UNIVERSIDADE FEDERAL DE SANTA MARIA
GRADUATION PROGRAM IN SOIL SCIENCE

UNIVERSITÉ DE POITIERS
ÉCOLE DOCTORALE THÉODORE MONOD

TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE

DOCTORAL THESIS IN JOINT SUPERVISION

ANDRÉ SOMAVILLA

Santa Maria, RS, Brazil
2021

PPGCS/UFSM, RS SOMAVILLA, André Doutor 2021

TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE

André Somavilla

Thesis submitted to the Doctoral Program of the Graduate Program in Soil Science, concentration area in Chemical Processes and Elements Cycling, at the Federal University of Santa Maria, Brazil, as a partial requirement for the degree of **Doctor in Soil Science**, and to the Doctoral School in Chemistry Ecology Geosciences Agrosciences Theodore Monod, at University of Poitiers, France, as a partial requirement for the degree of **Doctor in Structure et évolution de la Terre et autres planètes**, research area in Terre Solide et Enveloppes superficielles.

Advisor at UFSM: Pr. Dr. Danilo Rheinheimer dos Santos
Advisor at University of Poitiers: Pr. Dr. Laurent Caner

Santa Maria, RS, Brazil
2021

Somavilla, André
TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE / André Somavilla.- 2021.
257 p.; 30 cm

Orientador: Danilo Rheinheimer
Coorientador: Laurent Caner
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Ciência do Solo, RS, 2021

1. Phosphorus fertilization 2. Grassland 3. Phosphorus depletion 4. Grassland 5. Cropland I. Rheinheimer , Danilo II. Caner, Laurent III. Título.

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, ANDRé SOMAVILLA, 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.

E-mail:somavillaa@gmail.com

Universidade Federal de Santa Maria
Graduation Program in Soil Science

Université de Poitiers
École Doctorale Théodore Monod

The Examining Committee, undersigned,
approved the Doctoral Thesis

TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE

elaborated by
André Somavilla

as a partial requirement for the degree of **Doctor in Soil Science** by the Federal University of Santa Maria and **Doctor in Structure et évolution de la Terre et autres planets,** research area in Terre Solide et Enveloppes superficielles, by University of Poitiers.

Examining Committee:

Dr. Pr. Danilo Rheinheimer dos Santos
Universidade Federal de Santa Maria, Brazil (President/Advisor/Examiner)

Dr. Laurent Caner
Université de Poitiers, France (Advisor/Examiner)

Paulo Pavinato

Dr. Paulo Sergio Pavinato
University of São Paulo, Brazil (Rapporteur/Examiner)

Dr. Christian Morel
INRAE, France (Rapporteur/Examiner)

Dr. Abad Chabbi
INRAE, France (Examiner)

Dra. Bruna Raquel Winck
Federal University of Rio Grande do Sul, Brazil (Examiner)

Tales Tiecher
Federal University of Rio Grande do Sul. Brazil (Examiner)

Santa Maria, July 21, 2021

Dedicatória

Dedico aos meus pais Lucas Antonio Somavilla e Izabel Liberalesso Somavilla, pelo amor, carinho, paciência, educação, ensinamentos, incentivo, apoio, confiança e exemplo de família e união.

À minha irmã Paula, pelo amor fraterno, apoio e incentivo.

À minha esposa Isley, pelo amor, companheirismo, apoio e confiança, um exemplo de mulher e de esposa. Você chegou em minha vida e me fez um novo ser.

À meu filho Luís, que com sua ingenuidade trouxe novos desafios e alegrias. Com seu olhar inocente e esperançoso me fortalece a cada dia em nossa caminhada.

AGRADECIMENTOS

REMERCIEMENTS

ACKNOWLEDGEMENT

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Ciência do Solo.

Aos meus orientadores, Prof. Dr. Danilo Rheinheimer dos Santos e Prof. Dr. Laurent Caner, pela oportunidade, confiança, paciência, orientações, ensinamentos e pelo exemplo de dedicação à ciência e ao trabalho.

Ao CNPq pelo financiamento da bolsa de estudos nacional e internacional.

Aos colegas pós-graduandos de grupo de pesquisa, em especial Gilmar Luiz Scheffer, Rodrigo Hammerschmitt, Roque Bellinaso, Vanessa Dias, Jocelina Vargas, Graciele Fernandes, Jaqueline Spricigo, Deonilce Retka, Andressa Santos, Leticia Moro e Marilia Camotti, pela ajuda e apoio nas atividades laboratoriais e de campo.

Aos inúmeros alunos de pós graduação e bolsistas de iniciação científica que contribuíram para a manutenção do experimento “Campo Nativo” durante os 21 anos de sua execução.

Aos amigos Fabio José Andres Schneider e Bruno Chaves pelas brasagens realizadas na garagem de casa. Sem vocês minha visão sobre “fazer cerveja” certamente seria outra.

Aos colegas de PPGCS e professores pelas inúmeras discussões nos corredores, salas de aula e laboratório que, por vezes calorosas, ajudaram a lapidar minha formação científica e pessoal.

Aos membros da banca que dedicaram seu tempo na leitura e contribuições para melhora de meu trabalho.

L'Université de Poitiers et l'École doctorale Théodore Monod pour l'accueil et la possibilité d'accomplissement du doctorat en cotutelle. L'équipe IC2MP-HydrASA pour l'opportunité et le soutien pour l'analyse des échantillon et l'utilisation des équipements du laboratoire. A l'INRAE Poitou-Charentes, en particulier à Abad Chabbi et Marie-Laure Decau, pour leur disponibilité et leur aide dans la réalisation de la plupart des travaux sur les sols de l’ORE ACBB de la présente thèse.

À Céline Boissard, Natalie Dauger et Claude Fontaine pour le soutien technique scientifique au laboratoire pendant mon séjour en France.

A mon ami Laurent Caner et sa famille pour leur attention, leur volonté et, surtout, pour l'accueil pendant la période à Poitiers.

A mes amis doctorants Jérémie Aubineau, Marine Bonnet, Thomas Dabat, Grâce Guiyeligou, Florian Martin, Laurent Pallas qui ont partagé avec moi des moments très importants au laboratoire et, pourquoi pas, dans les bars et les parcs de Poitiers. Aux amis de Master, Floriane Jamoteau, Martin, Lina Varon, Benjamin pour leur amitié et leur accompagnement lors des soirées au parc.

À Claire Guichard et à sa famille pour les innombrables conversations, messages, promenades en ville et invitations à son domicile. Vous m'avez certainement apporté beaucoup sur le plan personnel et culturel et vous avez rendu mes journées de Noël et de matchs de l'équipe de France beaucoup plus heureuses.

A Sylvie Petagna pour les plusieurs cafés dégustés en ville, toujours avec beaucoup de conversation et de connaissances culturelles et de langue française (même avec mes nombreuses gaffes). Sans vous, ma capacité à parler français ne serait pas la même.

Au Palais de La Bière (PLB) pour avoir toujours offert la dégustation d'une bonne bière et un environnement accueillant pour rencontrer des amis.

A todos que contribuíram de alguma forma no desenvolvimento deste trabalho e de minha formação profissional e pessoal.

Obrigado a todos!

Merci à tous !

“Porque perseverei?

Sabia onde estava meu lar e que eu sempre poderia voltar à ele!”

List of Figures

Chapter II - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation.

Chapter III - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation.

Chapter IV - P-Legacy effect of soluble fertilizer added with limestone and phosphate rock on grassland soil in subtropical climate region.

Chapter V – P stock depletion and soil C:N:P stoichiometry by annual crop or grassland management systems over 13 years.

Chapter VI - Soil P microbial biomass and coupling of C:N:P: a soil aggregate scale approach.

List of Tables

Chapter II - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation.

Chapter III - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation.

Chapter IV - P-Legacy effect of soluble fertilizer added with limestone and phosphate rock on grassland soil in subtropical climate region.

Chapter V – P stock depletion and soil C:N:P stoichiometry by annual crop or grassland management systems over 13 years.

Chapter VI - Soil P microbial biomass and coupling of C:N:P: a soil aggregate scale approach.

Summary

RESUMO

Tese de Doutorado em Co-tutela
Programa de Pós-Graduação em Ciência do solo – Universidade Federal de Santa Maria, Brasil
Escola Doutoral: Chimie Ecologie Géosciences Agrosciences Théodore Monod – Université de Poitiers, Franca

DINÂMICA TEMPORAL DE P EM DIFERENTES SISTEMAS DE MANEJO DE SOLOS EM CLIMA TEMPERADO E SUBTROPICAL

Autor: André Somavilla
Orientador na UFSM: Pr. Danilo Rheinheimer dos Santos
Orientador na Université de Poitiers: Laurent Caner
Data e local da defesa: 21 de julho de 2021, Santa Maria.

As perspectivas de aumento da população mundial exigem que a produção de alimentos seja potencializada e melhor distribuída pelo mundo. Uma alternativa sustentável para alcançar altas produções de alimentos é o uso eficiente das áreas já cultivadas. O fósforo (P) é um nutriente essencial para a produção vegetal e suas reservas naturais estão ameaçadas de esgotamento em um futuro próximo. Assim, este trabalho se insere no cenário de aumento da eficiência de fertilizantes, aumento da produção agrícola e manutenção do equilíbrio dinâmico de nutrientes visando a sustentabilidade dos sistemas agrícolas, segurança alimentar e mitigação de mudanças climáticas. Portanto, o objetivo principal desta tese foi estabelecer uma estratégia mais eficaz de fertilização com P no aumento da produção de forragem, manutenção da comunidade de plantas e uso eficiente de P. Bem como, testar o efeito de sistemas de manejo na disponibilidade e esgotamento de P do solo e sua estequiometria com carbono e nitrogênio no solo. Para atingir nossos objetivos, analisamos dois experimentos de longo prazo. O primeiro foi estabelecido em 1997 em uma pastagem natural do bioma Pampa, Rio Grande do Sul, Brasil. O solo da área experimental é pobre em fósforo, com baixos teores totais e disponíveis de P. Neste local foi avaliado o desempenho de diferentes fontes de P por meio do histórico de produção de matéria seca, eficiência de uso de P, alterações botânicas da vegetação, disponibilidade de P no solo e dinâmica das diferentes frações de P do solo. O segundo experimento foi estabelecido em 2005 em um solo agrícola antropizado situado em Lusignan, França. O solo experimental possui altos estoques de P total e disponível devido ao histórico de fertilização anteriores ao experimento. Neste local foi analisado o efeito de longo prazo de dois sistemas de manejo (lavoura permanente e pastagem permanente) por meio da exportação de P, redução do estoque de P, C e N do solo, redução da disponibilidade de P, mudanças na estequiometria C:N:P do solo, alterações nos teores de P contidos na biomassa microbiana do solo e alteração na estequiometria C:N:P e disponibilidade de P em diferentes tamanhos dos agregados do solo. Com isso concluímos que em solos com baixo estoque de P, o uso de fontes solúvel de P (como superfosfato simples e triplo) associado à calagem é a melhor estratégia para melhorar a produção de matéria seca e a eficiência do uso de P no campo nativo do bioma Pampa.

Esses fertilizantes levam a uma pequena mudança na riqueza de espécies. Entretanto, houve maior substituição de espécies, principalmente gramíneas *tussock* e *forbes* e aumento na contribuição de leguminosas para a produção de forragem. Para manter o rendimento de matéria seca, é necessário um aporte regular de fósforo com no máximo três anos de intervalo. O uso de fosfato natural não melhorou as condições de fertilidade e produção de matéria seca de forma satisfatória. No solo com alto estoque de P, o cultivo permanente de pastagem proporcionou maior redução dos estoques totais e lábeis de P devido à sua maior capacidade de exportar P em comparação ao cultivo permanente com culturas anuais. Esse comportamento proporcionou uma redução nos valores da relação C:N:Po e, consequentemente, um acoplamento entre os ciclos C, N e P no solo. Além disso, observamos que o P_{mic} é um importante reservatório de P biodisponível em ambos os sistemas de manejo e é responsável por até 47% do conteúdo de P biodisponível (P Olsen) no sistema de manejo com culturas anuais. Pela técnica de ^{31}P-RMN foi observado que o cultivo permanente de pastagem teve um efeito mais significativo na redução do α-glicerofosfato e no aumento de *mio*-IHP e de monofosfato de adenosina do que culturas anuais.

Palavras-chave: Pastagens naturais; Culturas anuais; Manejo do solo; Labilidade do fósforo; Fertilização fosfatada; Fertilizante mineral; Esgotamento de Fósforo.

RÉSUMÉ

Thèse de doctorat en co-tutelle

École doctorale : Chimie Ecologie Géosciences Agrosciences « Théodore Monod »
Université de Poitiers, France
Programme de post-graduation en Science du Sol - Université Fédérale de Santa Maria, Brésil

DYNAMIQUE TEMPORELLE DU P DANS DES SOLS CULTIVES PAR DIFFÉRENTS SYSTÈMES DES CULTURE EN CLIMAT TEMPERE ET SUBTROPICAL

Auteur : André Somavilla
Directeur à l'Université de Poitiers : Laurent Caner
Directeur à l'UFSM : Danilo Rheinheimer dos Santos
Date de soutenance : 21/07/2021

La perspective d'une augmentation de la population mondiale implique que la production alimentaire soit améliorée et mieux répartie dans le monde. Une alternative durable pour atteindre une production alimentaire élevée est une utilisation efficace des surfaces déjà cultivées. Le phosphore (P) est un élément nutritif essentiel à la production végétale dont les réserves naturelles sont menacées d'épuisement dans les prochaines décennies. Ainsi, ce travail s'inscrit dans le scénario d'augmentation de l'efficacité des engrais et de la production agricole et de maintien de l'équilibre dynamique des nutriments en visant la durabilité des systèmes agricoles, la sécurité alimentaire et la réduction du changement climatique. Par conséquent, l'objectif principal de ces travaux était d'établir une stratégie plus efficace de fertilisation au P pour augmenter la production de fourrage, le maintien de la communauté végétale et l'utilisation efficace du P. De même, nous avons testé l'effet des systèmes de gestion du sol sur la disponibilité et l'épuisement du P dans le sol et sa stœchiométrie avec le carbone et l'azote. Pour atteindre nos objectifs, nous avons analysé deux expériences de long terme. La première a été établie en 1997 dans une prairie naturelle du biome de la Pampa dans l'état du Rio Grande do Sul au Brésil. Le sol du site expérimental est un sol avec de faibles stocks de P total et disponible. Sur ce site, l'effet de différentes sources de P a été testé sur la production de matière sèche, l'efficacité de l'utilisation du P par les plantes et les changements botaniques. Le second site, établi en 2005, est situé sur le site expérimental de l'ORE ACBB de l'INRAE de Lusignan en France. Le sol du site expérimental présentait des stocks élevés de P total et disponible en raison d'une fertilisation historique avant l'expérience. Aucune fertilisation P n'a été réalisée depuis l'installation du site expérimental. Sur ce site, l'effet à long terme de deux systèmes de gestion (culture permanente et prairie permanente) a été mesuré par l'exportation de P, l'épuisement des stocks totaux de P, C et N du sol, le suivi de la disponibilité du P et les changements dans la stœchiométrie C:N:P du sol. La dynamique du P dans la biomasse microbienne du sol et les modifications du couplage P, C et N dans la taille des agrégats du sol ont également été suivies. Nous avons conclu que l'utilisation de sources de P soluble (comme le superphosphate simple et triple) associée au chaulage est une meilleure stratégie pour améliorer la production de matière sèche et l'efficacité d'utilisation du P dans les prairies de la Pampa. Ces engrais ont entraîné un léger changement dans la richesse des espèces ; cependant, il y a eu un plus grand renouvellement des espèces, principalement dans les groupes de graminées et de plantes fourragères et dans la contribution des légumineuses à la production de fourrage. Pour maintenir le rendement en matière sèche, un apport régulier de P, ne dépassant pas trois ans, est nécessaire. L'utilisation de phosphates naturels (roches phosphatées) n'a pas permis d'améliorer la fertilité et la production de matière sèche de manière satisfaisante en raison de leur non-dissolution à long terme (plus de six ans). D'autre part, les prairies permanentes ont entraîné un plus grand épuisement des stocks totaux et P-labile en comparaison des cultures permanentes. De même, les prairies permanentes ont favorisé une transformation du P inorganique en P organique dans le pool le plus labile, accompagnant l'augmentation des stocks de C et de N dans le sol. La technique RMN ^{31}P a permis d'observer que les prairies permanentes avaient un effet plus significatif sur la réduction de l'α-glycérophosphate et l'augmentation du myo-IHP et de l'adénosine monophosphate que les terres cultivées permanentes.

Mots clés : Prairies ; terres cultivées ; gestion des sols ; labilité du phosphore ; fertilisation au phosphore ; engrais minéral ; épuisement du phosphore.

ABSTRACT

Doctoral Thesis in Joint Supervision
Graduation Program in Soil Science – Federal University of Santa Maria, Brazil
Doctoral School in Chemistry Ecology Geosciences Agrosciences Théodore Monod – University of Poitiers, France

TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE

Author: André Somavilla
Advisor at UFSM: Pr. Danilo Rheinheimer dos Santos
Advisor at University of Poitiers: Laurent Caner
Place and date of defense: Santa Maria, July 21, 2021

The prospect of world population increases requires that the production of food to be potentialized and better distributed around the world. One sustainable alternative to achieve high food production is an efficient use of the areas already cultivated. In this sense, appropriated management of nutritional inputs and soil management systems are fundamentals. Phosphorus (P) is an essential nutrient for plant production whose natural reserves are threatened with depletion in the coming decades. Thus, this work is placed in the scenario of increasing fertilizer efficiency, raising agricultural production, and maintaining the dynamic balance of nutrients aiming at the sustainability of agricultural systems, food security and reduction of climate change. Therefore, the main objective of this These was establishing a more effective strategy of P fertilization on increase forage production, maintenance of plant community and efficiency use of P. As well as, testing the effect of soil management systems on soil P availability and depletion and their stoichiometry with carbon and nitrogen. To achieve our objectives, we analyzed two long-term experiments. The first one was established in 1997 in a natural grassland of Pampa biome, Rio Grande do Sul, Brazil. The soil of this experiment is a phosphorus-poor soil with low total and available P stocks. In this site the effect different P sources were tested by analysing historical dry-matter production, P use efficiency and botanical changes, as well soil P availability and P pools dynamic. The second one was established in 2005 in an agricultural soil situated in l’ORE ACBB at l’INRAE - Lusignan, France. The experimental soil had high total and available P stocks due to an historic fertilization before experiment. The studies on this site were focused on the long term effect of two management systems (permanent cropland and permanent grassland) by field, P exportation, soil P, C and N total stocks depletion, P availability depletion and changes in soil C:N:P stoichiometry. The dynamic of P in soil microbial biomass and alteration of P, C and N coupling in soil aggregate sizes. We concluded that the use of soluble P sources (such as simple and triple superphosphate) associated with liming is a better strategy to improve dry matter production and P use efficiency in Pampa grassland. These fertilizers lead to a small change in species richness; however, there was higher species turnover, mainly in the tussock grass and forbs groups and in the legume contribution to forage production. To maintain dry matter yield, a regular phosphorus input with no more than three years is necessary. Though, the use of natural phosphates (phosphate rocks) did not improve fertility conditions and dry matter production satisfactorily due to their long term non dissolution (more than six years). In soil with high P stock, permanent grassland provided a greater depletion of total and P-labile stocks. As well, permanent grassland supports a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. Additionally, we observed that the P_{mic} is an important bioavailable P pool in both management systems and accounted for up to 47% of the available P content (P Olsen) in a permanent cropland system. By ^{31}P-NMR technique was observed that permanent grassland had a more significant effect in reducing α–glycerophosphate and increasing *myo*-IHP and adenosine monophosphate than permanent cropland.

Keywords: Grassland; Cropland; Soil management; Phosphorus lability; Phosphorus fertilization; Mineral fertilizer; Phosphorus depletion.

Long summary

TEMPORAL DYNAMIC OF P IN DIFFERENT SOIL MANAGEMENT SYSTEMS IN TEMPERATE AND SUBTROPICAL CLIMATE

Introduction

The increase of world population requires that the production of food to be potentialized (Ray et al., 2013). Which should be achieved by more efficient use of the areas already cultivated. However, the agriculture practiced around the world in recent decades is contrary to achieving a sustainability. Mainly due to the intensive and inadequate use of agrochemicals such as phytosanitaires, industrial fertilizers, and fossil fuels.

The world consumption of industrial fertilizers, for example, increased 17.7% between 2006-2016 (FAO, 2019). In this amount, P and K are essentially from finite reserves in mines and there is no substitute for agricultural production (Cordell et al., 2009; U.S. Geological Survey, 2019). The high demand of P, makes worrying about the projections of possible depletion of the natural phosphate rock reserves in the next 50-100 years (Cordell et al., 2009; Steen, 1998). Moreover, there are a high annual addition of P to soils and water bodies, causing serious environmental and social problems, as a eutrophication of water sources (Goyette et al., 2018; Schilling et al., 2018; Schoumans et al., 2014; Withers et al., 2017).

At global level, the most efficient use of phosphate fertilizers is essential to achieve the sustainability of production systems without the loss of productive potential of food (MacDonald et al., 2011; Marin et al., 2016; McDonald et al., 2019; Tian et al., 2019). This will be obtained through the interaction of factors such as: soil management practices; fertilization strategies; use of organic fertilizers and cultivation of plants adapted to the conditions of production systems.

In this sense, rock powder or crushed rocks are considered P source with low solubility with potential to use in agriculture due to gradual release of P in the soil (Oliveira et al., 2015; Szilas et al., 2007; Tiecher et al., 2014). However, the fertilization strategy to use less soluble P sources (i.e. phosphate rock) in subtropical soils has demonstrated a greater increase of less available forms of P than soluble sources (Soltangheisi et al., 2018). In the case of phosphate rock, the diversity of conclusions in the literature indicates the need for further studies on the subject.

Apart from fertilization strategy, the soil management systems alter the dynamic of plants nutrients uptake by changing nutrients cycling and their chemical forms in the soil. As example, no-till systems, with crop rotation or perennial pasture cultivation, where there is straw maintenance and no soil plowing, have significant levels of organic P, either in the organic matter or in the soil microbial biomass (Boitt et al., 2018a, 2018b; Condron et al., 2005; Tiecher et al., 2012a). In contrast, in systems with soil plowing there is an mineralization of soil organic matter and, consequently, increasing a inorganics P pools with possibility of P immobilization by soil constituents, causing an increase in the less labile forms of P (Rodrigues et al., 2016; Tiecher et al., 2012b). Therefore, it is evident that understanding the dynamics of P in medium and long-term production systems and soil management systems are essential for improving the P use efficiency in a sustainable agriculture.

In this thesis, seven chapters will be developed that deal with the dynamics of soil P in areas of forage production and annual crop. These seven chapters are characterized by 3 main moments of the material.

The first moment, composed of chapter I, is addressed to the literature review regarding the themes that will be addressed in the other chapters. Such as, an approach to characterize the chemical element P, its origin, location and importance for the life cycle on Earth. Moreover, approaches will be carried out on the dynamics, stocks and use of P sources in agricultural soils, with emphasis on areas managed with pastures in regions of subtropical and temperate climates. To conclude the first moment, an approach will be made regarding the coupling of the biogeochemical cycle of P and elements C and N and its importance for increasing the levels of organic matter in the soil.

The second moment, composed by chapters II to VI, is addressed to the presentation of the five articles that make up this thesis. These articles are based on two long-term experiments located in Brazil and France. The use of two experiments located in these countries aims to carry out studies in both places involved in the realization of this doctorate, such as carried out in joint supervision. Chapters II, III and IV deal with three articles already published in international scientific journals and are from the experiment started in 1997, located in Santa Maria – Brazil. The main objectives of the experiment are to test the use of different rates of P sources, associated or not with liming, in natural grassland of the Pampa biome. In chapter II, published in the European Journal of Agronomy (Somavilla et al., 2021c), a historical approach of the experiment, analyzing data of dry matter productivity of more than 21 years, botanical composition of native vegetation and soil fertility in the 0-10 cm topsoil layer was carried out.

In chapter III, published in the Soil and Tillage Research (Somavilla et al., 2021b), data on nutritional content and the effect of liming in stratified layers at a depth of 0-10 cm topsoil layer are analyzed, as well as data on concentration and P export via dry matter. In chapter IV, also published in Soil and Tillage Research (Somavilla et al., 2021a), an analysis of soil P legacy due to the use of different P sources was carried out. As well as the presence in the soil of undissolved apatite minerals from the rock phosphate P source.

Chapters V and VI are being prepared for publication in international journals, with submission scheduled for 2021. These works are related to an experiment initialized in 2005, located in Lusignan - France, which aimed to test the effect of different management systems (annual crops or perennial pasture) on the depletion and legacy of P in the soil and its stoichiometry C:N:P. In chapter V, historical dataset of export and depletion of P by plants, changes in soil P pools and the coupling of organic P in the dynamics of C and N at 0-30 cm topsoil layer were analyzed. In chapter VI, the participation of the P from soil microbial biomass in the bioavailable soil P content during the barley growth season was analyzed. As well as, the distribution of P pools and their stoichiometry C:N:P in different classes of soil aggregates according to management systems with annual crops and perennial pasture.

The third moment, composed of chapter VII, is addressed to the general discussion of the thesis. In this chapter, approaches are made about the dynamics and availability of P in agricultural soils, methods of quantification and characterization of the forms of P in soils. In addition to an agri-environmental approach on the use of phosphate fertilizers in agricultural areas.

At the end, we have a conclusion that takes up the observations made in the previous chapters.

Context

The present thesis is the final doctoral manuscript carried out in joint supervision between the Federal University of Santa Maria (Brazil) and the Université de Poitiers (France). Furthermore, the present thesis is part of a research project titled "Long-term effect of soil use and management practices on soil fertility: changes in mineralogy and availability of phosphorus and potassium". This project was funded by the Brazilian government and is linked to MEC/MCTI/CAPES/CNPq/FAPs - Special Visiting Researcher - PVE 2014 Nº 03/2014. The project is carried out in partnership between the Universidade Federal de Santa Maria - Brazil and the Université de Poitiers - France and aims to develop studies to understand the long-term effect of soil use and management on fertility, with emphasis on mineralogical changes and availability of phosphorus and potassium to crops. As well as the production and dissemination of scientific knowledge, this project aims to strengthen Franco-Brazilian scientific cooperation and capacitating people through the exchange of researchers and students between universities. Moreover, this work also has the collaboration of researchers from the Institute National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement - INRAE Nouvelle Aquitaine SOERE ACBB, located at Lusignan – France, where a considerable part of this work was developed.

Chapter II - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation.

Phosphorus (P) corresponds to approximately 0.2% of plant dry matter (Schachtman et al., 1998); it is a key component for protein and nucleic acid production, as well as for enzymatic activation and energy transfer (Wang et al., 2017) and plant growth is directly linked to P availability in the soil. Overall, soils in Southern Brazil grasslands (the so-called Pampa grasslands) are intensely weathered, rich in iron oxides and kaolinite, and formed from parent materials presenting low P concentration, which implies low natural P availability to plants. Moreover, they are naturally acid soils (pH 4.4–5.1) and this feature leads to high-energy P adsorption onto soil clay minerals and oxides, as well as reduces natural soil P availability to plants (e.g., Mehlich-1 method), which often ranges from 2 mg kg^{-1} to 8 mg kg^{-1} (Oliveira et al., 2011; Rheinheimer et al., 1997).

Vegetation growing in these soils comprises species adapted to local conditions such as low P availability and soil acidity (Oliveira et al., 2018; Marques et al., 2019). Approximately 3,000 plant species can be found in the Pampa grassland ecosystem (Pampa biome), 450 of them are grass species used for forage production (Boldrini, 2009). The vegetation composing the Pampa grasslands has high potential for forage production when it is properly managed. Grassland improvement through soil fertility correction and exogenous winter species' inclusion can lead to forage dry matter production of 14 Mg ha^{-1} year^{-1} and increase meat production to approximately 900 kg ha^{-1} year^{-1} of cattle body weight (Oliveira et al., 2015). It is necessary to implement proper plant and soil fertility management processes in order to achieve high forage production rates. Limestone and phosphate fertilizers are fundamental strategies used to improve soil fertility, increase nutrient availability and, consequently, to improve plant development (Prestes et al., 2016).

The current study aims to investigate (i) whether soluble P sources (triple and simple superphosphate) and limestone use result in greater dry matter production than the use of lesser soluble P sources (phosphate rock) or the no use of liming; and (ii) in which ways P fertilization and liming affects the botanical composition and grassland plant species richness. Soil and vegetation data from a 21-year-old trial conducted in Southern Brazilian Pampa grassland managed with different phosphate sources, liming, fertilization rates and plant species' overseeding were analyzed.

Phosphate fertilization and liming application in Southern Brazil Pampa grasslands soil helped improving dry matter production. However, Pampa grasslands responded to phosphorus sources in different ways over 21 years. Soluble fertilizer using, such as triple and simple superphosphate, led to higher dry matter production. Phosphate fertilization led to small changes in species richness; thus, it is possible maintaining floristic biodiversity, despite the use of phosphorus fertilizers . However, there was higher species turnover, mainly in the tussock grass and forbs groups, which may contribute to change in ecosystem services. Legume species' contribution to dry matter production can increase due to soluble phosphate fertilizer and limestone using.

Although species turnover and increased proportion of legumes can enable improved bromatological features, they can lead to reduced soil C stock and decrease Pampa biome' ability to adapt to climate change, in the long-term. Thus, more conclusive studies should be conducted to analyze changes in ecosystem services and soil carbon increase in fertilized Pampa biome soil.

Regular phosphorus input is necessary to maintain dry matter yield and legume species' contribution in dry matter production higher than without phosphorus fertilization; in the present case, four years after phosphorus input resulted in dry matter yields similar between treatments; in the condition of the present study (i.e., with dry matter exportation) such a input cannot surpass three years.

The highest available soil P content after phosphate rock application at rate of 250 kg ha^{-1} of P (six years before soil sampling) may be an artifact that occurred during P extraction with Mehlich-1. This assumption suggests low phosphate rock dissolution over time and justifies the low increase in Pampa grassland dry matter production response.

Chapter III - Chemical pattern of vegetation and topsoil of rangeland fertilized over 21 years with phosphorus sources and limestone.

The Pampa ecosystem of South America is an ecological region consisting of grasslands and rangelands, covering 500,000 km^2 in Argentina, Brazil, Uruguay, and Paraguay (Pallarés et al., 2005). The great potential of the Pampa natural vegetation leads it to be used as the main source of animal food in these regions (rangeland) (Carvalho et al., 2006). In Brazil, the Pampa rangelands are the alimentary base for almost 13 million head of cattle (IBGE, 2016) which are predominantly managed in the extensive system. Historically, landowners in Pampa have not made investments to improve pasture productivity. Since the end of the 18th century, grasslands have been used to produce beef on large farms without the application of any agricultural inputs; hence, nowadays, these areas are cultivated in an inadequate way, resulting in overgrazing, low productivity, and consequently, low economic returns (Borges et al., 2016; Carvalho and Batello, 2009; Fedrigo et al., 2018).

The improvement of the economic efficiency of cattle production in rangelands implies an increase in dry matter production. Well managed rangelands of Pampa can reach a dry matter production around 14 Mg ha^{-1} year^{-1} (Oliveira et al., 2015). The principal causes of low productivity is high soil acidity (pH ~ 4.4) and low fertility, such as reduced available P (2.6 to 7.6 mg of P kg^{-1}). In the scenario of low fertility, an increase of nutrients availability by limestone and phosphorus fertilizers are important strategies to support plant development (Gatiboni et al., 2000; Prestes et al., 2017, 2016).

The dolomite rock (magnesian limestone) is abundant and is almost the unique material used to neutralize soil potential acidity in Southern Brazil. The very low solubility of dolomite demands its incorporation into the soil profile. Phosphorus fertilizers should also be incorporated into the soil because of very low phosphorus mobility. However, in grasslands, liming and fertilization are carried out by deposition on the soil surface. Nevertheless, the fertilization in soil surface can restrict the improvement of soil fertility to topsoil layers and, consequently, rangeland productivity. The objective of this chapter was to test whether long-term surface application of phosphorus fertilizers and limestone in a rangeland allow for an increase in the phosphorus availability and aluminum neutralization in deep soil layers and increase in the rangeland dry matter production.

This study investigated the chemical properties of rangeland fertilized over 21 years with different phosphorus sources and liming. The highest rate of phosphorus (249 kg ha^{-1} of P applied until 2012) provides the highest levels of nutrient availability in the soil and in the plant shoot, but there is no more response in dry matter production of rangeland after more than four years since the last fertilization. That indicates the necessity of periodic phosphorus fertilization in a time-space lower than four years.

The use of phosphate rock in the soil surface provides a greater deepening of phosphorus in the soil profile by mass flow phenomenon (achieving 7.5 cm after 21 years and a rate of 249 kg ha^{-1} of P) compared to soluble fertilizers. Moreover, further studies are needed to understand the apatite mineral dissolution in rangelands soils and the P availability to plants.

After 21 years of surface deposition of limestone in rangeland, the reacidification was not significant, and the neutralizing front reached depths greater than 10 cm. In these conditions, the levels of exchangeable Ca and Mg, pH, exchangeable Al, and the saturation by Al are better than without limestone for plant development.

Chapter IV - P-Legacy effect of soluble fertilizer added with limestone and phosphate rock on grassland soil in subtropical climate region.

In the last 50 years, Southern Brazilian grassland - the so-called Pampa grassland - had its natural vegetation area decreased by 26% in order to open room for annual and forest crops capable of providing higher financial return (Oliveira et al., 2017). One of the possibilities to slow down the pace of destruction of this biome is to increase forage production and, consequently, beef and sheep meat. However, most of these areas require mandatory use of P fertilizer added with limestone to increase dry matter yield (Oliveira et al., 2015; Tiecher et al., 2014).

Water soluble fertilizers are the main P source used worldwide. They play an essential role in agriculture because they enable fast increase in P available in soil solution and in P bioavailability (Bolan et al., 2003), as well as favors P uptake by plants, which, consequently, improves crop yield in the short-term (Pavinato et al., 2017; Silveira et al., 2018). Alternatively, to soluble fertilizers use, phosphate rocks (RP) have been used as fertilizer at global scale (Dias et al., 2015; Oliveira et al., 2015; Pufahl and Groat, 2017; Soltangheisi et al., 2020, 2018). Igneous and sedimentary phosphate rocks are the two main RP types in the world (Ptáček, 2016; Pufahl and Groat, 2017). The structure of igneous phosphate rocks is coarser, lesser porous, and significantly lesser soluble/reactive than that of sedimentary phosphate rocks. RP dissolution enables synchronism between P release to soil solution and plants' needs. This behavior can lead to RP-legacy particle effect and to lesser P inner-sphere immobilization by soil clay minerals and oxides; this process favors P recovery by plants in the long-term (Pavinato et al., 2017; Szilas et al., 2007).

In this work, the aims of the current chapter were to evaluate (i) whether there is no long-term dissolution of P-bearing minerals when phosphate rock is used as fertilizer in Pampa grassland; (ii) analyzing its P-legacy response and plant dry matter production in comparison to those of grassland subjected to fertilization based on soluble phosphate added with limestone.

The current study has evaluated soil P-legacy and low apatite dissolution in an experiment comprising 20 years of fertilization with P sources and limestone. P-legacy was differentiated based on the solubility of the used P source. The use of soluble P source and limestone (triple superphosphate at rate of 250 kg ha^{-1} of P add with limestone at rate of 3.2 Mg ha^{-1}) resulted in greater nutrient use efficiency (five times greater than that of phosphate rock) and in higher dry matter yield (22%). Consequently, there was also greater P exportation through plant tissue and lesser residual effect of P fertilizer on the topsoil (0-2.5 cm).

In addition, the use of phosphate rock (at a rate of 250 kg ha^{-1} of P) has led to higher total P and moderate-lability P levels in the soil. It happened due to low apatite dissolution (even under favorable soil thermodynamics conditions) in coarse sand and coarse silt particle size fractions, which was analytically checked and subjected to Scanning Electron Microscopy (SEM-EDS). Apatite stability has exceeded six years under the evaluated soil conditions, although it lasted shorter than 20 years. Therefore, the lower grassland dry matter production can be partly attributed to low phosphate rock dissolution. Apparently, the use of sedimentary phosphate rock from Gafsa – Tunisia was not a satisfactory alternative to increase South American Pampa grassland yield.

Chapter V - P stock depletion and soil C:N:P stoichiometry by annual crop or grassland management systems over 13 years.

Phosphorus (P) is a key element for all life cycles (Elser 2012). The plant's P uptake occurs via root system and therefore, the maintenance of satisfactory soil available P contents is indispensable for plant growth (Lun et al. 2018; Tian et al. 2019). The soil P occurs in inorganic (Pi) or organic (Po) forms. The inorganic forms of P are orthophosphate (HPO_4^{-2} and $H_2PO_4^{-}$) in soil solution; bound to cations, such as calcium, forming geogenic or anthropogenic calcium phosphates; adsorbed by inner-sphere at functional groups of clay minerals and Fe and

Al oxi-hydroxides (Walker and Syers 1976; Helfenstein et al. 2018). The organic P is somehow bound to at least one C atom, forming mainly orthophosphate monoesters (e.g., inositol phosphates and adenosine monophosphate) and orthophosphate diesters (e.g., nucleic acids, phospholipids) constituting the soil organic matter and soil microbial biomass (George et al. 2018; Li et al. 2018; Liu et al. 2018).

Both P forms occur simultaneously in soil and have different availability for plants uptake (P lability). The quantification of P availability is commonly assessed by sequential extractions with chemical reagents of increasing extraction force (e.g., sequential extraction proposed by Hedley et al. (1982)) and grouped into P pools called P labile, P moderately labile and P non-labile. Naturally, the P uptake by plants comes from the most available pool (labile), which are buffered by the one with less lability (Tiecher et al. 2018). In addition, the turnover of each P pool decreases with a reduction in P lability, ranging from minutes to hundreds of years (Helfenstein et al. 2020). Thus, it is expected that the depletion of soil P by agricultural activities will be perceived in the short term in most labile pools and in medium and long term in less labile pools. The buffering of the available P varies according to the soil type and the management adopted (Fink et al. 2016).

Although the P is absorbed by plants predominantly in inorganic form (Schachtman et al. 1998), the presence of Po in the soil has remarkable importance in the reduction of P inner-sphere adsorption to clay minerals and oxides and in mobilizing recalcitrant P (Guppy et al. 2005; Chassé and Ohno 2016; Rodrigues et al. 2016; Menezes-Blackburn et al. 2018). The transformation of Pi into Po occurs when plants and microorganisms incorporate Pi into their tissues. After death, the P returns to the soil in organic form into SOM. Therefore, the presence of Pi or Po in the soil is determined by the content of SOM and can be changed by the soil management system adopted. In the soil, there is a balance between the cycles of C, N and P, which are interconnected in SOM. Before the uptake of P by plants, a step of Po mineralization is necessary and, this one proceeds differently according to the organic compound's recalcitrance. In the soil, the orthophosphate monoesters have higher turnover than the orthophosphate diesters due to chemical bonding to soil colloids (Turner et al. 2005; Menezes-Blackburn et al. 2018). This comportment may induce in higher accumulation of Po monoesters form than in diesters form in the SOM.

In general, soil with high P stock, from fertilizers inputs or soil parent material, may supply plant demand and support plant yields for some period, even without P fertilization. However, the absence of phosphate fertilization associated to constant P exportation generates a negative soil P balance resulting in a decrease of soil P stocks and availability (Boitt et al. 2018; Tyson et al. 2020). This can cause restrictions on crop yields and the maintenance or accumulation of SOM in a time scale dependent of the P export. In this work, the aim was to test if the soil management system alters the temporal depletion of P stock and its coupling with C and N cycle.

The results showed a greater export of P and, consequently, a greater depletion of total P stock in the treatment with permanent grassland. The depletion of P mainly impacted the P pool with greater lability (P-labile). However, the depletion that occurred after 13 years was not enough to cause an evident reduction in plant yields productivity due to the buffering capacity of the P pools with less lability (P-mod-labile).

With permanent grassland, there was a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. This behavior provided a reduction in the C:N:Po atomic ratio values and, consequently, a coupling between the C, N and P cycles in the soil. ^{31}P-NMR technique revealed that permanent grassland had a more significant effect in reducing *α*–glycerophosphate and increasing *myo*-IHP and adenosine monophosphate than permanent cropland.

Chapter VI - Soil P microbial biomass and coupling of C:N:P: a soil aggregate scale approach.

Global guidelines have been proposed to increase the stock of soil organic carbon (SOC) as a strategy to counterbalance the emission of CO_2 by use of fossil fuels and thereby minimize the effect of global warming (Dignac et al., 2017). The SOC is defined mainly by the soil organic matter content - SOM (plant or animal waste, live or dead micro-organisms and humus) and it can be profoundly altered by soil management systems, especially the soil fertility (Assunção et al., 2019; Dignac et al., 2017; Panettieri et al., 2017; Poeplau et al., 2018). Soils occurring in temperate regions originally contain a large stock of bioavailable P, however long periods of cultivation without addition of this nutrient have also led to the depletion of labile forms and even may limit the productivity and the increase of SOM (Blake et al., 2003; Boitt et al., 2018; Le Noë et al., 2020).

In France, for example, until the 1970s there was a significant increase in the use of phosphate fertilizers, increasing total P contents in the country's soils. However, from the 1970s onwards, the consumption of phosphate fertilizers in French agriculture was drastically reduced through public policies (Le Noë et al., 2020). This strategy was justified due to the high annual P inputs and the high levels of P in the soils. However, without the addition of phosphate fertilizers, the P budget in many agricultural areas became negative, causing possible decreased production due to plant nutritional restrictions (Éveillard & Saby, 2018; Saby et al., 2016).

Increasing the content of C and N into soil also means immobilizing P. Therefore, the recalcitrance of organic compounds defines the recovery of Po by plants and microorganisms. Organic P must be converted into mineral form before being used again by plants. This process is carried out almost exclusively by soil microorganisms through mineralization of the organic matter. In addition, plants can synthesize and exude phosphatases that can assist in organic P bioavailability (Hayes et al., 1999). Accordingly, P stored in labile organic forms can be reused by plants (Menezes-Blackburn et al., 2018) and represent a temporal stock of P in the soil (Rheinheimer et al., 2019)

The recalcitrance of SOM is closely related to the complexity of organic compounds and to the physical protection provided by soil inorganic constituents (Assunção et al., 2019). In this perspective, the relationship between soil aggregation and SOM is a two-way path, while the SOM act like a glue to soil aggregation, the aggregation act as an armor to physical protection and non-mineralization of SOM (Kleber & Johnson, 2010; Tisdall & Oades, 1982). The variation of the SOM levels as function of soil aggregate size is an indicator of the P contents variation, mainly Po fractions. A considerable fraction of the Po is composed of P stored in the microbial biomass (P_{mic}), and therefore the P will be prevented from reacting with functional groups of the clay minerals and oxides. The size distribution of soil inorganic and organic constituents and the degree of mineralization and turnover of SOM varies with the size of the aggregates (Liao et al., 2018; Panettieri et al., 2017), and thus there are major changes in the stock and bioavailability of P in soils depending of the aggregate size distribution and stability. Thus, the availability of P to plants (Fontana et al., 2008; Pereira et al., 2010) and C: N: P stoichiometry should be differentiated for each aggregate size.

In this work, our objectives were to evaluate (i) whether soil microbial biomass is an important stock of P in organic form in soils of temperate climate region and its significance is larger in management systems that favors SOM stabilization; (ii) test if soil management systems alter P bioavailability and its coupling with C and N differently in each soil aggregate size.

Our results show that the soil management systems did not change the total levels of P in the soil after 13 years, but the inorganic P / organic P ratio was higher with the practice of soil plowing and annual plant cultivation. Concomitantly, the forage management system favored the accumulation of organic C, N and P in the soil. During the growing season (barley / mix forage - 2018/2019), the levels of P contained in soil microbial biomass were an important pool of bioavailable P in both management systems and represented up to 47% of the available P content (P Olsen) in a soil plowing and annual plant cultivation system.

The practice of soil plowing and annual crop cultivation reduced the percentage and the levels of total soil C, N and P in soil microbial biomass of large aggregates compared to the permanent cultivation of forages. Furthermore, the permanent cultivation of forage species maintained a higher bioavailability of P (P labile) in aggregates greater than 0.050 mm, mainly due to the soil Pi labile contents. In contrast, for the soil plowing and annual plant cultivation, the highest bioavailability of P was obtained in smaller aggregates (<0.200 mm), mainly due to the higher levels of Po labile in these aggregate classes. Stoichiometry C:N:Po was little changed by the 13 years of cultivation in the management systems.

Conclusion

In this Thesis, we analyzed two long-term experiments in terms of impacts of the use of phosphate fertilizers on the efficiency of phosphorus use by plants and its availability, P legacy, yields and coupling with carbon and nitrogen in the soil organic matter.

We concluded that in soil with low P stock value, the use of soluble P sources (such as simple and triple superphosphate) associated with liming is a better strategy to improve dry matter production and P use efficiency in Pampa grassland. These fertilizers lead to a small change in species richness; however, there was higher species turnover, mainly in the tussock grass and forbs groups and in the legume contribution to forage production. To maintain dry matter yield, a regular phosphorus input with no more than three years interval is necessary. Though, the use of phosphate rock did not improve fertility conditions and dry matter production satisfactorily due to their long term non dissolution in the soil (more than six years).

In soil with high P stock value, permanent grassland provided a greater depletion of total and P-labile stocks due to its higher capacity to P export than permanent cropland. As well, permanent grassland supports a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. This behavior provided a reduction in the C:N:Po atomic ratio values and, consequently, a coupling between the C, N and P cycles in the soil. Additionally, we observed that the P_{mic} is an important bioavailable P pool in both management systems and accounted for up to 47% of the available P content (P Olsen) in a permanent cropland system. By ^{31}P-NMR technique was observed that permanent grassland had a more significant effect in reducing α–glycerophosphate and increasing *myo*-IHP and adenosine monophosphate than permanent cropland.

Resumo Longo

DINÂMICA TEMPORAL DE P EM DIFERENTES SISTEMAS DE MANEJO DE SOLO EM CLIMA TEMPERADO E SUBTROPICAL

Introdução

O aumento da população mundial exige que a produção de alimentos seja potencializada (Ray et al., 2013). O que deve ser alcançado por meio do uso mais eficiente das áreas já cultivadas. Porém, a agricultura praticada em todo o mundo nas últimas décadas vai no caminho contrário da sustentabilidade ambiental. Principalmente devido ao uso intensivo e inadequado de agroquímicos, como pesticidas, fertilizantes químicos e combustíveis fósseis.

O consumo mundial de fertilizantes químicos, por exemplo, cresceu 17,7% entre 2006-2016 (FAO, 2019). Neste montante, P e K são essencialmente provindos de reservas finitas e não há qualquer substituto para eles na produção agrícola (Cordell et al., 2009; U.S. Geological Survey, 2019). A alta demanda de P na agricultura, torna preocupante as projeções de possível esgotamento das reservas naturais de rocha fosfática nos próximos 50-100 anos. (Cordell et al., 2009; Steen, 1998). Além disso, há uma alta adição anual de P aos solos e corpos d'água, causando sérios problemas ambientais e sociais, como a eutrofização dos mananciais d’água (Goyette et al., 2018; Schilling et al., 2018; Schoumans et al., 2014; Withers et al., 2017).

A nível global, o uso mais eficiente de fertilizantes fosfatados é essencial para alcançar a sustentabilidade dos sistemas de produção sem a perda do potencial produtivo das lavouras (MacDonald et al., 2011; Marin et al., 2016; McDonald et al., 2019; Tian et al., 2019). Isso será possível por meio do manejo conjunto de fatores como: práticas de manejo do solo; estratégias de fertilização; uso de fertilizantes orgânicos e cultivo de plantas adaptadas às condições dos sistemas de produção.

Nesse sentido, fosfato natural (rocha fosfatada moída) são considerados fonte de P com reduzida solubilidade, liberação gradual de P ao solo e com potencial de uso na agricultura (Oliveira et al., 2015; Szilas et al., 2007; Tiecher et al., 2014). No entanto, a fertilização do solo com fontes de P menos solúveis (como fosfato natural) em solos subtropicais demonstrou um incremento de formas de P com menor labilidade em comparação a fontes P solúveis (Soltangheisi et al., 2018). No caso do uso de fosfato natural como fonte de P, a diversidade de conclusões na literatura indica a necessidade de mais estudos sobre o assunto.

Além da estratégia de fertilização, os sistemas de manejo do solo alteram a dinâmica de absorção de elementos químicos pelas plantas e, com isso, alteram o ciclo de nutrientes e suas formas químicas no solo. Como exemplo, os sistemas de plantio direto, com rotação de culturas ou cultivo permanente com pastagem, onde há manutenção da palhada e não há revolvimento do solo, apresentam teores significativos de P orgânico, tanto na matéria orgânica quanto na biomassa microbiana do solo (Boitt et al., 2018a, 2018b; Condron et al., 2005; Tiecher et al., 2012a). Em contrapartida, o revolvimento do solo em sistema de cultivo convencional causa mineralização da matéria orgânica do solo e, consequentemente, aumento dos teores de P inorgânico, tornando possível maior imobilização do P pela matriz do solo e redução das formas mais lábeis de P (Rodrigues et al., 2016; Tiecher et al., 2012b). Portanto, fica evidente que o entendimento da dinâmica do P em sistemas de produção em médio e longo prazo e sistemas de manejo do solo são essenciais para melhorar a eficiência do uso do P para uma agricultura sustentável.

Nesta tese, serão desenvolvidos sete capítulos que tratam sobre a dinâmica do P do solo em áreas de produção forrageira e de cultura anual. Os sete capítulos que compõem esta tese, estão caracterizam 3 principais momentos do material.

O primeiro momento, composto pelo capitulo I, está endereçado para a revisão de literatura referente aos temas que serão abordados nos demais capítulos. Neste capítulo será realizada uma abordagem de caracterização do elemento químico P, sua origem, localização e importância para o ciclo de vida no planeta Terra. Além disso, serão realizadas abordagens sobre a dinâmica, os estoques e o uso de fontes de P em solos agrícolas, com destaque para áreas manejadas com pastagens em regiões de clima subtropical e temperado. Para finalizar o primeiro momento, será feita uma abordagem referente ao acoplamento do ciclo biogeoquímico do P e dos elementos C e N e sua importância para o aumento dos teores de matéria orgânica do solo.

O segundo momento, composto pelos capítulos II ao VI, está endereçado para a apresentação dos cinco artigos que compõem esta tese. Estes artigos são provenientes de dois experimentos de longa duração localizados no Brasil e na França. A utilização de dois experimentos localizados nestes países tem por objetivo realizar estudos em ambos locais envolvidos na realização deste doutorado em regime de cotutela. Nos capítulos II, III e IV são apresentados três artigos já publicados em revistas científicas internacionais e são trabalhos do experimento iniciado em 1997, localizado em Santa Maria - Brasil. De modo geral, o objetivo do experimento é testar o uso de diferentes doses e fontes de P, associadas ou não à calagem, em pastagens naturais do bioma Pampa. No capítulo II, publicado na revista European Journal of Agronomy (Somavilla et al., 2021c), foi realizada uma abordagem histórica do experimento, analisando dados de produtividade de matéria seca de mais de 21 anos, composição botânica da vegetação nativa e fertilidade do solo na camada superficial do solo de 0-10 cm. No capítulo III, publicado na revista Soil and Tillage Research (Somavilla et al., 2021b), são analisados dados sobre o conteúdo nutricional e o efeito da calagem em camadas estratificadas a uma profundidade de 0-10 cm do solo; assim como, dados sobre concentração e exportação de P via matéria seca. No capítulo IV, publicado também na revista Soil and Tillage Research (Somavilla et al., 2021a), é realizada uma análise do estoque de P do solo devido ao uso de diferentes fontes de P; assim como a presença no solo de minerais apatita não dissolvidos provenientes do fosfato natural.

Os capítulos V e VI estão em processo de preparação para publicação em revistas internacionais, com submissão prevista ainda para o ano de 2021. Estes trabalhos são referentes a um experimento iniciado em 2005, localizado em Lusignan - França, que visa testar o efeito de diferentes sistemas de manejo (cultivo permanente de culturas anuais ou pastagem) sobre o esgotamento do estoque de P no solo e sua estequiometria C:N:P. No capítulo V, foi analisado um conjunto de dados históricos de exportação e esgotamento de P pelas plantas, alterações nas frações de P do solo e o acoplamento entre P orgânico e C e N na camada superficial de 0-30 cm do solo. No capítulo VI, foi analisada a participação do P contido na biomassa microbiana no teor de P biodisponível do solo durante a estação de crescimento da cevada. Bem como, a distribuição de frações de P e sua estequiometria C:N:P em diferentes classes de agregados do solo de acordo com sistemas de manejo com culturas anuais e pastagem perene.

O terceiro momento, composto pelo capítulo VII, está endereçado para a discussão geral da tese. Neste capitulo, são realizadas abordagens sobre a dinâmica e disponibilidade de P nos solos agrícolas, métodos de quantificação e caracterização das formas de P nos solos. Além de uma abordagem agroambiental sobre a utilização de fertilizantes fosfatados em áreas agrícolas.

Ao final, temos uma conclusão que retoma as observações realizadas nos capítulos anteriores

Contexto

A presente tese é o material final para conclusão do doutorado realizado em supervisão conjunta (co-tutela) entre a Universidade Federal de Santa Maria (Brasil) e a Université de

Poitiers (França). Além disso, a presente tese é parte de um projeto de pesquisa intitulado "Efeito de longo prazo do uso do solo e práticas de manejo na fertilidade do solo: mudanças na mineralogia e disponibilidade de fósforo e potássio". Este projeto foi financiado pelo governo brasileiro e está vinculado ao edital MEC/MCTI/CAPES/CNPq/FAPs – Pesquisador Visitante Estrangeiro - PVE 2014 Nº 03/2014. O projeto é realizado em parceria entre a Universidade Federal de Santa Maria - Brasil e a Université de Poitiers - França e visa desenvolver estudos para compreender o efeito de longo prazo do uso e manejo do solo na fertilidade, com ênfase nas mudanças mineralógicas e disponibilidade de fósforo e potássio para as plantas. Além da produção e divulgação do conhecimento científico, este projeto visa fortalecer a cooperação científica franco-brasileira e capacitar pessoas por meio do intercâmbio de pesquisadores e estudantes entre universidades. Além disso, este trabalho também conta com a colaboração de pesquisadores do Institute National de la Recherche Agronomique – INRA Nouvelle Aquitaine SOERE ACBB, localizado em Lusignan - França, onde uma parte considerável deste trabalho foi desenvolvida.

Capítulo II - Adubação fosfatada e calagem em ensaio realizado ao longo de 21 anos: levantamento para maior produção de forragem e conservação da pastagem do Pampa.

O fósforo (P) corresponde a aproximadamente 0,2% da matéria seca das plantas (Schachtman et al., 1998); é um componente chave para a produção de proteínas e ácidos nucléicos, bem como para ativação enzimática e transferência de energia (Wang et al., 2017) e o crescimento da planta está diretamente ligado à disponibilidade de P no solo. De maneira geral, grande parte dos solos dos campos do sul do Brasil (chamados campos do Pampa) são intensamente intemperizados, ricos em óxidos de ferro e caulinita, e formados a partir de materiais de origem com baixa concentração de P. Estas características implicam em baixa disponibilidade natural de P para as plantas. Além disso, são solos naturalmente ácidos (pH 4,4-5,1) o que proporciona maior adsorção específica de P em argilominerais e óxidos do solo, reduzindo ainda mais a disponibilidade de P para as plantas (a qual frequentemente varia de 2 a 8 mg kg^{-1} (Oliveira et al., 2011; Rheinheimer et al., 1997)).

A vegetação nativa do bioma Pampa compreende espécies adaptadas às condições locais de baixa disponibilidade de P e acidez do solo (Oliveira et al., 2018; Marques et al., 2019). Aproximadamente 3.000 espécies de plantas podem ser encontradas no ecossistema campestre do Pampa (bioma Pampa), 450 delas são espécies de gramíneas utilizadas para a produção de forragem (Boldrini, 2009). Esta vegetação possui alto potencial para produção de forragem quando devidamente manejada, seja com manejo de oferta ou melhoramento da pastagem. O melhoramento da pastagem por meio da correção da fertilidade do solo e da inclusão de espécies exóticas de inverno pode elevar à produção de matéria seca da forragem para 14 Mg ha^{-1} ano^{-1} e aumentar a produção de carne para aproximadamente 900 kg ha^{-1} ano^{-1} de peso vivo (Oliveira et al., 2015). Para isso, é necessário implementar processos adequados de manejo da fertilidade do solo e das plantas para atingir altas taxas de produção de forragem. A fertilização fosfatada e a calagem são estratégias fundamentais para melhorar a fertilidade do solo, aumentar a disponibilidade de nutrientes e, consequentemente, melhorar o desenvolvimento das plantas (Prestes et al., 2016).

O presente estudo tem como objetivo avaliar (i) se as fontes solúveis de P (superfosfato triplo e simples) e o uso de calcário resultam em maior produção de matéria seca do que o uso de fontes de P com menor solubilidade (fosfato natural); e (ii) de que forma a fertilização com P e a calagem afetam a composição botânica e a riqueza de espécies vegetais do campo nativo do bioma Pampa. Neste estudo foram analisados dados de solo e vegetação de um ensaio de 21 anos conduzido em pastagem nativa do bioma Pampa, no sul do Brasil, manejadas com diferentes fontes de P, doses de P, calagem e semeadura de espécies exóticas.

A fertilização fosfatada e a aplicação de corretivo de acidez do solo ajudaram a melhorar a produção de matéria seca da pastagem. No entanto, este aumento de produtividade da pastagem foi diferenciado ao longo de 21 anos em função das fontes de fósforo. O uso de fertilizantes solúveis, como o superfosfato triplo e simples, proporcionou maior produção de matéria seca. A fertilização com fosfato levou a pequenas mudanças na riqueza das espécies vegetais; assim, é possível manter a biodiversidade florística, apesar do uso de fertilizantes fosfatados. Entretanto, houve maior rotatividade de espécies, principalmente nos grupos de *tussock* e *forbs*, o que pode proporcionar alteração nos serviços ecossistêmicos da vegetação. A contribuição das espécies de leguminosas para a produção de matéria seca aumentou devido ao uso de fertilizantes fosfatados solúveis e calcário.

Embora a substituição de espécies e o aumento da contribuição de leguminosas possam permitir a melhoria das características bromatológicas, podem, simultaneamente, levar à redução do estoque de C do solo e diminuição a capacidade da vegetação em se adaptar às mudanças climáticas, a longo prazo. Assim, estudos mais conclusivos devem ser realizados para analisar as mudanças nos serviços ecossistêmicos e o aumento do carbono no solo fertilizado do bioma Pampa.

A fertilização regular com fósforo é necessária para manter o rendimento e a contribuição das espécies de leguminosas na produção de matéria seca do campo nativo. No presente estudo, quatro anos após a fertilização com fósforo os rendimentos de matéria seca foram semelhantes entre os tratamentos com e sem P; assim, na condição do presente estudo (com exportação de matéria seca) o período entre fertilizações não pode ultrapassar três anos.

O maior teor de P disponível no solo após a aplicação de 250 kg ha^{-1} de P na forma de fosfato natural (seis anos antes da amostragem do solo) pode ser um artefato ocorrido durante o processo de extração de P por meio do extrator Mehlich-1. Esta suposição sugere baixa dissolução do fosfato natural ao longo do tempo e justifica a baixa resposta da pastagem a fertilização.

Capítulo III - Padrão químico da vegetação e solo superficial de pastagens fertilizadas ao longo de 21 anos com fontes de fósforo e calcário.

O ecossistema do Pampa localizado na América do Sul é uma região ecológica composta por campos e pastagens, cobrindo 500.000 km^2 entre Argentina, Brasil, Uruguai e Paraguai (Pallarés et al., 2005). O grande potencial da vegetação natural do Pampa possibilita sua utilização como principal fonte de alimento para animal (ovinos e bovinos) nessas regiões (pastagens) (Carvalho et al., 2006). No Brasil, as pastagens do Pampa são a base alimentar de quase 13 milhões de cabeças de gado (IBGE, 2016) que são predominantemente manejadas no sistema extensivo. Historicamente, os proprietários de terras no Pampa não têm feito investimentos para melhorar a produtividade das pastagens. Desde o final do século 18, as pastagens têm sido usadas para produzir carne em grandes fazendas sem a aplicação de quaisquer insumos agrícolas; portanto, atualmente, essas áreas são cultivadas de forma inadequada, resultando em sobre pastoreio, baixa produtividade e, consequentemente, baixo retorno econômico (Borges et al., 2016; Carvalho e Batello, 2009; Fedrigo et al., 2018).

A melhoria da eficiência econômica da produção pecuária em pastagens do Pampa implica um aumento na produção de matéria seca. Estas pastagens, quando manejadas adequadamente, podem atingir uma produção de matéria seca em torno de 14 Mg ha^{-1} ano^{-1} (Oliveira et al., 2015). As principais causas da baixa produtividade são a alta acidez do solo (pH ~ 4,4) e a baixa fertilidade natural, como exemplo o baixo teor de P disponível (2,6 a 7,6 mg de P kg^{-1}). No cenário de baixa fertilidade, o aumento da disponibilidade de nutrientes pelos fertilizantes fosfatados e pela calagem são estratégias importantes para suportar o desenvolvimento das plantas (Gatiboni et al., 2000; Prestes et al., 2017, 2016).

A rocha dolomítica (calcário magnesiano) é abundante e o principal material utilizado para neutralizar a acidez do solo no Sul do Brasil. A baixíssima solubilidade desta rocha exige sua incorporação ao perfil do solo. Os fertilizantes fosfatados também devem ser incorporados ao solo por causa da baixíssima mobilidade do fósforo no solo. Porém, nas pastagens, a calagem e a fertilização são realizadas pela simples deposição na superfície do solo. Esta prática pode restringir a melhoria da fertilidade à camadas superficiais do solo e, consequentemente, restringir a produtividade das pastagens. O objetivo deste capítulo foi testar se a aplicação de fertilizantes fosfatados e calcário na superfície do solo a longo prazo permite um aumento na disponibilidade de fósforo e neutralização de alumínio em camadas mais profundas do solo e, consequentemente, aumento na produção de matéria seca da pastagem.

Este estudo investigou propriedades químicas do solo de pastagens fertilizadas ao longo de 21 anos com diferentes fontes de P e calagem. A maior dose de fósforo aplicada (249 kg ha^{-1} de P de 1997 até 2012) proporcionou os maiores níveis de disponibilidade de P no solo e na parte aérea da planta, mas não há mais resposta na produção de matéria seca de pastagem após mais de quatro anos desde a última fertilização. Isso indica a necessidade de fertilização periódica com P em um intervalo de tempo inferior a quatro anos.

O uso de fosfato natural em superfície proporciona um maior aprofundamento do P no perfil do solo pelo fenômeno do fluxo de massa (atingindo 7,5 cm após 21 anos e uma taxa de 249 kg ha^{-1} de P) em relação aos fertilizantes solúveis. Além disso, mais estudos são necessários para entender a dissolução do mineral apatita em solos de pastagens e a disponibilidade de P para as plantas.

Após 21 anos da aplicação superficial do calcário na pastagem, a reacidificação não foi significativa e a frente de neutralização atingiu profundidades superiores a 10 cm. Nessas condições, os níveis de Ca e Mg trocáveis, pH, Al trocável e a saturação por Al são melhores para o desenvolvimento das plantas do que sem calcário.

Capítulo IV – Efeito da adição de fertilizantes solúveis com calcário e fosfato natural sobre o legado do P em solo de pastagem em região de clima subtropical.

Nos últimos 50 anos, os campos do sul do Brasil - os chamados campos do Pampa - tiveram sua área de vegetação natural reduzida em 26% a fim de abrir espaço para lavouras de culturas anuais e florestais capazes de proporcionar maior retorno financeiro (Oliveira et al., 2017). Uma das possibilidades para diminuir o ritmo de destruição desse bioma é aumentar a produção de forragem e, consequentemente, de carne bovina e ovina. No entanto, a maioria dessas áreas exige o uso de fertilizante fosfatados e calcário para aumentar a produção de matéria seca (Oliveira et al., 2015; Tiecher et al., 2014).

Os fertilizantes solúveis em água são a principal fonte de P usada em todo o mundo. Eles desempenham um papel essencial na agricultura, pois permitem rápido aumento do P disponível na solução do solo e na biodisponibilidade do P (Bolan et al., 2003), além de favorecer a absorção de P pelas plantas, o que, consequentemente, melhora a produtividade da cultura a curto prazo (Pavinato et al., 2017; Silveira et al., 2018). Como alternativa ao uso de fertilizantes solúveis, fosfatos naturais (RP) têm sido usados como fertilizantes em escala global (Dias et al., 2015; Oliveira et al., 2015; Pufahl e Groat, 2017; Soltangheisi et al., 2020, 2018). Fosfatos naturais de origem ígnea e sedimentar são os dois principais tipos de RP no mundo (Ptáček, 2016; Pufahl e Groat, 2017). A estrutura cristalina dos minerais de origem ígnea é mais grosseira, menos porosa e significativamente menos solúvel/reativa do que os minerais de origem sedimentar. A dissolução de RP permite o sincronismo entre a liberação de P para a solução do solo e as necessidades das plantas. Esse comportamento pode levar a um efeito de RP-Legado e à menor adsorção de P por esfera interna a argilominerais e óxidos do solo; esse processo favorece a recuperação do P pelas plantas a longo prazo (Pavinato et al., 2017; Szilas et al., 2007).

Neste capítulo, os objetivos foram avaliar (i) a inexistência de dissolução de minerais contendo P quando da utilização de fosfato natural como fertilizante em pastagens do Pampa; (ii) analisar o legado do P e a produção de matéria seca da vegetação submetida a fertilização com fosfato natural em comparação à fertilização com fosfato solúvel e calcário.

O legado do P foi diferenciado com base na solubilidade da fonte de P usada. O uso da fonte de P solúvel e calcário (superfosfato triplo na dose de 250 kg ha^{-1} de P adicionado com calcário na dose de 3,2 Mg ha^{-1}) resultou em maior eficiência de uso de nutrientes (cinco vezes maior que com fosfato natural) e em maior rendimento de matéria seca (22%). Consequentemente, também houve maior exportação de P através do tecido vegetal e menor efeito residual do fertilizante fosfatado na camada superficial do solo (0-2,5 cm).

Além disso, o uso de fosfato natural (na dose de 250 kg ha^{-1} de P) tem levado a maiores níveis de P total e moderadamente lábil no solo. Isso ocorreu devido à baixa dissolução da apatita (mesmo em condições termodinâmicas favoráveis do solo) em frações granulométricas de areia grossa e silte grosseiro, analisada analiticamente e submetida à Microscopia Eletrônica de Varredura (SEM-EDS). A estabilidade da apatita, em sua forma cristalina, ultrapassou seis anos nas condições de solo avaliadas, porém foi inferior a 20 anos. Portanto, a menor produção de matéria seca na pastagem pode ser parcialmente atribuída à baixa dissolução do fosfato natural no solo. Aparentemente, o uso de fosfato natural de origem sedimentar de Gafsa - Tunísia não foi uma alternativa satisfatória para aumentar a produtividade da pastagem do bioma Pampa.

Capítulo V - Depleção do estoque de P e estequiometria C:N:P em solo cultivado por 13 anos em sistema de culturas anuais ou de pastagens.

O fósforo (P) é um elemento-chave para todos os ciclos de vida (Elser 2012). A absorção de P pelas plantas ocorre via sistema radicular e, portanto, a manutenção de teores satisfatórios de P disponíveis no solo é indispensável para o crescimento da vegetação (Lun et al. 2018; Tian et al. 2019). No solo, o P ocorre nas formas inorgânicas (Pi) ou orgânicas (Po). As formas inorgânicas do P são ortofosfato (HPO_4^{-2} e $H_2PO_4^-$) na solução do solo; ligado a cátions, como cálcio, formando fosfatos de cálcio geogênicos ou antropogênicos; adsorvido por esfera interna em grupos funcionais de argilominerais e oxi-hidróxidos de Fe e Al (Walker and Syers 1976; Helfenstein et al. 2018). Na forma orgânica o P está ligado a pelo menos um átomo de C, formando principalmente ortofosfato monoésteres (por exemplo, fosfatos de inositol e monofosfato de adenosina) e ortofosfato diésteres (por exemplo, ácidos nucleicos, fosfolipídeos) que constituem a matéria orgânica do solo e a biomassa microbiana do solo (George et al. 2018; Li et al. 2018; Liu et al. 2018).

Ambas as formas de P ocorrem simultaneamente no solo e têm diferentes disponibilidades para absorção pelas plantas (P labilidade). A quantificação da disponibilidade de P é comumente avaliada por extrações sequenciais com reagentes químicos de força de extração crescente (por exemplo, extração sequencial proposta por Hedley et al. (1982)) e agrupados em pools de P chamados P lábil, P moderadamente lábil e P não lábil. Naturalmente, o P absorvido pelas plantas provem das formas com maior disponibilidade (lábil), que é tamponado por aquele com menor disponibilidade (Tiecher et al. 2018). Além disso, a renovação de cada fração de P reduz com a diminuição da labilidade do P, variando de minutos a centenas de anos (Helfenstein et al. 2020). Assim, espera-se que o esgotamento do P do solo pelas atividades agrícolas seja percebido, a curto prazo, nas formas mais lábeis e, a médio-longo prazo, nas formas menos lábeis. O tamponamento dos teores de P disponível varia de acordo com o tipo de solo e o manejo adotado (Fink et al. 2016).

