UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
DEPARTAMENTO DE SOLOS

BALANÇO DE FÓSFORO EM 12 ANOS E ESTRATÉGIAS DE CULTURAS ANUAIS PARA ACESSAR RESERVAS DE FÓSFORO CONSTRUÍDAS AO LONGO DE 15 ANOS POR FONTES ORGÂNICAS E MINERAL

Doutoranda: Carina Marchezan

Orientador: Prof. Dr. Gustavo Brunetto
Coorientador: Prof. Dr. Paulo Ademar Avelar Ferreira

Santa Maria, RS

2022

Carina Marchezan

BALANÇO DE FÓSFORO EM 12 ANOS E ESTRATÉGIAS DE CULTURAS ANUAIS PARA ACESSAR RESERVAS DE FÓSFORO CONSTRUÍDAS AO LONGO DE 15 ANOS POR FONTES ORGÂNICAS E MINERAL

Tese apresentada ao curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo, da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para obtenção do grau de **Doutora em Ciência do Solo**.

Orientador: Gustavo Brunetto
Coorientador: Paulo Ademar Avelar Ferreira

Santa Maria, RS

2022

Carina Marchezan

BALANÇO DE FÓSFORO EM 12 ANOS E ESTRATÉGIAS DE CULTURAS ANUAIS PARA ACESSAR RESERVAS DE FÓSFORO CONSTRUÍDAS AO LONGO DE 15 ANOS POR FONTES ORGÂNICAS E MINERAL

Tese apresentada ao curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo, da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para obtenção do grau de **Doutora em Ciência do Solo**.

Aprovado em 31 outubro de 2022

Gustavo Brunetto (UFSM)
(Presidente/Orientador)

Paulo Ademar Avelar Ferreira (UFSM)
(Coorientador)

Carlos Alberto Ceretta (UFSM)
(Banca de Avaliação)

Djalma Eugênio Schmitt (UFSC)
(Banca de Avaliação)

Cláudio Roberto Fonseca Sousa Soares (UFSC)
(Banca de Avaliação)

Glaciela Kaschuk (UFPR)
(Banca de Avaliação)

Santa Maria, RS

2022

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior - Brasil (CAPES) - Finance Code 001

Marchezan, Carina
BALANÇO DE FÓSFORO EM 12 ANOS E ESTRATÉGIAS DE CULTURAS ANUAIS PARA ACESSAR RESERVAS DE FÓSFORO CONSTRUÍDAS AO LONGO DE 15 ANOS POR FONTES ORGÂNICAS E MINERAL / Carina Marchezan.- 2022.
154 p.; 30 cm

Orientador: Gustavo Brunetto
Coorientador: Paulo Ademar Avelar Ferreira
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência do Solo, RS, 2022

1. dejetos de animais 2. absorção de P 3. atividade enzimática 4. morfologia radicular 5. rizosfera I. Brunetto, Gustavo II. Avelar Ferreira, Paulo Ademar III. Titulo.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, CARINA MARCHEZAN, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Tese) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

Santa Maria, RS
2022

Aos meus pais Amauri e Aneide Marchezan

Dedico esse trabalho!

Santa Maria, RS

2022

AGRADECIMENTOS

Primeiramente, agradeço a Deus por sempre me ofertar boas oportunidades e colocar boas pessoas em meu caminho.

Aos meus pais, Amauri Marchezan e Aneide Dal’Ongaro Marchezan, os quais sempre se empenharam ao máximo me fornecendo suporte para que eu alcançasse meus objetivos pessoais e profissionais. Também os agradeço aos meus irmãos Fábio, Heleno, Leila, Adriana e Simone, por nunca deixarem faltar amor e carinho.

Ao Professor Gustavo Brunetto, pela orientação, e confiança depositada. Muito obrigada, por estar presente, acompanhando e insistindo no meu desenvolvimento pessoal e científico, sempre me motivando nas horas mais difíceis. Agradeço pelas conversas, por ouvir meus desabafos e principalmente, por me fazer acreditar em mim e no meu potencial. Você fez muito mais que me orientar, me preparou para a vida!

Ao Professor Paulo Ademar Avelar Ferreira, pela coorientação e por me ensinar os primeiros passos dentro da pesquisa científica e me acolher (literalmente) em momentos de dificuldade. A aprendizagem é um processo individual, mas ela se torna mais fácil quando temos alguém que sabe como ensinar. Sua amizade e seu apoio foram fundamentais na elaboração, condução e na obtenção dos resultados desse trabalho.

Ao Professor Carlos Alberto Ceretta, mesmo encerrado sua careira como docente, sempre me incentivou e esteve presente em todas as etapas da minha formação. Você teve grande importância no meu crescimento pessoal e profissional, e com certeza sua postura e conduta serviu como inspiração a todos seus alunos.

À banca examinadora, Dr. Carlos Alberto Ceretta, Dr. Djalma Eugênio Schmitt, Dr. Cláudio Soares e Dra. Glaciela Kaschuk por aceitarem o convite para revisão do material, e contribuições realizadas.

Aos alunos de iniciação científica, Ricardo Fagan Vidal, Gustavo Scopel, Natália Moreira Palermo, Ana Laura Thoma, Vanessa Marques e Amanda Charoppen pela intensa colaboração no planejamento e execução das atividades. Graças a vocês temos uma Tese!

Aos professores do Departamento de Solos, especialmente, Celso Aita, Danilo Rheinheimer dos Santos, Fabrício Pedron, Leandro da Silva, Ricardo Dalmolin, Rodrigo Jacques, Sandro Giacomini e Zaida Antoniolli, pelas aulas ministradas e pela oportunidade de aprendizado durante o curso.

Santa Maria, RS

2022

À Universidade Federal de Santa Maria (UFSM) e ao Programa de Pós-Graduação em Ciência do Solo (PPGCS) pela oportunidade de realização do Curso de Mestrado.

Ao CNPq, Fapergs, e Agrisus pela bolsa e auxílio financeiro.

Aos colegas e colaboradores do GEPACES pela amizade e trocas de conhecimento.

A todos aqueles que não foram citados, mas, que de alguma contribuíram para minha formação pessoal e profissional, e para a realização deste estudo.

Santa Maria, RS

2022

A Rosa e o pequeno Príncipe.

“[...] não estou assim tão resfriada... O ar fresco da noite me fará bem.

Eu sou uma flor.

- Mas os bichos...

- É preciso que eu suporte duas ou três larvas se quiser conhecer as borboletas.”

Pequeno Príncipe - Antoine de Saint-Exupéry, 1943

Santa Maria, RS

2022

RESUMO

BALANÇO DE FÓSFORO EM 12 ANOS E ESTRATÉGIAS DE CULTURAS ANUAIS PARA ACESSAR RESERVAS DE FÓSFORO CONSTRUÍDAS AO LONGO DE 15 ANOS POR FONTES ORGÂNICAS E MINERAL

AUTOR: Carina Marchezan
ORIENTADOR: Gustavo Brunetto
COORIENTADOR: Paulo Ademar Avelar Ferreira

O sistema de produção agrícola brasileiro e de outros países são altamente dependentes das importações de fertilizantes fosfatados. A quantificação e o impacto a longo prazo de fontes secundárias de P, como resíduos da produção pecuária e o acesso as reservas de P nos solos agrícolas pelas plantas é de interesse comum e é uma alternativa para diminuir o uso dos fertilizantes fosfatados minerais. Esta Tese objetivou avaliar o impacto, a longo prazo, de diferentes fontes de adubação e alterações nas reservas de P no solo e investigar as estratégias utilizadas por plantas anuais em acessar e aumentar a disponibilidade de P na rizosfera. Para isso, conduzimos três estudos (**Capítulos I, II e III**) em um experimento de longa duração, implantado em 2004, sob um Argissolo Vermelho Distrófico arênico, em Santa Maria (RS). Os tratamentos utilizados foram dejetos líquidos de suínos, dejetos líquidos de bovinos, cama sobreposta de suínos fertilizante mineral e um tratamento controle, sem a aplicação de nutrientes. No **Capítulo I**, determinamos o Balanço de P no sistema, por meio da contabilidade das entradas, saídas e estoque desse elemento no solo ao longo de 12 anos de cultivo. As formas de P acumuladas no perfil do solo foram determinadas por meio da técnica do fracionamento químico sequencial de P. No **Capítulo II**, selecionados 3 tratamentos (dejetos líquidos de suínos, fertilizante mineral e controle) e investigamos alteração do sistema radicular de raízes de milho e sua relação com a eficiência absorção e utilização de P. Para isso, instalamos tubos de minirrizotron acrílico e monitoramos, *in situ*, a dinâmica de crescimento radicular. Os parâmetros morfológicos de raízes foram determinados e correlacionados com as varáveis de aproveitamento fisiológico do P e produtividade das culturas. Por último, no **Capítulo III**, submetemos os tratamentos abordados no **Capítulo II** a inoculação de FMA com a espécie *Rhizophagus intraradices* (Rootella BR). No solo foram determinados os teores de P, N e C contidos na biomassa microbiana e no solo, bem como a atividade das enzimas fosfatase ácida e β-glucosidade. Essas variáveis foram relacionadas à disponibilidade de P na rizosfera e sua absorção pelas plantas de milho e aveia. As aplicações de dejetos de animais, especialmente os de suínos, geram grandes reservas de P em solos. O grande acúmulo de P nesses solos diminuí a eficiência do balanço de massas de P. As plantas cultivadas em solos com reservas de P construídas com aplicação de dejetos de suínos apresentaram menores valores de variáveis morfológicas de raízes. Por outro lado, no solo rizosférico apresentaram maior disponibilidade de P, atividade enzimática e fluxo de C, N e P na biomassa que estão altamente correlacionados com a maior absorção de P e produção de biomassa pelas plantas. As plantas cultivadas em solo com a aplicação de fertilizante mineral

Santa Maria, RS

2022

apresentaram maiores valores de variáveis morfológicas de raízes, porém não apresentou diferença na disponibilidade de P entre o solo rizosférico e bulk. As plantas cultivadas no solo controle apresentaram valores morfológicos semelhantes aos apresentados pelas plantas cultivadas no solo com aplicações de fertilizante mineral. No entanto a produção de biomassa dessas plantas foi bem inferior.

Palavras-chaves: Dejetos de animais; sistema de plantio direto; absorção de P; atividade enzimática; morfologia radicular; técnica de minirhizotron

Santa Maria, RS

2022

ABSTRACT

12-YEAR PHOSPHORUS BALANCE AND ANNUAL CROP STRATEGIES TO ACCESS PHOSPHORUS RESERVES BUILT UP OVER 15 YEARS BY ORGANIC AND MINERAL SOURCES

AUTHOR: Carina Marchezan
GUIDELINE: Gustavo Brunetto
COORIENTATOR: Paulo Ademar Avelar Ferreira

The agricultural production system of Brazil and other countries is highly dependent on imports of phosphate fertilizers. The quantification and long-term impact of secondary P sources, such as residues from livestock production and the access to P reserves in agricultural soils by plants is of common interest and is an alternative to decrease the use of mineral phosphate fertilizers. This Thesis aimed to evaluate the long-term impact of different fertilizer sources and changes in soil P reserves and to investigate the strategies used by annual plants to access and increase P availability in the rhizosphere. To this end, we conducted three studies (**Chapters I, II and III**) in a long-term experiment, implemented in 2004, under an arhenic dystrophic red Argissolo Vermelho, in Santa Maria (RS). The treatments used were liquid swine manure, liquid bovine manure, overlapping pig litter mineral fertilizer and a control treatment, without the application of nutrients. In **Chapter I**, we determined the efficiency of the P balance in the system, by accounting the inputs, outputs and stock of this element in the soil over 12 years of cultivation. The accumulated P forms in the soil profile were determined by means of the sequential chemical P fractionation technique. In **Chapter II**, we selected 3 treatments (liquid swine manure, mineral fertilizer and control) and investigated changes in the root system of corn roots and their relationship with P uptake and utilization efficiency. For this, we installed acrylic minirizotron tubes and monitored, *in situ*, the root growth dynamics. Root morphological parameters were determined and correlated with physiological P utilization and crop productivity variables. Finally, in **Chapter III**, we subjected the treatments discussed in **Chapter II** to AMF inoculation with the species *Rhizophagus intraradices* (Rootella BR). In the soil were the contents of P, N and C contained in the microbial biomass and soil, as well as the activity of the enzyme acid phosphatase and β-glucosidase. These variables were related to the availability of P in the rhizosphere and its uptake by corn and oat plants. Animal waste applications, especially those of pigs, generate large reserves of P in soils. The large accumulation of P in these soils decreases the efficiency of the P mass balance. Plants grown in soils with P reserves built up with swine manure application showed lower values of root morphological variables. On the other hand, in the rhizospheric soil they presented higher P availability, enzyme activity and flux of C, N and P in biomass that are highly correlated with higher P uptake and biomass production by plants. Plants grown in soil with the application of mineral fertilizer showed higher values of morphological variables of roots but showed no difference in P availability between rhizospheric and bulk soil. The plants grown in the control soil presented morphological values close to those presented by the plants grown in the soil with application of mineral fertilizer. However, the biomass production of these plants was much lower.

Keywords: Animal manure; no-till system; P uptake; enzyme activity; root morphology; minirhizotron technic

Santa Maria, RS

2022

SUMÁRIO

Santa Maria, RS

2022

Santa Maria, RS

2022

LISTA DE TABELAS

CHAPTER I - PHOSPHORUS BALANCE IN SANDY SOIL SUBJECTED TO 12 YEARS OF SUCCESSIVE APPLICATIONS OF ANIMAL MANURE AND MINERAL PHOSPHATE FERTILIZER IN SUBTROPICAL CLIMATE

CAPÍTULO II - CRESCIMENTO DE RAÍZES E EFICIÊNCIA NA ABSORÇÃO DE P EM MILHO CULTIVADO EM SOLO COM 15 ANOS SUBMETIDO À APLICAÇÕES DE FERTILIZANTES ORGÂNICO E MINERAL

Santa Maria, RS

2022

CAPÍTULO III – ATIVIDADES BIOLÓGICA E BIOQUÍMICA COMO ESTRATÉGIAS PARA AUMENTAR A DISPONIBILIDADE DE P EM SOLOS RIZOSFÉRICOS DE MILHO E AVEIA MICORRIZADAS, CULTIVADAS EM SOLO COM HISTÓRICO DE 15 ANOS DE APLICAÇÕES DE DEJETOS LÍQUIDOS DE SUÍNOS E FERTILIZANTE MINERAL.

Santa Maria, RS

2022

LISTA DE FIGURAS

INTRODUÇÃO

CHAPTER I - PHOSPHORUS BALANCE IN SANDY SOIL SUBJECTED TO 12 YEARS OF SUCCESSIVE APPLICATIONS OF ANIMAL MANURE AND MINERAL PHOSPHATE FERTILIZER IN SUBTROPICAL CLIMATE

CAPÍTULO II - CRESCIMENTO DE RAÍZES E EFICIÊNCIA NA ABSORÇÃO DE P EM MILHO CULTIVADO EM SOLO COM 15 ANOS SUBMETIDO À APLICAÇÕES DE FERTILIZANTES ORGÂNICO E MINERAL

CAPÍTULO III – ATIVIDADES BIOLÓGICA E BIOQUÍMICA COMO ESTRATÉGIAS PARA AUMENTAR A DISPONIBILIDADE DE P EM SOLOS RIZOSFÉRICOS DE MILHO E AVEIA MICORRIZADAS, CULTIVADAS EM SOLO COM HISTÓRICO DE 15 ANOS DE APLICAÇÕES DE DEJETOS LÍQUIDOS DE SUÍNOS E FERTILIZANTE MINERAL.

Santa Maria, RS

2022

1 INTRODUÇÃO

Uma das maiores conquistas da Ciência do Solo no Brasil e em outros países do Mundo foi a transformação de solos ácidos e com baixa fertilidade natural em terras com alta capacidade produtiva. A criação de procedimentos técnicos e o desenvolvimento de sistemas de recomendações de calagem e adubação, contribuiu para aplicações de corretivos da acidez e fertilizantes em quantidades e formas apropriadas para cada sistema de produção. A maioria dos solos brasileiros são ácidos, altamente inteperizados, de baixa fertilidade natural e necessita quantidades consideráveis de fertilizantes para atender a demanda das culturas. Diante dessa demanda, no ano de 2020, Brasil se tornou o maior importador de fertilizantes do Mundo – responsável por 12,5% (7,8 bilhões de dólares) das importações mundiais de fertilizantes (Fertilizers | OEC - The Observatory of Economic Complexity, 2022). Em 2022, o Brasil é responsável por 8% do consumo global de fertilizantes e é o quarto maior importador de fertilizantes do Mundo, atrás da China (24%), Índia (14,6%) e Estados Unidos (10,3%) (COLUSSI; SCHNITKEY; ZULAUF, 2022).

A maior parte (75%) do fertilizante fosfatado utilizado na agricultura nacional é importado (COLUSSI; SCHNITKEY; ZULAUF, 2022). As minas de rocha fosfática do Brasil fornecem apenas quantidades limitadas de P devido à baixa solubilidade de P da rocha ígnea e altos custos de processamento. Somado a isso, a alta adsorção de P nos solos brasileiros, requer a aplicação de grandes quantidades de fertilizante fosfatado, para suprir a demanda das plantas e a rápida imobilização de P inorgânico que ocorre em solos ricos em ferro (Fe) e alumínio (Al) (PAVINATO et al., 2017; WITHERS et al., 2018). Diante desse cenário, nós constatamos que produção nacional de alimentos é altamente dependente do fertilizante mineral fosfatado de outros países, gerando preocupações quanto escassez futura de P e flutuações no valor da rocha fosfática. Assim, Brasil, bem como outros países, precisam encontrar alternativas para melhorar a eficiência e sustentabilidade do uso de P, diminuindo a dependência pelos fertilizantes inorgânicos solúveis derivados da rocha fosfática.

Segundo Withers et al. (2018), uma estratégia chave de administração de P é reutilizar (recuperar, reciclar ou clicar) fontes secundárias de P. A agricultura produz vários recursos biológicos ou resíduos de processamento que podem ser potencialmente reciclados de volta ao solo como fontes secundárias de P. A utilização de resíduos orgânicos da produção pecuária, pode ser uma alternativa para reciclar e aumentar os teores de P em solos. A produção nacional de suínos e de bovino de leite encontra-se em expansão. Os Estados da Região Sul do Brasil (Rio Grande do Sul - RS, Santa Catarina - SC e Paraná - PR) são responsáveis por, aproximadamente, 71% da produção nacional de suínos e exportam mais de 92% da carne destes animais, sendo que 440 mil toneladas de carne (20.7% da produção nacional total) são produzidas no RS (ABPA, 2022). Somado a isso, o RS contribuiu com 13% da produção nacional de leite (3,2 bilhões de litros em 2021) (Embrapa, 2022). Estas atividades, quando conduzidas em sistema confinado – o que representa a quase totalidade para a produção de suínos – geram um grande volume de dejetos de animais ricos em nutrientes, incluindo o fósforo (P) que pode ser reaproveitado na adubação das culturas (BOITT et al., 2018, 2022; FERREIRA et al., 2022).

No geral, os solos agrícolas brasileiros receberam mais fertilizantes P do que o necessário para as necessidades das culturas, inclusive em solos com aplicação de dejetos de animais e, portanto, acumularam reservas significativas de P no solo (WITHERS et al., 2018) . Essas reservas de P em solos também são conhecidas como *“P legacy”* = “legado P” (HAYGARTH et al., 2014; SHARPLEY et al., 2013). O legado de P estocados em solos compõe a segunda maior fonte de P do Mundo, com potencial para sustentar os sistemas de produção agrícola por décadas (SATTARI et al., 2012; ZHU; LI; WHELAN, 2018a). No entanto, infelizmente a maior parte dessas reservas de P não está facilmente disponível para a absorção das plantas. Isso porque, a dinâmica do P no solo é muito complexa e envolve processos de imobilização-mineralização, adsorção-dessorção e precipitação-solubilização (PARFITT, 1989) (Figura 1).

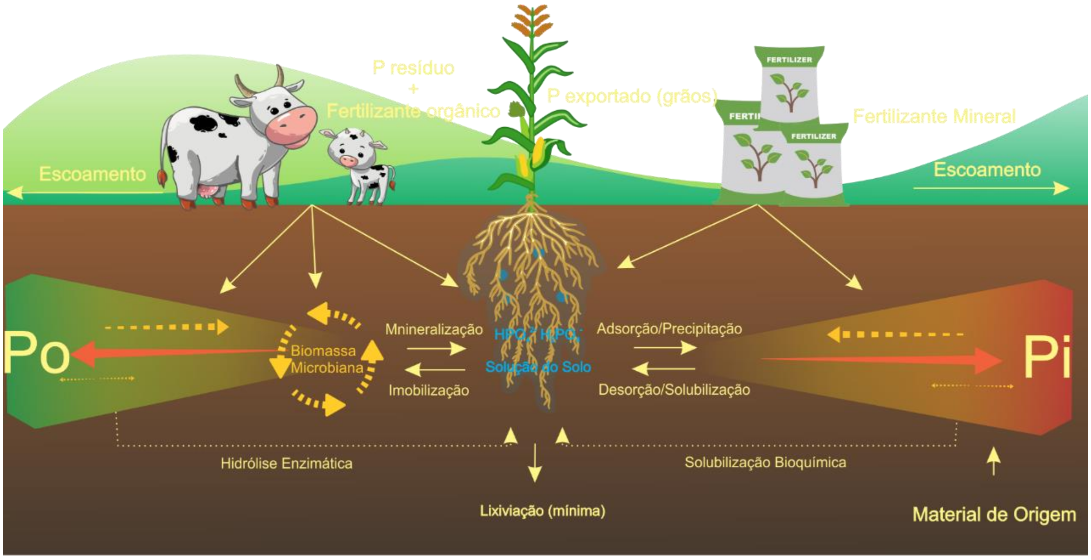


Figura 1. Ciclo do fósforo no sistema animal-solo-planta. O tamanho dos pools representados na figura não está em escala. Figura adaptada de Pierzynski et al. (2005). Fonte: Carina Marchezan

Assim, as formas de P presentes em solos possuem diferentes capacidades de dessorção e abastecimento da solução do solo, segundo sua natureza química e energia de ligação (BARROW, 2015). O acúmulo constante de P em solos pode aumentar os riscos de eutrofização da água devido as perdas desse elemento por escoamento superficial (MCDOWELL; SHARPLEY, 2001; RISSMAN; CARPENTER, 2015; SHARMA; BELL; WONG, 2015). Dentro dessa perspectiva, no **Capítulo I**, quantificamos a acumulação, distribuição e destino do P após a aplicação de diferentes tipos de esterco animal e fertilizante mineral fosfatado em um solo de plantio direto de longa duração. Somado isso avaliamos as formas de P predominantes e seu grau de labilidade em cada solo. Os resultados da quantificação do P, após 12 anos de aplicações de dejetos de animais e fertilizantes mineral, serviram como base para investigar possíveis estratégias das plantas para se adaptar em solos com diferentes acúmulos de P no solo (**Capítulos II e III**).

Nos **Capítulo II e III**, investigamos o crescimento radicular e alterações na rizosfera das plantas cultivadas em solos com 15 anos de aplicação de dejetos de suínos e fertilizantes minerais. As plantas alteram a arquitetura e o crescimento do sistema radicular solos com diferentes teores de P (HALLAMA et al., 2019; HANEKLAUS; SCHNUG, 2016). No entanto, a magnitude desse fenômeno pode diferir entre as espécies e genótipos de plantas, que pode apresentar variação na área, volume, comprimento e diâmetro de raízes (EREL et al., 2017; HODGE et al., 2009; NGUYEN; STANGOULIS, 2019). Por exemplo, as plantas dicotiledôneas podem utilizar a estratégia de modificar bioquimicamente a zona da rizosfera, para solubilizar o P, por exemplo, pela exsudação de compostos orgânicos e enzimas extracelulares; enquanto as gramíneas podem fazer uso do extenso sistema radicular para adquirir o P do solo (HINSINGER et al., 2018; LÓPEZ-ARREDONDO et al., 2014).

Além disso, às plantas podem exsudar ânions orgânicos de baixo peso molecular (exemplo, carboxilatos), com o objetivo de dissolver precipitados e cátions metálicos quelatados, que tornam o fosfato indisponível (RICHARDSON et al., 2009) (Figura 2). A exsudação de carboxilato proporciona a liberação do P sorvido por meio da troca de ligantes, ocupando os sítios de ligações de superfície em partículas reativas do solo. A exsudação de H^+ ou OH^-/HCO_3^- modifica o pH da solução do solo, determinando o comportamento das cargas variáveis dos minerais e da matéria orgânica do solo, podendo dissolver compostos que contenham P e/ou dessorver o P ligado a fase sólida do solo (ADELEKE; NWANGBURUKA; OBOIRIEN, 2017; HALLAMA et al., 2019;

HINSINGER, 2001). A liberação de prótons aumenta a disponibilidade de P em solos calcários, por causa da dissolução do fosfato-Ca (HINSINGER et al., 2003; RADERSMA; GRIERSON, 2004).

No entanto, é difícil prever o efeito da mudança do pH na disponibilidade de P, por exemplo, em solos com longo histórico de aplicações de fertilizantes orgânicos, onde parte do P também pode ser observado em formas inorgânicas (Pi), com diferentes energias de ligação. Mas também, parte do P pode estar em formas orgânicas (Po), normalmente em menor es quantidades no solo (NASH et al., 2014). Além disso, a liberação de ácidos orgânicos não diminui substancialmente o pH da rizosfera, pois são liberados principalmente na forma de ânions orgânicos normalmente acompanhados de cátions e prótons como íons balanceadores (HALLAMA et al., 2019).

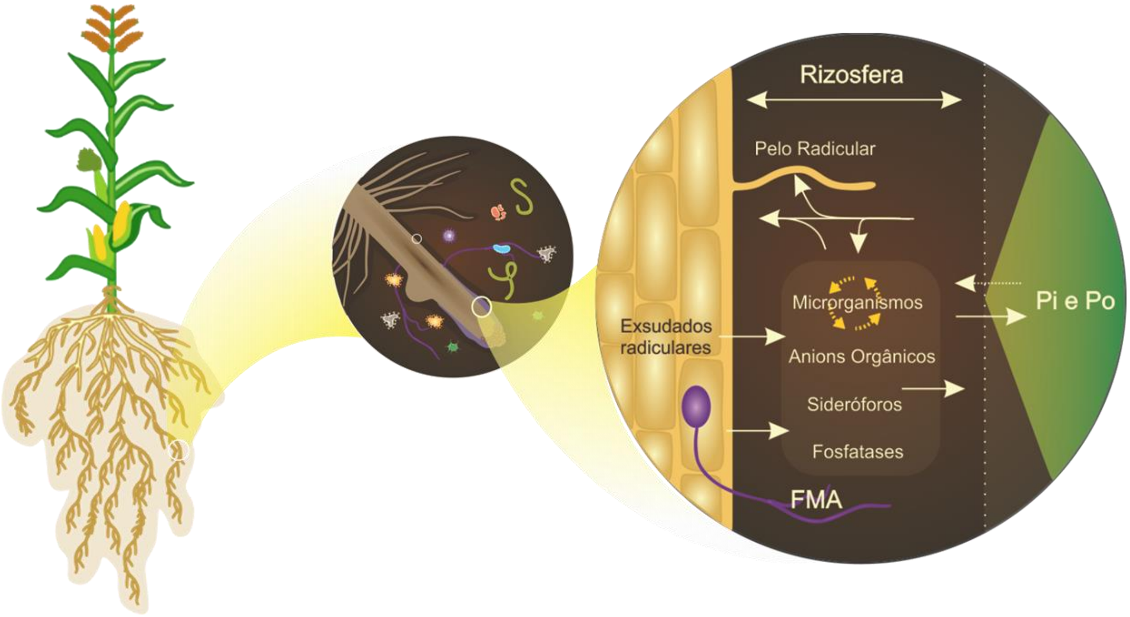


Figura 2. Representação fisiológica e bioquímica dos processos que interferem na disponibilidade e transformação do P na rizosfera e na micorrizosfera (adaptado de Richardson, 2009). Fonte Carina Marchezan

Os microrganismos também podem solubilizar e/ou mineralizar compostos contendo P, em especial, os Fungos Micorrízicos Arbusculares (FMAs), que podem ser inoculados em plantas, como aquelas anuais produtoras de grãos. Os microrganismos solubilizadores de P são um alvo para o desenvolvimento de inoculantes microbianos, que podem ser usados em larga escala na agricultura extensiva, possibilitando assim a mobilização do P presente no solo, reduzindo o uso de fertilizante fosfatado (LÓPEZ-CARMONA et al., 2019; WANG; FENG, 2021; ZHU; LI; WHELAN, 2018b). A associação de plantas e microrganismos podem modificar as propriedades bio(químicas) da rizosfera, contribuindo para a mobilização do P na solução do solo (WANG; FENG, 2021) (Figura 2). A maioria das plantas fazem associações com FMAs para aumentar a área de exploração do solo e absorção de P. Embora, essa associação seja variável com o tipo de FMAs, espécie vegetal e teor de P do solo, ela também pode potencializar as estratégias das plantas em adquirir P, podendo aumentar a acidificação nos solos neutros a alcalinos, exsudando maiores quantidades de ácidos orgânicos e incrementando a atividade da fosfatase (DELLA MÓNICA et al., 2020; WANG; FENG, 2021). Pelo exposto, é possível observar que plantas podem exsudar enzimas, como fosfatase ácida e ácidos orgânicos, o que poderá ser mais intenso em plantas inoculadas com FMAs, já que é esperado maior volume do sistema radicular.

2 HIPÓTESE

A revisão da literatura revelou que o sistema de produção agrícola brasileiro e de outros países são altamente dependentes das importações de fertilizantes fosfatados. A quantificação e o impacto a longo prazo de fontes secundárias de P, como resíduos da produção pecuária e das reservas de P nos solos agrícolas é de interesse comum e é uma alternativa para diminuir o uso dos fertilizantes fosfatados minerais. As reservas de P são construídas no solo ao longo dos anos por meio da adubação seja por fontes orgânicas ou mineral. As plantas e microrganismos do solo tem papel fundamental em acessar as reservas de P presentes no solo. No entanto as estratégias utilizadas não são suficientemente conhecidas em nível de campo em solos com diferentes tipos de manejo de adubação. As estratégias das plantas para acessar e aumentar a eficiência de absorção de P envolvem as alterações na arquitetura radicular, aproveitamento interno e na rizosfera. O ambiente rizosférico sofre forte influência das raízes, mas os microrganismos do solo também contribuem para aumentar a eficiência de absorção de P pelas plantas nessa região. A eficiência de absorção de P pode estar relacionada com a exsudação de enzimas, e ácidos orgânicos que contribuem para a maior disponibilidade de P no solo, seja por meio direto das raízes ou pelo aumento da atividade microbiana ocasionado pelo incremento do C orgânico na rizosfera. De acordo com a literatura acreditamos que a interação com fungos micorrizos pode contribuir para aumentar a absorção de P pelas plantas, mas ainda não é bem conhecido qual é sua contribuição em disponibilizar o P na rizosfera.

Assim, a presente Tese possui a hipótese de que:

“Reservas de P construídas com fontes orgânicas e mineral fazem com que plantas e microrganismos da rizosfera desenvolvam estratégias à aquisição de P.”

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o impacto, a longo prazo, de fontes de adubação e alterações nas reservas de P no solo e investigar as estratégias utilizadas por plantas anuais em acessar e aumentar a disponibilidade de P na rizosfera.

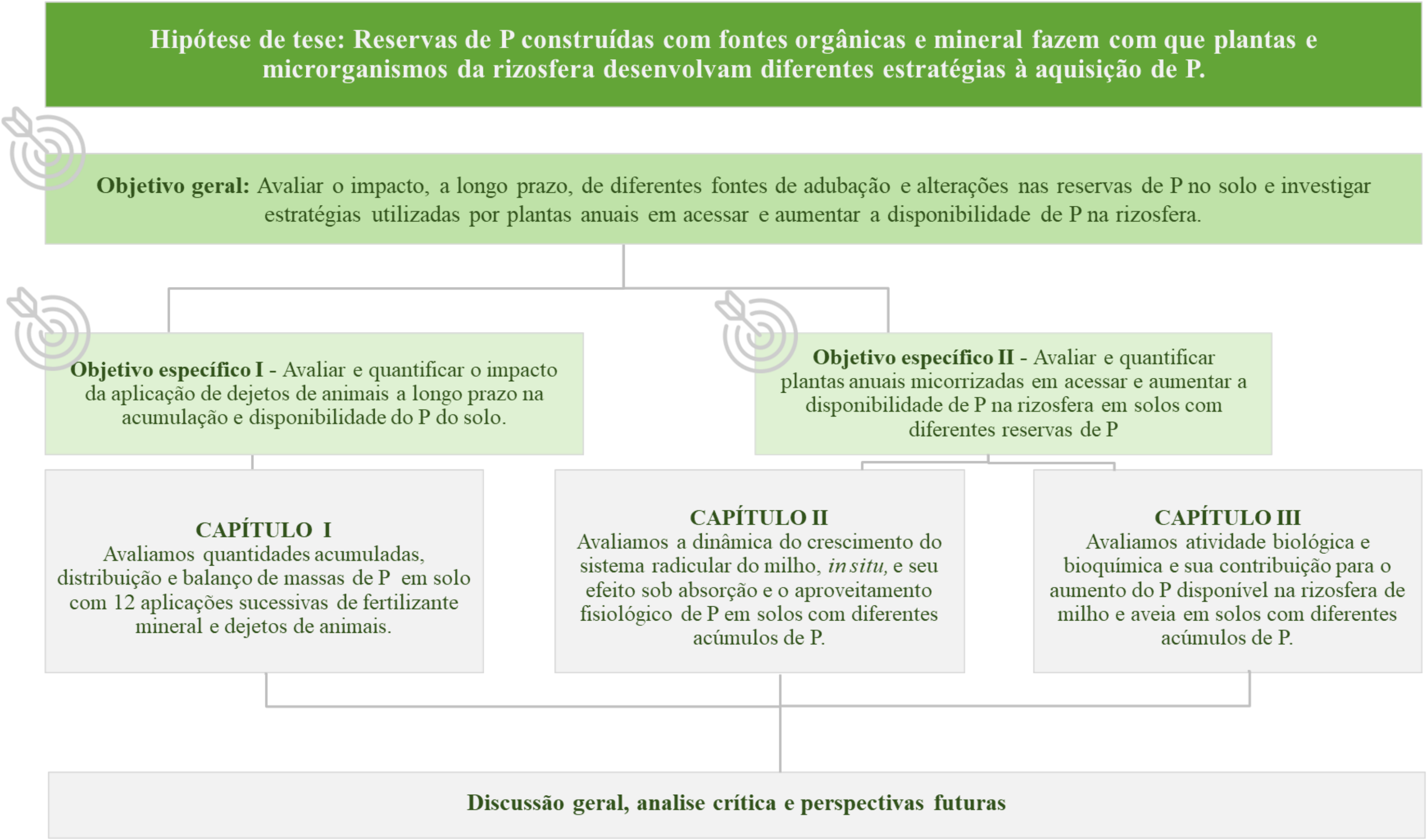
3.2 OBJETIVOS ESPECÍFICOS

a) Avaliar e quantificar o efeito da aplicação de dejetos de animais a longo prazo na acumulação e disponibilidade do P do solo.

Para isso elaboramos um estudo (Capítulo I) onde quantificamos balanço de massas de P e os efeitos de aplicações sucessivas de fertilizante mineral e dejetos de animais por 12 anos, nas quantidades acumuladas, distribuição de P no solo.

b) Avaliar e quantificar estratégias de plantas anuais micorrizadas em acessar e aumentar a disponibilidade de P na rizosfera em solos com diferentes reservas de P.

Para isso elaboramos dois estudos (Capítulo II e Capítulo III). No primeiro estudo nós avaliamos a dinâmica do crescimento do sistema radicular do milho *in situ* e seu efeito sob absorção e o aproveitamento fisiológico de P. No segundo estudo, avaliamos a atividade biológica e bioquímica e sua contribuição para o aumento do P disponível na rizosfera de milho e aveia cultivados em solo com histórico de 15 anos de aplicação de dejetos líquidos de suínos e fertilizante fosfatado.



Hipótese de tese: Reservas de P construídas com fontes orgânicas e mineral fazem com que plantas e microrganismos da rizosfera desenvolvam diferentes estratégias à aquisição de P.

Objetivo geral: Avaliar o impacto, a longo prazo, de diferentes fontes de adubação e alterações nas reservas de P no solo e investigar estratégias utilizadas por plantas anuais em acessar e aumentar a disponibilidade de P na rizosfera.

Objetivo específico I - Avaliar e quantificar o impacto da aplicação de dejetos de animais a longo prazo na acumulação e disponibilidade do P do solo.

Objetivo específico II - Avaliar e quantificar plantas anuais micorrizadas em acessar e aumentar a disponibilidade de P na rizosfera em solos com diferentes reservas de P

CAPÍTULO I
Avaliamos quantidades acumuladas, distribuição e balanço de massas de P em solo com 12 aplicações sucessivas de fertilizante mineral e dejetos de animais.

CAPÍTULO II
Avaliamos a dinâmica do crescimento do sistema radicular do milho, *in situ,* e seu efeito sob absorção e o aproveitamento fisiológico de P em solos com diferentes acúmulos de P.

CAPÍTULO III
Avaliamos atividade biológica e bioquímica e sua contribuição para o aumento do P disponível na rizosfera de milho e aveia em solos com diferentes acúmulos de P.

Discussão geral, analise crítica e perspectivas futuras

Figura 3. Organograma da Tese.

4 CHAPTER I - PHOSPHORUS BALANCE IN SANDY SOIL SUBJECTED TO 12 YEARS OF SUCCESSIVE APPLICATIONS OF ANIMAL MANURE AND MINERAL PHOSPHATE FERTILIZER IN SUBTROPICAL CLIMATE

4.1 ABSTRACT

Animal wastes and mineral phosphate fertilizers can be applied in agroecosystems to increase phosphorus (P) contents in soils. However, most of the P is rapidly adsorbed on functional groups of reactive soil particles, generating a P *legacy*. But, over the years, part of the functional groups of the reactive particles can be saturated, increasing the availability of P to plants and potentiating its transfer by runoff, which may even contaminate surface waters. Therefore, it is necessary to determine the P balance in soils, in order to rationalize the use of P sources, to adequately nourish plants, and to reduce the potential for contamination. The study aimed to evaluate the effect of the application of different animal wastes on the distribution of P in the profile of an Argissolo submitted for 12 years to successive applications of animal wastes and mineral phosphate fertilizer, in crop rotation, under no-till farming system. The study was conducted from 2004 to 2016 in the South region of Brazil. The treatments were the applications of liquid pig slurry (PS), liquid cow slurry (CS), pig deep-litter (PD), mineral fertilizer (MF) and a control treatment without application. The highest accumulation of P and its movement was observed in the 0-40 cm layer, in the soil submitted to applications of all P sources. The inputs of P via MF CS, PS and PD promoted the accumulation of 18, 42, 48 and 100 kg P ha^{-1} year^{-1}. The P mass balance showed that between 77 and 98% of the P added by animal manure and MF were accounted for in grain exports (17-34%), soil storage (41-72%), post-harvest residues (<1%), with the remaining 2-33%, unaccounted for, which was attributed, especially, to P transfer at the soil surface.

Key-words: P mass balance, P accumulation, animal manure, no-till farming system, *P Legacy*.

4.2 INTRODUCTION

Phosphorus (P) is one of the most important nutrients for maintaining or increasing the productivity of plants in agroecosystems. The demand for this nutrient for food and bioenergy production has intensified over the years as the world's population increases, raising concerns about its supply since P sources are limited and non-renewable (SCHOLZ AND WELLMER 2013; SCHOLZ et al. 2013).

Global swings in the price of phosphate fertilizers happen to accompany the depletion and scarcity of phosphate rock (CORDELL et al. 2009; GEISSLER et al. 2019). Therefore, it is critical to find viable alternatives that make it possible to meet food demand without relying solely on mineral phosphate fertilizers. An alternative could be the use of liquid animal waste, such as from pigs and cattle, or solid, such as pig deep-litter, as P sources in cropping systems (CORDELL et al. 2011; SCHNEIDER et al. 2019).

The states in the southern region of Brazil (Rio Grande do Sul, Santa Catarina, and Paraná) are responsible for approximately 70% of Brazil's swine production and export more than 84% of the meat from these animals, with 695,000 tons of meat (18.5% of total national production) produced in Rio Grande do Sul (ABPA 2019). In addition, Rio Grande do Sul contributed 13% of the national milk production (812 thousand liters in the first quarter of 2019) (IBGE 2019). These activities, when conducted in confined system, which represents almost the totality for swine production, generate a large volume of waste rich in nutrients, including P. This large volume of waste is used in agricultural soils as an alternative of disposal, but also as a way to reuse nutrients. However, successive applications of manure as fertilizer, in amounts commonly greater than those exported by crops, cause accumulation of P in soils over time (HAYGARTH et al. 2014).

However, long-term studies quantifying the role of legacy P in sustaining agricultural systems for food production are still scarce, especially in soils with higher adsorption capacity, such as those in tropical and subtropical regions. A field trial was established in Rio Grande do Sul, Brazil, in 2004 to evaluate the agronomic responses of grain with different types of animal wastes, along with changes in soil properties and associated potential environmental impacts. The objective of this study was to quantify the accumulation, distribution and indicators of potentially adverse environmental impacts following the application of different types of animal manure and mineral phosphate fertilizer in a long-term no-till soil. Due to the surface application and high adsorption capacity of the soil, most of the P added via animal manure and mineral fertilizer was retained in the topsoil layers.

4.3 MATERIAL AND METHODS

4.3.1 Location of experiment and treatments

The study was performed in a long-term experiment, with more than 16 years of conduction, located in the experimental area of the Federal University of Santa Maria (UFSM), in Santa Maria, Rio Grande do Sul (RS), southern region of Brazil (29°43'12"S and 53°43'4"W). The climate of the region is humid subtropical (Cfa 2), according to Koppen's classification, with annual averages of temperature, precipitation, and relative humidity of 19.3°C, 1,561 mm, and 82%, respectively. The soil is classified as Arenic Dystrophic Red Argissolo, according to (EMBRAPA, 2018), corresponding, to Typic Hapludalf (SOIL SURVEY STAFF, 2014), with 108 g kg^{-1} of clay; 183 g kg^{-1} of silt and 709 g kg^{-1} of sand. The chemical properties of the soil before the installation of the experiment (2004) and at the time of the study (2016) are presented in Table 1.

