (Mendes et al., 2019). Crop residues attenuates soil temperature (Shen et al., 2018), decreases water losses by evaporation, increases water retention capacity after conversion into SOM (Hashimi et al., 2023), and make a more favorable environment for the microbial community (Williams et al., 2023), resulting in longer survival, and higher activity and biomass. That is the reason why higher MB-C and MB-N were observed following maize, ruzigrass, and wheat, the off-season crops that left higher straw amounts on the soil surface. In fact, MB-C and straw were positively correlated in 2020/2021, which agrees with the results from previous studies (Bastos et al., 2023; Santos et al., 2022). The microbial biomass is the living fraction of the SOM and is considered an important indicator of soil health as it serves as a reservoir of readily available nutrients to crops (Cardoso et al., 2013). In this sense, higher MB-N found with the cultivation of ruzigrass indicates higher N-cycling capacity, in agreement with studies that have related the use of ruzigrass in the cropping system management for N cycling by retaining N in the plant biomass, and gradually releasing it to the system (Coelho et al., 2023; Souza et al., 2024). Studying soybean off-season diversification in the Cerrado biome, Souza et al. (2024) observed a faster half-life time for showy rattlebox biomass and N (mean of 82 and 65 days over three cropping seasons, respectively) compared with ruzigrass (mean of 132 and 211 days, respectively). In addition to the slow biomass production, the fast decomposition rate of the legume residues quickly releases N to the soil that can be released during the soybean cycle, but do not persist after the cash crop, corroborating the lowest MB-N and total available N in the plots with showy rattlebox.

The off-season crops as soil cover result in substrate availability that create a more favorable environment for microbial activity. This is clear from the positive correlation between straw left on the soil surface and soil microbial attributes like BR, and the activities of the enzymes BG, ARYL, PHOS, and GLUT in both cropping seasons. The effect of organic substrates on the soil microbial activity involves the supply of carbon, energy, and nutrients that undergo mineralization, leading to the release of carbon dioxide (CO<sub>2</sub>) into the atmosphere and minerals into the soil (Cardoso et al., 2013). Consequently, higher respiration rates were observed after off-season ruzigrass, followed by wheat, and maize. In contrast, the low input of organic carbon in showy rattlebox and fallow plots have resulted in lower soil microbial respiration and less MB-C. High  $q\text{CO}_2$  indexes are typically linked to unfavorable conditions for microbial growth, as a result of a low efficiency of the soil microorganisms in using organic carbon as substrate for maintenance and growth (Anderson and Domsch, 1993). However, the interpretation of the  $q\text{CO}_2$  index requires caution, as higher values can also be consequence of

easily degradable organic carbon inputs that promptly stimulate the microbial activity (Wardle and Ghani, 1995), and might explain the higher  $q\text{CO}_2$  found in the soil grown with ruzigrass and wheat.

The activity of soil enzymes is very sensitive to soil management and is an indicator of SOM turnover, nutrient availability, and soil health (Chaer et al., 2023; Mendes et al., 2021; 2019). The four soil enzymes assessed in this study (BG, ARYL, PHOS, and GLUT) responded to the off-season crops, highlighting their suitability as bioindicators of soil health. Overall, there was an increase on the activities from 2020/2021 to 2022/2023, likely due to (i) the lower occurrence of water deficit during the second cropping season, (ii) the quantitative and qualitative differences on the organic residues produced by the off-season crops, and (iii) the accumulation of abiotic exoenzymes (Lopes et al., 2021).