Embora o P seja absorvido pelas plantas predominantemente na forma inorgânica (Schachtman et al. 1998), a presença de Po no solo tem notável importância na redução da adsorção de P por esfera interna aos argilominerais e óxidos e na mobilização de P recalcitrante (Guppy et al. 2005; Chassé e Ohno 2016; Rodrigues et al. 2016; Menezes-Blackburn et al. 2018). A transformação de Pi em Po ocorre quando plantas e microrganismos incorporam Pi em seus tecidos. Após a morte, o P retorna ao solo na forma orgânica na matéria orgânica do solo (MOS). Portanto, a presença de Pi ou Po no solo é determinada pelo teor de MOS e pode ser alterada pelo sistema de manejo do solo adotado. No solo, há um equilíbrio entre os ciclos de C, N e P, que estão interligados na MOS. Antes da absorção de P pelas plantas, uma etapa de mineralização de Po é necessária e, esta prossegue de forma diferente de acordo com a recalcitrância dos compostos orgânicos. No solo, os ortofosfatos monoésteres têm maior *turnover* do que os ortofosfato diésteres devido à ligação química aos coloides do solo (Turner et al. 2005; Menezes-Blackburn et al. 2018). Este comportamento pode induzir a um maior acúmulo da forma de Po monoésteres do que na forma de diésteres na MOS.

Em geral, o solo com alto estoque de P, provindo de fertilizantes ou material de origem do solo, pode suprir a demanda da planta e suportar altas produtividade por algum período, mesmo sem fertilização com P. No entanto, a ausência de fertilização associada a exportação constante de P gera um balanço negativo do elemento no solo, resultando em uma diminuição de seu estoque e disponibilidade (Boitt et al. 2018; Tyson et al. 2020). Isso pode causar restrições na produtividade das culturas e na manutenção ou acúmulo de MOS em uma escala de tempo dependente da exportação de P. Neste trabalho, o objetivo foi testar se o sistema de manejo do solo altera a depleção temporal do estoque de P e seu acoplamento com o ciclo do C e N.

Nossos resultados evidenciam maior exportação de P e, consequentemente, maior esgotamento do estoque total de P no tratamento com pastagem permanente. A depleção de P impactou principalmente na fração de P com maior labilidade (P-lábil). No entanto, o esgotamento ocorrido após 13 anos não foi suficiente para causar uma redução evidente na produtividade das culturas devido à capacidade tamponante das frações de P com menor labilidade (P-mod-lábil).

Com pastagens permanentes, houve uma transformação de P inorgânico em P orgânico no reservatório mais lábil, acompanhando o aumento dos estoques de C e N no solo. Esse comportamento proporcionou uma redução nos valores da razão atômica C:N:Po e, consequentemente, um acoplamento entre os ciclos C, N e P no solo. A técnica de ^{31}P-NMR revelou que as pastagens permanentes tiveram um efeito mais significativo na redução do α-glicerofosfato e aumentaram o mio-IHP e o monofosfato de adenosina do que a utilização permanente com culturas anuais.

Capítulo VI - P na biomassa microbiana do solo e acoplamento de C:N:P: uma abordagem à escala de agregados do solo.

Diretrizes globais foram propostas para aumentar o estoque de carbono orgânico do solo (SOC) como uma estratégia para contrabalançar a emissão de CO_2 pelo uso de combustíveis fósseis e, assim, minimizar o efeito do aquecimento global (Dignac et al., 2017). O carbono orgânico do solo é definido principalmente pela SOM (resíduos vegetais ou animais, micro-organismos vivos ou mortos e húmus) e pode ser profundamente alterado pelos sistemas de manejo do solo, em especial a fertilidade do solo (Assunção et al., 2019; Dignac et al., 2017; Panettieri et al., 2017; Poeplau et al., 2018). Em solos que ocorrem em regiões temperadas há originalmente um grande estoque de P biodisponível, porém longos períodos de cultivo sem reposição desse nutriente também têm levado ao esgotamento das formas lábeis podendo limitar a produtividade e o aumento da SOM (Blake et al., 2003; Boitt et al., 2018; Le Noë et al., 2020).

Na França, por exemplo, até a década de 1970 houve um aumento significativo no uso de fertilizantes fosfatados, aumentando os teores de P total nos solos do país. No entanto, a partir da década de 1970, o consumo de fertilizantes fosfatados na agricultura francesa foi drasticamente reduzido por incentivo de políticas públicas (Le Noë et al., 2020). Esta estratégia foi justificada devido riscos causados pelos elevados aportes anuais de P e aos altos níveis de P nos solos. Porém, sem a adição de fertilizantes fosfatados, o balanço de P em muitas áreas agrícolas tem sido negativo, causando preocupação com a possibilidade de diminuição da produção devido às restrições nutricionais (Éveillard & Saby, 2018; Saby et al., 2016).

Imobilizar C e N no solo também significa imobilizar P. Portanto, a recalcitrância de materiais orgânicos define a recuperação de Po por plantas e microrganismos. O íon fosfato deve ser retirado da matéria orgânica para ser reaproveitado pelas plantas. Este processo é realizado quase exclusivamente por microrganismos do solo por meio da mineralização da matéria orgânica. Além disso, as plantas podem sintetizar e exsudar enzimas fosfatases que podem auxiliar na biodisponibilidade de P orgânico (Hayes et al., 1999). Consequentemente, o

P armazenado em formas orgânicas lábeis pode ser reutilizado pelas plantas (Menezes-Blackburn et al., 2018) e representa um estoque temporal de P no solo (Rheinheimer et al., 2019)

A recalcitrância da MOS está intimamente relacionada à complexidade das estruturas orgânicas e à proteção física fornecida pelos colóides do solo (Assunção et al., 2019). Nessa perspectiva, a relação entre a agregação do solo e a MOS é um caminho de mão dupla, enquanto a MOS atua como uma cola para a agregação do solo, a agregação atua como uma armadura para proteção física e não mineralização da MOS (Kleber & Johnson, 2010; Tisdall e Oades, 1982). A variação dos níveis de MOS em função do tamanho do agregado do solo é também um indicador da variação dos teores de P, principalmente das frações de Po. Uma fração considerável do Po é armazenado na biomassa microbiana do solo (P_{mic}) e, portanto, impedido de reagir com grupos funcionais dos argilominerais e óxidos do solo. A distribuição de tamanho das partículas inorgânicas e orgânicas do solo e seu grau de alteração variam com o tamanho dos agregados (Liao et al., 2018; Panettieri et al., 2017). Assim, a disponibilidade de P para as plantas (Fontana et al., 2008; Pereira et al., 2010) e a estequiometria C: N: P devem ser diferenciadas para cada tamanho de agregado.

Neste trabalho, nossos objetivos foram avaliar (i) se a biomassa microbiana do solo é um importante estoque de P na forma orgânica em solos de região de clima temperado e sua significância é maior em sistemas de manejo com mais MOS; (ii) testar se os sistemas de manejo do solo alteram a biodisponibilidade de P e seu acoplamento com C e N de forma diferente em cada tamanho de agregado do solo.

Nossos resultados evidenciam que os sistemas de manejo do solo não alteraram os teores totais de P no solo após 13 anos, porém a relação P inorgânico / P orgânico foi maior com a prática de revolvimento do solo e cultivo de cereais. Concomitantemente, o sistema de manejo com forrageiras favoreceu acúmulo de C, N e P orgânico no solo. Durante toda estação de crescimento (cevada/mix forrageiro- 2018/2019) os teores de P contido na biomassa microbiana do solo foram um importante *pool* de P biodisponível em ambos sistemas de manejo e representaram até 47 % do teor de P disponível (P Olsen) em sistema de cultivos com cereais e revolvimento do solo.

A prática de revolvimento do solo e cultivo de cereais reduziu a porcentagem e os teores de C, N e P na biomassa microbiana do solo em agregados grandes em comparação ao cultivo permanente de forrageiras. Além disso, o cultivo permanente de espécies forrageiras manteve maior biodisponibilidade de P (P lábil) em agregados maiores que 0.050 mm, devido principalmente aos teores de Pi lábil do solo. Diferentemente, para o solo cultivado com cereais e anualmente revolvido, a maior biodisponibilidade de P foi obtida em agregados menores (< 0.200 mm), principalmente devido aos maiores teores de Po lábil nestas classes de agregados. A estequiometria C:N:Po foi pouco alterada pelos 13 anos de cultivo nos sistemas de manejo.

Conclusão

Nesta Tese, analisamos dois experimentos de longa duração quanto aos impactos do uso ou não de fertilizantes fosfatados na eficiência do uso do P e sua disponibilidade, no legado do P, produtividade das culturas e acoplamento com C e N na matéria orgânica do solo.

Concluímos que em solos com baixo estoque de P, o uso de fontes de P solúvel (como superfosfato simples e triplo) associado à calagem é a melhor estratégia para melhorar a produção de matéria seca e a eficiência do uso de P no campo nativo do bioma Pampa. Esses fertilizantes levam a uma pequena mudança na riqueza de espécies; entretanto, houve maior rotatividade de espécies, principalmente nos grupos *tussock* e *forbes* e na contribuição de leguminosas na matéria seca produzida. Para manter elevada produção de matéria seca, é necessário um aporte regular de fósforo com intervalos menores que três anos. Porém, o uso de fosfato natural não melhorou as condições de fertilidade e produção de matéria seca de forma satisfatória devido à sua não dissolução no solo a longo prazo (mais de seis anos).

Em solo com alto estoque de P, o cultivo permanente de pastagens proporcionou um esgotamento maior dos estoques totais e lábeis de P, devido à sua maior capacidade de exportar P em comparação a culturas anuais. Da mesma forma, cultivo permanente de pastagens proporcionou uma transformação de P inorgânico em P orgânico no reservatório mais lábil, acompanhando o aumento dos estoques de C e N no solo. Esse comportamento proporcionou uma redução nos valores da relação atômica C:N:Po e, consequentemente, um acoplamento entre os ciclos C, N e P no solo. Além disso, observamos que o P_{mic} é uma importante fração de P biodisponível em ambos os sistemas de manejo e foi responsável por até 47% do conteúdo de P disponível (P Olsen) em um sistema de cultivo com culturas anuais. Pela técnica de ^{31}P-RMN foi observado que o cultivo permanente de pastagens teve um efeito mais significativo na redução do α-glicerofosfato e aumentaram o *mio*-IHP e o monofosfato de adenosina do que o cultivo de culturas anuais.

Résumé long

DYNAMIQUE TEMPORELLE DU P DANS DES SOLS CULTIVES PAR DIFFÉRENTS SYSTÈMES DES CULTURE EN CLIMAT TEMPERE ET SUBTROPICAL

Introduction

L'augmentation de la population mondiale nécessite que la production alimentaire soit optimisée (Ray et al., 2013). Ceci pourrait être en partie réalisé par une utilisation plus efficace des surfaces déjà cultivées. Cependant, l'agriculture pratiquée dans le monde au cours des dernières décennies ne tend pas vers durabilité, principalement en raison de l'utilisation intensive et inadéquate de produits agrochimiques tels que les phytosanitaires, les engrais chimiques et les combustibles fossiles.

La consommation mondiale d'engrais chimiques, par exemple, a augmenté de 17,7 % entre 2006 et 2016 (FAO, 2019). Le P et le K utilisés proviennent essentiellement de réserves finies/épuisables dans les mines et il n'y a pas de substitut pour la production agricole (Cordell et al., 2009 ; U.S. Geological Survey, 2019). La forte demande en P rend préoccupantes les projections d'un possible épuisement des réserves naturelles de roche phosphatée dans les 50 à 100 prochaines années (Cordell et al., 2009 ; Steen, 1998). En outre, il y a un fort apport annuel de P dans les sols et les masses d'eau, ce qui cause de graves problèmes environnementaux et sociaux, comme l'eutrophisation des ressources en eau (Goyette et al., 2018 ; Schilling et al., 2018 ; Schoumans et al., 2014 ; Withers et al., 2017).

Au niveau mondial, une utilisation plus efficace des engrais phosphatés est essentielle pour parvenir à la durabilité des systèmes de production sans perte du potentiel de production des aliments (MacDonald et al., 2011 ; Marin et al., 2016 ; McDonald et al., 2019 ; Tian et al., 2019). Ce résultat sera obtenu par l'interaction de facteurs tels que : les pratiques de gestion des sols ; les stratégies de fertilisation ; l'utilisation d'engrais organiques et la culture de plantes adaptées aux des systèmes de production et aux conditions locales.

Dans ce sens, la poudre de roche ou les roches concassées sont considérées comme des sources de P à faible solubilité avec un potentiel d'utilisation en agriculture dû à la libération progressive de P dans le sol (Oliveira et al., 2015 ; Szilas et al., 2007 ; Tiecher et al., 2014). Cependant, la stratégie de fertilisation visant à utiliser des sources de P moins solubles (c'est-à-dire la roche phosphatée) dans les sols subtropicaux a démontré une plus grande augmentation des formes de P moins disponibles que les sources solubles (Soltangheisi et al., 2018). Dans le cas de la roche phosphatée, la diversité des conclusions dans la littérature indique la nécessité de poursuivre les études sur le sujet.

Outre la stratégie de fertilisation, les systèmes de gestion des sols modifient la dynamique d'absorption des nutriments par les plantes en changeant le cycle des nutriments et leurs formes chimiques dans le sol. Par exemple, les systèmes sans labour, avec rotation des cultures ou des pâturages pérennes et un maintien de la paille sur le sol ont des niveaux significatifs de P organique, soit dans la matière organique, soit dans la biomasse microbienne du sol (Boitt et al., 2018a, 2018b ; Condron et al., 2005 ; Tiecher et al., 2012a). En revanche, dans les systèmes avec labour, il y a une minéralisation de la matière organique du sol et, par conséquent, une augmentation des formes inorganiques de P avec une possibilité de sorption du P par les constituants du sol, provoquant une augmentation des formes moins labiles (Rodrigues et al., 2016 ; Tiecher et al., 2012b). Par conséquent, il est évident que la compréhension de la dynamique du P dans les systèmes de production à moyen et long terme et des systèmes de gestion du sol sont essentiels pour améliorer l'efficacité d'utilisation du P dans une agriculture durable.

Dans cette thèse, sept chapitres traitent de la dynamique du P du sol dans les zones de production de fourrage et de culture annuelle. Ces sept chapitres sont caractérisés par 3 parties principales de la thèse.

La première partie, correspondant au chapitre I, est consacrée à une revue de la littérature concernant les thèmes qui seront abordés dans les autres chapitres, tels que, les approches pour caractériser l'élément chimique P, son origine, sa localisation et son importance pour le cycle de vie sur Terre. En outre, des approches seront réalisées sur la dynamique, les stocks et l'utilisation des sources de P dans les sols agricoles, en mettant l'accent sur les zones gérées avec des pâturages dans les régions de climats subtropicaux et tempérés. Pour conclure la première partie, une approche concernant le couplage du cycle biogéochimique du P et des éléments C et N et son importance pour augmenter les niveaux de matière organique dans le sol sera présentée.

La seconde partie correspondant aux chapitres II à VI est consacrée à la présentation des cinq articles qui constituent la thèse. Ces articles sont basés sur deux expériences à long terme situées l’une au sud du Brésil et l’autre en France, région Poitou-Charentes. Les chapitres II, III et IV présentent trois articles déjà publiés dans des revues scientifiques internationales et proviennent d'une expérience située dans une parcelle expérimentale installée à l’Université Fédérale de Santa Maria (Rio Grande do Sul, Brésil) mise en place en 1997. Les principaux objectifs de l'expérience sont de tester l'utilisation de différentes fertilisations phosphatée (sources et quantité), associés ou non au chaulage, dans une prairie naturelle du biome de la Pampa. Dans le chapitre II, publié dans le European Journal of Agronomy (Somavilla et al., 2021c) une approche historique de l'expérience, analysant les données de production de la matière sèche depuis 21 ans, la composition botanique de la végétation naturelle et la fertilité du sol dans la couche 0-10 cm a été réalisée. Dans le chapitre III, publié dans la revue Soil and Tillage Research (Somavilla et al., 2021b), les données sur le contenu nutritionnel et l'effet du chaulage dans la couche 0-10 cm du sol sont analysées, ainsi que les données sur la concentration et l'exportation de P via la matière sèche. Dans le chapitre IV, également publié dans la revue Soil and Tillage Research (Somavilla et al., 2021a), une analyse de l'héritage en P du sol dû à l'utilisation de différentes sources de P a été réalisée. La présence de résidus d’apatite non dissoute provenant du phosphate naturel dans le sol a été recherchée.

Les chapitres V et VI correspondent à des projets d’articles en cours de rédaction pour être publié dans des revues internationales, la soumission étant prévue pour 2021. Ces travaux sont liés à une expérience initialisée en 2005, située à l’INRAE de Lusignan - France, qui vise à tester l'effet de différents systèmes de gestion (cultures annuelles ou pâturages pérennes) sur l'épuisement et l'héritage du P dans le sol et sa stœchiométrie C:N:P. Dans le chapitre V, des données historiques sur l'exportation et l'épuisement du P par les plantes, les changements dans les pools de P du sol et le couplage du P organique dans la dynamique du C et du N dans la couche 0-30 cm du sol ont été analysés. Dans le chapitre VI, la contribution du P de la biomasse microbienne du sol au P biodisponible du sol (méthode Olsen) pendant la saison de croissance de l'orge a été analysée. De même, la distribution des pools de P et leur stœchiométrie C:N:P dans différentes classes d'agrégats de sol selon les systèmes de gestion avec des cultures annuelles et des pâturages pérennes a été analysée.

La troisième partie correspond au chapitre VII de discussion générale de la thèse. Dans ce chapitre, les résultats sur la dynamique et la disponibilité du P dans les sols agricoles, les méthodes de quantification et de caractérisation des formes de P dans les sols sont discutées. La fin de la discussion concerne une approche agro-environnementale de l'utilisation des engrais phosphatés dans les zones agricoles.

La conclusion générale reprend les principaux résultats et interprétations présentés dans les chapitres précédents.

Contexte

La présente thèse est le manuscrit final du doctorat réalisé en cotutelle entre l'Université Fédérale de Santa Maria (Brésil) et l'Université de Poitiers (France). Ce doctorat est inclus dans un projet de recherche intitulé "Long-term effect of soil use and management practices on soil fertility : changes in mineralogy and availability of phosphorus and potassium". Ce projet a été financé par le gouvernement Brésilien et est lié au programme Science Sans Frontières (MEC/MCTI/CAPES/CNPq/FAPs - Special Visiting Researcher - PVE 2014 Nº 03/2014). Le projet est réalisé en partenariat entre l'Universidade Federal de Santa Maria - Brésil et l'Université de Poitiers en France et vise à développer des études pour comprendre l'effet à long terme de l'utilisation et de la gestion des sols sur la fertilité, en mettant l'accent sur les changements minéralogiques et la disponibilité du phosphore et du potassium pour les cultures. Outre la production et la diffusion de connaissances scientifiques, ce projet vise à renforcer la coopération scientifique franco-brésilienne et la formation des personnes par l'échange de chercheurs et d'étudiants entre universités. Ce travail bénéficie également de la collaboration de chercheurs de l'Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement - INRAE Nouvelle Aquitaine - Système d’Observation et d’Expérimentation sur le long terme pour la Recherche en Environnement - Agroécosystème, Cycle Biogéochimique et Biodiversité (SOERE ACBB), situé à Lusignan - France, où une partie considérable de ce travail a été réalisée.

Chapitre II - Fertilisation phosphatée et chaulage dans un essai mené sur 21 ans : un suivi pour une plus grande production de fourrage et la conservation des pâturages de la Pampa

Le phosphore (P) correspond à environ 0,2 % de la matière sèche des plantes (Schachtman et al., 1998) ; c'est un composant clé pour la production de protéines et d'acides nucléiques, ainsi que pour l'activation enzymatique et le transfert d'énergie (Wang et al., 2017). La croissance des plantes est directement liée à la disponibilité du P dans le sol. Globalement, les sols des prairies du sud du Brésil (les prairies dites de la Pampa) sont intensément altérés, riches en oxydes de fer et en kaolinite, et formés à partir de matériaux parentaux présentant une faible concentration en P. Ceci implique une faible disponibilité naturelle du P pour les plantes. En outre, ce sont des sols naturellement acides (pH 4,4-5,1), ce qui entraîne une adsorption de

P à haute énergie sur les minéraux argileux et les oxydes de fer du sol. La disponibilité naturelle de P du sol pour les plantes (par exemple, méthode Mehlich-1) est faible avec des valeurs souvent comprises entre 2 mg kg^{-1} et 8 mg kg^{-1} (Oliveira et al., 2011 ; Rheinheimer et al., 1997).

La végétation qui pousse sur ces sols comprend des espèces adaptées aux conditions locales telles que la faible disponibilité du P et l'acidité du sol (Oliveira et al., 2018 ; Marques et al., 2019). On trouve environ 3 000 espèces végétales dans l'écosystème des prairies de la Pampa (biome de la Pampa), dont 450 sont des espèces de graminées utilisées pour la production de fourrage (Boldrini, 2009). La végétation qui compose les prairies de la Pampa a un fort potentiel de production de fourrage lorsqu'elle est correctement gérée. L'amélioration des prairies par la correction de la fertilité du sol et l'inclusion d'espèces hivernales exogènes peut conduire à une production de matière sèche fourragère de 14 Mg ha^{-1} an^{-1} et permettre la production de viande à environ 900 kg ha^{-1} an^{-1} de masse corporelle de bovins (Oliveira et al., 2015). Il est nécessaire de mettre en œuvre des techniques de gestion de la fertilité et des sols appropriés afin d'atteindre des taux élevés de production de fourrage. Les amendements calcaires et les engrais phosphatés sont des stratégies fondamentales utilisées pour améliorer la fertilité du sol, augmenter la disponibilité des nutriments et, par conséquent, améliorer le développement des plantes (Prestes et al., 2016).

La présente étude vise à déterminer (i) si les sources de P solubles dans l’eau (superphosphate triple et simple) et l'utilisation de calcaire entraînent une plus grande production de matière sèche que l'utilisation de sources de P moins solubles (roche phosphatée) ou l'absence de chaulage ; et (ii) de quelles manières la fertilisation P et le chaulage affectent la composition botanique et la richesse botanique des prairies. Les données sur le sol et la végétation d'un essai de 21 ans mené dans une prairie de la pampa du sud du Brésil, gérée avec différentes sources et taux de fertilisation phosphatée, chaulage, et sur-semis d'espèces végétales, ont été analysées.

La fertilisation phosphatée et le chaulage des prairies de la pampa du sud du Brésil ont permis d'améliorer la production de matière sèche. Cependant, les prairies de la Pampa ont réagi de différentes manières aux sources de phosphore pendant 21 ans. L'utilisation d'engrais solubles, tels que le superphosphate triple et simple, a conduit à une production plus élevée de matière sèche. La fertilisation phosphatée a entraîné de faibles changements dans la richesse des espèces ; il est donc possible de maintenir la biodiversité floristique avec l'utilisation d'engrais phosphatés. Cependant, le renouvellement des espèces a été plus élevé, principalement dans les groupes graminées à touffes et de plantes fourragères, ce qui peut contribuer à la modification des services écosystémiques. La contribution des espèces de légumineuses à la production de matière sèche peut augmenter en raison de l'utilisation d'engrais phosphatés solubles et de calcaire.

Bien que le renouvellement des espèces et la proportion accrue de légumineuses puissent permettre d'améliorer les caractéristiques bromatologiques, ils peuvent conduire à une réduction du stock de carbone dans le sol et diminuer la capacité du biome de la Pampa à s'adapter à long terme au changement climatique. Ainsi, des études plus concluantes devraient être menées pour analyser les changements dans les services écosystémiques et l'augmentation du carbone du sol dans les sols fertilisés du biome de la Pampa.

Un apport régulier de P est nécessaire pour maintenir le rendement en matière sèche et la contribution des espèces de légumineuses à la production de matière sèche à un niveau plus élevé que sans fertilisation phosphorée. Dans le cas présent, quatre années après la fertilisation, l'apport de P a permis d'obtenir des rendements en matière sèche similaires au témoin sans P. Dans les conditions de la présente étude (c'est-à-dire avec exportation de matière sèche), l'effet d'un tel apport ne peut dépasser trois ans.

La teneur la plus élevée en P disponible (extrait par Mehlich-1) dans le sol après l'application de roche phosphatée à un taux de 250 kg ha^{-1} de P (six ans avant l'échantillonnage du sol) peut être un artefact pendant le processus d'extraction du P basé sur la solution d'extraction Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). Cette hypothèse suggère une faible dissolution des roches phosphatées au fil du temps et justifie la faible augmentation de la réponse de la production de matière sèche des prairies de la Pampa.

Chapitre III - Profil chimique de la végétation et de la couche arable d'une prairie fertilisée pendant 21 ans avec des sources de phosphore et du calcaire.

La Pampa en Amérique du Sud est un écosystème composé de prairies et de pâturages, couvrant 500 000 km^2 en Argentine, au Brésil, en Uruguay et au Paraguay (Pallarés et al., 2005). Le grand potentiel fourrager de la végétation naturelle de la Pampa l'amène à être utilisée comme principale source d'alimentation animale dans ces régions (pâturage) (Carvalho et al., 2006). Au Brésil, les pâturages de la Pampa constituent la base alimentaire de près de 13 millions de têtes de bétail (IBGE, 2016) qui sont majoritairement gérées dans un système d'élevage extensif. Historiquement, les propriétaires fonciers de la Pampa n'ont pas réalisé d'investissements pour améliorer la productivité des pâturages. Depuis la fin du XVIIIe siècle, les prairies ont été utilisées pour produire de la viande bovine dans de grandes exploitations sans l'application d'aucun intrant agricole. De nos jours, ces zones sont cultivées de manière inadéquate entraînant un surpâturage, une faible productivité et, par conséquent, de faibles rendements économiques (Borges et al., 2016 ; Carvalho et Batello, 2009 ; Fedrigo et al., 2018).

L'amélioration de l'efficacité économique de la production bovine dans les pâturages implique une augmentation de la production de matière sèche. Les pâturages de la Pampa bien gérés peuvent atteindre une production de matière sèche d'environ 14 Mg ha^{-1} an^{-1} (Oliveira et al., 2015). Les principales causes de la faible productivité sont une forte acidité du sol (pH ~ 4,4) et une faible fertilité comme par exemple de faibles teneurs en P disponible (2,6 à 7,6 mg de P kg^{-1}). Une augmentation de la disponibilité des nutriments par des amendements calcaires et engrais phosphorés est une stratégie importante pour soutenir le développement des plantes (Gatiboni et al., 2000 ; Prestes et al., 2017, 2016).

La dolomie (calcaire magnésien) constitue presque le seul amendement utilisé pour neutraliser l'acidité potentielle des sols dans le sud du Brésil. La très faible solubilité de la dolomite exige son incorporation dans le profil du sol. Les engrais phosphatés doivent également être incorporés au sol en raison de la très faible mobilité du P. Cependant, dans les prairies, le chaulage et la fertilisation sont réalisés par dépôt à la surface du sol. Cette fertilisation en surface peut limiter l'amélioration de la fertilité du sol aux couches supérieures et, par conséquent, la productivité des pâturages. L'objectif de ce chapitre était de tester si l'application en surface à long terme d'engrais phosphatés et d’amendements calcaires dans un pâturages permet d'augmenter la disponibilité du phosphore et la neutralisation de l'aluminium dans la couche profonde du sol et d'augmenter la production de matière sèche du pâturage.

Cette étude a examiné les propriétés chimiques de pâturages fertilisés pendant 21 ans avec différentes sources de phosphore et chaulés. Le taux le plus élevé de phosphore (249 kg ha^{-1} de P appliqué jusqu'en 2012) fournit les niveaux les plus élevés de disponibilité des nutriments dans le sol et dans les plantes. Cependant, il n'y a plus de réponse dans la production de matière sèche du pâturage au-delà de quatre ans après la dernière fertilisation. Cela indique la nécessité d'une fertilisation périodique au phosphore avec une période inférieure à quatre ans.

L'utilisation de roche phosphatée à la surface du sol permet une diffusion plus profonde du phosphate dans le sol par un phénomène de flux de masse (atteignant 7,5 cm après 21 ans et un taux de 249 kg ha^{-1} de P) par rapport aux engrais solubles. Cependant, d'autres études sont nécessaires pour comprendre la dissolution de l’apatite dans les sols de pâturages et la disponibilité du P pour les plantes.

21 ans après d’application de calcaire en surface des sols de pâturages la ré-acidification n'était pas significative, et le front de neutralisation a atteint des profondeurs supérieures à 10 cm. Dans ces conditions, les teneurs en Ca et Mg échangeables, le pH, l'Al échangeable et la saturation en Al sont plus favorables au développement des plantes que dans le sol sans apport de calcaire.

Chapitre IV - Effet de l'engrais soluble additionné de calcaire et de roche phosphatée sur le sol des prairies dans une région au climat subtropical.

Au cours des 50 dernières années, la superficie de la prairie naturelle du sud du Brésil - dite prairie de la Pampa - a diminuée de 26% et a été remplacée par des cultures annuelles (Soja, Mais), et forestières capables de fournir un meilleur rendement financier (Oliveira et al., 2017). L'une des possibilités de ralentir le rythme de destruction de ce biome est d'augmenter la production de fourrage et, par conséquent, de viande bovine et ovine. Cependant, la plupart de ces zones nécessitent l'utilisation obligatoire d'engrais phosphaté et d’amendements calcaires pour augmenter le rendement en matière sèche (Oliveira et al., 2015 ; Tiecher et al., 2014).

Les engrais phosphatés solubles dans l’eau (e.g. superphosphates et phosphates d’ammoniaque) sont les principales sources de P utilisées dans le monde. Ils jouent un rôle essentiel en agriculture car ils permettent une augmentation rapide du P disponible dans la solution du sol et ainsi la biodisponibilité du P (Bolan et al., 2003). Ceci favorise l'absorption du P par les plantes, et par conséquent, améliore le rendement des cultures à court terme (Pavinato et al., 2017 ; Silveira et al., 2018). Alternativement à l'utilisation d'engrais solubles, les roches phosphatées (RP) ont été utilisées comme engrais à l'échelle mondiale (Dias et al., 2015 ; Oliveira et al., 2015 ; Pufahl et Groat, 2017 ; Soltangheisi et al., 2020, 2018). Les roches phosphatées ignées et sédimentaires sont les deux principaux types de RP dans le monde (Ptáček, 2016 ; Pufahl et Groat, 2017). La structure des roches phosphatées ignées est plus grossière, elles sont moins poreuses et nettement moins solubles/réactives que les roches phosphatées sédimentaires. En théorie, la dissolution des RP permet un synchronisme entre la libération de P dans la solution du sol et les besoins des plantes. Ce comportement peut conduire à l'effet de particules héritées de RP et à une moindre immobilisation du P sous forme de complexe de sphère interne par les minéraux argileux et les oxydes du sol. Ce processus favorise la réutilisation du P par les plantes à long terme (Pavinato et al., 2017 ; Szilas et al., 2007).

Dans ce travail, les objectifs du présent chapitre étaient d'évaluer (i) la dissolution à long terme des minéraux porteurs de P (hydroxyapatite/fluorapatite) lorsque la roche phosphatée est utilisée comme engrais dans la prairie de la Pampa ; (ii) en analysant la réponse sur les teneurs en P et la production de matière sèche des plantes en comparaison avec celles de la prairie soumise à une fertilisation à base de phosphate soluble avec du calcaire.

L'étude actuelle a évalué les réserves du sol en P et la faible dissolution de l'apatite dans une expérience de 20 ans de fertilisation avec des sources de P et du calcaire. Le P-legacy en P a été différencié en fonction de la solubilité de la source de P utilisée. L'utilisation d'une source de P soluble et de calcaire (superphosphate triple à un taux de 250 kg ha^{-1} de P ajouté à du calcaire à un taux de 3,2 Mg ha^{-1}) se traduit par une plus grande efficacité d'utilisation des nutriments (cinq fois supérieure à celle du phosphate naturel) et par un rendement plus élevé en matière sèche (22%). Par conséquent, l'exportation de P par les tissus végétaux est également plus importante et l'effet résiduel de l'engrais P dans la couche arable (0-2,5 cm) est moindre.

En outre, l'utilisation de roche phosphatée (à raison de 250 kg ha^{-1} de P) a conduit à des niveaux plus élevés de P total et de P modérément disponible dans le sol. La faible dissolution de l'apatite (même dans des conditions thermodynamiques du sol favorables) dans les fractions de sable grossier et de limon grossier a été vérifiée analytiquement et en microscopie électronique à balayage (MEB-EDS). La stabilité de l'apatite a dépassé six ans dans les conditions du sol étudié, mais elle a duré moins de 20 ans. Par conséquent, la production plus faible de matière sèche des prairies peut être partiellement attribuée à la faible dissolution des roches phosphatées. Apparemment, l'utilisation de roche phosphatée sédimentaire de Gafsa - Tunisie n’est pas une solution satisfaisante pour augmenter le rendement des prairies de la Pampa sud-américaine.

Chapitre V - Épuisement des stocks de P et stœchiométrie C:N:P du sol en fonction des systèmes de gestion des cultures annuelles ou des prairies sur 13 ans.

Le phosphore (P) est un élément clé pour tous les organismes vivants (Elser 2012). L'absorption du P par les plantes est effectuée par le système racinaire et, par conséquent, le maintien d'une teneur satisfaisante en P disponible dans le sol est indispensable à leur croissance (Lun et al. 2018 ; Tian et al. 2019). Le P du sol se présente sous forme inorganique (Pi) ou organique (Po). Les formes inorganiques du P sont les ions orthophosphate (HPO_4^{2-} et $H_2PO_4^-$) dans la solution du sol ; associé à des cations tels que le calcium, formant des phosphates de calcium géogéniques ou anthropiques ; adsorbé sous forme de complexe de sphère interne sur les groupes fonctionnels des minéraux argileux et des oxy-hydroxydes de Fe et Al (Walker et Syers 1976 ; Helfenstein et al. 2018). Le P organique est lié à au moins un atome de C, formant principalement des monoesters d'orthophosphate (par exemple, phosphates d'inositol et monophosphate d'adénosine) et des diesters d'orthophosphate (par exemple, acides nucléiques, phospholipides) constituant la matière organique du sol et la biomasse microbienne du sol (George et al. 2018 ; Li et al. 2018 ; Liu et al. 2018).

Les deux formes de P sont présentes simultanément dans le sol et ont une disponibilité différente pour l'absorption par les plantes (labilité du P). La quantification de la disponibilité du P est généralement évaluée par des extractions séquentielles avec des réactifs chimiques de force d'extraction croissante (par exemple, l'extraction séquentielle proposée par Hedley et al. (1982)). Le P est distribué dans différents compartiments appelés P labile, P modérément labile et P non labile. Naturellement, l'absorption de P par les plantes provient du compartiment le plus disponible (labile), qui est alimenté par le compartiment de P moins labile (Tiecher et al. 2018). En outre, le renouvellement de chaque compartiment de P est réduit avec une diminution de la labilité du P, allant de quelques minutes à des centaines d'années (Helfenstein et al. 2020). Ainsi, on s'attend à ce que l'épuisement du P du sol par les activités agricoles soit perçu à court terme dans les compartiments les plus labiles et à moyen-long terme dans les compartiments moins labiles. Le pouvoir tampon du P disponible varie en fonction du type de sol et de la gestion adoptée (Fink et al. 2016).

Bien que le P soit absorbé par les plantes principalement sous forme orthophosphates (Schachtman et al. 1998), la présence de Po dans le sol a une importance remarquable dans la diminution de l'adsorption du P sous forme de complexe de sphère interne sur les minéraux argileux et les oxydes et dans la mobilisation du P récalcitrant (Guppy et al. 2005 ; Chassé et Ohno 2016 ; Rodrigues et al. 2016 ; Menezes-Blackburn et al. 2018). La transformation du Pi en Po se produit lorsque les plantes et les microorganismes incorporent le Pi dans leurs tissus. Après leur mort, le P retourne au sol principalement sous forme organique dans la SOM. Par conséquent, la présence de Pi ou de Po dans le sol est déterminée par la teneur en SOM et peut être modifiée par le système de gestion du sol adopté. Dans le sol, il existe un équilibre entre les cycles de C, N et P, qui sont interconnectés dans la MOS. Avant l'absorption du P par les plantes, une étape de minéralisation du P est nécessaire et, celle-ci se déroule différemment selon la récalcitrance des composés organiques. Dans le sol, les monoesters d'orthophosphate ont un taux de renouvellement moins élevé que les diesters d'orthophosphate en raison de leur liaison chimique aux colloïdes du sol (Turner et al. 2005 ; Menezes-Blackburn et al. 2018). Ce comportement peut induire une accumulation plus importante de monoesters de P que de diesters dans la MOS.

En général, un sol avec un stock élevé de P disponible, provenant des apports d'engrais ou du matériau parental du sol, peut répondre à la demande des plantes et soutenir les rendements des plantes pendant une certaine période, même sans fertilisation phosphatée. Cependant, l'absence de fertilisation phosphatée associée à une exportation constante de P génère un bilan négatif de P dans le sol, ce qui entraîne une diminution des stocks et de la disponibilité de P dans le sol (Boitt et al. 2018 ; Tyson et al. 2020). Cela peut entraîner des baisses sur les rendements des cultures et le maintien ou l'accumulation de la SOM dans une échelle de temps dépendante de l'exportation de P. Dans ce travail, l'objectif était de tester si le système de gestion du sol modifie l'épuisement temporel du stock de P et son couplage avec le cycle du carbone et de l’azote.

Les résultats dans le dispositif SOERE-ACBB ont montré une plus grande exportation de P et, par conséquent, un plus intense épuisement du stock total de P dans le traitement avec des prairies permanentes par rapport à l’utilisation du système des culture permanent. L'épuisement du P a principalement affecté le compartiment de P avec une plus grande labilité (P-labile). Cependant, l'épuisement qui s'est produit après 13 ans n'a pas été suffisant pour provoquer une réduction évidente de la productivité des plantes en raison de la capacité tampon des réserves de P avec moins de labilité (P-mod-labile).

Avec les prairies permanentes, il y a eu une transformation du P inorganique en P organique dans le compartiment le plus labile, accompagnant l'augmentation des stocks de C et N dans le sol. Ce comportement a permis une réduction des valeurs du rapport atomique C:N:Po et, par conséquent, un couplage entre les cycles C, N et P dans le sol. La technique ^{31}P-NMR a révélé que les prairies permanentes avaient un effet plus significatif sur la réduction de l'α-glycérophosphate et l'augmentation du myo-IHP et de l'adénosine monophosphate que les terres cultivées permanentes.

Chapitre VI - Biomasse microbienne P du sol et couplage C:N:P : une approche à l'échelle des agrégats.

Des directives mondiales ont été proposées pour augmenter le stock de carbone organique du sol (COS) comme stratégie pour contrebalancer l'émission de CO_2 par l'utilisation de combustibles fossiles et ainsi minimiser l'effet du réchauffement climatique (Dignac et al., 2017). Le COS est défini principalement par la teneur en matière organique du sol - MOS (déchets végétaux ou animaux, micro-organismes vivants ou morts et humus) et il peut être profondément modifié par les systèmes de gestion des sols (Assunção et al., 2019 ; Dignac et al., 2017 ; Panettieri et al., 2017 ; Poeplau et al., 2018). Les sols des régions tempérées contiennent à l'origine, en générale, un stock important de P biodisponible, mais de longues périodes de culture sans ajout de remplacement de ce nutriment ont également conduit à l'épuisement des formes labiles et peuvent même limiter la productivité et l'augmentation de la MOS (Blake et al., 2003 ; Boitt et al., 2018 ; Le Noë et al., 2020).

En France, par exemple, jusqu'aux années 1970, il y a eu une augmentation significative de l'utilisation des engrais phosphatés, rendant les teneurs croissantes en P total élevées dans les sols du pays. Cependant, à partir des années 1970, la consommation d'engrais phosphatés dans l'agriculture française a été drastiquement réduite par des politiques publiques (Le Noë et al., 2020). Cette stratégie était justifiée en raison de l'importance des apports annuels de P et des teneurs élevées en P dans les sols. Cependant, sans l'ajout d'engrais phosphatés, le bilan de P dans de nombreuses zones agricoles est devenu négatif, ce qui suscite des inquiétudes quant à la possibilité d'une diminution de la production en raison de restrictions nutritionnelles des plantes (Éveillard & Saby, 2018 ; Saby et al., 2016).

L'immobilisation du C et du N dans le sol signifie également l'immobilisation du P. Par conséquent, la récalcitrance des composés organiques définit le recyclage du Po par les plantes et les microorganismes. Le P organique doit être converti en forme minérale avant d'être réutilisé par les plantes. Ce processus est réalisé presque exclusivement par les microorganismes du sol par minéralisation de la matière organique. En outre, les plantes peuvent synthétiser et exsuder des phosphatases qui peuvent contribuer à la biodisponibilité du P organique (Hayes et al., 1999). Par conséquent, le P stocké dans des formes organiques labiles peut être indirectement réutilisé par les plantes (Menezes-Blackburn et al., 2018) et représente un stock temporaire de P dans le sol (Rheinheimer et al., 2019).

La récalcitrance de la MOS est étroitement liée à la complexité des composés organiques et à la protection physique assurée par les constituants inorganiques du sol (Assunção et al., 2019). Dans cette perspective, la relation entre l'agrégation du sol et la MOS est à double sens, tandis que la MOS agit comme un agent d’agrégation du sol, l'agrégation agit comme une armure pour la protection physique et contre la minéralisation de la MOS (Kleber & Johnson, 2010 ; Tisdall & Oades, 1982). La variation des niveaux de MOS en fonction de la taille des agrégats du sol est un indicateur de la variation de la teneur en P, principalement des fractions de Po. Une fraction considérable du P est composée de P stocké dans la biomasse microbienne (P_{mic}), et le P sera donc protégé contre une réaction avec les groupes fonctionnels des minéraux d'argile et des oxydes de fer. La distribution granulométrique des constituants inorganiques et organiques du sol et le degré de minéralisation et de renouvellement de la MOS varient en fonction de la taille des agrégats (Liao et al., 2018 ; Panettieri et al., 2017), ce qui peut induire des changements majeurs dans le stock et la biodisponibilité du P dans les sols. Ainsi, la disponibilité du P pour les plantes (Fontana et al., 2008 ; Pereira et al., 2010) et la stœchiométrie C : N : P doivent être différenciées pour chaque taille d'agrégat.

Dans ce travail qui a utilisé le dispositif SOERE-ACBB, nos objectifs étaient d'évaluer (i) si la biomasse microbienne du sol est un stock important de P sous forme organique dans les sols des régions à climat tempéré et si son importance est plus grande dans les systèmes de gestion qui favorisent la stabilisation de la MOS ; (ii) tester si les systèmes de gestion du sol modifient la biodisponibilité du P et son couplage avec le C et le N différemment dans chaque taille d'agrégat du sol.

Nos résultats montrent que les systèmes de gestion du sol (avec prairie ou culture permanent) n'ont pas changé les niveaux totaux de P dans le sol après 13 ans, mais le rapport P inorganique / P organique était plus élevé avec la pratique de labour du sol et la culture de plantes annuelles. Parallèlement, le système de production de fourrage a favorisé l'accumulation de C, N et P organique dans le sol. Pendant la saison de croissance (orge / mélange fourrager - 2018/2019), les niveaux de P contenus dans la biomasse microbienne du sol constituaient un réservoir important de P biodisponible dans les deux systèmes de gestion et représentaient jusqu'à 47 % de la teneur en P disponible (P Olsen) dans un système de labour du sol et de culture de plantes annuelles.

La pratique du labour du sol et de la culture annuelle a réduit le pourcentage et les niveaux de C, N et P totaux du sol dans la biomasse microbienne du sol des grands agrégats par rapport à la prairie permanente. En outre, la prairie permanente a maintenu une biodisponibilité plus élevée du P (P labile) dans les agrégats supérieurs à 0,050 mm, principalement en raison de la teneur en P labile du sol. En revanche, pour le traitement avec le labour du sol et la culture de plantes annuelles, la biodisponibilité la plus élevée de P a été obtenue dans des agrégats plus petits (<0,200 mm), principalement en raison des niveaux plus élevés de Po labile dans ces classes d'agrégats. La stœchiométrie C:N:Po a été peu modifiée par les 13 années de culture dans les systèmes de gestion.

Conclusion générale

Dans cette thèse, nous avons analysé deux expériences à long terme sur les impacts de l'utilisation ou non d'engrais phosphatés sur l'efficacité de l'utilisation du phosphore par les plantes et sa disponibilité, le P-legacy, les rendements et le couplage avec le carbone et l'azote dans la matière organique du sol.

Nous avons conclu que dans les sols à faibles stocks de P dans les prairies de la Pampa, l'utilisation de sources de P soluble (comme le superphosphate simple et triple) associée au chaulage est une meilleure stratégie pour améliorer la production de matière sèche et l'efficacité d'utilisation du P. Ces engrais entraînent un faible changement dans la richesse botanique de la prairie naturelle. Cependant, il y a eu un plus grand renouvellement des espèces, principalement dans les groupes de graminées à touffes et de plantes fourragères et dans la contribution des légumineuses à la production de fourrage. Pour maintenir le rendement en matière sèche, un apport régulier de phosphore à un intervalle de trois ans au maximum est nécessaire. L'utilisation de roches phosphatées n'a pas amélioré la fertilité du sol et la production de matière sèche de manière satisfaisante en raison de leur faible dissolution à long terme dans le sol (plus de six ans).

Dans les sols à forte teneur en P, les prairies permanentes ont entraîné un plus grand épuisement des stocks totaux et P-labile en raison de leur capacité d'exportation de P légèrement supérieure à celle des terres cultivées permanentes. De plus, les prairies permanentes favorisent une transformation du P inorganique en P organique dans le compartiment le plus labile, accompagnant l'augmentation des stocks de C et de N dans le sol. Ce comportement a entraîné une réduction des valeurs du rapport atomique C:N:Po et, par conséquent, un couplage entre les cycles C, N et P dans le sol. De plus, nous avons observé que le Pmic est un important réservoir de P biodisponible dans les deux systèmes de gestion et qu'il représente jusqu'à 47% de la teneur en P disponible (P Olsen) dans un système de culture permanente. Par la technique ^{31}P-NMR, nous avons observé que les prairies permanentes avaient un effet plus significatif sur la réduction de l'α-glycérophosphate et l'augmentation du myo-IHP et de l'adénosine monophosphate que les terres cultivées permanentes.

1. Introduction

The prospect of the world population reach 9.6 billion of people by the year 2050 (UN, 2013) requires that the production of food to be potentialized (Ray et al., 2013). Which only would be possible by expanding the agricultural areas or by more efficient use of the areas already cultivated. However, the agriculture practiced around the world in recent decades is contrary to achieving a sustainability. Mainly due to the intensive and inadequate use of agrochemicals such as phytosanitaires, industrial fertilizers, and fossil fuels. The disproportionate use of agrochemicals led to environmental contamination, depletion of natural reserves, climate change and put in risk the food security.

The world consumption of industrial fertilizers, for example, increased 17.7% between 2006-2016 (FAO, 2019). In this amount, we highlight the use of nitrogen, phosphate and potassium fertilizers that aim to supply nitrogen (N) phosphorus (P) and potassium (K) nutrients, respectively. The supply of N to the plants is practically unlimited due to the industrial production of urea (Dawson and Hilton, 2011) and by the biological fixation of N performed by leguminous plants (Herridge et al., 2008).

On the other hand, P and K fertilizers are essentially from finite reserves in mines and there is no substitute for agricultural production (Cordell et al., 2009; U.S. Geological Survey, 2019). Therefore, the high demand, especially for P, has triggered a number of concerns (Bennett and Elser, 2009). The second refers to the high annual addition of P to soils and water bodies, causing serious environmental and social problems, as a eutrophication of water sources (Goyette et al., 2018; Schilling et al., 2018; Schoumans et al., 2014; Withers et al., 2017). Therefore, at the global level, the most efficient use of phosphate fertilizers is essential to achieve the sustainability of production systems without the loss of productive potential of food (MacDonald et al., 2011; Marin et al., 2016; McDonald et al., 2019; Tian et al., 2019). The most efficient use of phosphate fertilizers is obtained through the interaction of factors such as: soil management practices; fertilization strategies, use of organic fertilizers and cultivation of plants adapted to the conditions of production systems.

For strategies of fertilization, the use of nanotechnology in fertilizers, such as polymers, biopolymers, nanoclays, among others, are pointed out as strategies for smarter fertilization (Calabi-Floody et al., 2018). These technologies provide a gradual release of nutrients to the soil, which meets the need of plants and avoids P immobilization by soil matrix (high stable P). In addition to these technologies, the use of rock powder and crushed rocks are also considered products of low solubility with potential use for gradual release of P in the soil (Oliveira et al., 2015; Szilas et al., 2007; Tiecher et al., 2014). However, the use of less soluble sources (as phosphate rock) in subtropical soils has demonstrated a greater increase of less available forms of P than soluble sources (Soltangheisi et al., 2018). In the case of phosphate rock, the diversity of conclusions in the literature indicates the need for further studies on the subject.

Soil management practices may also alter the dynamics of plants nutrients uptake by changing nutrients cycling and the chemical forms in the soil. No-till systems, with crop rotation or perennial pasture cultivation, where there is straw maintenance and no soil plowing, have significant levels of organic P, either in the organic matter or in the soil microbial biomass (Boitt et al., 2018a, 2018b; Condron et al., 2005; Tiecher et al., 2012a). This reduces the probability of P sorption into soil mineral matrix. In contrast, in systems with soil plowing there is an increase of the P sorption by soil matrix, causing an increase in the less labile forms of P (Rodrigues et al., 2016; Tiecher et al., 2012b), besides the loss of organic matter and the release of CO_2 to atmosphere. Due to the issues discussed, it is evident that understanding the dynamics of P in medium and long-term production systems and soil management systems are essential for improving the efficiency of phosphate fertilizers.

Thus, this work is placed in the scenario of increasing fertilizer efficiency, raising agricultural production, and maintaining the dynamic balance of nutrients aiming at the sustainability of agricultural systems, food security and reduction of climate change. The present doctoral thesis is being presented for dual degrees (cotutelle) at the Federal University of Santa Maria (Brazil) and at the Université de Poitiers (France). Furthermore, the present thesis is part of a research project titled "Long-term effect of soil use and management practices on soil fertility: changes in mineralogy and availability of phosphorus and potassium". This project was funded by the Brazilian government and is linked to MEC/MCTI/CAPES/CNPq/FAPs - Special Visiting Researcher - PVE 2014 N° 03/2014. The project is carried out in partnership between the Federal University of Santa Maria - Brazil and the Université de Poitiers - France and aims to develop studies to understand the long-term effect of soil use and management on fertility, with emphasis on mineralogical changes and availability of phosphorus and potassium to crops. As well as the production and dissemination of scientific knowledge, this project aims to strengthen Franco-Brazilian scientific cooperation and capacitating people through the exchange of researchers and students between universities. Moreover, the present work also has the collaboration of researchers from the Institute National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement – INRAE Nouvelle

Aquitaine SOERE ACBB, located at Lusignan – France, where a considerable part of this work was developed.

1.1.Approaches made in the thesis

In this thesis, seven chapters will be developed that deal with the dynamics of soil P in areas of forage production and annual crop. These seven chapters are characterized by 3 main moments of the material.

The first moment, composed of chapter I, is addressed to the literature review regarding the themes that will be addressed in the other chapters. Such as, an approach to characterize the chemical element P, its origin, location and importance for the life cycle on Earth. Moreover, approaches will be carried out on the dynamics, stocks and use of P sources in agricultural soils, with emphasis on areas managed with pastures in regions of subtropical and temperate climates. To conclude the first moment, an approach will be made regarding the coupling of the biogeochemical cycle of P and elements C and N and its importance for increasing the levels of organic matter in the soil.

The second moment, composed by chapters II to VI, is addressed to the presentation of the five articles that make up this thesis. These articles are based on two long-term experiments located in Brazil and France. The use of two experiments located in these countries aims to carry out studies in both places involved in the realization of this doctorate, such as carried out in joint supervision. Chapters II, III and IV deal with three articles already published in international scientific journals and are from experiments started in 1997, located in Santa Maria – Brazil. The main objectives of the experiment are to test the use of different rates of P sources, associated or not with liming, in natural grassland of the Pampa biome. In chapter II, published in the European Journal of Agronomy, a historical approach of the experiment, analyzing data of dry matter productivity of more than 21 years, botanical composition of native vegetation and soil fertility in the 0-10 cm topsoil layer was carried out. In chapter III, published in the Soil and Tillage Research, data on nutritional content and the effect of liming in stratified layers at a depth of 0-10 cm topsoil layer are analyzed, as well as data on concentration and P export via dry matter. In chapter IV, also published in Soil and Tillage Research, an analysis of soil P legacy due to the use of different P sources was carried out. As well as the presence in the soil of undissolved apatite minerals from the rock phosphate P source.

Chapters V and VI are being prepared for publication in international journals, with submission scheduled for 2021. These works are related to an experiment initialized in 2005, located in Lusignan - France, which aimed to test the effect of different management systems (annual crops or perennial pasture) on the depletion and legacy of P in the soil and its stoichiometry C:N:P. In chapter V, an historical dataset of P export and depletion by plants, changes in soil P pools and the coupling of organic P in the dynamics of C and N at 0-30 cm topsoil layer were analyzed. In chapter VI, the participation of the P from soil microbial biomass in the bioavailable soil P content during the barley growth season was analyzed. As well as, the distribution of P pools and their stoichiometry C:N:P in different classes of soil aggregates according to management systems with annual crops and perennial pasture.

The third moment, composed of chapter VII, is addressed to the general discussion of the thesis. In this chapter, approaches are made about the dynamics and availability of P in agricultural soils, methods of quantification and characterization of the forms of P in soils. In addition to an agri-environmental approach on the use of phosphate fertilizers in agricultural areas.

To conclude, we take up the observations made in the previous chapters. In the end, we want the reader to understand the importance and challenges that permeate the theme “Temporal dynamic of P in different soil management systems in temperate and subtropical climate”. As well as, the need and importance of doing science, wherever it is. Good Reading!

2. Hypotheses

We tested five hypotheses. Each one discussed and analyzed in one chapter, such as:

Chapter II - We hypothesized that triple and simple superphosphate and limestone using would lead to greater increase in dry matter production and to changes in the botanical composition and richness of Pampa grassland plant species than the use of lesser soluble P sources (phosphate rock) or no liming treatment.

Chapter III – We hypothesized that long-term surface application of phosphorus fertilizers and limestone in a rangeland allow for an increase in the phosphorus availability and aluminum neutralization in deep soil layer and increase in the rangeland dry matter production.

Chapter IV - We hypothesized that there is no long-term dissolution of P-bearing minerals when phosphate rock is used as fertilizer in Pampa grassland. As well as, this no P-bearing minerals dissolution change the P-legacy response and plant dry matter production in comparison to those of grassland subjected to fertilization based on soluble phosphate added with limestone.

Chapter V – We hypothesized that the soil cultivated with permanent grassland have a greater depletion of P stock due to the P exportation via dry matter, and a great increase of soil organic P pool, due to its coupling with C and N in the SOM than the soil cultivated with annual plowing and permanent crop rotation.

Chapter VI - We hypothesized that: a) Soil microbial biomass is an important stock of P in organic form in soils of temperate climate region and its significance is greater in management systems with more SOM. b) soil management systems alter the total P contents and their availability to plants differently in each soil aggregate size, due to changes in: aggregates size distribution, content of SOM and P_{mic}; c) the C:N:P stoichiometry is differentiated according to the aggregate size.

3. Objectives

3.1.General objectives

Our main objective is establishing a more effective strategy of P fertilization allowing increase in forage yield, maintenance of the plant community and efficiency use of P. Besides, testing the effect of soil management systems on soil P availability and depletion and their stoichiometry with C and N in soil.

3.2.Specific objectives

The specifics objectives were divided in the different chapters, such as:

Chapter II – The aims was investigated (i) whether the use soluble P sources (triple and simple superphosphate) with limestone result in greater dry matter production than the use of lesser soluble P sources (phosphate rock) or the absence of liming; and (ii) in which ways P fertilization and liming affects the botanical composition and grassland plant species richness.

Chapter III - The objective of this chapter was to test whether long-term surface application of P fertilizers and limestone in a rangeland allow for an increase in the P availability and aluminum neutralization in deep soil layer and increase in dry matter production.

Chapter IV - The aims of the current chapter were to evaluate (i) whether there is long-term dissolution of P-bearing minerals when phosphate rock is used as fertilizer in Pampa grassland; (ii) analyzing its P-legacy response and plant dry matter production in comparison to those of grassland subjected to fertilization based on soluble phosphate added with limestone.

Chapter V – The aim of this chapter was to test if the soil management system alters the temporal depletion of P stock and its coupling with C and N cycle.

Chapter VI – The objectives of this chapter were to evaluate (i) whether soil microbial biomass is an important stock of P in organic form in soils of temperate climate region and its significance in management systems with more SOM content; (ii) test if soil management systems alter P bioavailability and its coupling with C and N differently in each soil aggregate size.

4. Chapter I - Background: Literature Review

The origin of phosphorus (P) in the universe goes back to the massive regions of star formation and, on Earth, goes back to the appearance of the planet and its first years of formation. During the emergence of the solar system, massive stars containing P were formed and incorporated into the ProtoSolar Nebula, and consequently also on Earth. Furthermore, the shock of stellar bodies to the Earth's surface, in the first years of its formation, allowed a greater amount of the element to be incorporated into the planet (Rivilla et al., 2020). P has atomic number 15, atomic mass 30.97 and its electronic conformation results in 5 electrons in the valence shell, which gives it high reactivity. In the environment, P is present predominantly coordinating four oxygen atoms with chemical bonds of a covalent character (68%) and highly energetic, thus forming the phosphate oxyanion (PO_4^{3-}).

Due to the chemical characteristics of phosphate and its high energy potential, the P is an indispensable element for all life cycles (Elser, 2012). In plant tissues, P represents approximately 0.2 % of the plants dry-matter (Schachtman et al., 1998). It is a key component for the production of proteins, nucleic acids, enzymatic activation and energy transfer (Wang et al., 2017). Together with nitrogen, P is the macronutrient most limiting of photosynthetic production in aquatic or terrestrial environments (Elser et al., 2007; Fay et al., 2015).

In the Earth's crust, phosphate is found naturally linked to Ca, forming minerals called "calcium phosphates" or mainly "apatite". The presence of isomorphic and ionic substitutions in the crystal of these minerals by other chemical elements gives extensive variation to the group of apatite. The apatite minerals mainly occur in the form of carbonate-fluorapatite ($Ca_{10}(PO_4, CO_3)_6F_2$), fluorapatite ($Ca_{10}(PO_4)_6F_2$), hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) or chlorapatite ($Ca_{10}(PO_4)_6Cl_2$) which have different characteristics of crystallinity, water solubility, porosity and chemical compositions (Chien, 1977; Toledo and Pereira, 2001). Most of the rocks on the Earth's surface have small amounts of apatite minerals in their composition. However, in some of them, the concentration is higher (called phosphate rocks) and, therefore, they represent the most important P source on the planet.

In the soil, geogenic P comes from weathering and dissolution of the apatite minerals present in the parent materials (Frossard et al., 1995; Tiessen et al., 1984), thus, the soil P concentration vary considerably worldwide according to soil type and bedrock (Fairhurst et al., 1999; Walker and Syers, 1976). As an example, in Brazilian native soils, the total P content varies largely from <25 mg kg^{-1} to >500 mg kg^{-1} (Pavinato et al., 2020) and in France soils the total P content arrive to 4,000 mg kg^{-1} (Delmas et al., 2015). During the pedogenesis process, the released P can remain in inorganic form (Pi) or be transformed into organic forms (Po) by living organisms. The more common inorganic forms of soil P are: (i) orthophosphate (HPO_4^{-2} and $H_2PO_4^-$); P bound to cations, such as calcium, inducing neoformation of calcium phosphates with low solubility (Beck and Sanchez, 1994); (ii) P adsorbed by inner-sphere to the functional groups of clay minerals and Fe and Al oxides (Bortoluzzi et al., 2015; Kim et al., 2011). In the organic forms, P is somehow bound to at least one C atom, forming mainly orthophosphate monoesters (e.g. inositol phosphates) and orthophosphate diesters (e.g. nucleic acids, phospholipids) constituting soil organic matter and soil microbial biomass (George et al., 2018; Li et al., 2018; Liu et al., 2018).

One of the best accepted models on the evolution of pedogenesis and the consequence on the levels and availability of P in the soil was presented by Walker and Syers (1976). According to the model, at zero weathering time, all P present in the soil is in primary minerals in soil parent material, mainly in apatite form. With the pedogenesis evolution, the primary minerals are solubilized, and the P is released to the soil solution. From the soil solution, P can be adsorbed onto functional groups of clay minerals or oxides (mineral matrix of the soil) or incorporated into the living biomass of the soil and, consequently, incorporated in the soil organic matter (organic matrix of the soil). In more intensely weathered soils, almost all the primary minerals are weathered, and a large part of the P is adsorbed to the soil inorganic constituents. This adsorption is temporarily strengthened and P becomes extremely difficult to desorb, to the point that it becomes practically unavailable to plants. In these cases, the organic P pool and its recycling play a fundamental role in maintaining the availability of P to plants and microorganisms (Turner and Condron, 2013).

In intensely weathered soils and with low levels and / or availability of P, the native plant species that grow in are adapted to low fertility environments and are able to develop through strategies capable of maximizing the nutrients uptake. These adaptive strategies can be based on the increase and arrangement of the root system, plant-organism association (e.g. plants - mycorrhizal fungi), increase in the efficiency of P transporters in tissues or on the reduction of the plant's metabolic cost (Ramaekers et al., 2010; Wen et al., 2019).

In these soils, when there is the presence of apatite minerals, whether of geogenic or anthropogenic origin, the plants act in the P solubilization at the root-soil interface, mainly by lowering the rhizosphere pH (Arcand and Schneider, 2006). Among the processes that can favor the lowering of pH, the release of proton (H^+) and the release of organic acids (e.g. citric, oxalic and malic acid) stand out. The reduction of the rhizosphere pH due to root proton exudation occurs when the cations uptakes is superior to anions uptakes. This behavior can occur in plants that have high Ca uptake, such as white clover (Hinsinger and Gilkes, 1995) or due to the absorption of N in ammonium form, as in ryegrass and clover. In addition to impacting the pH reduction, the greater absorption of Ca also favors the solubilization of apatite due to a chemical imbalance caused by the deficit of the element in solution (equations 1 and 2) (Hinsinger and Gilkes, 1997, 1995).

$$\mathrm{Ca}_5(PO_4)_3OH + 7H^+ = 5Ca^{2+} + 3H_2PO_4^- + H_2O \qquad \text{Equation (1)}$$

$$\mathrm{Ca}_5(PO_4)_3F + 6H^+ = 5Ca^{2+} + 3H_2PO_4^- + F^- \qquad \text{Equation (2)}$$

Plant species have different potential for apatite solubilization. Plants of the genus *Lolium* and *Lupinus* have a high potential to solubilize apatite. As a consequence, they can be used as cover crops for green manure, in order to solubilize mineral P and immobilize it in organic form in plant tissues (Arcand and Schneider, 2006; Soltangheisi et al., 2020). Conversely, leguminous plants have less potential for solubilization since biological N fixation and the proton exudation process presented above occurs with less intensity.

In areas of native vegetation, such as the natural grassland of the Pampa biome, located in South America and which are the subject of this thesis, the low natural levels of total P and available P of the soil (Oliveira et al., 2011; Pavinato et al., 2020; Rheinheimer et al., 1997) highlight the importance of (re)cycling organic P to maintain a minimum P availability for maintaining vegetation (de Oliveira et al., 2014; Oliveira et al., 2011). In the Pampa biome, there are more than 3000 plant species identified (Boldrini, 2009). This great genetic variability allows a synergism between the plant species, where some of them, have part of the mechanisms mentioned above and are capable of “mining” P from the soil.

In addition to plant species, soil microbiota (fungi and bacteria) can also play a fundamental role in the bioavailability and temporary storage of P present in apatite minerals or adsorbed to soil clay minerals. Among the microorganisms with the greatest potential are: fungi of the genus *Aspergillus*, *Penicillium* and *Rhizophagus* (arbuscular mycorrhizal fungi) (Souchie and Abboud, 2007; Taktek et al., 2015); and bacteria of the genus *Pantoea*, *Pseudomonas*, *Bacillus* and *Rhizobium* (Kaur and Reddy, 2015; Rodríguez and Fraga, 1999).

These microorganisms can act individually or in plant / fungus / bacteria association. For example, when there is an association between plants, arbuscular mycorrhizal fungi, and P solubilizing bacteria. Since arbuscular mycorrhizal fungi act by increasing the volume of soil explored by the plant's root system and P-solubilizing bacteria act in increasing P availability (Taktek et al., 2015).

Similar to plant species, the main mechanisms involved in P solubilization by bacteria are the release of organic anions and the consequent lowering of the pH (to solubilize apatite minerals) and phosphatase enzymes and acid phytases (to mineralize organic soil phosphorus) (Chen et al., 2006; Pande et al., 2017; Rodríguez and Fraga, 1999; Taktek et al., 2015). Moreover, other mechanisms are also reported to increase the availability of inorganic P. For example: chelation of P-linked cations; competition with P for soil adsorption sites; formation of soluble complexes with metal ions associated with insoluble P (Ca, Al, Fe) (Sharma et al., 2013).

Chen et al. (2006) identified the presence of eight different organic acids responsible for the solubilization of mineral P in the soil. Among them: citric, gluconic and lactic acid. Likewise, Taktek et al. (2015) identified the presence of gluconic and 2-ketogluconic acid generated by P solubilizing bacteria. The solubilization of anthropogenic inorganic P immobilized in clay minerals and oxides is not reported. What seems to be more consistent is the solubilization of P bound to Ca. Whether it comes from external sources (such as natural phosphates) or precipitated from the soil solution in the form of calcium phosphate with low crystallinity.

Besides the solubilization of mineral P from the soil, fungi and bacteria act fundamentally in the cycling of the Po contained in SOM. The conversion of Po to orthophosphate ions triggered by these microorganisms is fundamental for the reuse of Po by plants. Since, P is absorbed by plants predominantly in inorganic form (Schachtman et al., 1998). In this regard, the main strategy adopted is the use of enzymes capable of hydrolyzing the organic compounds of P, such as acid and alkaline phosphatases and phytases (Rodríguez et al., 2006; Rodríguez and Fraga, 1999; Sharma et al., 2013). The mineralization of organic compounds containing P occurs mainly in organic matter with a lower degree of humification and with less physical-chemical protection of soil colloids.

Unlike natural areas, where a dynamic balance is experienced between ecosystem agents, when there is a need to explore vegetation, whether native or exotic vegetation is implanted, the native soil P content and its cycling through biomass can limit high yields levels.

For this reason, in soils with low Pi availability, a restriction is observed in the growth and productivity of agricultural crops (Gatiboni et al., 2000; Mai et al., 2018; Oliveira et al., 2015; Soltangheisi et al., 2018; Tiecher et al., 2014). Thus, the existence of satisfactory contents of Pi in the soil is extremely important for plant development (Lun et al., 2018; Tian et al., 2019) and crucial to maintain acceptable levels of plant productivity. Therefore, it is necessary the use of P inputs to improve the soil fertility and provide the nutritional needs of cultivated crops (Lun et al., 2018; Tian et al., 2019).

Nevertheless to improve the soil P contents, the phosphate fertilizers currently commercialized come from the exploitation of the non-renewable natural P reserves (Bennett and Elser, 2009; Cordell et al., 2009). These reserves can be formed by igneous or sedimentary phosphate rocks. Igneous phosphate rocks take place in large deposits in intrusive masses formed by the solidification of silicate melted at high temperatures. The slow cooling of the magma favors the growth of the crystalline structure, making the larger crystals, less porous, and more resistant rocks. Often the P content in these deposits is around 5-15% of P_2O_5 (Pufahl and Groat, 2017). On the other hand, the sedimentary phosphate rocks take place in marine, metamorphic or biogenic deposits. Due to the sedimentary origin, the presence of clay, quartz, carbonates and heavy metal contaminants is recurrent (Ptáček, 2016; Pufahl and Groat, 2017; Toledo and Pereira, 2001). Often the P content in these deposits is around 15-35% of P_2O_5 (Pufahl and Groat, 2017). Nowadays, more than 85 % of the P reserves are located in Morocco and Western Sahara, Tunisia, China, Algeria, Syria, Brazil and South Africa and their exploitation supplied approximately 67 % of the phosphate rock mined in 2018 (U.S. Geological Survey, 2019). The P reserves have the expectative of depletion in the future years (Cordell et al., 2009; Steen, 1998) and the concentration of the world's reserves in few countries makes P a key element for global food security and strategic for the economy of nations (Bennett and Elser, 2009).

Due to the P concentration and low water-solubility of rocks phosphate when applied to agricultural soils, mostly of the phosphate fertilizers currently used in agriculture, such as single superphosphate (SP), triple superphosphate (TP), monoammonium and diammonium phosphate (MAP and DAP, respectively) are the product of acid chemical treatment of phosphate rocks. The acid treatment increases the water soluble P concentration (Pufahl and Groat, 2017) for rapid dissolution and release to the soil.