The experiment was initiated in 2004, under no-till farming system. The experimental design was randomized blocks, with four repetitions. The plots had dimensions of 5 x 5 m. The treatments were pig slurry (PS), cattle slurry (CS), pig deep-litter (PD), mineral fertilizer (urea + triple superphosphate - SFT + potassium chloride - KCl) (MF) and a control treatment, without the application of nutrients.

The applications of PS, CS, PD and mineral fertilizer were made on the surface, over the cultural residues of the previous crop and without incorporation. The treatments were applied only once a year and before summer crop sowing, in the period from 2004 to 2009. The treatments were not applied in winter. Starting in 2010, the treatments were applied twice each year, prior to winter and summer crop establishment. The crop succession used until August 2016 was black oats (*Avena strigosa S.*), corn (*Zea mays),* forage turnips (*Raphanus sativus),* black beans (*Phaseolus vulgaris),* and wheat (*Triticum* spp.). Further details about the experiment can be obtained in Couto et al. (2018), Lourenzi et al. (2014), Marchezan et al. (2020).

4.3.2 Collecting soil samples

In June 2016, soil samples were collected in the layers 0-4, 4-10, 10-20, 20-30, 30-40 and 40-50 cm. For this, in each plot, two trenches with dimensions of 40 x 40 x

40 cm were opened. In each trench, two soil samples were collected, one in the longitudinal direction and the other transversal to the sowing line. To correct the soil attributes and concentrations in units of mass per area in hectares, in each sampled layer the bulk density of the soil was determined by means of metal volumetric rings (78 cm^3), as described by (Embrapa, 1997).

4.3.3 P fractionation in soil

The soil was air dried, ground and passed through a 2 mm mesh sieve. It was then prepared and subjected to sequential P chemical fractionation with ammonium chloride (NH_4 Cl 1 mol l^{-1}), sodium bicarbonate ($NaHCO_3$ 0.5 mol l^{-1} , pH 8.5), sodium hydroxide (NaOH 0.1 mol l^{-1}), hydrochloric acid (HCl 1 mol l^{-1}), and a second extraction with NaOH (0.1 mol l^{-1}) (HEDLEY et al. 1982; CONDRON et al. 1996; CONDRON AND NEWMAN 2011). Total soil P was determined in a soil subsample after digestion with concentrated sulfuric acid (H_2 SO_4) and hydrogen peroxide (H_2 O_2 at 30% v/v), according to (OLSEN AND SOMMERS, 1982). The inorganic P in the alkaline extracts of $NaHCO_3$ and NaOH was assigned to the reactive P-molybdate analyzed by the method of (DICK AND TABATABAI, 1977). In the alkaline extracts, total P was determined by digestion with ammonium persulfate + sulfuric acid in an autoclave (USEPA 1971). Unreacted P-molybdate was considered as organic P and was obtained by the difference between total P and inorganic P. The P in acid extracts was determined according to the method of Murphy and Riley (1962).

Total organic P extracted was determined as the sum of the organic P extracted with $NaHCO_3$ and the first and second NaOH extractions. Total extracted inorganic P was calculated as the sum of inorganic P in all extracted fractions. The difference between total soil P and total extracted P (inorganic + organic) was considered as residual P. Soil P fractions were grouped into four P pools according to their relative lability: labile P (sum of NH_4 Cl and $NaHCO_3$ fractions [inorganic and organic]), moderately labile P (first NaOH [inorganic and organic]); stable P (HCl + second NaOH [inorganic and organic]); and residual P (non-extractable P) (CROSS; SCHLESINGER 1995). The amounts of total P, total inorganic P, total organic P up to 50 cm were expressed in units of kg ha $^{-1}$.

The potential risk of P transfer was evaluated by P concentration values (mg P kg^{-1}) determined in the first extraction of sequential P fractionation (NH_4 Cl-P). For this, we considered the most superficial layers (0-4 and 4-10 cm), following the recommendations of McDowell and Sharpley (2001). Easily extractable P in soil, can be used as an indicator of environmental risk, because this P fraction has a high potential for transfering from the soil by erosion processes and surface runoff (SHARPLEY et al. 2001; GATIBONI et al. 2015A, 2020). When the reactive functional groups of soil surfaces can no longer adsorb P, the equilibrium point is established and, as a consequence, large amounts of weakly bound P will be present in the soil solution (in this study represented by NH_4 Cl-P), thus increasing potential P transfers (MCDOWELL AND SHARPLEY 2001; BOITT et al. 2018).

4.3.4 P added by manure and mineral fertilizer

Liquid waste derived from pig and cattle farming was collected in open effluent tanks. The overlapping pig litter was collected in covered environments on the pig farms. The general physicochemical properties of the manure were analyzed on a representative sample before each application on all crops performed. The applied dose of each organic waste was defined based on its N concentration and the N requirement needed by the crops, according to the regional recommendation of the Commission for Soil Chemistry and Fertility (CQFS RS/SC, 2016). Therefore, the P inputs varied among the treatments. The total P inputs in the manure were calculated based on the P concentrations (kg m^{-3} for liquid manure and kg kg^{-1} for solid manure), present in the manure, multiplied by the amount of manure applied (m^3 manure ha^{-1} for CS and PS and kg manure ha^{-1} for PD) and, therefore, were expressed in kg P ha^{-1} . For MF the P inputs were made according to the P contents present in the soil and the nutritional requirement of the crop. The P inputs (in kg P ha^{-1}) in the manure and MF, by crop season/year are presented in Fig. 1.

4.3.5 P in residue and exported by crops

Plants were collected at flowering (winter and summer crops). For this, the shoot of the plants was cut close to the soil surface. Plants were dried in a forced air oven at ±65°C to constant mass for dry matter determination. The tissue was ground, prepared and submitted to nitric-perchloric digestion (ratio 4:1) (CLAESSEN et al., 1997). P was determined in the extract according to Murphy and Riley (1962). This procedure was repeated for the determination of P present in grains and cereals. For the calculation of the P balance in the system, the $P_{residue,}$ consists of the P present in the cultural residues after harvest. For this, we used the data of P contained in the dry matter of the oat crop established at the time of soil collection.

4.3.5 P Balance

The P balance in the soil-plant system (kg ha^{-1}) was estimated considering the 50 cm layer, the amount of the nutrient added in fertilization (kg ha^{-1}), the amount of P exported by grain crops (kg ha^{-1}), and the change in the amount of P in the soil. To determine the P budget of the system, we used a similar approach described by Boitt et al. (2018).

The P mass balance was calculated empirically as follows (units in kg P ha^{-1}) (Equation 1):

$$P_{added} - \Delta P_{soil} - P_{exported} - P_{residue} - P_{unaccountedfor} = 0 \qquad \textit{(Equação 1)}$$

Where: P_{added} is the cumulative sum of P added by the treatments; ΔP_{soil} is the change in P storage in the soil profile ($P_{initial}$ - P_{final}) calculated by summing the amounts of P determined in each soil layer (0 to 50 cm layer); *Pexported* is P exported by grains and cereals; $P_{residue}$ is the P content of crop residues and $P_{unaccounted\ for}$ *is* the difference between P_{added} and the sum of Pcounted for pools, which was attributed to some accumulated uncertainties in P accounting in the mass balance. For the control treatment, P_{added} was zero and $P_{unaccountedfor}$ was considered insignificant, given the absence of P addition and minimal soil losses through erosion and runoff in this experiment (Lourenzi et al., 2014a). Therefore, the soil from the control treatment was used to calculate $P_{initial}$ and was considered equal throughout the experiment area. This made it possible to determine the ΔP_{soil} of all treatments.

Total P inputs accounted for (*Paccountedfor*; Equation 2) in the treatments fertilized with manure and mineral fertilizer are given by the difference in the sum of all P pools (given by $\sum P_{Pool}$; Equation 3), minus the sum of the P pools of the control treatment (no P inputs) as follows:

$$P_{contabilizado}(kgha^{-1}) = \sum P_{pools} trat.adubados - \sum P_{pools} controle \qquad \textit{(Equation 2)}$$

Being,

$$\sum P_{pools} = P_{solo} + P_{exportado} + P_{residuo} \qquad \textit{(Equation 3)}$$

Thus, the P accounted for in the system (for each treatment that received fertilization) was calculated according to Equation 4:

$$P_{contabilizado}(\%) = \frac{P_{contabilizado}[kgha^{-1}]}{P_{adicionado}[kgha^{-1}]} \times 100 \qquad \textit{(Equation 4)}$$

The cumulative agronomic efficiency of the P balance of the system was calculated according to Equation 5:

$$Pbalanceefficiency(\%) = \frac{P_{exportado}}{(P_{exportado}+\Delta P_{solo})} \times 100 \quad (\textit{Equation 5})$$

Where: the ΔP_{soil} , in units of kg P ha^{-1}, is the change in soil P storage in the 0 to 50 cm soil layer was calculated using Equation 6:

$$\Delta P_{soil}(kgha^{-1}) = P_{final} - P_{initial} \qquad (\textit{Equation 6})$$

4.3.6 Statistical analysis

Differences in mean soil P fractions between treatments and depths, as well as crop yields, were determined using ANOVA and when significant were compared by Scott Knot's 5% test. In addition, linear correlations (Pearson correlation coefficients) between the data were determined using R software (R DEVELOPMENT CORE TEAM 2015).

4.4. RESULTS

4.4.1 P in soil

The additions of CS, PS, PD and MF over 12 years increased the amounts of P in all fractions (Fig.2 and Table S1). The highest values of labile P were observed in the superficial layers in soils submitted to animal manure applications (CS, PS, PD). But, among the manure sources, the highest values of labile P up to 40 cm layer were observed in the soil with PD applications (Fig. 2a). The moderately labile P fractions increased up to 40 cm, especially in soils with animal manure applications, and the highest amounts were observed in soils with PD application (Fig. 2b). The highest values of stable P were observed up to 30 cm, in soils submitted to residue and mineral fertilizer applications (Fig. 2c). The highest values of residual P (not extractable by chemical P fractionation, Fig. 2d) were observed up to 30 cm in the control and MF soils.

The highest values of inorganic P were observed in the soil fertilized with PD, followed by PS, CS and MF, with increases of 73, 54, 50 and 40%, relative to the control soil (0-50 cm layer) (Fig. 3a). The additions of PD, CS, PS and MF increased surface inorganic P contents by 79, 69, 65 and 52%, respectively, relative to the control (0-10 cm layer), with observed amounts greater than 580 kg P ha^{-1} in the soil with PD addition. The contribution of inorganic P to total soil P varied from 26% in the control soil to 54, 41, 39 and 37% in the soils with PD, PS, CS and MF applications, respectively (layer 0-50 cm, Fig. 3a, 3c and Table S2). On the other hand, the highest amounts of organic P were observed in soil with PD, followed by CS, PS and MF, with increments of 36, 30, 29 and 6%, relative to the control (layer 0-50 cm Fig. 3b). The additions of PD, PS, CS and MF increased the amounts of organic P on the surface with values 50, 47, 43 and 42% higher than the control (layer 0-10 cm). The proportion of soil organic P varied from 37% in the soil with PD and MF additions, to 42% in the control soil and with PS and CS applications (layer 0-50 cm, Fig. 3b, 3c and Table S2).

The increase of total P and its distribution in the soil profile were proportional with the amounts of P added by the treatments. The highest amounts of total P were observed in the soil with PD applications, followed by PS, CS and FM, with increases of 79, 41, 37.16%, respectively, relative to the control soil (layer 0-50cm, Fig. 3c and Table 2). The highest values of total P in depth were seen in the soil with PD applications, with increment until the 40 cm layer. On average, applications of PD, PS, CS and MF increased the amounts of total P by 91, 48, 42 and 18 kg P ha^{-1} year^{-1} in the soil up to the 50 cm layer, relative to the control.

4.4.2 P mass balance

Cumulative P inputs were 746, 938, 1431, 538, kg P ha^{-1} in soils with CS, PS, PD and MF applications, respectively (Table 2). On average, applications of CS, PS, PD and MF corresponded to inputs of 62, 78, 119 and 45 kg P ha^{-1} year^{-1}, respectively. The variation of P inputs among the crops (Fig. 1) was correlated with the dry matter concentrations in the manure (Pearson's correlation coefficient = 0.41, P value < 0.001) and also by the N content present in the manure, since the amounts of animal manure applied were established considering the N concentration.

Average grain and cereal yields (corn, beans and wheat) over 12 years increased significantly in soils with PD, PS, CS and FM applications, relative to the control (Fig. 4a). On average, corn yield (10 crops) grown on soils with FM, PS, CS and PD applications were 1.8, 2.2, 2.4, 2.0 and 2.5 times higher respectively, relative to yield on the control soil. The yield of beans grown on soils with MF, PS, CS and PD applications increased by 1.5; 2.7, 3.0 and 4.1 times respectively, relative to the control. The highest wheat yield was observed in soils grown with MF applications followed by PS, PD and CS with increments of 2.6, 2.3, 2.3 and 2.2 times, respectively, relative to the control. More information on the grain yield responses of the crops in this study can be seen in (Bacca et al., 2020) and Couto et al. (2018). The highest cumulative P exports (sum of corn, bean, and wheat exports) over 12 years were observed in crops grown on soils subjected to manure and mineral phosphate fertilizer applications (Fig. 4b). Exports of P by crops grown on fertilized soils averaged 150, 143, 119 and 85 kg P ha^{-1}, higher than that observed in the control soil (Fig. 4b and Table 2).

The applications of MF, CS, PS, PD promoted the accumulation of 18, 42, 48 and 100 kg P ha^{-1} year^{-1} (Table 2). The mass balance calculations showed that between 41 and 72% of the total P added by manure and MF was accumulated in the 50 cm layer. Furthermore, most of the P added in the cropping system can be explained by soil storage, exports by grains and crop residues (98, 89, 89 and 77%, for treatments CS, PS, PD and MF, respectively). The P balance efficiency was highest in the soil with FM, at 40%, decreasing to 30, 29 and 19% in soils with CS, PS and PD applications, respectively.

4.5 DISCUSSION

4.5.1 Soil P Balance

Additions of CS, PS and PD and MF in no-till system for several years, increased the amounts of P in the soil, with its vertical movement, proportional to the amount of P applied by each source (BOITT et al. 2018; FILHO et al. 2020; OYELEKE AZEEZ et al. 2020). The difference in the increment in the amounts of total P compared to the control were observed in all treatments up to 40 cm depth, with a higher proportion in the upper soil layer (0-20 cm). This happened because, in the no-till farming system, the soil is not disturbed, and the fertilizer sources are applied on the surface. In addition, the rapid adsorption of the phosphate ion by the functional groups of reactive soil particles decreases the availability of P in solution, preventing it from moving to depth (PARFITT 1989; BARROW 2015).

The fraction with which P was accumulated in the soil differed among treatments and in depth, with preferential accumulation of inorganic P in PD. In the soil layers with PS, CS and MF applications, the accumulated amounts of organic and inorganic P were similar. The accumulation of P in the inorganic fraction in PD may have happened, especially, because of the higher concentration of P per kilogram of PD. This is because, PD waste has animal feces and urine, and food leftovers, which are deposited for several months in pig breeding stalls (OLIVEIRA; SOARES, 2001).

Therefore, PD has a high concentration of nutrients, among them, P. Moreover, the concentration of inorganic P in swine manure is higher in relation to the manure from other livestock farms, due to the absence of the enzyme phytase in the digestive tract, which prevents the degradation and utilization of compounds that contain P, excreting in the feces, the vast majority of the P consumed by grains (BAXTER et al., 2003; WIENHOLD; MILLER, 2004).

The higher amounts of organic P observed in the topsoil layers with CS, PS, PD and MF applications can be explained by the higher biomass production of the soil cover plant species and grain crops (FILHO et al. 2020). Plants during their growth and development absorb P only in the orthophosphate (Pi) form and at the end of the crop cycle the P, after being incorporated into the tissues, returns to the soil in the form of organic P (PAGLIARI; LABOSKI 2012; HALLAMA et al. 2019; FILHO et al. 2020). The replacement of P to the soil happens because of deposition and decomposition of the aerial part of the crops, but also, by root senescence.

The successive inputs of P by CS, PS and PD significantly increased the labile P fractions, especially the concentrations of weakly bound P (P-NH_4 Cl) in the topsoil (0-4 and 4-10 cm), with the highest values observed in the soil with PD, because of the greater amount of P added (Fig. 1 and Fig. 5). The increase in P-NH_4 Cl concentrations in the topsoil layers may potentiate possible transfers of soluble P or P adsorbed to functional groups of solid particles in the surface runoff solution (PIERZYNSKI et al. 2005; MCDOWELL et al. 2014). Because of the high affinity of the phosphate ion to reactive soil particles and the small movement of P in the profile observed in this study, we believe that it is unlikely that P was transferred by leaching (MCDOWELL; SHARPLEY 2001; CERETTA et al. 2010; TIECHER et al. 2020).

In a study conducted in southern Brazil, Ceretta et al. (2010) and collaborators observed losses ranging from 6.4 and 14.4 kg P ha^{-1} by surface runoff, in an experiment conducted on sandy soil (53% sand and 4% slope), subjected to applications of doses from 20 to 80 m^3 ha^{-1} of liquid pig manure for 8 years. In the same experiment, Tiecher et al. (2020), quantified during 6 crops (2.5 years), the forms of P transferred by leaching. The authors concluded that less than 0.2 kg P ha^{-1} year^{-1} applied at a dose of 80 m^3 ha^{-1} of liquid pig manure was leached. When compared to P losses by surface runoff, leaching losses are economically and agronomically negligible. However, due to the numerous precipitations and high volume of rainfall in subtropical climate, which may increase the water flux in the profile, the leached P concentrations may exceed the limits recommended by the environmental legislation, especially in soils with high P contents. Thus, although the amounts of P transferred by leaching are small, these losses can have a significant environmental impact in groundwater contamination, especially, when the water table is shallow (GIROTTO et al. 2013; TOOR; SIMS 2016; KHAN et al. 2018; TIECHER et al. 2020).

Soil P accumulation in the inorganic fractions and P movement in the soil profile are similar to other studies that evaluated the effects of long-term P fertilization by phosphate fertilizers and different animal manure sources (GUARDINI et al. 2012; VANDEN NEST et al. 2014; REQUEJO;EICHLER-LÖBERMANN 2014; TIAN et al. 2017; BOITT et al. 2018). Studies conducted on soils and climatic conditions similar to those of the present study (<20% clay), with a history of swine manure application, also observed a movement of labile P greater than 30 cm (LOURENZI et al. 2014, 2015; DE CONTI et al. 2015). While in more clayey soils (>40% clay), P movement observed in experiments with long application history was less than 20 cm (CASSOL et al. 2012; BOITT et al. 2018). The lower migration of P to deeper layers in clayey soils compared to sandy soils is directly related to the higher capacity of P adsorption in soils, especially, in free functional groups on the surfaces of clays and Fe and Al oxides and hydroxides (RHEINHEIMER et al. 2003; GATIBONI et al. 2015B).

Most of the P added by CS, PS, PD and MF was accounted for by mass balance calculations. However, it was not possible to calculate 0.8, 8.0, 12 and 10 kg P ha^{-1} year^{-1} in soils that received applications of CS, PS, PD and MF, respectively (these values correspond respectively to 1, 13, 10 and 22% of the P added annually). The highest amount of unaccounted P in the PD soil (144 kg P ha^{-1}) may be related to the highest concentration of weakly bound P among the treatments evaluated, which indicate potential P losses (Table 1 and Fig. 5).

Accumulated P in the soil represented the main P compartment in this agricultural system, even in a sandy soil (10.8% clay), which has low P adsorption capacity (DE CONTI et al. 2015; TIECHER et al. 2017). In the soils submitted the additions of CS, PS and PD may have happened because of the constant additions of P many times higher than the demands of the crops. Added to this, the maintenance of cultural residues on the surface and the cultivation of cover crops over the years may have collaborated to the cycling and maintenance of P in this compartment. As discussed above, the transfer of P by leaching may be insignificant in this soil and, therefore, from 2.5 to 23% of the P inputs, which could not be accounted for by the mass balance, may represent P lost by surface runoff.

4.5.2 Environmental and agronomic implications

The applications, especially of liquid (CS and PS) and solid (PD) animal manure, significantly increased the P content of the soil, causing concern about the continued use of these nutrient sources. Over 12 years, the amounts of animal manure were defined considering the efficiency index of each manure, the concentration of N and the need of each crop, as established by the regional recommendation on the use of animal manure (CQFS-RS/SC, 2016). Probably, these variables are efficient in defining the amount of manure in the first years of applications. However, when the manure is applied repeatedly over several years, it can greatly increase the P contents in the soil, exceeding the extraction capacity of the plants, causing significant accumulation of this nutrient in the soil (BOITT et al., 2018c; FILHO et al., 2020; GUARDINI et al., 2012b). A study by Gatiboni et al. (2020) established the environmental safety limits for P in soil in the southern region of Brazil. The method is based on the concentrations of bioavailable P in soil (Mehlich-1 extractor) in the 0-10 cm layer, soil P adsorption capacity and clay content, according to the equation: *P threshold (mg P kg*$^{-1}$ *) = 20 + clay content (%)*. When employing this prediction for the soil of the present study (clay content of 10.8%), the P threshold was exceeded by 79, 135, 148 and 202%, for MF, PS, CS and PD applications, respectively. These values correspond to the significant increment in the concentrations of weakly bound P (NH_4 Cl-P Fig.5), in the topsoil layer (0-4), representing potential risk of P losses by surface runoff (MCDOWELL; SHARPLEY 2001; GATIBONI et al. 2020). If applications continue and no strategy to mitigate excess soil P is implemented, the management used may potentiate several environmental risks, among them, declining surface water quality (SHARPLEY et al. 2001; CHEN AND GRAEDEL 2016; PENN; BOWEN 2017).

The grain yield data obtained for corn, beans, and wheat over the years showed the increase in productivity achieved through applications of CS, PS, and PD. The improvement in soil nutrient availability demonstrates the potential of using animal manure as a source of fertilizer (GROHSKOPF et al. 2016; MARCHEZAN et al. 2020; ADEMAR et al. 2022). Although constant monitoring of critical soil P levels is necessary. The P mass balance results presented in this study showed the imbalance in P additions among treatments, causing the significant accumulation of P in the soil compartment and the low efficiency of P applied to plants (SHARPLEY et al. 2013; WIRONEN et al. 2018; KHAN et al. 2018). In addition, the differences in the amounts of P added by the manure over the seasons may have been a consequence of the composition of the manure, especially the effect of dry matter concentration per kilogram of manure added. These factors contributed to the excessive addition of P in the PD treatment, thus decreasing the overall P mass balance efficiency of the culture system to unsatisfactory levels. These results raise concerns not only about the amount of animal waste that can be applied per area, but also about the chemical composition of the added manure in agricultural areas.

Continued additions of P and the accumulation of P legacy in soils after long periods of animal manure applications in the same areas can present a serious environmental risk, as was widely demonstrated in this long-term study. Similarly, applications of mineral phosphate fertilizers (MF) also implied a worrisome accumulation of P in the soil, especially in the topsoil. However, the amounts of plant-available P (Mehlich-1 extractor) is within the adequate range (30-50 mg P kg^{-1}), requiring annual maintenance fertilization. We observed that the addition of MF increased the fractions of P not available to plants, with 51% of the total P in the stable and residual form in the surface layer (Fig. 2d). This may have happened because of the high affinity of the phosphate ion that is rapidly adsorbed to the surface functional groups of reactive soil particles (Parfitt 1989). This effect was more pronounced in soil with MF applications, probably because of the lower pH value, caused by acidification, caused by the release of H ions^{+} coming from the solubilization of nitrogen fertilizers (PERYEA; BURROWS 1999; SCHRODER et al. 2011). The decrease in pH, causes the protonation of functional groups of reactive soil particles, thus increasing the binding energy of P with the soil solid phase, increasing the more stable P fractions (BARROW 2017). Low efficiency and accumulation of P in the surface layers in soils with mineral phosphate fertilizer additions is very common in most highly weathered soils.

Considering obtained results, research is needed to find strategies to mobilize recalcitrant P fractions present in the soil and decrease the dependence on constant phosphate fertilizations (HALLAMA et al., 2019; HAYGARTH et al., 2013; SATTARI et al., 2012b; SHARPLEY et al., 2013b).

4.6. CONCLUSIONS

The additions of PD, PS, CS, and MF to the soil over 12 years caused the accumulation of 100, 48, 42, and 18 kg P ha^{-1} $year^{-1}$, respectively.

All P sources caused significant P accumulation in the surface layers, especially in the inorganic P fraction. But, in the soil with PD the P moved up to 40 cm and with applications of CS, PS and MF, the movement was observed up to 30 cm.

Soils with animal manure applications, especially PD, have a worrisome potential for P transfer. Thus, threshold levels of P should be constantly monitored, and the fertilizer recommendation system should consider the history of nutrient source applications in the cropping system to establish the need and dose for a new application of a P-containing source.

The P mass balance revealed that 77 and 98% of P input by animal manure and MF were accounted for in grain exports (17-34%), soil storage (41-72%), post-harvest residues (<1%), with the remaining 2-33%, unaccounted for because of transfers by surface runoff. This reinforces the need for conservation farming practices to avoid runoff and soil losses.

4.7. REFERENCES

ABPA. **Produção brasileira de proteína animal**. Brasilia: [*s. n.*], 2019.

ADEMAR, Paulo *et al.* Long-Term Effects of Animal Manures on Nutrient Recovery and Soil Quality in Acid Typic Hapludalf under No-Till Conditions. **Agronomy 2022, Vol. 12, Page 243**, [*s. l.*], v. 12, n. 2, p. 243, 2022. Disponível em: https://www.mdpi.com/2073-4395/12/2/243/htm. Acesso em: 23 jan. 2022.

BACCA, Alessandra *et al.* Residual and immediate effect after 16 applications of organic sources on yield and nitrogen use efficiency in black oat and corn. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], v. 44, p. 1–15, 2020. Disponível em: https://doi.org/10.36783/18069657rbcs20190013. Acesso em: 18 ago. 2020.

BARROW, N. J. A mechanistic model for describing the sorption and desorption of phosphate by soil. **European Journal of Soil Science**, [*s. l.*], v. 66, n. 1, p. 9–18, 2015. Disponível em: http://doi.wiley.com/10.1111/ejss.12198_2. Acesso em: 16 ago. 2019.

BARROW, N. J. The effects of pH on phosphate uptake from the soil. **Plant and Soil**, [*s. l.*], v. 410, n. 1–2, 2017.

BAXTER, Christopher A. *et al.* Phytase, High-Available-Phosphorus Corn, and Storage Effects on Phosphorus Levels in Pig Excreta. **Journal of Environment Quality**, [*s. l.*], v. 32, n. 4, p. 1481, 2003.

BOITT, Gustavo *et al.* Fate of phosphorus applied to soil in pig slurry under cropping in southern Brazil. **Geoderma**, [*s. l.*], v. 321, p. 164–172, 2018.

CASSOL, Paulo Cezar *et al.* Disponibilidade de macronutrientes e rendimento de milho em latossolo fertilizado com dejeto suíno. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], v. 36, n. 6, p. 1911–1923, 2012.

CERETTA, Carlos Alberto *et al.* Nutrient transfer by runoff under no tillage in a soil treated with successive applications of pig slurry. **Agriculture, Ecosystems & Environment**, [*s. l.*], v. 139, n. 4, p. 689–699, 2010.

CHEN, Minpeng; GRAEDEL, T. E. A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. **Global Environmental Change**, [*s. l.*], v. 36, p. 139–152, 2016.

CLAESSEN, Maria Elisabeth Christine *et al.* Manual de Métodos de Análise de Solo, 2a Edição. **Embrapa - Centro Nacional de Pesquisa de Solos**, [*s. l.*], 1997.

COMISSAO DE QUÍMICA E FERTILIDADE DO SOLO RIO GRANDE DO SUL E SANTA CATARINA CQFS RS/SC. **Manual de Adubação e de Calagem para os estados do Rio Grande so Sul e Santa Catarina**. 11^{a} editioed. [*S. l.: s. n.*], 2016.

CONDRON, L. M. *et al.* Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. **Biology and Fertility of Soils**, [*s. l.*], v. 21, n. 1–2, p. 37–42, 1996.

CONDRON, Leo M.; NEWMAN, Susan. Revisiting the fundamentals of phosphorus fractionation of sediments and soils. **Journal of Soils and Sediments**, [*s. l.*], v. 11, n. 5, p. 830–840, 2011.

CORDELL, D. *et al.* Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. **Chemosphere**, [*s. l.*], v. 84, n. 6, p. 747–758, 2011.

CORDELL, Dana; DRANGERT, Jan-Olof; WHITE, Stuart. The story of phosphorus: Global food security and food for thought. **Global Environmental Change**, [*s. l.*], v.

19, n. 2, p. 292–305, 2009. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S095937800800099X. Acesso em: 6 maio 2019.

COUTO, R. R. *et al.* Health risk assessment and soil and plant heavy metal and bromine contents in field plots after ten years of organic and mineral fertilization. **Ecotoxicology and Environmental Safety**, [*s. l.*], v. 153, 2018.

CROSS, Anne Fernald; SCHLESINGER, William H. A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. **Geoderma**, [*s. l.*], v. 64, n. 3–4, p. 197–214, 1995.

DE CONTI, Lessandro *et al.* Effects of Pig Slurry Application and Crops on Phosphorus Content in Soil and the Chemical Species in Solution. **Revista Brasileira de Ciência do Solo**, [*s. l.*], v. 39, n. 3, p. 774–787, 2015. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-06832015000300774&lng=en&tlng=en. Acesso em: 2 out. 2018.

DICK, W. A.; TABATABAI, M. A. Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds1. **Journal of Environment Quality**, [*s. l.*], v. 6, n. 1, p. 82, 1977. Disponível em: https://www.agronomy.org/publications/jeq/abstracts/6/1/JEQ0060010082. Acesso em: 6 maio 2019.

EMBRAPA. **Manual de Métodos de Análise de Solo**. [*S. l.: s. n.*], 1997-. ISSN 1517-2627. v. 2

EMBRAPA. **Sistema brasileiro de classificação de solos**. 5ªed. Rio de Janeiro, RJ - Brasil: [*s. n.*], 2018.

FILHO, José de Souza Oliveira *et al.* Phosphorus accumulation in soil after successive applications of swine manure: a long-term study in Brazil. **Environmental Earth Sciences**, [*s. l.*], v. 79, n. 2, p. 1–12, 2020. Disponível em: https://doi.org/10.1007/s12665-019-8805-z.

GATIBONI, Luciano Colpo *et al.* Establishing environmental soil phosphorus thresholds to decrease the risk of losses to water in soils from rio grande do Sul, brazil. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], v. 44, p. 1–14, 2020.

GATIBONI, Luciano Colpo *et al.* **Soil Phosphorus Thresholds in Evaluating Risk of Environmental Transfer To Surface Waters in Santa Catarina, Brazil**. [*S. l.*]: scielo, 2015a.

GATIBONI, Luciano Colpo *et al.* Soil phosphorus thresholds in evaluating risk of environmental transfer to surface waters in Santa Catarina, Brazil. **Revista Brasileira de Ciência do Solo**, [*s. l.*], v. 39, n. 4, p. 1225–1234, 2015b. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-06832015000401225&lng=en&tlng=en. Acesso em: 27 mar. 2019.

GEISSLER, Bernhard; MEW, Michael C.; STEINER, Gerald. Phosphate supply security for importing countries: Developments and the current situation. **Science of the Total Environment**, [*s. l.*], 2019. Disponível em: Acesso em: 30 jul. 2019.

GIROTTO, Eduardo *et al.* Nutrient transfers by leaching in a no-tillage system through soil treated with repeated pig slurry applications. **Nutrient Cycling in Agroecosystems**, [*s. l.*], v. 95, n. 1, p. 115–131, 2013.

GROHSKOPF, Marco André *et al.* Soil solution nutrient availability, nutritional status and yield of corn grown in a typic hapludox under twelve years of pig slurry fertilizations. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], v. 40, 2016.

GUARDINI, Renato *et al.* Accumulation of phosphorus fractions in typic Hapludalf soil after long-term application of pig slurry and deep pig litter in a no-tillage system. **Nutrient Cycling in Agroecosystems**, [*s. l.*], v. 93, n. 2, p. 215–225, 2012.

HALLAMA, Moritz *et al.* Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. **Plant and Soil**, [*s. l.*], v. 434, n. 1–2, p. 7–45, 2019. Disponível em: http://link.springer.com/10.1007/s11104-018-3810-7. Acesso em: 22 jul. 2019.

HAYGARTH, Philip M. *et al.* **Sustainable phosphorus management and the need for a long-term perspective: The legacy hypothesis**. [*S. l.: s. n.*], 2014.

HEDLEY, M J; STEWART, J W B; CHAUHAN, B S. Changes in inorganic and organic soil phosphorus fractions induced by cultivation pratices and by laboratory incubations. **Soil Science Society of American Journal**, [*s. l.*], v. 46, p. 970–976, 1982.

IBGE. **Pesquisa Trimestral do Leite - Primeiros resultados Dados da quantidade de leite cru processado pela indústria.** [*S. l.: s. n.*], 2019.

KHAN, Asif *et al.* Phosphorus efficiency, soil phosphorus dynamics and critical phosphorus level under long-term fertilization for single and double cropping systems. **Agriculture, Ecosystems & Environment**, [*s. l.*], v. 256, p. 1–11, 2018.

LOURENZI, Cledimar Rogério *et al.* Forms of phosphorus transfer in runoff under no-tillage in a soil treated with successive swine effluents applications. **Environmental Monitoring and Assessment**, [*s. l.*], v. 187, n. 4, p. 209, 2015.

LOURENZI, Cledimar Rogério *et al.* Teor disponível, escoamento superficial e percolação de formas de fósforo em argissolo com an adição de fontes orgânicas e mineral de nutrientes. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], v. 38, n. 2, p. 544–556, 2014.

MARCHEZAN, Carina *et al.* Nitrogen Availability and Physiological Response of Corn After 12 Years with Organic and Mineral Fertilization. **Journal of Soil Science and Plant Nutrition**, [*s. l.*], 2020. Disponível em: Acesso em: 9 mar. 2020.

MCDOWELL, Rich; MCDOWELL, Richard W; SHARPLEY, Andrew N. Soil phosphorus fractions in solution: Influence of fertiliser and manure , filtration and method of determination Soil phosphorus fractions in solution : in ¯ uence of fertiliser and manure , ® ltration and method of determination. [*s. l.*], v. 45, n. October 2014.

MCDOWELL, R.W.; SHARPLEY, A.N. Approximating Phosphorus Release from Soils to Surface Runoff and Subsurface Drainage. **Journal of Environment Quality**, [*s. l.*], v. 30, n. 2, p. 508, 2001. Disponível em: https://www.agronomy.org/publications/jeq/abstracts/30/2/508. Acesso em: 22 jul. 2019.

MURPHY, J.; RILEY, J.P. A modified single solution method for the determination of phosphate in natural waters. **Analytica Chimica Acta**, [*s. l.*], v. 27, p. 31–36, 1962.

OLIVEIRA, Pav; SOARES, Wr. Produção e manejo de dejetos de suínos. **A Produção Animal na Visão dos Brasileiros**, [*s. l.*], v. d, p. 72–90, 2001.

OLSEN, S.R; SOMMERS, L.E. Phosphorus. *Em*: A.L. PAGE, R.H. MILLER, D.R. Keeney (org.). **Methods of Soil Analysis, Part 2. Chemical and Microbiological**

Properties. 2ªed. Madison, WI: [*s. n.*], 1982. p. 403–430. *E-book*. Disponível em: https://dl.sciencesocieties.org/publications/books/abstracts/agronomymonogra/methodsofsoilanb/1059. Acesso em: 31 mar. 2019.

OYELEKE AZEEZ, Musibau *et al.* Phosphorus in an arable coarse sandy soil profile after 74 years with different lime and P fertilizer applications. [*s. l.*], 2020.

PAGLIARI, Paulo H.; LABOSKI, Carrie A. M. Investigation of the Inorganic and Organic Phosphorus Forms in Animal Manure. **Journal of Environmental Quality**, [*s. l.*], v. 41, n. 3, p. 901–910, 2012.

PARFITT, R.L. Phosphate reactions with natural allophane, ferrihydrite and goethite. **Journal of Soil Science**, [*s. l.*], v. 40, p. 359–369, 1989.

PENN, Chad; BOWEN, James M. Design and construction of phosphorus removal structures for improving water quality. **Design and Construction of Phosphorus Removal Structures for Improving Water Quality**, [*s. l.*], p. 1–228, 2017.

PERYEA, F. J.; BURROWS, R. L. Soil acidification caused by four commercial nitrogen fertilizer solutions and subsequent soil pH rebound. **Communications in Soil Science and Plant Analysis**, [*s. l.*], v. 30, n. 3–4, p. 525–533, 1999.

PIERZYNSKI, G M; MCDOWELL, R W; SIMS, J T. **Chemistry, cycling and potential movement of inorganic phosphorus in soils**. Madison: Soil Science Society of America, 2005.

R DEVELOPMENT CORE TEAM. R Internals. **R Development Core Team**, [*s. l.*], v. 1, p. 63, 2015.

REQUEJO, María Isabel; EICHLER-LÖBERMANN, Bettina. Organic and inorganic phosphorus forms in soil as affected by long-term application of organic amendments. **Nutrient Cycling in Agroecosystems**, [*s. l.*], v. 100, n. 2, p. 245–255, 2014.

RHEINHEIMER, Danilo dos Santos *et al.* Dessorção de fósforo avaliada por extrações sucessivas em amostras de solo provenientes dos sistemas plantio direto e convencional. **Ciência Rural**, [*s. l.*], v. 33, n. 6, p. 1053–1059, 2003.

SCHNEIDER, Kimberley D. *et al.* Options for Improved Phosphorus Cycling and Use in Agriculture at the Field and Regional Scales. **Journal of Environmental Quality**, [*s. l.*], v. 48, n. 5, p. 1247–1264, 2019. Disponível em:

https://onlinelibrary.wiley.com/doi/abs/10.2134/jeq2019.02.0070. Acesso em: 16 jul. 2020.

SCHOLZ, Roland W. *et al.* Sustainable use of phosphorus: A finite resource. **Science of the Total Environment**, [*s. l.*], v. 461–462, p. 799–803, 2013.

SCHOLZ, Roland W.; WELLMER, Friedrich Wilhelm. Approaching a dynamic view on the availability of mineral resources: What we may learn from the case of phosphorus? **Global Environmental Change**, [*s. l.*], v. 23, n. 1, p. 11–27, 2013.

SCHRODER, Jackie L. *et al.* Soil Acidification from Long-Term Use of Nitrogen Fertilizers on Winter Wheat. **Soil Science Society of America Journal**, [*s. l.*], v. 75, n. 3, p. 957–964, 2011.

SHARPLEY, Andrew *et al.* Phosphorus Legacy: Overcoming the Effects of Past Management Practices to Mitigate Future Water Quality Impairment. **Journal of Environment Quality**, [*s. l.*], v. 42, n. 5, p. 1308, 2013.

SHARPLEY, Andrew N.; MCDOWELL, Richard W.; KLEINMAN, Peter J.A. Phosphorus loss from land to water: Integrating agricultural and environmental management. *Em*: 2001. **Plant and Soil**. [*S. l.: s. n.*], 2001. p. 287–307.

SOIL SURVEY STAFF. Keys to soil taxonomy. **Soil Conservation Service**, [*s. l.*], v. 12, p. 410, 2014.

TIAN, J. *et al.* Accumulation and distribution of phosphorus in the soil profile under fertilized grazed pasture. **Agriculture, Ecosystems and Environment**, [*s. l.*], v. 239, p. 228–235, 2017.

TIECHER, Tales *et al.* Chemical, biological, and biochemical parameters of the soil P cycle after Long-Term pig slurry application in No-Tillage system. **Revista Brasileira de Ciencia do Solo**, [*s. l.*], 2017.

TIECHER, Tadeu L. *et al.* Phosphorus forms leached in a sandy Typic Hapludalf soil under no-tillage with successive pig slurry applications. **Agricultural Water Management**, [*s. l.*], v. 242, n. July, p. 106406, 2020.

TOOR, Gurpal S.; SIMS, J. Thomas. Phosphorus Leaching in Soils Amended with Animal Manures Generated from Modified Diets. **Journal of Environmental Quality**, [*s. l.*], v. 45, n. 4, p. 1385–1391, 2016.

USEPA, United States Environmental Protection Agency. **Methods of chemical analysis for water and wastes.** Cincinnati: [*s. n.*], 1971.

VANDEN NEST, T. *et al.* Effect of organic and mineral fertilizers on soil P and C levels, crop yield and P leaching in a long-term trial on a silt loam soil. **Agriculture, Ecosystems and Environment**, [*s. l.*], v. 197, p. 309–317, 2014.

WIENHOLD, Brian J.; MILLER, Phillip S. Phosphorus Fractionation in Manure from Swine Fed Traditional and Low-Phytate Corn Diets. **Journal of Environmental Quality**, [*s. l.*], v. 33, n. 1, p. 389–393, 2004.

WIRONEN, Michael B.; BENNETT, Elena M.; ERICKSON, Jon D. Phosphorus flows and legacy accumulation in an animal-dominated agricultural region from 1925 to 2012. **Global Environmental Change**, [*s. l.*], v. 50, n. February, p. 88–99, 2018.

4.8 TABLES AND FIGURES

Table 1. Chemical properties of soil collected at 0-10 cm before experiment implant (2004) and after 18 applications of organic and mineral N sources (August 2016).

Chemical Properties	Initial Soil (2004)*	Treatments (2016)				
		Control	CS	PS	PD	FM
pH_{H2O} (1:1)	4.9	5.2	5.3	4.9	5.7	4.5
SOM (g kg^{-1}) (1)	19	14.0	21.9	16.9	20.9	15.9
Available P (mg kg^{-1}) (2)	20.3	9.7	76.5	72.9	93.2	55.1
Exchangeable K (mg kg^{-1}) (2)	60	29.0	117.0	71.8	116.8	75.0
Exchangeable Ca ($cmol_c$ kg^{-1}) (3)	0.8	2.2	3.3	2.1	4.4	1.4
Exchangeable Mg ($cmol_c$ kg^{-1}) (3)	0.3	1.8	2.6	1.7	2.8	1.0
Exchangeable Al ($cmol_c$ kg^{-1}) (3)	0.03	0.5	0.2	0.6	0.1	1.3
H+Al ($cmol_c$ kg^{-1})	3.7	3.7	3.7	3.3	1.9	4.1
$CTC_{effective}$ ($cmol_c$ kg^{-1})	1.3	4.6	6.4	4.5	7.6	3.9
$CTC_{pH\ 7.0}$ ($cmol_c$ kg^{-1})	5	7.8	9.9	7.2	9.5	6.7
Base Saturation (%)	25.4	53.7	63.5	56.3	80.0	38.8
Aluminum Saturation (%)	2	11.0	2.9	12.9	1.3	33.2

* Data from (Lourenzi et al, 2014) (1) Determined by TOC + Van Bemmelen factor; (2) Extracted by Mehlich-1; (3) Extracted by KCl 1 mol·L^{-1}; Control = no fertilization; CS= Cattle Slurry; PS = Pig Slurry; PL = Pig Deep-Litter; FM = mineral fertilizer.