In the sampling in the fifth year of off-season diversification (2020/2021), the activities of BG, ARYL, PHOS, and GLUT were higher with the use of ruzigrass compared with fallow, while maize, wheat, and showy rattlebox usually showed intermediate activities. Nevertheless, in the seventh year (2022/2023), ruzigrass stood out in stimulating the soil enzymes. Conversely, the soil cropped with showy rattlebox exhibited the lowest soil enzyme activity, along with fallow. Ruzigrass provided the highest aboveground biomass, and is also recognized for its capacity to produce high and persistent root biomass that exudates compounds that stimulate the soil microbial growth (Balbinot Junior et al., 2017; Rosolem et al., 2019), what may explain the stimulation on the soil enzyme activities. Positive and significant correlations between the amount of straw and the soil enzyme activities corroborate this statement and agrees with other studies on the effects of crop diversification on soil enzyme activities. Higher enzyme activities mean more active biogeochemical cycles in the soil, making it more effective in cycling carbon and nutrients, as a result of a more active microbial community (Cardoso et al., 2013) as a consequence of a healthier soil (Baldwin-Kordick, 2022; Chaer et al., 2023; Wang et al., 2022). In a global meta-analysis on the effects of cover crops on the soil microbial activity, Kim et al. (2020) observed that cover crops enhanced the activity of soil enzymes (BG and PHOS) compared with fallow. Higher activity of PHOS was found in agricultural soils amended with organic P, indicating a correlation between PHOS and the availability of organic P (Janes-Bassett et al., 2022). ARYL acts on the mineralization of sulfur from organic compounds (Sobucki et al., 2021) and is well correlated with mulching on the soil (Bastos et al., 2023) and SOM (Lopes et al., 2013). GLUT has proven to be a good discriminator between soil management with off-season crops, being higher when black oats were used as winter cover crop in NT systems compared with fallow (Bini et al., 2014).

Following the 4-quadrant model on the relationship between SOC and the average activities of arylsulfatase and  $\beta$ -glucosidase per unit of SOC (ASEA) proposed by Chaer et al. (2023), an increase in BG and ARYL in the soil with ruzigrass could mean an increase of soil health with an increase in the soil biological activity. On the other hand, plots under fallow or the low-biomass producing showy rattlebox presented a decrease in SOC and ASEA, suggesting a decrease in soil health. For the soybean/maize succession, ASEA did not differ from showy rattlebox and fallow in 2020/2021 and showy rattlebox in 22/23, showing that this strategy of crop succession may lead to losses in soil health in the long term. Thus, sensitive indicators of soil health like ASEA must be monitored to prevent soil degradation.

#### *4.4 Soybean grain yield*

Ruzigrass as off-season crop increased soybean grain yield by 19% on average when compared with soybean after fallow, which exhibited the lowest yield. Off-season wheat, maize, and showy rattlebox resulted in intermediate soybean yield. Previous studies have reported increase in soybean yield due to previous cropping of ruzigrass in off-season, mainly attributed to their roots (Balbinot Junior et al., 2017), as well as enhancements in soil physical quality (Bertollo et al., 2021). Moreover, nutrient cycling by off-season crops may have a lasting effect on the following crop (Souza et al., 2024). Therefore, the highest amount of mineral N and K found in the ruzigrass soil might have played a role in the increase of soybean yield. Volf et al. (2023) also demonstrated the capacity of ruzigrass to cycle and provide K for soybean, which agrees with the highest concentration of K in the soil cropped with ruzigrass (Supplementary table S1). Finally, ruzigrass stimulated the soil microbial and biochemical activity, boosting the C, P, S, and N cycling, and contributing for soybean yield. Previous studies have reported positive correlations between microbial indicators and crop yields (Lopes et al., 2013; Mendes et al., 2019), consistent with our findings in which basal respiration and the activity of soil enzymes positively correlated with soybean yield.

The effects of off-season crops on soybean yield were more pronounced in 2020/2021, a season that presented strong water deficit during the soybean growth (Figure 1). In the Brazilian Cerrado biome, Mendes et al. (2018) assessed soil biochemical attributes in soybean production systems cultivated with ruzigrass in the off-season, or left under fallow. During the eighth year of the study, following a period of severe drought, the soybean yield after ruzigrass was much higher than after fallow, despite no differences in the soil chemical attributes. However, the activities of the enzymes  $\beta$ -glucosidase, arylsulfatase, and acid

phosphatase were much higher in the soil with ruzigrass, highlighting the importance of a biologically active soil for facing drought without compromising the cash crop yield. In a similar condition of this trial, Balbinot Junior et al. (2024) observed more stable soybean yield after ruzigrass and wheat in comparison with fallow, after 7 years of study.

#### *4.4 Global data analysis and implications*

Due to the complex nature of soil and the interplay between soil physics, chemistry, and biology, the assessment of soil biological health is challenging. Despite the importance and the level of response of individual attributes in evaluating the effects of soil management on soil health, a comprehensive overview considering multiple attributes contributes for a more robust analysis (Bünemann et al., 2018; Cardoso et al., 2013). The PCA multivariate approach showed that ruzigrass as off-season cover crop provided better soil conditions (e.g.: soil cover, mild soil temperature, and the availability of water and availability of organic substrates) to improve the soil microbial activity and nutrient cycling over time, which have contributed to increase soybean yield. Conversely, showy rattlebox and fallow were mostly similar each other, demonstrated that the use of the legume of low-biomass production as off-season cover crop resulted in worse condition for the soil microbial traits and, consequently, resulting in lower soybean yield.