The use of water soluble phosphate fertilizers result in rapid availability of P in inorganic form in the soil solution by phosphate compounds dissolution (i.e. monocalcium phosphate

(Equation 3)) (Bolan et al., 2003). The presence of Pi in the soil solution favoring the plants uptake, as discussed above. However, the fertilizer potential to generate protons provide the increase of H^+ in soil solution. The high concentration of H^+ promote dissolution of Al and Fe soil inorganic constituents and modifies the minerals functional groups, which may result in easier P inner-sphere adsorption (Bolan et al., 2003). Moreover, intensely weathered soils naturally have functional groups of clay minerals and oxides capable of P inner-sphere adsorption with high energy (Bortoluzzi et al., 2015; Kim et al., 2011). This highly energetic adsorption makes the P unavailable or available at long-term for crops uptake (as legacy P) (Rodrigues et al., 2016; Roy et al., 2017). Thus, the mineral soil constituents, which were initially a source of P, acts as sink of P from soil solution (Pearse et al., 2007).

$$Ca(H_2PO_4)_2 + H_2O \rightarrow CaHPO_4\, H_2O + H_2PO_4{}^- + H^+ \qquad \text{Equation (3)}$$

This highly energetic adsorption considerably reduces the recovery of P added via fertilizer, especially in intensely weathered soils (Freitas et al., 2013; Roy et al., 2016). This can be evidenced by the low P recovery in tropical soils, around 54% (Resende et al., 2006) and between 48 and 69% in subtropical soils (Tiecher et al., 2012b). The low recovery of P, reported in tropical and subtropical soils, corroborates to the reduced efficiency use of P worldwide (0.46 in croplands and 0.72 in pasture) and also means that there is excess fertilization (P input) for the production volume (P exported) (Lun et al., 2018; Tian et al., 2019).

In soils located in the Brazilian Cerrado region with tropical climate, the total P content has almost doubled in the last three to four decades due to the high supply of phosphate fertilizers by the practiced agriculture. However, much of the P is stored in forms with moderate to low availability, forming a P pool not available (Rodrigues et al., 2016). On the contrary, in France - a country with temperate climate - until the early 1970s, there was a high consumption of phosphate fertilizers and a massive application of animal manure in agriculture soils, which resulted in high values of total and available P content in the soils. However, in recent years, the drastic reduction in phosphate fertilization has led to decreases in the levels of available P in soils (SABY et al., 2016). To optimize these two scenarios, knowledge of the dynamics and balance of P in the soil becomes of great importance for the sustainability of agricultural areas intended for food production.

Besides the problems of reducing P recovery by crops, the adsorption of P by clay minerals and oxides facilitates their loss with surface runoff and erosion. During the runoff process, P is lost with fine particles (clay minerals and oxides) transported as particulate form by the water transfer (soil erosion) to water bodies (Bender et al., 2018; Goyette et al., 2018; Zafar et al., 2017). This implies problems of eutrophication of water resources (Schilling et al., 2018; Withers et al., 2017, 2014). Moreover, the chemical treatments of the natural rocks provide a considerable increase in the cost of fertilizer, great emission of C to the atmosphere during the manufacturing process (Cordell et al., 2009), contamination and environmental eutrophication by industrial discards at P exploitation sites (Kateb et al., 2018).

As an alternative to reduce production costs, environmental pollution and increase the recovery of P added through the gradual dissolution of fertilizer, crushed rocks have been directly used as P source. It is known that small acidulated apatite crystals found in hydrothermalized basalts present high P-release, although this mineral is found in low quantities in these rocks - less than 0.5% (Korchagin et al., 2019). Similar phenomenon is observed for silicate rocks, whose composition often encompasses very low P contents (Silva et al., 2012). Nevertheless, phosphate rocks (RP) present higher P content (5-30%), with potential to be used as fertilizer at global scale (Dias et al., 2015; Oliveira et al., 2015; Pufahl and Groat, 2017; Soltangheisi et al., 2020, 2018). In these cases, by lower solubility of natural phosphates, it is expected that there will be a synergy among the phosphate rock dissolution, the release of the nutrient from the fertilizer and plants uptake. This synergy provide a less immobilization of the P with high energy by clay minerals and oxides present in the soil (Szilas et al., 2007)

However, in addition to the characteristics of the phosphate rock, chemical and physical soil properties play an important role on the dissolution rate of the apatite mineral and the release of P to plants uptake. In this scenario, the same factors that act by accelerating or reducing the dissolution of geogenic apatite minerals present in the soil parent material, also act in the dissolution of apatite added to the soil as a fertilizer. Therefore, soil pH (Chien, 1977), water content (Heindel et al., 2018) and soil organic matter content (Alloush, 2003) alter the reaction equilibrium of the apatite dissolution due to protonation (H^+) and nutrient adsorption by soil matrix. Furthermore, high Ca soil content is the main factor that retard the solubilization of apatite minerals, even when there is P deficiency and availability of H^+ in the medium (Robinson and Syiers, 1990). Due to this, if the soil has characteristics that disfavor the dissolution of the apatite, the mineral will remain in crystalline state and there will be low P release to the soil solution (Kumar et al., 1994). Furthermore, even with favorable thermodynamic conditions, the dissolution of apatite minerals in the soil can be prevented or delayed by self-inhibition processes. This occurs when, after the start of the apatite particle dissolution, there is a formation of zones around the granules with high levels of Ca, P and pH, resulting in a microregion with unfavorable thermodynamic conditions for the continuity of the process (Dorozhkin, 2012; Mafé et al., 1992).

A typical case of non-solubilization of apatite minerals from phosphate rock (RP) fertilization is observed in Soltangheisi et al. (2018). The soil sampling was performed soon after 6 years of fertilization. The authors observed values of P extracted by HCl much higher with RP than with single superphosphate. This is because the acid extractor solubilizes the apatite crystals and, in this case, evidences the existence of apatite minerals from fertilization.

Based on this observation and on the amplitude of factors that influence the dissolution of the apatite minerals, the minimum and maximum period of P supply to the plants when RP is used is uncertain. In general, the use of RP is indicated mainly for crops with longer cultivation time, such as perennial crops (Oliveira et al., 2015; Tiecher et al., 2014). However, there are also positive results for annual crops (Szilas et al., 2007).

Inorganic forms of P, even essential for absorption by plants (Schachtman et al., 1998) may favor numerous soil P sink, as discussed above. On the other hand, the organic forms of P have remarkable importance in the reduction of inner-sphere P adsorption and in mobilizing legacy P (Chassé and Ohno, 2016; Guppy et al., 2005; Menezes-Blackburn et al., 2018). The transformation of Pi into Po occurs through the incorporation of Pi into the plants and microorganism’s biomass. After death, organic compounds containing P return to the soil. Thus, the proportion of Po in the soil is determined by chemical properties and mineralogical composition of the soil, management practices and cultures used.

As an example, soils cultivated with pastures, in general, have high input and maintenance of organic matter in the first centimeters of the topsoil, favoring the accumulation of organic forms of P together with elements of C and N (Poeplau et al., 2018; Rumpel et al., 2015). Moreover, rotation with forage species, such as legumes with high nutritional demand or mining plants of P less available in the soil, favor the incorporation of Pi into organic molecules (Crème and Rumpel, 2016; Soltangheisi et al., 2020, 2018; Tiecher et al., 2012b). That represent a coupling of the cycles of P, C and N in the organic phase of the soil (Rumpel and Crème, 2015). The plant tissues, soil microbial biomass also contribute to temporarily store large amounts of P in the organic form (soil organic P represent between 30 and 65% of total soil P) (Condron et al., 2005; Tiecher et al., 2012a). After microorganisms death there is a synchronization between availability of P in the soil and the demand of plants uptake (Conte et al., 2002; Rheinheimer et al., 2019; Rheinheimer and Anghinoni, 2003).

Apart of SOM contain in its chemical composition Po, it recovers the soil matrix and competes with Pi for the same reactive functional groups of the clay minerals and oxides (Yang et al., 2019). From this coating there is no direct contact between molecules of Pi and reactive functional groups, which avoids inner-sphere adsorption (Guppy et al., 2005) and improves the P availability to plants (Fontana et al., 2008; Pereira et al., 2010).

In contrast, management practices, such as soil plowing, can cause loss of SOM through the mineralization of organic compounds (Rumpel et al., 2015). In the organic P compounds, orthophosphate monoesters have higher turnover than orthophosphate diesters due mainly to molecular structure and chemical relationship with soil colloids (Menezes-Blackburn et al., 2018). As well as, the chemical relationships, the physical protection of SOM alters the mineralization rate of organic compounds (Panettieri et al., 2017; Virto et al., 2010). In this sense, soil aggregation provides physical-chemical protection for SOM. What is denoted by the greater recalcitrance of SOM associated with small aggregates or clay particles (Mangalassery et al., 2019; Panettieri et al., 2017; Sarker et al., 2018; Virto et al., 2010, 2008).

Further the direct effects on the Pi and Po equilibrium in soil, the mineralization of the SOM provides the decoupling of the C, N and P cycles and consequent losses of C and N of the system. In the soil, there is an equilibrium between the cycles of C, N and P, defined in the SOM. This balance is characteristic of each ecosystem and variable in the medium and long term. For example, in ocean studies, it was observed similarity between C:N:P stoichiometry of water and plankton biomass (values close to 106:16:1) (Redfield, 1958). What became known as “Redfield Ratio”. Based on these observations, researchers have sought to establish representative C:N:P stoichiometry for terrestrial ecosystems. Thus, the C:N:P atomic ratio identified globally for the soil is 186:13:1 (Cleveland and Liptzin, 2007). However, locally this value can vary a lot depending on the soil management adopted. For example, Xue et al. (2019) identified an C:N:P atomic ratio of 66:5:1 for soils in China. Values quite different from those observed worldwide, but characteristic for the country's ecosystem. Even if there are differences between ecosystems, the C:N:P atomic ratio is a useful tool to identify the levels of C, N and P and possible nutrient limitations for choosing a better management strategy for these ecosystems (Cleveland and Liptzin, 2007; George et al., 2018).

In terrestrial environments, numerous factors alter the biogeochemical cycles of C, N and P and consequently the coupling / uncoupling between them. Among them, there are soil fertilization, alteration of native vegetation, soil management practices, alteration of water and temperature regimes and change in the concentration of gases in the atmosphere. Understanding how each one affects individually, or through their interactions, the coupling of C, N and P biogeochemical cycles in soil is essential to predict and reduce the harmful effects of climate change and land use on terrestrial ecosystems (Soares et al., 2019).

In soil, C:N:P stoichiometry is space-dependent due to the different accumulation of SOM with different nutritional contents (Tipping et al., 2016). This differentiation occurs due to the chemical complexity of the organic structures (Assunção et al., 2019) and the physical and physic-chemical protection that soil particles exert on organic compounds (George et al., 2018; Kleber and Johnson, 2010). In this sense, soil aggregation has a significant relationship to the accumulation and recalcitrance of SOM (Mangalassery et al., 2019; Panettieri et al., 2017; Sarker et al., 2018; Virto et al., 2010, 2008). Panettieri et al. (2017) demonstrated that in areas of permanent grassland there is a greater accumulation of SOM in large aggregates and a more efficient storage of lignin than systems with permanent cropland. Thus, management systems with soil revolving tend to reduce aggregation (Acar et al., 2018; Pinheiro et al., 2004) and increase the exposure of organic compounds to mineralization, thereby decoupling C:N:P (Crème et al., 2018; Nascente et al., 2015).

In the subsequent chapters (chapters II to VI), the five articles that make up this thesis will be presented. Chapters II, III and IV refer to articles already published and, therefore, presented in the publication version of the respective scientific journals and chapters V and VI referring to articles in the process for submission.

5. Chapter II - Phosphate fertilization and liming in a trial conducted over 21 years: a survey for greater forage production and Pampa pasture conservation

André Somavilla; Anderson Cesar Ramos Marques; Laurent Caner; Leandro Bittencourt de Oliveira; Fernando Luiz Ferreira de Quadros; Abad Chabbi; Tales Tiecher; Danilo Rheinheimer dos Santos

Published in : **European Journal of Agronomy**
DOI: 10.1016/j.eja.2021.126259

Enviado em 8 de outubro de 2020.
Aceito para publicação em 8 de fevereiro de 2021.

Abstract

Phosphorus fertilization and liming are alternatives used to increase forage production in Southern Brazil grasslands (Pampa grasslands) and to avoid its replacement by cultivated exotic crops. However, changes in natural soil fertility can significantly affect the equilibrium of natural vegetation communities. We hypothesized that triple and simple superphosphate and limestone using would lead to greater increase in dry matter production and to changes in the botanical composition and richness of Pampa grassland plant species than the use of lesser soluble P sources (phosphate rock) or no liming treatment. To test our hypotheses, we assessed a 21-year-old field trial to identify its forage production pattern, based on P sources and liming application. The vegetation was studied in 2009 and 2018, based on the following variables: Shannon and Pielou indices, species richness, dry matter and plant groups (species grouped based on growth form, life period, photosynthetic pathway, morphology and growing season). Soil chemical properties in the 0–10 cm layer were also evaluated in 2018. According to the long-term evaluation, two high dry matter yield periods were observed after P fertilization (55% and 25 % higher dry matter yield, on average). Each of these periods was followed by decreased dry matter yield, regardless of the P source. Species richness did not show changes overtime, although there was high turnover of plant species. Soluble fertilizer usage resulted in increased dry matter production and in the replacement of large proportions of species, mainly in tussock grasses and forbs groups. The contribution of legume species for dry matter production increased due to the combined effect of phosphate fertilizers and limestone. However, maintaining forage production levels higher than without phosphorus fertilization requires regular supply of soluble phosphorus fertilizers, which, in the present case, cannot exceed three years.

Keywords – Grassland, Phosphate rock, Dry matter, Hierarchical approach, Botanical composition.

5.1.Introduction

Phosphorus (P) corresponds to approximately 0.2% of plant dry matter (Schachtman et al., 1998); it is a key component for protein and nucleic acid production, as well as for enzymatic activation and energy transfer (Wang et al., 2017). Nitrogen and P are the macronutrients mostly limiting photosynthetic production in aquatic or terrestrial environments

(Elser et al., 2007; Fay et al., 2015). Consequently, plant growth is directly linked to P availability in the soil.

Soil P availability for plants grown in natural environments results from the formation of P forms available in primary minerals during soil formation processes. Parent material composition and weathering intensity play fundamental roles in soil nutrient availability for plants (Turner et al., 2018). Overall, soils in Southern Brazil grasslands (the so-called Pampa grasslands) are intensely weathered, rich in iron oxides and kaolinite, and formed from parent materials presenting low P concentration, which implies low natural P availability to plants. Moreover, they are naturally acid soils (pH 4.4–5.1) and this feature leads to high-energy P adsorption onto soil clay minerals and oxides, as well as reduces natural soil P availability to plants (e.g., Mehlich-1 method), which often ranges from 2 mg kg^{-1} to 8 mg kg^{-1} (Oliveira et al., 2011; Rheinheimer et al., 1997).

Vegetation growing in these soils comprises species adapted to local conditions such as low P availability and soil acidity (Oliveira et al., 2018; Marques et al., 2019). Approximately 3,000 plant species can be found in the Pampa grassland ecosystem (Pampa biome), 450 of them are grass species used for forage production (Boldrini, 2009). Pampa grasslands produce from 3.7 Mg ha^{-1} year^{-1} (Soares et al., 2005) to 9.8 Mg ha-1 year-1 (Pellegrini et al., 2010) of dry matter under natural conditions, which enables mean potential production of 60–70 kg ha^{-1} year^{-1} of cattle body weight under extensive livestock management (Carvalho et al., 2006). However, Pampa grasslands are often poorly managed due to production system intensification processes that lead to overgrazing, low forage and beef production and, consequently, to low profitability (Carvalho and Batello, 2009; Borges et al., 2016; Fedrigo et al., 2018). Pampa grassland areas have decreased by 26 % in the last 50 years to give room to annual crops and cultivated forests capable of providing higher revenues (Oliveira et al., 2017).

Nevertheless, the vegetation composing the Pampa grasslands has high potential for forage production when it is properly managed. Grassland improvement through soil fertility correction and exogenous winter species' inclusion can lead to forage dry matter production of 14 Mg ha^{-1} year^{-1} and increase meat production to approximately 900 kg ha^{-1} year^{-1} of cattle body weight (Oliveira et al., 2015). This means that it is possible intensifying these production systems, increasing farmers' income and protecting the natural biome, at the same time. It is necessary implementing proper plant and soil fertility management processes in order to achieve high forage production rates. Limestone and phosphate fertilizers are fundamental strategies used to improve soil fertility, increase nutrient availability and, consequently, to improve plant development (Prestes et al., 2016).

Low-solubility phosphate fertilizers, such as phosphate rock (RP), have been suggested as better alternative than soluble phosphates to increase P contents in grasslands (Tiecher et al., 2014; Oliveira et al., 2015). Slow fertilizer solubility enables the theoretical synchronism between P availability and plant demand (Szilas et al., 2007), which gradually leads to increased dry matter production in the long-term. However, RP's ability to release P is uncertain due to several factors that control its dissolution, such as soil pH (Robinson and Syers, 1990), moisture level (Heindel et al., 2018) organic matter content (Alloush, 2003) and Ca content (Robinson and Syers, 1990). Therefore, the RP effect on Pampa grasslands' dry matter production in the long-term remains unknown.

Although soil fertility improvement has beneficial effect on dry matter production, it can also change grassland' dynamics. For instance, increasing soil P availability can favor the development of more nutritionally-demanding plants, such as leguminous plants, and make them more abundant and even dominant in the vegetation community (Blanck et al., 2011; Ceulemans et al., 2013; Harpole et al., 2016). Balanced nitrogen and phosphate fertilizer using can be an alternative to reduce the effect of grassy or leguminous species dominance on fertilized areas (Harpole et al., 2016). Species dominance is also not desired in Pampa grasslands because it can reduce the wide coexistence of plant species, which is one of the main features of Pampa vegetation communities. Changes in the botanical composition of grasslands subjected to P fertilization are contradictory. Some studies presented decreased species richness (Zhao et al., 2019), whereas other studies did not show changes in the number of plant species (Tiecher et al., 2014; Oliveira et al., 2015). These last cases can be explained by the analyzed time scale: 12 years (Tiecher et al., 2014) and 16 years (Oliveira et al., 2015). Longer follow-up time is suggested to analyze changes in botanical composition more reliably (Schellberg et al., 1999).

The current study aims to investigate (i) whether soluble P sources (triple and simple superphosphate) and limestone use result in greater dry matter production than the use of lesser soluble P sources (phosphate rock) or the use of no liming; and (ii) in which ways P fertilization and liming affects the botanical composition and grassland plant species richness. Soil and vegetation data from a 21-year-old trial conducted in Southern Brazilian Pampa grassland managed with different phosphate sources, liming, fertilization rates and plant species' overseeding were analyzed.

5.2.Materials and Methods

Site location and initial soil and vegetation conditions

The trial was implemented in 1997, in soil type classified as Ultisol with natural grassland, which is commonly referred to as Pampa biome. It was conducted in the experimental area of the Soil Science Department of Federal University of Santa Maria. The grassland at the trial site is classified as Mesic Pampa Grassland subtype 5b (Andrade et al., 2019). Climatic features of the trial site comprise humid subtropical climate type with annual rainfall of approximately 1,770 mm; the mean annual temperature in the hottest months (December–February) reaches 24.2 ºC, and 14.5 ºC in the coldest months (June–August) (Fig. 1). The overall chemical features of the topsoil layer (0-20 cm) in 1997 comprised: 170 g kg^{-1} of clay (pipette method); pH (H_2O extraction) of 4.5; 10.4 g kg^{-1} of C (Walkley-Black method); 2.5 mg kg^{-1} and 60 mg kg^{-1} of P and K, respectively (extracted by Mehlich-1); and 1.30 $cmol_c$ kg^{-1}, 1.17 cmolc kg^{-1} and 0.75 $cmol_c$ kg^{-1} of Al, Ca and Mg, respectively (extracted by using 1 mol L^{-1} KCl).

The botanical composition of the area mainly comprised *Paspalum notatum* (45.3% of pasture biomass), *Eryngium ciliatum* (21.2% of pasture biomass), *Andropogon ternatus* (1.0% of pasture biomass), *Paspalum plicatulum* (0.5% of pasture biomass), *Chloris polydactyla*, and *Schizachyrium microstachyum* (0.3% of pasture biomass), as well as *Eryngium horridum, Aristida laevis, Piptochaetium montevidense* and *Saccharum angustifolius* (each with 0.2% of pasture biomass). Senescent material contribution corresponded to 29.2% of pasture biomass (Bandinelli et al., 2005).

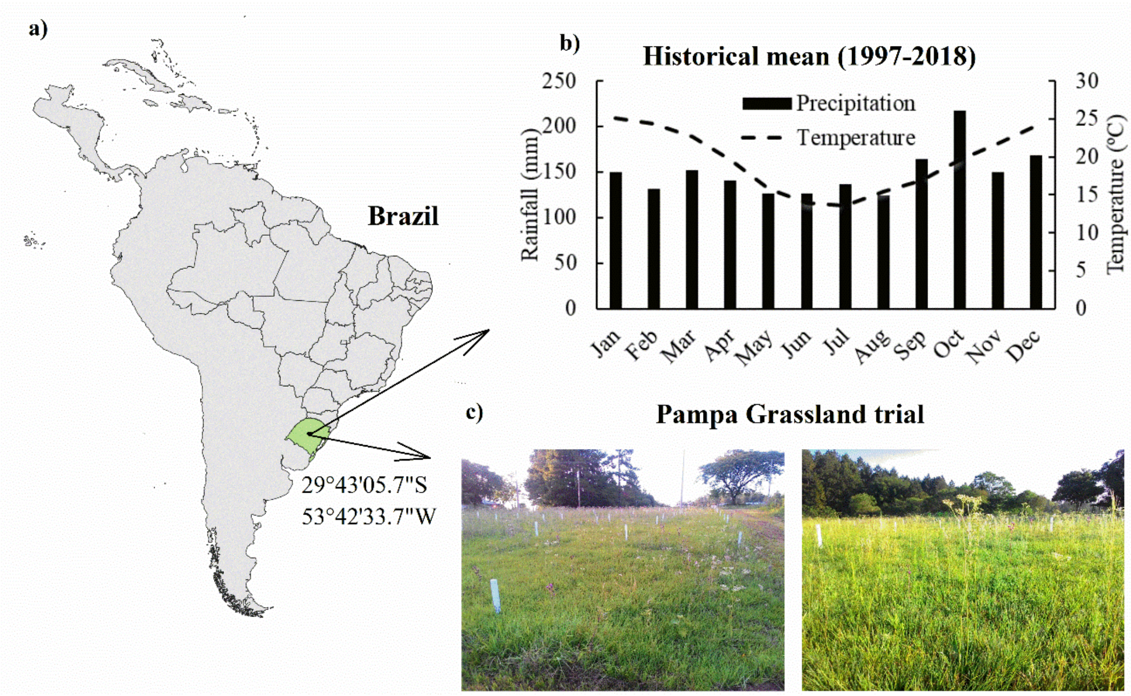


Fig. 1– 21-year-olf field trial conducted in a Pampa grassland site (a); mean rainfall and temperature (1997–2018) (b) and experiment's overview.

Experimental history and treatments

The trial was carried out based on a two-factorial (6 × 3) split-plot complete randomized block design, with three replications. The main factor consisted in the application of different P sources and limestone, as well as in *Lolium multiflorum* Lam. cv. 'Comum' and *Trifolium vesiculosum* Savi. cv. 'Yuchi' overseeding, namely: TPCa—triple superphosphate + limestone + overseeding; TP—triple superphosphate + overseeding, SPCa—single superphosphate + limestone + overseeding; RP—phosphate rock from Gafsa + overseeding; OS—without P but with overseeding; and Control—without P and without overseeding (Control). The second factor (split-plot) was P fertilization rate: 118 kg ha^{-1} of P, applied until 1998; 206 kg ha^{-1} of P, applied until 2010; and 250 kg ha^{-1} of P, applied until 2012. Phosphorus inputs in each treatment over time are shown in Fig. 2.

Liming was applied to the soil surface at a rate of 3.2 Mg ha^{-1} in 1997 (the amount necessary to achieve pH 5.5). Potassium (108 kg ha^{-1} of K) and nitrogen (70 kg ha^{-1} of N, in its urea form) were applied to the soil surface in all treatments, except for the Control, in 1997. *L. multiflorum* and *T. vesiculosum* overseeding was carried out with 30 kg ha^{-1} and 12 kg ha^{-1} of seeds, respectively, in the cool season of 1997 and 2002.

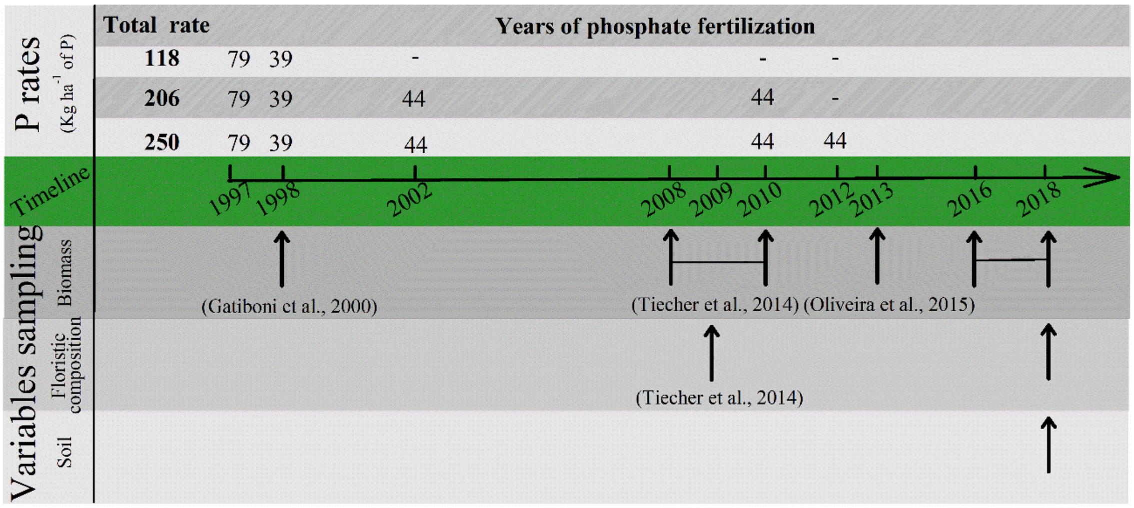


Fig. 2- 21-year-old field trial conducted in Pampa grasslands - framework of treatments and variable sampling.

Biomass production and floristic composition

Dry matter production values recorded in 1998, 2008–2010 and 2013 were taken from (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015) and reanalyzed in association with dry matter measurements taken between 2016 and 2018, as described below. In addition to biomass production, data about floristic composition in 2009 were taken from Tiecher et al. (2014) and reanalyzed in association with results of the botanical sampling performed in 2018 - exclusive to the present study.

Dry matter production was measured between 2016 and 2018 by cutting the shoot biomass in area covering 0.25 m^2 per plot, as carried out by (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015). Next, plant material was oven-dried at 60°C, for 48 h. After the cutting procedure performed to estimate dry matter production was over, all areas were mowed and plant material was removed from the site. The vegetation was not grazed by animals throughout the 21-year experiment and tractor trimmer was used to mow the pasture at 5 cm (in height), throughout the experimental period.

Data sets about quantified dry matter production were taken from the literature and normalized in comparison to the treatment without P fertilization (OS). OS was used as standard treatment because, unlike the Control treatment, it had yield data of all analyzed years available. Relative values recorded for each analysis period were achieved through the following equation:

$$RDm = \frac{Dm_x}{Dm_{OS}} 100 \qquad \text{Equation 1}$$

where RDm (%) is the relative dry matter, Dm_x (kg ha^{-1}) is the dry matter of treatment x, and Dm_{OS} (kg ha^{-1}) is the dry matter of treatment OS.

Floristic composition was quantified based on the BOTANAL method, in January 2009 (67-day growth period) (Tiecher et al., 2014) and in February 2018 (75-day growth period)—i.e., 12 and 21 years after the beginning of the experiment, respectively. Briefly, the three most dominant species were ranked based on dry weight, in a fixed frame (0.25 m2), and species composition rate was estimated. All other species were identified and 1% of total dry matter was attributed to each one of them (Tothill et al., 1978).

Species identified in both botanical samplings (2009 and 2018) were classified into nine groups, according to Oliveira et al. (2015), by taking into consideration a hierarchical approach (Lavorel et al., 1997) based on growth form (grasses, legumes, forbs, sedges); life period (annual, perennial); photosynthetic pathway (C3 or C4); morphology (tussock, prostrate); and growing season (cool or warm-season) (Appendix A). These nine groups comprised warm-season C4 prostrate perennial grasses (WPPG), warm-season C4 tussock perennial grasses (WTPG), warm-season C4 annual grasses (WAG), warm-season legumes (WL), cool-season C3 annual grasses (CAG), cool-season C3 perennial grasses (CPG), cool-season legumes (CL), forbs, and sedges.

The vegetation in the plots was analyzed based on the diversity, richness, equitability and maintenance of the observed species. Species diversity was estimated based on the Shannon index (H') provided by equation 2.

$$H' = -\sum_{i=1}^{S} \frac{ni}{N} ln \frac{ni}{N} \qquad \text{Equation 2}$$

wherein S is the number of species in the sampled area, ni is the dry matter of each species (kg ha^{-1}), N is the total dry matter (kg ha^{-1}), and ln is the natural logarithm.

Species richness (SR) was based on the total number of species found in the sampled area of each plot. Species equitability (evenness) was analyzed was analyzed based on the Pielou index (J) (Pielou, 1966) provided by equation 3.

$$J = \frac{H'}{\ln{(SR)}} \qquad \text{Equation 3}$$

The maintenance of species was analyzed by the rate of persistence (i.e., the number of species observed in both floristic composition quantification), disappearance (i.e., the number of species identified in 2009 but not identified in 2018) and emergence (i.e., the number of species not identified in 2009 but identified in 2018) of then.

Soil sampling and analysis

Soil was sampled in the 0–10 cm layer in October 2018. Sampled soil was oven-dried at 65ºC, sieved at 2 mm mesh size and stored for chemical analyses.

Soil pH was measured at soil/water ratio of 1:1 (v/v). Phosphorus (P) and K contents available in the soil were extracted through Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1953) and quantified through spectrophotometry (Murphy and Riley, 1962) and atomic emission techniques, respectively. Exchangeable Ca, Mg, and Al contents were extracted with 1 mol L^{-1} KCl and quantified through atomic absorption spectrophotometry (Ca and Mg), whereas Al was quantified by titration with 0.0125 mol L^{-1} NaOH. Effective cation exchange capacity (CECef, $cmol_c$ kg^{-1}), cation exchange capacity at pH 7.0 ($CEC_{pH\ 7.0}$, $cmol_c$ kg^{-1}), Al (Al_{sat}, %) and cation saturation (V, %) were estimated.

Statistical analyses

Dry matter production pattern over 21 years was graphically analyzed after values were standardized, as described above. Soil variables were analyzed based on a two-factorial split-plot complete randomized block design with three replications, where each plot corresponded to P sources and split-plot corresponded to the P fertilizer rate. Analysis of variance and mean test (Scott-Knott test, $p \leq 0.05$) were carried out in the SISVAR 5.6 software (Ferreira, 2015).

Dry matter production and botanical variables of Shannon diversity index, Pielou equitability index and species richness were analyzed through randomization test ($p \leq 0.05$). In addition, principal component analysis (PCA) was applied to the same variables, which were standardized in the Multiv software (Pillar, 1997) based on their marginal total and subjected to ordination multivariate analysis in the Past software (Hammer et al., 2001).

5.3.Results

Soil chemical properties

Residual limestone effect persisted in the soil after 21 years. Significant effects between P sources and P fertilizer rate were only observed for available soil P content (Table 1). Liming (TPCa and SPCa) treatments decreased soil acidity, as shown through higher soil pH-H2O, cations saturation (V) and exchangeable Ca and Mg values, as well as through low Al and Alsat contents, and low potential acidity values (H+Al, Table 2).

There was no residual effect 20 years after the application of 118 kg ha^{-1} of P; mean soil P content of 15 mg kg^{-1} were observed for all treatments (Table 3). The application of additional 44 kg ha^{-1} of P in 2002 and 2010 resulted in mean increase by 13% in available soil P content in soil subjected to treatments with P inputs, regardless of the P source. The application of additional 44 kg ha^{-1} of P in 2012 maintained the difference in available soil P content in the soil between treatments that were not treated with P (Control and OS) and those that were treated with it (RP, TP, TPCa, and SPCa) (Table 3).

Table 1–Significance of the effects of experimental factors and their interactions on soil chemical properties, as resulting from analysis of variance (ANOVA).

FV	**pH**	**Al**	**Ca**	**Mg**	**K**	**P**	**(H+Al)**	**CEC$_{ef}$**	**CEC$_{pH7.0}$**	**Al$_{sat}$**	**V**
	H_2O	**--- cmol$_c$ kg^{-1} ---**			**- mg kg^{-1} -**		**--------- cmol$_c$ kg^{-1} ---------**			**--- % ---**	
T[a]	**	**	**	**	ns	*	**	**	ns	**	**
S	ns	ns	ns	ns	ns	**	ns	ns	*	ns	ns
T*S	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
CV 1 (%)	3.2	33.4	25.4	24.1	19.5	19.8	13.0	10.8	8.9	27.5	12.2
CV 2 (%)	3.2	24.9	15.3	14.4	15.4	17.2	10.9	8.6	5.5	23.0	12.4

[a] T—Treatments (phosphorus sources); S—Split-plot (rate of P fertilizer). *Significant at $p \leq 0.05$; ** Significant at $p \leq 0.01$; ns—Not significant at $p \leq 0.05$.

Table 2–Mean values of soil chemical properties measured in October 2018 in the 0–10 cm soil layer.

Treat	**pH**	**Al**	**Ca**	**Mg**	**K**	**(H+Al)**	**CEC$_{ef}$**[c]	**CEC$_{pH7.0}$**[d]	**Al$_{sat}$**[e]	**V**[f]
	H_2O	**--------- cmol$_c$ kg^{-1} ---------**			**- mg kg^{-1} -**	**--------- cmol$_c$ kg^{-1} ---------**			**------ % ------**	
Control[a]	4.8 c[b]	0.81 a	0.92 c	0.68 b	76.20 a	4.05 a	2.60 b	5.84 a	36.6 a	29.6 c
OS	4.7 c	0.87 a	0.81 c	0.62 b	63.37 a	4.13 a	2.46 b	5.72 a	39.8 a	26.9 c
RP	4.7 c	0.77 a	1.04 c	0.66 b	69.18 a	4.13 a	2.65 b	6.00 a	35.8 a	29.7 c
TP	4.7 c	0.77 a	0.93 c	0.56 b	57.98 a	4.27 a	2.42 b	5.91 a	35.8 a	27.6 c
TPCa	5.5 a	0.13 b	1.93 a	0.85 a	69.08 a	2.60 b	3.08 a	5.55 a	4.8 b	51.8 a
SPCa	5.3 b	0.23 b	1.57 b	0.93 a	65.15 a	2.92 b	2.91 a	5.59 a	10.1 b	46.4 b

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding. [b]Means followed by the same letter did not differ statistically by Scott-Knott test ($p \leq 0.05$). [c]Effective cations exchange capacity. [d]Cations exchange capacity at pH 7.0. [e]Al saturation. [f]Cations saturation.

Table 3– Available soil P contents by Mehlich-1, measured in 2018 in the 0–10 cm topsoil layer in plots that received 118, 206 and 250 kg ha^{-1} of P from different P sources.

Treat	**P^{b} (mg kg^{-1})**		
	118 kg ha^{-1} (1998^{d})	**206 kg ha^{-1} (2010)**	**250 kg ha^{-1} (2012)**
Control[a]	14.8 aA[c]	12.3 bA	11.7 cA
OS	13.2 aA	12.6 bA	11.8 cA
RP	16.1 aB	16.9 aB	27.7 aA
TP	15.0 aB	20.7 aA	24.5 aA
TPCa	13.4 aB	16.8 aB	20.5 bA
SPCa	18.0 aA	16.3 aA	21.9 bA

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding. [c]Means fallowed by the same letter, upper case letters in the line and lower-case letters in the column, did not differ statistically by Scott-Knott test (*p*≤0.05). [d]Year of the last phosphorus fertilization.

Historical pattern of dry matter production

Two high yield periods identified throughout the 21 experimental years corresponded to post-fertilizer application times. Each of these periods was succeeded by decreased yield rates over time. The RDm of Pampa grassland in 1998, soon after the first fertilization with soluble P (SPCa, TPCa, and TP), was 55% higher than that of the OS treatment, on average, whereas the RDm of the control treatment was 25% lower than that of OS. Low solubility phosphate (RP) using provided RDm 34% higher than that of OS (Fig. 3).

In the period from 2016 to 2018 (i.e., 4 and 6 years after the fertilization carried out in 2012, respectively – treatment based on 250 kg ha^{-1} of P) the P fertilization did not result in significant difference in RDm between treatments (mean test not shown). Fig. 3 presents the decrease line drawn based on means recorded for treatments with soluble P in order to show yield decrease over time.

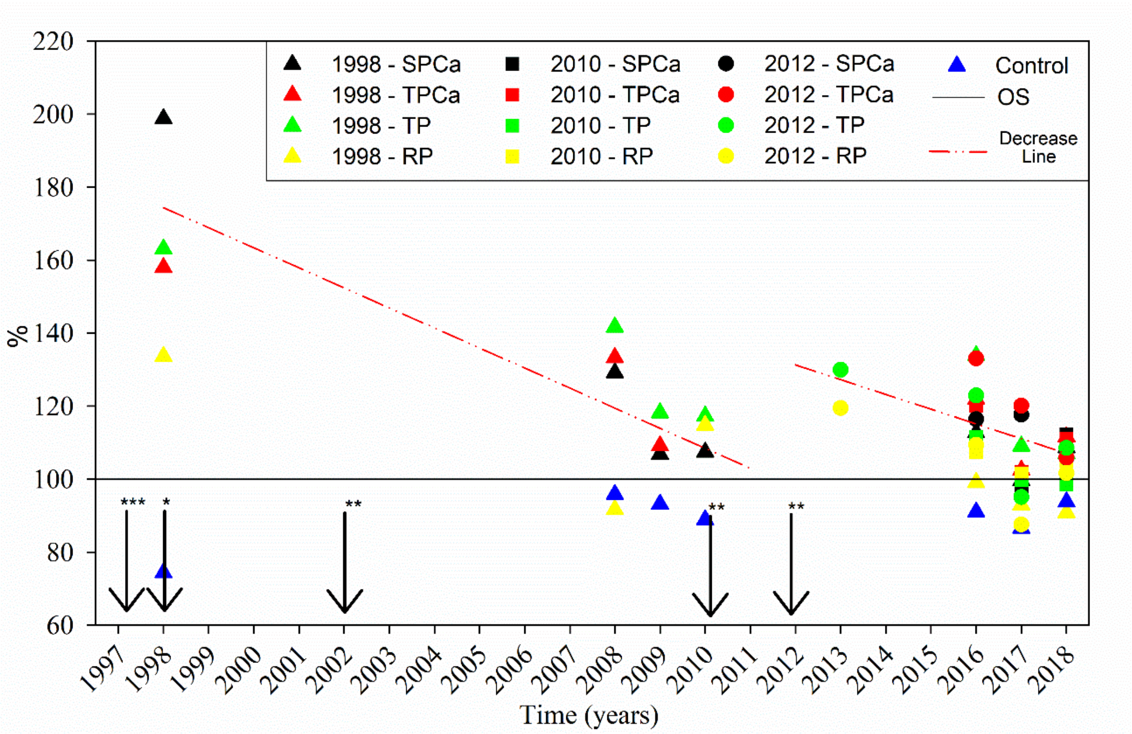


Fig. 3– Dry matter production over time, normalized by OS treatment. Values recorded in 1998, 2008-2010 and 2013 were adapted from Gatiboni et al. (2000), Oliveira et al. (2015) and Tiecher et al. (2014), respectively. Arrows represent fertilization applications at the following rates: ***79 kg ha^{-1} of P, ** 44 kg ha^{-1} of P and *39 kg ha^{-1} of P. Treatments: Control— treatment without P application or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.

Floristic composition and plant dry matter production accessed in 2009 and 2018.

Based on the evaluation performed in 2009, the experiment only consisted in two P fertilizer rates. The first one comprised the application of 118 kg ha^{-1} of P until 1998, whereas the second one encompassed the application of 162 kg ha^{-1} of P until 2002. The application of 162 kg ha^{-1} of P until 2002 - for chronological purposes - gave rise to treatments based on the application of 205 kg ha^{-1} of P until 2010 and 2012 (Figure 2).

Dm in the 2009 botanical sampling was higher in plants treated with soluble P fertilizer, whereas RP was the less productive treatment. Dm values recorded in the 2018 botanical sampling were similar between P sources (Table 4). Overall, botanical sampling Dm values were higher in 2018 than in 2009. This pattern corroborates the significance of differences in dry matter production between 2007 and 2009 (Tiecher et al., 2014) and the non-significance of it between 2016 and 2018 (Fig. 3) (mean test not shown).

Shannon dominance index was different between RP (1.39) and TP (1.20) in 2009 (Table 4). Species richness and Pielou equitability index (J) did not show differences between treatments. These variables recorded different outcome in the 2018 botanical sampling, since Shannon index was lower in RP (0.65) and TP (0.77) and higher in OS (1.08) and SPCa (1.09). Pielou equitability (J) index was lower in RP (0.297) and higher in TP and OS (0.50 and 0.54, respectively). Species richness only differed in TP, since it recorded values lower than the ones recorded in the other treatments. Differences between P fertilizer rates were not observed in any vegetation sampling (2009 and 2018, Table 4).

The comparison between the two botanical sampling years was based on principal component analysis and it is discussed below. However, there were reductions in Shannon and Pielou indices and species richness, as well as increase in Dm between 2009 and 2018. This reduction, together with the similarity observed in 2018, may suggest homogenization in values recorded for the analyzed variables due to temporal reduction in treatments' effect. On the other hand, different P sources may lead to species turnover without causing changes in botanical indices and species richness (Oliveira et al., 2015).

Plant diversity and vegetation dynamics

Sixty-seven (67) plant species distributed in 15 families were identified throughout the experiment. Poaceae, Asteraceae and Fabaceae prevailed among them; they encompassed 25, 14 and 10 species, respectively. Overall, forbs, WTPG and WPPG were the groups of plants recording the largest number of observed species, regardless of the adopted P source (Fig. 4). Botanical sampling carried out during summer (October–March) - the season recording the highest temperatures in the year (Fig. 1) - accounted for the prevalence of warm-season species.

Forbs and WTPG were the species that mostly disappeared between the two analyzed years. Species disappearance rate ranged from 64% to 85% in forbs, and from 75% to 100% in WTPG (Fig. 4). In this plant groups (forbs and WTPG), the highest species disappearance values were observed for TP and TPCa treatments. SPCa recorded the lowest species disappearance rate. With respect to warm-season leguminous (WL) and warm-season prostrate grasses (WPPG), the lowest species disappearance rate was associated with P and limestone application. There were 50% and 67% of WL species disappearance, as well as 33% and 40% of WPPG species disappearance in plants subjected to TPCa and SPCa treatments, respectively.

The highest species emergence rate was observed for the forbs and WTPG groups; values ranged from 64% to 85% for forbs, and from 50% to 100% for WTPG. The highest species emergence rate recorded for these two groups was observed for plants subjected to TP and TPCa treatments. TP and TPCa accounted for the highest species turnover rate recorded for the forbs and WTPG groups. It resulted in forbs maintenance and in decrease (-25%) richness of WTPG plant groups in TP treatment from 2009 to 2018. TPCa treatment led to species richness increase (100%) in forbs and decrease (-50%) in WTPA plant groups from 2009 to 2018.

Overall, there was incidence of WTPG species, which led to increased species richness, mainly in treatments with RP, SPCa and TP. Plants belonging to the legume group (WL) had a greater appearance in the RP and TPCa treatments (25 and 125%, respectively), and it increased their participation in species richness of these treatments.

Table 4–Randomization test to Shannon dominance (H') and Pielou equitability (J) indexes, richness and dry matter production (Dm) accessed by vegetation sampling in 2009 and 2018.

2009

	Factor treatments							
Treat	**Dm (kg ha^{-1})**		**Shannon**		**Pielou**		**Richness**	
Control[a]	2120	ab[b]	1.33	ab	0.55	ns	11.50	ns
OS	2018	ab	1.28	ab	0.56		10.00	
RP	1380	b	1.39	a	0.61		10.00	
TP	2582	a	1.20	b	0.57		10.00	
SPCa	2985	a	1.30	ab	0.53		10.50	
TPCa	2520	a	1.25	ab	0.52		10.83	
	Factor rate of P fertilization (kg ha^{-1} of P)							
118	2102	a	1.22	a	0.53	ns	10.06	ns
162	2433	a	1.37	a	0.58		10.89	

2018

	Factor treatments							
Treat	**Dm (kg ha^{-1})**		**Shannon**		**Pielou**		**Richness**	
Control	2831	b	0.95	ab	0.42	bc	9.56	a
OS	3247	a	1.08	a	0.50	ab	9.00	a
RP	3096	a	0.65	b	0.29	c	9.00	a
TP	3381	a	0.77	b	0.54	ab	7.33	b
TPCa	3547	a	1.00	ab	0.40	bc	9.22	a
SPCa	3909	a	1.09	a	0.45	b	7.78	a
	Factor rate of P fertilization (kg ha^{-1} of P)							
118	3287	ns	0.97	ns	0.45	ns	9.00	ns
206	3390		0.88		0.41		8.50	
250	3329		0.92		0.44		8.44	

[a] Treatments: Control— treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding. [b]Means fallowed by the same letter did not differ statistically ($p \leq 0.05$). [ns]not-significant ($p \leq 0.05$).

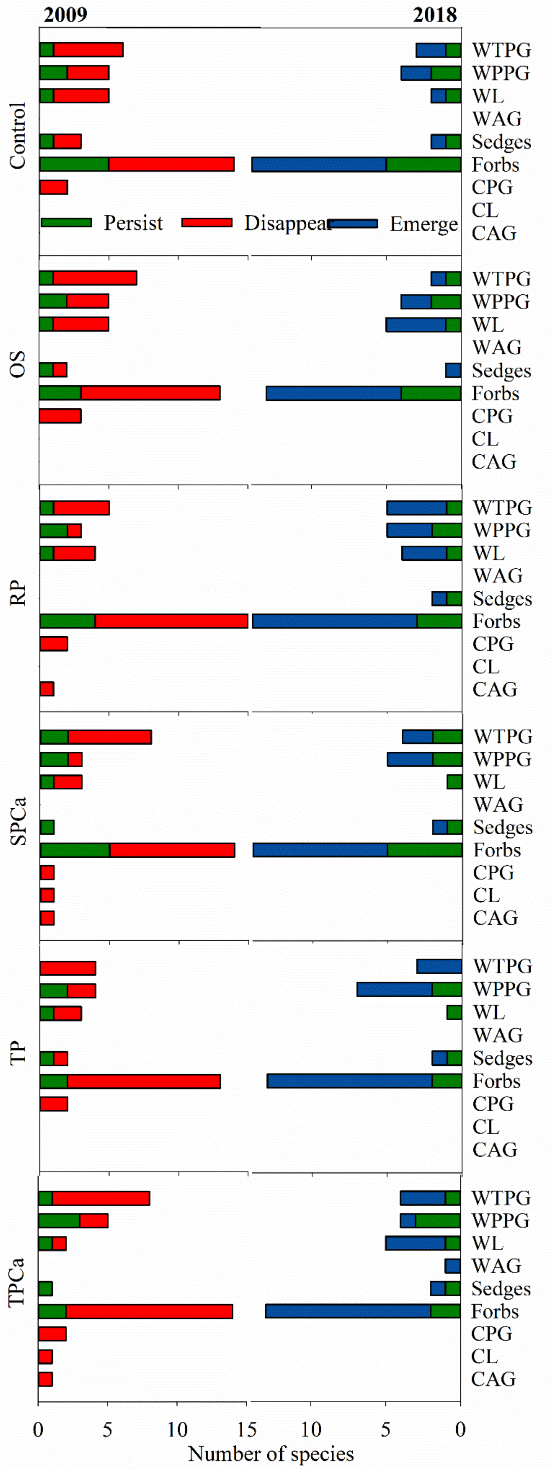


Fig. 4–Species replacement between 2009 and 2018 by functional groups. WPPG—warm-season C4 prostrate perennial grasses; WTPG—warm-season C4 tussock perennial grasses, WAG—warm-season C4 annual grasses; WL—warm-season legumes; CAG—cool-season C3 annual grasses; CPG—cool-season C3 perennial grasses; CL—cool-season legumes; forbs and sedges; Treatments: Control—treatment without P application or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.

Principal components analysis (PCA) was carried out to feature changes in botanical variables, plant groups and RDm from 2009 to 2018 (Fig. 5). Plant groups were represented by the dry matter produced in each treatment. The CPG, CL and CAG groups were removed from the analysis because they were not observed in any of the treatments in 2018 sampling. The first two principal components in PCA explained 64.4% of total variance.

Species richness and Shannon diversity index (H') were negatively associated with TP and SPCa, in both evaluated years. Species richness in 2009 was associated with the Control, OS and TPCa treatments, and this behavior remained the same in 2018. Nevertheless, H' was associated with RP in 2009 and presented large shift towards TPCa in 2018. Pielou equitability index (J) was positively associated with RP, TP and SPCa, and negatively associated with TPCa, Control and OS, in 2009. However, J was not positively associated with RP in 2018, but it was positively associated with SPCa and TPCa. RDm location in PCA did not change from 2009 to 2018. RDm was positively associated with SPCa, TP and TPCa, and negatively associated with RP, Control and OS, in the two evaluated years.

Groups composed of grasses and legumes (WL, WPPG and WTPG) suffered greater effect of treatments over time. WPPG was strongly correlated to TPCa in 2009, whereas its correlation to RP and TP was higher in 2018. WTPG behaved in a different way; it was positively correlated to TP and SPCa in 2009 and presented correlation to OS, TPCa and SPCa in 2018. Warm-season legumes (WL) were positively associated with SPCa and TP in 2009, although they were more closely associated with TPCa in 2018. Sedges and forbs groups were slightly affected by treatments. Overall, sedges and forbs were only associated with SPCa and TP in 2009. In 2018, they were correlated to RP, as well. Forbs and sedges groups were negatively related to TPCa in both evaluations.

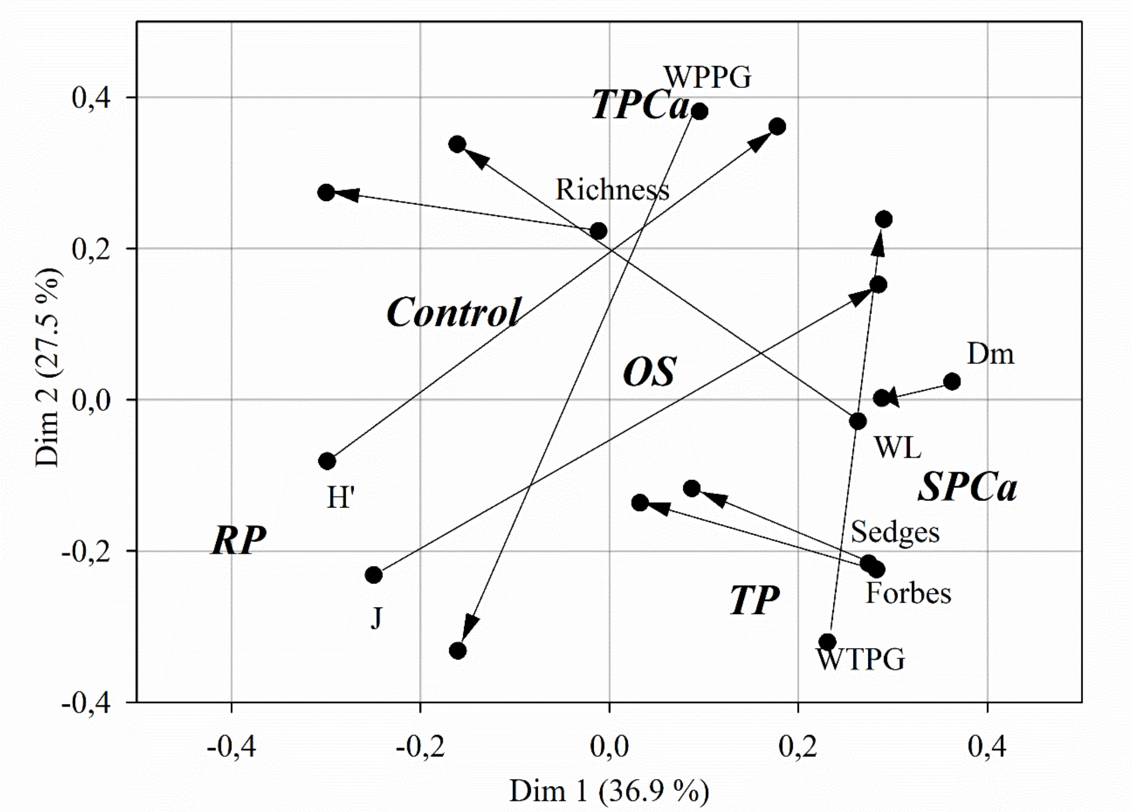


Fig. 5–Bi-plot of vegetation parameters and dry matter produced by the main plant groups accessed in 2009 and 2018, based on fertilization with different P sources. Arrows represent the 2009→2018 temporal change in the behavior of the assessed variables. H'—Shannon diversity index; J—Pielou equitability index; Dm—Dry matter field production; WPPG—warm-season C4 prostrate perennial grasses; WTPG—warm-season C4 tussock perennial grasses, WAG—warm-season C4 annual grasses; WL—warm-season legumes; CAG—cool-season C3 annual grasses; CPG—cool-season C3 perennial grasses; CL—cool-season legumes; forbs and sedges; Treatments: Control—treatment without P or overseeding; OS—without P application, although with overseeding; RP—phosphate rock of Gafsa + overseeding; TP—triple superphosphate + overseeding; TPCa—triple superphosphate + limestone + overseeding; SPCa—single superphosphate + limestone + overseeding.

5.4.Discussion

Phosphate fertilization addition and soil acidity correction increased dry matter production and Pampa grassland quality. These practices enable increasing the economic return of livestock activity carried out in managed natural fields, as well as avoid replacing Pampa grasslands by annual or forest crops. Concurrent with the increase in dry matter production we found that soluble phosphate fertilizer usage led to a long-term plant species replacement.

Soil chemical features

Limestone application (TPCa and SPCa) played key role to differentiate the treatments after 21 years. Even after this time, it was possible observing significant residual effect of limestone on the 0–10 cm soil layer. Similar results were observed for the same soil in cropland areas. In these cases, residual limestone effect was observed after twelve and eighteen years (Rheinheimer et al., 2018; Vargas et al., 2019). In the present case, superficial liming provided TPCa and SPCa cations saturation levels higher than the reference value (40%, SBCS, 2016) in Pampa grassland areas. This means that renewed liming application is not compulsory.

Slightly higher Ca content in RP treatment may be associated with low dissolution of apatite minerals or carbonates found in phosphate rock fertilizer. The phosphate rocks from sedimentary deposits, such as the one used in the current study, have low CaCO3 contents (Robinson and Syers, 1990; Lefires et al., 2014). However, difference observed in soil Ca content is more likely associated with apatite dissolution than with CaCO3 incidence in phosphate rock fertilizer, since the small increase in Ca content was not followed by changes in soil pH and Al contents.

Time was a key factor in soil P availability. There were no differences between treatments in the long-term and there was decrease in available soil P content. This reduction may explain the sharp yield decrease and differences in RDm between treatments (Fig. 3). Moreover, the reduced difference between treatments and available soil P content have evidenced P adsorption as inner-sphere complex by soil clay minerals and iron oxi-hydroxides (Kim et al., 2011; Bortoluzzi et al., 2015), as well as high nutrient exportation by vegetal biomass (Oliveira et al., 2014).

The highest available soil P content observed after the application of 250 kg ha^{-1} of P was in compliance with the higher fertilizer rate application (Fig. 3). However, RP application at rate of 250 kg ha^{-1} of P resulted in the highest available soil P content. This behavior may be indicative of gradual P dissolution. It was not observed after the application of 118 (until 1998) and 205 (until 2010) kg ha^{-1} of P, but it was observed after the application of 250 kg ha^{-1} of P (until 2012 - Table 3).

Nevertheless, higher soil P availability did not lead to higher dry matter production in RP. Thus, the highest available P values recorded for RP may be an artifact from P extraction process based on the adopted method. The Mehlich-1 extractor used to obtain available P is composed of acid solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) that can promote dissolution of residual apatite minerals from RP fertilizer in the soil. In that case extracted P value may partly correspond to apatite dissolution and may not exactly correspond to bioavailable P (Freitas et al., 2013). Using acid extractors to quantify available P in soils subjected to RP fertilization is only recommended after 2-year fertilization (SBCS, 2016). Therefore, if future studies can prove that non-solubilized apatite remains in the soil 6 years after the last RP fertilization, it will be necessary recommending another method to quantify P availability in the soil.

Association between produced dry matter and P fertilization

Control and OS treatments recorded the lowest RDm, mainly in periods following fertilizer application. This feature highlights the limitations of Pampa grassland soils to achieve higher dry matter production under natural conditions, due to reduced natural P availability level in these areas, as also reported in other studies (Rizo et al., 2004; Soares et al., 2005; Pellegrini et al., 2010). Soluble phosphate fertilization is an alternative to quickly provide nutrients to soil solution. Improvements in soil chemical features due to soluble fertilizer using played key role in increasing dry matter yield (Gatiboni et al., 2000). Soluble P sources have historically led to the highest dry matter production response over time (Gatiboni et al., 2000; Tiecher et al., 2014; Oliveira et al., 2015). However, the rapid plant response to fertilization with single and triple superphosphate was followed by a decrease of forage production over time.

Yield decrease over time can be associated with negative balance in P availability in the soil. This balance is defined by leaving available soil P through dry matter exportation or immobilization by P inner-sphere adsorption onto clay minerals and/or soil oxides (P non-labile) and through no P replacement via fertilization. This process can be intensified due to low natural P availability (±13 mg kg^{-1}, Table 3) at pH ± 4.7 (Table 2) and to the presence of kaolinite, goethite and hematite at the topsoil layer (0–10 cm) (Bortoluzzi et al., 2015, 2007; Moterle et al., 2016). Thus, P availability in the soil may have shown higher exhaustion level in the first half of the investigated period (1998–2010) than in the second half of it (2010–2018); moreover, it could be the cause of greater slope in the RDm decreasing line between 1998 and 2010 (Fig. 3).

Constant P input into the system tends to saturate functional groups of inorganic constituents accounting for P sorption (Roy et al., 2017). The amount of P added to the soil until 2010 (162 kg ha^{-1} of P) may have been enough to cause small reduction in future P immobilization rates and, consequently, greater maintenance of soil P availability over time in order to avoid high dry matter production decrease rates. Therefore, periodic phosphate fertilizer applications should be conducted to counteract losses of available soil P by sorption in soil matrix and plant P exportation, as well as to maintain high dry matter production rates.

RP application has generated lower Pampa grassland yield response between P-fertilized plots due to lower P release by RP in the short-term than that observed for soluble P sources (TPCa, TP and SPCa). However, Pampa grassland response to RP was always lower than that observed for soluble P sources; the time elapsed after fertilization day led to RDm decrease. The temporal pattern of RDm reduction soon after RP application was not expected. Gradual dissolution of apatite minerals and fertilizer reapplication in the long-term were expected to keep productivity constant over time. RDm observed over time due to RP using may be associated with two main factors. The first one is the likelihood that RP is poorly solubilized in the soil, even in the long-term, and consequently, there is low P release for plant uptake purposes. Low P dissolution and maintenance in its apatite form has been evidenced in studies carried out worldwide (Kumar et al., 1994; Soltangheisi et al., 2018) and in preliminary results of the present trial. Finally, the temporal change in the botanical composition of the investigated site is the second factor, which, consequently, had effect on dry matter production.

Changes in vegetation composition

The smaller difference in vegetation indices in 2009 and larger differences in 2018 have indicated that the botanical composition has changed due to treatments applied within the evaluated period. Despite differences reported in vegetation indices, greater differences may have been avoided by cutting the vegetation on a regular basis, over 21 years. Vegetation cuts were performed in a simultaneous and uniform way throughout the treatments in order to simulate rotational grazing. Rotational grazing systems can provide plant species richness increase in the long-term, even within a mid-term period (six years) (Boavista et al., 2019). Therefore, the effect of periodic cuts may have mitigated, to some extent, the effect of soil fertility treatments on species richness and botanical indices.

However, there was small temporal variation in the Shannon (H’) and Pielou (J) indices, species richness and Dm from 2009 to 2018 (Fig. 5). Significant reductions due to P fertilization over time, mainly in species richness, have been reported in grasslands investigated in the Americas, Europe and Asia (Blanck et al., 2011; Ceulemans et al., 2013; Zhao et al., 2019). Small variation in botanical features between the two surveys performed in the current study was associated with different P sources, which were capable of promoting species turnover, although without changing botanical indices and species richness, as suggested by Oliveira et al. (2015). Species turnover may lead to changes in nutrient exportation rates and such a process may affect soil nutritional levels due to changes in bromatological features of forage plants, as well as in soil organic matter mineralization and accumulation.

The largest species turnover was recorded for forbs and WTPG plant groups at TP and TPCa. Although species turnover took place, species richness only decreased in the WTPG group, regardless of the treatments. This decrease may be associated with the fact that tussock growth habit is more affected by grazing than shorter or prostrate plants (Díaz et al., 2007). Grass species grown in South American grasslands play key role in forage production due to the large number of species and efficient dry matter production (Biganzoli and Zuloaga, 2015). This feature was observed in the present study, since the largest number of identified species belonged to family Poaceae and accounted for more than 65% of produced dry matter, regardless of the treatment (data not shown). Accordingly, treatments associated with the WTPG and WPPG groups were also associated with higher RDm (Fig. 5).

Unlike Poaceae plants, Asteraceae and Fabaceae plants - which composed the forbs and WL groups, respectively - are more nutritionally demanding and frequent in Pampa grasslands, but they provide little contribution to total dry matter production (Oliveira et al., 2015). Although some results have shown increased participation of leguminous plants in total forage dry matter when P fertilizers were applied (Rodríguez et al., 2007; Tiecher et al., 2014), this increase can be easily overcome through N supplementation (Oliveira et al., 2015). The higher dry matter production of forbs and WL groups in the current study was associated with soluble P sources (triple and simple superphosphate) and limestone application, since they led to high soil fertility levels. The greater participation of leguminous species, the higher dry matter yield in the long-term, and the higher P levels in plant tissues have contributed to greater P depletion and lower availability in the soil in treatments based on soluble fertilizer and limestone application.

In addition to the fact that leguminous plants were associated with SPCa treatment and, later on, with TPCa, grass species were mostly associated with limestone-free treatments (RP, TP, OS and Control), although RP and TP presented P contents similar to that of the TPCa and SPCa treatments. This behavior indicated that the simultaneous P and limestone application is more effective in increasing legume participation in dry matter production. Increasing the proportion of legume plants, either by favoring endemic leguminous species or sowing exotic legumes, helps enriching bromatological features (e.g., digestibility in vitro, crude protein and phosphorus content) (Gatiboni et al., 2008) and drought resistance in forage plants (Sanaullah et al., 2012).

In addition to changes in bromatological features, P fertilization can lead to changes in plant dry matter stoichiometric ratios (C:N:P). It mainly happens due to changes in botanical composition and increased P content in plant tissue (Gatiboni et al., 2008; Oliveira et al., 2014). Studies conducted worldwide have indicated reduced soil C and N stocks in managed pastures, even after fertilization and liming application (Eze et al., 2018). Furthermore, reduced C and N stock in pasture areas is enhanced by high plant material exportation due to increased grazing intensity (Zhou et al., 2017). However, Poeplau et al. (2018) have identified increased soil C stock, in the >10 cm soil layer, of fertilized pasture areas under temperate climate. The aforementioned authors have attributed such increase to reduced root C:N stoichiometry and to consequent increase in microbial C-use efficiency. Changes in soil C stocks in subtropical climate regions, such as the Pampa biome, can be observed after soil P fertilization. However, more conclusive studies should be conducted to analyze the soil C pattern in this scenario.

Cool-season species belonging to groups CPG, CL and CAG were identified even during the season presenting the highest temperatures in 2009. None of these groups was observed in 2018 (Fig. 4). *L. multiflorum* (CAG) and *T. vesiculosum* (CL), which are exotic species that were introduced in the region by overseeding conducted in 1997 and 2002, stand out among the cool-season species identified in 2009. Species *T. vesiculosum* was only identified in SPCa and TPCa. On the other hand, *L. multiflorum* was also observed in RP. This feature highlights the nutritional demands of the two exotic species, mainly for pH, Ca and P (Tables 2 and 3). The disappearance of groups CPG, CL and CAG in 2018 cannot be exclusively attributed to the effect of P sources, because environmental factors, such as temperature, may have played key role in the development of cool-season species. Moreover, the time after CAG and CL species introduction (last sowing in 2002, 16 years earlier) may have hindered the natural re-sowing of CAG and CL species. Therefore, the non-identification of these two species emphasizes the important role played by the periodic reintroduction of both *L. multiflorum* and *T. vesiculosum* in order to maintain satisfactory stocking levels.

5.5. Conclusion

Phosphate fertilization and liming application in Southern Brazil Pampa grasslands soil helped improving dry matter production. However, Pampa grasslands responded to phosphorus sources in different ways over 21 years. Soluble fertilizer using, such as triple and simple superphosphate, led to higher dry matter production. Phosphate fertilization led to small changes in species richness; thus, it is possible maintaining floristic biodiversity, despite phosphorus fertilizer using. However, there was higher species turnover, mainly in the tussock grass and forbs groups, which may contribute to change in ecosystem services. Legume species' contribution to dry matter production can increase due to soluble phosphate fertilizer and limestone using.

Although species turnover and increased proportion of legumes can enable improved bromatological features, they can lead to reduced soil C stock and decrease Pampa biome' ability to adapt to climate change, in the long-term. Thus, more conclusive studies should be conducted to analyze changes in ecosystem services and soil carbon increase in fertilized Pampa biome soil.

Regular phosphorus input is necessary to maintain dry matter yield and legume species' contribution in dry matter production higher than without phosphorus fertilization; in the present case, four years after phosphorus input resulted in dry matter yields similar between treatments; in the condition of the present study (i.e., with dry matter exportation) such an input cannot surpass three years. Lower species turnover may happen under rotational grazing conditions, and the effect of phosphorus fertilization can persist for longer due to lesser selective grazing and lower phosphorus exportation.

The highest available soil P content after phosphate rock application at rate of 250 kg ha-1 of P (six years before soil sampling) may be an artifact occurred during P extraction process based on Mehlich-1 extractor. This assumption suggests low phosphate rock dissolution over time and justifies the low increase in Pampa grassland dry matter production response.

5.6.Electronic Supplementary Material

Appendix A – Plants' hierarchical grouping.

5.7.Acknowledgment

The authors would like to thank all the students and researchers who helped keeping the field trial active over 21 years. We also thank Gilles Lemaire for his suggestions to improve the study.

5.8.Funding information:

The current research was supported by the National Council for Scientific and Technological Development - CNPq (process number PVE 400887/2014-2 and GM/GD 140270/2019-1).

5.9.References

Alloush, G.A., 2003. Dissolution and effectiveness of phosphate rock in acidic soil amended with cattle manure. Plant Soil 251, 37–46. https://doi.org/10.1023/A:1022987915057

Andrade, B.O., Bonilha, C.L., Overbeck, G.E., Vélez-Martin, E., Rolim, R.G., Bordignon, S.A.L., Schneider, A.A., Vogel Ely, C., Lucas, D.B., Garcia, É.N., dos Santos, E.D., Torchelsen, F.P., Vieira, M.S., Silva Filho, P.J.S., Ferreira, P.M.A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V.D., Boldrini, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. Appl. Veg. Sci. 22, 168–184. https://doi.org/10.1111/avsc.12413

Bandinelli, D.G., Gatiboni, L.C., Trindade, J.P.P., Quadros, F.L.F. de, Kaminski, J., Flores, J.P.C., Brunetto, G., Saggin, A., 2005. Composição floristica de pastagem natural afetada por fontes de fosforo, calagem e introdução de espécies forrageiras de estação fria. Ciência Rural 35, 84–91. https://doi.org/10.1590/S0103-84782005000100013

Biganzoli, F., Zuloaga, F., 2015. Análisis de diversidad de la familia Poaceae en la región austral de America del Sur. Rodriguesia 66, 337–351. https://doi.org/10.1590/2175-7860201566205

Blanck, Y.L., Gowda, J., Mårtensson, L.M., Sandberg, J., Fransson, A.M., 2011. Plant species richness in a natural Argentinian matorral shrub-land correlates negatively with levels of plant phosphorus. Plant Soil 345, 11–21. https://doi.org/10.1007/s11104-010-0671-0

Boavista, L. da R., Trindade, J.P.P., Overbeck, G.E., Müller, S.C., 2019. Effects of grazing regimes on the temporal dynamics of grassland communities. Appl. Veg. Sci. 22, 326–335. https://doi.org/10.1111/avsc.12432

Boldrini, I.I., 2009. A flora dos campos do Rio Grande do Sul, Campos Sulinos - conservação e uso sustentável da biodiversidade. Brasília.