Table 2. P mass balance in the no-tillage cropping system receiving long-term P inputs in cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF). The P pools presented are the cumulative amounts (in units of kg P ha ^{1}) after 18 successive application of the treatments from 2004-2016. Data presented are means (n = 4).

P budget in the system		Treatments				
		Control	CS	PS	PD	FM
		kg P ha^{-1}				
Inputs	P added via treatments[a]	0	747.5	938.2	1431.1	538.71
Pools	Soil P pools [b]	1383.9e*	1892.9c	1971.1b	2417.1a	1605.5d
	Crop P residue [c]	4.8d	10.3a	10.3a	9.5b	8.7c
Outputs	Grain P export [d]	98.88d	218.08b	242.28a	243.87a	184.22c
Total P accounted [e]		1487.6e	2121.3c	2223.7b	2670.5a	1798.4d
P unaccounted [f]		0	10.1b	98.4a	144.4a	124.2a
Accounted P in the system (%) [g]		0	98.6a	89.5b	89.9b	76.9c
Added P accumulated in soil (%) [h]		0	68.1b	62.6c	72.2a	41.1d
P balance efficiency (%) [i]		0	30.0b	29.2b	19.1c	45.5a

*Letters identify significant differences in the means between treatments for a given P pool (Scott Knott test at 5%).

[a] Total P added via pig slurry after 12 years (2004-2016).

[b] Total soil P in the layer 0-50 cm depth.

[c] Amounts of P stored in crop residues

[d] Total amount of P exported in grain (corn and beans [in 2016]) in 12 years as a function of P concentration in grain (% P) and total quantities of produced grain (kg ha ^{1}).

[e] Sum of P accounted for in pools (soil and residues) and P outputs (P exported in grain)

[f] Punaccounted (kg P ha^{-1}) = Pinput – (ΔPsoil+Poutput+Presidue)

[g] Accounted P (%) = [1-(Punaccounted:Pinput)]×100

[h] Accumulated P in soil (%) = (ΔPsoil:Pinput)×100

[i] P balance efficiency (%) = [Poutput:(Poutput+ΔPsoil)] ×100

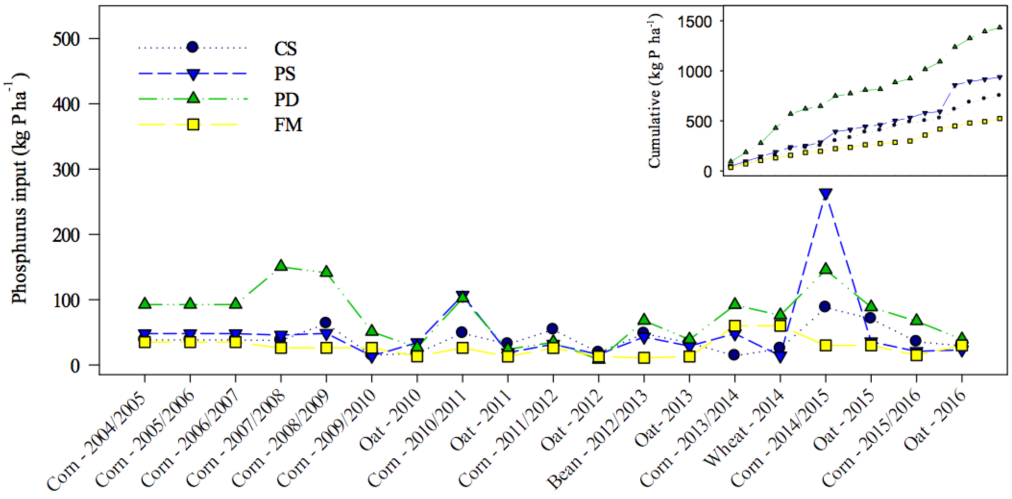


Figure 1. Total P applied (expressed in kg P ha-1) to field plots receiving cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF) from 2004 to 2012 under no-tillage system.

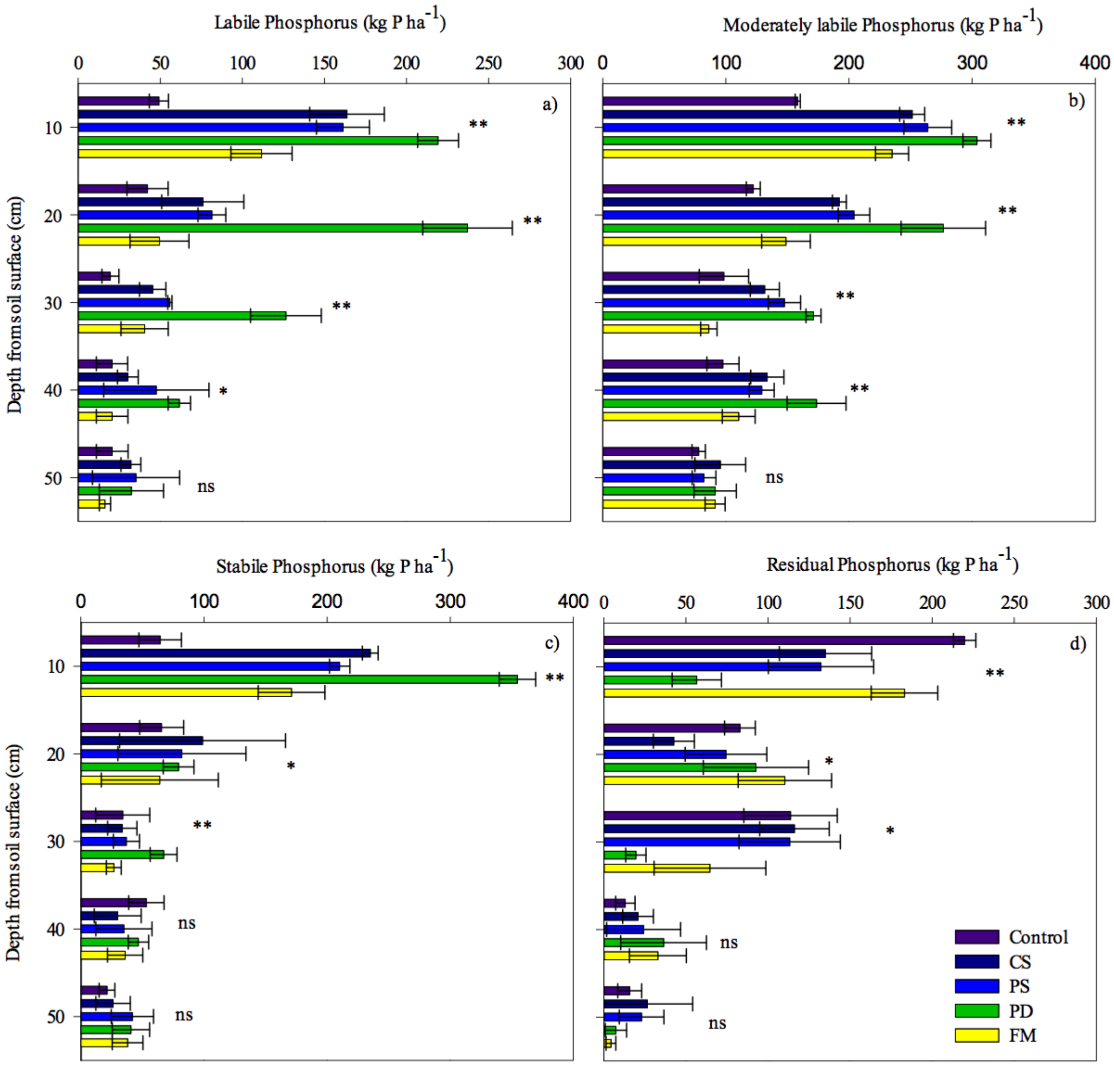


Figure 2. Average P quantities (kg P ha^{-1}) determined in P fractions for soils sampled from different depths after 12 years under applications cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF): labile P (PiAM + Pibic + Pobic), moderately labile P (PiOH_I + PoOH_I), stable P (PiHCl + PiOH_II + PoOH_II), and residual P (Pres). *P<0.05; **P<0.01 and ns: not significant (Scott Knott test at 5%). Standard deviation is presented on the surface of the bars. Data presented are means (n = 4).

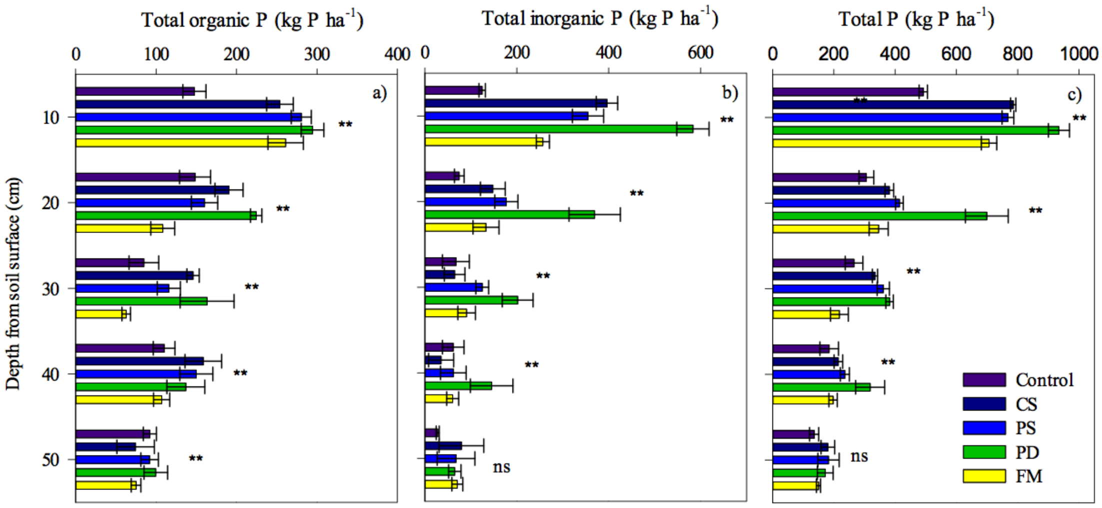


Figure 3. Average quantities (kg P ha^{-1}) of total extracted inorganic P (Pi), total extracted organic P (Po), and total P determined for soils sampled from different depths after 12 years under applications cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF) (total extracted inorganic P = sum of PiAM, Pibic, PiOH_I, PiHCl, PiOH_II, total extracted organic P = sum of Pobic + PoOH_I + PoOH_II). *P<0.05; **P<0.01 and ns: not significant (Scott Knott test at 5%). Standard deviation is presented on the surface of the bars. Data presented are means (n = 4).

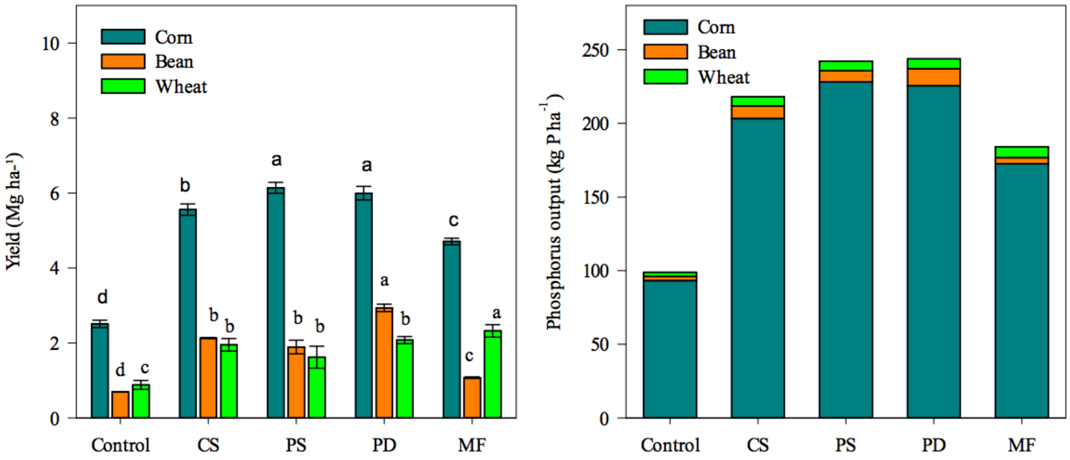


Figure 4. Response of the production of grains of corn, beans and wheat (a) and dry mass maize, beans, wheat, oats and turnips (b), depending on the successive applications cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF). The results represent an average of 12 years (2004-20016) of crop rotation management with 18 treatment applications. Corn was cultivated in 10 seasons, while beans and wheat were grown in 2012/2013 and 2014 cropping seasons, respectively. For cover crops, oats was grown for 8 cropping seasons and O. Radish in three cropping seasons (2005, 2007 and 2009). Different letters between treatments at the same depth indicate significant difference (Scott Knott test at 5%). Standard deviation was presented the surface of the bars.

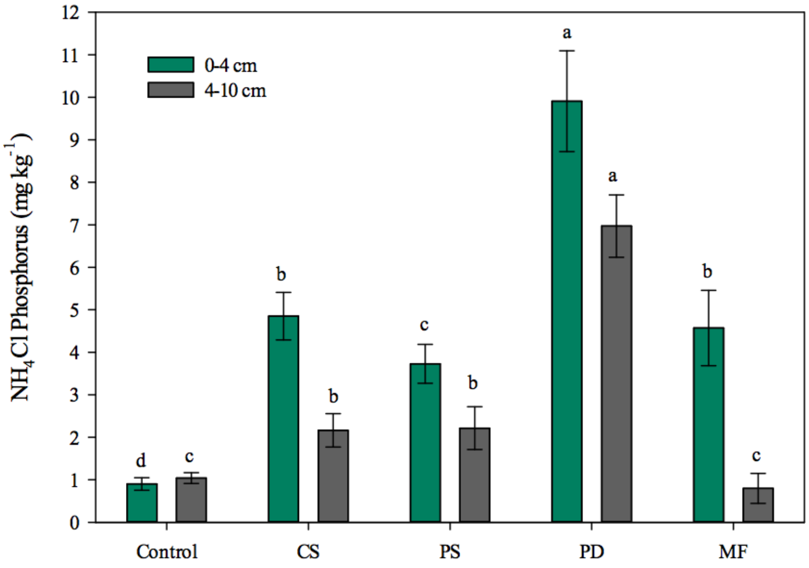


Figure 5. Representation weakly bound P extracted with 1 mol L^{-1}NH$_4$Cl after long-term animal slurry and mineral fertlizer additions, in the topmost soil layers (0–10cm). Substantial increases in this labile fraction impose potential environmental risks mainly following soil erosion by runoff. Vertical bars represent standard deviation of averages. The arrow represents a ‘change- point’ (at slurry input rate of 50 m^{-3} ha^{-1} y^{-1}), where large amounts of P are at increased risk of movement by surface runoff (McDowell et al., 2001).

4.9 TABELAS E FIGURAS SUPLEMENTARES

Table S1. Distribution of P pools in the soil profile after 18 successive application (2004-2016) cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF) additions. Data presented are means (n = 4).

Treatments	Depht (cm)					
	0-10	10-20	20-30	30-40	40-50	0-50
Labile P (kg ha^{-1})						
Control	49.13D*a**	42.21Ca	19.63Bb	16.51Bb	20.67Ab	148.15
CS	163.74Ba	73.33Bb	58.15Bc	33.49Bc	37.30Ac	366.01
PS	161.24Aa	81.43Bb	55.81Bc	60.11Ac	35.22Ac	394.15
PD	219.18Aa	245.71Aa	109.42Ab	61.56Ac	32.40Ac	668.35
MF	111.65Ba	49.51cb	40.45Bc	20.65Bc	16.27Ac	238.52
Moderately P (kg ha^{-1})						
Control	158.40Ca	119.74Db	107.04Cc	102.43Cc	79.98Ac	567.58
CS	251.38Ba	175.200Bb	131.60Bc	131.60Bc	82.55Ad	772.38
PS	264.02Ba	204.11Bb	168.86Bc	127.99Bc	89.93Ad	854.91
PD	303.96Aa	276.63Ab	171.23Ac	161.75Ac	94.22Ad	1007.79
MF	234.98Ca	148.85Cb	86.17Cc	106.70Cc	91.28ac	667.99
Stable P (kg ha^{-1})						
Control	64.45Da	53.34Ba	38.30Ba	53.20Ab	21.26Ab	230.54
CS	235.07Ba	74.51Ab	42.19Bc	35.25Ac	34.50Ac	421.53
PS	210.21Ba	51.74Bb	41.19Bb	22.26Ab	42.10Ab	367.50
PD	354.53Aa	80.22Ab	67.22Ab	46.73Ac	36.47Ac	585.17
MF	171.09Ca	42.58Bb	26.79Bb	40.18Ab	29.59Ab	310.23
Residual P (kg ha^{-1})						
Control	219.85Ca	95.07Ab	100.84Ab	12.43Ac	13.80Ac	442.00
CS	135.08Ba	57.96Bb	102.29Aa	18.56Ab	36.02Ab	349.91
PS	132.32Ba	76.32Ab	95.21Ab	25.58Ac	15.11Ac	344.54
PD	56.65Aa	83.65Ab	38.43Bb	26.58Ab	11.68Ab	216.98
MF	188.65Ca	105.28Ab	64.63Cc	32.90Ad	12.71Ad	404.17

(*) Means within columns at the same depth followed the same capital letter, and (**) means within rows at different depths followed the same lower-case letter, are not significantly different according to the Scott-Knott test ($p<0.05$)

Table S2. Quantities of inorganic, organic and total P (kg P ha-1) accumulated in the soil profile after 18 successive application (2004-2016) cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF) additions. Data presented are means (n = 4).

Tratamentos	Depht (cm)					
	0-10	10-20	20-30	30-40	40-50	0-50
Inorganic P (kg ha^{-1})						
Control	124.43D9*a**	78.02Cb	75.89Bb	62.23Bb	29.65Ab	370.23
CS	396.24Ba	145.16Bb	86.01Bc	50.37Bc	64.82Ac	742.61
PS	355.00Ba	177.07Bb	133.10Bc	64.66Bd	75.15Ad	805.32
PD	583.27Aa	378.15Ab	201.60Ac	124.79Ad	67.72Ae	1355.52
MF	256.62Ca	132.71Bb	90.61Bc	64.90Bc	62.11Ac	606.94
Organic P (kg ha^{-1})						
Control	147.55Ca	137.26Ca	89.07Cb	109.91Bb	92.25Ab	576.04
CS	253.94Ba	177.88Bb	145.93Ac	150.04Ac	89.52Ab	817.31
PS	280.47Aa	160.20Cb	132.76Bc	145.70Ac	92.11Ac	811.25
PD	294.40Aa	224.4Ab	146.27Ac	145.33Ac	99.37Ad	905.78
MF	261.11Ba	108.23Db	62.80Cb	102.64Bc	75.03Ac	609.80
Total P (kg ha^{-1})						
Control	491.83Da	310.36Cb	265.81Cb	184.57Bc	135.70Ad	1388.26
CS	785.26Ba	381.00Bb	334.23Ac	218.97Bd	176.10Ad	1895.55
PS	767.79Ba	413.94Bb	361.07Ac	235.94Bd	182.37Ae	1961.11
PD	934.31Aa	686.11Ab	386.30Ac	296.70Ad	175.97Ae	2479.49
MF	706.38Ca	346.22Cb	218.04Bc	197.52Bc	149.85Ad	1613.78

(*) Means within columns at the same depth followed the same capital letter, and (**) means within rows at different depths followed the same lower-case letter, are not significantly different according to to the Scott-Knott test ($p<0.05$)

Table S3. P contents in the different forms of Hedley fractionation and sum of the P contents extracted from the soil profile after 18 successive application (2004-2016) cattle slurry (CS), pig slurry (PS), pig deep-litter (PD) and mineral fertilizer (MF) additions. Data presented are means (n = 4).

Tratamentos	Depht (cm)					
	0-4	4-10	10-20	20-30	30-40	40-50
NH4Cl (Pi) (mg kg^{-1})						
Control	0.90D*a**	1.15Ca	0.61Ba	1.18Aa	0.95Aa	1.18Aa
CS	4.85Ba	2.16Bb	0.41Bc	1.07Ac	0.99Ac	1.18Ac
PS	3.73Ca	2.21Bb	0.47Bc	0.72Ac	0.72Ac	1.18Ac
PD	9.66Aa	6.97Ab	3.43Ac	1.13Ad	0.05Ae	0.92Ad
MF	4.57Ba	0.80Cb	0.66Bb	1.06Ab	1.08Ab	1.18Ab
NaHCO3 (Pi) (mg kg^{-1})						
Control	26.01Ca	12.56Cb	19.82Ca	5.51Bb	4.43Bb	1.95Ab
CS	59.84Bb	83.35Aa	42.56Bc	6.68Bd	5.71Bd	9.34Ad
PS	41.18Ba	88.15Ab	45.44Bb	31.46Ab	14.34Ac	13.52Ac
PD	101.49Ab	91.28Ab	113.89Aa	41.17Ac	36.37Ac	13.84Ad
MF	60.29Ba	35.41Bc	27.40Cc	22.06Bc	10.63Bc	8.25Ac
NaOH I (Pi) (mg kg^{-1})						
Control	24.56Ca	15.38Ca	8.46Cb	14.43Ba	3.58Ab	2.30Ab
CS	45.20Ba	33.80Bb	16.50Cc	13.56Bc	3.83Ad	5.24Ad
PS	52.44Ba	28.03Bb	29.91Bb	21.36Bb	4.79Ac	5.74Ac
PD	85.03Aa	59.82Ab	51.50Ab	36.73Ac	11.02Ad	4.29Ad
MF	32.32Ca	23.91Ba	25.07Ba	16.04Bb	5.02Ab	10.47Ab
HCl (Pi) (mg kg^{-1})						
Control	7.50Ea	2.91Da	1.47Aa	0.44Aa	0.15Aa	0.11Aa
CS	185.26Ba	96.28Bb	3.35Ac	0.99Ac	0.11Ac	0.11Ac
PS	137.55Ca	89.69Bb	3.99Ac	1.37Ac	0.14Ac	0.27Ac
PD	277.82Aa	180.24Ab	8.69Ac	0.73Ac	0.92Ac	1.21Ac
MF	90.84Da	67.77Cb	1.70Ac	0.27Ac	0.27Ac	0.27Ac
NaOH II (Pi) (mg kg ^{1})						
Control	19.82Ba	44.52Ac	12.78Bc	17.56Bc	30.88Ab	12.21Ac
CS	21.76Ba	22.38Ba	24.06Ba	15.50Ba	13.66Ba	14.84Aa
PS	18.47Ba	8.93Bb	23.14Ba	18.39Bb	18.36Bb	24.29Aa
PD	41.84Aa	11.57Bb	37.35Aa	38.14Aa	26.31Ab	22.80Ab
MF	7.17Ca	9.83Ba	21.88Ba	13.25Ba	18.90Aa	22.02Aa
NaHCO3 (Po) (mg kg^{-1})						

Control	14.44Da	10.22Ba	3.98Ba	4.73Ca	6.80Aa	9.24Aa
CS	63.90Ba	14.22Bb	1.66Bc	18.76Bb	10.88Ab	8.70Ac
PS	71.14Aa	7.61Bb	1.43Bb	0.66Cb	13.09Ab	6.39Ab
PD	57.32Ba	47.06Ab	20.54Ac	31.69Ac	0.45Bd	4.64Ad
MF	41.07Ca	10.80Bb	0.56Bb	0.40Cb	0.51Bb	0.31Ab
NaOH I (Po) (mg kg^{-1})						
Control	88.94Ba	73.91Db	62.26Cc	42.80Cd	57.78Cc	45.59Ad
CS	143.24Aa	124.66Bb	96.56Bc	63.40Bd	77.72Bd	35.19Ae
PS	144.35Aa	128.26Bb	88.76Bc	65.47Ad	73.44Bd	48.11Ae
PD	136.92Aa	148.08Aa	109.33Ab	63.41Bd	80.84Ac	54.63Ad
MF	133.97Aa	109.84Cb	60.97Cc	34.07Cd	60.62Cc	44.19Ad
NaOH II (Po) (mg kg^{1})						
Control	0.52Bb	0.17Ab	19.61Aa	1.76Ab	0.45Ab	0.41Ab
CS	7.85Bb	6.57Ab	13.91Aa	3.18Ab	3.64Ab	0.71Ab
PS	13.03Aa	18.24Ba	2.95Ab	1.96Ab	2.18Ab	0.49Ab
PD	1.15Bb	16.89Ba	0.60Ab	0.44Ab	0.74Ab	0.34Ab
MF	19.83Aa	21.77Ba	1.03Ab	2.05Ab	2.10Ab	0.43Ab
Residual P (mg kg^{-1})						
Control	162.22Aa	121.05Ab	54.96Ac	58.63Ac	7.35Ad	8.26Ad
CS	142.71Aa	60.54Bb	34.09Ac	59.82Ab	10.79Ac	13.02Ac
PS	138.42Aa	53.97Bb	44.37Ab	56.01Ab	15.14Ad	9.05Ad
PD	48.31Ba	34.96Ba	48.63Aa	22.47Bb	15.92Ab	7.71Ab
MF	136.00Aa	105.84Ab	60.86Ac	37.58Bc	15.24Ad	7.61Ad
Total P (mg kg^{-1})						
Control	344.92Ea	281.86Db	176.90Cc	154.54Cc	109.21Bd	81.26Ae
CS	674.61Ba	443.95Bb	224.12Bc	195.45Ad	124.81Be	107.95Ae
PS	620.29Ca	425.10Bb	240.66Bc	212.39Ad	139.61Be	109.20Af
PD	759.54Aa	596.87Ab	406.46Ac	223.40Ad	190.66Ae	102.87Af
MF	526.06Da	385.97Cb	200.13Cc	126.77Bd	116.88Bd	89.73Ae

(*) Means within columns at the same depth followed the same capital letter, and (**) means within rows at different depths followed the same lower case letter, are not significantly different according to the Scott-Knott test ($p<0.05$).

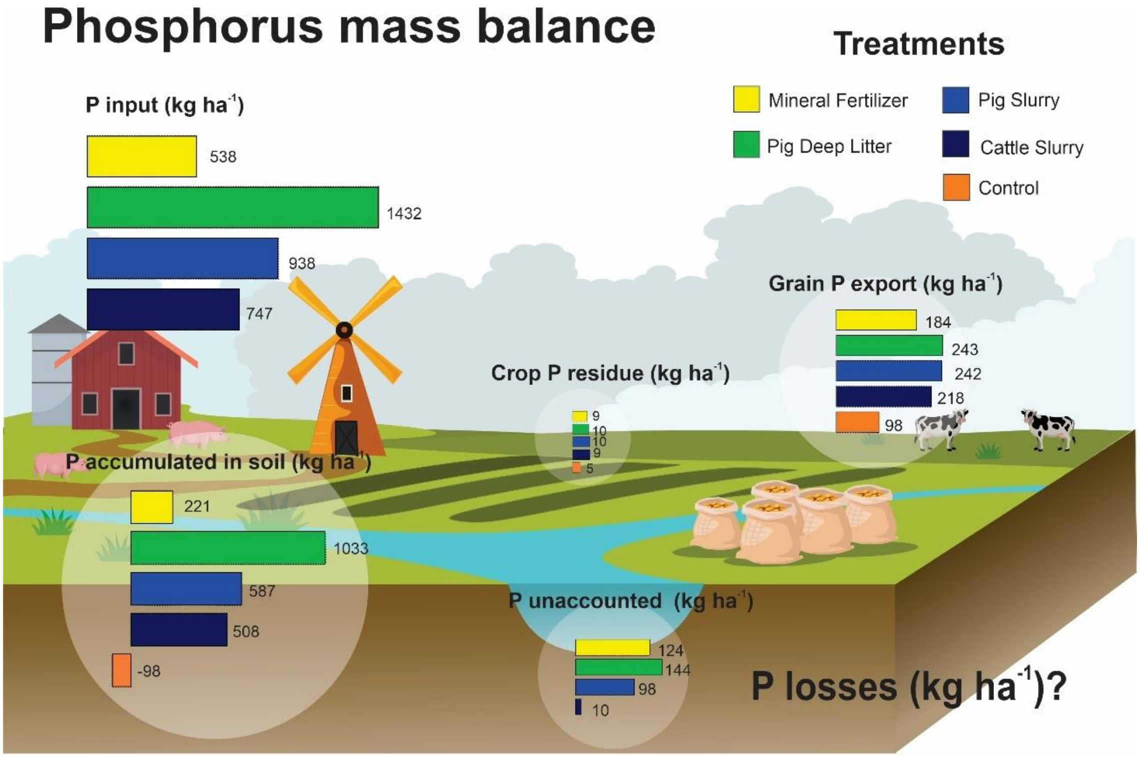


Figure 6. Graphic abctract.

5 CAPÍTULO II - CRESCIMENTO DE RAÍZES E EFICIÊNCIA NA ABSORÇÃO DE P EM MILHO CULTIVADO EM SOLO COM 15 ANOS SUBMETIDO À APLICAÇÕES DE FERTILIZANTES ORGÂNICO E MINERAL

5.1 RESUMO

A aplicações de fontes de P industrializadas e orgânicas em solos altamente intemperizados, como os tropicais e subtropicais, tendem a apresentar baixa eficiência de absorção às plantas. As plantas, em solos com baixa disponibilidade de P, desenvolvem um conjunto de respostas fisiológicas e bioquímicas de adaptação. A resposta mais comum é a diminuição da taxa fotossintética e o aumento da razão raiz/parte aérea (biomassa). Somado a isso, é possível observar alteração em variáveis morfológicas do sistema radicular como o comprimento , diâmetro, volume e área superficial em plantas com restrição de P. O estudo objetivou avaliar a alteração do sistema radicular de raízes de milho e sua relação com a eficiência absorção e utilização de P em condições de campo. Para isso, o ensaio foi conduzido em um experimento de longa duração, com teores contrastantes de P cultivado sob sistema plantio direto, submetido a 15 anos de aplicações de dejetos líquidos de suínos e fertilizante fosfatado mineral. O experimento de longa duração implantado em 2004, localizado em Santa Maria (RS), foi realizado durante a cultura do milho nas safras de 2019/20 e 2020/21. O delineamento utilizado foi blocos ao acaso, com 3 tratamentos e 4 repetições cada. Os tratamentos utilizados foram dejetos líquidos de suínos; fertilizante mineral e sem aplicação de fertilizante (controle). Para investigar a dinâmica do crescimento radicular, em julho de 2018 foram instalados tubos de minirhizotron no solo próximo as linhas de semeadura até a camada de 60 cm, para escaneamento do crescimento radicular da cultura do milho. Os parâmetros morfológicos de raízes analisados foram a distribuição em profundidade do comprimento, área e volume. Os escaneamentos das raízes e as análises fisiológica e químicas da parte aérea das plantas foram realizados nos estágios fenológicos, vegetativo (V8) e florescimento (R1). As plantas cultivadas no solo com histórico de aplicações de dejetos de suínos obtiveram os menores parâmetros morfológicos de raízes, contrapartida foram observados maiores valores de eficiência de absorção de P, produção de biomassa e grãos. As plantas cultivas no solo controle e com aplicação de fertilizante mineral demostraram um comportamento muito semelhante nos parâmetros morfológicos de raízes. No entanto a produção de biomassa e grãos foi diferente. O crescimento radicular e a eficiência de aproveitamento de P apresentaram relação negativa. Em plantas que com maiores valores de comprimento, área e volume de raízes foram observadas as menores produções de biomassa e grãos.

Palavras chaves: morfologia de raízes; minirhizotron; fisiologia; fosforo; dejeto líquido de suínos

5.2 INTRODUÇÃO

Os solos tropicais e subtropicais no Mundo não fornecem a quantidade necessária de fósforo (P) requerida pelas culturas. Assim, em todos os cultivos é recomendado realizar aplicações de fertilizantes fosfatados, especialmente, as fontes de P industrializadas. Mas, as reservas de P no Mundo são limitadas e não são distribuídas de maneira uniforme entre os países (EL ATTAR et al., 2022; GEISSELER; SCOW, 2014**).** Isso tem contribuído para o aumento do custo dos fertilizantes fosfatados nas últimas décadas, especialmente, para os países que possuem pequenas reservas de P e necessitam importar (LANGHANS et al., 2021). Por isso, torna-se necessário a recuperação e reutilização de outras fontes de P, como os dejetos de animais, entre eles, os dejetos líquidos de suínos (SCHNEIDER et al., 2019; WITHERS et al., 2018).

Mas, as aplicações de fontes de P minerais e orgânicas em solos altamente intemperizados, como os tropicais e subtropicais (MACDONALD et al., 2011), tendem a apresentar baixa eficiência de absorção às plantas (LUN et al., 2018; ROBERTS; JOHNSTON, 2015). Estudos demonstram que a eficiência de absorção de P dificilmente ultrapassa a 40% e em alguns casos apenas 10 a 20% do P aplicado é usado diretamente pelas plantas no ano de aplicação (FAGERIA; MOREIRA; DOS SANTOS, 2013; MACDONALD et al., 2011). Isso acontece porque parte do P aplicado forma complexo de esfera interna com os grupos funcionais de partículas inorgânicas reativas, como óxidos de ferro (Fe), alumínio (Al) e manganês (Mn) (ROBERTS; JOHNSTON, 2015), diminuindo a disponibilidade e favorecendo o acúmulo em frações mais recalcitrantes, que pouco podem contribuir com a nutrição das plantas (COELHO et al., 2020; NEGASSA; LEINWEBER, 2009**).**

As plantas, em solos com baixa disponibilidade de P, desenvolvem um conjunto de respostas fisiológicas e bioquímicas de adaptação (LÓPEZ-ARREDONDO et al., 2014). A resposta mais comum é a diminuição da taxa fotossintética e o aumento da razão raiz/parte aérea (biomassa) (HERMANS et al., 2006; LIU, 2021). Em condições de deficiência de P, o aumento da razão raiz/parte aérea acontece principalmente pela diminuição do crescimento de brotos e folhas e ao aumento da atribuição do C a emissão de novas raízes (HERMANS et al., 2006). Somado a isso, é possível observar alteração em variáveis morfológicas do sistema radicular como o comprimento , diâmetro, volume e área superficial em plantas com restrição de P (BHOSALE et al., 2018; LIU, 2021).

A deficiência de P pode aprimorar o aproveitamento interno e a aquisição de P (CAMPOS et al., 2018; LIU, 2021). Isso pode acontecer com o aumento da produção de fatores de transcrição e transportadores de P nas raízes das plantas (LIU et al., 2011). Para a maioria das plantas, em maior ou menor grau, a deficiência de P desencadeia exsudados radiculares que incluem fosfatases, carboxilatos e prótons (LAMBERS et al., 2008b; WANG; LAMBERS, 2019). Estes exsudatos ajudam a solubilizar Pi que é fixado em grupos funcionais de superfície do solo e libera Pi de compostos organofosforados, aumentando assim a disponibilidade de Pi para a absorção de plantas (LAMBERS et al., 2008a; RICHARDSON et al., 2011).

A interação solo-raiz interfere diretamente na morfologia e arquitetura de raízes e no desempenho fisiológico das plantas (HINSINGER et al., 2003). As propriedades químicas e físicas do solo podem variar consideravelmente a curtas distancias dentro de uma mesma área. Em particular, os nutrientes são desigualmente distribuídos no perfil do solo, além disso propriedades físicas como porosidade interferem na quantidade de água no solo e tem grande impacto no sistema radicular. Essas interações são a principal causa da diferença no desempenho do sistema radicular das plantas cultivadas em condições de campo e em casa de vegetação (EREL et al., 2017). Isso sugere a investigação de estratégias de adaptação do sistema radicular das plantas em condições específicas de solo (LAMBERS et al., 2008b).

O estudo objetivou avaliar a alteração do sistema radicular de raízes de milho e sua relação com a eficiência absorção e utilização de P em condições de campo. Para isso, o ensaio foi conduzido em um experimento de longa duração, com teores contrastantes de P cultivado sob sistema plantio direto, submetido a 15 anos de aplicações de dejetos líquidos de suínos e fertilizante fosfatado mineral.

5.3.2MATERIAL E MÉTODOS

5.3.1 Descrição do Experimento

O estudo foi realizado em um experimento de longa duração, com 15 anos de condução, localizado na área experimental da Universidade Federal de Santa Maria (UFSM), em Santa Maria, Rio Grande do Sul, Brasil (29°43’12’’S e 53°43’4’’W) (Figura 1). O clima da região é subtropical úmido (Cfa), conforme a classificação de Koppen, com médias anuais de temperatura, precipitação e umidade relativa de 19,3ºC, 1.561 mm e 82%, respectivamente. O solo é classificado como Argissolo Vermelho Distrófico arênico de acordo com Embrapa (2018), correspondente ao Typic Hapludalf (SOIL SURVEY STAFF, 2014), com 108 g kg^{-1} de argila; 183 g kg^{-1} de silte e 709 g kg^{-1} de areia.

O experimento foi iniciado em 2004, sob sistema de plantio direto, com delineamento de blocos ao acaso com quatro repetições e parcelas com dimensões de 5 x 5 m (25 m^2). Cinco tratamentos foram implantados: dejetos líquidos de suínos (DLS), dejetos líquidos de bovinos (DLB), cama sobreposta de suínos (CSS), fertilizante mineral (ureia + superfosfato triplo + cloreto de potássio) (NPK) e um tratamento controle, sem a aplicação de nutrientes. A dose aplicada de cada resíduo orgânico é definida com base na sua concentração de N e na exigência de N requerida pela cultura do milho e trigo, conforme recomendação regional da Comissão de Química e Fertilidade do Solo Rio Grande do Sul e Santa Catarina (CQFS RS/SC, 2004). As aplicações de dejetos líquidos de suínos e fertilizante mineral foram realizadas em superfície, sobre os resíduos culturais da cultura antecedente e sem incorporação. Os tratamentos foram aplicados apenas uma vez ao ano e antes da semeadura das culturas de verão, no período de 2004 a 2009, sem aplicação dos tratamentos no período de inverno. A partir de 2010 foram realizadas duas aplicações ao ano, antes da implantação das culturas de inverno e de verão. A sucessão de culturas utilizada até agosto de 2020 foi aveia preta (*Avena strigosa S.*), milho (*Zea mays*), nabo forrageiro (*Raphanus sativus*), feijão preto (*Phaseolus vulgaris*) e trigo (*Triticum* spp.). Maiores detalhes sobre o experimento podem ser obtidos em Ferreira et al. 2022) e Lourenzi et al. (2021). Para a condução deste estudo nós selecionamos três tratamentos com diferentes teores de P no solo, controle, dejetos líquidos de suínos e fertilizante mineral. Os detalhes dos atributos químicos do solo são apresentados na Tabela 1. As avaliações foram realizadas durante o cultivo do milho safras 2019/20 e 2020/21.

5.3.2 Avaliação da dinâmica do crescimento de raízes

Para investigar a dinâmica do crescimento de raízes do milho foram instalados tubos de minirhizotron no solo próximo às linhas de semeadura, para escaneamento do crescimento radicular da cultura do milho. Em cada tratamento foi analisada uma linha semeadura por parcela, totalizando 4 tubos por tratamentos. Inicialmente foi realizada uma abertura localizada 10 cm entre a linha de semeadura e o centro da abertura com inclinação de 45º, em relação a superfície do solo. As aberturas para alocação dos tubos foram feitas através de uma broca espiral, de 70 mm de diâmetro e 1,5 m de comprimento, tracionada por perfurador de solo movido à gasolina (Branco, Bps 52). Após a perfuração do solo e retirada do solo foi inserido em cada abertura um tubo de acrílico transparente. A instalação dos tubos em 45º de inclinação permitiu a realização de quatro imagens ao longo do tubo, pois as imagens foram capturadas com as dimensões fixas 21,6 x 19,6 cm. O tubo media 7 cm de diâmetro externo e 105 cm de comprimento, para realização de futuros escaneamentos (Figura 2). Internamente os tubos foram protegidos por um espaguete de polietileno plástico atóxico, leve, flexível e impermeável. A proteção externa do tubo foi confeccionada a partir de canos de PVC (10 cm), cuja finalidade foi evitar possíveis danos causados pelo clima, manejo, ou ataque de pragas. Devido ao distúrbio causado ao solo e a planta no momento da instalação dos canos de acrílicos serão aguardados três meses de estabilização do sistema (solo-planta-tubo), para o início das atividades de escaneamento.

5.3.3 Coleta e análise de imagens de raízes

As imagens (Resolução 600 DPI) foram coletadas nos estágios fenológicos, V8 (25 DAE - vegetativo) e R1 (75 DAE – pleno florescimento) da cultura do milho. As imagens foram geradas por um scanner de sistema radicular *in situ* (CI-600 Growth Monitoring System, CID, EUA). As imagens foram convertidas para camadas de solo de 0-15, 15-30, 30-45 e 45-60 cm e analisadas separadamente. O scanner CI-600 foi alimentado e controlado por um computador, conectado por um cabo USB. As imagens capturadas (formato TIFF e resolução 600 DPI) em cada repetição e em cada profundidade foram analisadas em software computacional RootSnap, que permite o desenho automático e/ou manual de raízes finas. Os parâmetros avaliados nas imagens digitais foram as medidas de comprimento radicular e diâmetro médio das raízes. O monitoramento destas variáveis facilitou o entendimento da dinâmica de crescimento radicular, emissão e senescência de raízes, conforme a adição de fertilizantes, bem como sua variabilidade no tempo.

5.3.4 Coleta de plantas e análise de P

Nas mesmas épocas em que foram feitas a avaliação do crescimento radicular da cultura do milho, três plantas foram selecionadas aleatoriamente no centro das parcelas e cortadas rente à superfície. As plantas foram secas em estufas de ar forçado a 65ºC, até a massa constante para a determinação da matéria seca. Posteriormente, as amostras do tecido vegetal foram moídas em moinho tipo Wiley, de aço inoxidável, passadas em peneira de 20 mesh (0,85 mm), acondicionadas em sacos de papel, para serem analisadas quimicamente. Para determinar os teores de P, as amostras do material vegetal foram submetidas à digestão nítrico-perclórica (proporção 4:1) (EMBRAPA, 1997), sendo os teores de P determinados por colorimetria de acordo com Murphy & Riley (1962).