### **5. Conclusions**

The results of this study support our hypothesis that off-season ruzigrass is a suitable cover crop for improving the soil microbial traits and yield of soybean cropping systems. However, the benefit of showy rattlebox as cover crop was not confirmed, as its cultivation led to microbial traits levels close to fallow, decreasing the soil biological health over time. Maize and wheat provided soybean yields close to ruzigrass; however, the lower soil enzyme activities under these off-season crops compared with ruzigrass in the seventh-year study suggests conditions leading to biological degradation that should be evaluated in the long-term.

This study endorses ruzigrass as a promising cover crop for diversification of soybean cropping systems and emphasizes the importance of selecting off-season crops to enhance microbial biomass and activity, and summer crop yield. Our findings contribute to a broader understanding of crop diversification as a sustainable agricultural practice that

significantly improves soil biological health, improving the sustainability of the soybean production system in the tropical/subtropical region.

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## ARTIGO B -Supplementary files

**Supplementary Table S1:** Soil chemical characteristics at the topsoil (0-0.1 m) of an Oxisol as affected by off-season crops after soybean harvest in 2020/2021 and 2022/2023 cropping seasons.

	pH (CaCl <sub>2</sub> )	P (mg kg <sup>-1</sup> )	K (cmolc dm <sup>-3</sup> )	Ca	Mg	H+Al	SB	CEC	V (%)
<b>20/21</b>									
Maize	5.53	60.4 a	0.80 ab	5.22	2.34	4.57	8.36	12.9	64.5
Wheat	5.31	54.8 a	0.82 ab	4.81	2.02	5.03	7.65	12.7	59.2
Ruzigrass	5.56	36.2 b	1.03 a	5.09	2.30	4.48	8.35	12.8	64.8
Showy rattlebox	5.60	55.7 a	0.76 b	5.43	2.31	4.42	8.51	12.9	65.6
Fallow	5.58	44.2 ab	0.68 b	5.53	2.59	4.39	8.59	13.0	65.6
P-value	0.355	<0.01	< 0.01	0.495	0.254	0.331	0.649	0.973	0.383
CV (%)	4.3	18.1	14.3	13.0	16.0	11.4	12.6	5.92	8.2
<b>22/23</b>									
Maize	5.7	34.1	0.69 ab	5.51	2.76	4.11	8.96	13.1	64.8
Wheat	5.36	38.9	0.61 b	4.76	2.34	4.72	7.7	12.4	59.6
Ruzigrass	5.7	25	0.85 a	5.29	2.75	4.11	8.81	12.9	64.8
Showy rattlebox	5.82	36.6	0.54 b	5.65	2.70	3.86	8.89	12.7	65.4
Fallow	5.79	27.7	0.54 b	5.17	2.55	3.88	8.26	12.1	65.6
P-value	0.249	0.08	<0.01	0.432	0.553	0.132	0.434	0.332	0.383
CV (%)	5.92	25.9	18.6	14.5	16.9	13.2	14.1	5.99	8.2

Means followed by the same letter do not differ one another by the Tukey's test ( $P < 0.05$ ). pH – soil pH; P: phosphorus; K - potassium; Ca – calcium; Mg – magnesium; H+Al – exchangeable acidity; SB - sum of bases; CEC – cation exchange capacity; V (%) – base saturation; CV – Coefficient of variation.

**Supplementary Table S2:** Correlation between variables and the components (Dim 1 and Dim2) and variability explained by the axes in principal component analysis (PCA) of soil microbiological and chemical attributes as affected by off-season crops in soybean production systems in two cropping seasons. Numbers in bold represent strong correlations with a given axis (dimension) in the PCA.