Borges, J.A.R., Tauer, L.W., Lansink, A.G.J.M.O., 2016. Using the theory of planned behavior to identify key beliefs underlying Brazilian cattle farmers’ intention to use improved natural grassland: A MIMIC modelling approach. Land use policy 55, 193–203. https://doi.org/10.1016/j.landusepol.2016.04.004

Bortoluzzi, E.C., Pérez, C.A.S., Ardisson, J.D., Tiecher, T., Caner, L., 2015. Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. Appl. Clay Sci. 104, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Bortoluzzi, E.C., Pernes, M., Tessier, D., 2007. Interestratificado caulinita-esmectita em um argissolo desenvolvido a partir de rocha sedimentar do sul do Brasil. Rev. Bras. Cienc. do Solo 31, 1291–1300.

Carvalho, P.C. d F., Batello, C., 2009. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: The natural grasslands dilemma. Livest. Sci. 120, 158–162. https://doi.org/10.1016/j.livsci.2008.04.012

Carvalho, P.C.F., Fisher, V., Dos Santos, D.T., L Ribeiro, A.M., F De Quadros, F.L., S Castilhos, Z.M., E C Poli, C.H., G Monteiro, A.L., Nabinger, C., Cristina Genro, T.M., A Jacques, A. V, 2006. Produção animal no bioma Campus Sulinos. Brazilian J. Anim. Sci. 35, 156–202.

Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2013. Plant species loss from European semi-natural grasslands following nutrient enrichment - is it nitrogen or is it phosphorus? Glob. Ecol. Biogeogr. 22, 73–82. https://doi.org/10.1111/j.1466-8238.2012.00771.x

CQFS-RS/SC, 2016. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina.

Díaz, S., Lavorel, S., McIntyre, S., Falczuk, V., Casanoves, F., Milchunas, D.G., Skarpe, C., Rusch, G., Sternberg, M., Noy-Meir, I., Landsberg, J., Zhang, W., Clark, H., Campbell, B.D., 2007. Plant trait responses to grazing - A global synthesis. Glob. Chang. Biol. 13, 313–341. https://doi.org/10.1111/j.1365-2486.2006.01288.x

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Eze, S., Palmer, S.M., Chapman, P.J., 2018. Soil organic carbon stock in grasslands: Effects of inorganic fertilizers, liming and grazing in different climate settings. J. Environ. Manage. 223, 74–84. https://doi.org/10.1016/j.jenvman.2018.06.013

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y.M., Chu, C., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G., Feng, X., Firn, J., Gruner, D.S., Hagenah, N., Hautier, Y., Heckman, R.W., Jin, V.L., Kirkman, K.P., Klein, J., Ladwig, L.M., Li, Q., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Risch, A.C., Schütz, M., Stevens, C.J., Wedin, D.A., Yang, L.H., 2015. Grassland productivity limited by multiple nutrients. Nat. Plants 1, 1–5. https://doi.org/10.1038/nplants.2015.80

Fedrigo, J.K., Ataide, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. Restor. Ecol. 26, 677–685. https://doi.org/10.1111/rec.12635

Ferreira, D.F., 2015. Sisvar. Versão 5.6. Lavras: UFLA/DEX. Available in: < http://www.dex.ufla.br/~danielff/programas/sisvar.html>. Accessed in: 1 jan. 2021.

Freitas, I.F., Ferreira Novais, R., Mercês de Albuquerque Villani, E., Vieira Novais, S., 2013. Phosphorus Extracted By Ion Exchange Resins and Mehlich-1 From Oxisols (Latosols) Treated With Different Phosphorus Rates and Sources for Varied Soil-Source Contact Periods. Rev. Bras. Ciência do Solo 37, 667–677. https://doi.org/http://dx.doi.org/10.1590/S0100-06832013000300013

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Aquino, J.E.R., 2008. Efeito da adubação fosfatada e da calagem sobre a qualidade bromatológica da forragem de pastagem natural com introdução de espécies forrageiras de inverno. Rev. Bras. Agrociência 14, 125–134.

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Brunetto, G., Saggin, A., Flores, J.P.C., 2000. Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. Pesqui. Agropecu. Bras. 35, 1663–1668. https://doi.org/10.1590/S0100-204X2000000800020

Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. Past: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 9.

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall 10, A.S., Seabloom, E.W., Williams, R., Bakker 12, J.D., Cadotte, M.W., Chaneton, J., Chu, C., Cleland, E.E., Antonio, C. D', Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., Mcculley, R.L., Moore, J.L., Morgan 25, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. Nat. Publ. Gr. 537, 28. https://doi.org/10.1038/nature19324

Heindel, R.C., Lyons, W.B., Welch, S.A., Spickard, A.M., Virginia, R.A., 2018. Biogeochemical weathering of soil apatite grains in the McMurdo Dry Valleys, Antarctica. Geoderma 320, 136–145. https://doi.org/10.1016/j.geoderma.2018.01.027

Kim, J., Li, W., Philips, B.L., Grey, C.P., 2011. Phosphate adsorption on the iron oxyhydroxides goethite (α-FeOOH), akaganeite (β-FeOOH), and lepidocrocite (γ-FeOOH): A31P NMR Study. Energy Environ. Sci. 4, 4298–4305. https://doi.org/10.1039/c1ee02093e

Kumar, V., Gilkes, R.J., Armitage, T.M., Bolland, M.D.A., 1994. Identification of residual P compounds in fertilized soils using density fractionation, X-ray diffraction, scanning and transmission electron microscopy. Fertil. Res. 37, 133–149. https://doi.org/10.1007/BF00748554

Lavorel, S., McIntyre, S., Landsberg, J., Forbes, T.D.A., 1997. Plant functional classifications: From general groups to specific groups based on response to disturbance. Trends Ecol. Evol. 12, 474–478. https://doi.org/10.1016/S0169-5347(97)01219-6

Lefires, H., Medini, H., Megriche, A., Mgaidi, A., 2014. Dissolution of Calcareous Phosphate Rock from Gafsa (Tunisia) Using Dilute Phosphoric Acid Solution. Int. J. Nonferrous Metall. 3, 1–7. https://doi.org/10.4236/ijnm.2014.31001

Marques, A.C.R., Piccin, R., Tiecher, T., Oliveira, L.B. De, Kaminski, J., Bellinaso, R.J.S., Krug, A.V., Gatiboni, L.C., Quadros, F.L.F. De, Carranca, C., Brunetto, G., 2019. Phosphorus fractionation in grasses with different resource-acquisition characteristics in natural grasslands of South America. J. Trop. Ecol. 35, 203–212. https://doi.org/10.1017/S0266467419000166

Mehlich, A., 1953. Determination of P, Ca, Mg, K, Na and NH4 by North Carolina Soil Testing Laboratoris. Raleigh Univ. North Carolina.

Moterle, D.F., Kaminski, J., dos Santos Rheinheimer, D., Caner, L., Bortoluzzi, E.C., 2016. Impact of potassium fertilization and potassium uptake by plants on soil clay mineral assemblage in South Brazil. Plant Soil 406, 157–172. https://doi.org/10.1007/s11104-016-2862-9

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. ACTA 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Oliveira, T.E. de, Freitas, D.S. de, Gianezini, M., Ruviaro, C.F., Zago, D., Mércio, T.Z., Dias, E.A., Lampert, V. do N., Barcellos, J.O.J., 2017. Agricultural land use change in the Brazilian Pampa Biome: The reduction of natural grasslands. Land use policy 63, 394–400. https://doi.org/10.1016/j.landusepol.2017.02.010

Oliveira, L.B., Marques, A.C.R., de Quadros, F.L.F., Farias, J.G., Piccin, R., Brunetto, G., Nicoloso, F.T., 2018. Phosphorus allocation and phosphatase activity in grasses with different growth rates. Oecologia 186, 633–643. https://doi.org/10.1007/s00442-018-4059-9

Oliveira, L.B., Soares, E.M., Jochims, F., Tiecher, T., Marques, A.R., Kuinchtner, B.C., Rheinheimer, D.S., De Quadros, F.L.F., 2015. Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil. Rangel. Ecol. Manag. 68, 445–452. https://doi.org/10.1016/j.rama.2015.07.012

Oliveira, L.B., Tiecher, T., de Quadros, F.L.F., Trindade, J.P.P., Gatiboni, L.C., Brunetto, G., dos Santos, D.R., 2014. Formas de fósforo no solo sob pastagens naturais submetidas à adição de fosfatos. Rev. Bras. Cienc. do Solo 38, 867–878. https://doi.org/10.1590/S0100-06832014000300018

Oliveira, L.B., Tiecher, T., Quadros, F.L.F., Santos, D.R., 2011. Fósforo microbiano em solos sob pastagem natural submetida a queima e pastejo. Rev. Bras. Cienc. do Solo 35, 1509–1515. https://doi.org/10.1590/S0100-06832011000500005

Pellegrini, L.G., Nabinger, C., Neumann, M., Carvalho, P.C. de F., Crancio, L.A., 2010. Produção de forragem e dinâmica de uma pastagem natural submetida a diferentes métodos de controle de espécies indesejáveis e à adubação. Rev. Bras. Zootec. 39, 2380–2388. https://doi.org/10.1590/S1516-35982010001100010

Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13, 131–144. https://doi.org/10.1016/0022-5193(66)90013-0

Pillar, V.D., 1997. Multivariate exploratory analysis and randomization testing with Multiv. Coenoses 12, 145–148.

Poeplau, C., Zopf, D., Greiner, B., Geerts, R., Korvaar, H., Thumm, U., Don, A., Heidkamp, A., Flessa, H., 2018. Why does mineral fertilization increase soil carbon stocks in temperate grasslands? Agric. Ecosyst. Environ. 265, 144–155. https://doi.org/10.1016/j.agee.2018.06.003

Prestes, N.E., Do Amarante, C.V.T., Pinto, C.E., Prestes, G., Zanini, G.D., De Medeiros-Neto, C., Sbrissia, A.F., 2016. Forage production in a natural grassland with limestone and phosphorus dosages. Semin. Agrar. 37, 3265–3275. https://doi.org/10.5433/1679-0359.2016v37n5p3265

Rheinheimer, D. dos S., Tiecher, T., Gonzatto, R., Santanna, M.A., Brunetto, G., da Silva, L.S., 2018. Long-term effect of surface and incorporated liming in the conversion of natural grassland to no-till system for grain production in a highly acidic sandy-loam Ultisol from South Brazilian Campos. Soil Tillage Res. 180, 222–231. https://doi.org/10.1016/j.still.2018.03.014

Rheinheimer, D.S., Santos, J.C.P., Kaminski, J., Mafran, A.L., 1997. Crescimento de leguminosas forrageiras afetado pela adição de fósforo, calagem do solo e micorrizas, em condições de casa de vegetação. Ciência Rural 27, 571–576. https://doi.org/10.1590/S0103-84781997000400008

Rizo, L.M., Moojen, E.L., Quadros, F.L.F. de, Côrrea, F.L., Fontoura Júnior, J.A., 2004. Desempenho de pastagem nativa e pastagem sobre-semeada com forrageiras hibernais com e sem glifosato. Ciência Rural 34, 1921–1926. https://doi.org/10.1590/S0103-84782004000600039

Robinson, J.S., Syers, J.K., 1990. A critical evaluation of the factors influencing the dissolution of Gafsa phosphate rock. J. Soil Sci. 41, 597–605.

Rodríguez, A.M., Jacobo, E.J., Scardoani, P., Deregibus, V.A., 2007. Effect of Phosphate Fertilization on Flooding Pampa Grasslands (Argentina). Rangel. Ecol. Manag. 60, 471–478. https://doi.org/10.2111/1551-5028(2007)60

Roy, E.D., Willig, E., Richards, P.D., Martinelli, L.A., Vazquez, F.F., Pegorini, L., Spera, S.A., Porder, S., 2017. Soil phosphorus sorption capacity after three decades of intensive fertilization in Mato Grosso, Brazil. Agric. Ecosyst. Environ. 249, 206–214. https://doi.org/10.1016/j.agee.2017.08.004

Sanaullah, M., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by 14C pulse labeling. Soil Biol. Biochem. 55, 132–139. https://doi.org/10.1016/j.soilbio.2012.06.004

Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus uptake by plants : From soil to cell. Mol. Gen. Genet. 116, 447–453. https://doi.org/10.1104/pp.116.2.447

Schellberg, Moseler, Kuhbauch, Rademacher, 1999. Long-term effects of fertilizer on soil nutrient concentration, yield, forage quality and floristic composition of a hay meadow in the Eifel mountains, Germany. Grass Forage Sci. 54, 195–207. https://doi.org/10.1046/j.1365-2494.1999.00166.x

Soares, A.B., Carvalho, P.C. de F., Nabinger, C., Semmelmann, C., Trindade, J.K. da, Guerra, E., Freitas, T.S. de, Pinto, C.E., Fontoura Júnior, J.A., Frizzo, A., 2005. Produção animal e de forragem em pastagem nativa submetida a distintas ofertas de forragem. Ciência Rural 35, 1148–1154. https://doi.org/10.1590/S0103-84782005000500025

Soltangheisi, A., Rodrigues, M., Coelho, M.J.A., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2018. Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res. 179, 20–28. https://doi.org/10.1016/j.still.2018.01.006

Szilas, C., Semoka, J.M.R., Borggaard, O.K., 2007. Can local Minjingu phosphate rock replace superphosphate on acid soils in Tanzania? Nutr. Cycl. Agroecosystems 77, 257–268. https://doi.org/10.1007/s10705-006-9064-4

Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. Grass Forage Sci. 69, 567–579. https://doi.org/10.1111/gfs.12079

Tothill, J.C., Hargreaves, J.N.G., Jones, R.M., McDonald, C.K., 1978. Botanal – a comprehensive sampling and computing procedure for estimating pasture yield and composition. 1. Field sampling. CSIRO-Tropical Agron. Tech. Memo. 21.

Turner, B.L., Wells, A., Andersen, K.M., Condron, L.M., 2018. Consequences of the physical nature of the parent material for pedogenesis, nutrient availability, and succession in temperate rainforests. Plant Soil 423, 533–548. https://doi.org/10.1007/s11104-017-3514-4

Vargas, J.P.R., dos Santos, D.R., Bastos, M.C., Schaefer, G., Parisi, P.B., 2019. Application forms and types of soil acidity corrective: Changes in depth chemical attributes in long term period experiment. Soil Tillage Res. 185, 47–60. https://doi.org/10.1016/j.still.2018.08.014

Wang, D., Lv, S., Jiang, P., Li, Y., 2017. Roles, Regulation, and Agricultural Application of Plant Phosphate Transporters. Front. Plant Sci. 8, 1–14. https://doi.org/10.3389/fpls.2017.00817

Zhao, Y., Yang, B., Li, M., Xiao, R., Rao, K., Wang, J., Zhang, T., Guo, J., 2019. Community composition, structure and productivity in response to nitrogen and phosphorus additions in a temperate meadow. Sci. Total Environ. 654, 863–871. https://doi.org/10.1016/j.scitotenv.2018.11.155

Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., Zhou, H., Hosseinibai, S., 2017. Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. Glob. Chang. Biol. 23, 1167–1179. https://doi.org/10.1111/gcb.13431

5.10. General comments

In chapter II was analyzed a historic dataset of dry matter production using different rates of phosphates fertilizers with or without limestone in Southern Brazil Pampa grasslands. As well as the soil fertility in a topsoil layer of 0-10 cm and changes in botanical composition due to the use of P fertilizers and limestone. With this analysis we found that Pampa grasslands responds better, in terms of dry matter production, with the use of soluble phosphate fertilizers (such as triple and simple superphosphate) plus limestone. This practice instigated small changes in species richness, although there has been an increase in the participation of leguminous species in the production of total dry matter. In addition, we see the need for periodic phosphate fertilization to maintain high levels of dry matter productivity in the natural grassland.

Periodic phosphate fertilization aims to maintain satisfactory levels of available P in the soil, especially in the layer with the greatest presence of roots. In the native field, it is estimated that this layer is at least 0-10 cm due to the predominance of grass species in the plant community. Furthermore, since they are mostly acidic soils, with the presence of toxic aluminum in the profile and with low levels of Ca and Mg, there is less vertical development of the root system of the vegetation.

In this case, the correction of soil fertility and acidity should be carried out in every explored layer. In the case of the natural grassland, this practice is not feasible since it requires soil plowing to incorporate the lime fertilizer. Therefore, there are two main possibilities for soil correction. The first one refers to the incorporation in line, with the use of a distributor equipped with discs. The second one refers to the simple deposition of fertilizers and limestone on the soil surface. The latter, being the practice employed in the analyzed experiment.

Due to the superficial application, there was a need to conduct a study to evaluate the depth effect, especially in the 0-10 cm topsoil layer, of the use of the phosphate fertilizers and limestone. In this study, we hypothesized that long-term surface application of phosphorus fertilizers and limestone in the analyzed soil allow for an increase in the phosphorus availability and aluminum neutralization in deeper soil layer and, therefore, there is a better vegetation response.

To test our hypothesis, in the next chapter will be made a quantification of the main macronutrients and the soil acidity in layers of 2.5 cm to a depth of 10 cm topsoil profile. As well as, a quantification of the nutrient’s exportation via natural grassland dry matter.

6. Chapter III - Chemical pattern of vegetation and topsoil of rangeland fertilized over 21 years with phosphorus sources and limestone

André Somavilla; Laurent Caner; Isley Cristiellem Bicalho da Silva; Marília Camotti Bastos; Letícia Moro; Gilmar Luiz Schaefer; Luciano Colpo Gatiboni; Danilo Rheinheimer dos Santos

Artigo publicado pela revista: **Soil & Tillage Research**
DOI: 10.1016/j.still.2020.104759

Enviado em 27 de março de 2020.
Aceito para publicação em 30 de julho de 2020.

Abstract

The Pampa's rangeland in South America has been underused or replaced by annual crops or forest cultivation with higher economic returns. However, fertilization and liming are suitable strategies to improve rangeland dry matter production and avoid the replacement of these natural areas. Nevertheless, it is necessary to analyze and understand the long-term consequences of the use of different fertilizers on soil chemical characteristics and the response of endemic vegetation. Thus, our goal was to test whether, in rangeland, the long-term surface application of phosphorus fertilizers and limestone allow for an increase in phosphorus availability and aluminum neutralization in deep soil layer and increase in rangeland productivity. For this, a 21-year, long-term field trial with limestone and phosphorus fertilizers was used. The treatments were different phosphorus sources (triple superphosphate, single superphosphate, and phosphate rock), applied in three rates over time (118, 205, and 249 kg ha^{-1} of P until the years of 1998, 2010, and 2012, respectively), combined or not with limestone. The dry matter (between 2016 and 2019) and nutrient accumulation in plant shoot were measured. The topsoil was divided in four layers (0–2.5, 2.5–5.0, 5.0–7.5, and 7.5–10.0 cm), and chemical properties were determined. The use of phosphate rock in soil surface provided a deepening of phosphorus content at 7.5 cm after 21 years in a rate of 249 kg ha^{-1} of P. After 21 years of liming, the reacidification was not significant and the neutralizing front reached depths greater than 10 cm. In these conditions, the contents of exchangeable Ca, Mg and Al and pH are greater than, and Al saturation is lower than without limestone. The effect of fertilization and limestone amendments are however limited in time and there was no response of rangeland dry matter production after more than four years after the last fertilization.

Keywords: Legacy—P, Dry matter production of Pampa Grassland, Neutralizing front

6.1.Introduction

The Pampa ecosystem of South America is an ecological region consisting of grasslands and rangelands, covering 500,000 km2 in Argentina, Brazil, Uruguay, and Paraguay (Pallarés et al., 2005). The Pampa ecosystem is composed of more than 3,000 plant species, of which approximately 450 have economic interests (Boldrini, 2009). The great potential of the Pampa natural vegetation leads it to be used as the main source of animal food in these regions (rangeland) (Carvalho et al., 2006). In Brazil, the Pampa rangelands are the alimentary base for almost 13 million head of cattle (IBGE, 2016) which are predominantly managed in the extensive system. To this point, maintaining or increasing forage productivity is extremely important to sustaining agricultural production systems and the biodiversity of the ecosystem.

Nevertheless, historically, landowners in Pampa have not made investments to improve pasture productivity. Since the end of the 18th century, grasslands have been used to produce beef on large farms without the application of any agricultural inputs; hence, nowadays, these areas are cultivated in an inadequate way, resulting in overgrazing, low productivity, and consequently, low economic returns (Borges et al., 2016; Carvalho and Batello, 2009; Fedrigo et al., 2018).

In the last 50 years, problems related to the chemical quality of the soils were responsible for a decrease in 26% of the native vegetation area (Borges et al., 2016; Oliveira et al., 2017; Ruviaro et al., 2016). Therefore, farmers ended up leaving pasture production and gave place to annual crops (e.g., soybean and corn) and cultivated forests (pine and eucalyptus) with better financial return (Oliveira et al., 2017). Thus, to avoid the conversion of native areas to other production systems, it is necessary to increase the economic efficiency of cattle production in order to make the activity economically competitive (Borges et al., 2016).

The improvement of the economic efficiency of cattle production in rangelands implies an increase in dry matter production. Well managed rangelands of Pampa can reach a dry matter production around 14 Mg ha^{-1} year^{-1} (Oliveira et al., 2015); in contrast, in areas with inadequate management, the productions achieve averages between 3.7 Mg ha^{-1} year^{-1} (Soares et al., 2005) and 9.8 Mg ha^{-1} year^{-1} (Pellegrini et al., 2010). The principal causes of low productivity is high soil acidity (pH ~ 4.4) and low fertility, such as reduced available P (2.6 to 7.6 mg of P kg^{-1}), especially in soils of Southern Brazil (Oliveira et al., 2011; Rheinheimer et al., 1997; Tiecher et al., 2014). In the scenario of low fertility, an increase of nutrients availability by limestone and phosphorus fertilizers are important strategies to support plant development (Gatiboni et al., 2000; Prestes et al., 2017, 2016).

The dolomite rock (magnesian limestone) is abundant and is almost the unique material used to neutralize soil potential acidity in Southern Brazil. The very low solubility of dolomite demands its incorporation into the soil profile. Phosphorus fertilizers should also be incorporated into the soil because of very low phosphorus mobility. However, in grasslands, liming and fertilization are carried out by deposition on the soil surface. Nevertheless, the fertilization in soil surface can restrict the improvement of soil fertility to topsoil layers and, consequently, rangeland productivity.

Surface application limits the neutralization of aluminum and the increase of available soil P to the topsoil layer (Nunes et al., 2011; Rodrighero et al., 2015; Soltangheisi et al., 2018), which may lead to a below-expected response of vegetation to soil fertility increase. Nevertheless, long term improvement of soil chemical characteristics can be also expected in-depth, as in agricultural areas, due to laminar water flow through a porous medium and turbulent water flow in macropores (Czachor, 2011; Rheinheimer et al., 2018; Vargas et al., 2019).

Finally, monitoring the soil chemical changes in the soil profile and the impact in dry matter production in a long-term experiment with limestone and phosphorus fertilizer application in the soil surface is crucial to showing a possible preservation of Pampa vegetation and make the farm economically viable. The objective of this study was to test whether long-term surface application of phosphorus fertilizers and limestone in an rangeland allow for an increase in the phosphorus availability and aluminum neutralization in deep soil layer and increase in the rangeland dry matter production.

6.2.Material and Methods

Local and soil properties

The field trial was established in Rio Grande do Sul state in Southern Brazil (29°43'05.7"S 53°42'33.7"W) on a soil classified as Ultisol. The regional climate was humid subtropical, type Cfa according to Köppen classification, with an average annual precipitation of 1,769 mm year-1 distributed over all months. The average monthly temperature of the hottest months (December–February) is 24.2ºC and the coldest months (June–August) is 14.5ºC. In 1997 the chemical properties of the soil were characterized at the layer of 0–20 cm and are shown in Table 1.

Table 1—Topsoil layer of 0-20 cm characterization of the field trial establishment in 1997.

Factor	**Values**
pH[*]	4.5
Phosphorus (mg kg^{-1}) [**]	2.5
Potassium (mg kg^{-1}) [**]	60
Calcium (cmolc kg^{-1}) [***]	1.17
Magnesium ($cmol_c$ kg^{-1}) [***]	0.75
Aluminum ($cmol_c$ kg^{-1}) [***]	1.3
Carbone (g kg^{-1})	10.4
Clay (g Kg^{-1})	170

[*]water pH (1:1 v/v); [**]Extracted by Mehlich-1; [***]extracted by 1 mol L^{-1} KCl.

Field trial treatments

The field trial is a two-factorial experiment with randomized complete block design in a split-plot and three replications. The main factor is P sources, applied in a plot of 56 m^2, and the secondary factor is three total rates of phosphorus, applied in a split-plot of 18.5 m^2. Briefly, the beginning of field trial was in 1997 with different P sources application, magnesian limestone, and the over-seeding of *Lolium multiflorum* cv. “Comum” and *Trifolium vesiculosum* cv. “Yuchi”. The main plots correspond to triple superphosphate + limestone + over-seeding (TPCa); triple superphosphate + over-seeding (TP); single superphosphate + limestone + over-seeding (SPCa); phosphate rock of Gafsa + over-seeding (RP); without P but with over-seeding (OS), and control without P neither over-seeding (Control). The split-plot consist in the total application of 118, 205, and 249 kg ha^{-1} of P, with the last P fertilization occurring in 1998, 2010, and 2012, respectively. The general fertilization scheme is presented in Figure 1. The liming was carried out only in 1997 at the rate of 3.2 Mg ha^{-1} on the soil surface (the amount necessary to achieve the pH 5.5).

All treatments, except the Control, were fertilized with potassium (108 kg ha^{-1} of K) and nitrogen (70 kg ha^{-1} of N in urea form) superficially. The over-seeding of *L. multiflorum* and *T. vesiculosum* were carried out in line onto the surface of grassland in the winter of 1997 and 2002 with 30 and 12 kg ha^{-1} of seeds, respectively. During the 21 years of the experiment, the rangeland was periodically harvested. Because it is a natural biome, no pesticide has ever been used. There is no need to control invasive plants. Botanical modifications resulting from the use of natural pasture are being periodically evaluated. Field trial and managements detail can be found in Gatiboni et al. (2000), Gatiboni et al. (2008), Tiecher et al. (2014) and Oliveira et al. (2015).

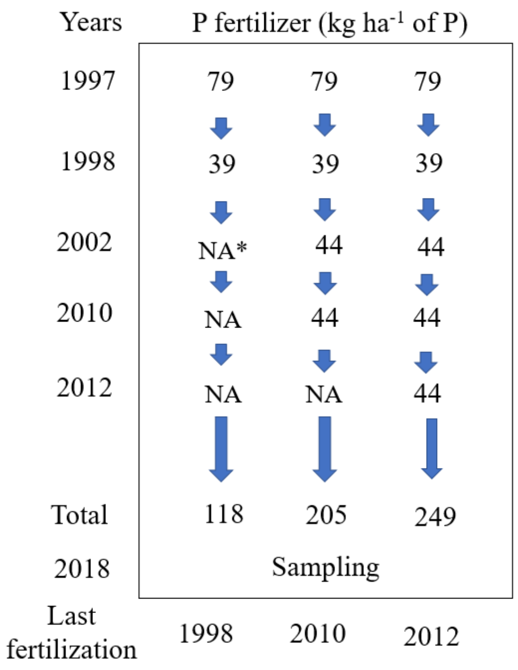


Figure 1—Rates and years of phosphorus fertilization. *Not phosphorus fertilizer application.

2.3. Soil sampling and analyses

Soil sampling was performed at layers of 0–2.5, 2.5–5.0, 5.0–7.5 and 7.5–10 cm in October 2018. The soil was oven dried at 65°C and passed through a 2 mm mesh sieve for posterior chemical characterization. The soil pH H_2O was measured with a 1:1 soil:solution ratio. The potential acidity (H + Al, $cmol_c$ kg^{-1}) was calculated according to SBCS (2016). The available soil P was quantified using acid extraction with Mehlich-1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1953). The available soil P content was also quantified by extraction with anion exchange resin - AER (AR 103 QDP 434 Ionics Inc. plates). Briefly, the AER membrane of 2 cm2 was saturated with 0.5 mol L^{-1} $NaHCO_3$ and washed with osmosed H_2O. The membrane was then maintained in contact with 0.5 g of soil + 10 ml of osmosed H_2O for 16 h in an end-over-end agitator at 25° C. The membrane was collected, rinsed to remove the adhering soil particles, and then eluted with 10 ml of 0.5 mol L^{-1} HCl. The P content from Mehlich-1 and AER extractions were both determined by colorimetry (Murphy & Riley 1962). The concentrations of exchangeable Ca, Mg and Al in the soil were quantified after extraction with 1 mol L^{-1} KCl solution (Tedesco et al., 1995). The Ca and Mg contents were quantified by atomic absorption spectrophotometry and Al content was quantified by titration with 0.0125 mol L^{-1} NaOH. The effective cations exchange capacity (CEC_{ef}, cmolc kg^{-1}), Al saturation (Al_{sat}, %), and base saturation (V, %) were calculated.

2.4. Dry matter production and chemical characterization

Between 2016 and 2019, the annual dry matter production was quantified by regular cutting of vegetation aboveground in an area of 0.25 m^2. The vegetation samples were oven dried at 60°C for 48 h. Soon after each vegetation sampling for dry matter quantification, all field area was mowed, and the plant material was removed from the site. The analysis of rangeland productivity was performed by the annual sum of the dry matter sampled in each plot. The values of annual dry matter production were normalized by the treatment without application of P as $RDm = (\Sigma Dmx / \Sigma Dm_{OS})*100$, with RDm (%) being the relative dry matter production; ΣDmx ($kg\ ha^{-1}$) the annual sum of dry matter of treatment x, and ΣDm_{OS} ($kg\ ha^{-1}$) the annual sum of dry matter of treatment OS.

The chemical composition of plant shoot was determined in the dry matter sampled in March 2019, after a time growth of 150 days. P, Ca, and Mg contents in plant shoot were quantified after nitric-perchloric digestion (Embrapa, 2009). The Ca and Mg were quantified by atomic absorption spectrophotometer and P by colorimetry (Murphy and Riley, 1962). Carbon (C) and nitrogen (N) were quantified by elemental analysis (Flash EA, Thermo Electron Corporation, Bremen, Germany).

2.5. Statistical analysis

An exploratory principal component analysis (PCA) was performed to identify the variables with the highest variance explanation and to guide other analysis. For PCA analysis, the data were transformed into a standardized normal distribution. To perform the PCA, R software, version 3.5.0, was utilized.

The analysis of variance of vegetation variables were performed as a two-factorial experiment with randomized complete block design in a split-plot. The main factor (plot) corresponds to the P sources and the secondary factor (split-plot) corresponds to the rate of phosphorus applied.

Differently of vegetation variables, the analysis of variance of soil variables were carried out as a three-factorial experiment with complete randomized block design in a split-split plot. The main and secondary factors also correspond to the P sources and the rate of phosphorus applied. The third factor (split-split plot) correspond to the topsoil layers.

When the effect of treatments and/or rate of phosphorus applied was significant by F test ($p \leq 0.05$), the differences between means were tested by Tukey or orthogonal contrast tests (p≤0.05). When the soil layer effect was significant by F test (p≤0.05), the means were submitted to regression analysis.

6.3.Results

Exploratory analysis

By exploratory PCA analysis (Figure 2), treatments were grouped mainly by the use of limestone and later by the use of P sources. The variance explained in the first two axes was 68.8%. The soil pH_{H2O}, Ca, Al, P-AER, and P-Mehlich-1 contents and V and Al_{sat} were the variables with the highest contribution to variance explanation. The treatments were separated mainly by PC1, which explain 51.4% of the total variance. The RP and TP were differentiated from the others treatments (OS, Control, SPCa, and TPCa) (PC2, 17.4%) due to the soil and plant variables related to P (Figure 2).

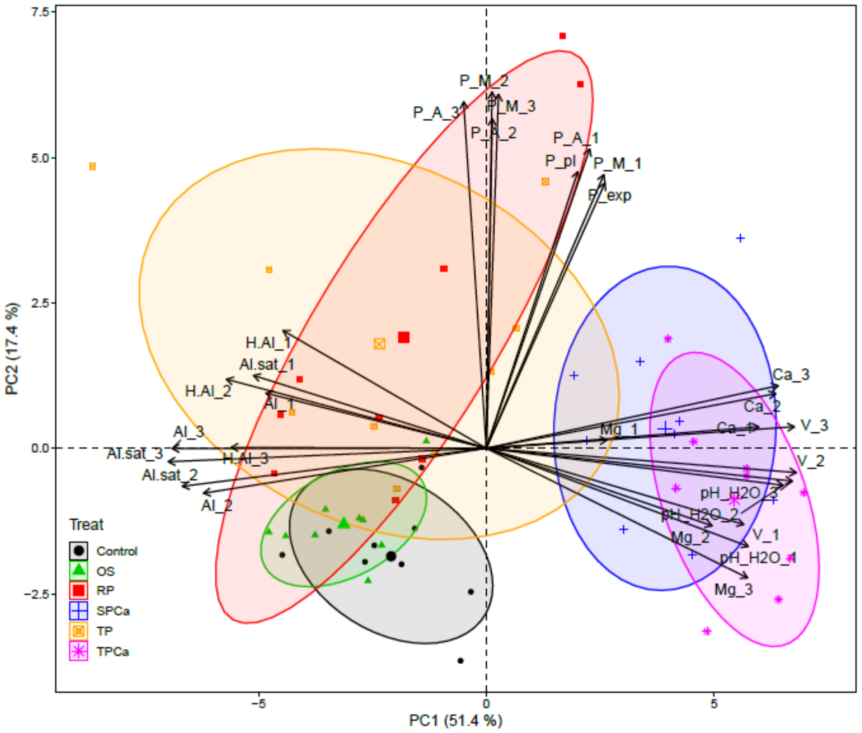


Figure 2—Exploratory principal component analysis of soil and plant dataset for the different P sources. Treatments: Control—control without P neither over-seeding; RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. Variables: P_M and P_A refer to P-Mehlich-1 and P-AER, respectively; Al.sat—aluminum saturation; H.Al—potential acidity; V—base saturation; The index 1, 2, and 3 refer to chemical characteristic in the layers of 0–2.5, 2.5–5.0, and 5.0–7.5 cm, respectively; P_exp—phosphorus exportation.

Soil chemical characteristics

Limestone addition

The soils with limestone addition (TPCa and SPCa) have the highest values of pH_{H2O}, exchangeable Ca and Mg, V, and CEC_{ef} and lower contents of exchangeable Al and Al_{sat} in the layers 2.5–5.0, 5.0–7.5, and 7.5–10 cm (Table 2 and Figure 3). The use of 3.2 Mg ha^{-1} of magnesian limestone in the soil surface was sufficient to deepen the neutralization front, increase the pH_{H2O} and V, and reduce exchangeable Al and Al_{sat} (Figure 3). For all topsoil layers, the difference between the groups with (TPCa and SPCa) and without (Control, OS, RP, and TP) limestone application was significant by the method of orthogonal contrasts (Figure 3).

The exchangeable Ca content in the RP treatment was similar to the levels of the treatments without limestone in all evaluated layers (Table 2), conversely to the expected result due to the RP dissolution on soil over the years.

Table 2—Depth chemical characterization of soil cultivated with rangeland after 21 years of phosphorus fertilization and liming.

Depth (cm)	Control[1]		OS		RP		SPCa		TPCa		TP		LSD[2]
	---------- pH_{H2O} ----------												
1.25	5.21	b[3]	5.03	b	5.05	b	5.53	a	5.6	a	5.03	b	0.29
3.75	4.77	b	4.77	b	4.7	b	5.25	a	5.48	a	4.83	b	
6.25	4.56	b	4.52	b	4.54	b	5.15	a	5.41	a	4.6	b	
8.75	4.48	b	4.44	b	4.52	b	5.09	a	5.36	a	4.5	b	
	---------- Al ($cmol_c$ kg^{-1}) ----------												
1.25	0.2	a	0.37	a	0.23	a	0.1	a	0.09	a	0.3	a	0.35
3.75	0.75	a	0.75	a	0.69	a	0.21	b	0.11	b	0.64	a	
6.25	1.07	a	1.1	a	1.01	a	0.3	b	0.16	b	0.98	a	
8.75	1.21	a	1.24	a	1.16	a	0.33	b	0.16	b	1.16	a	
	---------- Ca ($cmol_c$ kg^{-1}) ----------												
1.25	1.89	bc	1.52	c	2.03	bc	2.29	ab	2.81	a	1.63	c	0.52
3.75	0.81	b	0.82	b	0.96	b	1.55	a	1.91	a	0.94	b	
6.25	0.54	b	0.53	b	0.66	b	1.3	a	1.59	a	0.66	b	
8.75	0.42	b	0.39	b	0.5	b	1.14	a	1.4	a	0.5	b	
	---------- Mg ($cmol_c$ kg^{-1}) ----------												
1.25	1.44	abc	1.33	bc	1.6	ab	1.77	a	1.48	abc	1.2	c	0.37
3.75	0.52	b	0.51	b	0.48	b	0.95	a	0.95	a	0.46	b	
6.25	0.42	a	0.36	a	0.33	a	0.54	a	0.52	a	0.33	a	
8.75	0.34	a	0.27	a	0.24	a	0.49	a	0.45	a	0.26	a	
	---------- H+Al[3] ($cmol_c$ kg^{-1}) ----------												
1.25	3.19	ab	3.57	a	3.02	ab	2.48	b	2.25	b	3.76	a	1.04
3.75	3.91	ab	4	ab	4.28	a	3	bc	2.7	c	4.36	a	
6.25	4.53	a	4.33	a	4.55	a	3.07	b	2.77	b	4.2	a	
8.75	4.57	a	4.62	a	4.66	a	3.13	b	2.67	b	4.76	a	
	---------- CEC_{ef} ($cmol_c$ kg^{-1}) ----------												
1.25	3.94	bc	3.55	c	4.23	ab	4.48	ab	4.76	a	3.42	c	0.61
3.75	2.26	c	2.24	c	2.29	bc	2.88	ab	3.11	a	2.17	c	
6.25	2.15	a	2.08	a	2.1	a	2.24	a	2.37	a	2.08	a	
8.75	2.06	a	1.98	a	1.99	a	2.03	a	2.09	a	2	a	
	---------- Al_{sat} (%) ----------												
1.25	5.15	a	10.04	a	5.8	a	2.273	a	1.72	a	9.697	a	13.00
3.75	32.83	a	33.21	a	30.11	a	8.182	b	3.586	b	29.48	a	
6.25	49.4	a	53.04	a	48.63	a	13.69	b	6.485	b	46.42	a	
8.75	58.82	a	62.9	a	58.8	a	16.2	b	7.44	b	57.82	a	
	---------- V (%) ----------												
1.25	54.7	cd	47.4	ef	56.7	bc	64.0	ab	67.4	a	45.8	f	8.41
3.75	28.3	b	27.6	b	27.5	b	47.1	a	52.7	a	26.7	b	
6.25	19.6	b	19.0	b	19.9	b	39.1	a	44.7	a	21.7	b	
8.75	15.8	b	13.8	b	15.1	b	35.6	a	42.3	a	16.2	b	

[1] RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. [2] LSD—Least Significant Difference ($p \leq 0.05$). [3] Means followed by the same letter, in a row, did not differ statistically by Tukey test ($p \leq 0.05$). [3] H+Al—potential acidity; CEC_{ef}—effective cations exchange capacity; Al_{sat}—aluminum saturation; V—base saturation.

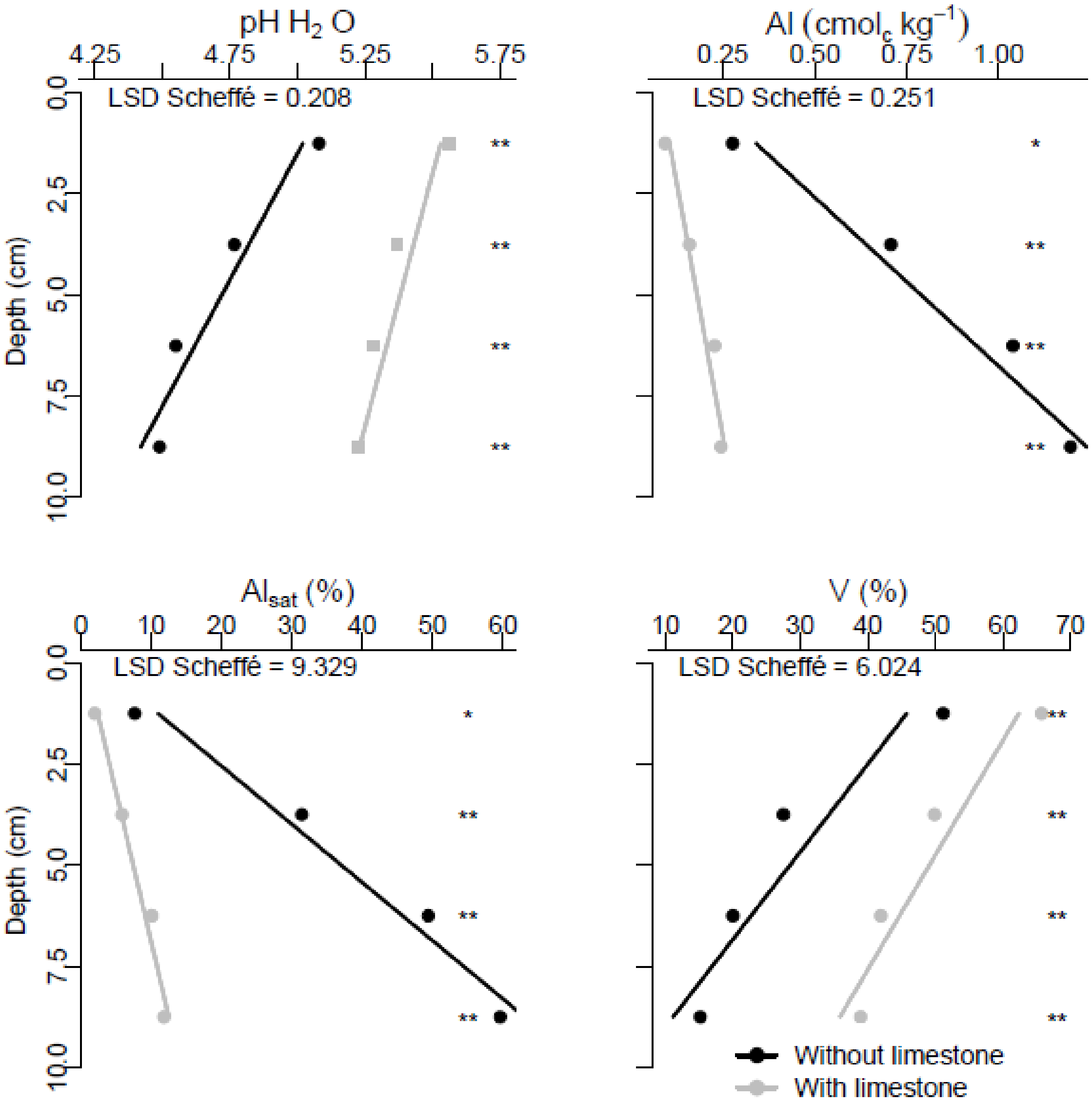


Figure 3—Chemical characteristics of soil due to the use of limestone 21 years before. Alsat—Aluminum saturation and V—Base saturation. *significant by orthogonal contrast test (p≤0.05). **significant by orthogonal contrast test (p≤0.01).

Phosphorus addition

For all treatments with P fertilization, available P contents in the 0–2.5 cm layer were higher in soils fertilized with 249 kg ha^{-1} of P (last fertilization in 2012) than with the other rates (Table 3). In this layer, the highest value of P was obtained in the treatment RP (42.1 and 51.8 mg kg^{-1} of P extracted by AER and Mehlich-1, respectively). For the other two rates, in the 0–2.5 cm layer, soils fertilized with TPCa and TP had greater available soil P contents than the others treatments (Table 3). In these cases, TPCa (118 kg ha^{-1} of P) had 28.1 and 40.6 mg kg^{-1} of P extracted by AER and Mehlich-1, respectively, and TP (205 kg ha^{-1} of P) had 33.1 and 41 mg kg^{-1} of P extracted by AER and Mehlich-1, respectively.

In deeper layers, although there was a decrease of the P-AER and P-Mehlich-1 contents (Figure 4), the rate of 249 kg ha^{-1} of P provided by RP resulted in the highest values of P-AER in the layers up to 7.5 cm (Table 3). For P-Mehlich-1, there was no significant difference between the P sources (Table 3), but the RP always had the highest absolute values.

The available P-Mehlich-1 values recommended for forage production in rangeland are above 15 mg kg^{-1} in the 0–10 cm soil layer (SBCS, 2016). However, when topsoil layers were analyzed for rates of 118 and 205 kg ha^{-1} of P, the recommended value of 15 mg kg^{-1} was generally attained only up to the depth of 4.5 cm. Nonetheless, at a rate of 249 kg ha^{-1} of P, this value was attained up to 10 and 7.4 cm in RP and TP, respectively, and up to 4.9 cm in the SPCa and TPCa treatments.

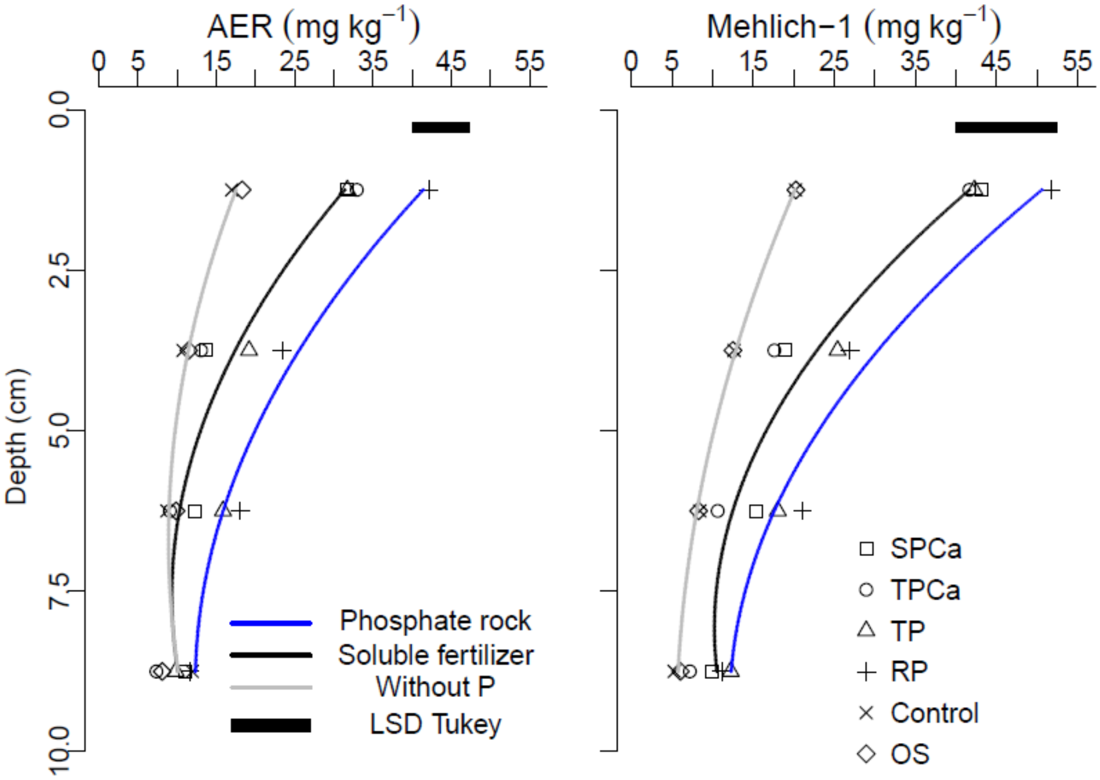


Figure 4—Phosphorus available contents by anion exchange resin (AER) and Mehlich-1 in soil cultivated with rangeland over 21 years and fertilized with 249 kg ha^{-1} of P. RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. LSD Tukey—Least significant difference by Tukey test ($p \leq 0.05$).

Table 3—Soil phosphorus contents by anion exchange resin (AER) and Mehlich-1 in soil cultivated with rangeland after 21 years of phosphorus fertilization and liming.

Depth	**Control**[1]						**RP**						**SPCa**					
(cm)	**118**[2] (1998)		**205** (2010)		**249** (2012)		**118** (1998)		**205** (2010)		**249** (2012)		**118** (1998)		**205** (2010)		**249** (2012)	
P- Anion Exchange Resin - AER																		
0-2.5	21.53	Ab[3]	21.56	Abc	16.95	Ac	24.33	Bab	27.18	Bab	42.1	Aa	21.94	Bab	23.87	Bb	31.63	Ab
2.5-5.0	11.97	Aa	12.06	Aa	10.73	Ac	16.23	Ba	16.05	Ba	23.4	Aa	15.67	Aa	16.36	Aa	13.72	Abc
5.0-7.5	10.03	Aa	8.62	Aa	8.68	Ab	11.53	Ba	10.61	Ba	18.02	Aa	8.31	Aa	12.99	Aa	12.21	Ab
7.5-10.0	8.29	Aa	6.37	Aa	11.92	Aa	8.73	Aa	7	Aa	11.64	Aa	5.28	Aa	8.58	Aa	10.98	Aa
P - Mehlich-1																		
0-2.5	29.23	Aab	26.03	Ab	20.24	Ab	31.95	Bab	33.54	Bab	51.76	Aa	36.22	Aba	32.34	Bab	43.24	Aa
2.5-5.0	14.29	Aa	12.17	Aa	12.67	Ab	14.56	Ba	16.48	Ba	26.94	Aa	17.83	Aa	16.81	Aa	19.03	Aab
5.0-7.5	9.39	Aa	6.45	Aa	8.43	Aab	8.68	Ba	11.19	ABa	21.1	Aa	13.14	Aa	10.31	Aa	15.34	Aab
7.5-10.0	6.15	Aa	4.49	Aa	5.29	Aa	9.32	Aa	6.43	Aa	11.18	Aa	4.94	Aa	5.75	Aa	10.01	Aa

Depth	**OS**						**TPCa**						**TP**					
(cm)	**118** (1998)		**205** (2010)		**249** (2012)		**118** (1998)		**205** (2010)		**249** (2012)		**118** (1998)		**205** (2010)		**249** (2012)	
P- Anion Exchange Resin - AER																		
0-2.5	20.22	Ab	16.03	Ac	18.27	Ac	28.08	ABa	25.23	Bb	32.92	Ab	20.47	Bb	33.09	Aa	31.71	Ab
2.5-5.0	15.09	Aa	11.84	Aa	11.46	Ac	14.03	Aa	11.37	Aa	13.03	Abc	15.63	Aa	15.06	Aa	19.17	Aab
5.0-7.5	9.6	Aa	8.48	Aa	9.84	Ab	9.43	Aa	7.64	Aa	9.06	Ab	10.85	Aa	11.81	Aa	15.84	Aab
7.5-10.0	6.93	Aa	5.98	Aa	8.08	Aa	7.2	Aa	5.64	Aa	7.33	Aa	6.61	Aa	10.37	Aa	9.81	Aa
P - Mehlich-1																		
0-2.5	26.41	Ab	23.65	Ab	20.29	Ab	40.65	Aa	33.5	Aab	41.67	Aa	28.94	Bab	41.02	Aa	42.29	Aa
2.5-5.0	14.48	Aa	13.9	Aa	12.53	Ab	11.48	Aa	12.98	Aa	17.63	Aab	15.46	Aa	20.45	Aa	25.42	Aa
5.0-7.5	7.99	Aa	7.24	Aa	8.28	Ab	9.41	Aa	6.94	Aa	10.65	Aab	9.96	Aa	12.29	Aa	18.06	Aab
7.5-10.0	4.07	Aa	5.57	Aa	6.06	Aa	5.03	Aa	5.55	Aa	7.22	Aa	5.76	Aa	8.86	Aa	12.25	Aa

[1] RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. [2] Kg ha^{-1} of P. [3] Means followed by the same letter, upper case letter between rate in the same treatment and lower-case letter between treatments, did not differ statistically by Tukey test ($p \leq 0.05$).

Dry matter production and plant shoot nutrient concentration

The RDm of rangeland developed under soils fertilized with different P sources was not significantly different for the four years of sampling (2016–2019) (Table 4). Differences in plant shoot P content and in P exportation by dry matter occurred only for the treatment fertilized with 249 kg ha^{-1} of P (2012). The RP and SPCa treatments had the highest levels of plant shoot P content (1.63 and 1.73 g kg^{-1}, respectively) and SPCa had the highest P exportation (7.8 kg ha^{-1}), although not statistically different from the TPCa and TP treatments (Table 5). Differences between the concentrations of C, N, K, Ca, and Mg in plant shoot were not significant ($p \leq 0.05$) and had an overall mean of 418, 10.9, 6.3, 5.8, and 4.5 g kg^{-1}, respectively (data not shown).

Table 4—Analysis of variance of relative dry matter production between 2016 and 2019.

	2016	**2017**	**2018**	**2019**
		--------------	% -----------------	
Treatments				
Control[1]	91	86	91	84
OS	100	100	100	100
RP	105	94	99	113
SPCa	117	105	114	127
TPCa	125	108	113	122
TP	123	101	110	116
Rate of phosphorus applied				
118	111	98	104	110
205	108	99	104	109
249	112	101	105	112
Effect				
Treat[2]	ns	ns	ns	ns
Rate[3]	ns	ns	ns	ns
Treat*Rate	ns	ns	ns	ns
CV1(%)	41.6	32.3	23.0	31.3
CV2(%)	13.9	16.0	9.6	21.1

[1] RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. [2] Treat—main plot (phosphorus sources); [3] Rate—split-plot (Rate of phosphorus applied). *significant at $p \leq 0.05$; ns—not significant at $p \leq 0.05$.

Table 5—Phosphorus contents in plant shoot and P exportation by dry matter of one cut carried out in mar/2019 after growth time of 150 days.

Treat	**118 kg ha^{-1} of P** (1998)		**205 kg ha^{-1} of P** (2010)		**249 kg ha^{-1} of P** (2012)	
	P shoot (g kg^{-1})	**P export** (kg ha^{-1})	**P shoot** (g kg^{-1})	**P export** (kg ha^{-1})	**P shoot** (g kg^{-1})	**P export** (kg ha^{-1})
Control[1]	0.89 Aa	2.4 Aa	0.80 Aa	2.3 Aa	0.89 Ab	2.3 Ab
OS	0.88 Aa	3.5 Aa	1.00 Aa	3.0 Aa	0.84 Ab	2.5 Ab
RP	0.87 Ba	3.0 Ba	0.99 Ba	4.0 ABa	1.63 Aa	5.6 Aab
SPCa	0.84 Ba	3.3 Ba	1.00 Ba	3.8 Ba	1.73 Aa	7.8 Aa
TPCa	0.77 Ba	3.1 Ba	1.18 ABa	4.2 ABa	1.26 Aab	5.4 Aab
TP	1.03 Aa	4.2 Aa	1.11 Aa	3.9 Aa	1.27 Aab	4.9 Aab

[1]RP—Gafsa phosphate rock + over-seeding; SPCa—single superphosphate + limestone + over-seeding; OS—without P but with over-seeding; TPCa—triple superphosphate + limestone + over-seeding; TP—triple superphosphate + over-seeding. [2]Means followed by the same letter, upper case letter in a line and lower-case letter in a column, did not differ statistically by Tukey test ($p \leq 0.05$).

6.4.Discussion

Soil properties

Limestone addition

The higher pH_{H2O} values and lower exchangeable Al and Al_{sat} observed in the 0–2.5 cm topsoil layer (Figure 3) are explained by the higher content of organic matter and root exudation in the soil superficial layers (Chen et al., 2017; Scavo et al., 2019).

In the 2.5–10 cm layer, the higher values of pH_{H2O}, exchangeable Ca and Mg, V and CEC_{ef}, as well as lower contents of exchangeable Al and Al_{sat}, denote a downward movement of the limestone neutralization effect. After 21 years of the limestone application, the neutralizing front reached a soil layer deeper than 10 cm. The deepening of the neutralizing front observed in the rangeland is similar to that observed in soil with annual crops under a no-tillage system, where the downward movement is governed by mass flow (Rheinheimer et al., 2018; Vargas et al., 2019). For agricultural areas with the same soil as in this experiment, the neutralization front exceeds 20 cm 12 years after liming (Vargas et al., 2019) and reaches 60 cm after 18 years (Rheinheimer et al., 2018). In the last case, the authors explained the variation of the chemical characteristics in soil depth by the high rainfall. The percolation of fine limestone particles is favored by water movement, such as a laminar water flow through a porous medium and turbulent water flow in macropores (Czachor, 2011). In the analyzed area, the presence of macropores is favored by the texture (170 g kg^{-1} of clay) and the absence of soil plowing.

In long-term, the difference in pH$_{H2O}$ values in the 0–10 cm layer between treatments with and without limestone has increased from 0.3 units, one year after liming (1998), to 1.2 units, 12 years after liming (2009) (Tiecher et al., 2014), and then, it reduced to 0.6 units, 21 years after liming (2018). This demonstrates the existence of a slow soil surface reacidification that is mainly due to the constant (re)cycling and plants exportation of Ca, Mg, and even Si and the downward migration with waterflow. However, the inexistence of surface erosion favors the permanence of limestone on the surface soil and prevents its loss by runoff.

The principal component analysis differentiated two groups on the principal component (PC1), with acidity variables and exchangeable Ca and Mg contents of the soil at a depth greater than 2.5 cm being the main contributors. This confirms that the application of limestone is the factor that most differentiated the treatments. The higher levels of exchangeable Ca content in all soil depths is attributed to the application of limestone in TPCa and SPCa. However, higher levels of Ca were also expected in the first soil layer with the RP treatment due to the dissolution of RP minerals in the soil. RP is composed of minerals of the apatite group (Ca3 (PO4)2(x)) as the main P source. Therefore, Ca is a product of apatite dissolution that, besides the increase P content, also favors an increase in Ca content. The calcium content increase due to phosphate rock dissolution is possible and was demonstrated in flooded soils (Gonçalves et al., 2008). However, in our experiment, no significant difference in Ca content was observed in the RP treatment compared to the control.

Phosphorus addition

The application of soluble P sources in soil surface (as in TPCa, SPCa, and TP) with or without limestone did not favor the nutrient downward movement in the soil profile. The available soil P gradients observed (Figure 4) may be due to nutrient cycling by plants (Tian et al., 2017) and the application of all the fertilizers onto soil surface. The rapid dissolution and release of P by soluble sources favor its adsorption by reactive functional groups of clay minerals and oxides of Fe and Al, that are abundant in tropical and sub-tropical soils (Arai and Sparks, 2001; Bolan et al., 2003; Bortoluzzi et al., 2015). Therefore, it is likely that the P from the soluble sources has been absorbed by the plants or by the soil colloids in the superficial layers. This limited the effect of fertilization in increasing P contents in depth. Higher levels of nutrients in deeper layers are crucial to increase the root system development and drought tolerance by plants.

In contrast, the use of RP at the highest rate (249 kg ha^{-1} of P) over the 21 years provided an increase in available soil P contents to a depth of 7.5 cm, contributing to the legacy-P of this soil. High P levels at depths greater than 2.5 cm were the factors that most contributed to the variance explained by the PC2 axis of PCA (Figure 2). This confirms the differentiation of RP from treatments without limestone application (Control, OS, and TP) due to the deepening of P in the soil profile.

The increase of P content to a depth of 7.5 cm, non-associated with an increase of soil Ca content, suggests low apatite dissolution rate (minerals present in phosphate rock fertilizer). The dissolution of apatite minerals occurs only when there are favorable thermodynamic conditions. In soil, the dissolution of apatite is controlled by factors like soil pH (Chien, 1977), water content (Heindel et al., 2018), soil organic matter content (Alloush, 2003), and mainly by the Ca contents (Robinson and Syers, 1990). Thus, when these factors are not favorable to the dissolution of mineral, apatite remains in its crystalline form (Kumar et al., 1994). In this case, the downward movement of P is possible by mass flow, in the same way that occurs with limestone, and also verified in the experiment (Figure 3).

However, agronomically, the absence of RP dissolution is not desired because it limits the release of P to the soil solution and plant uptake. In this experiment, the difference between P-AER and P-Mehlich-1 suggests some recalcitrance of the apatite minerals in the rangeland soil. Therefore, furthers studies are encouraged to better understand the apatite mineral recalcitrance in rangelands soils and its P availability to plants.

Vegetation analysis

The content of P in plant shoot corroborates the historic amount of P applied to the soil and to the available soil P content. In work carried out in the same experimental area, Oliveira et al. (2014) quantified the P content in the plant shoot in the order of 0.9, 1.5, and 1.9 g kg^{-1} (respectively for OS, RP, and TP) after 186 days of fertilization, in 2010. For these treatments, in our study, after approximately nine years of fertilization, lower values of P concentration were obtained even in the RP treatment (0.99 g kg^{-1}). This demonstrates that for all P sources tested, there is a reduction in P uptake with increasing time after fertilizer application. This is mainly due to the reduction of available soil P content, either through the uptake and export of the nutrient by forage (Oliveira et al., 2014; Pavlů et al., 2016) or by adsorption by functional groups of clays minerals and oxides present in the soil (Bortoluzzi et al., 2015; Kim et al., 2011).

On the other hand, in the treatment with fertilization at a rate of 249 kg ha^{-1} of P performed until 2012 (seven years before the sampling and quantification of P in plant shoot for this study), the levels of P in shoot were higher in the RP and lower in TP compared to the results obtained by Oliveira et al. (2014). The highest levels in the RP are due to the higher rate (249 kg ha^{-1} of P). However, in this case, there is also the possibility of the long-term effect of RP dissolution. This behavior was not identified after nine years of RP application (rate of 205 kg ha^{-1} of P) but possibly still exists seven years after the last application (rate of 249 kg ha^{-1} of P).

The P exportation obtained for the treatments was similar to values available in the literature for rangeland P fertilized and for the same growing season (March) but with a shorter period after the last fertilization (186 days) (Oliveira et al., 2014). Treatments with phosphorus fertilizers resulted in higher P exportation due to a greater soil P availability, P uptake and P contents in plant shoot. However, when the SPCa treatment was applied at a rate of 249 kg ha^{-1} of P, high dry matter production during the evaluated period also played a key role to the P exportation. The greater P exportation in SPCa contributes to a greater recovery of P applied. Therefore, SPCa does not have the highest levels of P in any soil layer analyzed. In addition, the increase in soil fertility levels may favor, in the long term, the alteration of the occurrence of plant species in the areas (Harpole et al., 2017, 2016; Oliveira et al., 2015). Among these species are legumes, which have greater nutritional requirements for their development.

Even with the significant difference in plant shoot P content, in response to the distinct soil available P levels (Table 3 and Table 5), there was no differences in rangeland dry matter production. Differences in dry matter production due to P fertilizer application were reported in previous studies carried out in the same experiment. In these cases, the treatments SPCa and TPCa had higher dry matter production (Gatiboni et al., 2000; Oliveira et al., 2015; Tiecher et al., 2014). Though, even without a statistical difference, it is possible to observe a pattern in the RDm values between 2016 and 2019. The lowest RDm was obtained in the treatment Control, OS, and RP. The productivity of rangeland grown on soils with soluble fertilizers was higher, reaching up to 27% of superiority in comparison to the other treatments.

Differences in Ca and Mg contents in the plant shoot were expected, especially in SPCa and TPCa, where soil Ca and Mg contents were increased due to the use of magnesian limestone. An increase in the concentration of Ca^{2+} in the soil solution often leads to an increase in the Ca concentration in the leaves (Hawkesford et al., 2012). However, in this experiment, differences in the content of Ca and Mg in plant shoot were not observed.

6.5.Conclusions

This study investigated the chemical properties of rangeland fertilized over 21 years with different phosphorus sources and liming. The highest rate of phosphorus (249 kg ha-1 of P applied until 2012) provides the highest levels of nutrient availability in the soil and in the plant shoot, but there is no more response in dry matter production of rangeland after more than four years since the last fertilization. That indicates the necessity of periodic phosphorus fertilization in a time-space lower than four years.

The use of phosphate rock in the soil surface provides a greater deepening of phosphorus in the soil profile by mass flow phenomenon (achieving 7.5 cm after 21 years and a rate of 249 kg ha-1 of P) compared to soluble fertilizers. Moreover, further studies are needed to understand the apatite mineral dissolution in rangelands soils and the P availability to plants.

After 21 years of surface deposition of limestone in rangeland, the reacidification was not significant, and the neutralizing front reached depths greater than 10 cm. In these conditions, the levels of exchangeable Ca and Mg, pH, exchangeable Al, and the saturation by Al are better than without limestone for plant development.

6.6.Author contributions

LCG and DR led the implementation of the experiment field. AS, DR, and LC participated in the conception of the work. AS, MC, LM, IBS, and GL participated in data acquisition and data interpretation. AS led the writing of the manuscript. All authors contributed to the manuscript correction, discussion, and interpretation of results.

6.7.Funding

This research was funded by National Council for Scientific and Technological Development (CNPq) (Projects number PVE 400887/2014-2 and GM/GD 140270/2019-1). The authors acknowledge financial support from the European Union (ERDF) and "Région Nouvelle Aquitaine".

6.8.Acknowledgments

We thank all the students and researchers who helped keep the experimental device active over 21 years.

6.9.References

Alloush, G.A., 2003. Dissolution and effectiveness of phosphate rock in acidic soil amended with cattle manure. Plant Soil 251, 37–46. https://doi.org/10.1023/A:1022987915057

Arai, Y., Sparks, D.L., 2001. ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface. J. Colloid Interface Sci. 241, 317–326. https://doi.org/10.1006/jcis.2001.7773

Bolan, N.S., Adriano, D.C., Naidu, R., 2003. Role of Phosphorus in (Im)mobilization and Bioavailability of Heavy Metals in the Soil-Plant System, in: Reviews of Environmental Contamination and Toxicology. Springer, New York, p. 177. https://doi.org/10.1007/0-387-21725-8_1

Boldrini, I.I., 2009. A flora dos campos do Rio Grande do Sul, Campos Sulinos - conservação e uso sustentável da biodiversidade. Brasília.

Borges, J.A.R., Tauer, L.W., Lansink, A.G.J.M.O., 2016. Using the theory of planned behavior to identify key beliefs underlying Brazilian cattle farmers’ intention to use improved natural grassland: A MIMIC modelling approach. Land use policy 55, 193–203. https://doi.org/10.1016/j.landusepol.2016.04.004

Bortoluzzi, E.C., Pérez, C.A.S., Ardisson, J.D., Tiecher, T., Caner, L., 2015. Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. Appl. Clay Sci. 104, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Carvalho, P.C. d F., Batello, C., 2009. Access to land, livestock production and ecosystem conservation in the Brazilian Campos biome: The natural grasslands dilemma. Livest. Sci. 120, 158–162. https://doi.org/10.1016/j.livsci.2008.04.012

Carvalho, P.C.F., Fisher, V., Dos Santos, D.T., L Ribeiro, A.M., F De Quadros, F.L., S Castilhos, Z.M., E C Poli, C.H., G Monteiro, A.L., Nabinger, C., Cristina Genro, T.M., A Jacques, A. V, 2006. Produção animal no bioma Campus Sulinos. Brazilian J. Anim. Sci. 35, 156–202.

Chen, Y.T., Wang, Y., Yeh, K.C., 2017. Role of root exudates in metal acquisition and tolerance. Curr. Opin. Plant Biol. 39, 66–72. https://doi.org/10.1016/j.pbi.2017.06.004

Chien, S.H., 1977. Dissolution oh phosphate rocks in a flooded acid soil. Soil Sci .Soc.Am.J. 41, 1106–1109.

Czachor, H., 2011. Laminar and turbulent flow in soils, in: Encyclopedia of Earth Sciences Series. Springer Netherlands, p. 413. https://doi.org/10.1007/978-90-481-3585-1_80

Embrapa, 2009. Manual de análises químicas de solos, plantas e fertilizantes, 2. ed. ed. Brasília.

Fedrigo, J.K., Ataide, P.F., Filho, J.A., Oliveira, L. V., Jaurena, M., Laca, E.A., Overbeck, G.E., Nabinger, C., 2018. Temporary grazing exclusion promotes rapid recovery of species richness and productivity in a long-term overgrazed Campos grassland. Restor. Ecol. 26, 677–685. https://doi.org/10.1111/rec.12635

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Aquino, J.E.R., 2008. Efeito da adubação fosfatada e da calagem sobre a qualidade bromatológica da forragem de pastagem natural com introdução de espécies forrageiras de inverno. Rev. Bras. Agrociência 14, 125–134.

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Brunetto, G., Saggin, A., Flores, J.P.C., 2000. Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. Pesqui. Agropecu. Bras. 35, 1663–1668. https://doi.org/10.1590/S0100-204X2000000800020

Gonçalves, G.K., Oliveira De Sousa, R., Vahl, C., Bortolon, L., 2008. Solubilização dos fosfatos naturais Patos de Minas e Arad em dois solos alagados, Revista Brasileira de Ciência do Solo. https://doi.org/10.1590/S0100-06832008000500036

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall 10, A.S., Seabloom, E.W., Williams, R., Bakker 12, J.D., Cadotte, M.W., Chaneton, J., Chu, C., Cleland, E.E., Antonio, C. D', Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., Mcculley, R.L., Moore, J.L., Morgan 25, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. Nat. Publ. Gr. 537, 28. https://doi.org/10.1038/nature19324

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall, A.S., Seabloom, E.W., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Hagenah, N., Kirkman, K., Pierre, K.J. La, Moore, J.L., Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., 2017. Out of the shadows: multiple nutrient limitations drive relationships among biomass, light and plant diversity. Funct. Ecol. 31, 1839–1846. https://doi.org/10.1111/1365-2435.12967

Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P., 2012. Functions of Macronutrients, in: Marschner, P. (Ed.), Mineral Nutrition of Higher Plants. Elsevier, London, pp. 135–189. https://doi.org/10.1016/B978-0-12-384905-2.00006-6

Heindel, R.C., Lyons, W.B., Welch, S.A., Spickard, A.M., Virginia, R.A., 2018. Biogeochemical weathering of soil apatite grains in the McMurdo Dry Valleys, Antarctica. Geoderma 320, 136–145. https://doi.org/10.1016/j.geoderma.2018.01.027

Instituto Brasileiro de Geografia e Estatística, I., 2016. Produção da Pecuária Municipal. Rio de Janeiro.