5.3.5 Trocas gasosas

Nas mesmas datas das avaliações do crescimento radicular, foram realizadas leituras de trocas gasosas na região mediana da última folha completamente expandida e totalmente exposta à radiação solar. Uma leitura foi realizada em três plantas, escolhidas aleatoriamente dentro de cada parcela. As determinações foram feitas a partir das 09:00 horas, utilizando um analisador de gás infravermelho em sistema aberto (LI-6400XT LI-COR, Inc., Lincoln, NE, EUA). A taxa fotossintética (A), concentração intercelular de CO_2 (Ci), taxa de transpiração (E) e condutância estomática de CO_2 (Gs) foram determinadas a uma concentração de CO_2 ambiente de 400 µmol mol^{-1}, temperatura de 20/25°C, umidade relativa de 50±5%, e uma densidade de fluxo de fótons de 1500 µmol m^{-2} s^{-1}.

5.3.6 Produção de matéria seca e grãos, eficiências fisiológicas e agrofisiológica de P

No final do ciclo produtivo das culturas foi determinada a produtividade média de grãos e umidade da massa de grãos foi ajustada para 13%.

A eficiência fisiológica do P (EFP) (SYERS et al., 2008) é definida como o rendimento biológico, obtido por unidade de absorção de nutrientes e foi determinada pela equação 1:

$$EFP(kgkg^{-1}) = (BY_f - BY_u)/(N_f - N_u)$$ *Equação 1*

Onde: BY_f é o rendimento biológico (grão + palha) da parcela fertilizada (kg); BY_u é o rendimento biológico da parcela não fertilizada (kg); N_f é a absorção de P (grão + palha) da parcela fertilizada (kg); e N_u é a absorção de P (grão + palha) da parcela não fertilizada (kg).

A eficiência agrofisiológica do P (EAFP) (SYERS et al., 2008) é definida como o rendimento de grãos obtido por unidade de absorção de nutrientes e será determinada pela equação 2:

$$EAFP(kgkg^{-1}) = (G_f - G_u)/(N_{uf} - N_{uu})$$ *Equação 2*

Onde: G_f é o rendimento de grãos da parcela fertilizada (kg); G_u é o rendimento de grãos da parcela não fertilizada (kg); N_{uf} é a absorção de P (grão + palha) da parcela fertilizada (kg); e N_{uu} é a absorção de P (grão + palha) da parcela não fertilizada (kg).

5.3.7 Análise estatística

A análise estatística utilizada para os resultados de produção de MSPA e grãos, parâmetros fisiológicos, aproveitamento de P e parâmetros morfológicos totais de raízes, foi em delineamento em blocos ao acaso. Os dados foram submetidos à análise de variância, as médias dos tratamentos dentro de cada estágio fisiológico em cada safra, quando significativas, foram comparadas pelo teste de Tukey 5% de significância. Para os parâmetros morfológicos de raízes a estatística foi realizada considerando o delineamento blocos ao acaso com parcelas subdivididas. As parcelas (fator principal) foram compostas pelos três tratamentos (Controle, Dejeto de Suínos e Fertilizante Mineral) e as subparcelas (fator secundário) foram compostas pelas quatro profundidades de amostragem (0-15, 15-30, 30-45 e 45 a 60 cm). A disposição das parcelas subdivididas permite inferir sobre a interação entre os tratamentos versus as profundidades. Os dados foram submetidos à análise de variância. As médias dos fatores principais (tratamentos), fatores secundários (profundidades) e suas interações (tratamentos × profundidades) foram comparadas pelo teste de Tukey com 5% de significância. As análises foram realizadas no software utilizando-se o programa estatístico Sisvar (FERREIRA, 2014). Posteriormente, os dados de planta e de solo foram submetidos a análise multivariada de componentes principais (PCA) foi realizada utilizando o pacote "Factoextra" disponível no ambiente estatístico R. A PCA foi realizada com base num conjunto de componentes principais (apenas os componentes 1 e 2 foram utilizados), que infere um conjunto de combinações lineares ortogonais padronizadas que, em conjunto, explicam 62,82% da variabilidade dos dados.

5.4 RESULTADOS

5.4.1 Morfologia de raízes

O comprimento de raízes, na safra 2019/20, no estágio vegetativo foi maior nas profundidades de 0-15 e 15-30 cm nos solos submetidos a aplicação de fertilizante mineral e Controle (Figura 3a, Tabela S1 e S2). O maior comprimento de raízes no solo submetido a aplicação de dejetos de suínos foi na camada de 30-45 cm (Tabela S1 e S2). O comprimento de raízes total foi maior no solo com aplicações de dejetos líquidos de suínos, seguido de fertilizante mineral, com comprimento de raízes 100% e 80% superior às plantas cultivadas no controle. No estágio do florescimento foi observado o maior comprimento de raízes em 30-45 cm em todos os tratamentos (Tabela S1 e S2). O comprimento de raízes total foi maior no solo com aplicações de fertilizante mineral, seguido do controle, com incremento médio de 30% em relação ao solo com aplicações de dejetos de suínos. Na safra 2020/21, no período vegetativo e florescimento, o maior comprimento de raízes foi observado em 15-30 e 30-45 cm de profundidade. Mas, nós não verificamos diferença do comprimento de raízes entre os tratamentos. O comprimento de raízes total no período vegetativo foi maior no solo com aplicações de fertilizante mineral, seguido do controle, com incremento de 25 e 44% em relação ao dejeto de suíno, respectivamente. No florescimento, o maior crescimento de raízes foi observado no solo com fertilizante mineral, sendo 21% superior ao valor observado nos solos com fertilizante mineral e dejetos de suínos.

A área superficial de raízes, na safra 2019/20, no estágio vegetativo foi maior em 30-45cm, nos solos submetidos a aplicação de fertilizante mineral e dejetos de suínos (Figura 3b). A maior área superficial de raízes no tratamento controle foi observada na profundidade de 0-15 e 15-30cm. A área superficial total foi maior nos solos com aplicações de dejetos líquidos de suínos e fertilizante mineral, em média 70% superior ao observado no solo controle. No estágio do florescimento foi observada a maior área superficial em 15-30 e 30-45 cm em todos os tratamentos. Os maiores valores de área superficial de raízes em profundidade (45-60cm) foram observados nos solos com aplicações de dejetos de suínos e fertilizante mineral. No florescimento, a área superficial de raízes total foi maior no solo com aplicações de fertilizante mineral, seguido do controle, com incremento de 27% e 10%, em relação ao solo com aplicações de dejetos de suínos. Na safra 2020/21, no período vegetativo e florescimento, os maiores valores de área superficial de raízes foram observados em 15-30 e 30-45 cm, no solo com aplicações de fertilizante mineral, seguido do dejeto de suíno. A área superficial de raízes total no período vegetativo foi maior no solo com fertilizante mineral, seguido do controle, com incremento em média 25% em relação ao dejeto de suíno. No florescimento, os maiores valores de área superficial de raízes foram observados no solo com aplicações de fertilizante mineral, 38% superior ao verificado nos solos controle e com dejetos de suínos.

O volume de raízes, na safra 2019/20, no período vegetativo foi maior em 30-45cm nos solos submetidos às aplicações de fertilizante mineral e dejetos de suínos (Figura 3c). No solo controle, o maior volume de raízes foi observado em 0-15 e 15-30cm. O volume de raízes total foi maior nos solos com aplicações de dejetos líquidos de suínos e fertilizante mineral. Os valores foram em média 70% superiores ao observado no solo controle. No período do florescimento, os maiores valores de volume de raízes foram verificados em 15-30 e 30-45 cm em todos os tratamentos. Os maiores volumes de raízes em profundidade (45-60cm) foram observados nos solos com dejetos de suínos e fertilizante mineral. No florescimento, o volume de raízes total foi maior no solo com aplicações de fertilizante mineral, seguido do solo controle, com incremento de 35% e 42%, em relação ao solo com aplicações de dejetos de suínos. Na safra 2020/21, nos períodos vegetativo e florescimento, o maior valor de volume de raízes foi observado em 15-30 e 30-40 cm, no solo com fertilizante mineral, seguido do dejetos de suínos. O maior volume de raízes total no período vegetativo foi observado no solo controle, seguido do fertilizante mineral com incremento. No florescimento, o maior volume de raízes total foi verificado no solo com aplicações de fertilizante mineral. O valor foi 74% superior ao observado no solo controle e 16% superior ao solo com dejetos de suínos.

O comprimento, área superficial e volume de raízes foram maiores no período de florescimento nas duas safras. O comprimento, área superficial e volume de raízes foram 53, 60 e 53% maiores na safra 2020/21, em relação à safra 2019/20.

O diâmetro médio de raízes não difere entre as profundidades e os tratamentos (Figura 4). No entanto, houve uma diminuição do diâmetro médio das plantas no florescimento, em relação ao período vegetativo nas duas safras (Figura 4). Também, nós observamos a diferença da distribuição do diâmetro média das raízes entre as duas safras, com incremento de aproximadamente 86 e 51%, nas raízes de menor diâmetro (<0.4 mm), nos períodos vegetativo e florescimento na safra 2020/21, em todos os tratamentos, em relação à safra 2019/20.

5.4.2 Produção de matéria seca e grãos, acúmulo e exportação de P

As plantas de milho cultivadas no solo com aplicações de dejetos de suínos, nas duas safras, no período vegetativo e florescimento apresentaram a maior produção de matéria seca da parte aérea (Figura 5a e 5b). A produção média de matéria seca da parte aérea no florescimento nas duas safras foi 30% e 130% maior no solo com dejetos de suínos, em relação ao fertilizante mineral e controle, respectivamente. A produção de matéria seca das plantas de milho em todos os tratamentos na safra 2020/21 foi em média 17% menor, em relação à safra 2019/20. A maior produção de matéria seca da parte aérea do milho e concentração de P no tecido, promoveram o maior acúmulo de P nas plantas cultivadas no solo com aplicações de dejetos de suínos. A maior produção de grãos e acúmulo de P nos grãos, nas duas safras foram observadas nas plantas cultivadas em solo com a aplicações de dejetos líquidos de suínos, seguido daquelas cultivadas em solo com a aplicação de fertilizante mineral (Figura 6a e 6b).

5.4.3 Trocas gasosas, Índices de eficiência fisiológica e agronômica do uso do P

As plantas de milho cultivadas no solo com dejetos de suínos e fertilizante mineral, nas duas safras, apresentaram taxas fotossintéticas maiores que as observadas nas plantas controle (Figura 7a). A taxa fotossintética das plantas foi maior no período vegetativo em todos os tratamentos nas duas safras. A maior taxa de transpiração foi observada nas plantas cultivadas no solo controle, em 2019/20. Em 2020/21, os maiores valores foram observados nas plantas cultivadas em solo com aplicações de fertilizante mineral, não diferindo estatisticamente do controle (Figura 7b). Não foi possível observar diferença da taxa de transpiração entre os períodos fenológicos nas duas safras. A maior eficiência do uso da água foi observada nas plantas cultivadas no solo com aplicações de dejetos de suínos e fertilizante mineral nas duas safras (Figura 7c). A eficiência do uso da água foi maior no período vegetativo na safra 2019/20, em todos os tratamentos, não diferindo entre os períodos na safra 2020/19. Os maiores valores de eficiência da enzima rubisco foram observados nas plantas cultivadas no solo com aplicações de dejetos de suínos e fertilizante mineral nas duas safras. Os maiores valores da enzima rubisco foi no período vegetativo em todos os tratamentos nas duas safras (Figura 6d). Os maiores valores de PPEI e APEI foram observados nas plantas cultivadas no solo com aplicações de dejetos de suínos nas duas safras (Figura 8a e 8b). Em média, a PPEI e APEI dos tratamentos com aplicações de fertilizante mineral e dejetos de suínos foram 16 e 126% menores na safra 2020/21, em relação a 2019/20.

5.4.4 Análise de componentes principais

Entre as atributos de solo, morfologia de raízes e precipitação, apenas os dois primeiros componentes foram retidos, de acordo com o limiar de autovalor (> 1), o que explica a variação de 62.82% nos resultados originais. Deste total, a variação de 40.82% foi explicada pela componente principal 1 (CP1) e 21.88% foi justificada pela componente principal 2 (CP2) (Figura 8). Entre os tratamentos, PC1 separou tratamento de dejetos de suínos de fertilizante mineral e PC2 separou o controle dos demais tratamentos (Figura 9a). O PC2 foi mais eficiente em separa as safras de cultivo (Figura 9b). Entre as camadas dos solos, PC1 foi mais eficiente em separar a camada 0-15 cm das demais, enquanto o PC2 foi mais eficiente em separar a camada 45-60 cm das demais (9c). As variáveis mais influentes no PC1 foram as variáveis de solo, saturação por Al, saturação por bases, pH, teor de P e as variáveis de morfologia de raiz como comprimento, área superficial e volume. As variáveis mais influentes no PC2 foram a precipitação, CTC_{pH7}, e diâmetro médio de raízes (9d).

5.5 DISCUSSÃO

5.5.1 Efeito das fontes de P na morfologia radicular do milho

Os maiores valores de comprimento, área superficial e volume de raízes foram observadas nas plantas cultivadas no solo controle e com adição de fertilizante mineral em relação aos dejetos de suínos (Figura 3a, 3b e 3c). Isso pode ter acontecido por causa dos menores teores de P no solo controle (5.5 mg kg^{-1}, camada de 0-15cm) e adubado com fertilizante mineral (47 mg kg^{-1}, camada de 0-15 cm), em relação ao solo adubado com dejetos de suínos (85.8 mg kg^{-1}, camada de 0-15 cm) (Tabela 1). O P pode ser facilmente absorvido em grupos funcionais de partículas inorgânicas, especialmente, em solos tropicais e subtropicais (ABDALA et al., 2020; BOITT et al., 2018), o que contribui para a diminuição da concentração de P na solução. Também, é baixa a concentração de formas químicas livres de P na solução do solo, por causa da grande afinidade de complexação a outros íons, como ferro (Fe), alumínio (Al), encontrados em solos intemperizados (LER; STANFORTH, 2003; VAN RIEMSDIJK; LYKLEMA, 1980). Assim, pequena porção de formas químicas livres de P na solução podem se aproximar a superfície externa de raízes por difusão (RUBIO et al., 2012). Neste sentido, as plantas podem emitir mais raízes para explorar um maior volume de solo (WANG; LAMBERS, 2019; WU et al., 2021), o que aumenta a probabilidade de contato com as raízes, aumentando a probabilidade de absorção de P. Também, as plantas em condição de baixa disponibilidade de P podem modificar a arquitetura do sistema radicular para explorar um maior volume de solo e aumentar a absorção de P (LYU et al., 2016; QI et al., 2012). Além disso, as plantas podem emitir com maior frequência raízes, aumentando a percentagem de raízes com menor diâmetro no solo (EREL et al., 2017; WANG et al., 2020). Este aumento na quantidade de raízes finas no solo pode aumentar a exsudação de compostos orgânicos de baixo peso molecular (HINSINGER et al., 2018). Os ácidos orgânicos podem encapsular a superfície de partículas reativas do solo, diminuindo a adsorção de formas de P, o que aumenta a sua disponibilidade às plantas (WANG; LAMBERS, 2019).

Destacamos que as plantas cultivadas no solo Controle apresentaram predominantemente valores de comprimento, área e volume de raízes similares aos observados nas plantas presentes no solo com aplicações de fertilizante mineral. Isso provavelmente aconteceu por causa da maior emissão de um número maior de raízes, na tentativa de se adaptar à menor disponibilidade de P (JIA; LIU; LYNCH, 2018; LAMBERS et al., 2008a). Isso acontece porque a concentração de auxina e a translocação de auxina para os tecidos radiculares, impactam as caracterizações do ritmo de divisão celular e a duração do alongamento celular das raízes (DE SMET et al., 2012; FUKAKI; TASAKA, 2009). Alguns estudos têm confirmado que os genes da família PIN-FORMED (PIN) atuam como mediadores críticos no controle da translocação de auxina celular e como reguladores importantes na definição de comportamentos arquitetura do sistema radicular (LI et al., 2014; PEI et al., 2012; ZHOU; LUO, 2018). Associado a essa translocação de auxina celular, a maior alocação de carbono fotoassimilado para o crescimento de raízes pode ter comprometido a produção de biomassa especialmente nesse tratamento. Isso porque, nós observamos forte correlação negativa entre o comprimento radicular e a produção de matéria seca da parte aérea (R^2 = -0.46, $P \leq 0.001$) (Table S4). Por isso, a manutenção de teores de P adequados no solo é importante, uma vez que, na tentativa de explorar maior volume de solo as plantas investem fotoassimilados antes destinados para a produção de biomassa e grão na produção de raízes (HERMANS et al., 2006).

A correlação negativa de comprimento, área e volume de raízes com teor de P no solo (R^2 = -0.46, R^2 = -0.46 e R^2 = -0.46, $P \leq 0.001$, respectivamente) ao longo do perfil, infere que em solo com menores teores de P (fertilizante mineral e controle) há uma menor produção de raízes ao longo do perfil do solo. No entanto, convém destacar que os maiores valores de comprimento, área e volume de raízes nas camadas 15-30 e 30-45 cm, pode ter acontecido pela demanda por água, uma vez que a camada superficial (0-15 cm) possui os maiores teores de nutrientes (Tabela 1, S1 e S2). Isso pode ter sido a principal causa do incremento na produção de raízes na safra 2020/21 em relação à safra 2019/20, onde o volume de chuvas foi menor, o que pode ter diminuído a disponibilidade de água no solo (Figura 1 e Figura 8b) (MA et al., 2019). Isso aconteceu mesmo sendo observado menores valores de pH, e altos teores de Al e saturação por Al em profundidade (Tabela 1). Isso aumenta a probabilidade de toxidez de Al^{+3} às raízes (ALLEONI et al., 2010; WRIGHT, 2008). Mas pode ser que isso não tenha acontecido em grande intensidade, porque as raízes também podem produzir compostos orgânicos que podem complexar o Al^{+3} na solução (SADE et al., 2016). Mas também, as raízes podem produzir mucilagem que pode complexar/adsorver o Al^{+3} na superfície externa das raízes, diminuindo a probabilidade de absorção e o seu efeito tóxico (SADE et al., 2016).

As raízes com menor diâmetro foram observadas no florescimento (Figura 4). Isso pode ter acontecido por causa do aumento da área foliar, que aumenta a taxa de transpiração e, consequentemente, a demanda por água e nutrientes. Por isso, é necessária a emissão mais frequentes de raízes. Mas isso também é possível porque a planta possui elevada taxa fotossintética. Assim, parte do carbono fotoassimilado também poderá ser destinado para a emissão de novas raízes (HERMANS et al., 2006; LIU, 2021).

5.5.2 Relação de atributos do solo, morfologia radicular e absorção e aproveitamento de P

As maiores produções de matéria seca da parte aérea e grãos de milho tenderam a ser observadas na safra 2019/20, provavelmente, por causa do maior volume de precipitação ao longo do ciclo da cultura, o que pode ter contribuído na disponibilidade de água no perfil do solo (Figura 4a). Isso pode ter favorecido o crescimento de raízes o que aumenta o potencial de absorção de água e nutrientes (LAMBERS et al., 2008b; MA et al., 2019). As maiores produções de matéria seca da parte aérea e grãos de milho foram observadas no solo com aplicações de dejetos de suínos, provavelmente por causa da maior disponibilidade de P e K (Tabela 1). Também, por causa da maior disponibilidade de Ca e Mg, que incrementam a saturação por bases, melhorando o ambiente químico para o crescimento de raízes (FERREIRA et al., 2022; CIANCIO et al., 2014; LAMBERS et al., 2008b) (Tabela 1). Outros estudos já realizados no mesmo experimento reportaram que aplicações de dejetos de suínos aumentaram a matéria orgânica no solo e os teores de formas de N no solo e solução ao longo do cultivo do milho, incrementando a produção das plantas (FERREIRA et al., 2022; BACCA et al., 2020; RODRIGUES et al., 2021). O incremento da matéria orgânica do solo pode aumentar a capacidade de troca de cânions, o que aumenta a adsorção de íons, diminuindo as perdas. Também, os grupos funcionais da matéria orgânica podem complexar o Al^{+3}, diminuindo o seu potencial de toxidez (ALLEONI et al., 2010).

Os maiores teores de P no tecido foram observados nas plantas cultivadas no solo com aplicações de dejetos de suínos, seguido de fertilizante mineral, nas duas safras. Isso aconteceu por causa da entrada anual de P no sistema milho-aveia durante 15 anos de aplicações das duas fontes de nutrientes. Isso aumentou o teor de P no solo, incrementando a disponibilidade e o potencial de absorção e transporte de P na planta (FERREIRA et al., 2022; BOITT et al., 2022). Como os novos híbridos de milho apresentam uma maior capacidade de absorção de P em relação aos híbridos mais antigos (Woli et al. 2018, 2019), este alto aporte de P ao solo contribuiu para o aumento do acúmulo de P no tecido e de P exportados pelos grãos (Figuras 5b e 6b).

A manutenção de teores adequados de P nas folhas da cultura do milho é essencial para o processo fotossintético (RYCHTER & RAO, 2005). Os teores de P nas folhas das plantas cultivadas no solo com aplicações de dejetos de suínos e fertilizante mineral estão adequados segundo Campbell (2000), o que explica os maiores valores de taxa fotossintética das plantas (Figura 7a). Os menores teores de P nas folhas das plantas cultivadas no solo Controle reduziram significativamente a capacidade fotossintética no período vegetativo e florescimento do milho. A fotossíntese é uma forma importante das culturas acumularem carboidratos e este processo está intimamente relacionado à produção (CHTOUKI et al., 2022; MALHOTRA et al., 2018). O P tem um papel fundamental na geração da coenzima adenosina trifosfato e participa do metabolismo fotossintético das células vegetais (DIETZ & HEILOS, 1990). Portanto, a fosforilação fotossintética, a atividade da enzima RuBP e o processo do ciclo de Calvin são significativamente inibidos sob condições de baixa disponibilidade de P no solo e, por consequência, menores teores no tecido (DING et al., 2017; KIRSCHBAUM & TOMPKINS, 1990) (Figura 7d). Em solo com menor disponibilidade de P plantas podem diminuir a condutância estomática e o rendimento quântico do fotossistema II, contribuindo de forma negativa para o crescimento e desenvolvimento das plantas (ZANGANI et al., 2021).

Também, nós destacamos que existe um trade-off entre a remobilização de nutrientes e a manutenção da fotossíntese durante o período reprodutivo (THOMAS E OUGHAM, 2014). A manutenção de P é benéfica para uma vida útil mais longa da folha o que permite uma maior captura de C, contribuindo para o enchimento de grãos (VENEKLAAS et al., 2012; THOMAS & OUGHAM, 2014). Segundo Wang & Ning, 2019, a prioridade da remobilização de P em plantas de milho acontece a partir do caule do que das folhas, sendo uma estratégia eficaz da planta, que em certa medida preserva a capacidade fotossintética da folha e permite um longo período de fixação de C durante o enchimento de grãos.

As plantas desenvolvem diversos mecanismos adaptativos em condições de deficiência de P. Uma estratégia é reduzir a taxa de crescimento e aumentar a eficiência do uso interno do P. Diferente da baixa mobilidade no solo, o P é um elemento relativamente móvel nas plantas, movendo-se facilmente entre os órgãos (FAGERIA; MOREIRA; DOS SANTOS, 2013; MARSCHNER, 2011). A deficiência de P altera a partição de P entre os órgãos; mas a preferência é em órgãos reprodutivos em detrimento do conteúdo de órgãos vegetativos (FAGERIA; MOREIRA; DOS SANTOS, 2013). Além disso, em períodos de baixa disponibilidade hídrica como na safra 2020/21 (Figura 1), as raízes podem produzir sinais como a síntese de ácido abscísico (ABA), aumentar o pH da seiva do xilema e alterar a tensão do xilema (KANG et al., 2004). Esses sinais podem ser transportados para a parte aérea das plantas e reduzir a abertura de estômatos, reduzindo substancialmente a taxa de transpiração, tendo pouco efeito sobre a fotossíntese e, portanto, pode melhorar a eficiência do uso da água apresentando poucos efeitos sobre o rendimento das culturas (KANG ET. AL., 2004; SEZEN et al., 2019; ABBOUD et al., 2021).

5.6 CONCLUSÕES

As plantas cultivadas no solo com histórico de aplicações de dejetos de suínos obtiveram os menores parâmetros morfológicos de raízes. Em contrapartida nós observamos maiores valores de eficiência de absorção de P, produção de biomassa e grãos.

As plantas cultivas no solo controle e com aplicação de fertilizante mineral demostraram um comportamento muito nos parâmetros morfológicos de raízes. No entanto a produção de biomassa e grãos foi diferente.

O crescimento radicular e a eficiência de aproveitamento de P apresentaram relação negativa. Em plantas que com maiores valores de comprimento, área e volume de raízes foram observadas as menores produções de biomassa e grãos.

5.7 REFERÊNCIAS

ABDALA, D. B. et al. Phosphorus speciation and iron mineralogy in an oxisol after 11 years of pig slurry application. **The Science of the total environment**, v. 743, 15 nov. 2020.

ADEMAR, P. et al. Long-Term Effects of Animal Manures on Nutrient Recovery and Soil Quality in Acid Typic Hapludalf under No-Till Conditions. **Agronomy 2022, Vol. 12, Page 243**, v. 12, n. 2, p. 243, 19 jan. 2022.

ALLEONI, L. R. F. et al. Acidity and Aluminum Speciation as Affected by Surface Liming in Tropical No-Till Soils. **Soil Science Society of America Journal**, v. 74, n. 3, p. 1010–1017, 1 maio 2010.

BACCA, A. et al. Residual and immediate effect after 16 applications of organic sources on yield and nitrogen use efficiency in black oat and corn. **Revista Brasileira de Ciencia do Solo**, v. 44, p. 1–15, 2020.

BHOSALE, R. et al. A mechanistic framework for auxin dependent Arabidopsis root hair elongation to low external phosphate. **Nature communications**, v. 9, n. 1, 1 dez. 2018.

BOITT, G. et al. Fate of phosphorus applied to soil in pig slurry under cropping in southern Brazil. **Geoderma**, v. 321, n. February, p. 164–172, 2018.

BOITT, G. et al. The fate of pig slurry phosphorus applied to a sandy loam soil under no-till cropping in southern Brazil. **Geoderma**, v. 422, 15 set. 2022.

CAMPBELL, C. Reference sufficiency ranges for plant analysis in the southern region of the United States. 2000.

CAMPOS, P. et al. Phosphorus acquisition efficiency related to root traits: Is mycorrhizal symbiosis a key factor to wheat and barley cropping? **Frontiers in Plant Science**, v. 9, p. 752, 5 jun. 2018.

CHTOUKI, M. et al. Interactive effect of soil moisture content and phosphorus fertilizer form on chickpea growth, photosynthesis, and nutrient uptake. **Scientific Reports 2022 12:1**, v. 12, n. 1, p. 1–13, 23 abr. 2022.

CIANCIO, N. R. et al. Crop response to organic fertilization with supplementary mineral nitrogen. **Revista Brasileira de Ciencia do Solo**, v. 38, n. 3, p. 912–922, 2014.

COELHO, M. J. A. et al. Soil phosphorus fate and its lability after a long-term phosphorus fertilizer strategy in Brazilian Oxisol. **Archives of Agronomy and Soil Science**, v. 0, n. 0, 2020.

COMISSAO DE QUÍMICA E FERTILIDADE DO SOLO RIO GRANDE DO SUL E SANTA CATARINA CQFS RS/SC. **Manual de Adubação e de Calagem para os estados do Rio Grande so Sul e Santa Catarina**. 11ª editio ed. [s.l: s.n.].

DE SMET, I. et al. Analyzing Lateral Root Development: How to Move Forward. **The Plant Cell**, v. 24, n. 1, p. 15, 2012.

EL ATTAR, I. et al. Phosphorus Availability and its Sustainable Use. **Journal of Soil Science and Plant Nutrition 2022**, v. 1, p. 1–13, 1 set. 2022.

EMBRAPA. **Manual de Métodos de Análise de Solo**. [s.l: s.n.]. v. 2

EMBRAPA. **Sistema brasileiro de classificação de solos**. 5ª ed. Rio de Janeiro, RJ - Brasil: [s.n.].

EREL, R. et al. Soil type determines how root and rhizosphere traits relate to phosphorus acquisition in field-grown maize genotypes. **Plant and Soil**, v. 412, n. 1–2, p. 115–132, 2017.

FAGERIA, N. K.; MOREIRA, A.; DOS SANTOS, A. B. PHOSPHORUS UPTAKE AND USE EFFICIENCY IN FIELD CROPS. **Journal of Plant Nutrition**, v. 36, n. 13, 2013.

FERREIRA, D. F. Sisvar: a guide for its bootstrap procedures in multiple comparisons. **Ciencia e Agrotecnologia**, v. 38, n. 2, p. 109–112, 2014.

FUKAKI, H.; TASAKA, M. Hormone interactions during lateral root formation. **Plant molecular biology**, v. 69, n. 4, p. 437–449, mar. 2009.

GEISSELER, D.; SCOW, K. M. **Long-term effects of mineral fertilizers on soil microorganisms - A review**. **Soil Biology and Biochemistry**Elsevier Ltd, 1 ago. 2014.. Acesso em: 31 maio. 2020

HERMANS, C. et al. How do plants respond to nutrient shortage by biomass allocation? **Trends in Plant Science**, v. 11, n. 12, p. 610–617, 1 dez. 2006.

HINSINGER, P. et al. **Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A reviewPlant and Soil**. [s.l: s.n.]. Acesso em: 10 jan. 2021.

HINSINGER, P. et al. Impact of Roots, Microorganisms and Microfauna on the Fate of Soil Phosphorus in the Rhizosphere. Em: **Annual Plant Reviews online**. Chichester, UK: John Wiley & Sons, Ltd, 2018. p. 377–407.

JIA, X.; LIU, P.; LYNCH, J. P. Greater lateral root branching density in maize improves phosphorus acquisition from low phosphorus soil. **Journal of Experimental Botany**, v. 69, n. 20, p. 4961–14790, 2018.

LAMBERS, H. et al. Plant nutrient-acquisition strategies change with soil age. **Trends in Ecology and Evolution**, v. 23, n. 2, p. 95–103, 2008a.

LAMBERS, H. et al. Plant nutrient-acquisition strategies change with soil age. **Trends in Ecology & Evolution**, v. 23, n. 2, p. 95–103, fev. 2008b.

LANGHANS, C. et al. Phosphorus for Sustainable Development Goal target of doubling smallholder productivity. **Nature Sustainability 2021 5:1**, v. 5, n. 1, p. 57–63, 22 nov. 2021.

LER, A.; STANFORTH, R. Evidence for Surface Precipitation of Phosphate on Goethite. **Environmental Science and Technology**, v. 37, n. 12, p. 2694–2700, 15 jun. 2003.

LI, X. et al. Overexpression of VP, a vacuolar H+-pyrophosphatase gene in wheat (Triticum aestivum L.), improves tobacco plant growth under Pi and N deprivation, high salinity, and drought. **Journal of experimental botany**, v. 65, n. 2, p. 683–696, 2014.

LIU, D. Root developmental responses to phosphorus nutrition. **Journal of Integrative Plant Biology**, v. 63, n. 6, p. 1065–1090, 1 jun. 2021.

LIU, F. et al. Comprehensive Sequence and Whole-Life-Cycle Expression Profile Analysis of the Phosphate Transporter Gene Family in Rice. **Molecular Plant**, v. 4, n. 6, p. 1105–1122, 1 nov. 2011.

LÓPEZ-ARREDONDO, D. L. et al. Phosphate Nutrition: Improving Low-Phosphate Tolerance in Crops. **Annual Review of Plant Biology**, v. 65, n. 1, p. 95–123, 2014.

LOURENZI, C. R. et al. Forms of nitrogen and phosphorus transfer by runoff in soil under no-tillage with successive organic waste and mineral fertilizers applications. **Agricultural Water Management**, v. 248, 1 abr. 2021.

LUN, F. et al. Global and regional phosphorus budgets in agricultural systems and their implications for phosphorus-use efficiency. **Earth System Science Data**, v. 10, n. 1, p. 1–18, 8 jan. 2018.

LYU, Y. et al. Major Crop Species Show Differential Balance between Root Morphological and Physiological Responses to Variable Phosphorus Supply. **Frontiers in Plant Science**, v. 7, n. DECEMBER2016, p. 1939, 21 dez. 2016.

MA, L. et al. Effects of varied water regimes on root development and its relations with soil water under wheat/maize intercropping system. **Plant and Soil**, v. 439, n. 1–2, p. 113–130, 15 jun. 2019.

MACDONALD, G. K. et al. Agronomic phosphorus imbalances across the world's croplands. **Proceedings of the National Academy of Sciences**, v. 108, n. 7, p. 3086–3091, 2011.

MALHOTRA, H. et al. Phosphorus nutrition: Plant growth in response to deficiency and excess. Em: **Plant Nutrients and Abiotic Stress Tolerance**. [s.l.] Springer Singapore, 2018. p. 171–190.

MARSCHNER, H. **Mineral nutrition of higher plants.** 3. ed. London: 2011, 2011.

MURPHY, J.; RILEY, J. P. A modified single solution method for the determination of phosphate in natural waters. **Analytica Chimica Acta 31**, v. 27, n. 27, p. 31–36, 1962.

NEGASSA, W.; LEINWEBER, P. **How does the hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: a review**. **Journal of Plant Nutrition and Soil Science**, jun. 2009. Disponível em: <http://doi.wiley.com/10.1002/jpln.200800223>. Acesso em: 6 maio. 2019

PEI, L. et al. Overexpression of Thellungiella halophila H+-pyrophosphatase Gene Improves Low Phosphate Tolerance in Maize. **PLOS ONE**, v. 7, n. 8, p. e43501, 30 ago. 2012.

QI, W. Z. et al. Morphological and physiological characteristics of corn (Zea mays L.) roots from cultivars with different yield potentials. **European Journal of Agronomy**, v. 38, n. 1, p. 54–63, abr. 2012.

RICHARDSON, A. E. et al. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. **Plant and Soil**, v. 349, n. 1–2, p. 121–156, 3 dez. 2011.

ROBERTS, T. L.; JOHNSTON, A. E. Phosphorus use efficiency and management in agriculture. **Resources, Conservation and Recycling**, v. 105, p. 275–281, 1 dez. 2015.

RODRIGUES, L. A. T. et al. Short- and long-term effects of animal manures and mineral fertilizer on carbon stocks in subtropical soil under no-tillage. **Geoderma**, v. 386, p. 114913, 15 mar. 2021.

RUBIO, G. et al. Rhizosphere phosphorus depletion by three crops differing in their phosphorus critical levels. **Journal of Plant Nutrition and Soil Science**, v. 175, n. 6, p. 810–871, dez. 2012.

SADE, H. et al. Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. **BioMetals 2016 29:2**, v. 29, n. 2, p. 187–210, 21 jan. 2016.

SCHNEIDER, K. D. et al. Options for Improved Phosphorus Cycling and Use in Agriculture at the Field and Regional Scales. **Journal of Environmental Quality**, v. 48, n. 5, p. 1247–1264, set. 2019.

SHARP, R. E.; DAVIES, W. J. Root Growth and Water Uptake by Maize Plants in Drying Soil. **Journal of Experimental Botany**, v. 36, n. 9, p. 1441–1456, 1 set. 1985.

SOIL SURVEY STAFF. Keys to soil taxonomy. **Soil Conservation Service**, v. 12, p. 410, 2014.

VAN RIEMSDIJK, W. H.; LYKLEMA, J. The reaction of phosphate with aluminum hydroxide in relation with phosphate bonding in soils. **Colloids and Surfaces**, v. 1, n. 1, p. 33–44, 1 jan. 1980.

WANG, X. et al. Heterogeneous phosphate supply influences maize lateral root proliferation by regulating auxin redistribution. **Annals of Botany**, v. 125, n. 1, p. 119–130, 8 jan. 2020.

WANG, Y.; LAMBERS, H. Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. **Plant and Soil**, v. 2050, n. Fao 2009, 2019.

WITHERS, P. J. A. et al. Transitions to sustainable management of phosphorus in Brazilian agriculture. **Scientific Reports**, v. 8, n. 1, p. 1–13, 1 dez. 2018.

WRIGHT, R. J. Soil aluminum toxicity and plant growth. **https://doi.org/10.1080/00103628909368163**, v. 20, n. 15–16, p. 1479–1497, 1 set. 2008.

WU, A. et al. Root morphology and rhizosheath acid phosphatase activity in legume and graminoid species respond differently to low phosphorus supply. **Rhizosphere**, v. 19, p. 100391, 1 set. 2021.

ZHOU, J. J.; LUO, J. The PIN-FORMED Auxin Efflux Carriers in Plants. **International Journal of Molecular Sciences**, v. 19, n. 9, 14 set. 2018.

5.8 TABELAS E FIGURAS

Tabela 1. Caracterização dos atributos químicos do solo submetido à aplicações de dejetos de animais sob sistema plantio direto, coletado em agosto de 2019 (*n* = 4).

Treatment	**Layer**	**pH in water**	**SMP Index**	**P**	**K**	**Ca**	**Mg**	**H+Al**	**S**	**CTC ef.**	**CTC pH 7.0**	**V**	**m**
	(cm)			———mg kg^{-1}--------		———cmol$_c$ kg^{-1}--------						-----------%------------	
Control	0-15	5.1±0.1	5.9±0.2	5.5±0.6	36.8±1.3	1.6±0.3	1.2±0.3	0.8±0.2	2.9±0.6	3.3±0.5	4.1±0.4	70.4±7.7	13.3±3.7
	15-30	4.6±0.1	5.4±0.2	2.1±0.7	11.1±2.6	1.2±0.1	1.1±0.1	1.6±0.3	2.3±0.2	3.2±0.3	4.8±0.4	48.9±3.1	28.4±2.7
	30-45	4.6±0.1	5.2±0.4	1.1±0.2	7.5±3.5	0.7±0.3	0.8±0.4	2.5±0.6	1.6±0.6	2.5±0.3	4.9±1.3	35.6±7.2	38.3±7.1
	45-60	4.7±0.2	5.2±0.1	1.2±0.4	9.5±5.0	0.5±0.2	1.0±0.5	2.0±0.5	1.5±0.7	2.4±0.7	4.5±0.6	32.1±3.0	41.4±4.4
Pig Slurry	0-15	5.1±0.1	5.8±0.1	85.8±1.7	103.6±4.2	2.1±0.2	1.2±0.2	0.8±0.1	3.5±0.1	3.8±0.3	4.6±0.3	74.3±3.2	9.9±1.9
	15-30	4.8±0.1	5.9±0.3	25.2±2.7	43.8±6.2	1.3±0.3	1.5±0.2	0.8±0.4	2.9±0.1	3.6±0.5	4.4±0.5	65.7±8.1	19.5±2.3
	30-45	4.8±0.1	5.9±0.1	9.9±1.4	25.3±6.1	0.9±0.3	1.2±0.4	0.7±0.1	2.2±0.1	2.9±0.7	3.6±0.7	60.1±6.0	23.9±6.0
	45-60	4.8±0.1	5.6±0.1	3.2±1.7	27.5±6.2	0.6±0.1	1.4±0.4	1.1±0.3	2.0±0.1	2.8±0.3	3.8±0.4	52.4±2.0	27.5±3.2
Mineral Fertilizer	0-15	4.6±0.1	5.1±0.2	46.9±1.9	89.9±5.8	0.9±0.2	0.5±0.1	2.4±0.8	1.6±0.4	2.6±0.3	5.1±0.7	34.0±9.0	39.3±6.3
	15-30	4.4±0.1	5.0±0.3	8.7±1.9	36.3±0.7	0.7±0.3	0.7±0.3	2.8±1.3	1.4±0.6	3.1±0.4	5.8±1.3	26.3±9.0	55.2±11.0
	30-45	4.6±0.1	5.1±0.1	2.2±0.6	24.8±3.7	0.5±0.2	0.4±0.2	2.4±0.3	1.0±0.4	2.4±0.4	4.8±0.6	20.2±8.0	60.6±13.9
	45-60	4.6±0.1	5.5±0.4	2.3±0.3	24.8±8.5	0.3±0.2	0.5±0.2	1.4±0.7	0.9±0.4	2.1±0.2	3.5±0.9	26.6±8.7	55.7±14.7

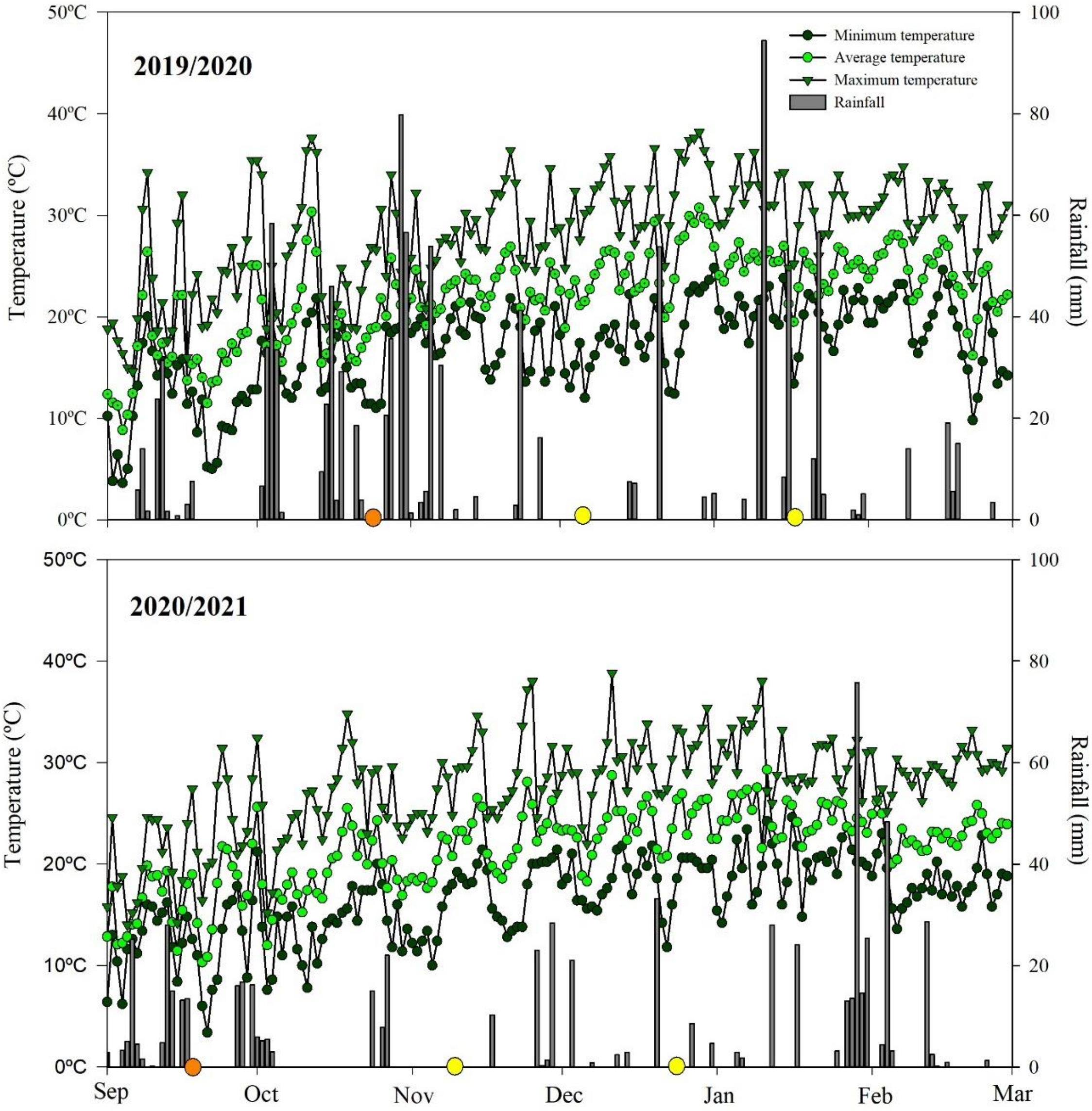


Figura 1. Mean monthly temperature and rainfall in Santa Maria, Rio Grande do Sul, Southern Brazil. Orange circles represent the sowing date of the corn crop and yellow circles represent the collection dates at the vegetative and flowering stages.