	2020/2021		2022/2023	
	Dim1	Dim2	Dim1	Dim2
BG	0.621	0.562	<b>0.940</b>	0.208
ARYL	<b>0.717</b>	0.258	<b>0.807</b>	0.237
PHOS	0.381	<b>0.703</b>	<b>0.760</b>	0.533
GLUT	0.631	-0.092	<b>0.724</b>	-0.047
MB-C	-0.095	<b>0.839</b>	0.650	-0.231
BR	0.660	0.598	<b>0.822</b>	0.307
SOC	0.650	0.190	<b>0.794</b>	-0.387
SEOC	-0.285	<b>0.709</b>	-0.404	0.508
qMic	-0.535	0.696	0.432	-0.168
q-CO <sub>2</sub>	<b>0.769</b>	0.320	0.138	0.512
MB-N	-	-	<b>0.671</b>	0.351
P	0.262	-0.241	-0.237	0.027
pH	<b>0.758</b>	-0.471	0.173	<b>-0.807</b>
Ca	<b>0.734</b>	-0.608	0.333	<b>-0.765</b>
Mg	0.686	-0.352	0.378	<b>-0.720</b>
K	<b>0.742</b>	0.340	<b>0.718</b>	-0.606
NH <sub>4</sub>	-	-	-0.148	0.367
NO <sub>3</sub>	-	-	<b>0.919</b>	0.207
% of variance	36.5	26.5	38.4	18.3
Cummulative	36.5	62.9	38.4	56.7

Microbial attributes: MB-C – microbial biomass carbon; BR – basal respiration; SOC – soil organic carbon; SEOC – salt extractable organic carbon; qMic – microbial quotient; qCO<sub>2</sub> – metabolic quotient; BG –  $\beta$ -glucosidase; ARYL: arylsulfatase; PHOS; acid phosphatase; GLUT: glutaminase; MB-N – microbial biomass nitrogen. Chemical attributes: pH – soil pH; Ca – calcium; Mg – magnesium; K – potassium; NH<sub>4</sub>- ammonium; NO<sub>3</sub> – nitrate.

## 5. CONSIDERAÇÕES FINAIS

Microrganismos e processos por eles desempenhados são importantes para a saúde do solo, participando de funções essenciais para a agricultura e a sustentabilidade dos ecossistemas. Os experimentos conduzidos neste trabalho demonstraram que a diversificação da entressafra da soja com culturas de interesse econômico e culturas de cobertura na segunda ou terceira safra influenciou positivamente atributos do solo como a biomassa e atividade microbiana no curto e médio prazo.

Independentemente do estudo, a inclusão de culturas de cobertura em sucessão (aveia preta ou braquiária) à soja ou como terceira safra (aveia preta) entre o final do ciclo do milho e a semeadura da soja promoveu a atividade microbiana, com efeitos mais proeminentes com o aumento do tempo de adoção. O estímulo à comunidade microbiana pelas culturas de cobertura foi principalmente ligado ao maior aporte de biomassa vegetal com alta relação C:N, e ao aumento de frações lábeis de carbono orgânico no solo.

As tendências de aumento da atividade microbiana observadas nos solos sob tratamentos com maior diversificação de culturas no experimento de Cafelândia demonstram que a inclusão de diferentes espécies no sistema de produção de soja influencia positivamente a comunidade microbiana no curto prazo (1 a 3 anos). O experimento conduzido em Londrina por 7 anos destacou o cultivo da braquiária (*Urochloa ruziziensis*) na entressafra como alternativa para promoção da saúde biológica do solo e aumento da produtividade da soja em sucessão.

Os atributos microbiológicos mostraram-se sensíveis às mudanças decorrentes da diversificação dos sistemas de produção de soja. Atributos relacionados ao ciclo do carbono foram eficientes em diferenciar modelos de produção para a diversificação da sucessão soja/milho após um ano de adoção, confirmando a sensibilidade de indicadores microbiológicos na avaliação da saúde do solo. Além disso, ferramentas desenvolvidas para monitoria da biologia do solo, como a Bioanálise do Solo (BioAS) foram responsivas à diversificação, mostrando-se importantes aliadas na busca por práticas de manejo visando a promoção da saúde do solo.

Este trabalho reforça a importância e a possibilidade da inclusão de atributos biológicos no monitoramento da saúde do solo no curto e longo prazo e apresenta sistemas de diversificação que promovem a biologia do solo em comparação à sucessão soja/milho e o pousio. A adoção de modelos diversificados, com maior produção de biomassa, é uma oportunidade para o aprimoramento dos sistemas de produção de grãos no Brasil. A utilização

de modelos de produção que propiciem melhoria na saúde do solo é fundamental para a evolução da agricultura em países tropicais, como o Brasil, contribuindo significativamente para o atendimento da demanda global por alimentos, fibras e bioenergia.