Kim, J., Li, W., Philips, B.L., Grey, C.P., 2011. Phosphate adsorption on the iron oxyhydroxides goethite (α-FeOOH), akaganeite (β-FeOOH), and lepidocrocite (γ-FeOOH): A31P NMR Study. Energy Environ. Sci. 4, 4298–4305. https://doi.org/10.1039/c1ee02093e

Kumar, V., Gilkes, R.J., Armitage, T.M., Bolland, M.D.A., 1994. Identification of residual P compounds in fertilized soils using density fractionation, X-ray diffraction, scanning and transmission electron microscopy. Fertil. Res. 37, 133–149. https://doi.org/10.1007/BF00748554

Mehlich, A., 1953. Determination of P, Ca, Mg, K, Na and NH4 by North Carolina Soil Testing Laboratoris. Raleigh Univ. North Carolina.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. ACTA 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nunes, R.S., Sousa, D.M.G., Goedert, W.J., Vivaldi, L.J., 2011. Distribuição De Fósforo No Solo Em Razão Do. R. Bras. Ci. Solo 877–888. https://doi.org/10.1021/JP047021Q

Oliveira, T.E. de, Freitas, D.S. de, Gianezini, M., Ruviaro, C.F., Zago, D., Mércio, T.Z., Dias, E.A., Lampert, V. do N., Barcellos, J.O.J., 2017. Agricultural land use change in the Brazilian Pampa Biome: The reduction of natural grasslands. Land use policy 63, 394–400. https://doi.org/10.1016/j.landusepol.2017.02.010

Oliveira, L.B., Soares, E.M., Jochims, F., Tiecher, T., Marques, A.R., Kuinchtner, B.C., Rheinheimer, D.S., De Quadros, F.L.F., 2015. Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil. Rangel. Ecol. Manag. 68, 445–452. https://doi.org/10.1016/j.rama.2015.07.012

Oliveira, L.B., Tiecher, T., de Quadros, F.L.F., Trindade, J.P.P., Gatiboni, L.C., Brunetto, G., dos Santos, D.R., 2014. Formas de fósforo no solo sob pastagens naturais submetidas à adição de fosfatos. Rev. Bras. Cienc. do Solo 38, 867–878. https://doi.org/10.1590/S0100-06832014000300018

Oliveira, L.B., Tiecher, T., Quadros, F.L.F., Santos, D.R., 2011. Fósforo microbiano em solos sob pastagem natural submetida a queima e pastejo. Rev. Bras. Cienc. do Solo 35, 1509–1515. https://doi.org/10.1590/S0100-06832011000500005

Pallarés, O.R., Berretta, E.J., Maraschin, G.E., 2005. The South American Campos ecosystem, in: Suttie, J., Reynolds, S.G., Batello, C. (Eds.), Grasslands of the World. Rome, pp. 171–219.

Pavlů, L., Gaisler, J., Hejcman, M., Pavlů, V. V., 2016. What is the effect of long-term mulching and traditional cutting regimes on soil and biomass chemical properties, species richness and herbage production in Dactylis glomerata grassland? Agric. Ecosyst. Environ. 217, 13–21. https://doi.org/10.1016/j.agee.2015.10.026

Pellegrini, L.G., Nabinger, C., Neumann, M., Carvalho, P.C. de F., Crancio, L.A., 2010. Produção de forragem e dinâmica de uma pastagem natural submetida a diferentes métodos de controle de espécies indesejáveis e à adubação. Rev. Bras. Zootec. 39, 2380–2388. https://doi.org/10.1590/S1516-35982010001100010

Prestes, N.E., Do Amarante, C.V.T., Pinto, C.E., Prestes, G., Zanini, G.D., De Medeiros-Neto, C., Sbrissia, A.F., 2016. Forage production in a natural grassland with limestone and phosphorus dosages. Semin. Agrar. 37, 3265–3275. https://doi.org/10.5433/1679-0359.2016v37n5p3265

Prestes, N.E., Do Amarante, C.V.T., Pinto, C.E., Prestes, G., Zanini, G.D., Zanella, P.G., Sbrissia, A.F., 2017. Limestone and phosphorus application and forage production in natural pastures with sodseeding of cool-season species. Semin. Agrar. 38, 3681–3693. https://doi.org/10.5433/1679-0359.2017v38n6p3681

Rheinheimer, D. dos S., Tiecher, T., Gonzatto, R., Santanna, M.A., Brunetto, G., da Silva, L.S., 2018. Long-term effect of surface and incorporated liming in the conversion of natural grassland to no-till system for grain production in a highly acidic sandy-loam Ultisol from South Brazilian Campos. Soil Tillage Res. 180, 222–231. https://doi.org/10.1016/j.still.2018.03.014

Rheinheimer, D.S., Santos, J.C.P., Kaminski, J., Mafran, A.L., 1997. Crescimento de leguminosas forrageiras afetado pela adição de fósforo, calagem do solo e micorrizas, em condições de casa de vegetação.pdf. Ciência Rural 27, 571–576. https://doi.org/10.1590/S0103-84781997000400008

Robinson, J.S., Syers, J.K., 1990. A critical evaluation of the factors influencing the dissolution of Gafsa phosphate rock. J. Soil Sci. 41, 597–605.

Rodrighero, M.B., Barth, G., Caires, E.F., 2015. Aplicação superficial de calcário com diferentes teores de magnésio e granulometrias em sistema plantio direto. Rev. Bras. Cienc. do Solo 39, 1723–1736. https://doi.org/10.1590/01000683rbcs20150036

Ruviaro, C.F., da Costa, J.S., Florindo, T.J., Rodrigues, W., de Medeiros, G.I.B., Vasconcelos, P.S., 2016. Economic and environmental feasibility of beef production in different feed management systems in the Pampa biome, southern Brazil. Ecol. Indic. 60, 930–939. https://doi.org/10.1016/J.ECOLIND.2015.08.042

SBCS, 2016. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina.

Scavo, A., Abbate, C., Mauromicale, G., 2019. Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. Plant Soil. https://doi.org/10.1007/s11104-019-04190-y

Soares, A.B., Carvalho, P.C. de F., Nabinger, C., Semmelmann, C., Trindade, J.K. da, Guerra, E., Freitas, T.S. de, Pinto, C.E., Fontoura Júnior, J.A., Frizzo, A., 2005. Produção animal e de forragem em pastagem nativa submetida a distintas ofertas de forragem. Ciência Rural 35, 1148–1154. https://doi.org/10.1590/S0103-84782005000500025

Soltangheisi, A., Rodrigues, M., Coelho, M.J.A., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2018. Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res. 179, 20–28. https://doi.org/10.1016/j.still.2018.01.006

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., 1995. Análises de solo, plantas e outros materiais, 2 Ed. ed. Porto Alegre.

Tian, J., Boitt, G., Black, A., Wakelin, S., Condron, L.M., Chen, L., 2017. Accumulation and distribution of phosphorus in the soil profile under fertilized grazed pasture. https://doi.org/10.1016/j.agee.2017.01.022

Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. Grass Forage Sci. 69, 567–579. https://doi.org/10.1111/gfs.12079

Vargas, J.P.R., dos Santos, D.R., Bastos, M.C., Schaefer, G., Parisi, P.B., 2019. Application forms and types of soil acidity corrective: Changes in depth chemical attributes in long term period experiment. Soil Tillage Res. 185, 47–60. https://doi.org/10.1016/j.still.2018.08.014

6.10. General comments

As demand was raised in chapter II, in chapter III the effect of the superficial application of the P sources and limestone on soil chemical characteristics in the 0-10 cm topsoil layer (stratified every 2.5 cm) was evaluated and on the shoot nutritional contents. Here, we obtained as a result that in the highest rate of phosphate fertilizer, although there is no difference in yields, the P content and consequently its export are higher. Reinforcing the need for periodicity in the application of phosphate fertilizers. This period, as noted in the previous two chapters, must be less than three years.

Differently, the use of limestone on soil surface, only once, increased soil pH, neutralized toxic elements (such as Al^{+3}) and increased levels of Ca and Mg up to the maximum depth analyzed (10 cm). Moreover, the soil reacidification was not significant enough to demand a new application of limestone even after more than 20 years of the first liming.

Among the P sources, the use of rock phosphate favored, in the highest rate, the increase of P levels, obtained by an acid extractor (Mehlich-1), up to a depth of 7.5 cm. This deepening was attributed to the downward movement by mass flow of apatite particles. Since, the dissolution of these particles is known to be slow in the soil, which would make it possible to carry it through the water. When an acid extractor (Mehlich-1) is used to quantify the levels of available soil P, there would be a dissolution of apatite crystalline particles from the rock phosphate and, consequently, an overestimation of the levels of available P is obtained.

From these results and the question also raised in chapter II regarding the possibility of non-dissolution of apatite minerals in the soil and the unavailability of P for plants, the demand for chapter IV arose. In chapter IV, we hypothesized that there is no long-term dissolution of P-bearing minerals when phosphate rock is used as fertilizer in Pampa grassland. As well as, this no P-bearing minerals dissolution change the P-legacy response and plant dry matter production in comparison to those of grassland subjected to fertilization based on soluble phosphate added with limestone.

To test our hypothesis, in chapter IV an analysis of P legacy dynamics will be developed due to the use of triple superphosphate + limestone and rock phosphate, with emphasis on the possibility of non-dissolution of apatite minerals in the soil and less availability of P for plants. For this, we used analytical techniques for extracting soil P (e.g., Hedley fractionation, Mehlich-1 extractor and anion exchange resin) and scanning electron microscopy technique coupled to EDX to identify the presence of apatite minerals in its crystalline form in the soil.

7. Chapter IV - P-Legacy effect of soluble fertilizer added with limestone and phosphate rock on grassland soil in subtropical climate region

André Somavilla; Laurent Caner; Edson Campanhola Bortoluzzi; Maria Alice Santanna; Danilo Rheinheimer dos Santos

Artigo publicado pela revista: **Soil & Tillage Research**
DOI: 10.1016/j.still.2021.105021

Enviado em: 3 de dezembro de 2020
Aceito para publicação em 26 de março de 2021

Abstract

P-fertilization can be applied to cropped soil through two main ways, namely: by using soluble phosphates to increase P pools, rapid release and uptake by plants or by using less soluble rock phosphate powder to trigger synchronization between P solubilization and its uptake by plants. The aims of the current study were to evaluate whether there is no dissolution of P-bearing minerals in the long-term when phosphate rock is used, as well as to analyze its response associated with P-legacy and plant dry-matter production in comparison to that of fertilization based on soluble phosphate added with limestone. A 20-year-old field experiment is in progress at Pampa grassland, Southern Brazil. Two rates of phosphate rock and triple superphosphate plus limestone (118 and 250 kg ha^{-1} of P), as well as a P-free control, were tested. The P-legacy effect of fertilizers were analyzed by phosphorus lability and apatite minerals recalcitrance. Additionally, a historic data set of dry matter production was considered to access phosphorus use efficiency. The use of soluble P source and limestone (triple superphosphate at rate of 250 kg ha^{-1} of P add with limestone at rate of 3.2 Mg ha^{-1}) resulted in greater nutrient use efficiency (42 kg kg^{-1} - five times greater than that of phosphate rock) and in higher dry matter yield (22%). In addition, the use of phosphate rock (at rate of 250 kg ha^{-1} of P) has led to higher total P and moderate-lability P levels in the soil due to low apatite dissolution (even under favorable soil thermodynamics conditions). P-apatite minerals were found in sand and silt soil fractions subjected to phosphate rock treatment, which presented up to 6-year P dissolution and promoted low P bioavailability. Agronomic use of soluble phosphate added with limestone is the most suitable technique used to increase grassland yield and P-use efficiency.

***Keywords**:* Apatite dissolution; Dry matter production; Forage; Hydroxyapatite; Pampa biome; Phosphorus sources.

7.1.Introduction

In the last 50 years, Southern Brazilian grassland - the so-called Pampa grassland - had its natural vegetation area decreased by 26% in order to open room for annual and forest crops capable of providing higher financial return (Oliveira et al., 2017). One of the possibilities to slow down the pace of destruction of this biome is to increase forage production and, consequently, beef and sheep meat. However, most of these areas require mandatory use of P fertilizer added with limestone to increase dry matter yield (Oliveira et al., 2015; Tiecher et al., 2014).

Water soluble fertilizers are the main P source used worldwide. They play an essential role in agriculture because they enable fast increase in P available in soil solution and in P bioavailability (Bolan et al., 2003), as well as favors P uptake by plants, which, consequently, improves crop yield in the short-term (Pavinato et al., 2017; Silveira et al., 2018). Furthermore, soluble fertilizers favor fast increase in soil P pools due to P incorporation in soil microbial biomass or cover crop biomass (Gumiere et al., 2019; Houben et al., 2019; Oliveira et al., 2014; Rheinheimer et al., 2019; Soltangheisi et al., 2018; Tiecher et al., 2012); and to adsorption, mainly of clay size minerals and Fe and Al oxy-hydroxides, through inner-sphere high energetic P adsorption in inorganic-soil functional groups (Bortoluzzi et al., 2015; Kim et al., 2011; Rheinheimer et al., 2003). However, highly energetic P adsorption in soil matrix acts as P sink (Pearse et al., 2007) and considerably reduces the recovery of P added via fertilizer, mainly in intensively weathered acid soils such as the ones observed in tropical and subtropical regions (Conte et al., 2003; Freitas et al., 2013; Roy et al., 2016). Thus, it is essential the use of fertilizer added with limestone to avoid P inner-sphere immobilization and to improve P recovery in Pampa grasslands.

Alternatively, to soluble fertilizers use, phosphate rocks (RP) have been used as fertilizer at global scale (Dias et al., 2015; Oliveira et al., 2015; Pufahl and Groat, 2017; Soltangheisi et al., 2020, 2018). Igneous and sedimentary phosphate rocks are the two main RP types in the world (Ptáček, 2016; Pufahl and Groat, 2017). The crystal structure of igneous phosphate rocks is coarser, lesser porous, and significantly lesser soluble/reactive than that of sedimentary phosphate rocks. Depending on RP origin, P can be found as mineral belonging to the apatite group, such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), carbonate-fluorapatite ($Ca_{10}(PO_4,CO_3)_6F_2$), fluorapatite ($Ca_{10}(PO_4)_6F_2$) or chlorapatite ($Ca_{10}(PO_4)_6Cl_2$), which have different crystallinity and solubility degree (Toledo and Pereira, 2001).

RP dissolution enables synchronism between P release to soil solution and plants' needs. This behavior can lead to RP-legacy particle effect and to lesser P inner-sphere immobilization by soil clay minerals and oxides; this process favors P recovery by plants in the long-term (Pavinato et al., 2017; Szilas et al., 2007). However, immediate and residual RP effects remain unclear. Several scholars have shown the potential of RP use in agriculture (Dias et al., 2015; Pavinato et al., 2017). Nevertheless, dry matter production was lower in Pampa grassland treated with RP than in the one treated with soluble fertilizer added with limestone (Gatiboni et al., 2000; Oliveira et al., 2015).

At this point, the key question about P fertilizer use in Pampa grasslands is: do soluble P fertilizers added with limestone used to increase lesser available P pools and, consequently, P release meet plants' nutritional needs or do lesser soluble phosphate rocks enable direct synchronization between apatite solubilization and P uptake by plants? Therefore, in the first case, P may not be released from soil matrix, even in the long-term and, in the second case, phosphate rocks may not dissolve in the soil (Kumar et al., 1994). The current hypothesis, is that dry matter production in Pampa grassland treated with RP is lower than that of grassland treated with soluble fertilizer added with limestone. This outcome is consistent with crystalline minerals deriving from RP due to environmental conditions.

7.2.Materials and methods

Local conditions and treatments

The experiment was installed in experimental field belonging to the Southern Brazilian Pampa biome (29°43'05.7"S 53°42'33.7"W), in 1997. Vegetation in the area is classified as Mesic Pampa grassland subtype 5b (Andrade et al., 2019). Climate in the region belongs to the subtropical humid type - Cfa - according to Köppen's classification, and its mean annual rainfall reaches 1,769 mm y^{-1}. The soil in the experimental field is of Typic Paleudults (USDA, 2014); its topsoil layer (0-20 cm) is featured by 170 g kg^{-1} of clay, pH H_2O of 4.5, 10.4 g kg^{-1} of C; 2.5 mg kg^{-1} and 60 mg kg^{-1} of P and K, respectively (extracted by Mehlich-1 - 0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H2SO4), as well as 1.30 cmolc kg^{-1}, 1.17 $cmol_c$ kg^{-1} and 0.75 $cmol_c$ kg^{-1} of Al, Ca and Mg, respectively (extracted by 1 mol L^{-1} KCl).

The experiment has followed a completely randomized block design, with five treatments and three repetitions. Treatments comprised the application of two P sources on soil surface (sedimentary phosphate rock - RP; and soluble fertilizer added with limestone - triple superphosphate added with dolomitic limestone – TP) at P rates of 118 kg ha^{-1} and 250 kg ha^{-1}, whereas the control treatment did not receive phosphorus fertilizer (WP). With respect to each P source, fertilizations in the treatment with 118 kg ha^{-1} of P were applied in 1997 and 1998, at doses of 79 kg ha^{-1} and 39 kg ha^{-1} of P, respectively; the last fertilization was performed 20 years before soil sampling in 2017 (herein called RP20 and TP20). In addition, fertilizations in the treatment with 250 kg ha-1 were performed in 1997 and 1998, at doses of 79 kg ha^{-1} and 39 kg ha^{-1} of P, respectively. They were followed by the application of 44 kg ha^{-1} of P in 2002, 2010 and 2012; the last fertilization was performed six years before soil sampling in 2017 (herein called RP6 and TP6).

The RP used in the current study derived from a sedimentary P deposit located at Gafsa-Tunisia. Total P concentration in phosphate rock (29% of P2O5) was used to calculate RP mass rates, whereas soluble P content in neutral ammonium citrate + water concentration in triple superphosphate (46% of P_2O_5) were used to calculate TP mass rates. Moreover, 3.2 Mg ha^{-1} of dolomitic limestone was applied on soil surface in TP20 and TP6 in order to increase soil pH to 5.5. All treatments were subjected to overseeding of *Lolium multiflorum* and *Trifolium vesiculosum* during Winter in 1997 and 2002 - 30 kg and 12 kg of seeds ha^{-1}, respectively. Further details about treatments can be seen in Table 1.

Forage growth was monitored, manually mowed with tractor trimmer and removed from soil surface throughout 20 experimental years. Further details about the experimental field and dry matter production are available at Gatiboni et al., (2000); Oliveira et al., (2015); Somavilla et al., (2021b) and; Tiecher et al., (2014).

Table 1 - Phosphate fertilizer and limestone surface broadcasting rates applied over 20 years.

Year	**Product**	**[1]RP20**	**RP6**	**TP20**	**TP6**	**WP**
1997	[2]Limestone (Mg ha^{-1})	-	-	3.2	3.2	-
	P (kg ha^{-1})	79	79	79	79	-
	[3]Over-seeding	Yes	Yes	Yes	Yes	Yes
1998	P (kg ha^{-1})	39	39	39	39	-
2002	P (kg ha^{-1})	-	44	-	44	-
	Over-seeding	Yes	Yes	Yes	Yes	Yes
2010	P (kg ha^{-1})	-	44	-	44	-
2012	P (kg ha^{-1})	-	44	-	44	-
2017	Soil Sampling	Yes	Yes	Yes	Yes	Yes
Total	Limestone (Mg ha^{-1})	0	0	3.2	3.2	0
	P (kg ha^{-1})	118	250	118	250	0
	Fertilizer (kg ha^{-1})	931	1965	587	1240	0

[1]RP-phosphate rock, TP-triple superphosphate, WP- no P fertilizer. 20 and 6 mean time intervals between the last fertilization and soil sampling. [2]limestone with total relative neutralizing power (TRNP) of 0.6. [3]Over-seeding of *Lolium multiflorum* and *Trifolium vesiculosum* with 30 kg ha^{-1} and 12 kg ha^{-1} of seeds, respectively.

Pampa grassland dry-matter production

Grassland dry-matter production (DM) was assessed based on literature dataset from 1998 (Gatiboni et al., 2000), 2008-2010 (Tiecher et al., 2014) and 2015-2019 (Somavilla et al., 2021b). DM production was analyzed based on the sum of historic DM production (ΣDM) in RP6, TP6 and WP. Phosphorus use efficiency (PUE, kg kg^{-1}) was estimated based on DM increase per unit of applied P:

$$PUE = \frac{(\Sigma DM_F - \Sigma DM_{WP})}{F} \qquad (1)$$

Wherein: ΣDM_F is the sum of fertilized DM (kg ha^{-1}); ΣDM_{WP} is the sum of non-fertilized DM (kg ha^{-1}) and F is the amount of applied P (kg ha^{-1}).

Phosphate rock featuring

Phosphate rock granulometry, mineralogy and morphology/chemical composition was featured. With respect to granulometry featuring, RP was fractionated into 2000–200, 200–50, 50–20, 20–2 and < 2 µm diameter. Briefly: 30 g of RP was dispersed by shaking in osmosed water (for 16h) and subjected to ultrasonification (three times, for two minutes). After the dispersion process was over, 2000–200 and 200–50 µm particles were obtained through sieving, whereas 50–20, 20–2 and < 2 µm particles were obtained through sedimentation in water at 20 ºC. RP bulk mineralogy was analyzed through X-ray diffraction (XRD) on randomly-oriented powders in order to identify apatite minerals. RP was milled, sieved (50-µm mesh) and analyzed in Bruker D8 Advance diffractometer (Cu Kα radiation, 40kV and 40 mA) equipped with Lynx eye detector - measurement range was set from 2.5 to 65°2θ, 0.012°2θ step size and 96 s counting time per step. Finally, particles' morphology and chemical mapping was performed in Scanning Electron Microscope (SEM JEOL/MP JSM-IT500, 15 kV, 1 nA, working distance 11 mm) coupled to Energy Dispersive X-ray Spectrometer (SEM-EDS Bruker Flash).

Soil sampling

Soil sampling was performed at layer 0-2.5 cm, in December 2017 (20 years after the assay was implemented). This layer was selected because fertilization was applied on soil surface, whose mineral dissolution response is highly likely to happen. Soil samples were oven dried at 65ºC and sieved (2 mm mesh) for subsequent analysis.

Soil phosphorus lability

Soil P in 0-2.5 cm bulk soil was divided into P - labile, P - moderately labile (P-mod-labile) and P – non-labile. P fractions were accessed through sequential P extraction using 0.5 mol L^{-1} $NaHCO_3$ (P-labile); 0.1 mol L^{-1} NaOH and 1 mol L^{-1} HCl (P-mod-labile) and 0.5 mol L^{-1} NaOH (P-non-labile), based on the protocol by Hedley et al. (1982) modified by Rheinheimer et al. (2000). With respect to alkaline extracts (NaHCO3 and NaOH), organic P was mineralized with ammonium persulfate (7.5%) + sulfuric acid (18.5%) in autoclave (USEPA, 1971) for total P quantification purposes. Total soil P (P_{total}) was quantified based on total soil digestion with concentrated $H_2SO_4 + H_2O_2 + MgCl_2$ (Brookes and Powlson, 1981). In all cases, P in the extracts was measured through colorimetry (at 882nm), after acid phospho-molybdate complex formation (Murphy and Riley, 1962).

Phosphate rock recalcitrance analysis

Soil calcium-phosphate stability diagram

Soil solution pH, as well as Ca and P content, were quantified in the WP treatment. Soil solution was obtained by following the centrifugation protocol (Somavilla et al., 2017). Briefly, 200 g of mowed dry soil (< 2 mm) were placed in PVC tube and saturated for 12h. Next, water excess was drained under absorbent paper for 12h. Samples were centrifuged at extraction energy of 100 kPa and the drained water was defined as soil solution. P content in the soil solution was quantified through colorimetry (Murphy and Riley, 1962), whereas Ca content in it was quantified through atomic absorption spectrophotometry (Tedesco et al., 1995).

Calcium-phosphate stability diagram was built based on hydroxyapatite (Eq. 2) and fluorapatite (Eq. 3) dissolution equations (Lindsay, 1979). Mean value of WP repetitions (0.0023 mmol L^{-1}) was the Ca^{2+} content used to develop this diagram. F^- activity used to fix the fluorapatite line was 0.0526 mol L^{-1}, which corresponded to the general fluoride level in natural soil solution (Yadav et al., 2018).

$$\mathrm{Ca}_5(PO_4)_3OH + 7H^+ = 5Ca^{2+} + 3H_2PO_4^- + H_2O \qquad Log\ K^0 = 14.46 \qquad (2)$$

$$\mathrm{Ca}_5(PO_4)_3F + 6H^+ = 5Ca^{2+} + 3H_2PO_4^- + F^- \qquad Log\ K^0 = -0.21 \qquad (3)$$

Soil physical fractionation

The 0-2.5 cm bulk soil of RP20, RP6 and WP were fractionated into five different particle sizes, namely: coarse sand (CSand, 2000–200 μm); fine sand (FSand, 200–50 μm); coarse silt (CSilt, 50–20 μm); fine silt (FSilt, 20–2 μm) and Clay (< 2 μm). The fractionation process was carried out with 30 g of soil dispersed by shaking in osmosed water (for 16h) and subjected to ultrasonification (three times, for two minutes) without soil organic matter (SOM) oxidation or chemical dispersant (i.e., NaCl). This protocol was adopted to avoid solubilizing the apatite mineral deriving from RP, in case it remained in the soil, otherwise it would hinder its identification. After the dispersion process was over, CSand and FSand were obtained through sieving, whereas CSilt, FSilt and Clay were obtained through sedimentation in water at 20 ºC.

Available phosphorus quantification

The available P was quantified in bulk soil of all treatments and in each particle size fraction of WP, RP6 and RP20 by using anion exchange resin – AER (AR 103 QDP 434 Ionics Inc. plates) and Mehlich-1 extractor. Particle size fraction analyses were carried out just for WP, RP6 and RP20 treatments, based on the result of available soil P in bulk soil, in order to enable apatite mineral identification.

With respect to P-AER, resins were previously saturated with 0.5 mol L^{-1} $NaHCO_3$, kept in contact with soil in end-over-end agitator at 25° C, for 16 h, and eluted with 0.5 mol L^{-1} HCl. As for P-Mehlich-1, the soil was subjected to Mehlich-1 extractor (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1953) at ratio 1:10 soil/solution (m/v), stirred for 5 min and left to rest for 16 h. Next, the solution was filtered through a 0.22 μm Nylon filter. Phosphorus contents were quantified through colorimetry in both cases (Murphy and Riley, 1962). Relative P contents were calculated by multiplying the P quantified in each particle fraction by the respective particle fraction contribution to the bulk soil. ΔP was calculate based on the difference in relative P content between AER and Mehlich-1.

Density fractionation of soil particles

Density fractionation was performed based on sodium polytungstate solution, whose density was set at 2.8-2.9 Mg m^{-3} to allow separating P compounds from silicates. The fractionation process was performed at CSand (2000–200 μm), FSand (200–50 μm), CSilts (50–20 μm) and FSilt (20–2 μm) fraction sizes. Separation was not performed in the clay (<2 µm) fraction. Fraction sizes were suspended in dense sodium polytungstate solution (100 ml) in separating funnel, which was thoroughly shaken and left to rest for sedimentation purposes for at least 16 hours, for sand and coarse silt, and for several days, for fine silts. The material deposited at the bottom of the funnel was separated and the procedure was repeated twice. Dense particles were recovered with filter membrane (0.45 µm) and thoroughly rinsed with osmosed water, before SEM-EDS analyses.

Scanning Electron Microscopy (SEM-EDS)

Apatite mineral identification was performed in Scanning Electron Microscope (SEM JEOL/MP JSM-IT500, 15 kV, 1 nA, working distance 11 mm) coupled to Energy Dispersive X-ray Spectrometer (SEM-EDS Bruker Flash). SEM-EDS analyses were applied to each particle size fraction deriving from density fractionation.

Statistical analysis

Treatments were considered a qualitative factor and subjected to analysis of variance and mean test. P lability in bulk soil and relative P contents in each particle size were analyzed based on two-factorial completely randomized block design (split-plot) with three repetitions (Eq. 4), wherein the plot corresponded to the treatment and the split-plot corresponded to P lability fraction or to particle fraction. Total soil P and bulk soil P extracted through AER and Mehlich–1 were analyzed based on completely randomized block design (Eq. 5). Analysis of variance and mean test (Scott-Knott test, p≤0.05) were carried out in SISVAR 5.6 software.

$$Y_{ijk} = \mu + B_i + T_j + error\ a(j,i) + R_k + (TR)_{ij} + error\ b(i,j,k) \qquad (4)$$

$$Y_{ijk} = \mu + B_i + T_j + error(j,i) \qquad (5)$$

Wherein *Y* is the observation; µ is the overall experimental mean; *B* represents the blocks (i = 1, 2, 3); *T* represents the treatments (j = 1, 2, 3, 4, 5); *R* is the particle fraction or P lability (k = 1, 2, 3) and *error* is the experimental error.

7.3.Results

Grassland vegetal growth

The ΣDM quantified throughout 20 experimental years was 22% and 4% higher in TP6 and RP6, respectively, than in WP. This outcome resulted in greater PUE (five times) due to TP than to RP use (Figure 1).

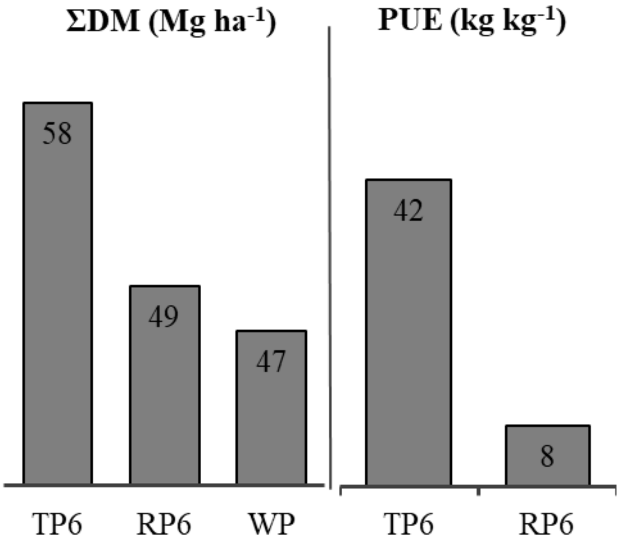


Figure 1 - Sum of dry-matter production (ΣDM) and phosphorus use efficiency (PUE) throughout 20 experimental years (adapted from Gatiboni et al. (2000); Tiecher et al. (2014); Somavilla et al. (2021b)). TP –Triple superphosphate; RP–Phosphate rock; WP– no phosphorus.

Phosphate rock featuring

RP is mainly composed of sand particle size (2000-200 and 200-50 µm, CSand and FSand, respectively) and just 4% of it corresponds to clay particle size (Figure 2a). The following minerals were identified in RP based on XRD: hydroxyapatite (d = 0.27 and 0.26 nm), carbonate fluorapatite (d = 0.26, 0.27 and 0.22 nm), gypsum (d = 0.42, 0.76 and 0.30 nm), calcite (d = 0.30, 0.18 and 0.22 nm) and quartz (d = 0.33 and 0.42 nm), as shown in Figure 2b. RP was featured as round and angular-shaped particles, based on SEM-EDS, and mapped for the presence of F, P and Ca (Figures 2c and d). Round and angular shapes resulted from the milling process the original sedimentary rock was subjected to in order to increase its specific surface area.

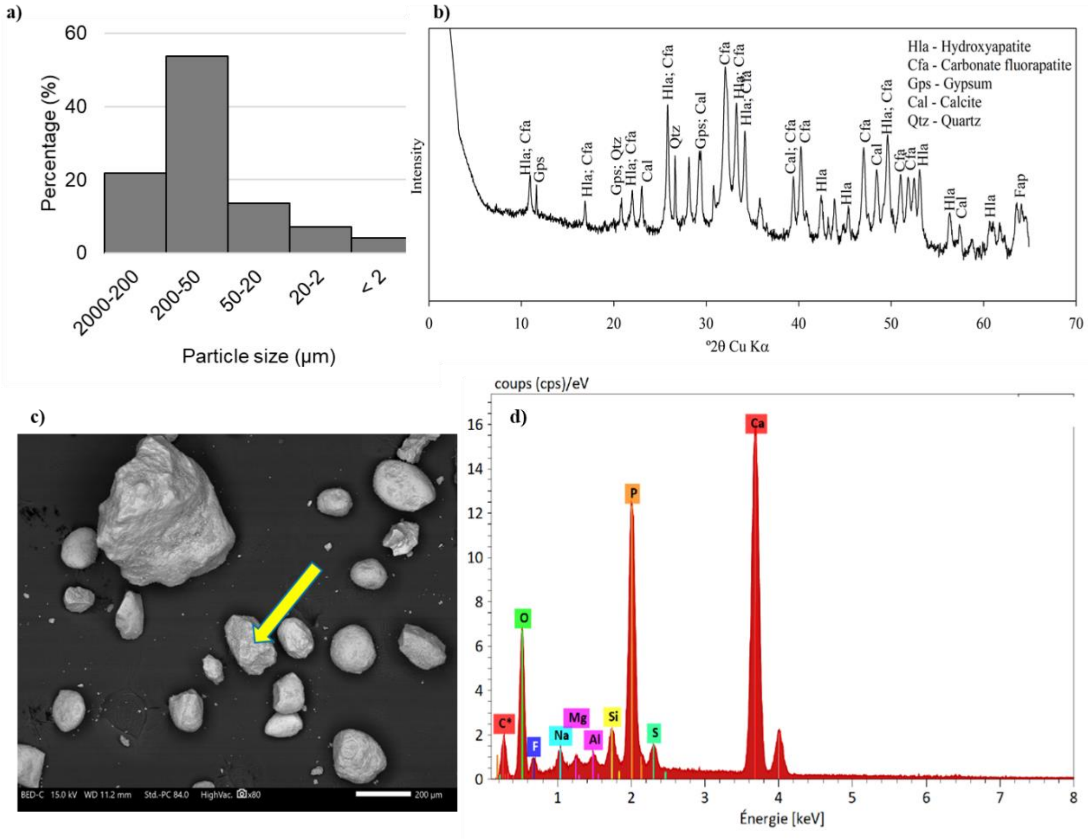


Figure 2 - Phosphate rock featuring through particle size distribution (a); X-ray diffraction pattern (b), scanning electron micrograph (c) and energy dispersive spectra (d). The yellow arrow indicates apatite particle analyzed in Energy Dispersive X-ray Spectrometer.

P-legacy based on different phosphorus sources in grassland

P_{total} and P lability have increased after phosphate fertilizers were used in different ways in each P source. P_{total} contents were always numerically higher when fertilizers were used, although only RP6 (440 mg kg^{-1}) recorded statistically significant difference in this variable (Figure 3a). Moderately labile P pool (extracted by using 0.1 mol L^{-1} NaOH and 1 mol L^{-1} HCl) was the dominant fraction in all treatments (Figure 3b). RP6 treatment presented the highest P-mod-labile content (132 mg kg^{-1}). Higher P-non-labile values were recorded for the highest RP fertilizer rate and for soluble fertilizer (RP6, TP6 and TP20) – mean value reached 33 mg kg^{-1}. There were no significant differences in P-labile pool between treatments.

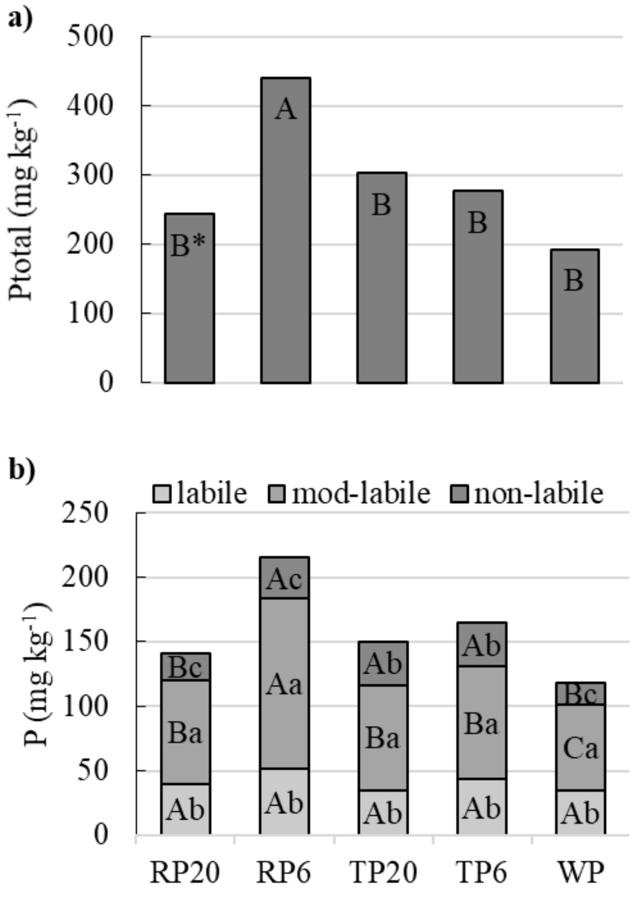


Figure 3 - Total soil phosphorus (a) and phosphorus lability (b) in 0-2.5 cm topsoil layer after 20 years of fertilization history. RP-phosphate rock, TP-triple superphosphate, WP-no P fertilizer. 20 and 6 represent the time interval between the last fertilization and soil sampling. *Means followed by the same letter, uppercase letter between treatments and lowercase letter between P labilities, did not statistically differ from each other in the Scott-Knott test ($p \leq 0.05$).

Recalcitrant apatite deriving from phosphate rock found in Pampa grassland soil

Hydroxyapatite and carbonate fluorapatite were identified as mineral P sources in RP powder. These minerals remained in the grassland soil, in their crystalline form, for at least six years after fertilization. However, P and Ca concentrations, as well as soil solution pH, have thermodynamically favored the dissolution of both hydroxyapatite and carbonate fluorapatite particles.

Calcium-phosphate stability diagram

Mean Ca^{2+} and P contents, and soil solution pH value observed for WP treatment were 0.0023 mmol L^{-1}, 0.0016 mmol L^{-1} and 6.1, respectively. Based on the stability diagram (Figure 4), the points featuring the WP treatment were below the hydroxyapatite/fluorapatite equilibrium line. This outcome indicates unfavorable natural thermodynamic conditions for hydroxyapatite and fluorapatite stability in the soil.

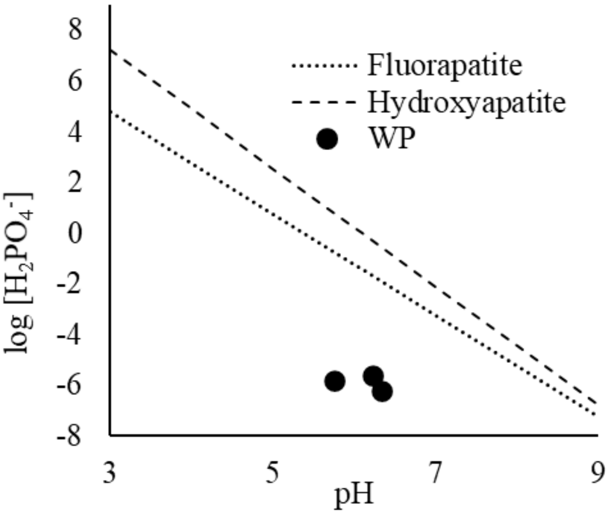


Figure 4 - Calcium-phosphate stability diagram when Ca^{2+} and F^- were fixed at 0.0023 mmol l^{-1} and 0.0526 mmol l-1, respectively. F- content in soil solution reported by Yadav et al. (2018). WP is assumed as soil natural condition receiving RP.

Phosphorus availability in the soil

Soil P contents – available in 0-2.5 cm bulk soil –extracted through AER and Mehlich-1 extractors were higher in RP6 than in RP20, WP, TP6 and TP20 (Figure 5a). P contents observed in RP6 (25.4 and 38.1 mg kg^{-1} of P, respectively, extracted through AER and Mehlich-1) have increased by 154% and 305% in comparison to those extracted through P-AER and P-Mehlich-1 in WP, respectively. Nevertheless, ΔP was not significantly higher in RP6 (12.7 mg kg^{-1} of P).

Particle size fractionation resulted in the distribution of CSand (43%), FSand (28%), CSilt (12%), FSilt (12%) and Clay (5%) particles. Relative P contents (relative P = [P]*% particle) extracted through AER and Mehlich-1, have increased as particle size decreased (Figures 5b and c). This behavior was similar in all treatments; however, RP6 was the only treatment recording significantly higher value for clay size fraction. Although RP6 presented the highest P contents in all particle sizes, significantly higher values (five and seven times higher in P-AER and P-Mehlich-1, respectively) were only observed in clay particles' size.

ΔP values recorded for particle sizes were always higher in RP6 (Figure 5d); in this case, ΔP were, on average, 2 mg kg^{-1} higher than in WP. Nevertheless, significant ΔP in RP6 were observed just in clay and CSand particle size. In addition, RP20 did not differ from WP in any of the particle fractions - ΔP values were approximately 0 mg kg^{-1}.

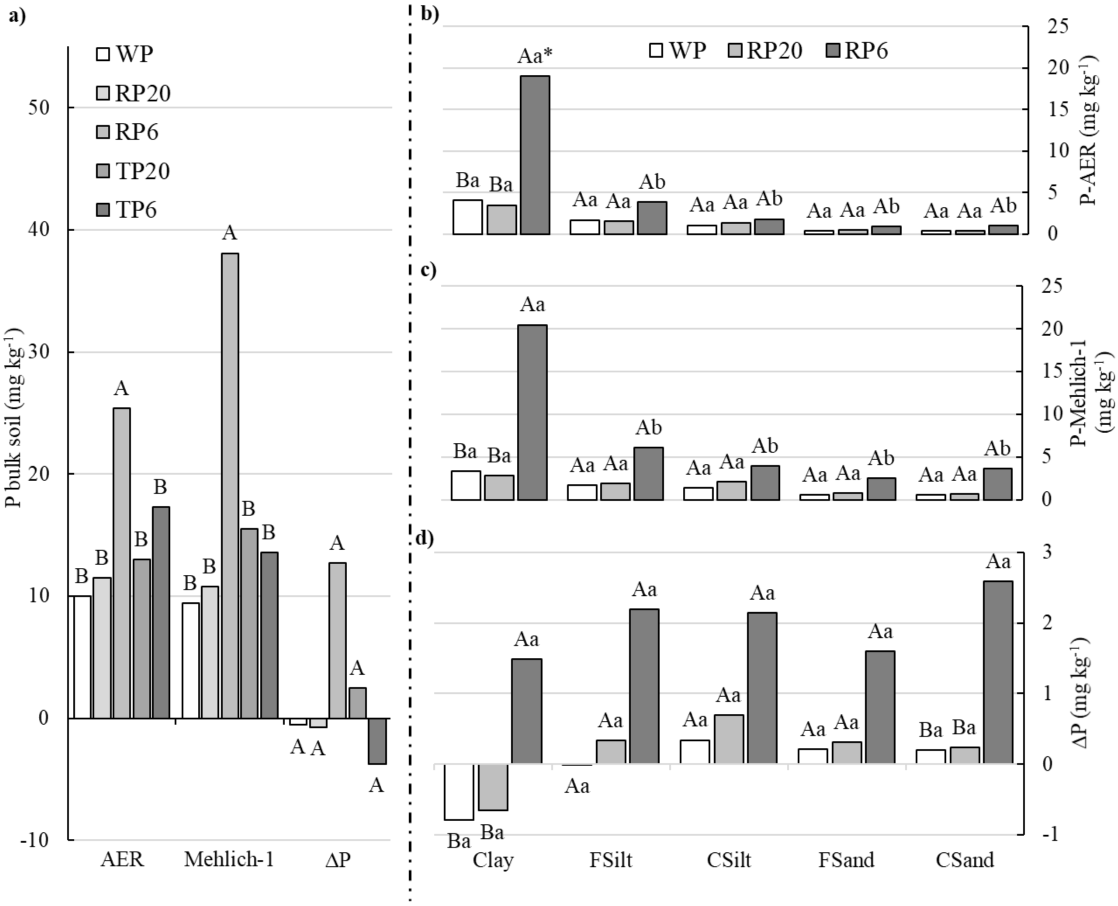


Figure 5 - P analysis in 0-2.5 cm bulk soil (a) and according to particle size assessed through AER and Mehlich-1 extractors (b, c and d). WP-no P fertilizer and; RP-phosphate rock. 20 and 6 represent the time interval between the last fertilization and soil sampling. CSand – coarse sand (Ø 2000 - 200 μm); FSand – fine sand (Ø 200 - 50 μm); CSilt – coarse silt (Ø 50 - 20 μm); FSilt – fine silt (Ø 20 - 2 μm) and Clay (Ø 2 > μm). *Means followed by the same lowercase letter between particle size and uppercase letter between treatments, did not statistically differ from each other in the Scott-Knott test ($p \leq 0.05$).

Apatite minerals identification in soil

Particle size fractions were analyzed through SEM-EDS, after densiometric fractionation. Apatite particles in CSilt (50-20 μm) and CSand (2000-200 μm) fractions were identified (Figures 6a1 and b1) based on their P and Ca contents (Figures 6a3 and b3). The other particles were composed of silica (quartz) or iron and/or titanium (iron oxides). These compositions were consistent with the hydroxyapatite particle composition featured in RP (Figure 2). Although particles identified in the soil presented different sizes, they had similar features such as round edges, worn surfaces and dissolution pits presenting partial dissolution in the long-term (Figures 6a2 and b2).

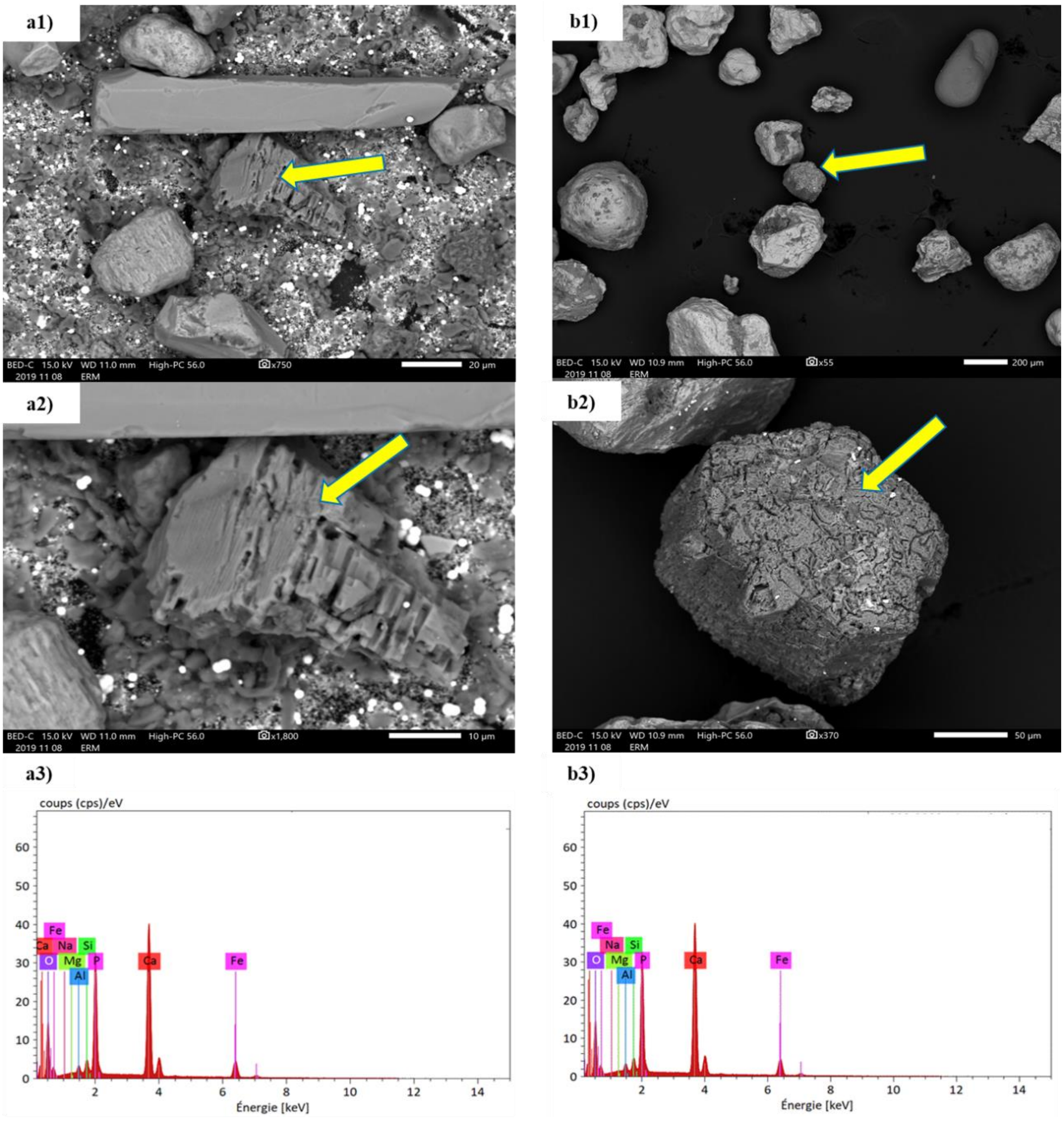


Figure 6 - Scanning electron micrograph of CSilt (a) and CSand (b) particle size fractions. The numbers represent the general view of the particles (1); detailed view of apatite particle pointed by yellow arrow (2) and; energy dispersive spectra of apatite particle pointed by yellow arrow (3). The yellow arrow indicates the analyzed apatite particle.

7.4.Discussion

Legacy soil P in grassland

Results accumulated over 20 years have indicated that dry matter yield was higher when soluble fertilizer added with limestone was used in the long-term (Figure 1). This outcome corroborated other studies carried out in the same field, although for a shorter period-of-time (Gatiboni et al., 2000; Oliveira et al., 2015). In addition to high yield, TP6 enabled greater PUE than RP6 throughout the investigated period. High dry matter yield and PUE have decreased P-legacy in TP6 due to P export from the system and to P accumulation forms.

Increased soil P availability due to P addition has led to high P content in grass leaves grown in Pampa grassland (Marques et al., 2020). With respect to South American Pampa grassland, P concentration in plant tissues can be 50% higher six months after P addition based on TP6 than on RP6, as well as 188% higher than that of treatments without P fertilizers (Oliveira et al., 2014). The difference in P concentration has decreased over time, although it remained numerically high six years after soluble fertilizer application (Somavilla et al., 2021a). Dry matter exportation leads to P stock depletion in soil system and changes P-legacy, as suggested by Boitt et al. (2018), who investigated a rangeland in New Zealand.

RP6 use as P source resulted in higher soil P_{total} values due to lesser P exportation by plants. Phosphorus release from RP6, as well as its uptake and exportation by plants, mainly depend on two factors. First, on rock powder granulometry, according to which, fast fine-particle fraction dissolution takes place, whereas coarse particles remain in the soil with less dissolution potential (Mackay and Syers, 1986; Syers et al., 1986). In the present case, 4% of RP presented particle size <2 µm and 25% of it was smaller than 50 µm. This outcome has indicated low RP reactivity and likely particle remaining in the soil. Second, soil pH, as well as Ca^{2+} and P contents, have played a key role in apatite dissolution and in P release (Guidry and Mackenzie, 2003; Mackay and Syers, 1986; Robinson and Syers, 1990), as addressed below. However, despite the use of soluble fertilizer added with liming at the rate of 250 kg ha^{-1} of P over 20 years, P exportation through dry matter and downward P movement deeper than 2.5 cm (Somavilla et al., 2021a) resulted in equivalent P_{total} contents at topsoil layer (0-2.5 cm) of TP6, TP20, WP and RP20.

Continuous P using over the years has favored P-non-labile and P-mod-labile increase in RP6, as well as P-non-labile increase in TP6, TP20 and RP20. This behavior corroborates the theory about the temporal strengthening of chemical bonds to the solid phase (Barrow,

1999) and about increase in P pools with lesser lability, mainly due to soluble fertilizer use (Soltangheisi et al., 2018). P-non-labile (extracted by using NaOH 0.5 mol L^{-1}) is the most recalcitrant form of inorganic P, bound to Fe and Al oxides, and to organic matter in aggregates (Condron et al., 1985). Thus, lack of differences in P-non-labile fraction among PR6, TP6 and TP20 has shown that the effect of P sorption by soil matrix depends more on the P content in the soil solution than on the adopted P source.

Moreover, RP6 has increased P levels in the mod-labile fraction because non dissolution of RP minerals that can be detected by the extraction performed with HCl (Freitas et al., 2013). The acid extractor accounted for breaking the bonds and for extracting P in Ca-P compounds (Cross and Schlesinger, 1995), which were also observed in the apatite minerals composing RP. Results similar to the ones recorded for P-mod-labile accumulation due to RP use were reported for clay soil subjected to no-tillage system and crop rotation (Soltangheisi et al., 2018). According to Soltangheisi et al. (2018), the P content obtained through HCl-based extraction was several times higher at RP than at soluble fertilizer application. This outcome was linked to the soil sampling forthwith RP application over six years. Although soil sampling in the current study was carried out six years after the last RP fertilization (RP6), samples presented higher P-mod-labile content. Furthermore, Soltangheisi et al. (2018) have calculated the fertilizer mass rate to be applied based on 9% soluble P content in comparison to the one adopted in the current study, whose total P content in RP was 29%.

Nevertheless, there was no significant P-mod-labile accumulation in RP20, mainly due to lower P rate (118 kg ha^{-1} of P) and to time interval since the last fertilization (20 years).

Although the lesser available P fractions were changed by treatments, the P-labile fraction was not influenced by P application. This outcome indicates that fractions presenting lesser lability were not capable of buffering the P extraction labile pool, mainly in RP6, likely due to time interval since the last fertilization (six years for TP6 and RP6, and 20 years for TP20 and RP20). The replacement of P deriving from fractions with minor-to-greater lability tends to reduce over time; therefore, higher P contents were no longer found in TP6 and RP6.

Phosphate rock recalcitrance in grassland soil

The thermodynamic conditions observed in the soil were favorable to the dissolution of both hydroxyapatite and carbonate-fluorapatite minerals identified in RP. In this case, theoretically, there would be P dissolution and release from RP into soil solution and, consequently, plant uptake or inner-sphere adsorption by soil mineral matrix. The favorable thermodynamic conditions of the soil result from moderately-acid soil solution pH (6.1) and from low Ca^{2+} and P contents at the topsoil layer (0-2.5 cm). Soil solution pH was higher than that observed at the beginning of the experiment (4.5 at 0-20 cm topsoil layer) or than the value (5.03) observed at the 0-2.5 cm topsoil layer (Somavilla et al., 2021a). Besides, Ca^{2+} and P levels in soil solution of superficial layers are often high due to constant nutrient cycling in the soil profile, as well as to chemical reactions and organic compound exudation in the rhizosphere zone (De Conti et al., 2018; Hinsinger et al., 2009). Nevertheless, pH, Ca^{2+} and P were low enough to provide thermodynamic conditions to dissolve carbonate-fluorapatite and hydroxyapatite, in the present case.

Even in case of favorable conditions, RP dissolution in the soil is not guaranteed at viable timescale, since physical-chemical phenomena associated with soil water regime, as well as with internal and external features of apatite particles (e.g., composition, solubility, specific surface area and surface defects) accounting for determining the timescale of dissolution processes, will take place (Dorozhkin, 2012). The RP used as P source in the current study had more than 75% coarse particles (2,000-50 µm) and 4% particles smaller than 2 µm. Larger particles have lesser specific surface area for chemical reactions; therefore, they are lesser reactive and more recalcitrant in the soil (Mackay and Syers, 1986; Rafael et al., 2018; Syers et al., 1986). Moreover, the self-inhibiting dissolution mechanism takes place when the surface of the mineral particle starts the process of dissolving and releasing Ca2+ and P, which raises soil pH. Low Ca^{2+}, P and H^{+} diffusion in soil solution enables the formation of a zone with high pH, and Ca^{2+} and P saturation levels around the particle. This zone changes the thermodynamic conditions and inhibits, or retards, the continuity of dissolution reactions (Dorozhkin, 2012; Mafé et al., 1992). In case the Ca^{2+}, P and soil pH in this region do not change under these conditions, RP particles do not meet the thermodynamic conditions for dissolution process and remain crystallized in the soil over time.

Available P extraction by AER and Mehlich-1 in bulk soil presented similarity among RP20, WP, TP6 and TP20, as well as difference towards RP6 (Figure 5a). This outcome is associated with the presence of apatite particles in the soil six years after fertilization (RP6) and with lack of it near 20 years after RP application. Mehlich-1 extractor enabled the dissolution of Ca-P compounds, such as hydroxyapatite and carbonate-fluorapatite from RP, due to its acidity (Freitas et al., 2013). Consequently, ΔP in RP6 resulted from apatite dissolution by the acid extractor. Mehlich-1 extraction results corroborated that observed for 0.5 mol L^{-1} HCl extraction. However, both solutions (Mehlich-1 and 0.5 mol L^{-1} HCl) have significantly differ in mineral dissolution power.

The bulk soil was subjected to physical fractionation in order to favor apatite particles' identification and to define P availability in each particle size fraction using RP. The experimental soil was a sandy soil comprising more than 70% of sand. However, the low rate of identified clay fractions (4% in RP treatments) was a major contribution to P content available in the soil, especially in RP6 (Figures 5b e c). This behavior mainly resulted from increased charge density and from a larger number of reactive functional groups with decreasing particle size. Moreover, clay particles in the analyzed soil comprised 1:1 clay minerals (kaolinite) and iron oxides (goethite and hematite), smaller amounts of 2:1 non-swelling clay minerals (illite and vermiculite interlayered with hydroxy aluminum) and lower amounts of 2:1 swelling clay minerals (Bortoluzzi et al., 2015, 2007; Moterle et al., 2016).

The ΔP of clay particles in WP and RP20 were negative. Phosphorus contents obtained through Mehlich-1 extractor application in clay soils were always lower than that extracted through AER due to extractor consumption and P re-adsorption during the extraction process (Freitas et al., 2013; Mumbach et al., 2018). This effect was stronger in the current study due to exclusive use of clay-sized soil particles. However, despite the likelihood of having Mehlich-1 extractor exhaustion, positive ΔP was observed in clay-sized particles from RP6. This outcome reinforces the likelihood of apatite mineral remaining in clay-sized particles.

The highest P-AER and P-Mehlich-1 values observed in RP6, mainly in the clay fraction of it, in association with positive ΔP values, enabled inferring that recalcitrant apatite remained in the grassland six years after RP fertilization. In addition, the non-differentiated ΔP in particle size fractions has indicated the presence of apatite in all particle sizes. However, such a presence was non-existent or could not be detected through the herein adopted techniques 20 years after fertilization (RP20). Similar results were observed in cropped clayey soil in Southern Brazil: P content extracted from soil by using 1 mol L^{-1} of HCl six years after RP was several times higher than that extracted from soil treated with soluble fertilizer (Soltangheisi et al., 2018). In Australia, the presence of apatite minerals was also identified five years after RP application on acidic soils (Kumar et al., 1994).

RP use in all cases has led to P-legacy effect due to soil P accumulation in low-lability forms (i.e., Ca-P compounds). This effect is not desirable in the long-term, since the applied P will no longer be available for plant uptake and there will be no increase in dry matter production. In some cases, specific plant species are capable of solubilizing Ca-P compounds and of mining P immobilized in low labile forms (Hallama et al., 2019; Soltangheisi et al., 2020). Strategies used by plants to get around P availability issues in the soil are mainly linked to changes in the rhizosphere environment due to H^+ exudation and Ca^{2+} absorption (Arcand and Schneider, 2006; Hinsinger and Gilkes, 1995) or to association with arbuscular mycorrhizal fungi or P-solubilizing bacteria (Magallon-Servin et al., 2020; Taktek et al., 2015). RP use is likely economically and environmentally viable for these plants.

Although RP distribution was chemically identified in all RP6 particle size fractions, apatite particles were only observed in the coarse sand and silt fractions. It may have happened due to two main factors. First, coarse particles (2000-50 µm) were more recalcitrant because their greater crystallinity and low specific surface area make it easier observing them at SEM; Second, CSand and CSilt particle fractionation during density fractionation of soil particles was favored when particles were fully dispersed. Moreover, clay, FSilt and CSilt fractions were sequentially fractionated through sedimentation (Stoke's law). This process may have favored apatite particle concentrations in the CSilt fraction, since apatite's density is higher than that of soil particles.

Nevertheless, the presence of RP crystalline apatite particles in RP6 was confirmed through SEM-EDS. Particles identified six years after RP application on soil surface presented few signs of dissolution pits, as reported in the literature (Kumar et al., 1994). This outcome suggests that the recalcitrance of these minerals has widely exceeded within six years, but it did not reach 20 years, as analytically tested in RP20.

Based on SEM-EDS, the analytical identification and confirmation of apatite minerals' persistence in their crystalline form have substantiated the current hypothesis, namely: it is the factor explaining the lower dry matter yield observed for RP-fertilized grassland than that of TP-fertilized grassland over the 20 years, even forthwith P fertilization (Somavilla et al., 2021b).In addition, lower P balance efficiency in field crops (Soltangheisi et al., 2020) or in flooded fields (Fukuda et al., 2013) and lower dry matter yield in pastures (Bolland et al., 1997; Oliveira et al., 2015; Soares et al., 2000) were reported as resulting from direct RP use as P fertilizer. Thus, it is evident that the use of phosphate rock without any treatment is not a good option to help increasing P use efficiency and forage yield. Based on the literature, some strategies used to "activate" phosphate rock, such as reducing phosphate rock particle to size smaller than 20 µm or phosphate rock acidulation by mixtures added with soluble P fertilizers, are suggested as options to maximize RP dissolution and overcome low P release and availability issues (Bindraban et al., 2020).

7.5.Conclusion

The current study has evaluated soil P-legacy and low apatite dissolution in experiment comprising 20 years of fertilization with P sources and limestone. P-legacy was differentiated based on the solubility of the used P source. The use of soluble P source and limestone (triple superphosphate at rate of 250 kg ha^{-1} of P add with limestone at rate of 3.2 Mg ha^{-1}) resulted in greater nutrient use efficiency (five times greater than that of phosphate rock) and in higher dry matter yield (22%). Consequently, there was also greater P exportation through plant tissue and lesser residual effect of P fertilizer on the topsoil (0-2.5 cm).

In addition, the use of phosphate rock (at rate of 250 kg ha^{-1} of P) has led to higher total P and moderate-lability P levels in the soil. It happened due to low apatite dissolution (even under favorable soil thermodynamics conditions) in coarse sand and coarse silt particle size fractions, which was analytically checked and subjected to Scanning Electron Microscopy (SEM-EDS). Apatite stability, in its crystalline form, has exceeded six years under the evaluated soil conditions, although it lasted shorter than 20 years. Therefore, the lower grassland dry matter production can be partly attributed to low phosphate rock dissolution. Apparently, the use of sedimentary phosphate rock from Gafsa – Tunisia was not a satisfactory alternative to increase South American Pampa grassland yield.

It is essential adopting P fertilizers and management practices considered environmentally friendly and capable of promoting Pampa Grassland yield in order to maintain ecosystem’s balance and to avoid replacing natural areas. Thus, further studies focused on identifying trends in C and N stock, ecosystem’s resilience and yield by use of P fertilizers and limestone in South American Pampa grassland should be conducted.

7.6.Conflicts of interest

The authors declare no conflict of interest.

7.7.Acknowledgment

We are grateful to Celine Boissard, for her assistance in SEM-EDS analysis, as well as to all students and researchers who helped keeping the experimental device active. The authors acknowledge the financial support granted by the European Union (ERDF) and by "Région Nouvelle Aquitaine".

7.8.Funding

This research was supported by the National Council for Scientific and Technological Development – CNPq-Brazil (Projects PVE 400887/2014-2; AS fellowship: GM/GD 140270/2019-1; DRS fellowship: 309515/2015-7; and ECB fellowship: 304676/2019-5).

7.9.References

Andrade, B.O., Bonilha, C.L., Overbeck, G.E., Vélez-Martin, E., Rolim, R.G., Bordignon, S.A.L., Schneider, A.A., Vogel Ely, C., Lucas, D.B., Garcia, É.N., dos Santos, E.D., Torchelsen, F.P., Vieira, M.S., Silva Filho, P.J.S., Ferreira, P.M.A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V.D., Boldrini, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. Appl. Veg. Sci. 22, 168–184. https://doi.org/10.1111/avsc.12413

Arcand, M.M., Schneider, K.D., 2006. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. An. Acad. Bras. Cienc. 78, 791–807. https://doi.org/10.1590/S0001-37652006000400013

Barrow, N.J., 1999. The four laws of soil chemistry: the Leeper lecture 1998. Aust. J. Soil Res. 37, 787–829. https://doi.org/10.1071/SR99114

Bindraban, P.S., Dimkpa, C.O., Pandey, R., 2020. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol. Fertil. Soils 56, 299–317. https://doi.org/10.1007/s00374-019-01430-2

Boitt, G., Black, A., Wakelin, S.A., McDowell, R.W., Condron, L.M., 2018. Impacts of long-term plant biomass management on soil phosphorus under temperate grassland. Plant Soil 1–12. https://doi.org/10.1007/s11104-017-3429-0

Bolan, N.S., Adriano, D.C., Naidu, R., 2003. Role of Phosphorus in (Im)mobilization and Bioavailability of Heavy Metals in the Soil-Plant System, in: Reviews of Environmental Contamination and Toxicology. Springer, New York, p. 177. https://doi.org/10.1007/0-387-21725-8_1

Bolland, M.D.A., Lewis, D.C., Gilkes, R.J., Hamilton, L.J., 1997. Review of Australian phosphate rock research. Aust. J. Exp. Agric. 37, 845–859. https://doi.org/10.1071/EA97144

Bortoluzzi, E.C., Pérez, C.A.S., Ardisson, J.D., Tiecher, T., Caner, L., 2015. Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. Appl. Clay Sci. 104, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Bortoluzzi, E.C., Pernes, M., Tessier, D., 2007. Interestratificado caulinita-esmectita em um argissolo desenvolvido a partir de rocha sedimentar do sul do Brasil. Rev. Bras. Cienc. do Solo 31, 1291–1300. https://doi.org/10.1590/S0100-06832007000600008

Brookes, P.C., Powlson, D.S., 1981. Preventing phosphorus losses during perchloric acid digestion of sodium bicarbonate soil extracts. J. Sci. Food Agric. 32, 671–674. https://doi.org/10.1002/jsfa.2740320707

Condron, L.M., Goh, K.M., Newman, R.H., 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. J. Soil Sci. 36, 199–207. https://doi.org/10.1111/j.1365-2389.1985.tb00324.x

Conte, E., Anghinoni, I., Rheinheimer, D.S., 2003. Frações de fósforo acumuladas em latossolo argiloso pela aplicação de fosfato no sistema plantio direto. Rev. Bras. Ciência do Solo 27, 893–900. https://doi.org/10.1590/S0100-06832003000500014

Cross, A.F., Schlesinger, W.H., 1995. A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64, 197–214.

De Conti, L., Ceretta, C.A., Tiecher, T.L., Silva, L.O.S., Tassinari, A., Somavilla, L.M., Mimmo, T., Cesco, S., Brunetto, G., 2018. Growth and chemical changes in the rhizosphere of black oat (Avena strigosa) grown in soils contaminated with copper. Ecotoxicol. Environ. Saf. https://doi.org/10.1016/j.ecoenv.2018.07.045

Dias, L.P.R., Gatiboni, L.C., Brunetto, G., Simonete, M.A., Bicaratto, B., 2015. Relative efficiency of rock phosphates in fertilization of planting seedlings Eucalyptus dunnii maiden and Eucalyptus benthamii maiden et cambage in soil with and without liming. Ciência Florest. 25, 37–48. https://doi.org/http://dx.doi.org/10.5902/1980509817443

Dorozhkin, S. V, 2012. Dissolution mechanism of calcium apatites in acids: A review of literature. World J. Methodol. 2, 1. https://doi.org/10.5662/wjm.v2.i1.1

Freitas, I.F., Novais, R.F., Villani, E.M.A., Novais, S.V., 2013. Phosphorus extracted by ion exchange resins and Mehlich-1 from Oxisols (Latosols) treated with different phosphorus rates and sources for varied soil-source contact periods. Rev. Bras. Ciência do Solo 37, 667–677. https://doi.org/http://dx.doi.org/10.1590/S0100-06832013000300013

Fukuda, M., Nagumo, F., Nakamura, S., Tobita, S., 2013. Ineffectiveness of directly applied Burkina Faso phosphate rock on rice growth. Soil Sci. Plant Nutr. 59, 403–409. https://doi.org/10.1080/00380768.2013.783770

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Brunetto, G., Saggin, A., Flores, J.P.C., 2000. Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. Pesqui. Agropecu. Bras. 35, 1663–1668. https://doi.org/10.1590/S0100-204X2000000800020

Guidry, M.W., Mackenzie, F.T., 2003. Experimental study of igneous and sedimentary apatite dissolution: Control of pH, distance from equilibrium, and temperature on dissolution rates. Geochim. Cosmochim. Acta 67, 2949–2963. https://doi.org/10.1016/S0016-7037(03)00265-5

Gumiere, T., Rousseau, A.N., Costa, D.P., Cassetari, A., Cotta, S.R., Andreote, F.D., Gumiere, S.J., Pavinato, P.S., 2019. Phosphorus source driving the soil microbial interactions and improving sugarcane development. Sci. Rep. 9, 1–9. https://doi.org/10.1038/s41598-019-40910-1

Hallama, M., Pekrun, C., Lambers, H., Kandeler, E., 2019. Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 434. https://doi.org/10.1007/s11104-018-3810-7

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Sci. Soc. Am. J. 46, 970. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321, 117–152. https://doi.org/10.1007/s11104-008-9885-9

Hinsinger, P., Gilkes, R.J., 1995. Root-induced dissolution of phosphate rock in the rhizosphere of lupins grown in alkaline soil. Aust. J. Soil Res. 33, 477–489. https://doi.org/10.1071/SR9950477

Houben, D., Michel, E., Nobile, C., Lambers, H., Kandeler, E., Faucon, M.P., 2019. Response of phosphorus dynamics to sewage sludge application in an agroecosystem in northern France. Appl. Soil Ecol. 137, 178–186. https://doi.org/10.1016/j.apsoil.2019.02.017

Kim, J., Li, W., Philips, B.L., Grey, C.P., 2011. Phosphate adsorption on the iron oxyhydroxides goethite (α-FeOOH), akaganeite (β-FeOOH), and lepidocrocite (γ-FeOOH): A31P NMR Study. Energy Environ. Sci. 4, 4298–4305. https://doi.org/10.1039/c1ee02093e

Kumar, V., Gilkes, R.J., Armitage, T.M., Bolland, M.D.A., 1994. Identification of residual P compounds in fertilized soils using density fractionation, X-ray diffraction, scanning and transmission electron microscopy. Fertil. Res. 37, 133–149. https://doi.org/10.1007/BF00748554

Lindsay, W.L., 1979. Chemical equilibria in soil. The Blackburn Press, Caldwell, New Jersey.