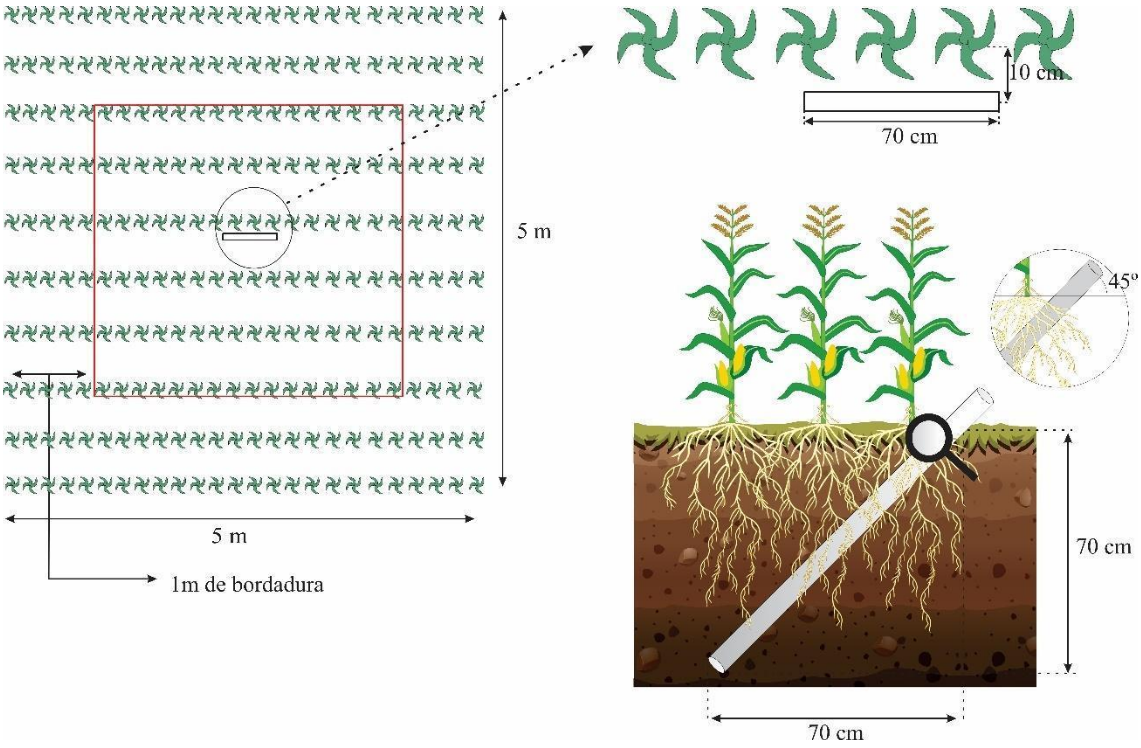


Figure 2. Experimental scheme, with detail for how to allocate the acrylic tubes in the soil for root system analysis.

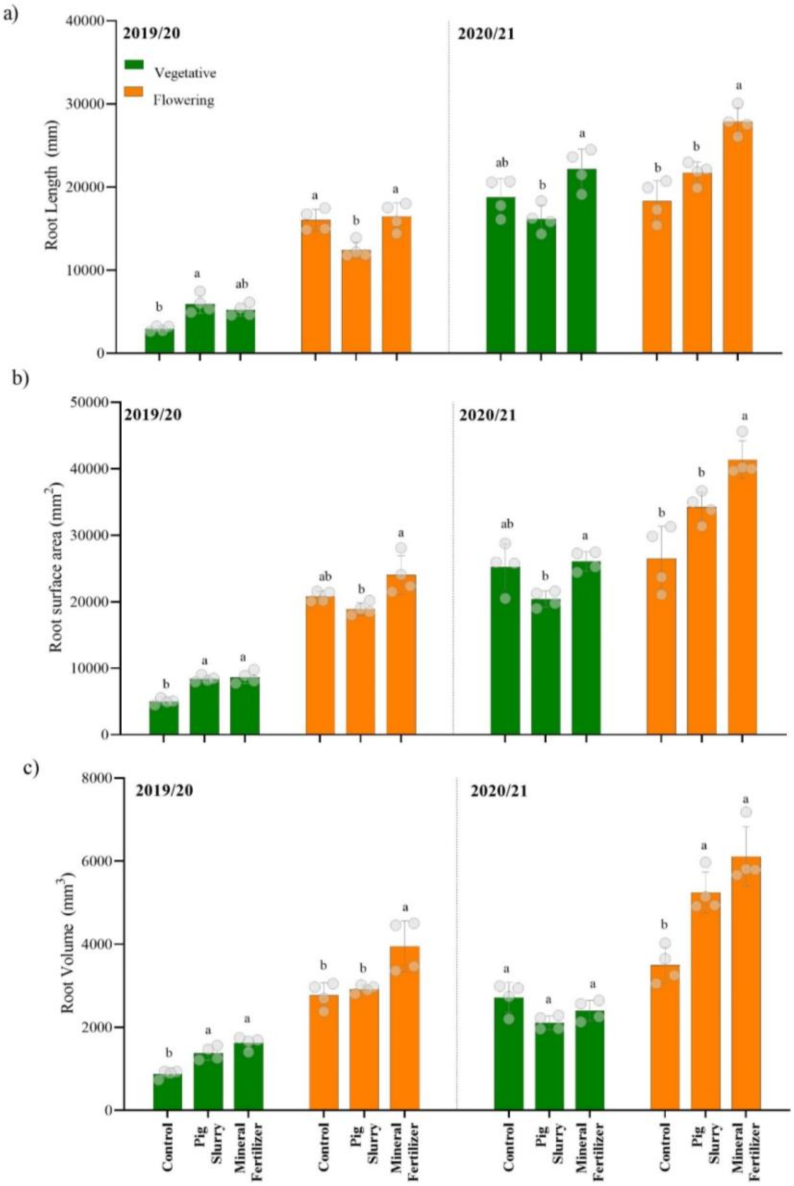


Figure 3. Total root length (a) surface area (b) and volume (c) observed in the 0-60 cm soil layer, in the vegetative and flowering stages of corn grown in the 2019/20 and 2020/2021 crops, in soils with a 15-year history of liquid swine manure and mineral fertilizer application. Lowercase letters compare the different fertilizer sources within each physiological stage within each crop based on Tukey's test ($p < 0.05$), since the interaction was significant at 5% based on the two-way analysis of variance. Horizontal bars represent the standard error of four repetitions.

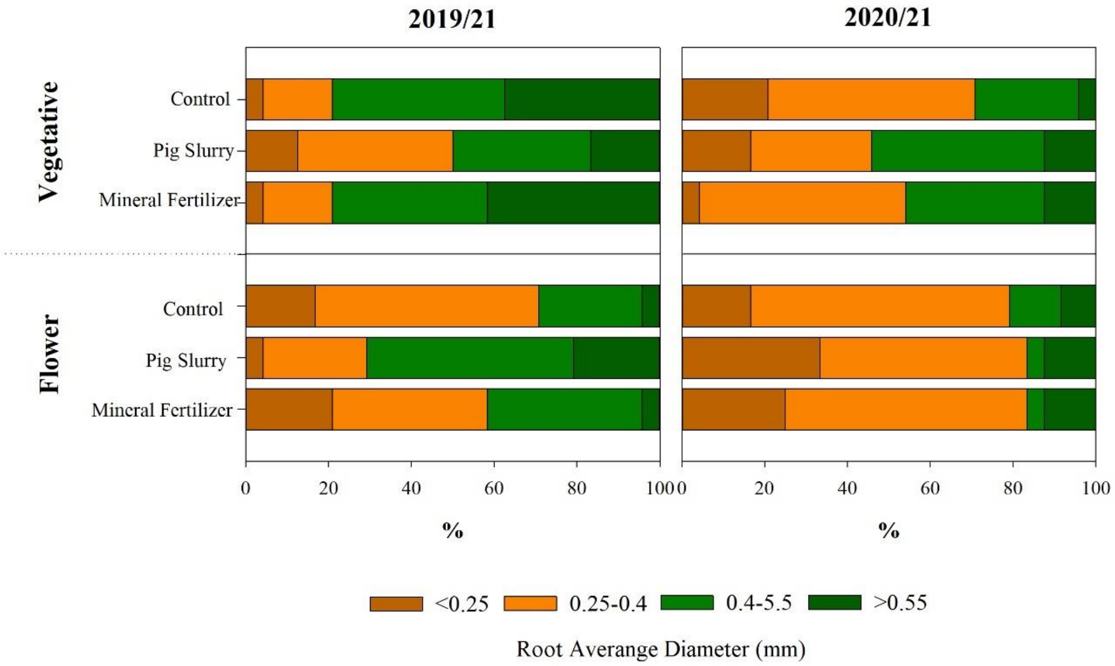


Figure 4. Distribution of mean root diameter observed in the 0 - 60cm soil layer in the vegetative and flowering stages of corn grown in 2019/20 and 2020/2021 crops in soils with a 15-year history of liquid swine manure and mineral fertilizer application.

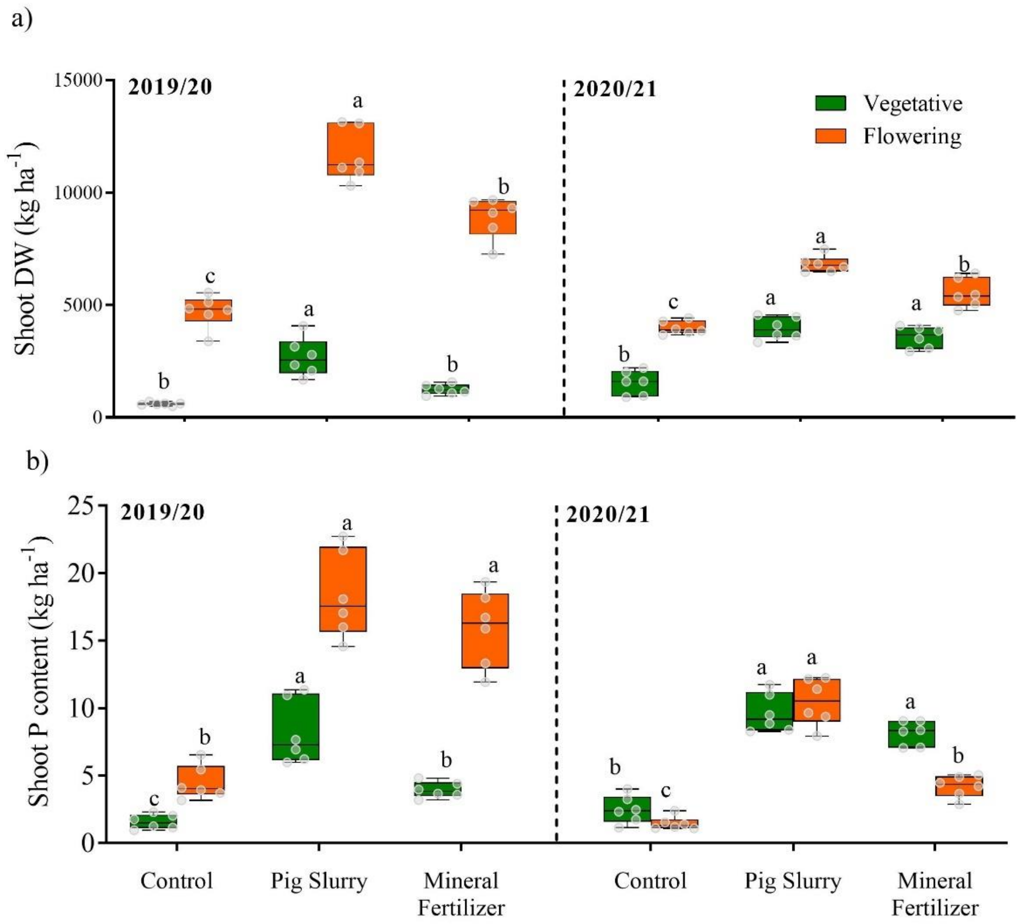


Figure 5. Dry matter production (kg ha^{-1}) (a) and shoot P content (kg ha^{-1}), of corn grown in 2019/20 and 2020/2021 crops, in soils with a 15-year history of application of liquid swine manure, mineral fertilizer, and no fertilizer application (Control). Observations (gray circles) correspond to the repetitions. Different letters indicate significant difference between treatments within each season by Tukey's test ($p < 0.05$).

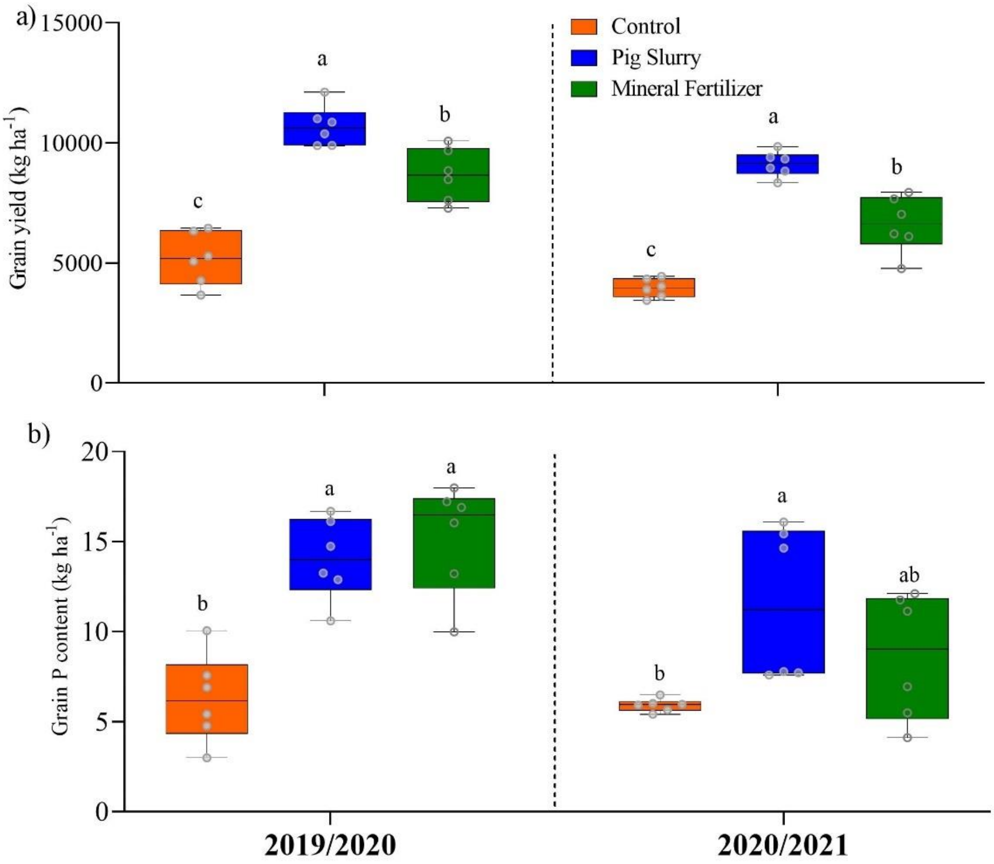


Figure 6. Grain yield (kg ha^{-1}) (a) and grain P content (kg ha^{-1}), of corn grown in 2019/20 and 2020/2021 crops, in soils with a 15-year history of application of liquid swine manure, mineral fertilizer, and no fertilizer application (Control). Observations (gray circles) correspond to the repetitions. Different letters indicate significant difference between treatments within each season by Tukey's test ($p < 0.05$).

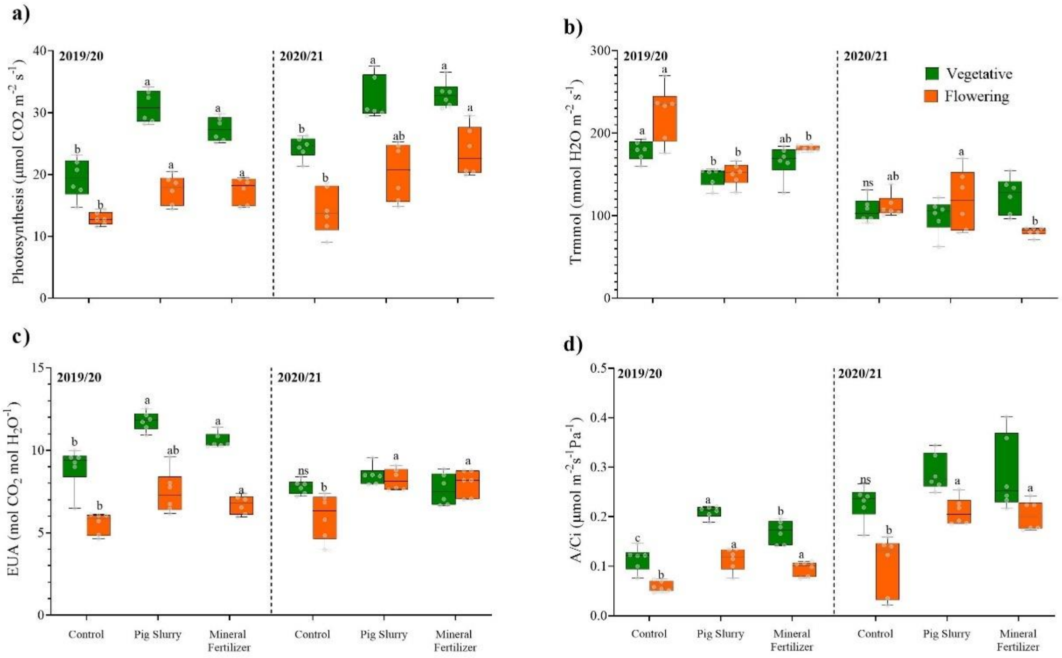


Figura 7. Photosynthetic rate (a), transpiration rate (b), water use efficiency (c), and rubisco enzyme efficiency (d), of corn grown in 2019/20 and 2020/2021 crops, in soils with a 15-year history of application of liquid swine manure, mineral fertilizer, and no fertilizer application (Control). Observations (gray circles) correspond to the repetitions. Different letters indicate significant difference between treatments within each season by Tukey's test ($p < 0.05$).

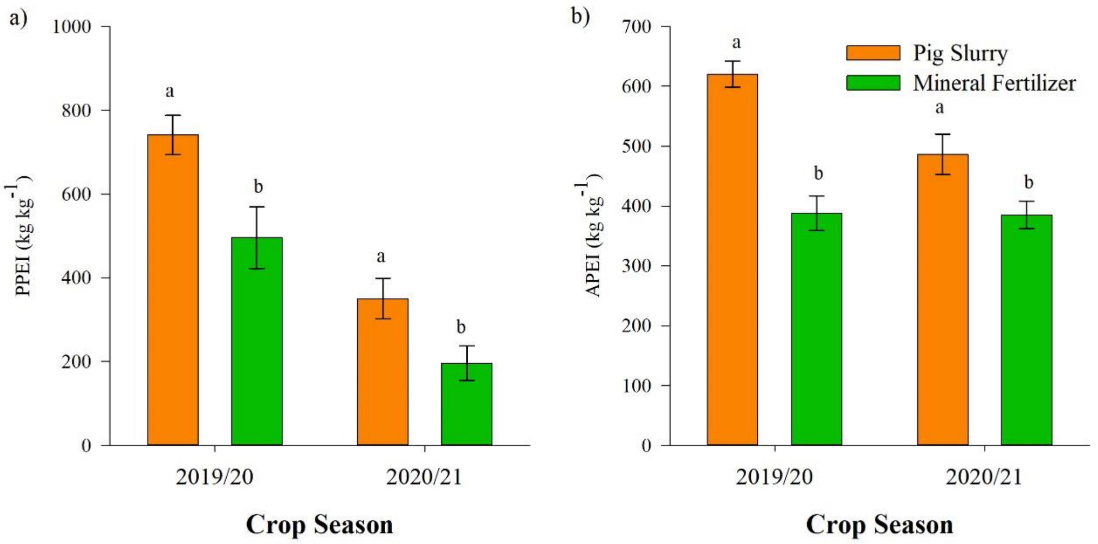


Figure 8. Physiological phosphorus efficiency index (a) and Agrophysiological phosphorus efficiency index (b), of corn grown in 2019/20 and 2020/2021 crops, in soils with a 15-year history of application of liquid swine manure, mineral fertilizer, and no fertilizer application (Control). Observations (gray circles) correspond to the repetitions. Different letters indicate significant difference between treatments within each season by Tukey's test ($p < 0.05$).

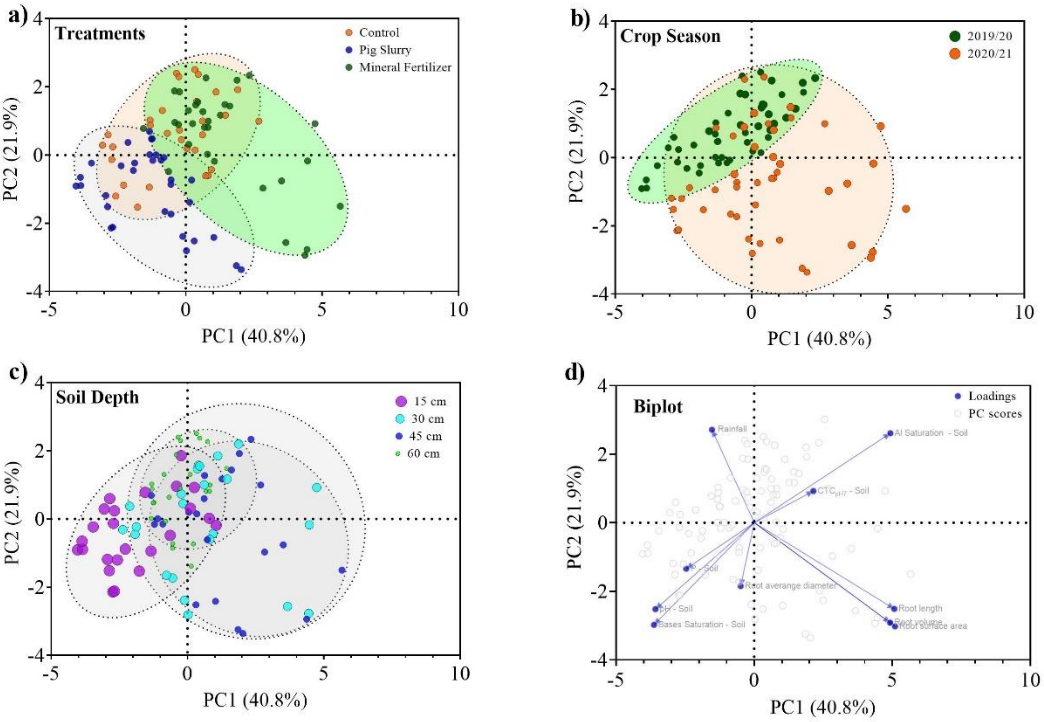


Figura 9. Relationship between principal component 1 (PC1) and principal component 2 (PC2) on maize root system morphological variables (length, surface area, volume and mean diameter), accumulated precipitation, main soil chemical characteristics (pH, base saturation, aluminum saturation, CTCPH7 and soil P content). Corn was grown in soil with 15-year history of pig slurry application, mineral fertilizer and no fertilization - Control (a), in 2019/20 and 2020/21 crops (b) root and soil morphology variables were evaluated.

5.9 TABELAS E FUGURAS SUPLEMENTARES

Table S1. Corn root morphological variables evaluated at the vegetative and flowering stages of corn grown in the 2019/20 crop in soils with a 15-year history of liquid swine manure and mineral fertilizer application.

Crop Season - 2019/20							
Time	**Treatment**	**Layer**	**Root number**	**Root length**	**Root surface area**	**Root Averange diameter**	**Root Volume**
		cm		mm	mm^2	mm	mm^3
Vegetative	Control	0-15	105,0Aa*	1421,3Aa	2098,7ABa	0,46 ns	317,2ABa
		15-30	49,8Aab	957,1Aab	1989,6Aa	0,46	415,6Aa
		30-45	24,3Bab	443,5Cab	676,9Bb	0,46	110,3Bb
		45-60	9,5Ab	124,5Bb	220,2Bb	0,49	34,7Bb
		0-60	**188,5B**	**2946,4B**	**4985,4B**	**0,47**	**877,8B**
	Mineral Fertilizer	0-15	106,3Aa	1703,1Aa	2694,1Aab	0,43	426,8Ab
		15-30	56,8Aab	1189,1Aab	2010,7Ab	0,44	377,5ABb
		30-45	75,3ABab	1739,1Ba	2987,8Aa	0,41	646,1Aa
		45-60	20,8Ab	560,3ABb	932,2Bb	0,46	176,0Bc
		0-60	**259,0B**	**5191,6AB**	**8624,8A**	**0,44**	**1626,3A**
	Pig Slurry	0-15	92,5Aab	1094,8Ab	1573,4Bb	0,39	237,6Bb
		15-30	68,0Aab	1045,0Ab	1243,3Ab	0,37	234,6Bb
		30-45	150,3Aa	2759,8Aa	3850,6Aa	0,39	571,8Aa
		45-60	41,3Ab	1025,6Ab	1740,4Ab	0,46	333,4Ac
		0-60	**352,0A**	**5925,2A**	**8407,8A**	**0,40**	**1377,5A**
Flowering	Control	0-15	189,8ns	2841,8ABb	2969,0Bb	0,34	375,4Bb
		15-30	264,8	4477,1Aab	6058,5Aa	0,38	905,7Aa
		30-45	312,5	5725,0Aa	7847,9Aa	0,39	987,9ABa
		45-60	176,3	2971,2Ab	3912,5Bb	0,40	504,9Bb
		0-60	**943,3A**	**16015,1AB**	**20787,9AB**	**0,38**	**2773,9B**
	Mineral Fertilizer	0-15	299,8	3658,3Aa	5305,7Aa	0,40	761,8Aa
		15-30	215,0	3581,3ABa	5802,6Aa	0,44	1073,8Aab
		30-45	239,5	5251,5Aa	7051,8ABa	0,35	1195,7Aa
		45-60	241,8	3965,3Aa	5838,7Aa	0,42	918,4Ab
		0-60	**996,0B**	**16456,4B**	**23998,7B**	**0,40**	**3949,6B**
	Pig Slurry	0-15	178,3	1814,5Bc	2984,9Bb	0,49	494,1ABb
		15-30	153,8	2490,8Bbc	4406,4Aab	0,49	794,7Aab
		30-45	294,5	4412,2Aa	6124,6Ba	0,34	833,0Ba
		45-60	195,3	3684,6Aab	5357,5ABa	0,46	800,4ABab
		0-60	**821,8A**	**12402,0A**	**18873,4A**	**0,45**	**2922,1A**

* The capital letters compared the different treatments in the same soil layer, while the lowercase letters compared the soil layers within each treatment based on the Tukey test ($p < 0.05$), since the interaction was significant in 5% based on the analysis variance (ns = not significant).

Table S2. Corn root morphological variables evaluated at the vegetative and flowering stages of corn grown in the 2020/21 crop in soils with a 15-year history of liquid swine manure and mineral fertilizer application.

Crop Season – 2020/21							
Time	**Treatment**	**Layer**	**Root number**	**Root length**	**Root surface area**	**Root Averange diameter**	**Root Volume**
		cm		mm	mm^2	mm	mm^3
Vegetative	Control	0-15	440,8ns	4140,1ns	5425,4Ab	0,37sn	510,2Ab
		15-30	284,8	3439,2	4696,2Bb	0,42	655,8Bb
		30-45	575,2	8875,9	11156,4Aa	0,37	1145,2Aa
		45-60	369,8	3824,5	3966,1Ab	0,28	408,3Aa
		0-60	**1670,5A**	**20279,7AB**	**25244,1AB**	**0,36**	**2719,4A**
	Mineral Fertilizer	0-15	351,5	4779,5	6397,7A	0,37	524,6B
		15-30	620,0	6926,2	7305,3AB	0,29	716,0A
		30-45	697,3	8019,4	9044,4AB	0,29	934,9B
		45-60	291,8	3450,8	3350,5A	0,27	223,6A
		0-60	**1960,5A**	**23175,9A**	**26097,8A**	**0,30**	**2399,1A**
	Pig Slurry	0-15	255,8	2444,1	2227,0Bc	0,28	171,9Bc
		15-30	456,8	5517,1	8068,8Aa	0,36	998,9Aa
		30-45	407,6	5613,3	6479,4Bab	0,29	424,6Bbc
		45-60	179,8	2605,8	3620,8Abc	0,37	514,5Ab
		0-60	**1299,9B**	**16180,3B**	**20395,9B**	**0,32**	**2109,9A**
Flowering	Control	0-15	238,8ns	3535,6ns	4822,7A	0,39	708,3Ab
		15-30	406,5	5609,5	7018,0B	0,33	955,1Aab
		30-45	510,8	7373,0	11525,5A	0,39	1490,3Ba
		45-60	190,5	2564,8	3118,8B	0,32	341,9Bb
		0-60	**1346,5B**	**19082,9B**	**26485,1B**	**0,36**	**3495,5B**
	Mineral Fertilizer	0-15	469,0	4751,1	7135,2Ab	0,41	934,5Ab
		15-30	688,8	9502,2	14988,0Ab	0,43	2116,3Ba
		30-45	803,3	9888,0	14029,3Aa	0,41	2400,4Aa
		45-60	354,1	3726,6	5221,5Ab	0,38	662,4ABb
		0-60	**2315,1A**	**27867,9A**	**41374,0A**	**0,41**	**6113,6A**
	Pig Slurry	0-15	330,0	3272,7	5133,0Ab	0,41	711,2Ac
		15-30	351,0	4956,0	9205,8Bab	0,48	1465,6Aab
		30-45	562,2	8601,1	12662,9Aa	0,42	1946,8Aa
		45-60	362,5	4876,7	7200,0Ab	0,40	1119,4Ac
		0-60	**1605,7B**	**21706,5B**	**34201,6B**	**0,43**	**5242,9A**

* The capital letters compared the different treatments in the same soil layer, while the lowercase letters compared the soil layers within each treatment based on the Tukey test ($p < 0.05$), since the interaction was significant in 5% based on the analysis variance (ns = not significant).

Table S3. Pearson correlation coefficients among root attributes and soil chemistry properties.

	Soil chemistry characteristics						**Root parameters**			
	Rainfall	**pH**	**P**	**CTC$_{pH7}$**	**Bases Saturation**	**Al Saturation**	**Length**	**Diameter**	**Surface area**	**Volume**
Rainfall		0,00	0,00	0,00	0,00	0,00	-0,41**	0,07	-0,46**	-0,39**
pH	0,00		0,42**	-0,34**	0,82**	-0,77*	-0,36**	0,17	-0,33**	-0,30**
P	0,00	0,42**		0,03	0,44**	-0,42 **	-0,30**	0,19	-0,26*	-0,24*
CTC$_{pH7}$	0,00	-0,34**	0,03		-0,33***	0,27**	0,08	0,07	0,17	0,17
Bases Saturation	0,00	0,82**	0,44**	-0,33**		-0,96**	-0,30**	0,19	-0,27**	-0,28**
Al Saturation	0,00	-0,77**	-0,42**	0,27**	-0,96**		0,31**	-0,17	0,29**	0,32**
Length	-0,41**	-0,36**	-0,30**	0,08	-0,30**	0,31**		-0,13	0,92	0,83**
Averange diameter	0,07	0,17	0,19	0,07	0,19	-0,17	-0,13		0,13	0,24*
Surface area	-0,46**	-0,33**	-0,26**	0,17	-0,27**	0,29**	0,92**	0,13		0,93**
Volume	-0,39**	-0,30**	-0,24**	0,17	-0,28**	0,32**	0,83**	0,24*	0,93**	

Correlation is significant if $p \leq 0.05$ (*) or $p \leq 0.01$ (**)

Tabela S4. Pearson's correlation coefficients between the evaluated parameters aerial part and roots of corn of all treatments in the two crops seasons.

		Shoot and Grain Parameters				Index		Physiological Parameters					Roots Parametes			
	Rainfall	**DW**	**P-DW**	**Yield**	**P-Grain**	**PPEI**	**APEI**	**A**	**Gs**	**Trmmol**	**EUA**	**A/Ci**	**Lenght**	**Diameter**	**Surface area**	**Volume**
Rainfall		0,56**	-0,34*	0,32	0,10	0,67**	0,23	-0,37*	0,58**	0,79**	-0,32	-0,65**	-0,62**	0,09	-0,64**	-0,43**
Shoot DW	0,56**		0,28	0,86**	0,38*	0,68**	0,43**	0,11	0,40*	0,14	0,32	-0,06	-0,46**	0,39*	-0,35*	-0,23
P-DW	-0,34*	0,28		0,55**	0,70**	-0,39*	-0,19	0,65**	-0,06	-0,49**	0,69**	0,72**	0,45**	0,36*	0,56**	0,49**
Yield	0,32	0,86**	0,55**		0,56**	0,40*	0,35*	0,34**	0,35*	0,02	0,47**	0,21	-0,16*	0,47**	-0,03	0,04
P-Grain	0,10	0,38*	0,70**	0,56**		-0,19	-0,44**	0,54**	0,37*	-0,02	0,48**	0,35	0,17	0,27	0,24	0,15
PPEI	0,67**	0,68**	-0,39*	0,40*	-0,19		0,63**	-0,38*	0,38*	0,43**	-0,17	-0,50**	-0,69**	0,07	-0,68**	-0,49**
APEI	0,23	0,43**	-0,19	0,35*	-0,44**	0,63**		-0,29	-0,09	0,14	0,07	-0,13	-0,43**	0,15	-0,40*	-0,29
A	-0,37*	0,11	0,65**	0,34**	0,54**	-0,38*	-0,29		0,21	-0,54**	0,66**	0,71**	0,51**	0,22	0,59**	0,28
Gs	0,58**	0,40*	-0,06	0,35*	0,37*	0,38*	-0,09	0,21		0,49**	-0,10	-0,18	-0,20	0,18	-0,20	-0,30
Trmmol	0,79**	0,14	-0,49**	0,02	-0,02	0,43**	0,14	-0,54**	0,49**		-0,54**	-0,67**	-0,49**	-0,08	-0,55**	-0,44**
EUA	-0,32	0,32	0,69**	0,47**	0,48**	-0,17	0,07	0,66**	-0,10	-0,54**		0,77**	0,17	0,28	0,30	0,10
A/Ci	-0,65**	-0,06	0,72**	0,21	0,35	-0,50**	-0,13	0,71**	-0,18	-0,67**	0,77**		0,43**	0,14	0,51**	0,34*
Lenght	-0,62**	-0,46**	0,45**	-0,16	0,17	-0,69**	-0,43**	0,51**	-0,20	-0,49**	0,17	0,43**		-0,05	0,94**	0,65**
Diameter	0,09	0,39*	0,36*	0,47**	0,27	0,07	0,15	0,22	0,18	-0,08	0,28	0,14	-0,05		0,21	0,26
Surface area	-0,64**	-0,35*	0,56**	-0,03	0,24	-0,68**	-0,40*	0,59**	-0,20	-0,55**	0,30	0,51**	0,94**	0,21		0,75**
Volume	-0,43**	-0,23	0,49**	0,04	0,15	-0,49**	-0,29	0,28	-0,30	-0,44**	0,10	0,34*	0,65**	0,26	0,75**	

Correlation is significant if p ≤ 0.05 (*) or p ≤ 0.01 (**)

Table S5. Analysis of variance of the analyzed data evaluating the different fertilizer sources (T) in the vegetative, flowering and grain stages of corn in the 2019/20 and 2020/21 crops.

	Crop Season 2019/2020						**Crop Season 2020/2021**					
	Vegetative		**Flowering**		**Grain**		**Vegetative**		**Flowering**		**Grain**	
	T	*CV (%)*	**T**	*CV (%)*	**T**	*CV (%)*	**T**	*CV (%)*	**T**	*CV (%)*	**T**	*CV (%)*
Grain Yeld (kg ha^{-1})	-	-	-	-	**	14.91	-	-	-	-	**	12.15
Grain P content (kg ha^{-1})	-	-	-	-	**	23.94	-	-	-	-	**	27.13
Shoot DW (kg ha^{-1})	**	31.97	**	10.92	-	-	**	16.09	**	9.21	-	-
Shoot P content (kg ha^{-1})	**	31.86	**	26.29	-	-	**	17.92	**	8.79	-	-
A (µmol CO_2 m^{-2}s^{-1})	**	12.14	**	11.6	-	-	**	7.94	**	8.21	-	-
GS (mol H_2O m^{-2}s^{-1})	**	19.79	ns	23.31	-	-	**	18.08	ns	15.29	-	-
CI (µmol mol^{-1})	ns	14.24	ns	17.85	-	-	**	9.97	ns	21.86	-	-
TRmmol (mmol H_2O m^{-2}s^{-1})	*	9.52	**	11.86	-	-	ns	19.63	*	19.24	-	-
EUA (mol CO_2 mol H_2O^{-1})	**	8.48	**	11.79	-	-	ns	7.98	**	12.72	-	-
CIA (µmol m^{-2} s^{-1} Pa^{-1})	**	13.03	**	16.03	-	-	ns	21.5	**	28.11	-	-

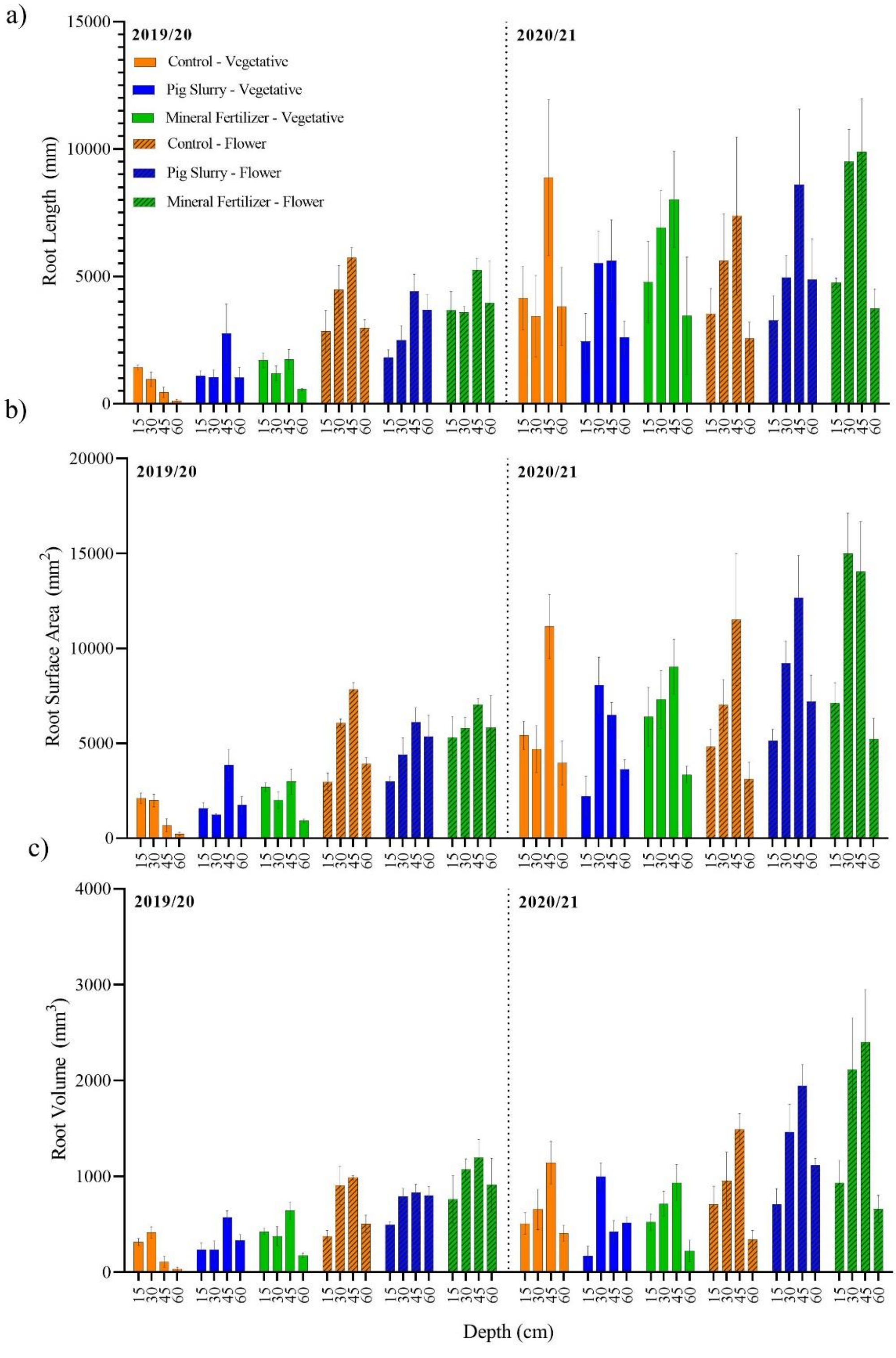


Figure S1. Length (a) surface area (b) and volume (c) of roots observed in soil layers 0-0.15, 0.15-0.30, 0.30-0.45 and 0.45-0.60 m in the vegetative and flowering stages of corn grown in the 2019/20 and 2020/2021 crops, in soils with a 15-year history of liquid swine manure and mineral fertilizer application. Uppercase letters compare the different fertilizer sources considering the same soil layer, while lowercase letters compare soil layers based on Tukey's test ($p < 0.05$), since the interaction was significant at 5% based on the two-way analysis of variance. Horizontal bars represent the standard error of four repetitions.

6 CHAPTER III – RHIZOSPHERE PHOSPHORUS DYNAMICS AND EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGUS IN A SUBTROPICAL CROPPING SYSTEM FOLLOWING LONG-TERM P INPUTS FROM DIFFERENT SOURCES

6.1 ABSTRACT

Phosphorus (P) is an essential nutrient for agricultural production, but its availability is generally very low in the vast majority of crops. The use of pig slurry as a source of nutrients can be an alternative to the P requirement of plants. In addition, soil growth promoting organisms, such as arbuscular mycorrhizal fungi (AMFs), can increase the volume of soil explored by hyphae and act in the solubilization of poorly soluble P forms and organic phosphates and increase their uptake by roots. Therefore, this study aimed to evaluate *in situ*, the effect of mycorrhizal symbiosis on chemical and biochemical parameters, and their relationship with P availability in the rhizosphere of annual crops in soil with 15 years of pig slurry and mineral fertilizer applications. The study was carried out in a long-term experiment (2004 to 2021), in Santa Maria (RS), Brazil. The experimental design was a randomized block design in a 3x2 bifactorial scheme with four repetitions. The treatments used were liquid pig slurry; mineral fertilizer and no fertilizer application (control), with and without AMF inoculation. The collections were performed during the cultivation of corn and oats, in rhizospheric and non-rhizospheric soils, between the 2019/20 and 2021 crops. The soil adhered to the plant roots was considered rhizospheric soil and the soil collected in the inter-row, was considered non-rhizospheric. In the soil samples, the activities of the enzymes acid phosphatase, β-glucosidase and the concentration of carbon, nitrogen and phosphorus in the microbial biomass (C-Bio, N-Bio and P-Bio) were analyzed over time in the availability of P. And in the plants, the production and uptake of P was evaluated. The values of enzyme activity and concentration of C-Bio, N-Bio and P-Bio were higher in the rhizospheric soils of corn and oat plants both in the control soil and in the soils with applications of pig slurry and mineral fertilizer. The higher P availability in the control soil and in the soils with pig slurry and mineral fertilizer applications is related to the phosphatase enzyme activity in the rhizosphere of corn and oats. We did not observe clear differences in P availability in the rhizospheric soil of inoculated and non-inoculated corn and oats with AMF. However, we observed in at least one crop higher P increment in the aerial part and biomass production of AMF-inoculated corn and oats.

Keywords: rhizosphere; arbuscular mycorrhizal fungi; acid phosphatase; β-glucosidase; microbial biomass

6.2 INTRODUCTION

Phosphorus (P) is an essential nutrient for agricultural production, but the concentrations of P (orthophosphate - Pi) most taken up by plants are generally very low in the vast majority of cultivated soils. The largest portions of P observed in soils are present in forms unavailable to plants, such as poorly soluble inorganic salts and organic phosphates. Thus, annual supplementation of plant-available phosphate fertilizers becomes necessary. However, with the rising cost of fertilizers and the predictions of phosphate rock depletion within the next few decades (CORDELL; DRANGERT; WHITE, 2009; GORMAN; BROCKMEIER; BOYSEN-URBAN, 2018) it is critical to find viable alternatives for sustainable food production without relying solely on mineral phosphate fertilizers. One alternative is the use of pig slurry as a source of nutrients, which in addition to supplying the P needs of the plants, over the years, can increase the stock of C and N, contributing to better soil fertility (BOITT et al., 2022; FERREIRA et al., 2022; RODRIGUES et al., 2021).

Also, plant symbiosis with soil growth-promoting organisms, such as arbuscular mycorrhizal fungi (AMFs), can contribute to the decreased use of phosphate fertilizers (RICHARDSON; SIMPSON, 2011). AMF enhance the growth of host plants by increasing the supply of water and nutrients, such as phosphate and nitrogen (N), and provide resistance against aluminum (Al) and heavy metal toxicity in the soil (AMBROSINI et al., 2015; SCHNEIDER et al., 2019; ZHU; LI; WHELAN, 2018). Plants and soil organisms are able to act on the solubilization (hydrolysis) of poorly soluble forms of P and of organic phosphates present in the soil in Pi (HINSINGER et al., 2003; RADERSMA; GRIERSON, 2004).

Enzymes play a key role in soil fertility and their activity is generally related to soil biological activity, thus acting as an indicator of soil quality. In addition, microbial biomass (BM) also plays a major role in soil nutrient cycling, decomposition of organic material, and acts as a source of nutrients (DICK; BREAKWELL; TURCO, 2015). Enzymes related to carbon decomposition and hydrolysis of P-containing organic compounds (e.g., β-glucosidase and phosphatases) can be secreted by plants and soil microorganisms, which act to increase the fractions of available Pi that can be taken up by crop plants, especially under conditions of low P (DICK; BREAKWELL; TURCO, 2015; HINSINGER et al., 2018).

Although the role of mycorrhizal symbiosis in the performance of different crops has been well documented, the contribution of roots and AMF under the enzymatic and microbial activity in the rhizospheric environment in soils with long history of pig slurry and mineral fertilizer applications, under field conditions, especially in soils with subtropical climate, is not well understood. The study aimed to evaluate *in situ*, the biological and biochemical activities as strategies to increase the availability of P in rhizospheric soils of mycorrhized corn, grown in soil with a 15-year history of pig slurry and mineral fertilizer application.

6.3 MATERIAL AND METHODS

6.3.1 Description of the experiment

The study was conducted in a long-term experiment, with more than 15 years of conduction, located in the experimental area of the Federal University of Santa Maria (UFSM), in Santa Maria, Rio Grande do Sul, Brazil (29°43'12"S and 53°43'4"W) (Figure 1). The region's climate is humid subtropical (Cfa), according to Koppen's classification, with annual averages of temperature, precipitation, and relative humidity of 19.3°C, 1,561 mm, and 82%, respectively. The soil is classified as Arenic Dystrophic Red Argissolo (EMBRAPA, 2018) corresponding to Typic Hapludalf (SOIL SURVEY STAFF, 2014) with 108 g kg^{-1} of clay, 183 g kg^{-1} of silt and 709 g kg^{-1} of sand.

The experiment was initiated in 2004, under no-tillage system, with randomized block design with four repetitions and plots with dimensions of 5 x 5 m (25 m^2). Five treatments were implemented: liquid pig slurry, liquid bovine manure, pig bedding, mineral fertilizer (urea + triple superphosphate + potassium chloride) and a control, without the application of nutrients. The applied dose of each organic waste is defined based on its N concentration and the N requirement required by the corn and wheat crops, according to the regional recommendation of the Commission of Chemistry and Soil Fertility Rio Grande do Sul and Santa Catarina (CQFS RS/SC, 2016). Pig slurry and mineral fertilizer applications were made on the surface, over the cultural residues of the preceding crop and without incorporation. The treatments were applied only once a year and before the sowing of the summer crops, in the period from 2004 to 2009, without applying the treatments in the winter period. As of 2010, two applications were made per year, before the implementation of winter and summer crops.

The crop succession used until August 2020 was black oats (*Avena strigosa S.),* corn (*Zea mays),* turnips (*Raphanus sativus),* black beans (*Phaseolus vulgaris*) and wheat (*Triticum* spp.). More details about the experiment can be obtained at Ferreira et al. (2022) and Lourenzi et al. (2021). For conducting this study, we selected three treatments with different soil P contents, control, DLS and NPK. Details of the soil chemical attributes are presented in Table 1. The evaluations were conducted during corn and oat cultivation between the years 2019 and 2021.

The plots of the selected treatments were divided into two parts. In one part, the plants were inoculated with AMF and in the other part the plants were not inoculated with AMF. The experimental design used was randomized blocks, with 6 treatments arranged in a 3x2 factorial scheme, with four repetitions in plots with dimensions of 2.5 x 5 m (12.5 m^2). The treatments were pig slurry, pig slurry + AMF, mineral fertilizer, mineral fertilizer + AMF, a control (without the application of nutrients) and a control + AMF. The inoculated AMF species was *Rhizophagus intraradices* (Rootella BR). The last application of the treatments was performed on the oat crop in July 2019. In order to consider only the P stock accumulated over the years, no new applications of the treatments were made during the 2 years of evaluation.

6.3.2 Rhizospheric soil sampling and sample preparation

Ten rhizospheric and non-rhizospheric soil collections were performed during two years in corn and oat crops. Soil was sampled before sowing of the corn crop and at the following crop development stages: V8 (vegetative), R1 (full flowering) and R6 (physiological maturity) and in the oat crop was collected only at R1 (flowering) stage (Figure 1). The collections were performed under well-drained conditions.

For the collection of rhizospheric soil from corn, within each plot three random plants were selected to compose a repetition (Figure 2). The roots were carefully removed from the soil with the help of a cutting shovel up to the 10 cm layer. They were then shaken to remove excess soil and taken immediately to the laboratory. The soil that remained adhered to the root system after this process was considered rhizospheric soil, according to the procedure described by Peiffer et al. (2013a and 2013b). The rhizospheric soil was separated from the roots in the laboratory using brushes and tweezers, passed through a 2 mm mesh sieve and stored in B.O.D at 4°C until the time of analysis. The non-rhizospheric soil was considered the soil collected from the 0-10 cm layer, located between the crop rows. For the oat rhizospheric soil, an area of 250 cm^2 was randomly selected within each plot to compose the repetition.

6.3.3 Soil analyses

In the stored wet soil, microbial C (C-Bio), microbial P (P-Bio) and microbial N (N-Bio) were extracted by the fumigation and extraction method (BROOKES; POWLSON; JENKINSON, 1982; VANCE; BROOKES; JENKINSON, 1987). The determination of P in the extracts was performed by the colorimetric method proposed by Dick & Tabatabai (1977). Carbon was determined by sulfochromic digestion, and the extract was titrated with ferrous ammonium sulfate solution 0.033 mol L^{-1}, according to (DE-POLLI; GUERRA, 1997). N was determined by sulfuric digestion, followed by distillation by the method of (TEDESCO; GIANELLO; BISSANI, 1995).

The activities of the acid phosphatase and β-glucosidase enzymes were determined by the colorimetry method with p-nitrophenol, after incubation with the specific substrate. Soil moisture was determined according to the methodology proposed by Embrapa (1997) for the correction of soil volume used in the analyses.

The pH was determined, and labile P extracted by Mehlich-1 and determined by the colorimetric method of Murphy & Riley (1962) in the dry soil. At the R1 phenological stage of the corn crop, total organic C (TOC) and total N (TN) contents were determined in a Shimadzu TOCV-TNM1 analyzer (Shimadzu Corp., Kyoto, Japan).

6.3.4 Plant sampling and P analysis

On the same dates as the soil samples, three plants were randomly selected in the center of the plots. They were cut close to the surface. The plants were dried in a forced air oven at 65ºC until constant mass to determine the dry matter. Subsequently, the plant tissue samples were ground in a stainless-steel Wiley mill, passed through a 20-mesh sieve (0.85 mm) and packed in paper bags. The tissue was submitted to nitric-perchloric digestion (ratio 4:1) and the P levels were determined by colorimetry according to Murphy & Riley (1965).

6.3.5. Statistical analysis

The statistical analysis used for the results of APM production and P content in the tissue was bifactorial in randomized blocks (3x2) with four repetitions: factor 1 - Sources of fertilization (Pig slurry, Mineral fertilizer and Control) and factor 2 - Inoculation with AMF (+AMF and -AMF). The data were submitted to variance analysis, the means of the treatments within each season, when significant, were compared using the Tukey test at 5% significance level. For the parameters of enzymes and C, N and P, the statistics were performed considering the randomized block design with subdivided plots. The plots (main factor) were composed of the three treatments (Control, Pig Manure and Mineral Fertilizer) and the subplots (Soil) were composed of the four sampling depths (Bulk Soil, Rhizosphere +AMF and Rhizosphere -AMF). The arrangement of the subdivided plots allowed inferring the interaction between the treatments versus the locations where the soil was collected. The data were submitted to variance analysis. The means of the main factors (treatments), secondary factors (soil), and their interactions (treatments × soil) were compared using Tukey's test at 5% significance level. The analyses were performed in software using the statistical program Sisvar (FERREIRA, 2014). Subsequently, the plant and soil variables were subjected to multivariate principal component analysis (PCA) for each crop (corn and oats), performed using the "Factoextra" package available in the R statistical environment. PCA was performed based on a set of principal components (only components 1 and 2 were used), which infers a set of standardized orthogonal linear combinations that together explained 62.55% and 66.6% of the variability of the corn and oats data, respectively. For the PCA of the soil chemical, biochemical, and microbiological variables for each crop, all sampled dates and their repetitions were considered with 240 observations for the corn crop and 74 for oats. Only the first two components were used, explaining 47.40 and 33.11% of the variability of the data for corn and oats respectively.

6.4 RESULTS

6.4.1 Temporal variation of available P and activity of the acid phosphatase and β-glucosidase enzymes

The highest levels of available P were observed in the soil submitted to pig slurry application, followed by mineral fertilizer, in all collection dates, with average levels of 146 and 83 mg P, respectively (Figure 3). In the control, the highest available P contents were observed in the rhizospheric soil, with and without AMF, with an average increment of 75%

in relation to the Bulk soil. The highest increment of available P was observed in the control soil and with pig slurry application, at the flowering of the oat crop 2021. In the soil with mineral fertilizer, we observed increment in available P in rhizospheric soils with and without AMF, at the vegetative (V8) and senescence (R6) stages of the 2020/21 corn crop. We observed no difference in available P in the oat crop in soil with mineral fertilizer. In the soil with pig slurry applications, we observed the lowest availability of P in the rhizospheric soil, during the senescence of the corn crop in crops 2019/20 and 2020/21.

The highest phosphatase enzyme activity was observed in the soil with pig slurry applications on all collection dates evaluated (Figure 4). In the control soil, in most of the collections, the highest phosphatase enzyme activity was observed in the rhizospheric soil, with and without inoculation of AMF, with an average increase of 100% in relation to the Bulk soil. The highest increment of phosphatase activity was observed at the flowering of the oat crop in 2021. During the corn crop, increment in phosphatase enzyme activity was observed at vegetative stage (V8) and flowering (R1) in 2019/20 and 2020/21 crops. In soil with mineral fertilizer, in most collections, we observed an increment in phosphatase enzyme activity in rhizospheric soil with and without inoculation with AMF, with an average increment of 125% compared to Bulk soil. The highest increment of phosphatase enzyme activity was observed in the vegetative stage and flowering of the corn crop in the 2019/20 crop and in the flowering of the oat crop in the 2021 crop. In the soil fertilized with pig slurry, in most of the collections, we observed an increase in phosphatase enzyme activity in the rhizospheric soil with and without inoculation with AMF. In the rhizospheric soil an average increment of 82% was observed in relation to the Bulk soil. The highest increment in the activity of this enzyme was observed in the vegetative stage and flowering of the corn crop in the 2019/20 crop and in the flowering of the oat crop in the 2021 crop.

The highest activity of β-glucosidase enzyme was observed in the soil with pig slurry applications, followed by mineral fertilizer on all collection dates (Figure 5). In the control soil, the highest β-glucosidase activity was observed in the rhizospheric soil with and without AMF inoculation, with an average increment of 2.7-fold compared to the Bulk soil. The highest β-glucosidase activity was observed in the oat crop flowering in 2020 and 2021. During corn cultivation the highest activity of β-glucosidase enzyme was observed at vegetative (V8), flowering (R1) and senescence stages in 2019/20 crop, and at vegetative and senescence stages in 2020/21. In soil fertilized with mineral fertilizer, we observed an increment in β-glucosidase activity in rhizospheric soil with and without inoculation with AMF, with an average increment of 2.3-fold compared to Bulk soil. The highest increment of β-glucosidase activity was observed at oat crop flowering in 2020 and 2021. During corn crop, increment in β-glucosidase activity was observed at vegetative (V8), flowering (R1) and senescence in 2019/20 and 2020/21 crops. In soil fertilized with mineral fertilizer, we found an increment in β-glucosidase activity in rhizospheric soil with and without inoculation with AMF, with an average increment of 2-fold compared to Bulk soil. The highest increment of β-glucosidase activity was observed at the flowering of the oat crop in 2020 and 2021. During the corn crop, increment in β-glucosidase activity was observed at vegetative (V8), flowering (R1) and senescence stages in 2019/20 and 2020/21 crops.

6.4.2 C, N and P of the microbial biomass

The highest concentrations of C-Bio were observed in the soil subjected to pig slurry application, followed by mineral fertilizer, but in 88% of the collections no difference was observed between the control treatment (Table 2). In the corn crop, the rhizospheric soil increased on average 18, 33 and 41% (average of all observations) the C-Bio in relation to the bulk soil, in the pig slurry, control and mineral fertilizer treatments, respectively. In the control soil, C-Bio contents differed between rhizospheric soils with and without AMF at only two collection times, at the vegetative stage of corn 2019/20 and flowering stage of corn 2020/21. In the soil with mineral fertilizer, an increase in C-Bio was observed in the rhizospheric soil during corn cultivation compared to the Bulk soil, with the greatest increase at vegetative and flowering stages in the 2019/20 and 2020/21 crops. In the soil fertilized with pig slurry the C-Bio contents differed between the rhizospheric soils with and without AMF in only two collection seasons, at vegetative and flowering stages of corn 2020/21. During corn cultivation, an increase in C-Bio was observed in the rhizospheric soil relative to the Bulk soil, with the greatest increment at vegetative and flowering stages in the 2019/20 and 2020/21 crops. The highest increment of C-Bio was observed at flowering in the 2021 oat crop in all treatments. The increment of C-Bio in the rhizosphere of oats was 98% in the control and 150% for mineral fertilizer and pig slurry, respectively compared to the bulk soil of each treatment.

The highest concentrations of N-Bio were observed in the soil subjected to pig slurry application, followed by mineral fertilizer, but in more than 85% of the collections no difference was observed between the control treatment (Table 2). In corn, rhizospheric soil increased N-Bio on average by 15, 55 and 128% (average of all observations) compared to bulk soil, in the treatments with mineral fertilizer, pig slurry and control respectively. In the control soil, N-Bio levels during corn cultivation were higher in the rhizospheric soil compared to the Bulk soil, with the highest increase observed in 87% of the collections. In the soil fertilized with mineral fertilizer, the levels of N-Bio were higher in the rhizospheric corn in relation to the bulk soil, with the highest values observed in the rhizospheres of the plants inoculated with AMF. In soil fertilized with pig slurry, N-Bio contents differed between rhizospheric soils with and without AMF at only two collection times: vegetative and flowering of the 2019/20 corn crop. The highest increment of N-Bio was observed at the oat crop flowering 2020 and 2021 in all treatments. The increment of N-Bio in the rhizosphere of oats was 32, 67, and 112% for the treatments with mineral fertilizer, pig slurry, and control applications, respectively compared to the bulk soil of each treatment.

The highest levels of P-Bio were observed in the soil subjected to pig slurry applications, followed by mineral fertilizer, on all collection dates, which was 3.4 times (average of all collections) higher than the control treatment (Table 2). We observed a decrease of P-Bio in the rhizosphere of corn and oats, 30% lower compared to Bulk soil, in the treatment with pig slurry applications and 35% in the control and mineral fertilizer treatments (average of all crops). In the control soil during corn cultivation P-Bio contents did not differ between rhizospheric soils with and without AMF. In the soil with mineral fertilizer, the highest P-Bio increment was observed in the rhizospheric soil of oats in 2020 and 2021 crops. In the soil fertilized with pig slurry, the highest increment of P-Bio was observed in the rhizospheric soil at the flowering of the corn crop 20120/21.

6.4.3 Aboveground dry matter production and P content

Corn plants grown in soil with applications of pig slurry, in both seasons, presented the highest production of dry matter of the shoot, on average 30% and 130% higher compared to mineral fertilizer and control, respectively (Figure 7a). No significant interaction was observed between inoculation with AMF and fertilizer sources. However, a 30% increase in dry matter production of AMF inoculated plants was observed in the 2019/20 crop. The highest P contents in corn tissue were observed in plants grown with pig slurry and mineral fertilizer applications in the 2019/20 crop, and in plants grown with mineral fertilizer applications in 2020/21 (Figure 7b). We verified a 15% increment in P content in the plants inoculated with AMF in the 2020/21 crop, in relation to the non-inoculated ones. Dry matter production of corn plants in all treatments in the 2019/20 crop was on average 17% higher, relative to corn plants in the 2020/21 crop.

Oats grown in soil with applications of pig slurry, in both seasons, showed the highest production of dry matter of the shoot, not significantly different from the mineral fertilizer in the 2021 season (Figure 7a). No significant interaction was observed between inoculation with AMF and fertilizer sources. However, an increment of 25% was observed in the dry matter production of the plants inoculated with AMF in the 2021 crop. The highest P contents in the oat tissue were observed in plants grown with pig slurry applications in both seasons, not differing from the treatment with mineral fertilizer in the 2021 season (Figure 7b). We observed no difference in the P content in the tissue of oats inoculated and not inoculated with AMF.

6.4.4 Principal Component Analyses

In corn crop, according to the eigenvalue threshold (>1), only the first two components were retained, which explains the variation of 62.55% in the original results. Of this total, the variation of 34.57% was explained by principal component 1 (CP1) and 27.98% was accounted for by principal component 2 (CP2) (Figure 8a). The variables with the highest contribution in CP1 were available P, grain yield, grain P contents, β-glucosidase enzyme activity, and soil moisture. The variables that contributed most to PC2 were precipitation, phosphatase enzyme activity, shoot dry matter, P content in dry matter, P in microbial biomass, and soil pH. PC1 was more efficient in separating the control treatment from the treatments with pig slurry and mineral fertilizer, while PC2 was more efficient in separating the 2019/20 and 2020/21 crops.

In the oat crop, the two retained principal components explained 66.6% of the variation in the original results. Of this total, 39.29% variation was explained by principal component 1 (CP1) and 27.32% was accounted for by principal component 2 (CP2) (Figure 8b). The variables with the highest contribution in CP1 were acid phosphatase enzyme activity, precipitation, and shoot dry matter production. The variables that contributed most to CP2 were tissue P content, P availability, phosphatase enzyme activity, pH, P in microbial biomass, and soil moisture. PC1 was more efficient in separating the 2020 and 2021 crops while PC2 was more efficient in separating the control treatments from the treatments with pig manure and mineral fertilizer applications.

In the soil cultivated with corn, the two principal components retained explained 47.40% of the variation in the original results. Of this total, 24.74% variation was explained by principal component 1 (PC1) and 22.65% was explained by principal component 2 (PC2) (Figure 9a). The variables with the highest contribution in PC1 were acid phosphatase enzyme activity, N in microbial biomass, precipitation and pH. The variables with the highest contribution in PC2 were available P, C and P in microbial biomass, moisture and phosphatase enzyme activity. PC1 was efficient in separating Bulk soil from rhizospheric and rhizospheric + AMF soils.

In the soil cultivated with oats, the two principal components retained explained 55.13% of the variation in the original results. Of this total, 32.11% variation was explained by principal component 1 (PC1) and 23.1% was accounted for by principal component 2 (PC2) (Figure 9b). The variables with the highest contribution in PC1 were acid phosphatase enzyme activity, C in microbial biomass, available P, pH and moisture. The variables with the highest contribution in PC2 were C and P in microbial biomass, β-glucosidase enzyme activity. PC1 and PC2 were efficient in separating Bulk soil from rhizospheric and rhizospheric + AMF soils.

6.5 DISCUSSION

6.5.1 Changes in the rhizosphere of plants grown in soils with different fertilizer sources

The higher P availability in soils with pig manure and mineral fertilizer applications is directly associated with the source and amount of this element added over the years (Figure 3). However, the difference in P availability in the rhizospheric soil of Bulk soils is associated with the ability of plants to modify the soil around the roots (rhizosphere), to favor P uptake, through carboxylate exudation, pH change, microbial activity and enzymes (HINSINGER, 2001; HINSINGER et al., 2015; SUN et al., 2020). The rhizosphere region processes the availability of P in the soil near the roots (HINSINGER et al., 2018; MIMMO et al., 2018). Plant uptake of P from the soil solution can deplete available P fractions in the rhizosphere region (RUBIO et al., 2012). This may explain the lower availability of P in the rhizospheric soil during flowering and senescence of corn plants when grown with pig slurry and mineral fertilizer.

In the control soil, the increase in available P in the rhizosphere region was observed for most of the corn crop. Moreover, P availability was also higher in the rhizospheric soil of oats in the control and pig slurry treatments. The pH change in the rhizospheric soil may have contributed to higher P availability in this region (BARROW, 2017; HINSINGER, 2001; SUN et al., 2020). Interestingly, in all treatments, in both corn and oat crops, we observed an increase in rhizosphere pH. Soil pH in the treatments varied on average from 4.6 to 5.2 in the Bulk soil, and from 5 to 5.5 in the rhizospheric soil. Generally, the rhizospheric pH is usually lower compared to the Bulk soil, because of the large exudation of H ions$^+$ from the plant roots, in response to the uptake of cations such as NH_4^+, Fe^{2+}, K^+, Mg^{2+} and Ca $^{2+}$ (LAMBERS et al., 2008; WANG; LAMBERS, 2019). In addition, root exudation and respiration may contribute to a decrease in the pH of the rhizosphere due to the release of CO_2, which in contact with water, may form carbonic acid (ADELEKE; NWANGBURUKA; OBOIRIEN, 2017; OBURGER et al., 2009) On the other hand, the plants can also exude OH ions$^-$ to balance the uptake of anions such as NO_3^- , $H_2PO_4^-$ and SO_4^-. Because these are two grasses with high N requirements, we perform urea applications during the corn and oat crops. These N inputs in the form of urea in soils with more acidic pH, below 5.5 (Table 1), may have resulted in higher concentration of nitrate, as the most predominant form of N in the soils in all treatments (MARCHEZAN et al., 2020). Therefore, in our study, we believe that nitrate uptake raised the pH in the rhizosphere region, enabled the desorption of phosphate ions strongly adsorbed on soil minerals, such as clays and oxides, and facilitated the dissolution of P-containing mineral compounds, such as P-Ca for example (HINSINGER et al., 2003). Phosphate once available in the soil solution is rapidly taken up by plants, and when not taken up, tends to be re-adsorbed to functional groups of reactive soil particles, but with lower binding energy (HINSINGER, 2001; WANG; LAMBERS, 2019). Thus, it does not leach, contributing to the increase in available P - extracted by Mehlich 1. The rapid uptake of phosphate by plants may explain the decrease in available P in rhizospheric soil with increasing pH (RUBIO et al., 2012). In corn cultivation, we observed negative correlation between pH and P availability in all treatments ($r= -0.21$ and $p>0.001$, Table S2), suggesting that P availability was lower in soil with higher pH – in our study, higher pH was observed in the rhizosphere region. This is characteristic of rhizospheric soils in all treatments. But, in the oat soil we did not observe a consistent correlation, which indicates that other root processes such as microbial activity and exudation of extracellular enzymes (DAO; SCHOMBERG; CAVIGELLI, 2015; GIANFREDA, 2015; NANNIPIERI et al., 2011) contributed to P availability (Figure 7 and Table S.3).

The enzymatic activity of rhizosphere soils is generally higher than those of bulk soil (Figure 4 and 5). The higher rhizosphere enzyme activity depends not only on the stimulation of root-associated microbial activity by rhizodeposition, but also on the release of enzymes by the roots or by root cell lysis (GIANFREDA, 2015). The enzyme acid phosphatase can elevate P availability by dephosphorylating soil organic P. Acid phosphatases are most active at pH below 7 and are produced by plants and soil microorganisms (ADETUNJI et al., 2017; NANNIPIERI et al., 2011). In our study, we found that phosphatase activity was higher in the rhizosphere when compared to the Bulk soil in all treatments, suggesting a direct contribution of roots in the production of this enzyme, although we cannot disregard acid phosphatase activity of microbial origin (Figure 4 and Table S.2). The increase in phosphatase activity related to the increase in P availability ($r = 0.34$, $p>0.001$) in all treatments. We observed an increase of phosphatase enzyme in soils with high P content, such as mineral fertilizer, pig slurry and control soil with low P content. This may be correlated with higher microbial activity in the rhizosphere region, especially in the soil with pig slurry applications.

The β-glycosidases have essential participation in soil C cycling, especially in the decomposition of plant cellulosic components and are synthesized mainly by soil organisms (ADETUNJI et al., 2017). The main reaction catalyzed by glycosidases is the hydrolysis of β-glycosidic bonds into low molecular weight (LMW) glycosides, disaccharides, oligosaccharides and glycosides releasing sugar (glucose) which are energy sources for organisms (GIANFREDA, 2015; WANG; LAMBERS, 2019). The rhizosphere of plants is rich in LMW root exudates that are actively secreted by roots as organic acids, amino acids, proteins, sugars, phenolics, and other secondary metabolites that are generally easily utilized by microorganisms (WU et al., 2021). The higher root biomass may contribute to increase the concentration of LMW root exudates in the soil. This may have stimulated the production of β-glucosidase by microorganisms in the rhizosphere of corn and oats throughout the growing season. Thus, we believe that the significant increase in β-glucosidase production in the rhizosphere at oat flowering is due to the larger volume of soil explored by roots, which potentiated the concentration of root exudates, thus increasing microbial activity in this region (ADETUNJI et al., 2017; ZHANG et al., 2022).

Comparing the two soils with high P content, with applications of liquid manure and mineral fertilizer, we can observe differences in the cycling of C, N and P in the biomass, suggesting that the microbial development was regulated by the availability of C in the soil (Table 2). Rodrigues et al. (2021) in this same experiment, observed a significant increase with the addition of liquid pig slurry compared to mineral fertilizer. The difference in soil C and N explains the difference in P cycling in the microbial biomass (CLEVELAND; LIPTZIN, 2007; HEUCK; WEIG; SPOHN, 2015). Added to this, plant roots also contributed to the increase in soil C and N, which was observed in the rhizosphere of corn plants (Figure 5). The changes in P availability, enzyme activity and cycling of C, N and P in the biomass in the rhizosphere suggest that the dominant means of P acquisition are different between soils with the application of mineral fertilizer and pig slurry.

Possibly, in this study the most dominant strategy in raising P availability in the rhizospheric soil of corn and oats was organic matter mineralization. Stimulation of the enzymes phosphatases and β-glucosidase may have contributed to increased P availability and higher uptake by plants. Since, we observed strong positive correlation between grain yield, dry matter and P content in grain and dry matter ($p>0.001$), with soil enzyme activities (Table S3 and S4).

6.5.2 Changes in rhizosphere and P uptake of AMF-inoculated plants

The AMF inoculation was efficient in colonizing corn and oat roots in all treatments. This suggests that the growth response of mycorrhized plants did not depend solely on soil P content (Figure S1). This contradicts numerous greenhouse and field studies that showed the highest AMF colonization in soils with low P (RAYA-HERNÁNDEZ et al., 2020; RICHARDSON; SIMPSON, 2011; WANG; FENG, 2021). We observed an increase in dry matter production of 2019/20 corn and 2021 oats, but no interaction between inoculation and different fertilizer sources was observed (Figure 6a). The AMF in this case may have acted on other fronts to promote better plant growth in these crops, since the P content in plant tissue did not differ between inoculated and non-inoculated plants (Figure 6b). One possibility is that AMF acted in the acquisition of N, since we observed differences in the content of this nutrient between plants (Figure S2). According to Johnson et al. (2015), the response of the host plant to AMF, not only depends on the P status of the plant, but also on the N status. Similar results were observed by Raya-Hernández et al. (2020), who showed that N limitation results in suppression in the growth of the shoot of AMF-induced corn plants. We emphasize that the AMF-induced corn growth responses observed during plant flowering were not converted into grain yield, where no AMF effects were observed.

The P availability, enzyme activity and fluxes of C, N and P were very similar in rhizospheric soil with AMF and without AMF throughout the corn and oat crop cycle (Figures 3 and 4, Table 2). The rhizospheric soil collection methodology used in this study did not allow the collection of thinner roots, which may have contributed to the loss of sensitivity of this factor (Figure 8). The study of functional characteristics of AMF in the field is difficult because of the interaction of plants with native soil fungi. In the present study, because it is a commercial fungus widely used on grain crops, we did not use any disinfection of the fungus (Stoffel et al., 2020a; Stoffel et al., 2020b) we did not use any soil disinfection and/or application of fungicides sensitive to MFA.

The similarity of rhizospheric soil characteristics of inoculated and uninoculated plants in our field experiments did not allow us to capture the ecosystem functionality of that provided by AMF. We only observed the potential benefits to crops. In the literature, studies show the interaction and functionality of AMF in the rhizosphere and their benefits in P acquisition by plants (LI; CAI, 2021; WAHID et al., 2019; WANG; FENG, 2021). However, these studies are conducted in controlled environments. In this scenario, it is important to consider the potential benefits of AMF in different agricultural contexts, in relation to agricultural practices such as soil conservation and fertilization, and how these practices affect AMF functionality (RAYA-HERNÁNDEZ et al., 2020).

6.5.3. Agronomic implications

We observed differences in soil C, N, and P flux and enzyme activity between the liquid pig slurry and mineral fertilizer treatments, which was reflected in P and N availabilities (Figure 7). The use of liquid pig slurry over the years stimulated the cycling of nutrients in the soil, conferring an increase in crop productivity and a greater contribution of C to the soil. The improvement in the physical, chemical, and biological qualities of the soil allowed a greater availability of nutrients in the soil, contributing to the expression of its productive potential (FERREIRA et al., 2021, 2022; LOSS et al., 2021). In environments favorable to plant development, the demand for photo assimilates for root production and exudation of organic compounds to facilitate nutrient uptake is lower, potentiating the increase in production. In view of the high cost of phosphate fertilizer, the use of pig slurry may be an efficient and sustainable alternative to supply the P needs of plants. In addition to this, the increase of organic matter in the soil as a result of manure applications also contributes to the accumulation of N, enhancing the cycling of nutrients and crop development (ANGERS et al., 2010; ZHANG et al., 2021).

In this study, we did not apply pig slurry and mineral fertilizer in the two years of evaluation, the plants grew and absorbed P from the stock built up in the soil over 15 years of applications. We observed that the plants grown in these soils did not suffer nutritional limitations of P. This suggests that the plants adapted differently between these two soils to meet their needs, since the dry matter production of the plants was different between these treatments. In unpublished results, we observed that plants grown in the soil with mineral fertilizer applications invested more photo-assimilates in root production in search of water and nutrients, whereas plants grown in the soil with animal manure applications invested in deep root emission, probably due to the higher demand for water. Added to this, precipitation between the 2019/20 and 2020/21 corn crops directly interfered with plant dry matter production and P uptake (Figure 1, 6a and 6b). This is because, soil moisture is directly correlated with soil microbial activity. The lack of soil moisture may have hindered the exudation of enzymes by soil microorganisms, limiting the mineralization of P and the uptake of this nutrient by plants.

Oat and corn plants inoculated at flowering in 2020 and 2020/21 with AMF did not show significant difference in dry matter production. Nevertheless, we observed an increase in P content in the tissue of inoculated corn plants. These seasons coincided with the period of lower rainfall. Thus, we believe that the efficiency of AMF was reduced by the lack of soil moisture but remained efficient in nourishing the plant with P and N, even under stress conditions. We observed the same increment of P and N in the grain of the corn crop. However, we observed no difference in grain yield.

6.6 CONCLUSIONS

The values of enzyme activity and microbial C, N, and P fluxes were higher in the rhizospheric soils of the corn and oat plants in both the control soil and the soils with applications of pig slurry and mineral fertilizer.

The higher availability of P in the control soil and with applications of pig slurry and mineral fertilizer is related to the phosphatase enzyme activity in the rhizosphere of corn and oats.

The highest ratio in the increase of available P in the rhizosphere relative to the bulk soil was observed in the rhizosphere of plants grown in the control treatment.

We did not observe clear differences, in enzyme activity and fluxes of C, N and P in microbial biomass, and P availability in rhizospheric soil of AMF-inoculated and non-inoculated corn and oats. However, we observed higher increment of P in the shoot and biomass production of AMF-inoculated corn and oats in at least one crop.

6.7 REFERENCES

ADELEKE, R.; NWANGBURUKA, C.; OBOIRIEN, B. Origins, roles and fate of organic acids in soils: A review. **South African Journal of Botany**, v. 108, p. 393–406, 2017.

ADETUNJI, A. T. et al. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: A review. **Journal of Soil Science and Plant Nutrition**, v. 17, n. 3, p. 794–807, 2017.

AMBROSINI, V. G. et al. Effect of arbuscular mycorrhizal fungi on young vines in copper-contaminated soil. **Brazilian Journal of Microbiology**, v. 46, n. 4, p. 1045–1052, 1 out. 2015.

ANGERS, D. A. et al. Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. **Nutrient Cycling in Agroecosystems**, v. 86, n. 2, p. 225–229, 2010.

BARROW, N. J. The effects of pH on phosphate uptake from the soil. **Plant and Soil**, v. 410, n. 1–2, p. 401–410, 1 jan. 2017.

BOITT, G. et al. The fate of pig slurry phosphorus applied to a sandy loam soil under no-till cropping in southern Brazil. **Geoderma**, v. 422, 15 set. 2022.

BROOKES, P. C.; POWLSON, D. S.; JENKINSON, D. S. Measurement of microbial biomass phosphorus in soil. **Soil Biology and Biochemistry**, v. 14, n. 4, p. 319–329, jan. 1982.

CAMARGO GOMES STOFFEL, S. et al. African Journal of Agricultural Research Yield increase of soybean inoculated with a commercial arbuscular mycorrhizal inoculant in Brazil. v. 16, n. 5, p. 702–713, 2020.

CLEVELAND, C. C.; LIPTZIN, D. C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? **Biogeochemistry**, v. 85, n. 3, p. 235–252, 8 ago. 2007.

COMISSAO DE QUÍMICA E FERTILIDADE DO SOLO RIO GRANDE DO SUL E SANTA CATARINA CQFS RS/SC. **Manual de Adubação e de Calagem para os estados do Rio Grande so Sul e Santa Catarina**. 11ª editio ed. [s.l: s.n.].

CORDELL, D.; DRANGERT, J.-O.; WHITE, S. The story of phosphorus: Global food security and food for thought. **Global Environmental Change**, v. 19, n. 2, p. 292–305, maio 2009.

DAO, T. H.; SCHOMBERG, H. H.; CAVIGELLI, M. A. Tillage and rotational effects on exchangeable and enzyme-labile phosphorus forms in conventional and organic cropping systems. **Nutrient Cycling in Agroecosystems**, v. 101, n. 2, p. 153–165, 2015.

DE-POLLI, H.; GUERRA, J. G. M. Ministério da Agricultura e do Abastecimento Empresa Brasileira de Pesquisa Agropecuária-EMBRAPA Centro Nacional de Pesquisa de Agrobiologia-CNPAB DETERMINAÇÃO DO CARBONO DA BIOMASSA MICROBIANA DO SOLO: MÉTODO DA FUMIGAÇÃO-EXTRAÇÃO. 1997.

DICK, R. P.; BREAKWELL, D. P.; TURCO, R. F. Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators. Em: [s.l.] John Wiley & Sons, Ltd, 2015. p. 247–271.

DICK, W. A.; TABATABAI, M. A. Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds1. **Journal of Environment Quality**, v. 6, n. 1, p. 82, 1977.

EMBRAPA. **Manual de Métodos de Análise de Solo**. [s.l: s.n.]. v. 2

EMBRAPA. **Sistema brasileiro de classificação de solos**. 5ª ed. Rio de Janeiro, RJ - Brasil: [s.n.].

FERREIRA, D. F. Sisvar: a guide for its bootstrap procedures in multiple comparisons. **Ciencia e Agrotecnologia**, v. 38, n. 2, p. 109–112, 2014.

FERREIRA, P. A. A. et al. Repeated Manure Application for Eleven Years Stimulates Enzymatic Activities and Improves Soil Attributes in a Typic Hapludalf. **Agronomy 2021, Vol. 11, Page: 2467**, v. 11, n. 12, p. 2467, 3 dez. 2021.

FERREIRA, P. A. A. et al. Long-Term Effects of Animal Manures on Nutrient Recovery and Soil Quality in Acid Typic Hapludalf under No-Till Conditions. **Agronomy**, v. 12, n. 2, 1 fev. 2022.

GIANFREDA, L. Enzymes of importance to rhizosphere processes. **Journal of soil science and plant nutrition**, v. 15, n. ahead, p. 0–0, 2015.

GORMAN, R.; BROCKMEIER, ; ; M; BOYSEN-URBAN, ; ; K. Phosphorus Scarcity: The Neglected Issue in the Modeling of Future Food Security. 2018.

HEUCK, C.; WEIG, A.; SPOHN, M. Soil microbial biomass C: N: P stoichiometry and microbial use oforganic phosphorus. **Soil Biology and Biochemistry**, v. 85, p. 119–129, 1 jun. 2015.

HINSINGER, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. **Plant and Soil**, v. 237, p. 173–195, 2001.

HINSINGER, P. et al. **Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A reviewPlant and Soil**. [s.l: s.n.]. . Acesso em: 10 jan. 2021.

HINSINGER, P. et al. **Impact of Roots, Microorganisms and Microfauna on the Fate of Soil Phosphorus in the Rhizosphere**. [s.l: s.n.]. v. 48

HINSINGER, P. et al. Impact of Roots, Microorganisms and Microfauna on the Fate of Soil Phosphorus in the Rhizosphere. Em: **Annual Plant Reviews online**. Chichester, UK: John Wiley & Sons, Ltd, 2018. p. 377–407.

JOHNSON, N. C. et al. Mycorrhizal phenotypes and the Law of the Minimum. **New Phytologist**, v. 205, n. 4, p. 1473–1484, 1 mar. 2015.

LAMBERS, H. et al. Plant nutrient-acquisition strategies change with soil age. **Trends in Ecology and Evolution**, v. 23, n. 2, p. 95–103, 2008.

LI, M.; CAI, L. Biochar and Arbuscular Mycorrhizal Fungi Play Different Roles in Enabling Maize to Uptake Phosphorus. **Sustainability 2021, Vol. 13, Page 3244**, v. 13, n. 6, p. 3244, 16 mar. 2021.

LOSS, A. et al. Carbon, nitrogen, and aggregation index in Ultisol with 11 years of application of animal manures and mineral fertilizer. **Journal of Soil and Water Conservation**, v. 76, n. 6, p. 547–557, 1 nov. 2021.

LOURENZI, C. R. et al. Forms of nitrogen and phosphorus transfer by runoff in soil under no-tillage with successive organic waste and mineral fertilizers applications. **Agricultural Water Management**, v. 248, 1 abr. 2021.