Mackay, A.D., Syers, J.K., 1986. Effect of phosphate, calcium, and pH on the dissolution of a phosphate rock in soil. Fertil. Res. 10, 175–184. https://doi.org/10.1007/BF01074371

Mafé, S., Manzanares, J.A., Reiss, H., Thomann, J.M., Gramain, P., 1992. Model for the dissolution of calcium hydroxyapatite powder. J. Phys. Chem. 96, 861–866. https://doi.org/10.1021/j100181a062

Magallon-Servin, P., Antoun, H., Taktek, S., Luz, E., 2020. Designing a multi-species inoculant of phosphate rock-solubilizing bacteria compatible with arbuscular mycorrhizae for plant growth promotion in low-P soil amended with PR. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-020-01452-1

Marques, A.C.R., Oliveira, L.B., Brunetto, G., Tavares, M.S., Quadros, F.L.F., Nicoloso, F.T., 2020. Interaction between growth strategies and phosphorus use efficiency in grasses from South America natural grasslands. Rev. Ceres 67, 62–69. https://doi.org/10.1590/0034-737x202067010009

Mehlich, A., 1953. Determination of P, Ca, Mg, K, Na and NH4 by North Carolina Soil Testing Laboratoris. Raleigh Univ. North Carolina.

Moterle, D.F., Kaminski, J., Rheinheimer, D.S., Caner, L., Bortoluzzi, E.C., 2016. Impact of potassium fertilization and potassium uptake by plants on soil clay mineral assemblage in South Brazil. Plant Soil 406, 157–172. https://doi.org/10.1007/s11104-016-2862-9

Mumbach, G.L., Oliveira, D.A., Warmling, M.I., Gatiboni, L.C., Mumbach, G.L., Oliveira, D.A., Warmling, M.I., Gatiboni, L.C., 2018. Quantificação de fósforo por Mehlich 1, Mehlich 3 e Resina Trocadora de Ânions em solos com diferentes teores de argila. Rev. Ceres 65, 546–554. https://doi.org/10.1590/0034-737x201865060010

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. ACTA 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Oliveira, T.E., Freitas, D.S., Gianezini, M., Ruviaro, C.F., Zago, D., Mércio, T.Z., Dias, E.A., Lampert, V.N., Barcellos, J.O.J., 2017. Agricultural land use change in the Brazilian Pampa Biome: The reduction of natural grasslands. Land use policy 63, 394–400. https://doi.org/10.1016/j.landusepol.2017.02.010

Oliveira, L.B., Soares, E.M., Jochims, F., Tiecher, T., Marques, A.R., Kuinchtner, B.C., Rheinheimer, D.S., Quadros, F.L.F., 2015. Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil. Rangel. Ecol. Manag. 68, 445–452. https://doi.org/10.1016/j.rama.2015.07.012

Oliveira, L.B., Tiecher, T., Quadros, F.L.F., Trindade, J.P.P., Gatiboni, L.C., Brunetto, G., Santos, D.R., 2014. Formas de fósforo no solo sob pastagens naturais submetidas à adição de fosfatos. Rev. Bras. Cienc. do Solo 38, 867–878. https://doi.org/10.1590/S0100-06832014000300018

Pavinato, P.S., Rodrigues, M., Soltangheisi, A., Sartor, L.R., Withers, P.J.A., 2017. Effects of cover crops and phosphorus sources on maize yield, phosphorus uptake, and phosphorus use efficiency. Agron. J. 109, 1039–1047. https://doi.org/10.2134/agronj2016.06.0323

Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A., Lambers, H., 2007. Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. New Phytol. 173, 181–190. https://doi.org/10.1111/j.1469-8137.2006.01897.x

Ptáček, P., 2016. Apatites and their Synthetic Analogues - Synthesis, Structure, Properties and Applications, Apatites and their Synthetic Analogues - Synthesis, Structure, Properties and Applications. InTech, Rijeka. https://doi.org/10.5772/59882

Pufahl, P.K., Groat, L.A., 2017. Sedimentary and igneous phosphate deposits: Formation and exploration: An invited paper. Econ. Geol. 112, 483–516. https://doi.org/10.2113/econgeo.112.3.483

Rafael, R.B.A., Fernandez-Marcos, M.L., Cocco, S., Ruello, M.L., Weindorf, D.C., Cardelli, V., Corti, G., 2018. Assessment of Potential Nutrient Release from Phosphate Rock and Dolostone for Application in Acid Soils. Pedosphere 28, 44–58. https://doi.org/10.1016/S1002-0160(17)60437-5

Rheinheimer, D.S., Fornari, M.R., Bastos, M.C., Fernandes, G., Santanna, M.A., Calegari, A., Beatriz, L., Canalli, S., Caner, L., Labanowski, J., 2019. Phosphorus distribution after three decades of different soil management and cover crops in subtropical region. Soil Tillage Res. 192, 33–41. https://doi.org/10.1016/j.still.2019.04.018

Rheinheimer, D.S., Anghinoni, I., Conte, E., 2003. Sorção de fósforo em função do teor inicial e de sistemas de manejo de solos. Rev. Bras. Ciência do Solo 27, 41–49. https://doi.org/10.1590/s0100-06832003000100005

Robinson, J.S., Syers, J.K., 1990. A critical evaluation of the factors influencing the dissolution of Gafsa phosphate rock. J. Soil Sci. 41, 597–605.

Roy, E.D., Richards, P.D., Martinelli, L.A., Coletta, L.D., Lins, S.R.M., Vazquez, F.F., Willig, E., Spera, S.A., VanWey, L.K., Porder, S., 2016. The phosphorus cost of agricultural intensification in the tropics. Nat. Plants 2, 2–7. https://doi.org/10.1038/NPLANTS.2016.43

Silveira, T.C., Pegoraro, R.F., Kondo, M.K., Portugal, A.F., Resende, Á.V., 2018. Sorghum yield after liming and combinations of phosphorus sources. Rev. Bras. Eng. Agric. e Ambient. 22, 243–248. https://doi.org/10.1590/1807-1929/agriambi.v22n4p243-248

Soares, W.V., Lobato, E., De Sousa, D.M.G., Rein, T.A., 2000. Avaliação do fosfato natural de gafsa para recuperação de pastagem degradada em latossolo vermelho-escuro. Pesqui. Agropecu. Bras. 35, 819–825. https://doi.org/10.1590/s0100-204x2000000400020

Soltangheisi, A., Rodrigues, M., Coelho, M.J.A., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2018. Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res. 179, 20–28. https://doi.org/10.1016/j.still.2018.01.006

Soltangheisi, A., Teles, A.P.B., Sartor, L.R., Pavinato, P.S., 2020. Cover Cropping May Alter Legacy Phosphorus Dynamics Under Long-Term Fertilizer Addition. Front. Environ. Sci. 8, 1–12. https://doi.org/10.3389/fenvs.2020.00013

Somavilla, A., Caner, L., Silva, I.C.B., Bastos, M.C., Moro, L., Schaefer, G.L., Gatiboni, L.C., Rheinheimer, D.S., 2021a. Chemical pattern of vegetation and topsoil of rangeland fertilized over 21 years with phosphorus sources and limestone. Soil Tillage Res. 205, 104759. https://doi.org/10.1016/j.still.2020.104759

Somavilla, A., Dessbesell, A., Rheinheimer, D.S., 2017. Centrifugation methodology to extract soil solution. Sci. Agrar. 18, 44. https://doi.org/10.5380/rsa.v18i3.52805

Somavilla, A., Marques, A.C.R., Caner, L., Oliveira, L.B., Quadros, F.L.F., Chabbi, A., Rheinheimer, D.S., 2021b. Phosphate fertilization and liming in a trial conducted over

21 years : A survey for greater forage production and Pampa pasture conservation. Eur. J. Agron. 125. https://doi.org/10.1016/j.eja.2021.126259

Syers, J.K., Mackay, A.D., Brown, M.W., Currie, L.D., 1986. Chemical and physical characteristics of phosphate rock materials of varying reactivity. J. Sci. Food Agric. 37, 1057–1064. https://doi.org/10.1002/jsfa.2740371102

Szilas, C., Semoka, J.M.R., Borggaard, O.K., 2007. Can local Minjingu phosphate rock replace superphosphate on acid soils in Tanzania? Nutr. Cycl. Agroecosystems 77, 257–268. https://doi.org/10.1007/s10705-006-9064-4

Taktek, S., Trépanier, M., Servin, P.M., St-Arnaud, M., Piché, Y., Fortin, J.-A., Antoun, H., 2015. Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus irregularis DAOM 197198. Soil Biol. Biochem. 90, 1–9. https://doi.org/10.1016/j.soilbio.2015.07.016

Tedesco, M.J., Gianello, C., Bissani, C.A., Bohnen, H., Volkweiss, S.J., 1995. Análises de solo, plantas e outros materiais, 2 Ed. ed. Porto Alegre.

Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. Grass Forage Sci. 69, 567–579. https://doi.org/10.1111/gfs.12079

Tiecher, T., Rheinheimer, D.S., Calegari, A., 2012. Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. Soil Tillage Res. 124, 57–67. https://doi.org/10.1016/j.still.2012.05.001

Toledo, M.C.M., Pereira, V.P., 2001. A variabilidade de composição da apatita associada a carbonatitos. Rev. do Inst. Geológico 22, 27–64.

USDA - United States Department of Agriculture, 2014. Keys to Soil Taxonomy, 12th ed. ed, USDA - United States Department of Agriculture. Washington, DC.

USEPA, 1971. Methods of chemical analysis for water and wastes. Cincinnati.

Yadav, N., Rani, K., Yadav, S.S., Yadav, D.K., Yadav, V.K., Yadav, N., 2018. Soil and Water Pollution with Fluoride, Geochemistry, Food Safety Issues and Reclamation-A Review. Int. J. Curr. Microbiol. Appl. Sci. 7, 1147–1162. https://doi.org/10.20546/ijcmas.2018.705.140

7.10. General comments

In chapter IV, we evaluate the soil P-legacy and low apatite dissolution in experiment comprising 20 years of fertilization with P sources and limestone due to the demand identified in chapters II and III. Here, we show a higher productivity of dry-matter and greater P use efficiency when using soluble fertilizer associated with limestone compared to the use of rock phosphate.

In addition, as suggested in chapters II and III, in chapter IV we prove that the highest levels of soil available P, obtained by Mehlich-1 extractor, when applying natural phosphate are, in fact, an artifact that occurred during the process of P extraction due to solubilization of recalcitrant apatite minerals. Moreover, apatite stability, in its crystalline form, has exceeded six years under the evaluated soil conditions, although it lasted shorter than 20 years. Therefore, the lower grassland dry matter production can be partly attributed to low phosphate rock dissolution. Another impact of this discovery is the need to reassess the methods currently used to quantify the soil available P when using rock phosphate as a fertilizer.

With the three articles discussed so far, it is evident that the use of sedimentary phosphate rock from Gafsa - Tunisia was not a satisfactory alternative to increase South American Pampa grassland yield. With the use of rock phosphates from other places, mainly phosphates from igneous core, it is unlikely that better results will be obtained, mainly due to the characteristics of greater crystallinity and less dissolution of the apatite minerals. Similarly, the use of rock phosphate in other soils has a high possibility of not showing great performances, since the tested soil is acidic and presented favorable conditions for the dissolution of the apatite minerals, which, even so, occurred only in a time scale greater than six years.Therefore, to increase soil fertility levels in Pampa biome in South America and increase the dry matter productivity of the grassland, it is recommended to use soluble sources of P (triple and simple superphosphate) associated with the use of dolomitic limestone. Thus, a poor soil, especially in P, will be able to withstand higher productivity, thereby avoiding the replacement of natural vegetation by exotic agricultural crops.

So far, three chapters have been presented referring to works carried out from a long-term experiment located in Brazil. In the sequence, two chapters will be presented referring to works carried out in a long-term experiment located in France. The realization of studies in two experiments, one in Brazil and the other in France, was a mutual choice between doctoral student and supervisors. This was chosen, due to the present thesis being carried out in a joint supervision (*cotutele*) between Brazilian and French university. Therefore, experimental data from both countries are being considered.

In the choice of experiments, it was recommended that both have in common the monitoring of forage production and the need to monitor the dynamics of P in the soil-plant system. Be it in soil with low content of total and available P, as is the case of the experiment in Brazilian soil, where it is necessary to introduce external sources of P; be it in soil with a high content of total and available P, as is the case of French soil experiment, in which, by government incentives, there has been a drastic reduction in the use of phosphate fertilizers in agriculture in recent decades.

Subsequently, chapters V aim to analyze historical dataset of export and depletion of P by plants, changes in soil P pools and the coupling of organic P in the dynamics of C and N at 0-30 cm topsoil layer. In chapter VI, we analyze the participation of the P from soil microbial biomass in the bioavailable soil P content during the barley growth season. As well as, we detail the distribution of soil aggregates and the different P pools in each of the different sizes of soil aggregates in order to characterize the microbial P turnover in the soil.

8. Chapter V - P stock depletion and soil C:N:P stoichiometry by annual crop or grassland management systems over 13 years

Abstract

Phosphorus nutrition is essential to plants yields and organic matter soil input. The maintenance of satisfactory soil available P contents is indispensable for plant growth. However, the constant extraction of P by plants leads to soil P stock depletion, reduced crop productivity and the entry of organic material into the soil. In this work, we monitored the temporal P stock depletion in a soil management system with forage and annual crop cultivation at a temperate soil with high P stock and without further P fertilization. As well as the relationship between soil organic P stocks, C and N in long-term. Thus, we analyzed trends in P, C and N stocks in the 0-30 cm topsoil layers of a Cambisol cultivated for 13 years with permanent cropland (CC) and permanent grassland (GG). We also characterized and evidenced changes in P organic forms by ^{31}P-NMR. The amount of P exported in 13 years was 10% greater in GG than CC (295 and 268 kg ha^{-1}, respectively). The total P stocks were decreased for both CC and GG management systems (0.30 and 0.25 Mg ha^{-1}, respectively). This depletion was perceived mainly in total Pi forms with a reduction of 0.75 and 0.29 Mg ha^{-1} in GG and CC, respectively. Differently, total Po stock increased 42.6% with GG. This corroborates with the increase of C and N stocks in GG (2.6 and 0.19 Mg ha^{-1}, respectively) and reduction in CC (-4.2 and -0.38 Mg ha^{-1}, respectively). A significant reduction was identified in P-labile forms (depletion of 32 and 23 % for GG and CC respectively). The C:N:Po atomic ratios reduced from 116:10:1 and 117:11:1 to 86:8:1 and 104:10:1 in 13 years of CC and GG cultivation, respectively. The results showed a greater export of P and, consequently, a greater depletion of total P stock in the treatment with permanent grassland. The depletion of P mainly impacted the P pool with greater lability (P-labile). However, the depletion occurred after 13 years was not enough to cause an evident reduction in plant yields due to the buffering capacity of the P pools with less lability (P-mod-labile). With permanent grassland, there was a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. This behavior provided a reduction in the C:N:Po atomic ratio values and, consequently, a coupling between the C, N and P cycles in the soil. ^{31}P-NMR technique revealed that permanent grassland had a more significant effect in reducing α–glycerophosphate and increase *myo*-IHP and adenosine monophosphate than permanent cropland.

Key-words – Forage; Dry matter; ^{31}P-NMR; P lability; P exportation; P pools; C and N stocks.

8.1.Introduction

Phosphorus (P) is a key element for all life cycles (Elser 2012). It corresponds to approximately 0.2 % of plants dry-matter (Schachtman et al. 1998) and is a constituent of proteins, nucleic acids and participates fundamentally in the transfer of energy in plants and animals (Elser 2012; Wang et al. 2017). Consequently, P nutrition is essential to the capacity of plants to incorporate carbon (C), produce dry matter and increase soil C stocks (i.e., soil organic matter-SOM). The plants P uptake occurs via root system and therefore, the maintenance of satisfactory soil available P contents is indispensable for plant growth (Lun et al. 2018; Tian et al. 2019).

The soil P occurs in inorganic (Pi) or organic (Po) forms. The inorganic forms of P are orthophosphate (HPO_4^{-2} and $H_2PO_4^{-}$) in soil solution; bound to cations, such as calcium, forming geogenic or anthropogenic calcium phosphates; adsorbed by inner-sphere at functional groups of clay minerals and Fe and Al oxi-hydroxides (Walker and Syers 1976; Helfenstein et al. 2018). The organic P is somehow bound to at least one C atom, forming mainly orthophosphate monoesters (e.g., inositol phosphates and adenosine monophosphate) and orthophosphate diesters (e.g., nucleic acids, phospholipids) constituting the soil organic matter and soil microbial biomass (George et al. 2018; Li et al. 2018; Liu et al. 2018).

Both P forms occur simultaneously in soil and have different availability for plants uptake (P lability). The quantification of P availability is commonly assessed by sequential extractions with chemical reagents of increasing extraction force (e.g., sequential extraction proposed by Hedley et al. (1982)) and grouped into P pools called P labile, P moderately labile and P non-labile. Naturally, the P uptake by plants comes from the most available pool (labile), which are buffered by the one with less lability (Tiecher et al. 2018). In addition, the turnover of each P pool decreases with a reduction in P lability, ranging from minutes to hundreds of years (Helfenstein et al. 2020). Thus, it is expected that the depletion of soil P by agricultural activities will be perceived in the short term in most labile pools and in medium-long term in less labile pools. The buffering of the available P varies according to the soil type and the management adopted (Fink et al. 2016). For example, in soils from tropical regions with larger weathering intensity, there may be high legacy-P but low availability to plants due to the characteristics of high P adsorption to the soil mineral matrix (Rodrigues et al. 2016; Withers et al. 2018). On the other hand, in soils from temperate regions, the P availability behavior tends to be facilitated due to the mineralogical characteristics of the soils resulting from the low pedogenetic evolution.

Although the P is absorbed by plants predominantly in orthophosphate ions form (Schachtman et al. 1998), the presence of Po in the soil has remarkable importance in the reduction of P inner-sphere adsorption to clay minerals and oxides and in mobilizing recalcitrant P (Guppy et al. 2005; Chassé and Ohno 2016; Rodrigues et al. 2016; Menezes-Blackburn et al. 2018). The transformation of Pi into Po occurs when plants and microorganisms immobilize Pi into their tissues. In soil with high P stock and major part of P adsorbed to inorganic constituent by inner-sphere complexation, some plant species (e.g., *Lolium sp* and *Lupinus sp*.) can mine and remobilize P by incorporation in the tissues (Kamh et al. 1999; Richardson et al. 2009). After death, the P returns to the soil mainly in organic form into SOM. Therefore, the presence of Pi or Po in the soil is determined by the content of SOM and can be changed by the soil management system adopted. For example, the cultivation of annual crops with soil plowing decreases the SOM (Bayer et al. 2002; Crème et al. 2018), even with NPK fertilization (Mathew et al. 2012; Abbas et al. 2020). However, the adoption of no-tillage systems and the variation of cultivated plant species, such as the introduction of legumes plants in the production system favor the SOM and the coupling of P, C and N cycles (Rumpel et al. 2015; Crème and Rumpel 2016; Abbas et al. 2020). Moreover, soils cultivated under pastures and NPK fertilized can also increase or preserve SOM stocks over time (Poeplau et al. 2018) and consequently tend to preserve Po contents.

The dynamics of SOM is a key factor that drives the cycles and the balance between C, N and P. Before the uptake of P by plants, a step of Po mineralization is necessary and, this process proceed differently according to the organic compound’s recalcitrance. In this case, the orthophosphate monoesters have low turnover than the orthophosphate diesters due to its chemical bonding to soil colloids (Turner et al. 2005; Menezes-Blackburn et al. 2018). This comportment may induce a higher accumulation of Po monoesters form than in diesters form in the SOM.

In general, soil with high P stock, from fertilizers inputs or soil parent material, may supply plant demand and maintain plant yields for long period, even without P fertilization. However, the absence of phosphate fertilization associated to constant P exportation generates a negative soil P balance resulting in a decrease of soil P stocks and availability (Boitt et al. 2018; Tyson et al. 2020). This can cause restrictions on crop yields and the maintenance or accumulation of SOM in a time scale dependent on the P export and on the level of initial plant-available P stock. Thus, the understanding of P depletion in soil under different management systems may be one of the main strategies that allow to bypass the negative effects of P deficiency in plant growth and produtivity. The main questions of this study are: How does the soil P stock change over time in forage and annual crop soil management system without P fertilization in a temperate climate agroecosystem? What are the changes in the relationships between the cycles and stocks of P, C and N in such a management system?

In a long-term field experiment, we addressed two hypotheses: 1) Soil growing with permanent grassland have a greater depletion of P stock due to the P exportation via dry matter. 2) There is a great increase of soil organic P pool in permanent grassland because of its coupling with C and N into SOM. For this, we analyzed the soil organic matter trends and the depletion of P stocks and its availability over time in a long-term field experiment carried out with contrasting soil management systems.

8.2.Material and methods

Experimental field

The experimental field was established at the environmental observatory of INRA center of Lusignan - France (Agroecosystems, Biogeochemical Cycles and Biodiversity, SOERE–ACBB; www.soere-acbb.com) (46°25'12,91"N; 0°07'29,35"E). The climate of the region is characterized by mean annual temperature of 12ºC and mean annual precipitation of 750 mm. The soil of the site is classified as Cambisol with two main characteristics of horizons: upper soil horizons with loamy texture and lower soil horizons with clayey rubefied horizons, rich in iron oxides (goethite and hematite) and kaolinite. Before the beginning of the experiment the area was homogenized by three annual crops (maize - wheat - barley). More details of the experimental field are disponible in Moni et al. (2010), Senapati et al. (2016) and Hubert et al.(2012).

The experiment was initialized in 2005 with complete randomized block design and four replicates. The treatments analyzed in this work were: permanent cropland (CC) with maize-wheat-barley rotation and, permanent grassland (GG) (Figure 1). In the permanent cropland *Zea mays* L. (maize) was sown with a density of 8.5 seeds m^{-2}; *Triticum aestivum* L. (wheat) with density of 150 seeds m^{-2} and *Hordeum vulgare* L. (barley) with density of 165 seeds m^{-2}. In the permanent grassland a mixture of *Dactylis glomerata L.* (cocksfoot), *Festuca arundinacea* Schreb (tall fescue) and *Lolium perenne* L. (ryegrass) was utilized.

In CC, annually soil plowing was carried out before sowing of each crop. The grassland was mowed 3-5 times a year with a tractor trimmer and all aerial biomass plants been removed. No animal grazing was performed in the area after the experiment beginning. Over time, no P and K fertilizations were performed, and N fertilization was performed according to biomass removal and soil analysis (Figure 1).

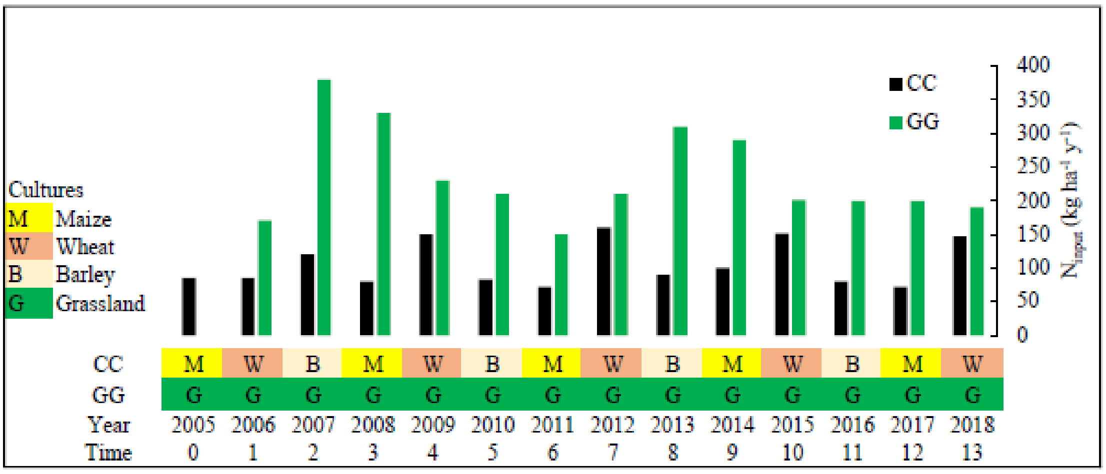


Figure 1 - Temporal progress of treatments and N fertilizer inputs in the field trial. CC–Permanent cropland and GG-permanent grassland.

Phosphorus and vegetation outputs

The vegetation biomass was measured just before harvest (once a year for cereals and 3-5 times a year for grassland) in 7.5 m^2. The biomass was oven dried at 60 °C to dry matter quantification. After, P dry matter concentration was analyzed by ICP-Radial. The P exportation was calculated by the product of element's concentration and the dry matter production.

Soil sampling

The soil sampling was carried out in 2005, 2011, 2014 and 2017 (i.e., at the beginning of the experiment and, 6, 9 and 12 years after) at a depth of 0-30 cm to quantify C and N. To quantify P, samples from 2005, 2011, 2014 and 2018 (i.e., at the beginning of the experiment and, 6, 9 and 13 years after) were taken in a depth of 0-30 cm. For each sample, five subsamples were sampled and mixed to obtain one representative field composite sample per plot. After sampling, the soil was oven dried at 65ºC, sieved at 2 mm and stored for the chemical analyzes.

In 2005, 2010 and 2016 the bulk density of the topsoil (0-30 cm) was determined by soil sampling with a cylinder of known volume. After drying at 105 ºC for 24h, the soil was weighted, and the density obtained by the ratio of soil mass and volume of the cylinder.

Soil chemical analyzes

Total P (Pt) was obtained by ignition method at 550 ºC during 2h (Walker and Adams 1958) and extraction with 1 mol L^{-1} H_2SO_4 during 24 h. The EnviroMAT SS-2 standard soil (nº 140-025-002) was used as a reference for the method and a recover of 96% of the total P was achieved. The total organic P (Po) was measured as follows: two subsamples were weighed, one of them was ignited at 550 ºC during 2h and the other one was not ignited. The P was then extracted in both subsamples with 10 mL of 0.5 mol L^{-1} H_2SO_4 by shaking for 24 h. The Po corresponds to the difference of P concentration in both subsamples. The total inorganic P (Pi) was quantified as a difference between Pt and Po.

The P lability was assessed using a five-step sequential extraction protocol (Hedley et al. 1982) modified by Rheinheimer et al. (2000). Briefly: 0.5 g of soil was weighted in a falcon tube and P was sequentially extracted by: a) Anion Exchange Resin (AER) membrane (AR 103 plate 434 QDP); b) 10 mL of 0.5 mol L^{-1} $NaHCO_3$ by shaking for 16 h; c) 10 mL of 0.1 mol L^{-1} NaOH by shaking for 16 h; d) 10 mL of 1 mol L^{-1} HCl by shaking for 16 h and, e) 10 mL of 0.5 mol L^{-1} NaOH by shaking for 16 h. In the alkaline extracts ($NaHCO_3$ and NaOH) the Pi was quantified by molybdate colorimetry method at 700 nm (Dick and Tabatabai 1977) and the Pt was determined after mineralization with ammonium persulfate (7.5 %) + H_2SO_4 (1:1; v/v) in autoclave at 121°C for two hours (USEPA 1971). The Po was obtained by the difference between Pt and Pi. The Pt of mineralized alkaline extractions and Pi in acid reagents were measured by colorimetry at 882nm after formation of an acid phospho-molybdate complex (Murphy and Riley 1962).

For P lability analyses were considered:

P-labile = Pi_{AER} + Pi_{NaHCO3} and Po_{NaHCO3};

P-moderately labile (P-mod-labile) = $Pi_{NaOH0.1}$ and $Po_{NaOH0.1}$ + Pi_{HCl};

P-non-labile = $Pi_{NaOH0.5}$ and $Po_{NaOH0.5}$;

The total soil C and N contents were determined by elementary analyzes (Flash EA, Thermo Electron Corporation, Bremen, Germany). For this, the soil was milled to homogenize and placed in tin capsules that were transferred to the combustion chamber at a temperature of 975 °C. As there is no carbonate in the soil and all the C was considered as organic C (Crème et al. 2018).

The P, C and N were analyzed as soil stock and obtained considering concentration and soil bulk density for the depth of 0–30 cm. The following soil atomic ratios C:Po and N:Po were obtained according to the equation described by (Xue et al. 2019):

$$\text{Atomic Ratio} = \frac{\text{Mass Ratio}}{\text{Atomic Weight Ratio}} \qquad \text{Equation 1}$$

With the following atomics weights: C: 12.0107, N: 14.0067 and P: 30.9738 g mol^{-1}.

^{31}P – Nuclear magnetic resonance (^{31}P-NMR)

A composite sample per treatment was used to perform the ^{31}P-NMR analyses. The ^{31}P-NMR analyses were just performed for the samples collected at the beginning of the experiment in 2005 and in 2018. The P was extracted with 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA (Cade-Menun and Liu 2014). Briefly: suspensions containing 1 g of soil from each replicate of treatments and 10 mL of 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA were prepared directly in centrifuge tubes. The tubes were shaken for 4 hours and centrifuged (3000 x g for 15 min). The extract from the four replications were homogenized and the Pt, Po and Pi contents of the extract were quantified using the above-mentioned methods. Afterwards, the extracts were freeze dried. To perform the ^{31}P-NMR analyses, the lyophilized extracts were re-dissolved in 2.7 mL 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA and 0.3 mL D_2O and stirred in vortex for 5 min. After 120 min of contact, the supernatant was separated by centrifugation (3000 x g for 15 min), filtered (<45 µm) and transferred into a 10 mm NMR tube. The ^{31}P-NMR spectrum was obtained with a Burker Advance DPX 400 spectrometer operating at a frequency of 162 MHz with proton decoupling. A pulse angle of 90º, temperature of 20 ºC, acquisition time of 0.5 seconds and relaxation time of 2 seconds were used. The number of scans ranged from 27.000 to 32.000. The peak area integration was carried out electronically using a baseline of 10 Hz using the TopSpin 3.6.2 Bruker software. The following phosphate compounds were identified: orthophosphate (7.10 ppm), monoester (6.33 to 5.09 ppm), diester (2.00 to -1.00 ppm), pyrophosphate (-3.14 to -3.58 ppm) and adenosine diphosphate (-9.36 to -9.41 ppm). The monoesters compounds were separated in *myo*- inositol hexakisphosphate (*myo*-IHP), *scyllo*-inositol hexakisphosphate (*scyllo*-IHP), α–glycerophosphate, β–glycerophosphate and adenosine monophosphate (Turner et al. 2003; Turner and Richardson 2004; Doolette et al. 2009; Cade-Menun 2015).

Statistical analyses

The variables were analyzed in randomized complete block design with split-plot in time and four replications. The soil management was considered as a main factor (plot) and the management time as a secondary factor (split-plot). The statistical model used is described at equation 2.

$$Y_{ijk} = \mu + B_i + T_j + error\ a(j,i) + R_k + error\ b(i,k) + (TR)_{ij} + error\ c(i,j,k)$$

Equation 2

Where Y is the observation; µ is the overall experimental average; B is the blocks (i = 1, 2, 3, 4); T is the soil managements (j = 1 and 2); R is the management time (k = 1, 2, 3, 4) and $error$ is the experimental error.

The soil management effects were tested by Scott-Knott test ($P \leq 0.05$) with use of complex variance. To test the time effects, the variables were submitted to regression analysis ($p \leq 0.05$).

8.3.Results

Yields and nutrients exportation

The mean of dry matter exportation was 8.0 and 7.9 Mg ha^{-1} y^{-1} for CC and GG, respectively, leading to a cumulated exportation of 112 and 110 Mg ha^{-1} (for CC and GG, respectively) at the end of the evaluated period (2005-2018). In the first years (2005- 2008) the dry matter exported by GG was 40% higher than for CC. However, after this period there was an inversion in the weight of dry matter exported. Over time there was a decrease in productivity in both treatments, especially in GG (Figure 2A).

The mean rate of P exportation was 19.1 and 21.0 kg ha^{-1} yr^{-1} for CC and GG, respectively. Consequently, the cumulated amount of P exported was 10% greater in GG than

CC (295 and 268 kg ha^{-1}, respectively) (Figure 2B). There was no tendency of reduction of P concentration in the dry matter, with means concentration of 0.23 and 0.28% for CC and GG, respectively.

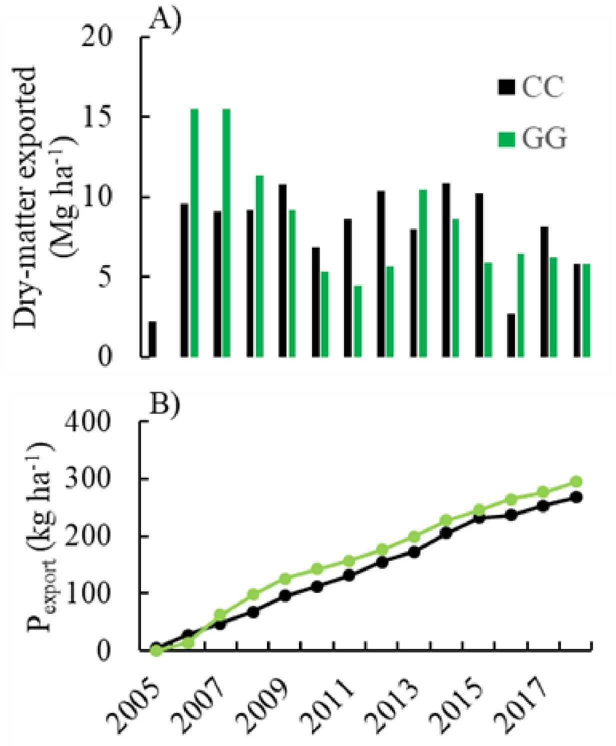


Figure 2 – Exportation of A) annual dry matter and B) cumulated phosphorus from trial field over 13 years of cultivation. CC-permanent cropland and GG-permanent grassland.

Total carbon, nitrogen, and phosphorus stocks

Similar temporal comportment in C and N stocks were observed for GG and CC (Figure 3). The GG improved 5.5% of C stock (increase of 2.6 Mg ha^{-1} in 12 years). Whereas CC promoted a reduction of 8.5% in C stock (decrease of 4.2 Mg ha^{-1} in 12 years). For N, the GG increased 3.7% (0.19 Mg ha^{-1}) and CC reduced 7.4% (0.38 Mg ha^{-1}) in the stocks.

The total P stocks decreased for both CC and GG management systems. Linear decreases of 0.0217 and 0.0159 Mg ha^{-1} yr^{-1} were identified for GG and CC, respectively (Figure 4A). The cumulated depletion of total soil P stock was 0.30 Mg ha^{-1} (9.4%) and 0.25 Mg ha^{-1} (8.0%), respectively for GG and CC, after 13 years. Further, total Pi stock was more sorely (2.3 times) depleted in GG than in CC (Figure 4B), which represent, at the end of 13 years, -0.75 and -0.29 Mg ha^{-1} for both treatments, respectively. Differently, Po stock increases of 42.6% in GG, with a rate of 32 kg ha^{-1} yr^{-1} and, was not changed in CC (Figure 4C).

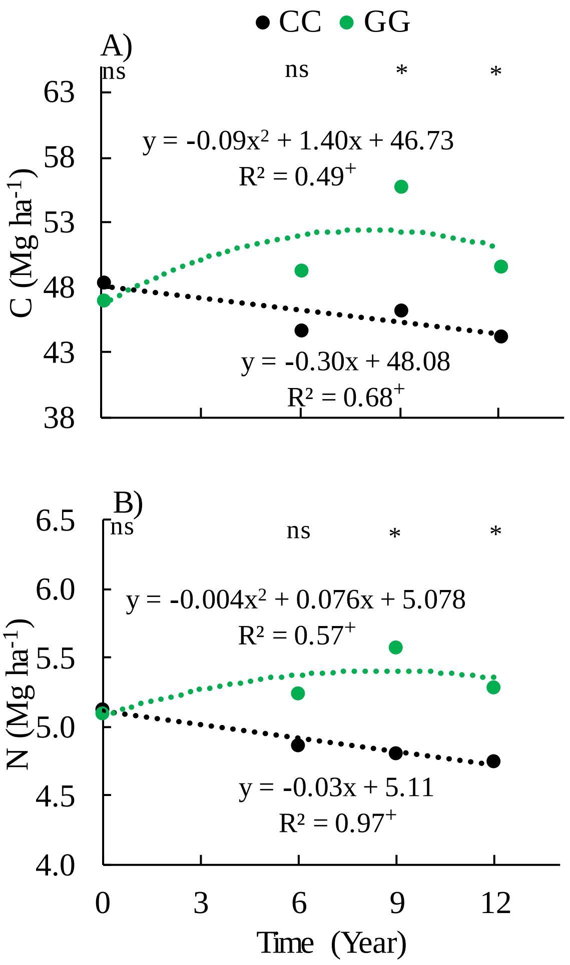


Figure 3 - Temporal change of A) Carbon and B) Nitrogen stocks in a 0-30 cm topsoil layer. CC-permanent cropland and GG-permanent grassland. ^{ns}Not-significative. *Significative by Scott-Knott test ($p \leq 0.05$). $^{+}$ Significative at $p \leq 0.01$.

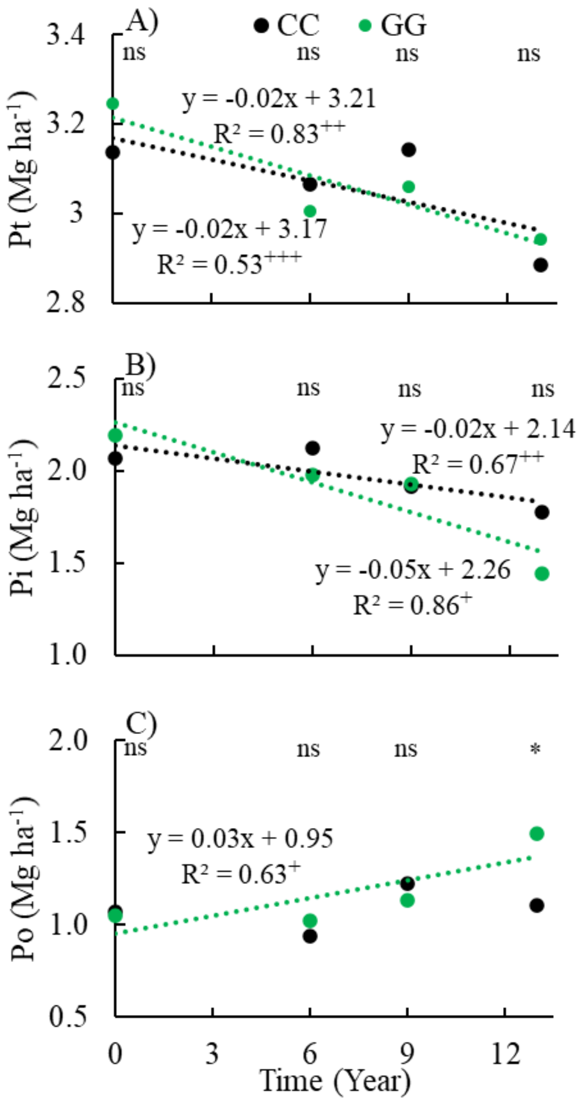


Figure 4 - Trends of A) total phosphorus, B) total inorganic phosphorus and, C) total organic phosphorus stocks in a 0-30 cm topsoil layer of different soil management systems. CC-permanent cropland and GG-permanent grassland. ^{ns}Not-significative. *Significative by Scott-Knott test ($p \leq 0.05$). $^{+}$Significative at $p \leq 0.01$. $^{++}$Significative at $p \leq 0.05$. $^{+++}$Significative at $p \leq 0.10$.

Phosphorus lability

Over time, significant reduction in P-labile fraction occurred in both GG and CC (Figure 5A). By contrast, the P-mod-labile (Figure 5B) and P-non-labile (Figure 5C) fractions were not altered by soil management systems. The P-mod-labile represents an overall mean of 44% of the total P stock in the 0-30 cm topsoil layer and, even statistically not significant, decreased in average of 0.13 Mg ha^{-1} in both treatments represent 47% of the total P stocks decrease. On the other hand, P-non-labile, assessed by 0.5 mol L^{-1} NaOH, represent an overall mean of 22% of the total P stock in the 0-30 cm topsoil layer. The depletion of P-labile was 0.13 and 0.17 Mg ha^{-1}, respectively for CC and GG that represent a reduction of 23 and 32 % of its initial content and 53 and 56% of the total P stocks depletion in the period (for CC and GG, respectively). Pi-labile and Po-labile present opposed trends over time. Whereas there was a depletion in Pi-labile of 0.16 and 0.21 Mg ha^{-1} at a rate of 0.012 and 0.016 Mg ha^{-1} y^{-1}, respectively for CC and GG (Figure 6A); Po-labile stocks increased by 49% (from 27 to 69 kg ha^{-1}) with GG soil management system and did not significantly vary in CC (Figure 6B).

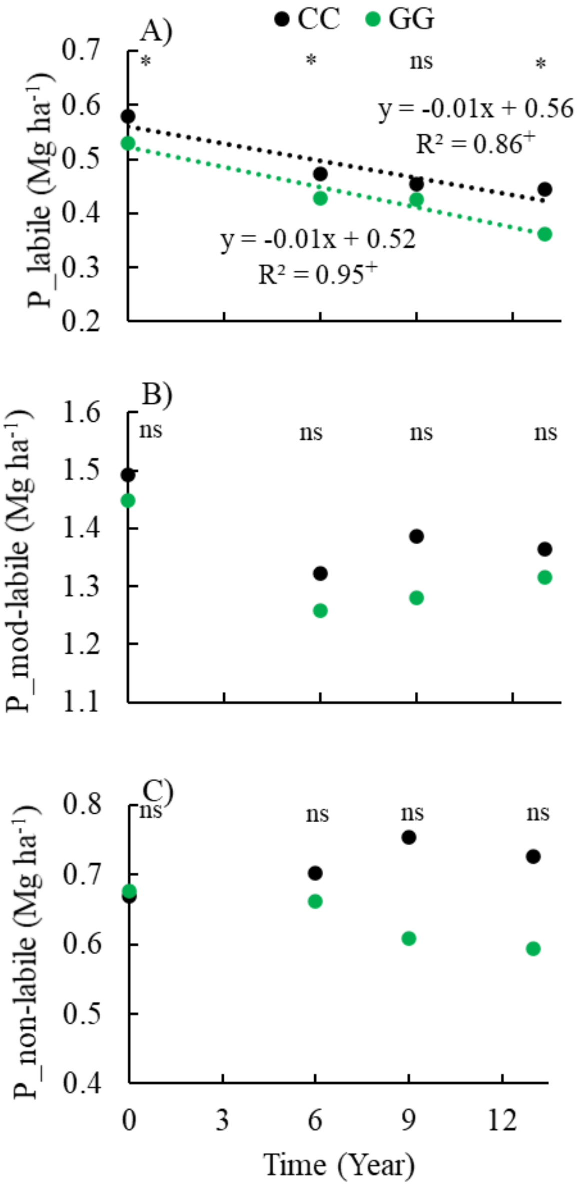


Figure 5 - Trends of A) labile, B) moderately labile and, C) non labile phosphorus of 0-30 cm topsoil layer of different soil management systems. CC-permanent cropland and GG-permanent grassland. ^{ns}Not-significative. *Significative by Scott-Knott test ($p \leq 0.05$). $^{+}$Significative at $p \leq 0.01$.

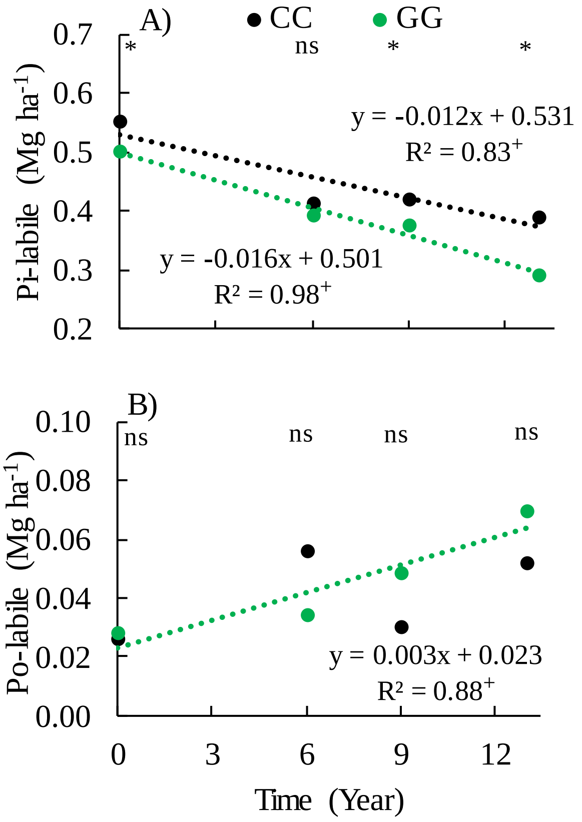
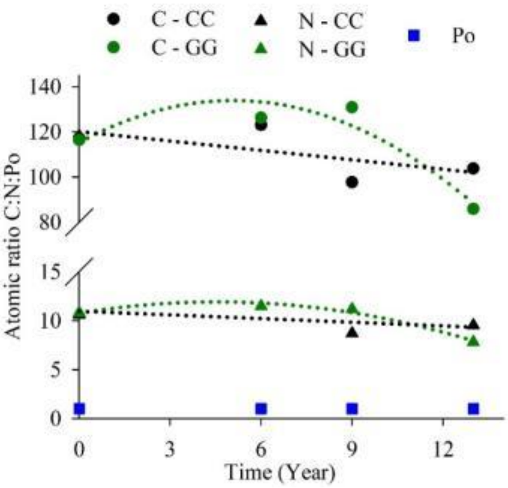


Figure 6 - Trends of A) inorganic P labile and B) organic P labile of 0-30 cm topsoil layer of different soil management systems. CC-permanent cropland and GG-permanent grassland. ^{ns}Not-significative. *Significative by Scott-Knott test ($p \leq 0.05$). $^{+}$Significative at $p \leq 0.01$.

Stoichiometry C:N:Po

Mainly due to changes in C and Po stocks, the stoichiometry of C, N and Po was modified by management systems (Figure 7). There was a significant reduction of C:Po atomic ratio over time in GG (from 116 to 86) and CC (from 117 to 104). The N:Po atomic ratio decreased from 11 to 8 in GG and from 11 to 10 in CC. At the beginning of the experiment the C:N:Po atomic ratios were 117:11:1 and 116:10:1, respectively for CC and GG while after 13 years they were 104:10:1 and 86:8:1, respectively for CC and GG.



	Treat	Equation	R^2
C:Po	CC	$Y=120.08-1.39x$	0.42^{++}
	GG	$Y=115.13+7.37x-0.73x^2$	0.90^{+}
N:Po	CC	$Y=10.96-0.12x$	0.32^{++}
	GG	$Y=10.75+0.51x-0.06x^2$	0.97^{+}

Figure 7- Trends of C:N:Po atomic ratio in a 0-30 cm topsoil layer of different soil management systems. $^{+}$Significative at $p \leq 0.01$. $^{++}$Significative at $p \leq 0.05$.

P characterization by ^{31}P-NMR

The soil Po and Pi forms at the beginning of the experiment (2005 - initial) and after 13 years of cultivation (2018) in different soil management systems were assessed after extraction with a solution of 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA and ^{31}P-NMR analysis. This solution extracted 57, 54 and 51 % of the total soil P and with a Pi / Po ratio of 2.7, 2.7 and 2.8 for Initial, CC and GG, respectively (Table 1). The Pi / Po ratio in the extraction solution was higher than the soil rate (2.0, 1.6 and 1.0 for Initial, CC and GG, respectively) indicating greater extraction of Pi compared to Po.

Orthophosphate, orthophosphate monoesters, orthophosphate diester, pyrophosphate and adenosine diphosphate were identified by ^{31}P-NMR (Figure 8). Although there was a small indication in the spectra, peaks assigned to diester compounds (2 to -1ppm) for the CC and GG samples were below the detection limit of the device. In all spectra there was a predominance of orthophosphate (72, 74 and 75 %), followed by monoester (23, 25 and 24 %) for Initial, CC and GG, respectively. Pyrophosphate and adenosine diphosphate had lower contribution in the three samples (Table 1). The monoester / diester ratio were higher after 13 years of cultivation (7.7, 52 and 50 for Initial, CC and GG, respectively) mainly due to the decrease adenosine diphosphate percentage (the only diester identified in CC and GG samples). In the monoester region: *myo*-IHP, *scyllo*-IHP, α – Glycerophosphate, *β* – Glycerophosphate and adenosine monophosphate were identified (Table 1 and Figure 8). The contribution of the last three stands out with 56, 61 and 62% of the phosphate's monoester. The Pi / Po ratio identified by the ^{31}P-NMR analysis was higher than that observed for the soil, but quite close to that observed for the NaOH + EDTA extraction solution. These features indicate that there were no large losses of Po during sample preparation and ^{31}P-NMR test.

Table 1 – Total, organic and inorganic P extracted by NaOH+EDTA and P forms analyzed by 31P-NMR in a Cambisol managed under permanent cropland (CC) and permanent grassland (GG) over 13 years in a temperate climate.

Parameter	**Initial**	**CC**	**GG**
P in soil			
Total soil P (mg kg^{-1})	719	637	657
Total Po (mg kg^{-1})	239	245	335
Total Pi (mg kg^{-1})	480	392	322
Pi / Po ratio	2.0	1.6	1.0
% of total Po	33	38	51
% of total Pi	67	62	49
P extracted by 0.25 mol L^{-1} NaOH + 0.05 mol L^{-1} EDTA			
Total P (mg kg^{-1})	407	342	338
P organic (mg kg^{-1})	111	92	88
P inorganic (mg kg^{-1})	296	250	250
Pi / Po ratio	2.7	2.7	2.8
% of total soil P	57	54	51
% Po	27	27	26
% Pi	73	73	74
P forms analyzed by ^{31}P-NMR			
Orthophosphate (%)	72	74	75
Monoester (%)	23	25	24
myo-IHP (%)	< 0.01	8	15
α - *Glycerophosphate (%)*	*27*	*23*	*19*
β - *Glycerophosphate (%)*	*15*	*18*	*15*
Adenosine monophosphate (%)	*14*	*19*	*29*
Scyllo-IHP (%)	*14*	*14*	*11*
Other monoesters (%)	*29*	*18*	*11*
Diester (%)	0.2	< 0.01	< 0.01
Pyrophosphate (%)	1.8	0.7	0.9
Adenosine diphosphate (%)	2.8	0.5	0.5
Monoester / Diester ratio	7.7	52	50
Pi / Po ratio	2.7	3.0	3.2

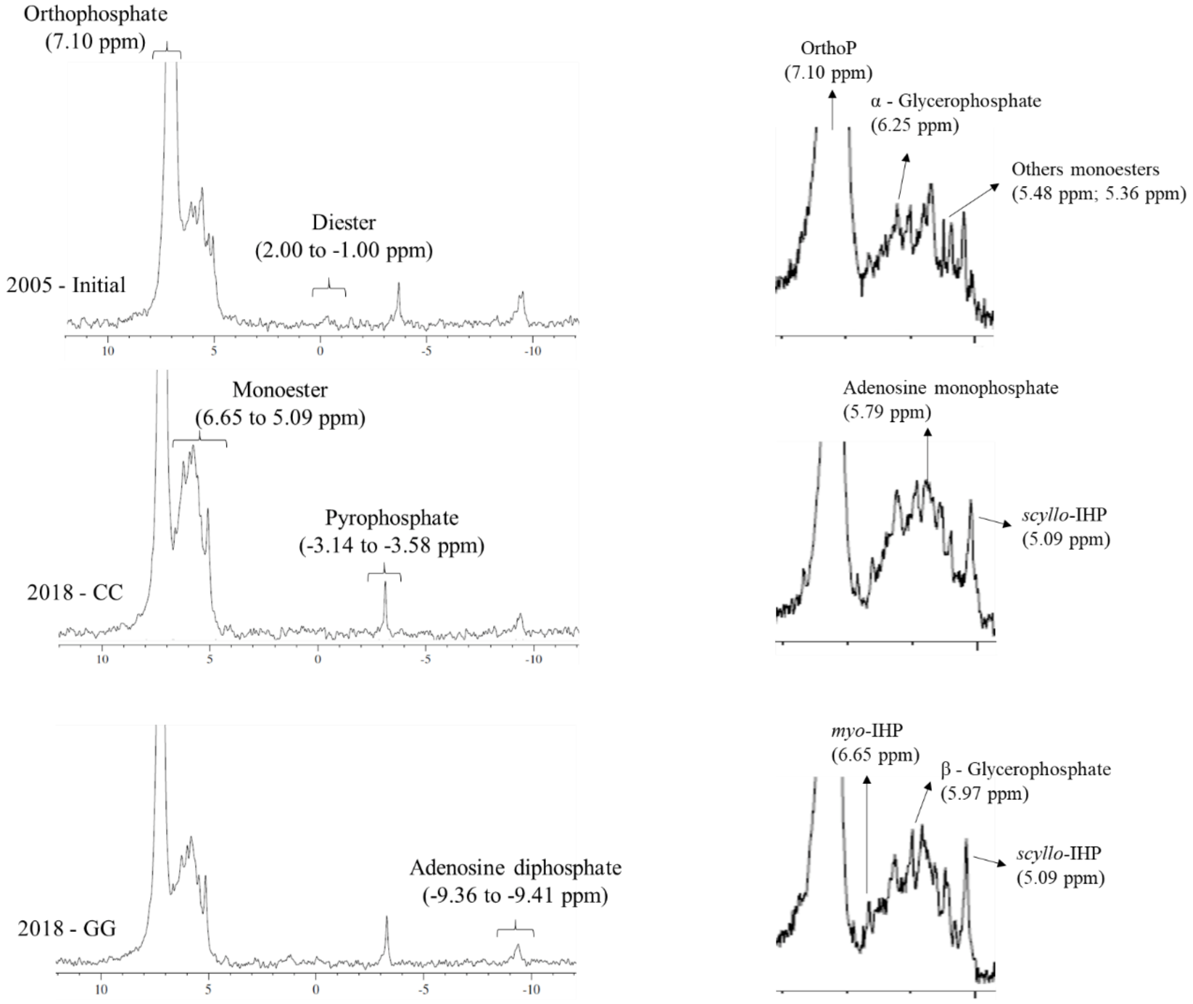


Figure 8 – ^{31}P-NMR spectrum of a representative composite soil samples of initial (2005) and after 13 years of CC and GG soil managements practices. CC-permanent cropland and GG-permanent grassland.

8.4.Discussion

Trends in C, N and P stocks

This study analyzed C, N and P stocks focusing on soil P lability changes over 13 years of cultivation (2005-2018) with contrasting soil management systems in a soil with high initial P stock (average of 3.2 Mg ha^{-1}), but without further P fertilization. The C and N stocks in the 0-30 cm topsoil layer were differentiated by management systems (increase of C and N stock in GG and decrease in CC). The constant input of plant residues (leaves, stems, and roots) and the absence of soil disturbance were fundamental to increase the C and N stocks in GG. Similar results were obtained for the 0-10 cm layer in the same experiment (Crème et al. 2018). In superficial soil layers with tillage management, the soil plowing favors the mineralization of SOM and losses of C and N to the atmosphere, fundamentally by potentiating the microbial activity through the greater residue-soil contact, system aeration and the reduction of physical protection of SOM (Six et al. 1998; Balesdent et al. 2000; Mathew et al. 2012; Abbas et al. 2020). In addition, soils managed with grassland have a significant supply of organic material, mainly in underground by rhizodeposition, which alter the soil's C stock (Rasse et al. 2005; Henneron et al. 2020).

The total P stock decreased in both treatments because of P exportation by plants (forage or grain) without P inputs by fertilizers (Figure 4A). This negative long-term balance can lead to a great reduction of P stocks (mainly available soil P) and make it restrictive to plants nutrition (Boitt et al. 2018; Soltangheisi et al. 2018; Azeez et al. 2020). Even though the amount of total dry matter exported was similar between the management systems (112 and 110 Mg ha^{-1} for CC and GG, respectively), the quantity of P exported differs between the treatments due to the nature of the exported plant material. In CC grains are harvested (maize-wheat-barley) with an average concentration of 0.23% of P while in GG, leaves and stems of grasses (*D. glomerata, F. arundinacea and L. perenne*) were exported as forage with a mean P concentration of 0.28%. This partly explains the difference of 27 kg ha^{-1} in the total P exported and the reduction of 0.3 Mg ha^{-1} of P from the soil in GG at after 13 years.

Although the depletion of total P stock is more pronounced in system managed with perennial pasture (GG), in both cases, it is slow because the annual exportation via dry matter is low with values of 19.1 and 21.0 kg ha^{-1} y^{-1} for CC and GG. Even with mowing management (3-4 times annually) and removal of aerial plant material from the perennial pasture. Differently, in areas where there is grazing with animals, the exportation of nutrients tends to be lower since there is a return of part of the nutrients exported by plants by animal urine and dung (Kayser and Isselstein 2005; Dubeux and Sollenberger 2019). Although generally low, the P exportation have a greater impact on soils with a low P stock and mainly low available P stock.

Simultaneously with the reduction of the total P stock, the Pi stock also decreased in both treatments. This is because plants uptake inorganic P and, later export (Schachtman et al. 1998) or return to the soil organic P forms. In GG, the decrease in the Pi stock was more accentuated (Figure 4B) because in addition to Pi exportation, there was a transformation and accumulation of Pi into the Po. The increase of 42% of Po denotes the high potential of grassland to transform Pi into Po. This transformation occurs when P is absorbed by plants, incorporated into essential plant metabolism, and returns to the soil by leaves, stems and roots and remains in the soil organic matter (Rheinheimer et al. 2020).

The accumulation of Po in grassland is a consequence of SOM increase, provided by input of organic matter into the soil and non-plowing during the period (Crème et al. 2018). In general, pastures soils have an increase of organic forms of P together with C and N due to accumulation of SOM (Rumpel et al. 2015; Poeplau et al. 2018). The SOM represent a coupling of P, C and N cycles in the soil organic phase (Rumpel and Crème 2015). Moreover, the incorporation of Pi into organic molecules in rangelands or croplands can be larger using mining plants (e.g., *Lolium sp* and *Lupinus sp.*), mixture of plants or rotation with more nutritional demanding plants (i.e., leguminous crops) (Tiecher et al. 2012; Crème and Rumpel 2016; Soltangheisi et al. 2018).

The C:N:Po atomic ratios were also modified by management systems due to changes in C and Po stocks (Figure 7). The stoichiometry values (104:10:1 and 86:8:1, respectively for CC and GG) are below the mean of 166:14:1 (Cleveland and Liptzin 2007) and 169:11:1 (Xu et al. 2013) for pasture and above the mean of 64:5:1 (Xu et al. 2013) for cropland values reported in the literature for bulk soil at global scale. The lower values observed in this work are mainly caused by the high soil Po contents. This is the result of the history of P input before the experiment and the increase in soil Po stock provided by permanent grassland cultivation.

P depletion

The decrease of total P stocks is first perceived by changes in P-labile and P-mod-labile stocks, since these fractions have turnover changing from minutes to months (Helfenstein et al. 2020). Moreover, P-labile is the most available P fraction to plants uptake. In our case, the decrease of 0.13 and 0.17 Mg ha^{-1} of P-labile (for CC and GG, respectively) represents most of the reduction in total P stocks (mean of 55%) and was greater in GG because it accompanied the largest export of P in this soil management system.

As for the total Pi and Po stock, Pi and Po-labile had the opposite behavior. In both treatments, Pi-labile reduced due absorption of inorganic P forms (i.e., HPO_4^{-2} and $H_2PO_4^-$) (Schachtman et al. 1998). On the other hand, Po-labile increased significantly in the GG treatment due to the accumulation of SOM in the soil. This behavior agrees with the increase of C, N and Po total soil stock (figures 3 and 4) and indicates an increase in the coupling C, N and Po in the P pool with greater availability. Po-labile corresponds to phosphorus from organic compounds most easily altered by soil management systems (soil microbial biomass, soluble organic molecules, and organic orthophosphates less adsorbed by soil colloids). Therefore, over the period analyzed (13 years), it was possible to identify more significant changes in the labile P pool.

The average decrease observed in the P-mod-labile (0.13 Mg ha^{-1}) represents the remaining portion of the reduction in the total P stock (mean of 47%). This behavior was similar for both soil management systems and shows the buffering effect of P-mod-labile on P-labile, also demonstrated for soils in the subtropical region (Tiecher et al. 2018).

These authors also pointed out that, in a cultivation system with soil revolving, Po with moderate lability (extracted by NaOH 0.1 mol L^{-1}), has a greater direct contribution (94%) to the buffering of the Pi-labile as plowing enhance mineralization. However, in our case, this effect was not observed since the reduction in the P-mod-labile was similar in both treatments and the reduction of Po-mod-labile was not significant in the CC treatment (data not shown). This behavior reinforces the effect of climatic conditions on the intensity of mineralization of SOM.

The P-non-labile stock was not altered by the soil management systems during the 13-year period evaluated. The P-non-labile extracted with NaOH 0.5 mol L^{-1} represents a more recalcitrant organic P pool (inside soil aggregates) and inorganic P pool (inner-sphere absorbed by iron and aluminum oxi-hydroxides) (Condron et al. 1985). So, there is a low sensitivity and minor turnover of this P pool, independent of the soil and the management system adopted (Helfenstein et al. 2020). Furthermore, the high stock of labile and mod-labile P, resulting from the fertilization history, was able to supply the demand of crops without need to access P non-labile pool.

Chemical nature of soil organic phosphorus

The extraction solution of NaOH + EDTA extracted more than 50% of the total P from the soil. In soils of temperate regions, this fact is recurrent due to the mineralogy and moderate pedogenetic evolution of the soil. The soil of the present work is classified as Cambisol and has upper soil horizons with loamy texture and high P content, mainly Pi (67, 62 and 49 % of total P is Pi in Initial, CC and GG respectively), due to the history of phosphate fertilizer use before the beginning of the experiment.

In a study carried out on soils with different uses and textural characteristics from New South Wales, South Australia, and Tasmania, Doolette et al. (2011) extracted between 21 and 89 % of the total soil P with the same solution of NaOH + EDTA (with a predominance of > 60 %). Abdi et al. (2019) extracted on average of 51% of total soil P for soils from Canada, with a clay texture and cultivated with grassland. In contrast, in subtropical soil, Rheinheimer et al. (2019) obtained much lower P recovery (average of 15.1%). In the latter, the authors attributed the percentage of P recovery due to the clayey texture and mineralogy dominated by kaolinite and iron oxides of the soil.

As in the present study, in all the cases mentioned above there was a predominance of orthophosphate and monoesters and small contribution of diesters and pyrophosphate in the analyzed extract. In the spectrum shift attributed to monoester, more complex organic compounds were identified, such as: *myo*-IHP, *scyllo*-IHP, α – glycerophosphate, β – glycerophosphate and adenosine monophosphate. These compounds have also been identified in soils cultivated with annual crops and grassland from tropical and temperate regions (McLaren et al. 2015; Deiss et al. 2016; Cade-Menun 2017; Abdi et al. 2019). The difference of our study was to evaluate the temporal effect of the permanent cultivation of cropland and grassland on chemical nature of soil organic P. This approach contemplates the need to analyze the temporal dynamics of organic P forms, as evidenced by Cade-Menun (2017).

The permanent cultivation of cropland and grassland for 13 years did not significantly alter the concentration of orthophosphate. Although a decrease in the total P stock and mainly in the total Pi stock has been identified (Figure 4). Likewise, no relevant temporal increase in the P organic forms in GG was observed by ^{31}P-NMR most probably to the high initial total Po stock, C and N contents.

No relevant changes in Po forms for GG were identified mainly because the percentage of Po in the NaOH + EDTA extract was similar between the treatments (mean of 27%), even in the GG treatment containing the highest content of total Po in the soil (Table 1). For Initial, CC and GG samples, there was selectivity for Pi extracting. Since the lower percentage of Pi (67, 62, 49% of Pi for Initial, CC and GG, respectively) was present in soil than in analyzed NaOH+EDTA extract (73, 73, 74% of Pi for Initial, CC and GG, respectively). Selectivity for Po compounds are more commonly with the use of NaOH + EDTA extraction solution and was reported by Rheinheimer et al. (2019) for soils from the subtropical region.

Another finding is that in the GG treatment, dry matter was removed annually in the order of 7.9 Mg ha^{-1} y^{-1} (reaching 110 Mg ha^{-1} in 13 years). The exportation of dry matter from pastures, whether manually or by animal grazing, tends to reduce the long-term Po content in the soil and mainly monoester forms detectable by ^{31}P-NMR. In temperate pastures from New Zealand, Boitt et al. (2018) obtained a decrease in the percentages of monoester compounds in the NaOH+EDTA extract when simulating grazing and exporting pasture dry matter for 20 years. Similarly, in winter forage of subtropical climate in Brazil, Deiss et al. (2016) observed a decrease of monoester compounds in NaOH+EDTA extract when grazing with animals in experiments of 7 to 12 years.

The main changes identified due to the cultivation of the soil with different management systems were the decrease in α – glycerophosphate, diesters, pyrophosphate and adenosine diphosphate forms and increase in *myo*-IHP and adenosine monophosphate forms in both treatments (Table 1). Greater differentiation between treatments occurred only in monoester compounds. In this case, cultivation with grassland had a more significant effect in reducing α – glycerophosphate and increase *myo*-IHP and adenosine monophosphate.

The monoester / diester ratio was profoundly altered by the time of cultivation of the soil and did not differ between treatments. The highest values of the monoester / diester ratio were defined due to the decrease of diester forms. Diester forms are considered more "labile"; whereas monoester compounds are strongly adsorbed to soil colloids and therefore are more recalcitrant (Cade-Menun 2017; Menezes-Blackburn et al. 2018). This behavior favors its mineralization by reason of agricultural cultivation practices, mainly in CC due to the annual soil plowing.

8.5.Conclusion

In this work we analyzed trends in soil organic matter, P stocks depletion and chemical nature of soil organic P and the stoichiometry C:N:Po in a long-term field experiment cultivated with contrasting soil management systems, such as permanent cropland and permanent grassland for 13 years.

Our results showed a greater export of P and, consequently, a greater depletion of total P stock in the treatment with permanent grassland. The depletion of P mainly impacted the P pool with greater lability (P-labile). However, the depletion occurred after 13 years was not enough to cause an evident reduction in plant yields productivity due to the buffering capacity of the P pools with less lability (P-mod-labile).

With permanent grassland, there was a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. This behavior provided a reduction in the C:N:Po atomic ratio values and, consequently, a coupling between the C, N and P cycles in the soil. ^{31}P-NMR technique revealed that permanent grassland had a more significant effect in reducing *α*–glycerophosphate and increase *myo*-IHP and adenosine monophosphate than permanent cropland.