MARCHEZAN, C. et al. Nitrogen Availability and Physiological Response of Corn After 12 Years with Organic and Mineral Fertilization. **Journal of Soil Science and Plant Nutrition**, v. 20, n. 3, p. 979–989, 1 set. 2020.

MIMMO, T. et al. Nutrient availability in the rhizosphere: a review. **Acta Horticulturae**, n. 1217, p. 13–28, 2018.

MURPHY, J.; RILEY, J. P. A modified single solution method for the determination of phosphate in natural waters. **Analytica Chimica Acta 31**, v. 27, n. 27, p. 31–36, 1962.

NANNIPIERI, P. et al. Role of Phosphatase Enzymes in Soil. Em: [s.l: s.n.]. p. 215–243.

OBURGER, E. et al. Interactive effects of organic acids in the rhizosphere. **Soil Biology and Biochemistry**, v. 41, n. 3, p. 449–457, 2009.

PEIFFER, J. A. et al. Supporting Information. **Pnas**, v. 2, n. 6, p. 1–14, 2013a.

PEIFFER, J. A. et al. Diversity and heritability of the maize rhizosphere microbiome under field conditions. **Proceedings of the National Academy of Sciences of the United States of America**, v. 110, n. 16, p. 6548–6553, 2013b.

RADERSMA, S.; GRIERSON, P. F. Phosphorus mobilization in agroforestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. **Plant and Soil**, v. 259, n. 1–2, p. 209–219, 2004.

RAYA-HERNÁNDEZ, A. I. et al. Field evidence for maize-mycorrhiza interactions in agroecosystems with low and high P soils under mineral and organic fertilization. **Applied Soil Ecology**, v. 149, p. 103511, 1 maio 2020.

RICHARDSON, A. E.; SIMPSON, R. J. Soil Microorganisms Mediating Phosphorus Availability Update on Microbial Phosphorus. **Plant Physiology**, v. 156, n. 3, p. 989–996, 1 jul. 2011.

RODRIGUES, L. A. T. et al. Short- and long-term effects of animal manures and mineral fertilizer on carbon stocks in subtropical soil under no-tillage. **Geoderma**, v. 386, p. 114913, 15 mar. 2021.

RUBIO, G. et al. Rhizosphere phosphorus depletion by three crops differing in their phosphorus critical levels. **Journal of Plant Nutrition and Soil Science**, v. 175, n. 6, p. 810–871, dez. 2012.

SCHNEIDER, K. D. et al. Options for Improved Phosphorus Cycling and Use in Agriculture at the Field and Regional Scales. **Journal of Environmental Quality**, v. 48, n. 5, p. 1247–1264, set. 2019.

SOIL SURVEY STAFF. Keys to soil taxonomy. **Soil Conservation Service**, v. 12, p. 410, 2014.

STOFFEL, S. C. G. et al. Yield increase of corn inoculated with a commercial arbuscular mycorrhizal inoculant in Brazil. **Ciência Rural**, v. 50, n. 7, 2020.

SUN, B. et al. The relative contributions of pH, organic anions, and phosphatase to rhizosphere soil phosphorus mobilization and crop phosphorus uptake in maize/alfalfa polyculture. **Plant and Soil**, v. 447, n. 1–2, p. 117–133, 1 fev. 2020.

SYERS, J. K.; JOHNSTON, A. E.; CURTIN, D. Efficiency of soil and fertilizer phosphorus use. **FAO Fertilizer and plant nutrition Bulletin,** p. 18-108, 2008.

TEDESCO, M.; GIANELLO, C.; BISSANI, C. Análises de solo, plantas e outros materiais. **UFRGS**, p. 174, 1995.

VANCE, E. D.; BROOKES, P. C.; JENKINSON, D. S. An extraction method for measuring soil microbial biomass C. **Soil Biology and Biochemistry**, v. 19, n. 6, p. 703–707, 1987.

WAHID, F. et al. Arbuscular mycorrhizal fungi improve the growth and phosphorus uptake of mung bean plants fertilized with composted rock phosphate fed dung in alkaline soil environment. **Journal of Plant Nutrition**, v. 42, n. 15, p. 1760–1769, 2019.

WANG, F.; FENG, G. Arbuscular Mycorrhizal Fungi Interactions in the Rhizosphere. p. 217–235, 2021.

WANG, Y.; LAMBERS, H. Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. **Plant and Soil**, v. 2050, n. Fao 2009, 2019.

ZHANG, D. et al. Carbon–Phosphorus Coupling Governs Microbial Effects on Nutrient Acquisition Strategies by Four Crops. **Frontiers in Plant Science**, v. 13, 5 jul. 2022.

ZHANG, Y. et al. Crop Production and Phosphorus Legacy with Long-Term Phosphorus- and Nitrogen-Based Swine Manure Applications under Corn-Soybean Rotation. **Agronomy 2021, Vol. 11, Page 1548**, v. 11, n. 8, p. 1548, 1 ago. 2021.

ZHU, J.; LI, M.; WHELAN, M. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. **Science of the Total Environment**, v. 612, p. 522–537, 2018.

6.7 TABLES AND FIGURES

Table 1. Chemical attributes of the soil in the 0-0.1 m layer, before the implementation of the experiment (2004) and after 25 applications of organic and mineral sources of nutrients (August 2019)

Chemical atributes	**Initial (2004)** (1)	**Treatments (2019)**		
		Control	**Pig Slurry**	**Mineral Fertilizer**
pH_{H20} (1:1)	4,9	5,5	5,6	5,0
C (g kg^{-1}) (2)	19	15,7	21,25	16,7
Available P (mg kg^{-1}) (3)	20,3	5,8	119,1	74,4
Exchangeable K (mg kg^{-1}) (3)	60	35	109	90
Exchangeable Ca ($cmol_c$ kg^{-1}) (4)	0,8	1,9	2,7	1,7
Exchangeable Mg ($cmol_c$ kg^{-1}) (4)	0,3	0,9	1,5	0,8
Exchangeable Al ($cmol_c$ kg^{-1}) (4)	0,03	0	0	0,5
H+Al ($cmol_c$ kg^{-1}) (4)	3,7	2,8	2,6	4,7
$CTC_{ecfetive}$ ($cmol_c$ kg^{-1})	1,3	3,05	4,5	3,2
$CTC_{pH\ 7,0}$ ($cmol_c$ kg^{-1})	5	5,8	7,1	7,4
Base saturation (%)	25,4	51,3	63,1	37,7
Al saturation (%)	2	0,0	0	16,2

(1)Data from Lourenzi et al. (2014); (2)Determined by TOC; (3)Extracted by Mehlich-1; (4)Extracted by KCl 1 $molL^{-1}$

Table 2. Carbon, nitrogen, and phosphorus in microbial biomass (C-BIO, N-BIO, P-BIO) in samples collected in rhizospheric and no rhizospheric soil of the black oat and maize inoculates and no inoculates with FMA, in no-tillage area after 25 applications of treatments, respectively, during fifteen years.

Coleta	**Data**	**Control**			**Mineral Fertilizer**			**Pig Slurry**		
		Rizo + FMA	**Rizo - FMA**	**Bulk Soil**	**Rizo + FMA**	**Rizo - FMA**	**Bulk Soil**	**Rizo + FMA**	**Rizo - FMA**	**Bulk Soil**
		Carbon – BIO (mg kg^{-1}soil)								
Fallow	Out- 2019			52,86B			75,87A			76,54A
V8 - Corn	Dez - 2019	47,97Ab	95,12Aa	50,73Ab	58,71Ab	100,12Ab	33,62Aa	57,91Aa	92,73Ab	31,63Aa
R1 - Corn	Jan - 2020	120,48Aa	74,23Ab	100,86Ab	85,79Bab	45,51Aa	93,33Ab	81,17Bab	63,49Ab	109,67Aa
R6 - Corn	Fev - 2020	26,05Ab	20,74Ab	72,49Aa	24,32Aa	19,07Aa	23,28Ba	24,93Aa	31,93Aa	24,32Ba
R1 - Oat	Ago - 2020	41,21Ba	17,02Aa	28,04Aa	106,72Aa	26,65Bb	38,54Ab	61,64Ba	41,89Ba	29,60Aa
Fallow	Set - 2020			49,80C			89,97A			68,96B
V8 - Corn	Nov - 2020	84,97Aa	111,61Aa	44,84Ab	93,01Aa	40,29Ba	59,91Aa	106,83Ba	52,60Bb	57,82Ab
R1 - Corn	Dez - 2020	60,21Bb	109,99Aa	7,83Ba	117,13Aa	109,29Aa	26,48Bb	125,11Aa	76,39Bb	59,87Ab
R6 - Corn	Fev - 2021	73,93Aa	87,14Aa	65,95Aa	81,77Aa	84,96Aa	71,25Aa	79,68Aa	49,42Ba	73,93Aa
R1 - Oat	Set – 2021	233,19Ca	265,29Ba	113,85ABb	276,04Ba	275,70Ba	96,02Bb	391,52Aa	366,23Aa	139,94Ab
		Nitrogen – BIO (mg kg^{-1}soil)								
Fallow	Out- 2019			56,56A			54,40A			57,61A
V8 - Corn	Dez - 2019	115,50Aa	96,04Aa	30,25Ab	37,37B	53,55B	44,87A	44,22B	71,51AB	19,48A
R1 - Corn	Jan - 2020	108,47Ba	76,74Ab	10,47C	107,32Bc	23,75B	40,37B	154,96A	90,78A	117,81A
R6 - Corn	Fev - 2020	10,69Bb	62,25Ab	25,32B	31,47B	30,61B	8,92B	58,89A	52,60A	75,84A
R1 - Oat	Ago - 2020	89,46B	89,73B	18,95B	128,75A	110,10AB	33,41AB	116,83A	181,99A	50,30A

Fallow	Set - 2020			5,40B			5,54B			9,26A
V8 - Corn	Nov - 2020	46,55A	14,45A	35,17A	47,13A	13,93A	22,84A	26,47A	26,30A	34,32A
R1 - Corn	Dez - 2020	11,25A	41,52A	24,03A	6,87A	9,89B	18,50A	14,59A	32,82AB	26,44A
R6 - Corn	Fev - 2021	101,31A	38,72A	29,72A	97,22A	58,93A	32,74A	31,84B	47,05A	13,90A
R1 - Oat	Set – 2021	50,74B	32,41B	58,18B	138,2Aa	121,53A	117,27A	100,81A	96,55A	66,19B
		Phosphorus – BIO (mg kg^{-1}soil)								
Fallow	Out- 2019			18,01B			25,53A			27,22A
V8 - Corn	Dez - 2019	3,86Ba	2,16Ba	3,07Ca	3,07Bb	4,29Bb	9,99Ba	12,69Aa	14,44Aa	13,46Aa
R1 - Corn	Jan - 2020	1,74Ba	2,60Aa	2,15Aa	6,83Aa	5,83Aab	3,12Aa	3,68Aa	5,83Aa	5,13Aa
R6 - Corn	Fev - 2020	2,37Aa	2,81Aa	1,95Aa	4,34Aa	3,21Aa	3,45Aa	1,56Aa	1,70Aa	2,43Aa
R1 - Oat	Ago - 2020	4,26Aa	1,87Ca	2,67Ca	4,42Ab	14,13Aa	6,17Bb	6,62Aa	9,57Bb	20,60Aa
Fallow	Set - 2020			1,60C			20,35A			13,58B
V8 - Corn	Nov - 2020	1,14Ba	0,94Ba	0,73Ca	2,14Bb	6,65Aa	7,09Ba	16,60Ab	9,52Ac	20,39Aa
R1 - Corn	Dez - 2020	1,71Ca	1,24Ca	0,40Ca	9,68Ba	8,80Bab	5,74Bb	18,12Ab	23,96Aa	13,58Ac
R6 - Corn	Fev - 2021	2,09Ca	1,72Ca	1,37Ca	11,87Ba	11,77Ba	9,60Ba	15,41Aa	17,38Aa	18,62Aa
R1 - Oat	Set – 2021	5,30Ba	5,77Ca	3,66Ba	11,78Ba	9,57Aa	8,90Aa	8,48ABa	6,59ABa	7,97Aa

Rhizo + AMF = Rhizospheric soil of plants inoculated with AMF; Rhizo - AMF = Rhizospheric soil of uninoculated plants; Bulk Soil = Soil collected between the crop lines (n=4). Different capital letters indicate significant difference between treatments within each soil and lowercase letters indicate significant difference between soils within each treatment by Tukey's test ($p < 0.05$).

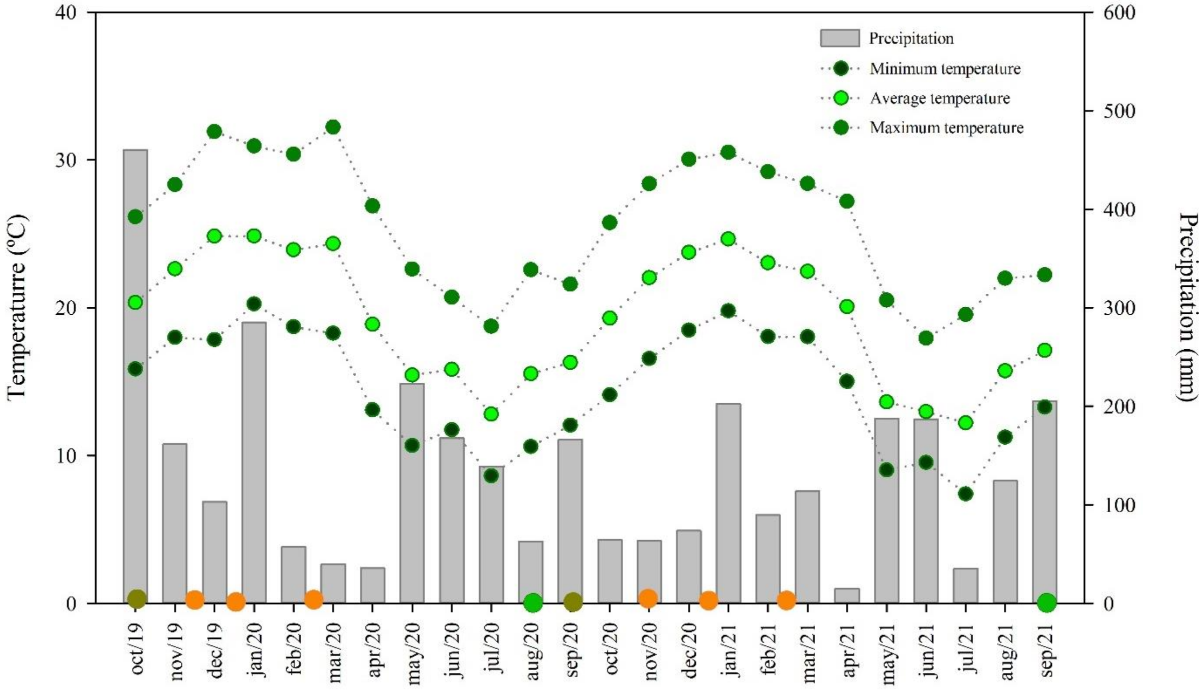


Figure 1. Average precipitation, maximum, minimum and average air temperature, in the agricultural year of 2019/2020, 2020, 2020/2021 and 2021 in Santa Maria (RS).

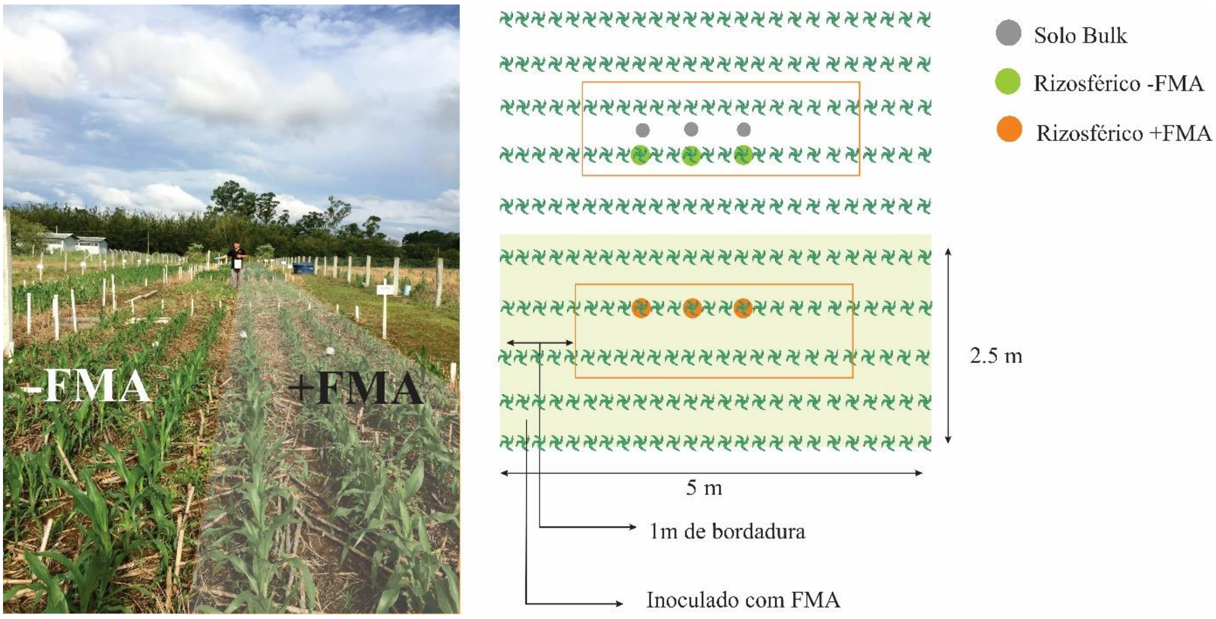


Figure 2. Experimental scheme, with details of how a rhizospheric soil sample was collected.

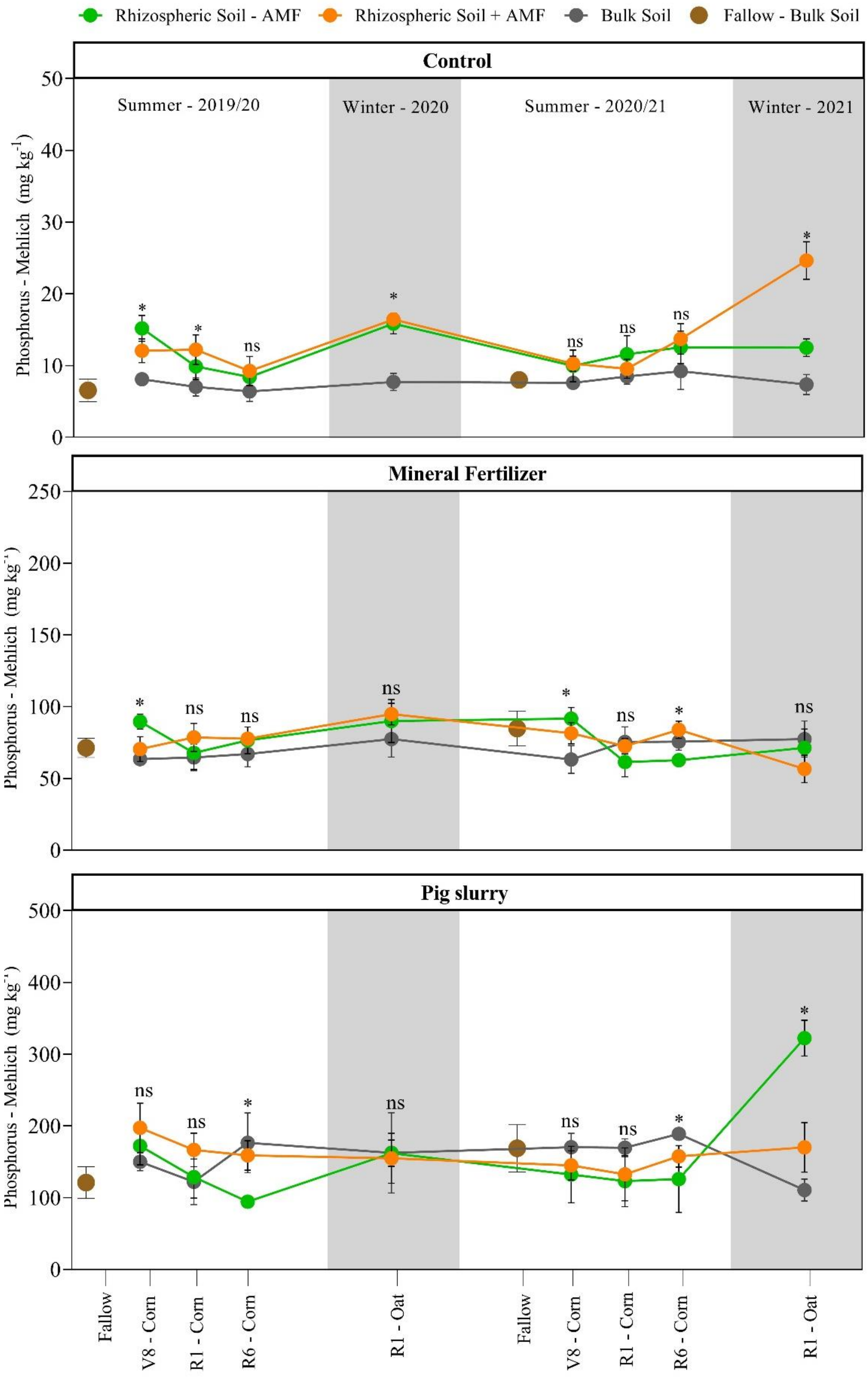


Figure 3. Available phosphorus measured on 10 occasions during the period from October 2019 to September 2021 in bulk soil and rhizospheric soil of corn inoculated and non-inoculated with FMA in soil cultivated in a no-tillage system with 15 years of application of swine manure, mineral fertilizer and no fertilizer application (control). Asterisks indicate significant treatment effect (*P<0.05; **P<0.01; ns = not significant).

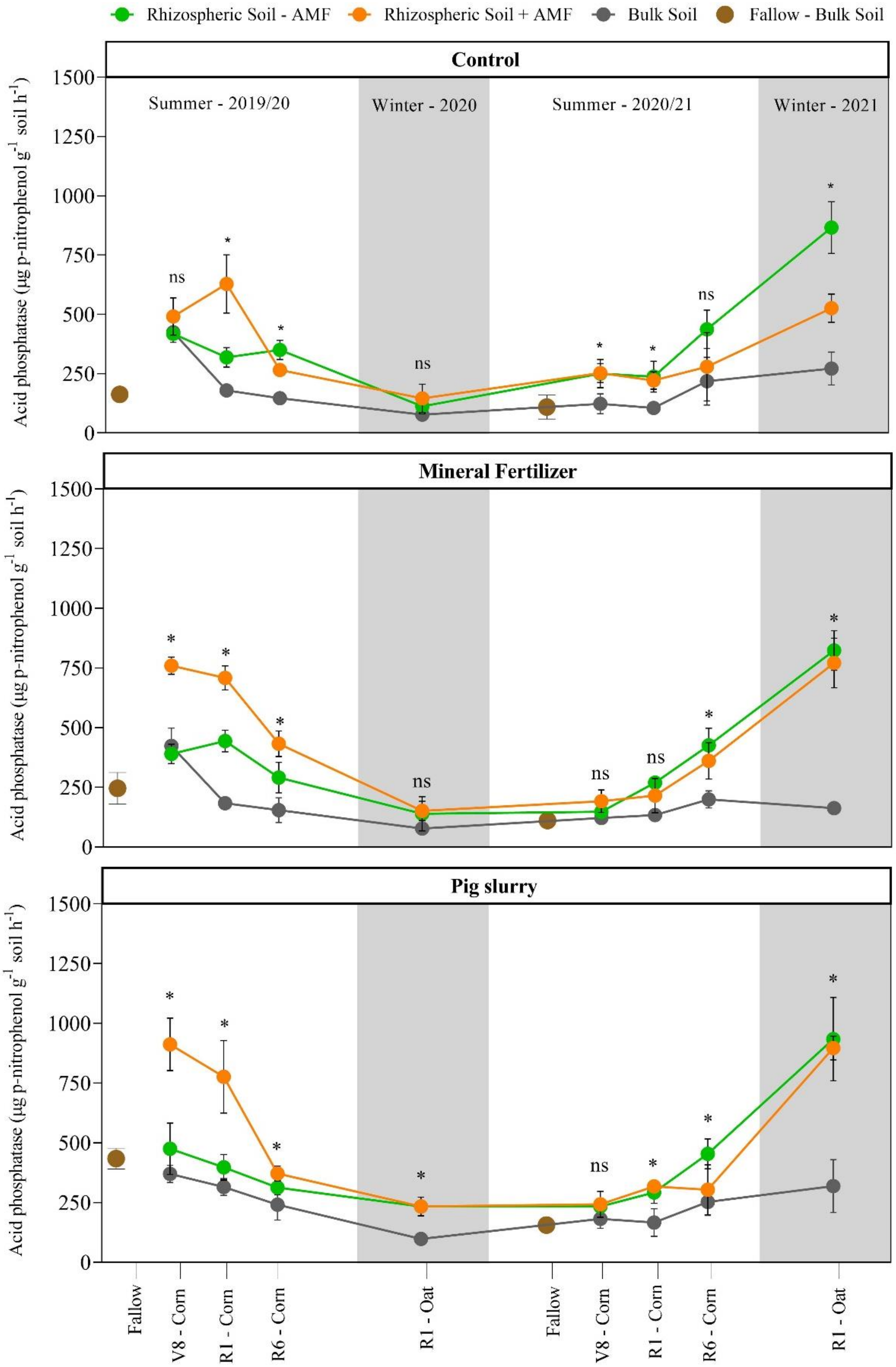


Figure 4. Acid phosphatase activity measured on 10 occasions during the period from October 2019 to September 2021 in bulk soil and rhizospheric soil of corn inoculated and non-inoculated with FMA in soil cultivated in a no-tillage system with 15 years of application of swine manure, mineral fertilizer and no fertilizer application (control). Asterisks indicate significant treatment effect (*P<0.05; **P<0.01; ns = not significant).

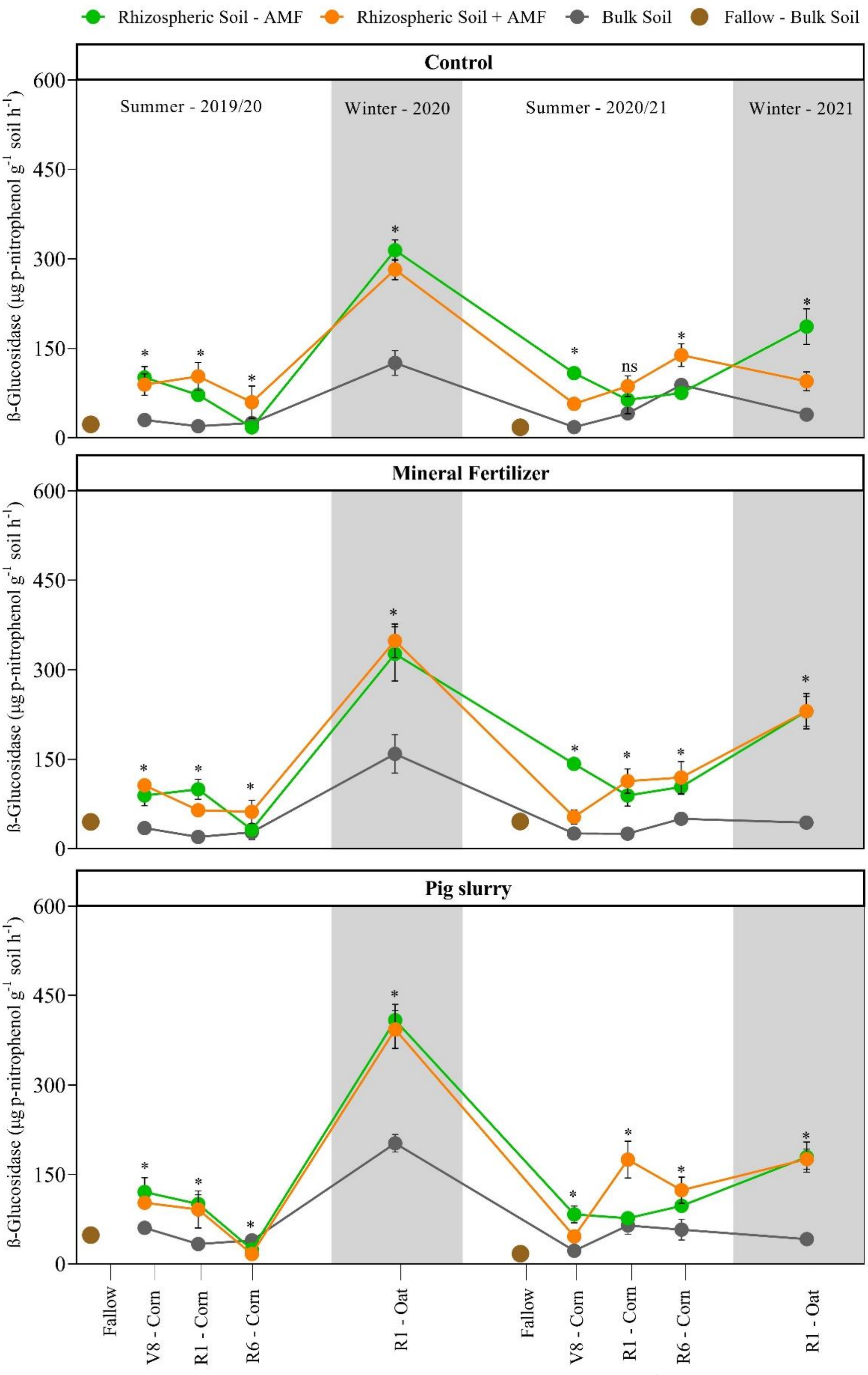


Figure 5. B-glucosidase activity measured on 10 occasions during the period from October 2019 to September 2021 in bulk soil and rhizospheric soil of corn inoculated and non-inoculated with FMA in soil cultivated in a no-tillage system with 15 years of application of swine manure, mineral fertilizer and no fertilizer application (control). Asterisks indicate significant treatment effect (*P<0.05; **P<0.01; ns = not significant).

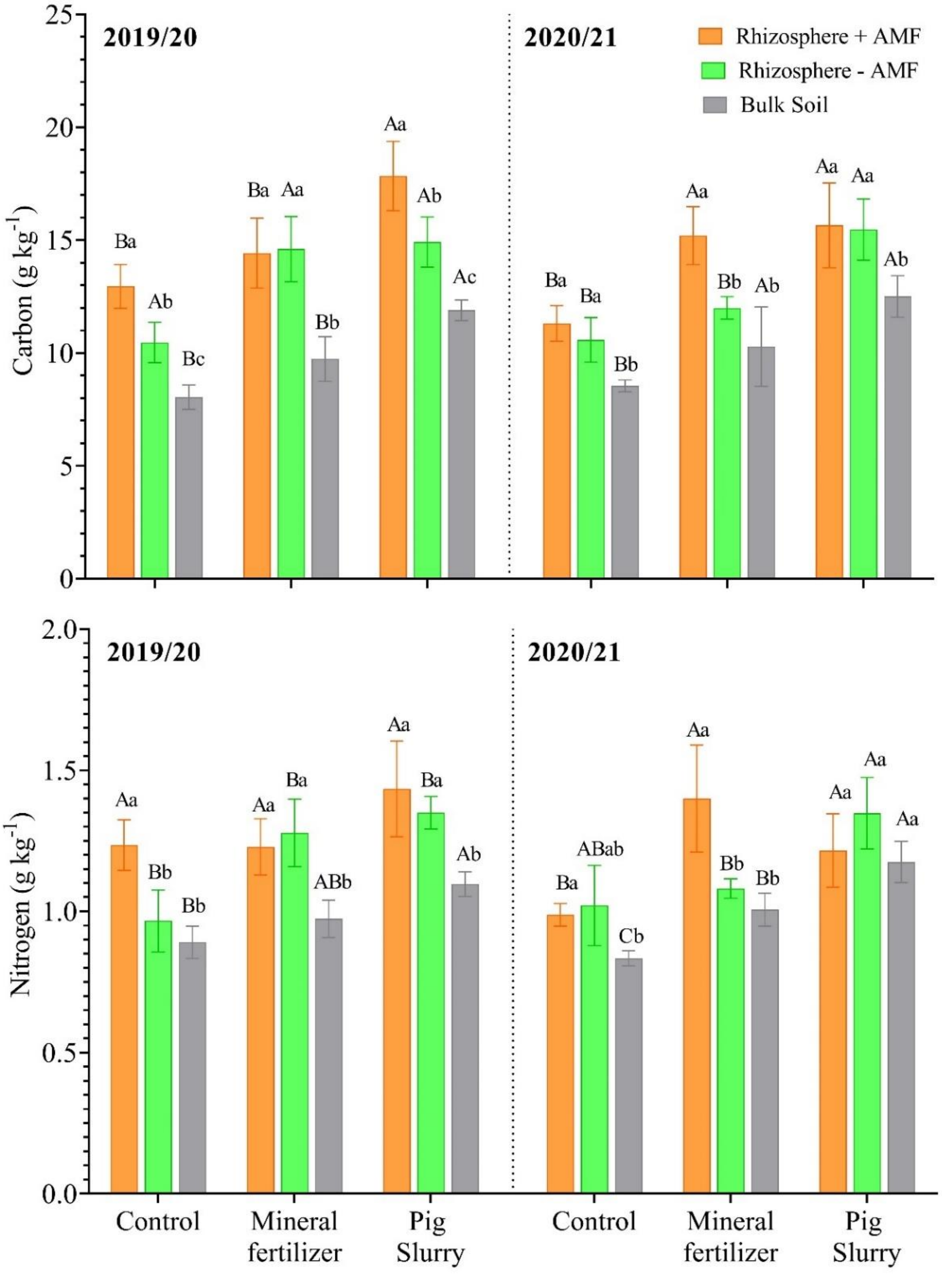


Figure 6. Total carbon and total nitrogen in bulk soil and rhizospheric soil of inoculated and non-inoculated maize with AMF grown during the 2019/20 (a and c) and 2020/21 (b and d) seasons in soil with 15 years of swine manure application and mineral fertilizer and without fertilizer application (control). Different letters between soil at the same treatment indicate a significant difference (Tukey test at 5%). Standard deviation is present the surface of the bar.

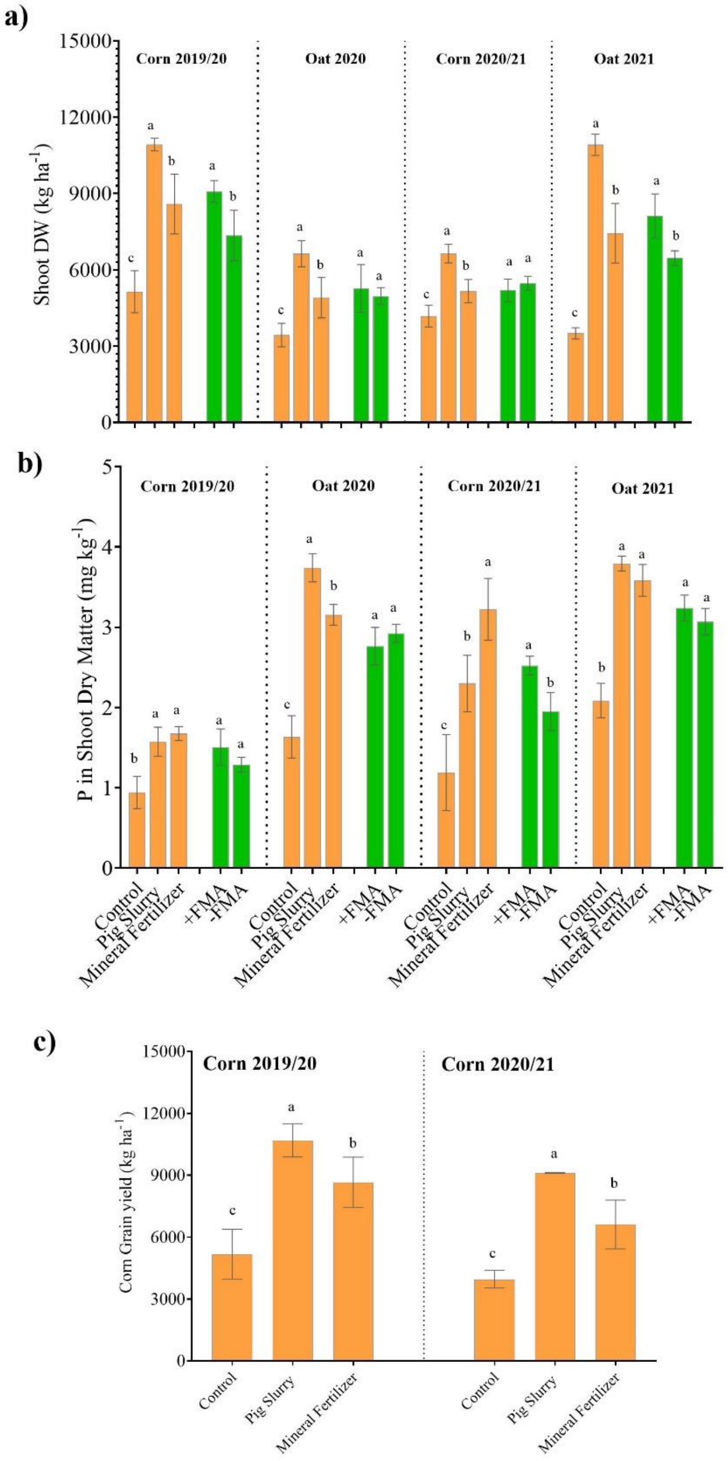


Figure 7. Dry matter production of the aerial part (a) and P concentration in dry matter (b) of inoculated and non-inoculated corn and oat with AMF in soil with 15 years of application of swine liquid jet and mineral fertilizer and without fertilizer application. Different letters between soil at the same treatment indicate a significant difference (Tukey test at 5%). Standard deviation is present the surface of the bar.

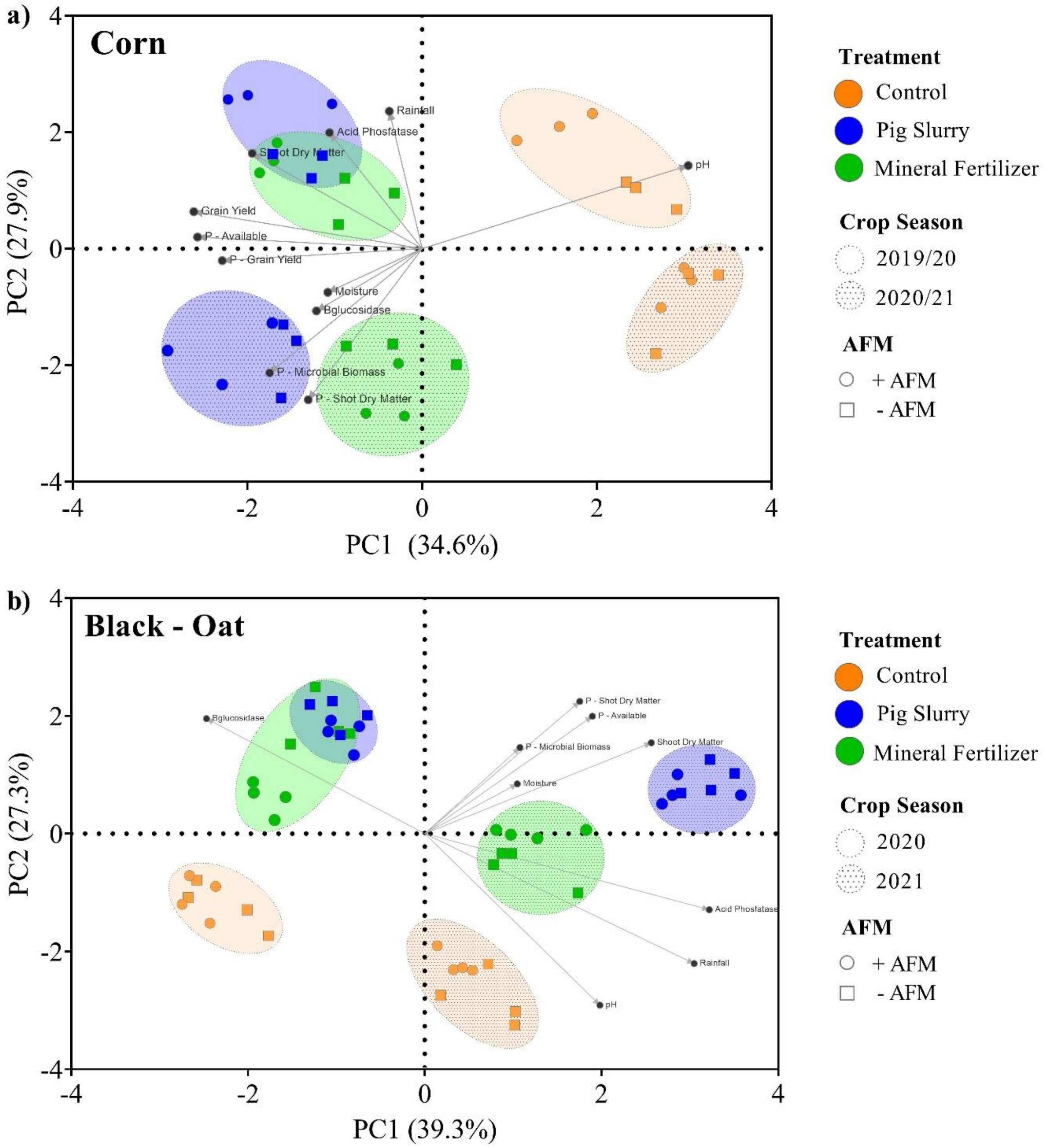


Figure 8. Relationship between principal component 1 (PC1) and principal component 2 (PC2) on yield variables of maize (a) and oats (b) (aerial part dry matter, grain yield and P concentration in tissue and grain), accumulated precipitation, main soil chemical and biochemical characteristics (microbial P, available P, pH, acid phosphatase and B-glucosidase enzymes and soil moisture). Corn and oats were grown on soils with a 15-year history of pig slurry application, mineral fertilizer, and no fertilization (Control), with and without arbuscular mycorrhizal fungus (AMF) inoculation. Data were obtained at flowering of corn crop 2019/20 and 2020/21 and flowering of oat crop 2020 and 2021.

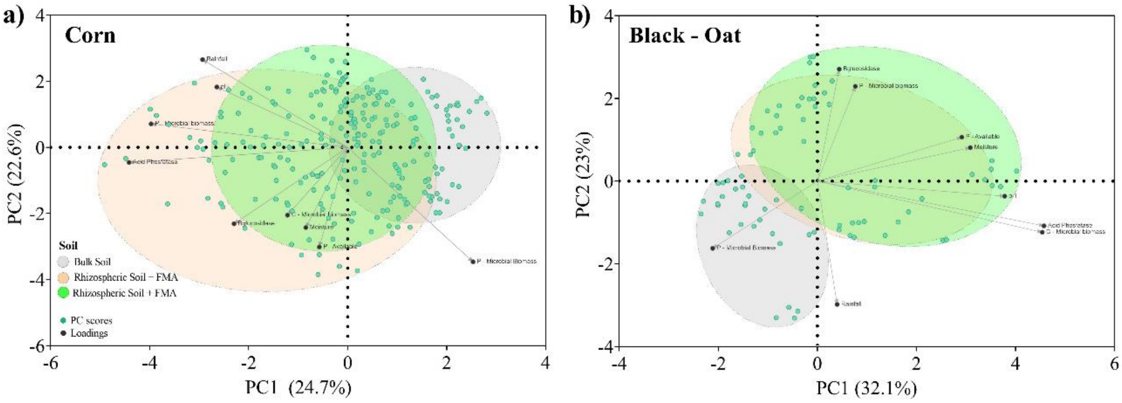


Figure 9. Relationship between principal component 1 (PC1) and principal component 2 (PC2) on chemical and biochemical variables in rhizospheric soil inoculated with arbuscular mycorrhizal fungi (AMF) and non-rhizospheric soil of maize (a) and oats (b), accumulated precipitation and soil moisture. Maize and oats were grown on soils with a 15-year history of pig slurry application, mineral fertilizer, and no fertilization (Control), with and without inoculation of arbuscular mycorrhizal fungi (AMF). Data were obtained at fallow, vegetative stage, flowering and senescence of the 2019/20 and 2020/21 corn crop and at flowering of the 2020 and 2021 oat crop. Loadings: 240 observations and 72 observations in the oat.

6.8 SUPPLEMENTARY TABLES AND FIGURES

Table S1. Analysis of variance of the analyzed data evaluating the different sources of fertilization (T) in the rhizospheric and non-rhizospheric soil of corn and oats inoculated and not inoculated with AMF (S) over time (C)

	Treatment (T)	Soil (S)	Collection time (C)	T*S	T*C	C*S	T*S*C	CV (%)
Soil moisture (%)	**	**	**	**	**	**	**	21.77
pH	**	**	**	ns	**	**	**	4.49
P disponível (mgkg^{-1})	**	**	**	ns	**	**	**	20.32
Carbon – BIO (mg kg^{-1}soil)	**	**	**	**	**	**	**	22.50
Nitrogen – BIO (mg kg^{-1}soil)	**	**	**	**	**	**	**	29.22
Phosphorus – BIO (mg kg^{-1}soil)	**	**	**	**	**	**	**	28.75
Acid Phosfatase (p-nitrofenol kg^{-1} soil h^{1})	**	**	**	*	**	**	**	19.09
B-Glicosidase (p-nitrofenol kg^{-1} soil h^{-1})	**	**	**	**	**	**	**	21.00

*P<0.05,**P<0.01

Table S2. Values of pH in H_2O and moisture in samples collected in rhizospheric and no rhizospheric soil of the black oat and maize inoculates and no inoculates with FMA in no-tillage area after 25 applications of the treatments, respectively, during fifteen years.

		Control			**Mineral Fertilizer**			**Pig Slurry**		
Coleta	**Ano**	**Rizo + FMA**	**Rizo - FMA**	**Bulk**	**Rizo + FMA**	**Rizo - FMA**	**Bulk**	**Rizo + FMA**	**Rizo - FMA**	**Bulk**
						pH in H_2O				
Fallow	Out- 2019			5,78A			4,84B			5,23A
V8 - Corn	Dez - 2019	5,31Aa	5,62Aa	5,32Aa	5,10Aa	4,93Bab	4,60Bb	5,29Aa	5,27ABa	5,01Aa
R1 - Corn	Jan - 2020	5,91Aa	5,80Aa	5,54Aa	5,00Bab	5,13Ba	4,72Bb	5,26Ba	5,32Ba	5,17Aa
R6 - Corn	Fev - 2020	5,88Aa	5,69Aab	5,33Ab	5,23Ba	4,91Bab	4,55Bb	5,39Ba	5,42Aa	5,22Aaa
R1 - Oat	Ago - 2020	4,94Ab	5,37Aa	4,80Ab	4,82Aa	4,83Ba	4,47Aa	5,05Aa	4,98Ba	4,71A
Fallow	Set - 2020			4,90A			4,90A			4,99A
V8 - Corn	Nov - 2020	5,57Aa	5,31Aa	5,23Aa	5,00Ba	4,77Bab	4,60Bb	5,01Ba	5,27Aa	5,08Aa
R1 - Corn	Dez - 2020	5,53Aa	5,41Aa	4,92ABb	4,76Ba	4,79Ba	4,66Ba	5,00Ba	5,28Aa	5,05Aa
R6 - Corn	Fev - 2021	5,28Aa	5,17ABab	4,82Ab	4,88Ba	5,02Ba	4,44Ab	5,57Aa	5,51Aa	4,72Ab
R1 - Oat	Set – 2021	5,40Aab	5,88Aa	5,08Ab	5,12Aa	5,36Ba	4,56Ba	5,43Aa	5,53ABa	5,00Ab
						Soil moisture (%)				
Fallow	Out- 2019			15,60A			8,53B			8,05B
V8 - Corn	Dez - 2019	18,77Aa	8,33Bb	16,37Aa	13,98Ba	14,37Aa	12,40Aa	5,45Ca	5,34Ba	7,39Ba
R1 - Corn	Jan - 2020	8,11Ba	11,32Ba	7,51Aa	14,39Aa	15,77Aa	6,90Aa	12,89Aa	11,46Ba	7,11Ab
R6 - Corn	Fev - 2020	8,64Aa	11,60Ba	10,77Ba	12,12Ab	17,71Aa	9,51Bb	9,35Aa	7,49Ba	15,04Ab
R1 - Oat	Ago - 2020	14,54Aa	8,39Bb	13,24Aa	11,80Aa	14,78Aa	13,04Aa	6,11Ba	5,22Ba	7,46Ba

Fallow	Set - 2020			8,95B			15,92A			6,13B
V8 - Corn	Nov - 2020	11,19Aa	6,51Bb	9,70Bab	18,89Ba	9,78Bb	17,05Aa	14,37Aa	17,92Aa	14,36Aa
R1 - Corn	Dez - 2020	11,64ABa	14,47Ba	7,21Ab	7,76Ba	5,30Ca	9,01Aa	14,87Ab	19,95Aa	9,13Ac
R6 - Corn	Fev - 2021	7,74Ba	7,84Ca	13,42Ab	7,55Bb	13,10Ba	4,63Bb	18,78Aa	17,59Aa	8,09Bb
R1 - Oat	Set – 2021	13,62Ba	8,05Bb	8,67Bb	17,16Ba	9,88Bb	13,28Aab	22,26Aa	22,57Aa	8,51Bb

Rhizo + AMF = Rhizospheric soil of plants inoculated with AMF; Rhizo - AMF = Rhizospheric soil of uninoculated plants; Bulk Soil = Soil collected between the crop lines (n=4). Different capital letters indicate significant difference between treatments within each soil and lowercase letters indicate significant difference between soils within each treatment by Tukey's test ($p < 0.05$).

Table S3. Pearson correlation coefficients between corn yield variables and soil chemical and biochemical parameters.

	Rainfall	Moisture	pH	P - Available	P - Bio	C - Bio	N - Bio	Acid Phosfatase	β-glucosidase	DM	P - DM	Yield	P - Grain
Rainfall		ns	**	ns	**	**	**	**	**	ns	ns	ns	ns
Moisture	-0,08		ns	**	**	ns	ns	*	**	ns	ns	ns	ns
pH	**0,25**	0,03		**	**	ns	**	**	ns	ns	**	ns	*
P - Available	0,00	**0,20**	**-0,21**		**	ns	ns	**	ns	**	**	**	**
P - Bio	**-0,33**	**0,40**	**-0,22**	**0,68**		*	*	ns	**	ns	**	*	*
C - Bio	**-0,23**	0,13	0,06	-0,02	**0,17**		*	ns	**	ns	ns	ns	ns
N - Bio	**0,36**	-0,02	**0,26**	0,06	**-0,16**	**0,17**		**	*	**	ns	ns	ns
Acid Phosfatase	**0,47**	**0,16**	**0,25**	**0,20**	0,05	0,11	**0,51**		**	*	ns	*	*
B-glucosidase	**-0,24**	**0,20**	0,10	0,12	**0,27**	**0,40**	**0,14**	**0,36**		ns	ns	ns	*
Shoot DM	0,13	-0,07	-0,08	**0,32**	0,18	-0,01	**0,28**	**0,24**	0,03		**	**	*
P - Shoot	0,07	0,20	**-0,31**	**0,29**	**0,27**	0,12	-0,08	0,08	0,18	**-0,37**		ns	*
Grain Yield	0,32	0,20	-0,21	**0,86**	**0,40**	-0,02	0,28	**0,37**	0,31	**0,78**	0,20		**
P - Grain Yield	0,10	0,31	**-0,40**	**0,47**	**0,41**	0,29	0,02	**0,38**	**0,39**	**0,37**	**0,42**	**0,56**	

Correlation is significant if $p \leq 0.05$ (*) or $p \leq 0.01$ (**)

Table S4. Pearson correlation coefficients between oat yield variables and soil chemical and biochemical parameters.

	Rainfall	**Moisture**	**pH**	**P - Available**	**P - Bio**	**C - Bio**	**N - Bio**	**Acid Phosfatase**	**β-glucosidase**	**DM**	**P - DM**
Rainfall		ns	**	ns	ns	**	**	**	**	ns	ns
Moisture	-0,03		ns	ns	ns	ns	ns	ns	ns	ns	ns
pH	**0,87**	0,10		ns	ns	**	**	**	**	ns	ns
P - Available	-0,08	0,45	-0,26		ns	ns	ns	ns	ns	**	**
P - Bio	-0,05	-0,28	-0,38	0,10		ns	ns	ns	ns	ns	**
C - Bio	**0,99**	0,07	**0,84**	0,08	-0,08		**	**	**	**	ns
N - Bio	**-0,90**	-0,07	**-0,91**	0,37	0,18	**-0,85**		**	**	ns	ns
Acid Phosfatase	**0,99**	0,02	**0,85**	0,01	-0,05	**0,99**	**-0,87**		**	ns	ns
B-glucosidase	**-0,98**	-0,08	**-0,93**	0,12	0,17	**-0,97**	**0,92**	**-0,97**		ns	ns
Shoot DM	0,51	0,26	0,19	**0,70**	0,30	**0,62**	-0,22	0,58	-0,42		**
P - Shoot	-0,14	-0,09	-0,49	**0,75**	**0,61**	-0,05	0,50	-0,09	0,26	**0,61**	

Correlation is significant if $p \leq 0.05$ (*) or $p \leq 0.01$ (**)

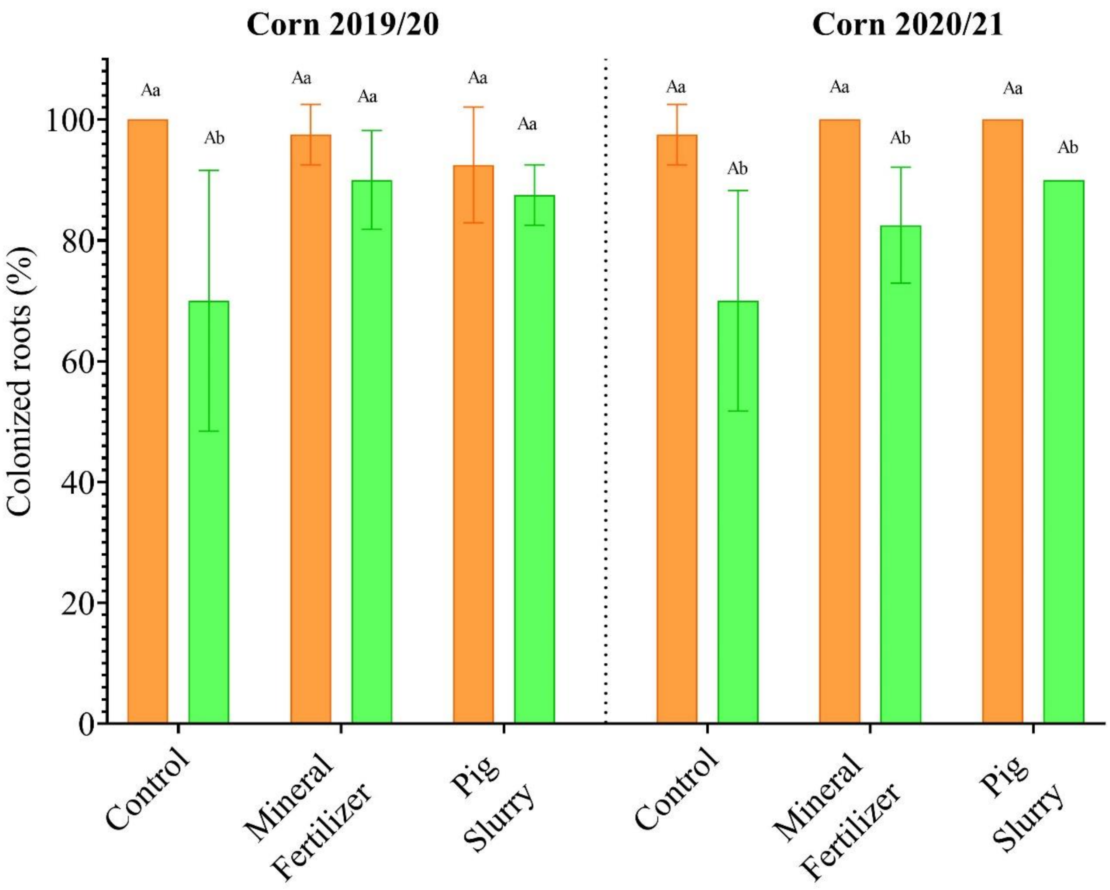


Figure S1. Percent of corn root colonized with AMF in soil with 15 years of application of swine liquid jet and mineral fertilizer and without fertilizer application. Different letters between soil at the same treatment indicate a significant difference (Tukey test at 5%). Standard deviation is present the surface of the bar.

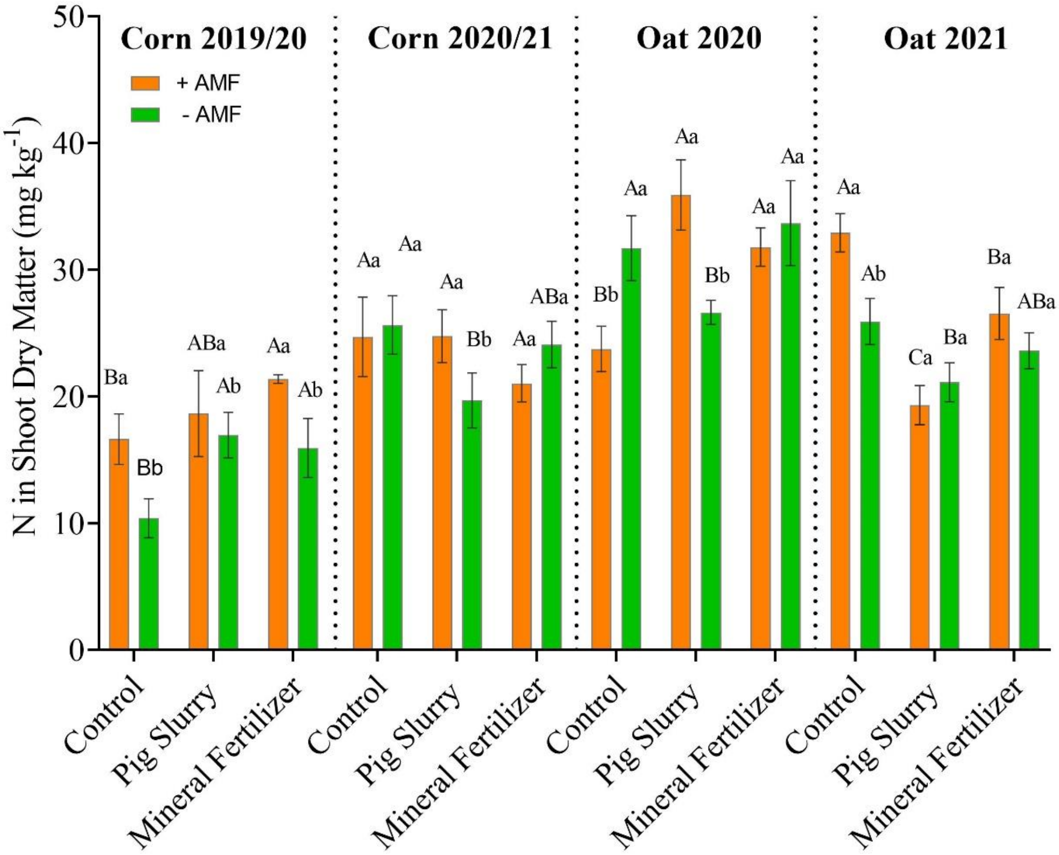


Figure S2. N concentration in dry matter of inoculated and non-inoculated corn and oat with AMF in soil with 15 years of application of swine liquid jet and mineral fertilizer and without fertilizer application. Different letters between soil at the same treatment indicate a significant difference (Tukey test at 5%). Standard deviation is present the surface of the bar.

7. DISCUSSÃO GERAL

7.1 ESTRATÉGIAS DE AQUISIÇÃO DE P

A adição continuada de fósforo (P), seja por meio de dejetos de animais ou por adubação fosfatada e seu acúmulo no solo a longo prazo, é uma condição comum na maioria dos solos de agroecossistemas altamente produtivos em todo o Mundo. Diante disso, no **Capítulo I** nós buscamos quantificar o impacto das adições sucessivas de P via dejetos de animais e fertilizante fosfatado, em solo arenoso cultivado sob sistema de plantio direto por 12 anos. Os resultados do balanço de massa de P nos mostraram que o fertilizante mineral, seguido dos dejetos líquidos, obtiveram maior eficiência no aproveitamento com menores perdas de P. As entradas de P via fertilizante mineral, dejetos de bovinos, dejetos de suínos e cama sobreposta de suínos promoveram a acumulação de 18, 42, 48 e 100 kg P ha^{-1} ano^{-1}. Neste estudo, quantificamos as reservas e as formas de P predominante em cada solo (Pi e Po).

Como discutido na introdução, as adições de P ao longo do tempo podem gerar reservas de P no solo, que pode sustentar a agricultura durante décadas. No entanto, grande parte do P acumulado em solos agrícolas se encontram em formas indisponíveis para a absorção das plantas. Por outro lado, as plantas, desenvolvem um conjunto de respostas fisiológicas e bioquímicas de adaptação em solos com diferentes disponibilidades de P. Diante disso, no **Capítulo II** investigamos as alterações do sistema radicular de raízes de milho e sua relação com a eficiência absorção, e utilização de P em condições de campo, em solo com diferentes reservas de P construídas com aplicações de dejetos de suínos e fertilizante mineral. Nesse estudo, nós observamos que plantas cultivadas no solo com histórico de aplicações de dejetos de suínos apresentaram os menores parâmetros morfológicos de raízes e maiores valores de eficiência de absorção de P, maior produção de biomassa e grãos. De maneira geral, observamos que em solos com baixo teor de P as plantas apresentaram maior crescimento radicular, que não necessariamente se converteu em maior produção de biomassa na parte aérea das plantas. Ou seja, a estratégia de investir em produção de raízes para aumentar o volume de solo explorado para aumentar a absorção de P, é uma estratégia onerosa que pode comprometer sua produção.

Outra estratégia das plantas e microrganismos em acessar as reservas de P no solo é alterar química e bioquimicamente a região próximas as raízes (rizosfera). Somado a isso, propomos a inoculação de FMAs como alternativa para aumentar a solubilização/mobilização do P do solo para formas mais lábeis, possibilitando assim, o maior aproveitamento de P pelas plantas. No entanto, o sistema radicular das plantas também possui papel importante nesse processo, pois pode acessar e aturar na solubilização/mobilização de P. Além disso, as plantas podem fazer outras associações com microrganismos do solo que podem contribuir para absorção de P. Diante disso, no **Capítulo III** avaliamos *in situ,* as atividades biológica e bioquímica como estratégias para aumentar a disponibilidade de P em solos rizosféricos de milho e aveia micorrizadas, cultivadas em solo com histórico de 15 anos de aplicações de dejetos líquidos de suínos e fertilizante mineral. Observamos em condições de campo diferença entre o solo rizosférico e bulk. Os valores de atividade enzimática e C, N e P microbiano foram maiores nos solos rizosférico das plantas de milho e aveia em todos os tratamentos. Observamos claramente que a maior disponibilidade de P em todos os tratamentos está relacionada com a atividade enzima fosfatase na rizosfera do milho e da aveia; e que a maior absorção de P pelas plantas está diretamente correlacionada com atividade da enzima fosfatase. Nas condições desse estudo não observamos diferenças claras entre as atividades bioquímica e microbiológicas na disponibilidade de P no solo rizosférico das plantas inoculadas e não inoculadas com FMA. Observamos benefícios na absorção de P e produção de matéria seca da parte aérea do milho e da aveia inoculados com FMA, porém não observamos diferença na produção de grãos de milho.

A utilização das reservas de P do solo como uma possível estratégia também foi considerada. Nos **Capítulos II e III** é possível aprofundar o conhecimento sobre o comportamento das plantas e das simbioses radiculares, e sua interação com a comunidade microbiana na disponibilidade de P na rizosfera de diferentes culturas agrícolas em solos com diferentes reservas de P. Ampliamos a visão solo-planta, para uma visão mais aprofundada, solo/rizosfera-planta (Figura 4).

Abordamos a forma de P predominante em solos com diferentes fontes de adubação e os processos internos de regulação e utilização de P pela planta que sinalizam e potencializam diferentes estratégias na rizosfera para aumentar a aquisição de P. Observamos diferença nas estratégias de aquisição de P entre as plantas cultivadas nos solos com aplicação de dejetos de suínos e fertilizante mineral, ambos os solos possuem alta disponibilidade de P.

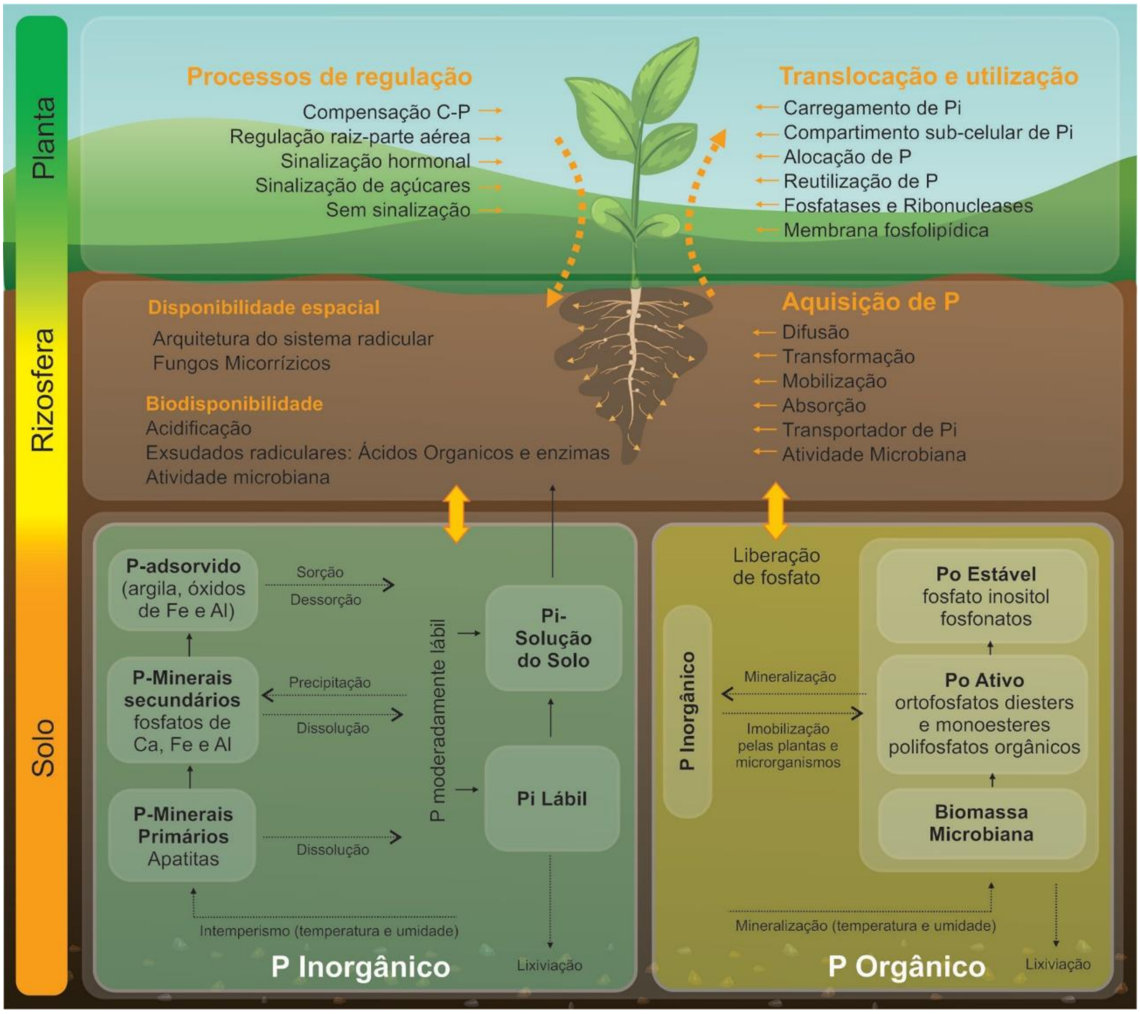


Figura 4. Resumo esquemático da dinâmica do P solo/rizosfera-planta. Adaptado de Shen et al. (2011). Fonte Carina Marchezan

Acreditamos que, as plantas cultivadas em solo com fertilizante mineral investiram em aumentar a disponibilidade espacial de P, apresentando maiores valores de comprimento área e volume de raízes. A similaridade na disponibilidade de P somado a menor atividade enzimática na rizosfera das plantas cultivadas no solo com aplicação de fertilizante mineral em relação as plantas cultivadas com dejetos de suínos, foi outro fator observado que contribui para essa afirmação. Por outro lado, as plantas cultivadas no solo com aplicação de dejetos de suíno a estratégia mais eficiente foi a de aumentar a biodisponibilidade de P. A aplicação de dejetos de animais ao longo dos anos incrementou C e N no solo possibilitando assim a maior atividade microbiana nesse solo. Observamos os menores valores de morfologia de raízes em relação ao fertilizante mineral, porém observamos maior atividade enzimática e maior fluxo de C, N e P no solo rizosférico dessas plantas. Infelizmente não conseguimos separar qual foi a contribuição dos microrganismos do solo e das raízes em aumentar a disponibilidade de P na rizosfera. Acreditamos a exsudação de C pelas raízes das plantas possibilitou maior atividade microbiana, e consequentemente de enzimas aumentando a disponibilidade de P.

Nas plantas cultivadas no solo controle observamos crescimento de raízes, com valores morfológicos muito próximos as plantas cultivadas no solo com aplicações de fertilizante mineral. Somado a isso observamos forte atividade enzimática e alta disponibilidade de P no solo rizosférico em relação ao solo bulk desse tratamento. Acreditamos que a maior contribuição da atividade enzimática nesse solo seja nas raízes das plantas, uma vez que a atividade microbiana nesse solo é inferior em relação aos solos com adubação. Por causa da baixíssima disponibilidade de P nesse solo acreditamos que as plantas investiram intensamente carbono para aumentara a disponibilidade espacial e a biodisponibilidade de P na rizosfera, o que pode ter prejudicado significativamente a produção de biomassa e grãos.

7.2 IMPLICAÇÕES TÉCNICAS E AMBIENTAIS

De maneira geral, nossos resultados fornecem informações importantes quanto ao uso de fontes secundárias de P como estratégias para diminuir a dependência da agricultura brasileira que também podem ser utilizadas por outros países tropicais e subtropicais. A utilização de dejetos de animais foi altamente eficiente em suprir a necessidade de P pelas culturas. Porém, em solos com a aplicações de dejetos de animais observamos incremento de formas de P solúveis nas camadas superficiais, o que representa um risco potencial de perdas de P por escoamento superficial. Caso as aplicações continuarem e nenhuma estratégia para mitigar o excesso de P no solo for implementada, a gestão utilizada pode potenciar vários riscos ambientais como a eutrofização de águas por exemplo. Isso sugere que precisamos de recomendações mais específicas para o uso de dejeto de animais como fonte de P, que leve em consideração a nutrição das plantas, mas também o fator ambiental atrelado ao se uso excessivo em solos com diferentes sistemas de cultivo. As estratégias de aquisição e P pelas plantas, em diferentes condições de solo, esclarece a contribuição das plantas e microrganismos da rizosfera na produção de biomassa e grãos. Esses resultados reforçam a necessidade da manutenção da atividade microbiológica do solo, como observamos no solo com aplicações de dejetos de suínos, foi de extrema importância no incremento da disponibilidade de P. Em nosso estudo não foi possível verificar a contribuição da FMA na rizosfera das plantas, no entanto observamos benefícios na produção de biomassa nas culturas da aveia e do milho pelo menos em uma safra, porém não se converteu em produtividade.

As informações geradas nesta tese sugerem um uso de dejetos de animais como fonte de P, mas faz alertas ao seu uso, especialmente, evitando a adição de P em excesso. Os resultados permitem diminuir o custo de produção, pela redução do fertilizante fosfatado mineral, bem como reflexos sociais pela maior satisfação do produtor, em função da melhoria na relação custo/benefício da atividade agropecuária. Isso porque, nossos resultados mostram que, nestas situações de uso intensivo de resíduos orgânicos, a atividade biológica e a biodisponibilidade de P na rizosfera possibilita que as plantas mantenham altas produtividade, acessando reservas de P construída ao longo dos anos.

8. CONSIDERAÇÕES FINAIS

As aplicações de dejetos de animais, especialmente os de suínos, geram grandes reservas de P em solos. O grande acúmulo de P nesses solos diminuí a eficiência do balanço de massas de P.

As plantas cultivadas em solos com reservas de P construídas com aplicação de dejetos de suínos apresentaram menores valores de variáveis morfológicas de raízes. Por outro lado, no solo rizosférico apresentaram maior disponibilidade de P, atividade enzimática e fluxo de C, N e P na biomassa.

As plantas cultivadas em solo com a aplicação de fertilizante mineral apresentaram maiores valores de variáveis morfológicas de raízes, porém não apresentou diferença na disponibilidade de P entre o solo rizosférico e bulk.

As plantas cultivadas no solo controlem apresentaram valores morfológicos próximos aos apresentados pelas plantas cultivadas no solo com aplicações de fertilizante mineral. No entanto a produção de biomassa dessas plantas foi bem inferior. A disponibilidade de P e a atividade enzimática na rizosfera foi superior ao solo bulk.

9. REFERÊNCIAS

ABPA - Associação Brasileira de Proteína Animal. Disponível em: <https://abpa-br.org/relatorios/>. Acesso em: 14 out. 2022.

ADELEKE, R.; NWANGBURUKA, C.; OBOIRIEN, B. Origins, roles and fate of organic acids in soils: A review. **South African Journal of Botany**, v. 108, p. 393–406, 2017.

ANUÁRIO Leite 2022: pecuária leiteira de precisão. - Portal Embrapa. Disponível em: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1144110/anuario-leite-2022-pecuaria-leiteira-de-precisao>. Acesso em: 14 out. 2022.

BARROW, N. J. A mechanistic model for describing the sorption and desorption of phosphate by soil. **European Journal of Soil Science**, v. 66, n. 1, p. 9–18, 1 jan. 2015.

BOITT, G. et al. Fate of phosphorus applied to soil in pig slurry under cropping in southern Brazil. **Geoderma**, v. 321, n. February, p. 164–172, 2018.

BOITT, G. et al. The fate of pig slurry phosphorus applied to a sandy loam soil under no-till cropping in southern Brazil. **Geoderma**, v. 422, 15 set. 2022.

COLUSSI, J.; SCHNITKEY, G.; ZULAUF, AND C. War in Ukraine and its Effect on Fertilizer Exports to Brazil and the U.S. **farmdoc daily**, v. 12, n. 34, 17 mar. 2022.

DELLA MÓNICA, I. F.; GODEAS, A. M.; SCERVINO, J. M. In Vivo Modulation of Arbuscular Mycorrhizal Symbiosis and Soil Quality by Fungal P Solubilizers. **Microbial Ecology**, v. 79, n. 1, p. 21–29, 1 jan. 2020.

EREL, R. et al. Soil type determines how root and rhizosphere traits relate to phosphorus acquisition in field-grown maize genotypes. **Plant and Soil**, v. 412, n. 1–2, p. 115–132, 2017.

FERREIRA, P. A. A. et al. Long-Term Effects of Animal Manures on Nutrient Recovery and Soil Quality in Acid Typic Hapludalf under No-Till Conditions. **Agronomy**, v. 12, n. 2, 1 fev. 2022.

Fertilizers | OEC - The Observatory of Economic Complexity. Disponível em: <https://oec.world/en/profile/hs/fertilizers?latestTrendsFlowSelector=flow1&latestTrendsViewSelector=latestTrendsViewOption1>. Acesso em: 14 out. 2022.

HALLAMA, M. et al. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. **Plant and Soil**, v. 434, n. 1–2, p. 7–45, 27 jan. 2019.

HANEKLAUS, S. H.; SCHNUG, E. Assessing the Plant Phosphorus Status. Em: **Phosphorus in Agriculture: 100 % Zero**. Dordrecht: Springer Netherlands, 2016. p. 95–125.

HAYGARTH, P. M. et al. Sustainable Phosphorus Management and the Need for a Long-Term Perspective: The Legacy Hypothesis. **Environmental Science & Technology**, v. 48, n. 15, p. 8417–8419, 5 ago. 2014.

HINSINGER, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. **Plant and Soil**, v. 237, p. 173–195, 2001.

HINSINGER, P. et al. **Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A reviewPlant and Soil**. [s.l: s.n.].. Acesso em: 10 jan. 2021.

HINSINGER, P. et al. Impact of Roots, Microorganisms and Microfauna on the Fate of Soil Phosphorus in the Rhizosphere. Em: **Annual Plant Reviews online**. Chichester, UK: John Wiley & Sons, Ltd, 2018. p. 377–407.

HODGE, A. et al. **Plant root growth, architecture and function**. **Plant and Soil**, 2009. Acesso em: 1 out. 2019

LÓPEZ-ARREDONDO, D. L. et al. Phosphate Nutrition: Improving Low-Phosphate Tolerance in Crops. **Annual Review of Plant Biology**, v. 65, n. 1, p. 95–123, 2014.

LÓPEZ-CARMONA, D. A. et al. Maize plant growth response to whole rhizosphere microbial communities in different mineral N and P fertilization scenarios. **Rhizosphere**, v. 9, p. 38–46, 1 mar. 2019.

MCDOWELL, R. W.; SHARPLEY, A. N. Approximating Phosphorus Release from Soils to Surface Runoff and Subsurface Drainage. **Journal of Environment Quality**, v. 30, n. 2, p. 508, 2001.

NASH, D. M. et al. Using organic phosphorus to sustain pasture productivity: A perspective. **Geoderma**, v. 221–222, p. 11–19, 1 jun. 2014.

NGUYEN, V. L.; STANGOULIS, J. Variation in root system architecture and morphology of two wheat genotypes is a predictor of their tolerance to phosphorus deficiency. **Acta Physiologiae Plantarum**, v. 41, n. 7, p. 109, 1 jul. 2019.

PARFITT, R. L. Phosphate reactions with natural allophane, ferrihydrite and goethite. **Journal of Soil Science**, v. 40, p. 359–369, 1989.

PAVINATO, P. S. et al. Effects of Cover Crops and Phosphorus Sources on Maize Yield, Phosphorus Uptake, and Phosphorus Use Efficiency. **Agronomy Journal**, v. 109, n. 3, p. 1039–1047, 1 maio 2017.

PIERZYNSKI, G. M.; MCDOWELL, R. W.; SIMS, J. T. Chemistry, cycling, and potatial movemet of inorganic phosphorus in soil. **Phosphorus: Agriculture and the Environment**, p. 53–86, 2005.

RADERSMA, S.; GRIERSON, P. F. Phosphorus mobilization in agroforestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. **Plant and Soil**, v. 259, n. 1–2, p. 209–219, 2004.

RICHARDSON, A. E. et al. **Plant mechanisms to optimise access to soil phosphorus**. Crop and Pasture Science. **Anais**...2009. Acesso em: 1 out. 2019

RISSMAN, A. R.; CARPENTER, S. R. Progress on nonpoint pollution: Barriers & opportunities. **Daedalus, the Journal of the American Academy of Arts & Sciences**, v. 144, n. 3, p. 35–47, 2015.

SATTARI, S. Z. et al. Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. **Proceedings of the National Academy of Sciences**, v. 109, n. 16, p. 6348 LP – 6353, abr. 2012.

SHARMA, R.; BELL, R. W.; WONG, M. T. F. Phosphorus forms in soil solution and leachate of contrasting soil profiles and their implications for P mobility. **Journal of Soils and Sediments**, v. 15, n. 4, p. 854–862, 2015.

SHARPLEY, A. et al. Phosphorus Legacy: Overcoming the Effects of Past Management Practices to Mitigate Future Water Quality Impairment. **Journal of Environmental Quality**, v. 42, n. 5, p. 1308–1326, 1 set. 2013.

WANG, F.; FENG, G. Arbuscular Mycorrhizal Fungi Interactions in the Rhizosphere. p. 217–235, 2021.

WITHERS, P. J. A. et al. Transitions to sustainable management of phosphorus in Brazilian agriculture. **Scientific Reports**, v. 8, n. 1, p. 1–13, 1 dez. 2018.

ZHU, J.; LI, M.; WHELAN, M. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. **Science of the Total Environment**, v. 612, p. 522–537, 2018a.

ZHU, J.; LI, M.; WHELAN, M. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. **Science of the Total Environment**, v. 612, p. 522–537, 15 jan. 2018b.

10. APÊNDICES

Apendice A - Apresentação da área experimental.

Apêndice B – Imagems do armazenamento (a) e da aplicação de dejetos líquidos (b).

Apêndice C – Imagem panorâmica da área experimental, Santa Maria, Rio Grande do Sul, Brasil, durante o cultivo do milho safra 2019/20.

Apêndice D – Imagem da área experimental, Santa Maria, Rio Grande do Sul, Brasil, durante o cultivo da Aveia safra 2021.

Apêndice E – Imagems da instalação dos tubos de minirhizotron (a) e escaneamento das raizes do milho (b).

Apêndice F – Imagems da inoculação (a) e semeadura do milho com FMAs.

Apêndice G – Imagems da separação do solo rizosferico em milho (a) separação das raízes (b) para a coloração (a) e determinação de colonização de raízes.

Apêndice G – Imagems de sintomas característicos de deficiencia de P no tratamento Controle (sem aplicação de fertilizanets) do milho safra 2020/21 e aveia safra 2021.

Apêndice F – ANÁLISE SENSORIAL DE LOMBO DE SUÍNO COM MOLHO DE MOSTARDA E MEL

(Adaptado de Marchezan L., 2010)

Materiais e equipamentos:

Liquidificador;
Panela de ferro grande;
Forno elétrico;
Forma de alumínio;
Papel alumínio;

Reagentes e substratos para o Lombo:

Uma peça de lombo de porco (1.2 kg);
3 colheres de mostarda tradicional;
3 colheres de azeite de oliva;
1 taça de vinho branco;
1 caldo bacon;
Pimenta do reino, alecrim, louro, alho e especiarias a gosto

Reagentes e substratos para o molho de mostarda e mel:

3 colheres de sopa de mostarda tradicional;
2 colheres de sopa de mostarda Dijon;
2 colheres de sopa de mel;
2 colheres de sopa de creme de leite;
50 ml de conhaque (opcional)

Procedimento de análise:

Um dia antes de cozinhar, bata no liquidificado a amostarda tradicional com azeite de oliva, vinho branco, caldo de bacon e os demais temperos. Em um recipiente junte a solução triturada no liquidificador com folhas de louro inteiras ao lombo e reserve na geladeira por 10 ou 12 horas.

Em uma panela de ferro bem quente, com um fio de azeite, sele todos os lados do lombo. Em seguida, transfira o lombo para uma forma e cubra com papel alumínio e leve ao forno 180°C por 30-40 min, para terminar o cozimento.

Em uma panela média, juntar o creme de leite, mostarda Dijon, mostarda tradicional, conhaque e por último o mel. Homogeneizar rapidamente a mistura em fogo baixo (para evitar de talhar o creme de leite).

Dica: essa de carne de porco assada pode ser consumida quente ou fria, e fica ótima acompanhada de batatas assadas ou farofa.

VITAE

Carina Marchezan, filha de Amauri Marchezan e Aneide Maria Dall’Ongaro, nascida em 4 de novembro de 1993, em Nova Palma, Rio Grande do Sul – Brasil. Sexta filha de um casal de pequenos agricultores, aos 4 anos ingressou na pré-escola e concluiu a 4ª série (1997-2003) na Escola Municipal de Ensino Fundamental São João na comunidade do Rincão dos Fréos. De 2003 a 2007, concluiu o ensino fundamental na Escola Municipal de Ensino Fundamental Cândida Zasso. Em 2010, concluiu o Ensino Médio na Escola Estadual de Ensino Básico Tiradentes.

Aos 17 anos saiu da casa dos pais para cursar faculdade de Agronomia na Universidade Federal de Santa Maria (UFSM). Logo no primeiro mês de graduação ingressou como bolsista voluntária na área de Melhoramento de Plantas no Departamento de Fitotecnia. Em 2012 ingressou como bolsista no grupo de pesquisa na área de química e fertilidade do solo no Departamento de Ciência do Solos – mais tarde nomeado GEPACES, onde permaneceu até o final da sua trajetória acadêmica.

Em 2016, concluiu a graduação e ingressou no Mestrado no Programa de Pós-graduação em Ciência do Solo (PPGCS) da UFSM, sob orientação do Professor Carlos Alberto Ceretta (título da Dissertação - Nitrogênio na solução do solo e aspectos nutricionais e fisiológicos do milho cultivado após 12 anos sob fontes orgânicas e mineral). Em agosto de 2018 finalizou o Mestrado e ingressou no Doutorado também no PPGCS da UFSM, sob orientação do Professor Gustavo Brunetto, onde obteve o título de Doutora, com a tese intitulada “Balanço de fósforo e estratégias de culturas anuais para acessar reservas de fósforo construídas ao longo de 15 anos por fontes orgânicas e mineral”.