8.6.References

Abbas F, Hammad HM, Ishaq W, et al (2020) A review of soil carbon dynamics resulting from agricultural practices. J Environ Manage 268:. https://doi.org/10.1016/j.jenvman.2020.110319

Abdi D, Cade-Menun BJ, Ziadi N, et al (2019) A31 P-NMR spectroscopic study of phosphorus forms in two phosphorus-fertilized grassland soils in Eastern Canada. Can J Soil Sci 99:161–172. https://doi.org/10.1139/cjss-2018-0125

Alewell C, Ringeval B, Ballabio C, et al (2020) Global phosphorus shirtage will be aggravated by soil erosion. Nat Commun 11:4546. https://doi.org/10.1038/s41467-020-18326-7

Azeez MO, Rubæk GH, Pedersen IF, Christensen BT (2020) Depletion, accumulation and availability of soil phosphorus in the Askov long-term field experiment. Soil Res 58:117–124. https://doi.org/10.1071/SR19203

Balesdent J, Chenu C, Balabane M (2000) Academic entrepreneurial orientation. Empirical evidence from life sciences. Soil Tillage Res 53:215–230. https://doi.org/https://doi.org/10.1016/S0167-1987(99)00107-5

Bayer C, Mielniczuk J, Martin-Neto L, Ernani PR (2002) Stocks and humification degree of organic matter fractions as affected by no-tillage on a subtropical soil. Plant Soil 238:133–140. https://doi.org/10.1023/A:1014284329618

Boitt G, Black A, Wakelin SA, et al (2018) Impacts of long-term plant biomass management on soil phosphorus under temperate grassland. Plant Soil 427:163–174. https://doi.org/10.1007/s11104-017-3429-0

Cade-Menun B, Liu CW (2014) Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy of Soils from 2005 to 2013: A Review of Sample Preparation and Experimental Parameters. Soil Sci Soc Am J 78:19–37. https://doi.org/10.2136/sssaj2013.05.0187dgs

Cade-Menun BJ (2015) Improved peak identification in 31P-NMR spectra of environmental samples with a standardized method and peak library. Geoderma 257–258:102–114. https://doi.org/10.1016/j.geoderma.2014.12.016

Cade-Menun BJ (2017) Characterizing phosphorus forms in cropland soils with solution 31P-NMR: past studies and future research needs. Chem Biol Technol Agric 4:1–12. https://doi.org/10.1186/s40538-017-0098-4

Chassé AW, Ohno T (2016) Higher Molecular Mass Organic Matter Molecules Compete with Orthophosphate for Adsorption to Iron (Oxy)hydroxide. Environ Sci Technol 50:7461–7469. https://doi.org/10.1021/acs.est.6b01582

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85:235–252. https://doi.org/10.1007/s10533-007-9132-0

Condron LM, Goh KM, Newman RH (1985) Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. J Soil Sci 36:199–207. https://doi.org/10.1111/j.1365-2389.1985.tb00324.x

Crème A, Rumpel C (2016) Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. Plant Soil. https://doi.org/10.1007/s11104-015-2740-x

Crème A, Rumpel C, Le Roux X, et al (2018) Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities. Soil Biol Biochem 122:203–210. https://doi.org/10.1016/j.soilbio.2018.04.018

Deiss L, de Moraes A, Dieckow J, et al (2016) Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil. Geoderma 274:88–96. https://doi.org/10.1016/j.geoderma.2016.03.028

Dick WA, Tabatabai MA (1977) Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds1. J Environ Qual 6:82. https://doi.org/10.2134/jeq1977.00472425000600010018x

Doolette AL, Smernik RJ, Dougherty WJ (2009) Spiking Improved Solution Phosphorus-31 Nuclear Magnetic Resonance Identification of Soil Phosphorus Compounds. Soil Sci Soc Am J 73:919–927. https://doi.org/10.2136/sssaj2008.0192

Doolette AL, Smernik RJ, Dougherty WJ (2011) A quantitative assessment of phosphorus forms in some Australian soils. Soil Res 49:152–165. https://doi.org/10.1071/SR10092

Dubeux JCB, Sollenberger LE (2019) Nutrient cycling in grazed pastures. Elsevier Inc.

Elser JJ (2012) Phosphorus: A limiting nutrient for humanity? Curr Opin Biotechnol 23:833–838. https://doi.org/10.1016/j.copbio.2012.03.001

EVEILLARD, P. & SABY, N. Diminution de la fertilisation minérale et conséquences sur la disponibilité des éléments phosphore et potassium dans les sols…In : Fertilisation et fertilité des sols. Académie d'Agriculture de France. Session Publique 7 mars 2018. Disponível em : https://www.academie-agriculture.fr/actualites/academie/seance/academie/fertilisation-et-fertilite-des-sols?070318. Acesso: 15 mars 2018.

Fink JR, Inda A V., Bavaresco J, et al (2016) Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. Soil Tillage Res 155:62–68. https://doi.org/10.1016/j.still.2015.07.017

George TS, Giles CD, Menezes-Blackburn D, et al (2018) Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant Soil 427:191–208. https://doi.org/10.1007/s11104-017-3391-x

Goyette JO, Bennett EM, Maranger R (2018) Low buffering capacity and slow recovery of anthropogenic phosphorus pollution in watersheds. Nat Geosci 11:921–925. https://doi.org/10.1038/s41561-018-0238-x

Grau O, Peñuelas J, Ferry B, et al (2017) Nutrient-cycling mechanisms other than the direct absorption from soil may control forest structure and dynamics in poor Amazonian soils. Sci Rep 7:1–11. https://doi.org/10.1038/srep45017

Guppy CN, Menzies NW, Moody PW, Blamey FPC (2005) Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust J Soil Res 43:189–202. https://doi.org/10.1071/SR04049

Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Sci Soc Am J 46:970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Helfenstein J, Pistocchi C, Oberson A, et al (2020) Estimates of mean residence times of phosphorus in commonly considered inorganic soil phosphorus pools. Biogeosciences 17:441–454. https://doi.org/10.5194/bg-17-441-2020

Helfenstein J, Tamburini F, von Sperber C, et al (2018) Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. Nat Commun 9:. https://doi.org/10.1038/s41467-018-05731-2

Henneron L, Cros C, Picon-Cochard C, et al (2020) Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. J Ecol 108:528–545. https://doi.org/10.1111/1365-2745.13276

Kamh M, Horst WJ, Amer F, et al (1999) Mobilization of soil and fertilizer phosphate by cover crops. Plant Soil 211:19–27. https://doi.org/10.1023/A:1004543716488

Kayser M, Isselstein J (2005) Potassium cycling and losses in grassland systems: A review. Grass Forage Sci 60:213–224. https://doi.org/10.1111/j.1365-2494.2005.00478.x

Li M, Cozzolino V, Mazzei P, et al (2018) Effects of microbial bioeffectors and P amendements on P forms in a maize cropped soil as evaluated by 31P–NMR spectroscopy. Plant Soil 427:87–104. https://doi.org/10.1007/s11104-017-3405-8

Liu J, Cade-Menun BJ, Yang J, et al (2018) Long-term land use affects phosphorus speciation and the composition of phosphorus cycling genes in agricultural soils. Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.01643

Lun F, Liu J, Ciais P, et al (2018) Global and regional phosphorus budgets in agricultural systems and their implications for phosphorus-use efficiency. Earth Syst Sci Data 10:1–18. https://doi.org/10.5194/essd-10-1-2018

MacDonald GK, Bennett EM, Potter PA, Ramankutty N (2011) Agronomic phosphorus imbalances across the world’s croplands. Proc Natl Acad Sci 108:3086–3091. https://doi.org/10.1073/pnas.1010808108

Mathew RP, Feng Y, Githinji L, et al (2012) Impact of No-tillage and conventional tillage systems on soil microbial communities. Appl Environ Soil Sci 2012:. https://doi.org/10.1155/2012/548620

McLaren TI, Smernik RJ, McLaughlin MJ, et al (2015) Complex Forms of Soil Organic Phosphorus-A Major Component of Soil Phosphorus. Environ Sci Technol 49:13238–13245. https://doi.org/10.1021/acs.est.5b02948

Menezes-Blackburn D, Giles C, Darch T, et al (2018) Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant Soil 427:5–16. https://doi.org/10.1007/s11104-017-3362-2

Moni C, Chabbi A, Nunan N, et al (2010) Spatial dependance of organic carbon-metal relationships. A multi-scale statistical analysis, from horizon to field. Geoderma 158:120–127. https://doi.org/10.1016/j.geoderma.2010.04.014

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chem ACTA 27:31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Oliveira LB, Tiecher T, Quadros FLF, Santos DR (2011) Fósforo microbiano em solos sob pastagem natural submetida a queima e pastejo. Rev Bras Cienc do Solo 35:1509–1515. https://doi.org/10.1590/S0100-06832011000500005

Panettieri M, Rumpel C, Dignac MF, Chabbi A (2017) Does grassland introduction into cropping cycles affect carbon dynamics through changes of allocation of soil organic matter within aggregate fractions? Sci Total Environ 576:251–263. https://doi.org/10.1016/j.scitotenv.2016.10.073

Poeplau C, Zopf D, Greiner B, et al (2018) Why does mineral fertilization increase soil carbon stocks in temperate grasslands? Agric Ecosyst Environ 265:144–155. https://doi.org/10.1016/j.agee.2018.06.003

Rasse DP, Rumpel C, Dignac MF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269:341–356. https://doi.org/10.1007/s11104-004-0907-y

Rheinheimer DS, Fornari MR, Bastos MC, et al (2019) Phosphorus distribution after three decades of different soil management and cover crops in subtropical region. Soil Tillage Res 192:33–41. https://doi.org/10.1016/j.still.2019.04.018

Rheinheimer DS, Somavilla A, Oliveira LB, Tiecher; T. (2020) Ciclo biogeoquímico do fósforo, diagnóstico de disponibilidade e adubação fosfatada. Porto Alegre. 71 p. Available in: https://www.researchgate.net/publication/345729958_Ciclo_biogeoquimico_do_fosforo_diagnostico_de_disponibilidade_e_adubacao_fosfatada.

Rheinheimer DS, Santos JCP, Kaminski J, Mafran AL (1997) Crescimento de leguminosas forrageiras afetado pela adição de fósforo, calagem do solo e micorrizas, em condições de casa de vegetação. Ciência Rural 27:571–576. https://doi.org/10.1590/S0103-84781997000400008

Rheinheimer DS, Anghinoni, I, & Conte, E (2000). Fósforo da biomassa microbiana em solos sob diferentes sistemas de manejo. Revista Brasileira de Ciência Do Solo, 24(3), 589–597. https://doi.org/10.1590/s0100-06832000000300012

Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci 60:124–143. https://doi.org/10.1071/CP07125

Rodrigues M, Pavinato PS, Withers PJA, et al (2016) Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. Sci Total Environ 542:1050–1061. https://doi.org/10.1016/j.scitotenv.2015.08.118

Rumpel C, Crème A, Ngo PT, et al (2015) The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. J Soil Sci Plant Nutr 15:353–371. https://doi.org/10.4067/s0718-95162015005000034

Saby, N. ; Gouny, L., Lemercier, B. F. ; Denoroy, P. ; Eveillard, P. 2016. Utilisation des données de la BDAT pour étudier l'évolution spatio-temporelle des teneurs en Magnésium échangeable, Potassium échangeable et Phosphore extractible dans les sols agricoles de France métropolitaine. [Contrat] auto-saisine. <hal-01581567>

Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiol 116:447–453. https://doi.org/10.1104/pp.116.2.447

Schilling KE, Streeter MT, Isenhart TM, et al (2018) Distribution and mass of groundwater orthophosphorus in an agricultural watershed. Sci Total Environ 625:1330–1340. https://doi.org/10.1016/j.scitotenv.2018.01.035

Schoumans OF, Chardon WJ, Bechmann ME, et al (2014) Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: A review. Sci Total Environ 468–469:1255–1266. https://doi.org/10.1016/j.scitotenv.2013.08.061

Senapati N, Jansson PE, Smith P, Chabbi A (2016) Modelling heat, water and carbon fluxes in mown grassland under multi-objective and multi-criteria constraints. Environ Model Softw 80:201–224. https://doi.org/10.1016/j.envsoft.2016.02.025

Six J, Elliott ET, Paustian K, Doran JW (1998) Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils. Soil Sci Soc Am J 62:1367–1377. https://doi.org/10.2136/sssaj1998.03615995006200050032x

Soltangheisi A, Rodrigues M, Coelho MJA, et al (2018) Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res 179:20–28. https://doi.org/10.1016/j.still.2018.01.006

Tian J, Boitt G, Black A, et al (2019) Mass balance assessment of phosphorus dynamics in a fertilizer trial with 57 years of superphosphate application under irrigated grazed pasture. Nutr Cycl Agroecosystems. https://doi.org/10.1007/s10705-019-09992-1

Tiecher T, Gomes MV, Ambrosini VG, et al (2018) Assessing linkage between soil phosphorus forms in contrasting tillage systems by path analysis. Soil Tillage Res 175:276–280. https://doi.org/10.1016/j.still.2017.09.015

Tiecher T, Santos DR dos, Kaminski J, Calegari A (2012) Forms of inorganic phosphorus in soil under different long term soil tillage systems and winter crops. Rev Bras Ciência do Solo 36:271–282. https://doi.org/10.1590/s0100-06832012000100028

Turner BL, Cade-Menun BJ, Condron LM, Newman S (2005) Extraction of soil organic phosphorus. Talanta 66:294–306. https://doi.org/10.1016/j.talanta.2004.11.012

Turner BL, Mahieu N, Condron LM (2003) Phosphorus-31 Nuclear Magnetic Resonance Spectral Assignments of Phosphorus Compounds in Soil NaOH–EDTA Extracts. Soil Sci Soc Am J 67:497. https://doi.org/10.2136/sssaj2003.0497

Turner BL, Richardson AE (2004) Identification of scyllo -Inositol Phosphates in Soil by Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy . Soil Sci Soc Am J 68:802–808. https://doi.org/10.2136/sssaj2004.8020

Tyson J, Corkrey R, Burkitt L, Dougherty W (2020) Modelling Changes in Soil Phosphorus When Phosphorus Fertiliser Is Reduced or Ceases. Front Environ Sci 8:1–11. https://doi.org/10.3389/fenvs.2020.00093

USEPA (1971) Methods of chemical analysis for water and wastes. Cincinnati

Virto I, Moni C, Swanston C, Chenu C (2010) Turnover of intra- and extra-aggregate organic matter at the silt-size scale. Geoderma 156:1–10. https://doi.org/10.1016/j.geoderma.2009.12.028

Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical forest. Annu Rev Ecol Syst Vol 17 137–167. https://doi.org/10.1146/annurev.es.17.110186.001033

Walker TW, Adams AFR (1958) Studies on soil organic matter: i. Influence of phos- phorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Sci 85:307–318

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wang D, Lv S, Jiang P, Li Y (2017) Roles , Regulation , and Agricultural Application of Plant Phosphate Transporters. 8:1–14. https://doi.org/10.3389/fpls.2017.00817

Weil, R. R.; Brady, N. C. Phosphorus and potassium. In: Weil, R. R.; Brady, N. C. The nature and properties of soils. 15th ed. Columbus, OH: Pearson, 2017. cap. 14, p. 643-695.

Withers PJA, Hodgkinson RA, Rollett A, et al (2017) Reducing soil phosphorus fertility brings potential long-term environmental gains: A UK analysis. Environ Res Lett 12:063001. https://doi.org/10.1088/1748-9326/aa69fc

Withers PJA, Rodrigues M, Soltangheisi A, et al (2018) Transitions to sustainable management of phosphorus in Brazilian agriculture. Sci Rep 1–13. https://doi.org/10.1038/s41598-018-20887-z

Xu X, Thornton PE, Post WM (2013) A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. 737–749. https://doi.org/10.1111/geb.12029

Xue H, Lan X, Liang H, Zhang Q (2019) Characteristics and environmental factors of stoichiometric homeostasis of soil microbial biomass carbon, nitrogen and phosphorus in China. Sustain 11:. https://doi.org/10.3390/su11102804

8.7.General comments

In chapter V, we used historical samples of the 0-30 cm topsoil layer of an experiment conducted with contrasting soil managements such as continuous cropland (maize-wheat-barley rotation) and permanent grassland (Dactylis + Festuca + Lolium) for 13 years. In our analysis, we identified a significant depletion in the total soil P stock, with emphasis on the treatment with the permanent cultivation of forage species. This depletion was mainly a reflection of the P exportation from pools with greater lability (P-Labil extracted with anion exchange resin + 0.5 mol L^{-1} $NaHCO_3$).

Furthermore, we identified a significant transformation from inorganic P to organic P in the most labile P pool, accompanying the increase of total C and N stocks in the soil. It was a result of the inorganic P uptake by plants and soil microorganisms, with consequent P accumulation in organic form in the soil organic matter over the 13 years of cultivation. This behavior provided a reduction in the C:N:Po atomic ratio values and, consequently, a coupling between the C, N and P cycles in the soil. Conversely, the practice of periodic soil plowing and the cultivation of annual crops provided a reduction in the total stock of C and N and did not alter the stock of soil organic P. By ^{31}P-NMR technique we identified that permanent grassland had a more significant effect in reducing α–glycerophosphate and increase *myo*-IHP and adenosine monophosphate than permanent cropland.

After analyzing the behavior of P dynamics in the soil during the 13 years of the experiment and identifying changes, mainly in the organic P levels of the labil fraction, we wondered about the influence of microbial biomass on this soil P pool and which the influence of the management system on the distribution of soil aggregates and the bioavailability of P in each one. Thus, in chapter VI we hypothesized that a) Soil microbial biomass is an important stock of P in organic form in soils of temperate climate region and its significance is greater in management systems with more SOM. b) soil management systems alter the total P contents and their availability to plants differently in each soil aggregate size, due to changes in: aggregates size distribution, content of SOM and P_{mic}; c) the C:N:P stoichiometry is differentiated according to the aggregate size.

Therefore, we decided to monitor during an annual crop cycle the participation of microbial biomass in the soil P bioavailability. Moreover, we detail the distribution of soil aggregates and the P pools in each of the different sizes of soil aggregates in order to characterize the microbial P turnover in the soil.

9. Chapter VI - Soil P microbial biomass and coupling of C:N:P: a soil aggregate scale approach

Abstract

The increase of soil organic matter (SOM) leads simultaneously to increase the soil organic phosphorus (Po) pools. Thus, soil management systems that improve SOM are favorable to Po stocks. Therefore, the microbial activity is essential to mineralize organic compounds, turn Po to Pi and, consequently, turn it plant available form. In this scenario, the access and the ability of microorganism to mineralize the organic compounds are fundamental to the reuse of Po by plants. This process is mainly governed by the chemistry of SOM, the physical protection by soil mineral matrix and the macrobian community. For this, we aimed to with this work better understand the effect of soil management systems in the importance of P in microbial biomass (P_{mic}) and the P pools in soil aggregates. To achieve our objectives, we analyzed a long-term experiment managed with permanent cropland (CC) and permanent grassland (GG) in a temperate soil. Here, we quantified P pools and C and N content in a bulk soil and in different soil aggregate sizes. As well as we monitored the P_{mic} changes and its contribution to available Olsen P during growth season of 2018/2019. Our results indicated a greater content of C, N and Po in GG than in CC. Overgrowth season of 2018/2019, P_{mic} had more importance in CC than in GG management. In this case, P_{mic} represented until 47 and 37 % of the Olsen P for CC and GG, respectively. The soil management systems changed the soil aggregate distribution, manly in larger aggregates sizes. This implies in greater content of C and N in larger aggregates (greater than 0.050 mm diameter) for GG and in macroaggregates (0.200 – 2 mm diameter) for CC. The P_{mic} was higher in aggregates larger than 0.200 mm diameter and exceed 35 and 60 mg kg^{-1} (for CC and GG, respectively). The C:N:P stoichiometry was greater in macroaggregates (169:14:1 and 147:12:1 for CC and GG, respectively) and lower in Silt+Clay fraction (94:10:1 and 98:11:1 for CC and GG, respectively). Our results show that the forage management system favored the accumulation of C, N and organic P in the soil. The P_{mic} content was an important pool of bioavailable P in both management systems throughout the growing season. The soil plowing, and cereal cultivation reduced the percentage and the levels of C, N and P_{mic} of large aggregates. The stoichiometry C:N:Po was little changed by the 13 years of cultivation in the management systems.

Key-words: Soil management systems; Stoichiometry C:N:P; P lability; Grassland; soil C and N.

9.1. Introduction

Global guidelines have been proposed to increase the stock of soil organic carbon (SOC) as a strategy to counterbalance the emission of CO_2 by use of fossil fuels and thereby minimize the effect of global warming (Dignac et al., 2017). The SOC is defined mainly by the soil organic matter content - SOM (plant or animal waste, live or dead micro-organisms and humus) and it can be profoundly altered by soil management systems (Assunção et al., 2019; Dignac et al., 2017; Panettieri et al., 2017; Poeplau et al., 2018).

The elimination of toxic elements and the maintenance of adequate levels of nutrients in the soil is one of the main factors that contributes to the accumulation of SOM in agricultural areas. In tropical and subtropical climatic environments, soils are deficient in available P that limit the productivity of commercial crops (Gatiboni et al., 2021). Conversely, soils occurring in temperate regions originally contain a large stock of bioavailable P. However long periods of cultivation without addition of this nutrient have also led to the depletion of labile forms and even may limit the productivity (Blake et al., 2003; Boitt et al., 2018; Le Noë et al., 2020).

In France, for example, until the 1970s there was a significant increase in the use of phosphate fertilizers, increasing total P contents in the country's soils. However, from the 1970s onwards, the consumption of phosphate fertilizers in French agriculture was drastically reduced through public policies (Le Noë et al., 2020). This strategy was justified due to the high annual P inputs and the high levels of P in the soils. However, without the addition of phosphate fertilizers, the P budget in many agricultural areas became negative, causing possible decrease production due to plant nutritional restrictions (Éveillard & Saby, 2018; Saby et al., 2016). In 2015, a mapping revealed 760 mg kg^{-1} as a mean concentration of total P in a French topsoil layer (Delmas et al., 2015). Nevertheless, unluckily only a small portion of the total P is available for plants.

The increase of SOM leads to the incorporation of Pi into the organic compounds (organic P - Po) by both plants and microorganisms, although most of the P added as fertilizer is distributed in pre-existing inorganic forms in the soil (Rheinheimer & Anghinoni, 2001). In cultivated soils, the P content in soil organic compounds can vary from 30 to 65% of the total soil P (Condron et al., 2005). Thus, SOM and soil microbial biomass are a significant pool of P for agricultural crops, independently of the soil type and climate. At global scale, C:N:P cycles are coupled in the SOM with a soil atomic ratio of 186:13:1 and a soil microbial biomass atomic ratio of 60:7:1 (Cleveland & Liptzin, 2007).

Increase content of C and N into soil also means immobilizing P. Therefore, the recalcitrance of organic compounds defines the recovery of Po by plants and microorganisms. Organic P must be converted into mineral form before being used again by plants. This process is carried out almost exclusively by soil microorganisms through mineralization of the organic matter. Although, simple removal of the phosphate ion by biochemical mineralization can occur without degradation of the organic compound (Mcgill & Cole, 1981). This occurs much more by the decrease of P contents in the leaves of the plants than by the organic P contents of the rhizosphere (Meeds et al., 2021) and the enzymatic activity associated to the soil solid phase (Jarosch et al., 2019). In addition, plants can synthesize and exude phosphatases that can increase its bioavailability and uptake (Hayes et al., 1999). Accordingly, P stored in labile organic forms can be reused by plants (Menezes-Blackburn et al., 2018) and represent a temporary stock of P in the soil (Rheinheimer et al., 2019)

The recalcitrance of SOM is closely related to the complexity of organic compounds and to the physical protection provided by soil inorganic constituents (Assunção et al., 2019). The soil aggregation has a significant impact on the recalcitrance of SOM (Mangalassery et al., 2019; Panettieri et al., 2017; Sarker et al., 2018; Virto et al., 2008, 2010). In this perspective, the relationship between soil aggregation and SOM is a two-way path, while the SOM act like a glue to soil aggregation, the aggregation act as an armor to physical protection and non-mineralization of SOM (Kleber & Johnson, 2010; Tisdall & Oades, 1982). The variation of the SOM levels as function of soil aggregate size is an indicator of the P contents variation, mainly Po fractions. A considerable fraction of the Po is composed of P stored in the microbial biomass (P_{mic}), and therefore the P will be prevented from reacting with functional groups of the clay minerals and oxides. Additionally, the functional groups of the organic matter can react directly with functional groups of the inorganic constituents, making it impossible to form inner-sphere complexes with inorganic phosphate (Guppy et al., 2005) and even with organic P monoesters.

The size distribution of soil inorganic and organic constituents and the degree of mineralization and turnover of SOM varies with the size of the aggregates (Liao et al., 2018; Panettieri et al., 2017). Consequently, the availability of P to plants (Fontana et al., 2008; Pereira et al., 2010) and C: N: P stoichiometry should be differentiated for each aggregate size.

Thus, our hypotheses are: a) Soil microbial biomass is an important stock of P in organic form in soils of temperate climate region and its significance is larger in management systems that favor SOM increase. b) soil management systems alter the total P contents and its availability to plants differently in each aggregate size; c) the C:N:P stoichiometry is differentiated according to the aggregate size. To validate our hypotheses, we monitored changes in C, N, and P stocks in different aggregate classes using a long-term experiment with contrasting management such as continuous grain-producing with soil plowing and with permanent grassland.

9.2. Material and methods

Field trial and sampling

The field trial was initialized in 2005 at the environmental observatory of INRA center of Lusignan - France (Agroecosystems, Biogeochemical Cycles and Biodiversity, SOERE–ACBB; www.soere-acbb.com) (46°25'12,91"N; 0°07'29,35"E). The region is characterized by annual mean temperature of 12 °C and a mean annual precipitation of 750 mm year^{-1}. The soil is classified as Cambisol with two distinct horizons. The first one is a loamy textured horizon and lower one is a clayey rubefied horizon rich in iron oxides (goethite and hematite) and kaolinite. More details of the experimental area were described by Crème et al. (2018), Moni et al. (2010) and Senapati et al. (2016).

After 2005, the experiment was carried out in a randomized complete block design with 4 replicates. The treatments analyzed in this work were: permanent cropland (CC) with maize-wheat-barley rotation and permanent grassland (GG) without P fertilization (Figure 1). The grassland is constituted by a mixture of *Dactylis glomerata* L. (cocksfoot), *Festuca arundinacea* Schreb (tall fescue) and *Lolium perenne* L. (ryegrass).

The soil was sampled from October 2018 to May 2019. The first sampling was carried out on 22 October 2018, shortly after CC soil plowing. After October 2018, four samplings were carried out (11/16/2018; 01/09/2019; 02/05/2019 and 05/16/2019) to assess the trends in microbial biomass and P Olsen of bulk soil. The soil samples were obtained by a mixing of five subsamples per treatment taken at depths of 0 - 30 cm. After sampling, the soil was taken to the laboratory and, immediately analyzed to estimate the P stored in the microbial biomass.

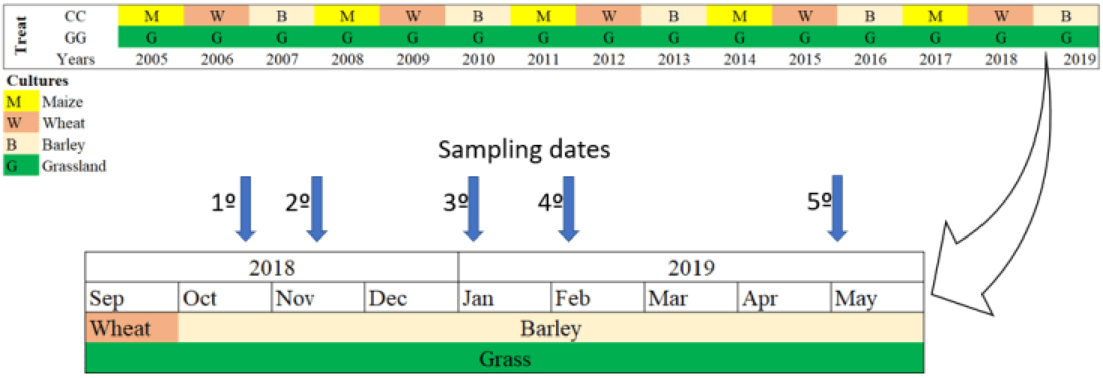


Figure 1 - Field trial design and sampling dates.

Soil fractionation by aggregates

The soil fractionation into aggregates classes was performed in samples from the first sampling (October/2018), according to the water stability by the method of Elliott (1986). Briefly, the soil at the field moisture was disintegrated in aggregates of diameter between 7.1 and 2 mm. Then, 50 g of 7.1-2.0 cm disintegrated soil was deposited on a 2 mm mesh sieve, submerged in deionized water and shaken vertically (3 cm amplitude) for 2 min at a rate of 25 oscillations min^{-1}. The procedure was repeated with the soil suspension in sieves of 0.200 and 0.050 mm mesh. After the procedure, a portion of aggregates still wet were stored and the aggregates remaining were oven dried at 65 °C. The aggregate size fractions were classified into: large macroaggregates (LMA, ø 2 – 7.1 mm), macroaggregates (MA, ø 0.200 – 2 mm), microaggregates (MI, ø 0.050 – 0.200 mm) and silt + clay (S+C, ø <0.050 mm).

P stored in the microbial biomass

The P stored in the microbial biomass (P_{mic}) was quantified in the bulk soil and in each aggregate size using the methodology described in Hedley et al. (1982) and modified by Rheinheimer et al. (2000). Briefly, 0.5 g of wet soil was weighed in triplicate (A, B and C). The subsample A was treated with 0.5 mL of chloroform ($CHCl_3$) under vacuum for 24 h. After, subsamples A and B were extracted by 10 mL of 0.5 mol L^{-1} $NaHCO_3$ and subsample C by 10 mL of 0.5 mol L^{-1} $NaHCO_3$ + 5 mg L^{-1} of P. All samples were kept under shaking for 16 h. Subsequently, the samples were centrifuged at 1000 x g for 15 min. An aliquot of 2 mL of the supernatant was mineralized in autoclave at 121 ° C and 103 KPa for two hours with 5 mL of 7.5% ammonium persulfate and 0.5 mL of H_2SO_4 1:1 (v/v) (USEPA, 1971). The total P in mineralized extracts was measured by colorimetry at 882 nm after formation of an acid phospho-molybdate complex (Murphy & Riley, 1962). The P_{mic} concentration was estimated by the equation 1:

$$P_{mic} = \frac{[P]*V*(A-B)}{(0.4*MSc*(C-B))} \qquad \text{Equation 1}$$

Where P_{mic} (mg kg^{-1}) is an estimative of P stored in the microbial biomass; [P] (mg L^{-1}) is the concentration of P inorganic added in the subsample C; V (mL) is the volume of extract solution; A, B and C (mg kg^{-1}) are the quantified P contents in each subsample; MSc (g) is the soil dry mass used in subsample C; 0.4 is a coefficient of P recovery from microbial biomass.

Soil P

The total P (Pt) in a bulk soil was quantified by ignition method (Walker & Adams, 1958). Briefly: 0.5 g of soil was weighed and ignited at 550 ºC during 2h. After calcination the P was extracted by 1 mol L^{-1} H_2SO_4 during 24h. The total organic P (Po) was measured as follows: two subsamples were weighed and one of them was ignited at 550 ºC during 2h. The P was extracted in both subsamples by 0.5 mol L^{-1} H_2SO_4 during 24 h. The Po was the difference between P concentration in both subsamples. The total inorganic P (Pi) was quantified by difference between Pt and Po.

The available P in a bulk soil (Olen P – P organic + P inorganic) was quantified after extraction with 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 (1:20 weight/volume) fallowed by mineralization with ammonium persulfate and H_2SO_4 in autoclave (USEPA, 1971).

The soil P fractionation was performed in the bulk soil and in the aggregate sizes by Hedley protocol (Hedley et al., 1982) modified by Rheinheimer et al. (2000). Briefly, a 0.5 g of soil was submitted to sequential extraction with: 1º - Anion Exchange Resin – AER (Pi); 2º - 0.5 mol L^{-1} $NaHCO_3$ (Po and Pi); 3º - 0.1 mol L^{-1} NaOH (Po and Pi); 4º - 1 mol L^{-1} HCl (Pi) and 5º - 0.5 mol L^{-1} NaOH (Po and Pi). At the end of P fractionation, the residual total P was quantified by soil digestion with H_2SO_4+H_2O_2 (Brookes & Powlson, 1982). The residual organic P was quantified by ignification method (Walker & Adams, 1958) and, the residual inorganic P was considered the difference between the residual total P and residual organic P.

In alkaline extracts, Pi was quantified by the molybdate colorimetry method (Dick & Tabatabai, 1977) and, Pt was determined by Murphy & Riley (1962) method after sample mineralization with ammonium persulfate and H_2SO_4 in autoclave (USEPA, 1971). The Po was obtained by difference between Pt and Pi. In acid extracts, Pi was directly measured by Murphy & Riley method.

For P lability analyses were considered:

a) P-labile = P_{AER} + P_{NaHCO3}

b) P-moderately labile (P-mod-labile) = $P_{NaOH0.1}$ + P_{HCl}

c) P-non-labile = $P_{NaOH0.5}$ and P-residual

Total C and N

The total C and N of bulk soil and each aggregate size was determined by elementary analyzer (Flash EA, Thermo Electron Corporation, Bremen, Germany). The samples were macerated until homogeneous consistency, placed in tin capsules and taken to the combustion chamber at a temperature of 975 ° C.

Stoichiometry C:N:P in soil aggregates size

The stoichiometry C:N:P was analyzed in all aggregation sizes. The total organic P was considered to calculate the atomic ratio C:Po and N:Po. The atomic ratios were obtained according to the equation described by (Xue et al., 2019):

$$\text{Atomic Ratio} = \frac{\text{Mass Ratio}}{\text{Atomic Weight Ratio}} \qquad \text{Equation 2}$$

Where the atomics weights used to C, N and P were 12.0107, 14.0067 and 30.9738. respectively.

Statistical analysis

The chemical variables from bulk soil and the aggregates size were analyzed in randomized complete block design in split-plot with 4 replications. The soil management is the main factor (plot) and the aggregate size is the split-plot. The statistical model used is described at equation 3.

$$Y_{ijk} = \mu + B_i + T_j + error\ a(j,i) + R_k + (TR)_{jk} + error\ b(i,j,k) \qquad \text{Equation 3}$$

Where Y is the observation; µ is the overall experimental average; B is the blocks (i = 1, 2, 3, 4); T is the soil managements (j = 1 and 2); R is the size of aggregate (k = 1, 2, 3, 4) and error is the experimental error.

The temporal pattern of P_{mic}, Olsen P and atomic ratio C:N:Po were analyzed in randomized complete block design in split-plot in time with 4 replications. According to equation 4.

$$Y_{ijk} = \mu + B_i + T_j + error\ a(j,i) + C_n + error\ b(i,n) + (TC)_{jn} + error\ c(i,j,n)$$

Equation 4

Where C is the time (n = 1, 2, 3, 4, 5).

The soil management effects in the variables analyzed were tested by Scott-Knott test ($P \leq 0.10$) with use of complex variance by SISVAR 5.6 software.

9.3.Results and Discussion

Bulk soil

Keeping the soil constantly covered with semi-perennial grass mix (*Dactylis* + *Festuca* + *Lolium*) for 13 years with mowing and removal of the aerial part or annual soil tillage by deep plowing and harrowing for cereal production (maize-wheat-barley rotation) with removal of the grain did not change the soil Pt content of the 0-30 cm layer (Figure 2g). Soil maintained for a long period under permanent grassland without disturbance increases significantly the soil organic matter stock compared to conventional continuous cereal cultivation (Crème et al., 2018). As a result, it is evident that organic compounds containing phosphate are mineralized in a rate higher than the bulk organic matter when the soil is constantly plowed and with lower amounts of residue input, including of rhizospheres' exudates. The Pi/Po total ratio was higher in CC (1.61) than in GG (0.96) (Figure 2d). The Pi / Po ratio in the 0-10 cm topsoil layer of soils under forests in France is, on average, 1.07 (i.e. Po represents 48% of the total phosphorus) (Achat et al., 2016). These values are similar to those observed in the 0-30 cm topsoil layer of soil cultivated in a management system with forage plants, which denotes a relationship close to values of non-agricultural natural areas.

Differences in Pi and Po content between treatments are linked to changes in the most bioavailable P pool (Pi_{AER} + Pi_{NaHCO3} + Po_{NaHCO3}) (Figure 2a and b). Phosphorus extracted by weak extractants or by resin exchange corresponds to an easily desorbable pool acting as a P source when plant demand is high and as a P sink when plant residues are mineralized or when phosphates fertilizers are added to the soil. Therefore, this pool is more sensitive to temporal variation by management systems and plant uptake/exportation (Helfenstein et al., 2020; Tiecher et al., 2018). The annual soil plowing to annual crop cultivation induces lower values of bioavailable Pi, especially P easily desorbable (Pi_{AER} + Pi_{NaHCO3} - 86.8 mg kg^{-1} in CC versus 65.1 mg kg^{-1} in GG) with concomitant smaller amount of weakly adsorbed Po to the clays and oxides and bioavailable after mineralization by microbial or vegetal enzymatic exudation (Po_{NaHCO3} – 11.4 mg kg^{-1} in CC versus 15.5 mg kg^{-1} in GG). Otherwise, in systems with the soil constantly covered by living plants there are larger amounts of P immobilization in plant shoot and root with consequent return to soil in organic compounds.

For the first sampling, higher values of P_{mic} were obtained in CC (32 mg kg^{-1}) compared to GG (11 mg kg^{-1}). These values represent 33 % of the labile P pool (Pi_{AER} + Pi_{NaHCO3} + Po_{NaHCO3}) and are an important source of available P. In contrast, P_{mic} represents only 14 % of labile P in GG. Differences in rapidly desorbable Po contents did not follow differences in the amount of P stored in the microbial biomass (Figure 3), contrary to what has been observed in subtropical soils (Rheinheimer et al., 2002).

The C and N percentage in the bulk soil were higher in GG (1.25 % and 0.13 %, respectively) than in CC (1.06 % and 0.11 %, respectively), supporting the increase in the SOM by permanent grassland management. This behavior was also reported to 0-10 cm topsoil layer in the same field trial for the year 2014 (Crème et al., 2018) and for 0-30 cm topsoil layer observed in a temporal analyze from 2005 to 2018 (Somavilla et al., unpublished).

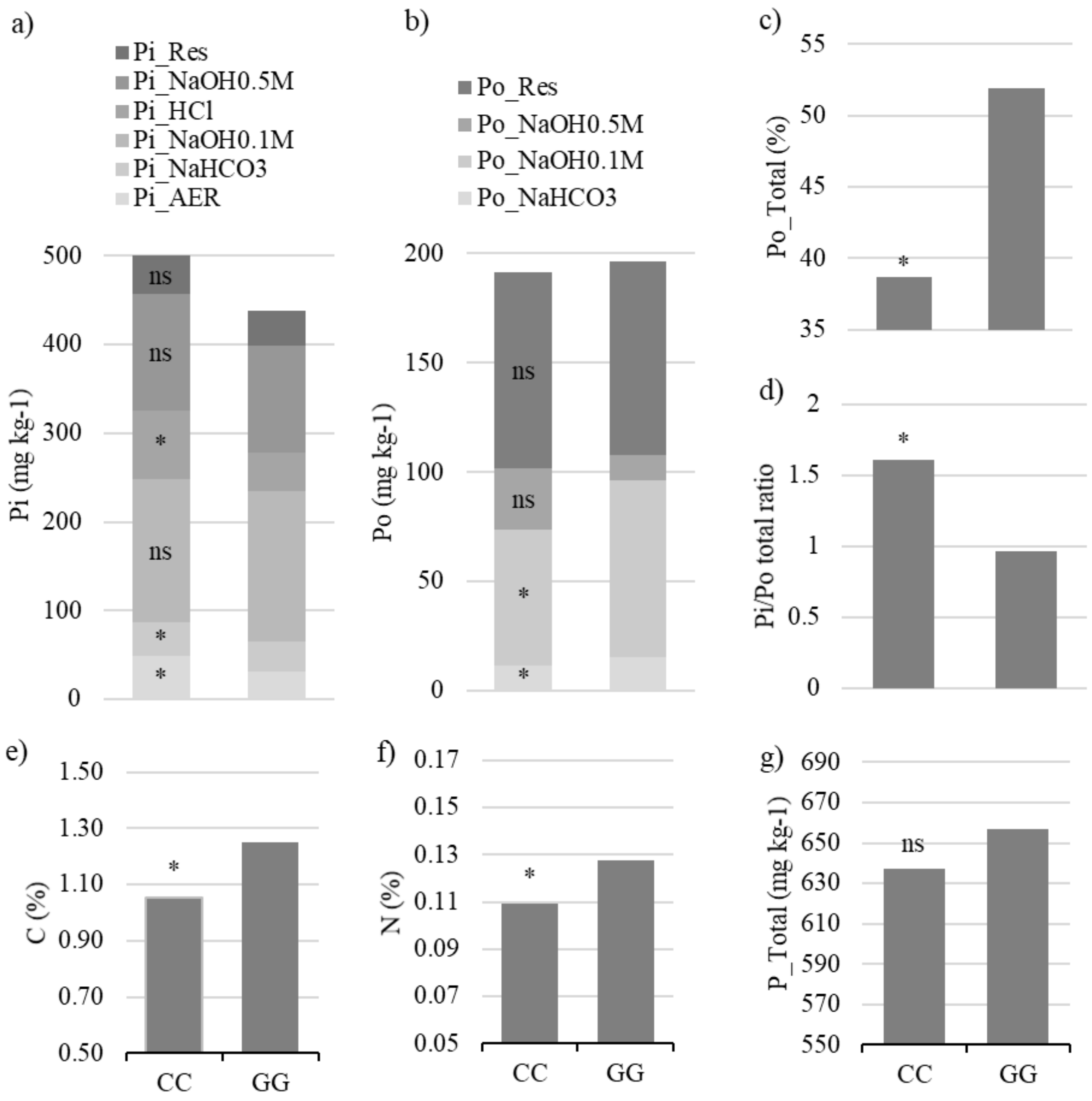


Figure 2 - Bulk soil P pattern in October 2018: a) and b) are P fractions obtained by sequential P fractionation; c) Total organic P; d) Inorganic and organic P ratio; e) and f) Total soil C and N content and; f) Total soil P concentration. *Means fallowed by the same letter did not differ statistically by Scott-Knott test ($p \leq 0.10$). ^{ns} Not significant difference by Scott-Knott test ($p \leq 0.05$).

We monitored during the growing season 2018/2019 the fluctuations in the contents of rapidly desorbable P (Olsen method) and the amount of P stored in the microbial biomass. The levels of P (Pi + Po) extracted by Olsen method were constantly higher in the plowed soil for cereal production (CC - average of 87 mg kg^{-1}) compared to the maintenance of the forage mix (GG - average of 74 mg kg^{-1}) (Figure 3). For both cases, Olsen P levels are above the critical values for low-demand crops (70 mg kg^{-1}), such as cereals (maize-wheat-barley) and forages (*Dactylis* + *Festuca* + *Lolium*) cultivated in the experiment (COMIFER, 2019).

Similar behavior was observed for the amount of P stored by microbial biomass, except in soil sampling performed on 02/05/2019 (barley growth), the only time when the CC system contained less P_{mic} than the system with permanent pasture (GG). The values of this Po pool represented 11 to 47% (for CC) and 11 to 37% (for GG) of the P extracted by the Olsen method (Figure 3).

In natural environments there is strong synchronism between plant demand, P stored by soil microbial biomass (SMB), and other bioavailable forms of Pi and Po. The values of P stored by SMB in the soil managed for 13 years under grassland remained remarkably close to the average (17 mg kg^{-1}), demonstrating a "near-equilibrium" state of the system. In contrast, we observed a positive relationship between incorporation of wheat crop residues and Pi immobilization by microbial biomass. Soil disturbance by plowing (0-30 cm) followed by harrowing, besides fragmenting and distributing the wheat crop residues, increased soil aeration. Consequently, we found P_{mic} values 2-fold higher as those measured in soil maintained under permanent grassland. The soil tillage effect persisted until early 2019 (± 90 days), when the P_{mic} values of the two contrasting land use and soil management systems stabilized around 15-20 mg kg^{-1}.

The temporal behavior of P_{mic} in the 2018/2019 growing season (Barley) denotes the importance of P_{mic} for the bioavailability of P in the soil, even in temperate soil and during the coldest and humid season of the year. In this case, P_{mic} assumed values close to 50% (47% in CC and 37% in GG) of the total bioavailable P content (by Olsen method). Though, between July and October (the warm and low rainfall months) the high evapotranspiration period considerably reduces soil moisture levels; while between November and January, soil moisture contents are often above field capacity (Senapati et al., 2016). Both conditions are limiting the microbial communities' development and therefore to the storage of P by soil microbial biomass.

For soils in regions of subtropical and tropical climate, extreme conditions of temperature and humidity are less common, which favors microbial development. Furthermore, in these regions, soils tend to have a high P adsorption capacity due to the mineralogical characteristics of the soil mineral matrix. Under these conditions, the bioavailable P stock stored in the soil's microbial biomass is even more important for plant supply.

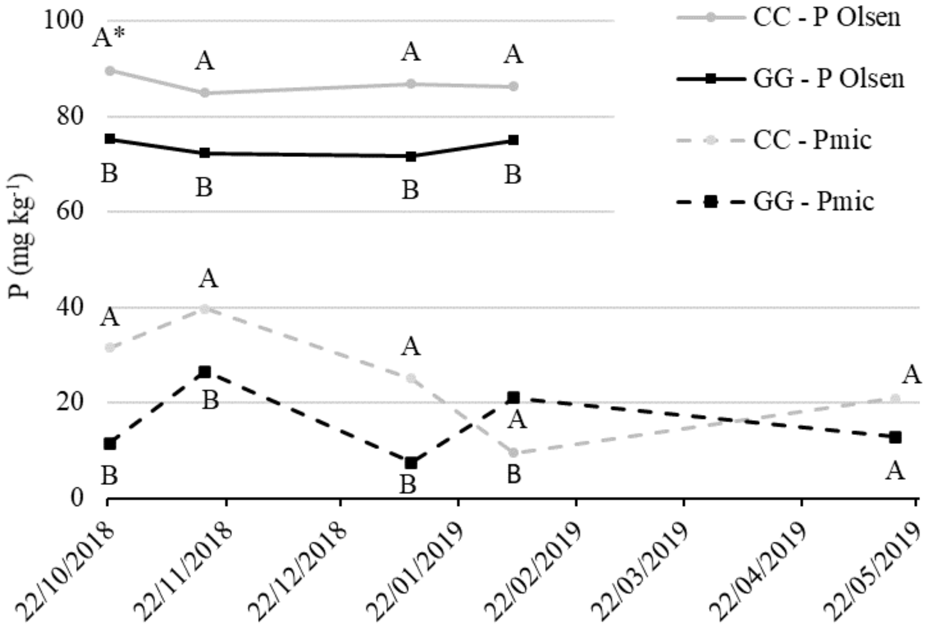


Figure 3 - Trends in Olsen P and P microbial biomass (P_{mic}) over the growth season of 2018/2019. *Means followed by the same letter, by date, did not differ statistically by Scott-Knott test ($p \leq 0.10$).

Soil aggregate analysis

Half of the soil mass in the 0-30 cm layer of soil maintained for 13 years under permanent grassland is organized into large macroaggregates (LMA, ø 2 – 7.1 mm). Another 33% of the soil mass is in macroaggregates between 0.2 and 2 mm (Figure 4). The aggregate size distribution model of this soil also demonstrates that there is little soil mass in microaggregates (MI, ø 0.050 – 0.200 mm). In contrast, the soil plowing in CC, performed on the same day of soil sampling, was sufficient to disrupt LMA and transforming them to MA (Figure 4a). The reduction in the aggregates average size and the disruption of the soil are unfavorable consequences of the soil plowing practice. Since, due to soil disruption, in the long term it may favor the increase of the soil density, the reduction of soil aeration and the water infiltration. Additionally, the effects of constant soil plowing can be seen by the increase in microaggregates of the silt + clay size.

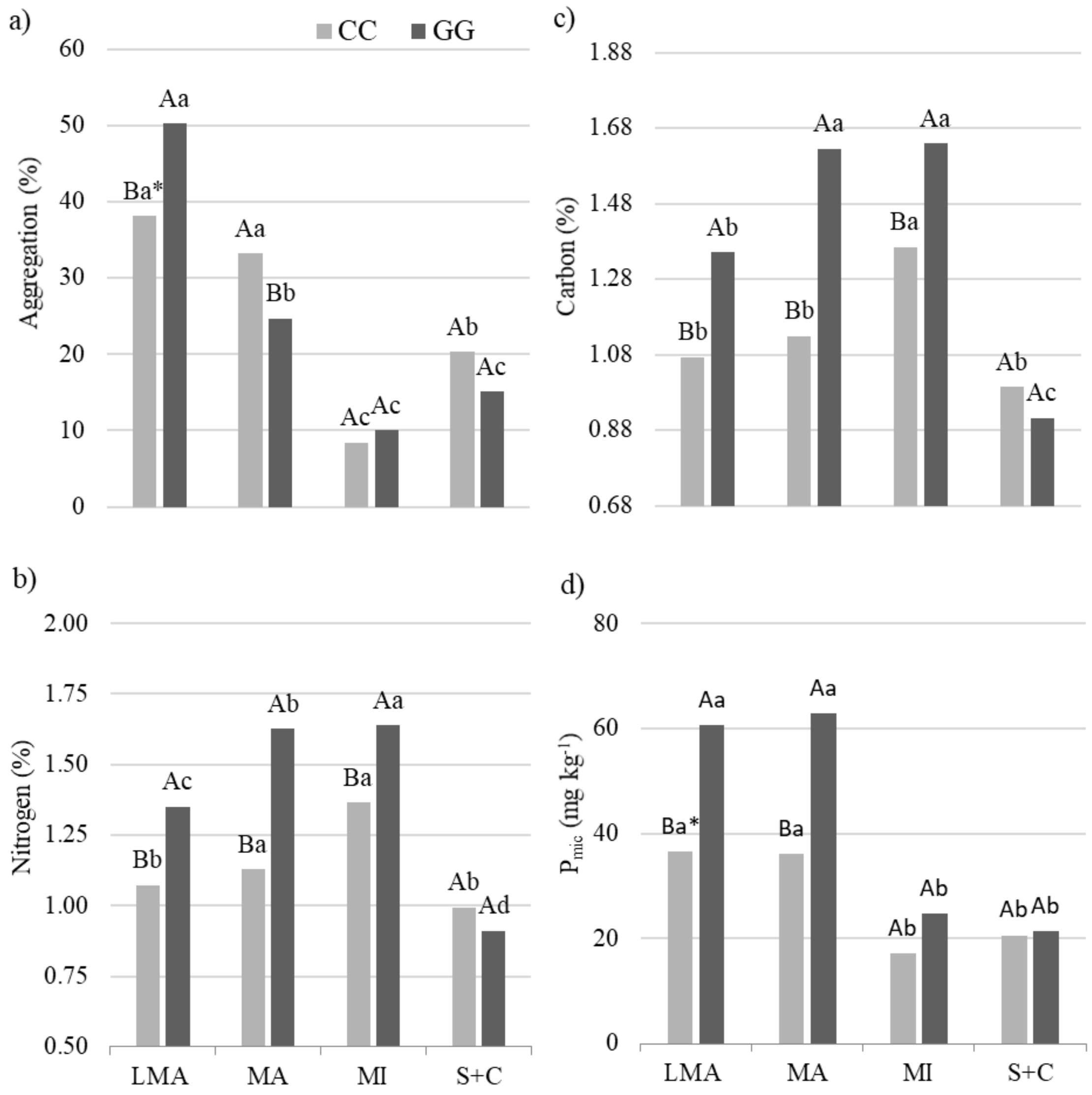


Figure 4 - Soil aggregate distribution (a) and contents of total N (b), total C (c) and P in microbial biomass – P_{mic} (d) in aggregates size from October/2018 soil sampling. *Means followed by the same letter, capital letter between treatments and tiny letter between aggregate size, did not differ statistically by Scott-Knott test ($p \leq 0.10$). LMA - large macroaggregates (ø 2 – 7.1 mm), MA - Macroaggregates (ø 0.200 – 2 mm), MI - microaggregates (ø 0.050 – 0.200 mm) and S+C - silt + clay (ø <0.050 mm).

The constant presence of vegetation in the soil favors the capture and stabilization of C in microaggregates, which support the formation of macroaggregates together with the performance of binding agents for stabilization, mainly of fungi (Liao et al., 2018). Thus, in addition to the aggregate's distribution, the permanent cultivation of forage species favored the accumulation of higher levels of N and C in larger aggregates. On the other hand, constant cultivation with permanent cropland and annual soil plowing reduced the percentage and the C and N content of large aggregates (Figure 4b and c). These results are also consistent with those evidenced for the year 2014, 9 years after the beginning of the same experiment (Panettieri et al., 2017).

Larger aggregates have a greater capacity for SOM accumulation, even with a lower degree of decomposition, mainly in systems with greater introduction of plant biomass, such as the cultivation of forage plants. The higher levels of SOM in these aggregates also favor microbial activity, due to the presence of decomposable substrate, and consequently the immobilization of P, C and N in their tissues. The highest P_{mic} values were obtained in aggregates larger than 0.050 mm (LMA and MA) and were more pronounced in GG (mean of 62 mg kg^{-1}) than CC (mean of 32 mg kg^{-1}) (Figure 4d). On the other hand, in smaller aggregates, in addition to containing compounds with a greater degree of decomposition, there is greater physical and chemical protection of organic matter by the soil mineral matrix (Virto et al., 2008, 2010). This fact hinders the performance of microorganisms (mainly bacteria - Liao et al. (2018)) in the mineralization of the substrate and, consequently, its development is compromised.

Moreover, larger aggregates have a faster N and C accumulation response due to the supply of organic matter in the soil. Instead, finer aggregates have a higher mean residence time of organic carbon. Higher levels of C, N and C: N atomic ratio in aggregates greater than 0.053 mm, especially in the class 0.250-0.050 mm were also identified by Liao et al. (2018). These authors attributed the greater presence of C and N in this aggregates class due to the fact that large aggregates provide a better microhabitat (especially for fungi) and with a better substrate for its development. Finally, the decrease of C, N and P_{mic} in larger aggregates under permanent cropland system suggest higher sensitivity to change in soil management systems and organic material mineralization.

The 13 years of contrasting soil management systems practices were not enough to show changes in the Pt content of the different aggregates sizes (Figure 5a). However, the constant cultivation of forages provided a higher concentration of Po, mainly in the largest aggregates (283, 287, 300 mg kg^{-1} for LMA, MA and MI, respectively). This result is consistent with the concentrations of C, N and P_{mic} (Figure 5b). In contrast, when soil plowing and annual crops are cultivated, Pi levels were increased in relation to forage cultivation, regardless of aggregate size (Figure 5c).

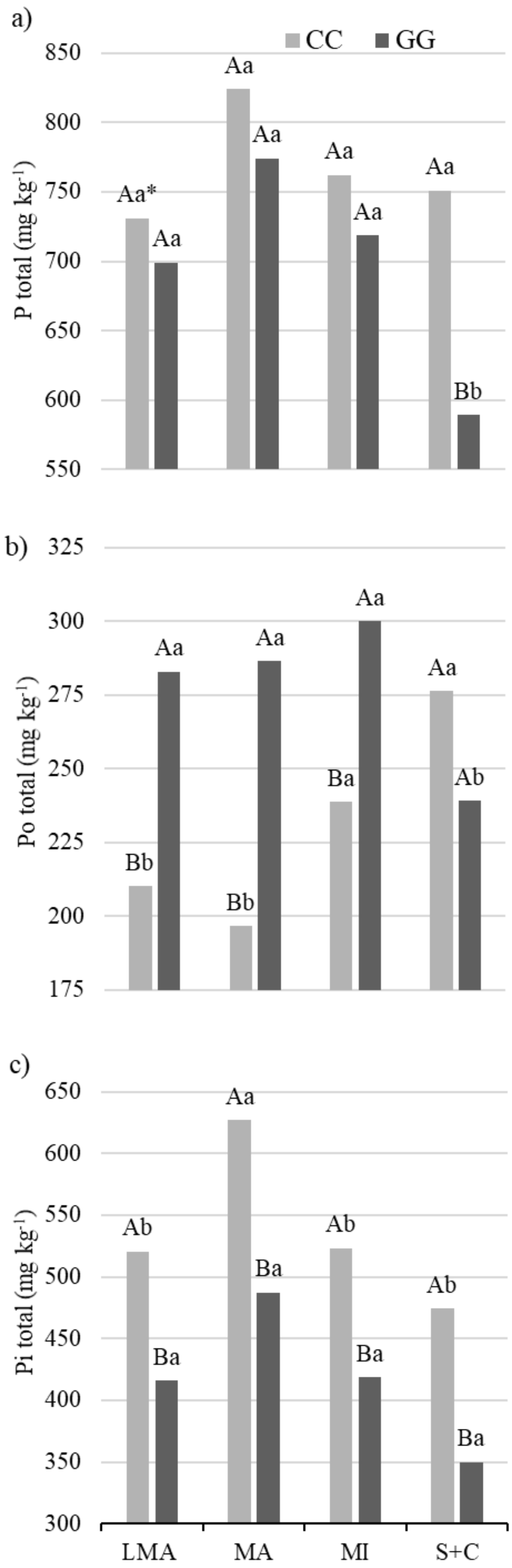


Figure 5 - Total P (a) and P organic (b) and inorganic (c) forms in different aggregates sizes from October/2018 soil sampling. *Means followed by the same letter, capital letter between treatments and tiny letter between aggregate size, did not differ statistically by Scott-Knott test ($p \leq 0.10$). LMA -

large macroaggregates (ø 2 – 7.1 mm), MA - Macroaggregates (ø 0.200 – 2 mm), MI - microaggregates (ø 0.050 – 0.200 mm) and S+C - silt + clay (ø <0.050 mm).

From the P chemical fractionation in the different aggregates sizes, it was possible to verify greater bioavailability of P (P labile) in aggregates greater than 0.050 mm when the soil was cultivated with forage species, mainly due to the contents of Pi labile in the soil (Table 1). Among P fractions with less lability, little difference was identified between the aggregates. Emphasized mainly by the highest levels of Po mod-labile and Po non-labile in aggregates of the intermediate size.

Differently, for the soil cultivated with cereals and annually plowed, the highest bioavailability of P was obtained in smaller aggregates (<0.200 mm), mainly due to the higher Po contents in these aggregate sizes (Table 1). This treatment also provided a higher content of less labile Pi (Pi mod-labile and Pi non-labile), in larger aggregates (> 0.200 mm).

Table 1 - Inorganic and organic soil P pools by different aggregates size in contrasting soil management systems.

Treat	**Size**	**P organic (mg kg-^{1})**			**P inorganic (mg kg^{-1})**		
		Labile	**Mod-labile**	**Non-labile**	**Labile**	**Mod-labile**	**Non-labile**
CC	LMA	5 Ab*	31 Ab	100 Ab	89 Aa	221 Ab	368 Ab
	MA	8 Ab	23 Bb	97 Ab	88 Aa	255 Aa	461 Aa
	MI	13 Aa	61 Aa	122 Aa	86 Aa	207 Ab	308 Ac
	S+C	12 Aa	55 Aa	135 Aa	93 Aa	213 Ab	321 Ac
GG	LMA	8 Aa	43 Ab	101 Ab	87 Aa	230 Aa	376 Aa
	MA	10 Aa	75 Aa	115 Aa	82 Aa	219 Ba	379 Ba
	MI	9 Aa	65 Aa	123 Aa	77 Aa	229 Aa	328 Aa
	S+C	13 Aa	65 Aa	99 Bb	60 Bb	164 Bb	336 Aa

CC – permanent cropland, GG – permanent grassland, LMA - large macroaggregates (ø 2 – 7.1 mm), MA - Macroaggregates (ø 0.200 – 2 mm), MI - microaggregates (ø 0.050 – 0.200 mm) and S+C - silt + clay (ø <0.050 mm). *Means followed by the same letter, capital letter between treatments and tiny letter between aggregate size, did not differ statistically by Scott-Knott test ($p \leq 0.10$).

The stoichiometry C:N:Po was little changed by the 13 years of cultivation. The main difference observed was the increase of C:Po atomic ratio with permanent cropland cultivation (Figure 6a). The highest values of the C:Po to CC are the result of the lowest Po values quantified in this treatment (Table 1 and Figure 5b). For the N:Po atomic ratio, there were no differences between treatments and aggregate classes (Figure 6b). The biggest differences were due to the atomic C:N atomic ratio. In this case, lower values were observed in S + C (9 in both treatments) and higher in MA (11.7 and 12.5 for CC and GG respectively).

The highest stoichiometry C:N:Po for both treatments were identified in macro and micro aggregates (169:14:1 and 158:14:1 in CC and 147:12:1 and 142:12:1 in GG respectively to MA and MI). Values very close to those identified for soils cultivated with pastures at global scale, mean of 166:14:1 (Cleveland & Liptzin, 2007) and 169:11:1 (Xu et al., 2013). Whereas, in S + C, stoichiometry C:N:Po was 94:10:1 and 98:11:1 for CC and GG, respectively. Value below those previously reported, but a consequence of lower C and N levels and higher Po values, mainly in CC treatment.

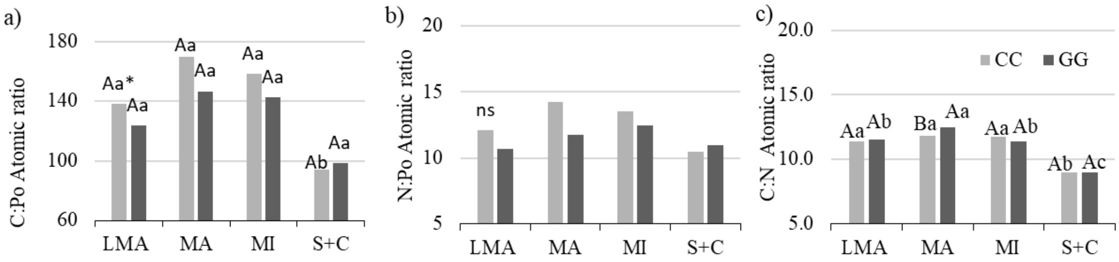


Figure 6 - Stoichiometry C:N:Po in aggregates from October/2018 soil sampling as: C:Po (a), N:Po (b) and C:N (c) atomic ratio. *Means followed by the same letter, capital letter between treatments and tiny letter between aggregate size, did not differ statistically by Scott-Knott test ($p \leq 0.10$). ^{ns} not significant. LMA - large macroaggregates (ø 2 – 7.1 mm), MA - Macroaggregates (ø 0.200 – 2 mm), MI - microaggregates (ø 0.050 – 0.200 mm) and S+C - silt + clay (ø <0.050 mm).

9.4.Conclusion

In this work we analyze the importance of the phosphorus stock present in the microbial biomass in the P bioavailability during the growing season of barley and forage crops in a temperate climate region. As well as, we evaluated changes in the total contents of P, C and N, in the content of P contained in the soil microbial biomass, in the lability of the P and in the C:N:P stoichiometry in different soil aggregates sizes due to the contrasting management systems.

Our results show that the soil management systems did not change the total levels of P in the soil after 13 years, but the inorganic P / organic P ratio was higher with the practice of soil plowing and annual crop cultivation. Concomitantly, the forage management system favored the accumulation of organic C, N and P in the soil. During the growing season (barley / mix forage - 2018/2019), the levels of P contained in the soil microbial biomass were an important pool of bioavailable P in both management systems and represented up to 47% of the available P content (P Olsen) in a crop cultivation system.

The practice of soil plowing and crops cultivation reduced the percentage and the levels of C, N and P in the soil microbial biomass in large aggregates compared to the permanent forage cultivation. Furthermore, the permanent forage cultivation maintained a higher P bioavailability (P labile) in aggregates greater than 0.050 mm, mainly due to the Pi labile contents. In contrast, for the soil with permanent cropland, the highest P bioavailability was obtained in smaller aggregates (<0.200 mm), mainly due to the higher levels of Po labile in these aggregate classes. The stoichiometry C:N:Po was little changed by the 13 years of cultivation in the management systems.

9.5. References

Achat, D. L., Pousse, N., Nicolas, M., Brédoire, F., & Augusto, L. (2016). Soil properties controlling inorganic phosphorus availability: general results from a national forest network and a global compilation of the literature. Biogeochemistry, 127(2–3), 255–272. https://doi.org/10.1007/s10533-015-0178-0

Assunção, S. A., Pereira, M. G., Rosset, J. S., Berbara, R. L. L., & García, A. C. (2019). Carbon input and the structural quality of soil organic matter as a function of agricultural management in a tropical climate region of Brazil. Science of The Total Environment, 658, 901–911. https://doi.org/10.1016/J.SCITOTENV.2018.12.271

Blake, L., Johnston, A. E., Poulton, P. R., & Goulding, K. W. T. (2003). Changes in soil phosphorus fractions following positive and negative phosphorus balances for long periods. Plant and Soil, 254(2), 245–261. https://doi.org/10.1023/A:1025544817872

Boitt, G., Black, A., Wakelin, S. A., McDowell, R. W., & Condron, L. M. (2018). Impacts of long-term plant biomass management on soil phosphorus under temperate grassland. Plant and Soil, 427(1–2), 163–174. https://doi.org/10.1007/s11104-017-3429-0

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? Biogeochemistry, 85(3), 235–252. https://doi.org/10.1007/s10533-007-9132-0

COMIFER. (2019). La fertilisation P – K – Mg - les bases du raisonnement. Available in : https://comifer.asso.fr/images/publications/brochures/COMIFER_RAPPORT_fertilisation_15102019.pdf.

Condron, L. M., Goh, K. M., & Newman, R. H. (1985). Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by 31P nuclear magnetic resonance analysis. Journal of Soil Science, 36(2), 199–207. https://doi.org/10.1111/j.1365-2389.1985.tb00324.x

Condron, L. M., Turner, B. L., & Cade-Menun, B. . (2005). Chemistry and dynamics of soil organic phosphorus. In J. T. Sims & A. S. Sharpley (Eds.), Phosphorus: Agriculture and the Environment. (pp. 87–121). https://doi.org/10.2134/agronmonogr46.c4

Crème, A., Rumpel, C., Le Roux, X., Romian, A., Lan, T., & Chabbi, A. (2018). Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities. Soil Biology and Biochemistry, 122(May), 203–210. https://doi.org/10.1016/j.soilbio.2018.04.018

Delmas, M., Saby, N., Arrouays, D., Dupas, R., Lemercier, B., Pellerin, S., & Gascuel-Odoux, C. (2015). Explaining and mapping total phosphorus content in French topsoils. Soil Use and Management, 31(2), 259–269. https://doi.org/10.1111/sum.12192

Dick, W. A., & Tabatabai, M. A. (1977). Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds1. Journal of Environment Quality, 6(1), 82. https://doi.org/10.2134/jeq1977.00472425000600010018x

Dignac, M.-F., Derrien, D., Barré, P., Barot, S., Cécillon, L., Chenu, C., Chevallier, T., Freschet, G. T., Garnier, P., Guenet, B., Hedde, M., Klumpp, K., Lashermes, G., Maron, P.-A., Nunan, N., Roumet, C., & Basile-Doelsch, I. (2017). Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies. A review. Agronomy for Sustainable Development, 37(2), 14. https://doi.org/10.1007/s13593-017-0421-2

Elliott, E. T. (1986). Aggregate Structure and Carbon, Nitrogen, and Phosphorus in Native and Cultivated Soils. Soil Sci .Soc. Am. J., 50(ii), 627–633. https://doi.org/10.2136/sssaj1986.03615995005000030017x

Elser, J. J. (2012). Phosphorus: A limiting nutrient for humanity? Current Opinion in Biotechnology, 23(6), 833–838. https://doi.org/10.1016/j.copbio.2012.03.001

Éveillard, P. & Saby, N. Diminution de la fertilisation minérale et conséquences sur la disponibilité des éléments phosphore et potassium dans les sols…In : Fertilisation et fertilité des sols. Académie d'Agriculture de France. Session Publique 7 mars 2018. Disponível em: https://www.academie-agriculture.fr/actualites/academie/seance/academie/fertilisation-et-fertilite-des-sols?070318. Acesso: 15 mars 2018.

Fontana, A., Pereira, M. G., Jarbas, T., & Cunha, F. (2008). Fósforo remanescente e correlação com as substâncias húmicas em um latossolo vermelho sob diferentes sucessões de cultura em plantio direto. Fósforo Remanescente e Correlação Com as Substâncias Húmicas Em Um Latossolo Vermelho Sob Diferentes Sucessões de Cultura Em Plantio Direto, 14(1), 161–166. https://doi.org/10.18539/cast.v14i1.1900

Guppy, C. N., Menzies, N. W., Moody, P. W., & Blamey, F. P. C. (2005). Competitive sorption reactions between phosphorus and organic matter in soil: A review. Australian Journal of Soil Research, 43(2), 189–202. https://doi.org/10.1071/SR04049

Hayes, J. E., Richardson, A. E., & Simpson, R. J. (1999). Phytase and acid phosphatase activities in extracts from roots of temperate pasture grass and legume seedlings. Australian Journal of Plant Physiology, 26(8), 801–809. https://doi.org/10.1071/PP99065

Hedley, M. J., Stewart, J. W. B., & Chauhan, B. S. (1982). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations. Soil Science Society of America Journal, 46(5), 970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Helfenstein, J., Pistocchi, C., Oberson, A., Tamburini, F., Goll, D. S., & Frossard, E. (2020). Estimates of mean residence times of phosphorus in commonly considered inorganic soil phosphorus pools. Biogeosciences, 17(2), 441–454. https://doi.org/10.5194/bg-17-441-2020

Jarosch, K. A., Kandeler, E., Frossard, E., & Bünemann, E. K. (2019). Is the enzymatic hydrolysis of soil organic phosphorus compounds limited by enzyme or substrate availability? Soil Biology and Biochemistry, 139, 107628. https://doi.org/10.1016/j.soilbio.2019.107628

Kleber, M., & Johnson, M. G. (2010). Advances in Understanding the Molecular Structure of Soil Organic Matter. Advances in Agronomy, 106(10), 77–142. https://doi.org/10.1016/S0065-2113(10)06003-7

Le Noë, J., Roux, N., Billen, G., Gingrich, S., Erb, K. H., Krausmann, F., Thieu, V., Silvestre, M., & Garnier, J. (2020). The phosphorus legacy offers opportunities for agro-ecological transition (France 1850-2075). Environmental Research Letters, 15(6). https://doi.org/10.1088/1748-9326/ab82cc

Liao, H., Zhang, Y., Zuo, Q., Du, B., Chen, W., Wei, D., & Huang, Q. (2018). Contrasting responses of bacterial and fungal communities to aggregate-size fractions and long-term fertilizations in soils of northeastern China. Science of the Total Environment, 635, 784–792. https://doi.org/10.1016/j.scitotenv.2018.04.168

Mangalassery, S., Kalaivanan, D., & Philip, P. S. (2019). Effect of inorganic fertilisers and organic amendments on soil aggregation and biochemical characteristics in a weathered tropical soil. Soil and Tillage Research, 187(November 2018), 144–151. https://doi.org/10.1016/j.still.2018.12.008

Mcgill, W. B., & Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma, 26, 267–286. https://doi.org/10.1016/0016-7061(81)90024-0

Meeds, J. A., Marty Kranabetter, J., Zigg, I., Dunn, D., Miros, F., Shipley, P., & Jones, M. D. (2021). Phosphorus deficiencies invoke optimal allocation of exoenzymes by ectomycorrhizas. ISME Journal, 1478–1489. https://doi.org/10.1038/s41396-020-00864-z

Menezes-Blackburn, D., Giles, C., Darch, T., George, T. S., Blackwell, M., Stutter, M., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Almeida, D. S., Wearing, C., Zhang, H., & Haygarth, P. M. (2018). Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant and Soil, 427(1–2), 5–16. https://doi.org/10.1007/s11104-017-3362-2

Moni, C., Rumpel, C., Virto, I., Chabbi, A., & Chenu, C. (2010). Relative importance of sorption versus aggregation for organic matter storage in subsoil horizons of two contrasting soils. European Journal of Soil Science, 61(6), 958–969. https://doi.org/10.1111/j.1365-2389.2010.01307.x

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytical Chemistry ACTA, 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Panettieri, M., Rumpel, C., Dignac, M. F., & Chabbi, A. (2017). Does grassland introduction into cropping cycles affect carbon dynamics through changes of allocation of soil organic matter within aggregate fractions? Science of the Total Environment, 576(November), 251–263. https://doi.org/10.1016/j.scitotenv.2016.10.073

Pereira, M. G., Loss, A., Beutler, S. J., & Torres, J. L. R. (2010). Carbono, matéria orgânica leve e fósforo remanescente em diferentes sistemas de manejo do solo. Pesquisa Agropecuaria Brasileira, 45(5), 508–514. https://doi.org/10.1590/S0100-204X2010000500010

Poeplau, C., Zopf, D., Greiner, B., Geerts, R., Korvaar, H., Thumm, U., Don, A., Heidkamp, A., & Flessa, H. (2018). Why does mineral fertilization increase soil carbon stocks in temperate grasslands? Agriculture, Ecosystems and Environment, 265(May), 144–155. https://doi.org/10.1016/j.agee.2018.06.003

Rheinheimer, D. dos S., Fornari, M. R., Bastos, M. C., Fernandes, G., Santanna, M. A., Calegari, A., Canalli, L. B. dos S., Caner, L., Labanowski, J., & Tiecher, T. (2019). Phosphorus distribution after three decades of different soil management and cover crops in subtropical region. Soil and Tillage Research, 192(January), 33–41. https://doi.org/10.1016/j.still.2019.04.018

Rheinheimer, D. S., Anghinoni, I., & Conte, E. (2000). Fósforo da biomassa microbiana em solos sob diferentes sistemas de manejo. Revista Brasileira de Ciência Do Solo, 24(3), 589–597. https://doi.org/10.1590/s0100-06832000000300012

SABY, N. ; GOUNY, L., LEMERCIER, B. F. ; DENOROY, P. ; EVEILLARD, P. 2016. Utilisation des données de la BDAT pour étudier l'évolution spatio-temporelle des teneurs en Magnésium échangeable, Potassium échangeable et Phosphore extractible dans les sols agricoles de France métropolitaine. [Contrat] auto-saisine. <hal-01581567>

Sarker, J. R., Singh, B. P., Cowie, A. L., Fang, Y., Collins, D., Badgery, W., & Dalal, R. C. (2018). Agricultural management practices impacted carbon and nutrient concentrations in soil aggregates, with minimal influence on aggregate stability and total carbon and nutrient stocks in contrasting soils. Soil and Tillage Research, 178(June 2017), 209–223. https://doi.org/10.1016/j.still.2017.12.019

Senapati, N., Jansson, P. E., Smith, P., & Chabbi, A. (2016). Modelling heat, water and carbon fluxes in mown grassland under multi-objective and multi-criteria constraints. Environmental Modelling and Software, 80, 201–224. https://doi.org/10.1016/j.envsoft.2016.02.025

Tiecher, T., Gomes, M. V., Ambrosini, V. G., Amorim, M. B., & Bayer, C. (2018). Assessing linkage between soil phosphorus forms in contrasting tillage systems by path analysis. Soil and Tillage Research, 175(March 2017), 276–280. https://doi.org/10.1016/j.still.2017.09.015

Tisdall, J. M., & Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. Journal of Soil Science, 33(2), 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x

USEPA. (1971). Methods of chemical analysis for water and wastes (United States Environmental Protection Agency (ed.)).

Virto, I., Barré, P., & Chenu, C. (2008). Microaggregation and organic matter storage at the silt-size scale. Geoderma, 146(1–2), 326–335. https://doi.org/10.1016/j.geoderma.2008.05.021

Virto, I., Moni, C., Swanston, C., & Chenu, C. (2010). Turnover of intra- and extra-aggregate organic matter at the silt-size scale. Geoderma, 156(1–2), 1–10. https://doi.org/10.1016/j.geoderma.2009.12.028

Walker, T. W., & Adams, A. F. R. (1958). Studies on soil organic matter: i. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur, and organic phosphorus in grassland soils. Soil Science, 85(6), 307–318.

Wang, D., Lv, S., Jiang, P., & Li, Y. (2017). Roles , Regulation , and Agricultural Application of Plant Phosphate Transporters. 8(May), 1–14. https://doi.org/10.3389/fpls.2017.00817

Xu, X., Thornton, P. E., & Post, W. M. (2013). R E S E A RC H PAPER A global analysis of soil microbial biomass carbon , nitrogen and phosphorus in terrestrial ecosystems. 737–749. https://doi.org/10.1111/geb.12029

Xue, H., Lan, X., Liang, H., & Zhang, Q. (2019). Characteristics and environmental factors of stoichiometric homeostasis of soil microbial biomass carbon, nitrogen and phosphorus in China. Sustainability (Switzerland), 11(10). https://doi.org/10.3390/su11102804

10.Chapter VII - General discussion

In this thesis, works were developed based on two long-term experiments carried out in the field. First, we have an experiment conducted for more than 20 years in a natural grassland of the Pampa biome, located in Rio Grande do Sul, Brazil. This experiment is implemented in an area with low content of total and available soil P due to the characteristics of the parental material and the non-application of any type of fertilization. The purpose of the treatments tested is to improve the level of nutrient availability, in particular P, by using phosphate fertilizers solely or simultaneously to limestone and, in response, to obtain greater productivity of dry matter from the grassland.

Second, we have an experiment conducted for more than 13 years in an area with an extensive history of fertilization and, consequently, high levels of total and available P in the soil. This experiment is located in Lusignan, France and with it we analyzed the effects of soil and plant management on the temporal behavior of the soil P stock depletion, changes in the C and N stocks, in the C:N:P stoichiometry and forms of bioaccumulation of P in the soil and in different aggregates size.

The realization of studies in two experiments, one in Brazil and the other in France, was a mutual choice between doctoral student and supervisors. This was chosen, due to the present thesis being carried out in a joint supervision (*cotutele*) between Brazilian and French universities. Therefore, experimental data from both countries are being considered. Comparatively, both experiments have important differences, such as: the soil P stock (result of the fertilization history) and climate (mainly water and temperature regime). However, both are managed for forage production. In addition, the chemical element/nutrient “phosphorus” is the same regardless of location and its biogeochemical cycle has the same steps, varying only the magnitude between them.

In addition, the problem involved in the phosphorus theme (i.e., exploitation of P reserves, unbalanced use of fertilizers and low P use efficiency in agriculture) is the same in both places, with predictable consequences in the contamination of natural areas (eutrophication of water bodies) and the depletion of world reserves of P and transcends local, national or continental boundaries. Thus, although a comparative analysis between the two studied sites is not feasible, a global approach to the biogeochemical behavior of P is appropriate.

The P element, in any ecosystem, is indispensable for establishing all life cycles (Elser, 2012). It is a key component for the production of proteins, nucleic acids, enzymatic activation and energy transfer (Wang et al., 2017). The plant’s P uptake occurs via the root system and, therefore, it is extremely necessary to have adequate P levels in agricultural soils for better crop productivity. Regardless of soil P content, the extraction and quantification of the plant-available P is commonly performed by soft extractors that vary depending on the soil acidity. Among the most common, we have the methods of Mehlich-1, Mehlich-3, anion exchange resin (AER) and Dyer for more acidic soils and methods of Olsen and Joret - Hébert for more alkaline soil. In addition to these methods, Morel et al. (2021) suggest the use of a process-based assessment that consider the content of orthophosphate ion in solution and the buffering soil capacity. In general, all the methods mentioned above are capable of extracting more bioavailable and accessible P pools by plants, resulting in a high correlation between extracted P and tissue P levels or crop productivity. For this, they are widely used as a criterion for recommending phosphate fertilizers.

In areas where the levels of total P and, mainly, available P to plants are low (such as the case of many areas in Pampa biome, Brazil), there is no other possibility than the anthropic P supply to improve soil P content and plants yields. In these cases, the use of P organic sources (animal waste, vegetable residues, industrial residues) or P inorganic sources (soluble fertilizers or phosphate rocks) are alternatives that are widespread in the agricultural environment. However, for each of these soil P inputs alternatives, there will be different consequences on its biogeochemical cycle and its use / uptake by plants.

In contrast, in soils with a large history of phosphate fertilization or from parent material with a higher P content and which have high content of total and available P there is no need for anthropic P input for increase crop productivity. In these cases, adequate soil management and replacement of exported nutrients are sufficient to maintain satisfactory levels of crop productivity and nutrient use efficiency (Le Noë et al., 2020).

The anthropic contribution of P to the soil to increase its plants availability or even the replacement of the P exported by the crops can be carried out via organic residues or via industrially treated fertilizers. In the first case, residues from poultry, swine or cattle rearing, plant material residue, agro-industrial residues, among others, are used. The use of organic waste as a nutrient source in agriculture is an old strategy, dating back to the time when it was believed that plants fed on organic matter. However, although old, it is an important alternative to the depletion of natural reserves of nutrients, especially P (Withers et al., 2018) and can be performed on any soil type worldwide. But, it should be noted that in regions with high waste production (e.g., pig, poultry and cattle producing regions) the soil becomes a means of disposal and, in these circumstances, limits of chemical elements excess in the soil (e.g., P) should be followed (Gatiboni et al., 2015).

Otherwise, industrially treated fertilizers come from the exploration of non-renewable natural reserves and undergo acid chemical treatments to increase solubility and P concentration (soluble fertilizers - triple and simple superphosphate) or just grinding to reduce particle size (low solubility fertilizers - rock phosphate). The use of soluble fertilizers provides quick release and P availability for crops with short-term vegetation response (Pavinato et al., 2017; Silveira et al., 2018). In contrast, the use of low soluble fertilizers tends to increase the levels of available P in the long term, due to the low solubility of mineral fertilizers in the soil.

The use of rock phosphate in agriculture is based on the proposal that the release and availability of P to crops occurs in a gradual and constant way, providing a synchronism between the presence of P in the soil solution and the plants uptake. Thus, there would be less immobilization of P to the soil mineral matrix and greater nutrient use in the long term. Though, the non-dissolution of these minerals makes the availability and uptake of P by plants impossible, resulting in less plant growth and productivity.

The dissolution of apatite minerals in the soil occurs from the existence of favorable thermodynamic conditions for this. These conditions are based on low pH values (Chien, 1977), and low contents of $H_2PO_4^-$ and Ca^{2+} in the soil (Robinson and Syiers, 1990). Characteristics existing in the studied soil, as well as in most soils of the Pampa biome in Brazil. However, even though favorable thermodynamic conditions to dissolution exist, some apatite minerals from the rock phosphate fertilizer remained in crystalline form for at least six years after fertilizer application. This non-dissolution was proven in an analytical way, by means of the difference in the extraction of P by the acid extractor Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1953) and by anion exchange resin (AER), and by the identification of apatite in its crystalline forms by scanning electron microscopy technique (SEM-EDS).

This finding, in addition to technical implications in the P fertilizer recommendation, suggests a reassessment of the laboratory techniques used to quantify the soil available P contents. Currently for soils in southern Brazil, the available P levels are obtained by an acid extractor (Mehlich-1) and for soil with a history of rock phosphate use, the use of Mehlich-3 extractor or anion exchange resin - AER are recommended for at least 2 years after application of the fertilizer (CQFS-RS/SC, 2016). With the identification of apatite minerals in the crystalline form remaining from the rock phosphate, this period should be revised to a minimum of 6 years, at the expense of overestimating the levels soil available P.

In soils from temperate regions (e.g., France) there is naturally the presence of considerable P levels in calcium phosphates of geogenic origin, not yet dissolved during the weathering of soils with a pH close to 7.0. For the soil analyzed in Lusignan - Fr, the P content attributed to calcium phosphates were 78 and 44 mg kg^{-1} when cultivating annual or forage crops, respectively (P extracted by 1 mol L^{-1} HCl). Comparatively, in the south of Brazil the P contents extracted by 1 mol L^{-1} HCl in areas of natural vegetation are lower than 5 mg kg^{-1} (Rheinheimer et al., 2020).

The presence of calcium phosphates in the soils represents a long-term P stock (as long as there is dissolution of P-Ca minerals) and does not compromise the quantification of the soil available P content due to the methodological procedures employed already being adjusted for these conditions. For French soils, there are three recommended procedures for quantifying the soil available P contents: 1º) Method of Olsen – extraction with 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5; 2º) Method of Joret-Hébert – extraction with 0.1 mol L^{-1} $(NH_4)_2C_2O_4$ at pH 7 e; 3º) Method of Dyer – extraction with 0.2 % citric acid. The first two are indicated for calcareous or high pH soils and the third for more acidic soils. The use of alkaline solutions for the extraction of soil available P (0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 and 0.2 mol L^{-1} $(NH_4)_2C_2O_4$ at pH 7) does not cause the dissolution of P-Ca compounds and, consequently, there is no overestimation of the available P content when there is presence of P-Ca compounds as occurs with the use of acid extraction solutions. Moreover, alkaline solutions provide extraction of more bioavailable organic P fractions, resulting in a good correlation between quantified P levels and crop productivity.

However, it is worth highlighting the problem involved in determining the P contents from acidic (i.e. Mehlich-1 and HCl) and alkaline extracts (i.e. Method Olsen, Joret-Hébert or even steps from Hedley P fractionation) even after more than 40 years of publication of the two main methodologies for quantifying P in solution (Dick and Tabatabai, 1977; Murphy and Riley, 1962). With alkaline solutions, it is possible to extract inorganic P and a considerable amount of organic P from the soil, whereas, with acidic solutions, inorganic P is primarily extracted. Thus, it is essential that in alkaline extracts, procedures that do not acidify the medium or that acidify for the shortest possible time are used for determining P content. Since, the acidification of the solution causes hydrolysis and mineralization of organic compounds, overestimating the levels of extracted inorganic P (Dick and Tabatabai, 1977). In the literature, is recurrent the misuse of the method Murphy and Riley (1962) (which permanently acidifies the medium for the phosphomolybdate complex formation) for quantification of inorganic P in alkaline extracts. Less often, it is possible to obtain authors who correctly use the method Dick and Tabatabai (1977) (which temporarily acidifies the medium for the phosphomolybdate complex formation) for quantification of inorganic P in alkaline extracts containing organic P. The correct use of these two techniques must be highlighted again, so that in future works this mistake can be corrected. In cases where there is no need for separately quantification of Pi and Po in alkaline extracts, an alternative adopted in recent years is the measure by ICP-OES.

Despite the different strategies used to quantify the levels of soil available P, the use of rock phosphate to increase available P contents or to restore the P exported by crops proved to be unfavorable in the Pampa biome, even though there were conditions of acidity and low levels of P and Ca in the soil. Likewise, the use of rock phosphate as a P source in soils with a higher pH and Ca and P levels (such as the case of some Franch soils) is unsustainable because the thermodynamic conditions are unfavorable for the solubilization of apatite minerals in the soil. Thus, it is preferable to treat phosphate rock industrially or to use some strategies to “activate” phosphate rock, such as reducing phosphate rock particle to size smaller than 20 µm or phosphate rock acidulation by mixtures added with soluble P fertilizers (Bindraban et al., 2020).

Regardless of the P source, the improvement in soil fertility levels provides a rearrangement of the balance experienced in the soil-plant system, mainly of endemic plant and animal species. In this sense, changes in soil available P contents provide changes in the composition of species in the plant community and may favor development of more nutritionally-demanding plants (Blanck et al., 2011; Ceulemans et al., 2013; Harpole et al., 2016). In this case, we identified that the use of soluble fertilizers associated with liming provided a greater contribution of leguminous plants in the total dry matter produced. The increase in the contribution of legumes can improve the bromatological quality of the forage produced (Gatiboni et al., 2008); though, in the long term, it can, at first, change ecosystem services of the plant community and, subsequently, reduce the capacity of soil C accumulation, due to changes in the C:N:P:S ratio of the plant tissue. Some strategies can be adopted to reduce the plant community alteration. Among them are the proper management of nitrogen and phosphate fertilization (Harpole et al., 2016; Oliveira et al., 2015) and management of animal cutting or grazing.

Unlike soils located in Brazil, which are mostly acidic and with an advanced weathering, mostly soils located in France have a high pH (Hu et al., 2021; Panettieri et al., 2017). In this case, the specific adsorption of P to the soil mineral matrix tends to be lower. Nevertheless, even so, there is specific adsorption of P to clay minerals and oxide-hydroxides of Fe and Al and the soil P stock is composed mainly of fractions with less availability for plants. As an example, we identified that 44 and 40% of the total soil P stock in the French experiment was in moderate and non-labile forms, respectively (obtained by Hedley fractionation: P-moderately labile = $Pi_{NaOH0.1}$ + $Po_{NaOH0.1}$ + Pi_{HCl}; P-non labile = $Pi_{NaOH0.5}$ + $Po_{NaOH0.5}$ + P residual). While, for the fertilized soil with Pampa natural grassland, the P values with moderate and non-labile forms were on average 30 and 56% of the total soil P stock, respectively.

Although with the chemical fractionation of P in the soil by the Hedley method it is not possible to accurately identify the different phosphate compounds, such as by the ^{31}P NMR or XANES techniques, it is possible to accurately infer about the P bioavailability to plants. Thus, Hedley fractionation is a fast, cheep and widely used tool in scientific work, which makes the results broadly comparable. Lately, there is a new proposition of P chemical fractionation (Gatiboni and Condron, 2021) with the proposition to further facilitate methodological procedures without loss of information on soil P bioavailability. However, its use is still incipient.

Regardless of the soil type, the accumulation of P will always occur in the fractions with the highest adsorption energy. Which makes much of the adsorbed P unavailable or barely available to plants. Thus, the adoption of strategies capable of storing the P added to the soil or mining the P inner-sphere adsorbed and keeping it in forms with less turnover and greater availability is extremely necessary in an environmentally sustainable agriculture. In this case, the maintenance of P in its organic forms is of special importance because it hinders the adsorption of P to clay minerals and Fe and Al oxi-hydroxides, besides to remobilizing the P already adsorbed by the mineral matrix of the soil. In soil with high P stock, some plant species (e.g. *Lolium sp* and *Lupinus sp.*) can mining and remobilize P by incorporation in the tissue (Kamh et al., 1999; Richardson et al., 2009). In addition to these species, in general the use of cover crops, forage species and management strategies that aim to increase the levels of organic matter and biological activity in the soil are alternatives to be considered to maintain higher levels of Po in the soil.

In our study, we identified a significant increase in the soil stocks of organic C, N and P when managed with permanent grassland. On the other hand, continuous cultivation with annual crops and soil plowing reduced the soil C and N stock, although it did not significantly reduce the total soil Po content. In an intermediate management system to those tested in this work, which alternate periods of annual crops and pasture, there is evidence of the possibility of increasing or at least maintaining SOM levels (Crème et al., 2018). The use of rotational systems between intensive annual and forage crops (integrated crop – livestock systems) is a promising option to increase the productivity of the areas while minimizing environmental impacts (Lemaire et al., 2015). So, it is possible to reconcile the benefits of forage systems (capture of C, low N emission, improve soil biological quality) with periods of intensive exploitation with annual crops.

Despite the importance of Po in the soil, plants primarily absorb P in its inorganic form. In this process, the microorganisms are the main agents that lead the transformation of Po into Pi through the decomposition of SOM (Menezes-Blackburn et al., 2018). Thus, the soil microbial biomass can, at first, be considered a P sink due to the incorporation of the element into the microbiological tissue and, subsequently, with death can be a soil P source. In several terrestrial ecosystems, the P cycling by microorganisms is the main way of supplying and maintaining the minimum soil available P levels for plants. This is the case of the Pampa biome, which in its natural state has a wide variety of plant species that cohabit soils with low available P levels and its nutritional maintenance is supplied by the bio cycling of nutrients in the soil.

In agricultural soils, the P contained in the soil's microbial biomass also plays a fundamental role in the bioavailability of P for crops. Its variation depends on time and on space, mainly due to the availability and access to the substrate (SOM and plant residues), the temperature and the soil moisture. Therefore, in periods with higher temperature, humidity and availability of decomposable residues, there will be greater immobilization of P in the soil's microbial biomass. Afterwards, with microorganism’s death, there will be bioavailability of the nutrient for the crops. In tropical and subtropical soils, the variation of P contents in soil's microbial biomass is more dynamic and in temperate soils, this variation is seasonal due mainly to variations in soil temperature and humidity. In addition, the mineralization rate of organic compounds is higher where there is a more easily biodegradable substrate. Consequently, in the soil there is a differentiated accumulation of organic compounds containing P, either by its chemical structure or by the relationship with the soil mineral matrix.

Through the technique of ^{31}P NMR, despite its limitations in the scope of soil P extraction, it is possible to identify and semi-quantify the different organic compounds containing P present in the soil. Mostly, there is presence of orthophosphate monoester compounds (e.g., inositol phosphates and adenosine monophosphate) and orthophosphate diesters (e.g., nucleic acids, phospholipids), with a predominance of orthophoshates monoester.

This is because orthophosphate monoesters have higher turnover than the orthophosphate diesters due to chemical bonding to soil colloids (Turner et al. 2005; Menezes-Blackburn et al. 2018). This comportment may induce in higher accumulation of Po monoesters form than in diesters form in the SOM. The monoester / diester ratio identified in our study was 7.7, 52, 50 for the initial sample of the experiment, CC and GG after 13 years of soil management, respectively. This relationship was altered by the treatments, but there was no difference between the management systems.

In addition to chemical relationships, the physical protection of SOM by soil aggregation is another factor that alters the mineralization rate of organic compounds (Panettieri et al., 2017; Virto et al., 2010), leading to a differentiation of P turnover in different soil aggregate sizes. In the study carried out in chapter VI, we identified higher levels of P contained in the soil microbial biomass in macroaggregates, indicating greater microbial activity in these aggregates size. Furthermore, the practice of soil plowing and crops cultivation reduced the content of P in the soil microbial biomass in large aggregates compared to the permanent forage cultivation.

In sequence, an agri-environmental approach will be carried out on the use of phosphate fertilizers in agricultural areas. Firstly, aspects related to changes in C:N:P stoichiometry due to climatic variation and land use will be discussed. Subsequently, environmental and agronomic aspects of overuse or lack of the phosphate fertilizers use will be discussed. Finally, prospects for future studies based on the different approaches carried out in this thesis will be addressed.

10.1. Land use management and global climate change affect soil C:N:P stoichiometry

The C:N:P stoichiometry represents the molar ratio between nutrients C, N and P either in plants and microorganisms or in soil organic matter (SOM). Ecologically the interactions between plants, microorganisms and soil are reciprocals and therefore the C:N:P stoichiometry of each of them has a broad relationship with the others. Worldwide, there is an identified C:N:P atomic ratio around 186:13:1 for soils and 60:7:1 for soil microbial biomass (Cleveland and Liptzin, 2007). However, these authors also suggest that there are wide variations in the stoichiometry between different ecosystems of the world. In China, for example, a C:N:P atomic ratio of 66:5:1 in soil and 66:8:1 in soil microbial biomass was identified (Xue et al., 2019). Values greatly different from those observed for the first authors, but characteristic for the country's ecosystem. In this work, we identified C:N:P atomic ratio of 104:10:1 for cultivation with annual crops and 86:8:1 for permanent grassland. These values are intermediate to those observed by the authors mentioned above and characteristic of the analyzed soil and crop systems.

In terrestrial environments numerous factors alter the biogeochemical cycles of C, N and P and consequently the coupling/decoupling between them. For example, the management practices adopted (CC and GG) and studied in this thesis changed C:N:P stoichiometry over the 13 years of cultivation. This change can be enhanced by changes in agricultural activities, fertilizer use, changes in plant photosynthetic efficiency due to increased levels of CO_2 in the atmosphere, global temperature, drought and rainfall excess (George et al., 2018; Huang et al., 2018; Panettieri et al., 2017; Sanaullah et al., 2014; Yuan and Chen, 2015). These factors alter the soil C:N:P stoichiometry by modifying the amount and composition of vegetal litter and the microbial activity for C mineralization. Thus, changes occurring in the coupling C, N and P, in the levels of organic matter and the soil C stock can promote or reduce the SOM decomposition process and consequently the emission of CO_2 to the atmosphere.

In general, the soil C and N stock and the C:N:P coupling is modified as follows:

- Soil Fertilization: Increasing dry matter yield. However, there may be imbalance in biogeochemical cycles, especially of N and P and result in increased microbial activity and acceleration of the litter decomposition rate.

Fertilization of agricultural areas can be useful for increasing dry matter production and soil carbon stock, especially in pasture areas (Poeplau et al., 2018). These authors estimated that for grassland temperate it takes 1.15 kg ha^{-1} of nitrogen fertilizer (associated with PK fertilizer) to sequester 1 kg ha^{-1} of soil organic carbon. However, worldwide studies indicate a reduction in soil C and N stock in managed pastures, even when fertilization and liming occur (Eze et al., 2018; Zhou et al., 2017). The reduction of C and N stock in pasture areas is enhanced with increased grazing intensity and can be explained by reduced litter return to soil (Zhou et al., 2017).

In addition, unbalanced fertilizers can cause cycle decoupling, especially N:P due to nutrient excess (Yuan and Chen, 2015). Another notable factor is that grasses respond positively to fertilizer use and thus species richness can be reduced in plant communities (Harpole et al., 2016; Stevens et al., 2004). Reduction in the number of species may reduce the increase in SOM due to the decrease in belowground biomass. (Fornara and Tilman, 2008). The production of belowground biomass is important as it provides increased SOM in deeper layers of soil profile.

- Land use: Land use can change the composition and the quantity of litter inputs to the soil system. Crop rotation and grass introduction are positive for increasing SOM. On the other hand, soil revolving systems tend to have less carbon stock.

In agriculture, the conversion of cropping systems is the main cause of change in the coupling of biogeochemical cycles of C, N, P and S. In their review, Kopittke et al. (2017) identified that the conversion of native vegetation to crops results in an average reduction of 43% of C stock, 42% of N and 27% of P. On the other hand, the conversion of native vegetation to pasture resulted in less reduction of C (11%) and increase of N (4.1%) and P (25%). These results demonstrate the importance of minimum soil tillage, maintenance of vegetation cover to produce dry matter and reduce variations in C, N and P stocks. Even in semiarid regions, the implementation of these practices in grassland systems increases the soil C and N stocks (Ghimire et al., 2019). In tropical and subtropical regions such as Brazil, the use of no-till and litter-maintenance cropping systems is essential for increasing soil C and N stocks and reducing soil losses. In addition, the use of grasses in rotation systems as cover crops results in improved SOM content and quality (Rosolem et al., 2016).

In general, alternatives can be thought to reduce the deleterious effect of climate change and land use managements on C:N:P stoichiometry and soil C stock. For example, the use of some climate-stable C4 plants (Filho et al., 2019), introduction of mixture of grasses and legumes plants in agroecosystems (Creme et al., 2017; Crème and Rumpel, 2016; Fornara and Tilman, 2008; Sanaullah et al., 2012), use of forage management systems (Panettieri et al., 2017) and NPK balanced fertilization of pastures (Poeplau et al., 2018).

- Climate change: There are changes in composition and quantity of litter inputs to the soil system. Mainly changes in protein content, lignin and C:N ratio. These changes are favorable to higher C mineralization and soil microbial activity.

Climate change, such as increased CO_2 in the atmosphere, global temperatures and the frequency of droughts, cause changes in the composition of labile metabolites and heteropolymer in plant tissues (Suseela and Tharayil, 2018), which results in alteration of the

C:N:P stoichiometry of the litter. Higher CO_2 concentration strongly impacts mineral and protein accumulation in plant tissues (Soares et al., 2019). Stress due to drought or high temperature in the growth season results in litter with higher N concentration and lower C:N and lignin:N ratio (Sanaullah et al., 2014). Higher temperatures, drought and N fertilization, increase litter N:P ratio and cause decoupling of P and N (Yuan and Chen, 2015).

Thus, in the climate change scenario there is a change in litter quality due to direct effects of drought, temperatures and CO_2 on plant metabolism. The entry of higher N content and less lignin in the soil favors the availability of energy and nutrients and consequently increase the decomposition rates of organic matter due to the higher soil microbial activity (Blagodatskaya et al., 2010; Sanaullah et al., 2014, 2010). Accelerated mineralization of litter by microorganisms may also result in priming effect on SOM. The priming effect represents the mineralization of native SOM by microorganisms (Sanaullah et al., 2014). Thus, besides the high N litter does not favor the accumulation of C, it drives the reduction of the native C stock in the soil.

10.2. Environmental aspects of P stock depletion

P depletion occurs when there is a negative balance between P inputs and outputs in the soil system. In natural areas without human interference, except in areas of constant deposition, there is no depletion or significant increase in the P contents in the system and the interaction between plant and animal species allows the cycling and reuse of nutrients to maintain the ecosystem balance. An example of this behavior is the natural grassland of the Pampa biome - South America (which are kept in soil with very low available P content - from 2 mg kg^{-1} to 8 mg kg^{-1} (Oliveira et al., 2011; Rheinheimer et al., 1997). Another case reported in the literature is in tropical and temperate forests that develop in poor soils and nutritionally rich litters (Grau et al., 2017; Vitousek, 1984; Vitousek and Sanford, 1986).

In agricultural areas destined for economic exploitation (production of grains, fibers or dry matter) nutritional exports will always be greater than in natural ones. In these cases, the P export by plants is inevitable and, as a result, there will be a depletion of soil P content in a speed depending on the concentration of the element in the soil, the degree of exploitation / export and the P inputs into the soil via fertilizer (Azeez et al., 2020; Tyson et al., 2020). In these factors, man has intervened fundamentally due to the increase in the economic exploitation of the areas and the greater export of agricultural products. Additionally, there is an imbalance in the inputs of P via fertilizer and in most of the cultivated areas there is a deficit of phosphate fertilization. For the year 2000, MacDonald et al. (2011) identified a deficit of phosphate fertilization worldwide, mainly in forage crops and particularly grasses, in several world regions, such as South America and Europe.

Apart from the P exportation via agricultural products, runoff is another factor that contributes to the loss and depletion of P in the soil system. By a study carried out on a global scale, Alewell et al. (2020) showed significant outflow of P via erosion in the order of 5.9 kg P ha^{-1} y^{-1}, with emphasis on Africa and South America. The removal of P from the soil via erosion is even more impacting environmentally than via exportation from agricultural crops due to extrafarm environmental and social problems caused by eutrophication of water bodies and water sources (Goyette et al., 2018; Schilling et al., 2018; Schoumans et al., 2014; Withers et al., 2017).

Regardless of how the P leaves the soil system, the reduction of the available P content has a significant impact on plant production and growth. Since, P is a key element for the production of proteins, genetic material, energy transfer, cell division, growth of meristematic tissues, photosynthesis, flowering and fruiting (Elser 2012; Wang et al. 2017; Weil & Brady, 2017). Consequently, insufficient supply of P to plants impacts in low productivity, low economic return for farmers and low carbon supply to the soil (i.e. soil organic matter / and C stocks). In soil cultivated with temperate grassland (mixture of *Trifolium pratense* L., *Trifolium repens* L., *Lolium perenne* L. *Dactylis glomerata* L), Boitt et al. (2018a) identified a reduction of 45% in plant production due to forage exports for 20 years without further P fertilizer use. As well as, the authors identified a reduction in the levels of soil organic C up to a depth of 10 cm. In the soil, there is a balance between the cycles of C, N and P, which are interconnected by SOM (C: N: P stoichiometry). Due to this, the achievement of C stock increase targets to minimize climate change and increase food security, such as those proposed by the 4 ‰ initiative (www.4p1000.org), has a close relationship with soil P dynamic.

In the soil analyzed in this work, the decrease in the P stock was 0.30 and 0.25 Mg ha^{-1}, respectively for permanent grassland and permanent cropland, at the end of 13 years of cultivation. This reduction was significant, mainly for grassland. However, the high soil P stock, a result of the fertilization history prior to the beginning of the experiment, contributed to the crops not suffering from P deficiency during the evaluated period. But, at the national level (France), until the early 1970s, there was a high consumption of phosphate fertilizers in French agriculture, which resulted in high values of P availability in soils (Le Noë et al., 2020). Though, in recent years there have been drastic decreases in the levels of soil P availability due to the policy of drastic reduction in phosphate fertilization (Saby et al., 2016; Le Noë et al., 2020). This decrease in fertilization has generated negative P balances in many agricultural areas, making worrying about the possibility of decreased production due to nutritional restrictions (Éveillard & Saby, 2018).

Unlike the French soil analyzed, where there are high levels of P in the soil, in the Pampa biome, the P exportation by dry matter is much more significant in view of the low levels of soil P. However, in this place it is essential to provide P via fertilizer to maintain satisfactory levels of growth and plant development. In our study, we identified that this contribution should be periodic with a time interval less than 3 years. Thus, except in the case of under-fertilization, it is unlikely that the P stock will be reduced over time.

10.3. Agronomic and environmental aspects of P fertilizer use in rangeland

The use of sedimentary phosphate rock or soluble P fertilizers in natural grassland (e.g., Pampa rangeland) or cultivated rangelands implies, to some degree, an increase of annual cuts or change in grazing pressure. Consequently, disbalance of natural ecosystem are plausible because great export of nutrients, changes in the botanical community and variation in the physical and biological properties of the soil. In addition to the possibility of environmental contamination with trace elements and heavy metal.

As well as P, elements such as K, Ca and Mg are also exported simultaneously by dry matter. In grazing fields, K is little exported because it returns to the soil through urine and dung. However, in mown rangeland the export is great and can quickly compromise dry matter production (Kayser and Isselstein, 2005). Calcium and Mg are also more intensively exported when there is total removal of plant material. Results from this work and studies carried out by Tiecher et al. (2014) demonstrate that, even during the period of liming effect, in 10 years there was a reduction of up to 1.2 and 0.5 $\text{cmol}_c\ \text{kg}^{-1}$ of Ca and Mg contents, respectively, in a topsoil layer (0-10cm) of Pampa grassland.

In continuous grazing fields, especially when the grazing rate is not adjusted to forage availability, a selection by animals of the less-consumed species occurs, changing the endemic botanical composition (Boavista et al. 2019). Moreover, the use of phosphate fertilizers favor competition between plant species and more nutritionally demanding plants (e.g. legumes) may become dominant in the plant community (Blanck et al. 2011; Ceulemans et al. 2013; Harpole et al. 2016; Harpole et al. 2017). In the experimental field of this study, replacement of tussock grass and forbs species and increase in leguminous species contribution to dry matter produced was identified with the use of soluble fertilizer and limestone. To counterbalance, N fertilizers management can be an alternative to improve grasses growth (Harpole et al., 2016). Rotational grazing is also an alternative to avoid grazing-resistant species (Boavista et al. 2019).

Changes in plant species associated with the P and, sometimes, N inputs in production systems, also alter the stoichiometric C:N:P with consequences on the nutrients cycling and storage of soil organic matter. Worldwide, studies indicate a depletion of soil C and N stocks in fertilized pastures (Eze et al. 2018) that can be potentialized by increasing grazing intensity (Zhou et al. 2017). For the Pampa biome, the soil C may be increased using the adequate grazing intensity (Conte et al., 2011). For the Pampa rangeland, it is mandatory adopt an adequate management system, which maintains the C and N soil stock because the large coverage area (500.000 km^2) may contribute significantly to the atmospheric C sequestration or release from soil.

Additionally to chemical and nutritional changes, great grazing pressure, when not supported by the increase of dry matter productivity, reduces soil biological properties, such as microbial and enzymatic activity (Vargas et al., 2015). There may also worsen soil physical properties such as increase soil density and reduce soil aggregation (Conte et al., 2011). To improve factors such as C stock, microbial activity and soil density and maintain satisfactory economic return, these authors suggest moderate stocking rate (i.e., 12 kg dry matter per 100 kg animal weight).

Although poorly addressed, phosphate fertilizers can have several trace and heavy metal contaminants (e.g. U, Cd, As, Pb, Cr) that concentrations depending of phosphate rock source used to P fertilizer production (Molina et al., 2009; Raven and Loeppert, 1997). Thus, it is expected that long-term phosphate fertilizers application may cause metal accumulation, especially Cd and As, which present high risks to the ecosystem equilibrium and food chain (Jiao et al., 2012). The phosphate rock used in the present study is from a sedimentary deposit located in Gafsa - Tunisia and contains levels of U, Cd and As of the 88, 38 and 4 mg kg^{-1}, respectively (Syers et al., 1986). The highest RP rate used in our study (1.9 Mg ha^{-1}) results in an approximate amount of 173 g ha^{-1} of U, 75 g ha^{-1} of Cd and 9 g ha^{-1} of As. For triple superphosphate highest rate (1.2 Mg ha^{-1}), considering mean values of Cd and As of 28.8 and 17.9 mg kg^{-1} (Molina et al., 2009), there are an approximate amount of 35 g ha^{-1} of Cd and 22 g ha^{-1} of As. Although the concentration of trace and heavy metal in soluble fertilizers is variable and, in some cases, higher than that found in phosphate rock, the rate of soluble fertilizer is lower because the high P concentration makes soluble fertilizers necessary at a lower rate than RP fertilizer.

10.4. Management practices irreversibly alter the ecosystem

Making an analogy to the 4th law of chemistry postulated by Barrow (1999) which says it is impossible to apply fertilizers twice to the same soil, the use of fertilizer (in our case phosphate and limestone fertilizers) will change the dynamics of the system components, such as vegetation, biological activity, soil chemistry / mineralogy and the biogeochemistry cycle of the elements and nothing will return to the initial state.

In terms of vegetation, the community of native plants in managed systems is altered by human activity due to two main factors. The first one refers to grazing/cutting vegetation (Coelho et al., 2018; Lemaire e Chapman, 1996) and the second one is the alteration of vegetative development due to an increase in soil nutritional levels (Harpole et al., 2017, 2016). The grazing / cutting of native vegetation alters the size and shape of the leaf blade, providing changes in the participation of species in the floristic composition, leaf area index, carbon assimilation rate and growth (Boavista, 2012; Goulart, 2014; Lemaire e Chapman, 1996; Nabinger, 2009). In addition to the direct effect of removing a significant part of the plants, animal grazing also provides soil physical changes due to the pressures exerted (Auler et al., 2017). This can lead to an increase in the soil compaction state and a consequent reduction of physical conditions for root growth and water infiltration.

The increase of soil nutritional levels through the use of phosphate, potassium or nitrogen fertilizers also alters the competition between endemic plant species. In the case of the Pampa natural grassland, the species are adapted to low fertility conditions and are able to develop through strategies capable of maximizing the nutrients uptake. For greater P uptake, for example, these strategies can be an increase and arrangement of the root system, association with mycorrhizae, increased efficiency of P transporters and reducing plant metabolic cost (Ramaekers et al., 2010). However, the use of fertilizers will always enhance the development of plants, and in a different way for each species. The increase in the P and K content favors the development of leguminous plants (Oliveira et al., 2015; Marques, 2017; Sallis e Siewerdt, 2000). In contrast, nitrogen fertilizers enhance the growth of grass species at the expense of legumes. As a result, in the long term there is competition between species, mainly for nutrients and light, and more efficient photosynthetic plants become dominant in the plant community (Harpole et al., 2017, 2016).

In addition to the possibility of a reduction in the richness of plant species due to the fertilization, previously studies carried out in the same experiment analyzed in this thesis showed that fertilization can maintain richness and floristic diversity, but with the substitution of species (Oliveira et al., 2015; Tiecher et al., 2014). These observations were also evidenced in our work. In our case, phosphate fertilization led to small changes in species richness. However, there was higher species turnover, mainly in the tussock grass and forbs plant groups, which may contribute to change in ecosystem services. Also, there was an increase in the contribution of leguminous species to the dry matter production. Although species turnover and increased proportion of legumes can enable improved bromatological features, they can lead to reduced soil C stock and decrease Pampa biome' ability to adapt to climate change, in the long-term.

In soil chemical and mineralogical terms, the use of fertilizers will have a decisive impact on the biogeochemical cycle and on the chemical characteristics of the soil. When added to the soil, phosphate fertilizers are solubilized and oxyanions are formed (HPO_4^{-2}, $H_2PO_4^-$) in soil solution. From this, both due to the structural arrangement of the molecule and the degree of protonation of the P oxyanions, HPO_4^{-2} is adsorbed by inner-sphere to the soil matrix. Inner-sphere adsorption occurs when the anion makes covalent bonds with the functional surface groups. The predominance of P inner-sphere adsorption occurs in the functional groups present in the particles of the soil clay fraction, especially clay minerals and Fe and Al oxy-hydroxides (Arai and Sparks, 2001; Bortoluzzi et al., 2015). The number of functional groups and, consequently, the soil P adsorption capacity is finite and variable depending on the chemical and mineralogical characteristics of each soil. Therefore, subsequent additions of phosphate fertilizers will result in less P adsorption by inner-sphere and greater availability for plants and microorganisms. In general, the soils where the Pampa Biome occur are acidic, with a pH around 4.0 to 5.0. This fact contributes to a greater specific adsorption of P to the soil mineral matrix. In contrast, soils in France are naturally more alkaline (pH between 6.0 and 7.0), which reduces the P adsorption by soil mineral matrix.

Therefore, it is evident that any human action on an ecosystem triggers a series of changes in the environment. In particular, in the analysis environment of this thesis, the phosphate fertilization or liming of the Pampa biome and the practices of soil and vegetation management in French agricultural areas modify the phytosociology of the populations of native plants and microorganisms; as well as the chemical and mineralogical properties of soils irreversibly.

10.5. Prospects for future studies

With the text and analyzes carried out for the present thesis, some necessary or possible analyzes to be conducted were left open. Following are some prospects for future studies.

1º – Quantification of soil carbon storage as a function of the rates and P sources applied in the Pampa native grassland. This work is justified, since the increase in the dry matter productivity of the aerial part can also be reflected in an increase in the contribution of organic material into the soil, either by above or underground biomass. Furthermore, the presence of several C4 plants species may favor the accumulation of soil organic matter due to the structure of the organic compounds released during their growth and subsequent decomposition. However, the increase in the P content in the plant shoot due to soil fertilization can change the mineralization rates of the plant material, causing a change in the carbon accumulation in the soil.

2º – Analysis of the mechanisms developed by plants for the acquisition of P in environments with low availability of the element. This work is justified, since the Pampa native grassland has great genetic variability, with more than 450 forage species with economic interest that develop in an environment with low nutritional levels, especially P. Thus, there is the possibility of selecting more "efficient" plants in the P uptake. This approach is aligned with the pursuit of increasing the P use efficiency in agricultural systems in scenarios of increasing the levels of P legacy and depleting world reserves.

3º – Analysis of rock phosphate dissolution in a controlled environment. For better establishment of dissolution time and potential for using rock phosphate, the solubility of the apatite minerals can be tested in a more controlled environment and with different levels of pH, humidity, SOM and Ca and P levels. In this analysis, the same operational variables analyzed in the field (P content - AER and Mehlich-1 and SEM-EDS) could be evaluated. In addition to the possibility of using XRD and XANES to identify the presence of apatite particles in soil samples.

4º - Analysis of the possibility to use basalt rock powder as a nutrient source for plants. In south Brazil, basalt rocks are a waste from amethyst mines. This waste has been used in recent years as a nutrients source for plants and a soil remineralizer. However, it is necessary to test the dissolution of rock phosphate in the soil, to validate the use of basalt rock powder as a nutrients source for plants. In this case, to analyze the efficiency of the use of the basalt rock powder, it would be possible to carry out a dissolution analysis in a controlled environment, with different levels of pH, humidity, SOM and Ca and P levels. In this analysis, the same operational variables analyzed to test rock phosphate dissolution (P content - AER and Mehlich-1 and SEM-EDS) could be evaluated. In addition to the possibility of using XRD and XANES to identify the recalcitrance of mineral particles.

5º – Analysis and identification of microorganisms with potential use as solubilizers of phosphate minerals. As well as there is a great genetic variability in the native vegetation of the Pampa biome, there is certainly also great variability in the soil microbial community. Therefore, in the same experiment used in this thesis, which is more than 20 years old, there is a great possibility that there are microorganisms specialized in solubilizing phosphate minerals and that have potential for agronomic use.

6º - Analysis of identification and quantification of soil contamination with heavy metals via phosphate fertilization. As discussed earlier in the text, phosphate fertilizers contain heavy metals (e.g., U, Cd, As, Pb and Cr) as contaminants that can accumulate in the soil. Thus, a quantitative analysis of the fertilized soil is necessary to verify the existence of a significant accumulation of heavy metals due to long-term phosphate fertilization.

11.Conclusion

In this Thesis, we analyzed two long-term experiments in terms of impacts by the use or not of phosphate fertilizers on the efficiency of phosphorus use and its availability, P legacy, yields and coupling with carbon and nitrogen in the soil organic matter. The first experiment has more than 20 years and is located in a Pampa grassland of Brazil; the second one has more than 13 years and is located in management systems trial (annual crops or perennial forage) in a temperate soil of France.

For each section of this thesis, we have the specific conclusion as fallow:

- Phosphate fertilization and liming application in Southern Brazil Pampa grasslands soil improved dry matter production. Soluble fertilizer using, such as triple and simple superphosphate, led to higher dry matter production. Phosphate fertilization led to small changes in species richness; thus, it is possible maintaining floristic biodiversity, despite phosphorus fertilization. However, there was higher species turnover, mainly in the tussock grass and forbs groups, which may contribute to change in ecosystem services. Legume species' contribution to dry matter production can increase due to soluble phosphate fertilizer and limestone using.

- The use of phosphate rock in the soil surface provides a greater deepening of phosphorus in the soil profile by mass flow phenomenon (achieving 7.5 cm after 21 years and a rate of 249 kg ha^{-1} of P) compared to soluble fertilizers. After 21 years of surface deposition of limestone in rangeland, the reacidification was not significant, and the neutralizing front reached depths greater than 10 cm. The highest rate of phosphorus (249 kg ha^{-1} of P applied until 2012) provides the highest levels of nutrient availability in the soil and in the plant shoot, but there is no more response in dry matter production of rangeland after more than four years since the last fertilization. That indicates the necessity of periodic phosphorus fertilization in a time-space lower than four years.

- The use of soluble P source and limestone (triple superphosphate at rate of 250 kg ha^{-1} of P add with limestone at rate of 3.2 Mg ha^{-1}) resulted in greater nutrient use efficiency (five times greater than that of phosphate rock) and in higher dry matter yield (22%). Consequently, there was also greater P exportation through plant tissue and lesser residual effect of P fertilizer on the topsoil (0-2.5 cm). In addition, the use of phosphate rock (at rate of 250 kg ha^{-1} of P) has led to higher total P and moderate-lability P levels in the soil. It happened due to apatite dissolution (even under favorable soil thermodynamics conditions) in coarse sand and coarse silt particle size fractions. Apatite stability exceeded six years under the evaluated soil conditions, although it lasted shorter than 20 years.

- We identified a greater export of P and, consequently, a greater depletion of total P stock in the treatment with permanent grassland. The depletion of P mainly impacted the P pool with greater lability (P-labile). However, the depletion occurred after 13 years was not enough to cause an evident reduction in plant yields productivity due to the buffering capacity of the P pools with less lability (P-mod-labile). With permanent grassland, there was a transformation from inorganic P to organic P in the most labile pool, accompanying the increase of C and N stocks in the soil. This behavior provided a reduction in the C:N:Po atomic ratio. ^{31}P-NMR technique revealed that permanent grassland had a more significant effect in reducing *α*–glycerophosphate and increase *myo*-IHP and adenosine monophosphate than permanent cropland.

- Management systems with annual or forage crops did not change the total P content in the soil. However, the inorganic P / organic P ratio was higher with the practice of soil plowing and annual crop cultivation. Concomitantly, the forage management system favored the accumulation of organic C, N and P in the soil. The P_{mic} contents were an important pool of bioavailable P in both management systems and represented up to 47% of the available P content (P Olsen) in a annual crop system. Soil plowing and annual crop cultivation reduced the percentage and the contents of C, N and P_{mic} in large aggregates in comparison to the permanent forage cultivation. In addition, the permanent cultivation of forage species maintained greater bioavailability of P (P labile) in aggregates greater than 0.050 mm. The stoichiometry C:N:Po was poorly changed by the 13 years of cultivation in the management systems.

12.References

Acar, M., Celik, I., Günal, H., 2018. Effects of long-term tillage systems on aggregate-associated organic carbon in the eastern Mediterranean region of Turkey. Eurasian J Soil Sci 7, 51–58. https://doi.org/10.18393/ejss.335329

Alewell, C., Ringeval, B., Ballabio, C., Robinson, D.A., Panagos, P., Borrelli, P., 2020. Global phosphorus shirtage will be aggravated by soil erosion. Nat. Commun. 11, 4546. https://doi.org/10.1038/s41467-020-18326-7

Alloush, G.A., 2003. Dissolution and effectiveness of phosphate rock in acidic soil amended with cattle manure. Plant Soil 251, 37–46. https://doi.org/10.1023/A:1022987915057

Arai, Y., Sparks, D.L., 2001. ATR-FTIR spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface. J. Colloid Interface Sci. 241, 317–326. https://doi.org/10.1006/jcis.2001.7773

Arcand, M.M., Schneider, K.D., 2006. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. An. Acad. Bras. Cienc. 78, 791–807. https://doi.org/10.1590/S0001-37652006000400013

Assunção, S.A., Pereira, M.G., Rosset, J.S., Berbara, R.L.L., García, A.C., 2019. Carbon input and the structural quality of soil organic matter as a function of agricultural management in a tropical climate region of Brazil. Sci. Total Environ. 658, 901–911. https://doi.org/10.1016/J.SCITOTENV.2018.12.271

Azeez, M.O., Rubæk, G.H., Pedersen, I.F., Christensen, B.T., 2020. Depletion, accumulation and availability of soil phosphorus in the Askov long-term field experiment. Soil Res. 58, 117–124. https://doi.org/10.1071/SR19203

Barrow, N.J., 1999. of Soil Research. Aust. J. Soil Res. Volume 36, 395–409. https://doi.org/10.1071/SR99114

Beck, M.A., Sanchez, P.A., 1994. Soil Phosphorus Fraction Dynamics during 18 Years of Cultivation on a Typic Paleudult. Soil Sci. Soc. Am. J. 58, 1424–1431. https://doi.org/10.2136/sssaj1994.03615995005800050021x

Bender, M.A., dos Santos, D.R., Tiecher, T., Minella, J.P.G., de Barros, C.A.P., Ramon, R., 2018. Phosphorus dynamics during storm events in a subtropical rural catchment in southern Brazil. Agric. Ecosyst. Environ. 261, 93–102. https://doi.org/10.1016/j.agee.2018.04.004

Bennett, E.M., Elser, J., 2009. A broken biogeochemical cycle. Nature 478, 29–31.

Bindraban, P.S., Dimkpa, C.O., Pandey, R., 2020. Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. Biol. Fertil. Soils 56, 299–317. https://doi.org/10.1007/s00374-019-01430-2

Blagodatskaya, E., Blagodatsky, S., Dorodnikov, M., Kuzyakov, Y., 2010. Elevated atmospheric CO2 increases microbial growth rates in soil: results of three CO2 enrichment experiments. Glob. Chang. Biol. 16, 836–848. https://doi.org/10.1111/j.1365-2486.2009.02006.x

Blanck, Y.L., Gowda, J., Mårtensson, L.M., Sandberg, J., Fransson, A.M., 2011. Plant species richness in a natural Argentinian matorral shrub-land correlates negatively with levels of plant phosphorus. Plant Soil 345, 11–21. https://doi.org/10.1007/s11104-010-0671-0

Boavista, L. da R., Trindade, J.P.P., Overbeck, G.E., Müller, S.C., 2019. Effects of grazing regimes on the temporal dynamics of grassland communities. Appl. Veg. Sci. 22, 326–335. https://doi.org/10.1111/avsc.12432

Boitt, G., Black, A., Wakelin, S.A., McDowell, R.W., Condron, L.M., 2018a. Impacts of long-term plant biomass management on soil phosphorus under temperate grassland. Plant Soil 427, 163–174. https://doi.org/10.1007/s11104-017-3429-0

Boitt, G., Simpson, Z.P., Tian, J., Black, A., Wakelin, S.A., Condron, L.M., 2018b. Plant biomass management impacts on short-term soil phosphorus dynamics in a temperate grassland. Biol. Fertil. Soils 54, 397–409. https://doi.org/10.1007/s00374-018-1269-6

Bolan, N.S., Adriano, D.C., Naidu, R., 2003. Role of phosphorus in (im)mobilization and bioavailability of heavy metals in the soil-plant system, Reviews of Environmental Contamination and Toxicology. https://doi.org/10.1007/0-387-21725-8_1

Boldrini, I.I., 2009. A flora dos campos do Rio Grande do Sul, Campos Sulinos - conservação e uso sustentável da biodiversidade. Brasília. 408p.

Bortoluzzi, E.C., Pérez, C.A.S., Ardisson, J.D., Tiecher, T., Caner, L., 2015. Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. Appl. Clay Sci. 104, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Calabi-Floody, M., Medina, J., Rumpel, C., Condron, L.M., Hernandez, M., Dumont, M., Mora, M. de la L., 2018. Smart Fertilizers as a Strategy for Sustainable Agriculture. Adv. Agron. 147, 119–157. https://doi.org/10.1016/bs.agron.2017.10.003

Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2013. Plant species loss from European semi-natural grasslands following nutrient enrichment - is it nitrogen or is it phosphorus? Glob. Ecol. Biogeogr. 22, 73–82. https://doi.org/10.1111/j.1466-8238.2012.00771.x

Chassé, A.W., Ohno, T., 2016. Higher Molecular Mass Organic Matter Molecules Compete with Orthophosphate for Adsorption to Iron (Oxy)hydroxide. Environ. Sci. Technol. 50, 7461–7469. https://doi.org/10.1021/acs.est.6b01582

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.-A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. 34, 33–41. https://doi.org/10.1016/J.APSOIL.2005.12.002

Chien, S.H., 1977. Dissolution of Phosphate Rocks in a Flooded Acid Soil. Soil Sci. Soc. Am. J. 41, 1106–1109. https://doi.org/10.2136/sssaj1977.03615995004100060018x

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? Biogeochemistry 85, 235–252. https://doi.org/10.1007/s10533-007-9132-0

Condron, L.M., Turner, B.L., Cade-Menun, B.., 2005. Chemistry and dynamics of soil organic phosphorus, in: Sims, J.T., Sharpley, A.S. (Eds.), Phosphorus: Agriculture and the Environment. Madison, pp. 87–121. https://doi.org/10.2134/agronmonogr46.c4

Conte, E., Anghinoni, I., Rheinheimer, D.S., 2002. Fósforo da biomassa microbiana e atividade de fosfatase ácida após aplicação de fosfato em solo no sistema plantio direto. Rev. Bras. Ciência do Solo 26, 925–930. https://doi.org/10.1590/S0100-06832002000400009

Conte, O., de Lima Wesp, C., Anghinoni, I., de Faccio Carvalho, P.C., Levien, R., Nabinger, C., 2011. Soil density, aggregation and carbon fractions of an Alfisol under natural pasture and different herbage allowance. Rev. Bras. Cienc. do Solo 35, 579–587. https://doi.org/10.1590/S0100-06832011000200027

Cordell, D., Drangert, J.O., White, S., 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Chang. 19, 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009

CQFS-RS/SC, 2016. Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina. 376 p.

Creme, A., Chabbi, A., Gastal, F., Rumpel, C., 2017. Biogeochemical nature of grassland soil organic matter under plant communities with two nitrogen sources. Plant Soil 415, 189–201. https://doi.org/10.1007/s11104-016-3158-9

Crème, A., Rumpel, C., 2016. Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. Plant Soil. https://doi.org/10.1007/s11104-015-2740-x

Crème, A., Rumpel, C., Le Roux, X., Romian, A., Lan, T., Chabbi, A., 2018. Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities. Soil Biol. Biochem. 122, 203–210. https://doi.org/10.1016/j.soilbio.2018.04.018

Dawson, C.J., Hilton, J., 2011. Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. Food Policy 36, S14–S22. https://doi.org/10.1016/J.FOODPOL.2010.11.012

de Oliveira, L.B., Tiecher, T., de Quadros, F.L.F., Trindade, J.P.P., Gatiboni, L.C., Brunetto, G., dos Santos, D.R., 2014. Formas de fósforo no solo sob pastagens naturais submetidas à adição de fosfatos. Rev. Bras. Cienc. do Solo 38, 867–878. https://doi.org/10.1590/S0100-06832014000300018

Delmas, M., Saby, N., Arrouays, D., Dupas, R., Lemercier, B., Pellerin, S., Gascuel-Odoux, C., 2015. Explaining and mapping total phosphorus content in French topsoils. Soil Use Manag. 31, 259–269. https://doi.org/10.1111/sum.12192

Dias, L.P.R., Gatiboni, L.C., Brunetto, G., Simonete, M.A., Bicaratto, B., 2015. Relative efficiency of rock phosphates in fertilization of planting seedlings eucalyptus dunnii maiden and eucalyptus benthamii maiden etcambagem in soil with and without liming. Ciência Florest. 25, 37–48. https://doi.org/10.1590/1980-509820152505037

Dick, W.A., Tabatabai, M.A., 1977. Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds1. J. Environ. Qual. 6, 82. https://doi.org/10.2134/jeq1977.00472425000600010018x

Dorozhkin, S. V, 2012. Dissolution mechanism of calcium apatites in acids: A review of literature. World J. Methodol. 2, 1. https://doi.org/10.5662/wjm.v2.i1.1

Elser, J.J., 2012. Phosphorus: A limiting nutrient for humanity? Curr. Opin. Biotechnol. 23, 833–838. https://doi.org/10.1016/j.copbio.2012.03.001

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Eze, S., Palmer, S.M., Chapman, P.J., 2018. Soil organic carbon stock in grasslands: Effects of inorganic fertilizers, liming and grazing in different climate settings. J. Environ. Manage. 223, 74–84. https://doi.org/10.1016/j.jenvman.2018.06.013

Fairhurst, T., Lefroy, R., Mutert, E., Batjes, N., 1999. The importance, distribution and causes of phosphorus deficiency as a constraint to crop production in the tropics. . Agrofor. Forum 9, 2–8.

FAO, 2019. FAOSTAT - Fertilizers [WWW Document]. Food Agric. Organ. Unites Nations.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y.M., Chu, C., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G., Feng, X., Firn, J., Gruner, D.S., Hagenah, N., Hautier, Y., Heckman, R.W., Jin, V.L., Kirkman, K.P., Klein, J., Ladwig, L.M., Li, Q., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Risch, A.C., Schütz, M., Stevens, C.J., Wedin, D.A., Yang, L.H., 2015. Grassland productivity limited by multiple nutrients. Nat. Plants 1, 1–5. https://doi.org/10.1038/nplants.2015.80

Filho, A.L.A., Lima, P.M.T., Sakita, G.Z., Dias e Silva, T.P., Da Costa, W.S., Ghini, R., Abdalla, A.L., Piccolo, M.C., 2019. CO 2 fertilization does not affect biomass production and nutritive value of a C 4 tropical grass in short timeframe 1–8. https://doi.org/10.1111/gfs.12443

Fontana, A., Pereira, M.G., Jarbas, T., Cunha, F., 2008. Fósforo remanescente e correlação com as substâncias húmicas em um latossolo vermelho sob diferentes sucessões de cultura em plantio direto. Fósforo remanescente e correlação com as substâncias húmicas em um latossolo vermelho sob Difer. sucessões Cult. em plantio direto 14, 161–166. https://doi.org/10.18539/cast.v14i1.1900

Fornara, D.A., Tilman, D., 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. J. Ecol. 96, 314–322. https://doi.org/10.1111/j.1365-2745.2007.01345.x

Freitas, I.F., Novais, R.F., Villani, E.M. de A., Novais, S.V., 2013. Phosphorus extracted by ion exchange resins and Mehlich-1 from Oxisols (Latosols) treated with different phosphorus rates and sources for varied soil-source contact periods. Rev. Bras. Ciência do Solo 37, 667–677. https://doi.org/http://dx.doi.org/10.1590/S0100-06832013000300013

Frossard, E., Brossard, M., HedleyM.J., A., M., 1995. Phosphorus in the global environmental: transfers, cycles and management, in: Tiessen, H. (Ed.), Phosphorus in the Global Environmental: Transfers, Cycles and Management. Chichester, U.K, pp. 107–137.

Gatiboni, L.C., Condron, L.M., 2021. A rapid fractionation method for assessing key soil phosphorus parameters in agroecosystems. Geoderma 114893. https://doi.org/10.1016/j.geoderma.2020.114893

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Aquino, J.E.R., 2008. Efeito da adubação fosfatada e da calagem sobre a qualidade bromatológica da forragem de pastagem natural com introdução de espécies forrageiras de inverno. Rev. Bras. Agrociência 14, 125–134.

Gatiboni, L.C., Kaminski, J., Pellegrini, J.B.R., Brunetto, G., Saggin, A., Flores, J.P.C., 2000. Influência da adubação fosfatada e da introdução de espécies forrageiras de inverno na oferta de forragem de pastagem natural. Pesqui. Agropecuária Bras. 35, 1663–1668. https://doi.org/10.1590/s0100-204x2000000800020

Gatiboni, L.C., Smyth, T.J., Schmitt, D.E., Cassol, P.C., de Oliveira, C.M.B., 2015. Limites críticos ambientais de fósforo no solo para avaliar seu risco de transferência para águas superficiais no estado de Santa Catarina, Brasil. Rev. Bras. Cienc. do Solo 39, 1225–1234. https://doi.org/10.1590/01000683rbcs20140461

George, T.S., Giles, C.D., Menezes-Blackburn, D., Condron, L.M., Gama-Rodrigues, A.C., Jaisi, D., Lang, F., Neal, A.L., Stutter, M.I., Almeida, D.S., Bol, R., Cabugao, K.G., Celi, L., Cotner, J.B., Feng, G., Goll, D.S., Hallama, M., Krueger, J., Plassard, C., Rosling, A., Darch, T., Fraser, T., Giesler, R., Richardson, A.E., Tamburini, F., Shand, C.A., Lumsdon, D.G., Zhang, H., Blackwell, M.S.A., Wearing, C., Mezeli, M.M., Almås, R., Audette, Y., Bertrand, I., Beyhaut, E., Boitt, G., Bradshaw, N., Brearley, C.A., Bruulsema, T.W., Ciais, P., Cozzolino, V., Duran, P.C., Mora, M.L., de Menezes, A.B., Dodd, R.J., Dunfield, K., Engl, C., Frazão, J.J., Garland, G., González Jiménez, J.L., Graca, J., Granger, S.J., Harrison, A.F., Heuck, C., Hou, E.Q., Johnes, P.J., Kaiser, K., Kjær, H.A., Klumpp, E., Lamb, A.L., Macintosh, K.A., Mackay, E.B., McGrath, J., McIntyre, C., McLaren, T., Mészáros, E., Missong, A., Mooshammer, M., Negrón, C.P., Nelson, L.A., Pfahler, V., Poblete-Grant, P., Randall, M., Seguel, A., Seth, K., Smith, A.C., Smits, M.M., Sobarzo, J.A., Spohn, M., Tawaraya, K., Tibbett, M., Voroney, P., Wallander, H., Wang, L., Wasaki, J., Haygarth, P.M., 2018. Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant Soil 427, 191–208. https://doi.org/10.1007/s11104-017-3391-x

Ghimire, R., Thapa, V.R., Cano, A., Acosta-Martinez, V., 2019. Soil organic matter and microbial community responses to semiarid croplands and grasslands management. Appl. Soil Ecol. 141, 30–37. https://doi.org/10.1016/j.apsoil.2019.05.002

Goyette, J.O., Bennett, E.M., Maranger, R., 2018. Low buffering capacity and slow recovery of anthropogenic phosphorus pollution in watersheds. Nat. Geosci. 11, 921–925. https://doi.org/10.1038/s41561-018-0238-x

Grau, O., Peñuelas, J., Ferry, B., Freycon, V., Blanc, L., Desprez, M., Baraloto, C., Chave, J., Descroix, L., Dourdain, A., Guitet, S., Janssens, I.A., Sardans, J., Hérault, B., 2017.

Nutrient-cycling mechanisms other than the direct absorption from soil may control forest structure and dynamics in poor Amazonian soils. Sci. Rep. 7, 1–11. https://doi.org/10.1038/srep45017

Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust. J. Soil Res. 43, 189–202. https://doi.org/10.1071/SR04049

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall 10, A.S., Seabloom, E.W., Williams, R., Bakker 12, J.D., Cadotte, M.W., Chaneton, J., Chu, C., Cleland, E.E., Antonio, C. D', Davies, K.F., Gruner, D.S., Hagenah, N., Kirkman, K., Knops, J.M.H., La Pierre, K.J., Mcculley, R.L., Moore, J.L., Morgan 25, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., Wragg, P.D., 2016. Addition of multiple limiting resources reduces grassland diversity. Nat. Publ. Gr. 537, 28. https://doi.org/10.1038/nature19324

Harpole, W.S., Sullivan, L.L., Lind, E.M., Firn, J., Adler, P.B., Borer, E.T., Chase, J., Fay, P.A., Hautier, Y., Hillebrand, H., Macdougall, A.S., Seabloom, E.W., Bakker, J.D., Cadotte, M.W., Chaneton, E.J., Chu, C., Hagenah, N., Kirkman, K., Pierre, K.J. La, Moore, J.L., Morgan, J.W., Prober, S.M., Risch, A.C., Schuetz, M., Stevens, C.J., 2017. Out of the shadows: multiple nutrient limitations drive relationships among biomass, light and plant diversity. Funct. Ecol. 31, 1839–1846. https://doi.org/10.1111/1365-2435.12967

Heindel, R.C., Lyons, W.B., Welch, S.A., Spickard, A.M., Virginia, R.A., 2018. Biogeochemical weathering of soil apatite grains in the McMurdo Dry Valleys, Antarctica. Geoderma 320, 136–145. https://doi.org/10.1016/j.geoderma.2018.01.027

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311, 1–18. https://doi.org/10.1007/s11104-008-9668-3

Hinsinger, P., Gilkes, R.J., 1997. Dissolution of phosphate rock in the rhizosphere of five plant species grown in an acid, P-fixing mineral substrate. Geoderma 75, 231–249. https://doi.org/10.1016/S0016-7061(96)00094-8

Hinsinger, P., Gilkes, R.J., 1995. Root-induced dissolution of phosphate rock in the rhizosphere of lupins grown in alkaline soil. Aust. J. Soil Res. 33, 477–489. https://doi.org/10.1071/SR9950477

Hu, B., Bourennane, H., Arrouays, D., Denoroy, P., Lemercier, B., Saby, N.P.A., 2021. Developing pedotransfer functions to harmonize extractable soil phosphorus content measured with different methods: A case study across the mainland of France. Geoderma 381, 114645. https://doi.org/10.1016/j.geoderma.2020.114645

Huang, J., Wang, P., Niu, Y., Yu, H., Ma, F., Xiao, G., Xu, X., 2018. Changes in C:N:P stoichiometry modify N and P conservation strategies of a desert steppe species Glycyrrhiza uralensis. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-30324-w

Jiao, W., Chen, W., Chang, A.C., Page, A.L., 2012. Environmental risks of trace elements associated with long-term phosphate fertilizers applications: A review. Environ. Pollut. 168, 44–53. https://doi.org/10.1016/j.envpol.2012.03.052

Kamh, M., Horst, W.J., Amer, F., Mostafa, H., Maier, P., 1999. Mobilization of soil and fertilizer phosphate by cover crops. Plant Soil 211, 19–27. https://doi.org/10.1023/A:1004543716488

Kateb, A. El, Stalder, C., Rüggeberg, A., Neururer, C., Spangenberg, J.E., Spezzaferri, S., 2018. Impact of industrial phosphate waste discharge on the marine environment in the Gulf of Gabes (Tunisia). PLoS One 13. https://doi.org/10.1371/journal.pone.0197731

Kaur, G., Reddy, M.S., 2015. Effects of Phosphate-Solubilizing Bacteria, Rock Phosphate and Chemical Fertilizers on Maize-Wheat Cropping Cycle and Economics. Pedosphere 25, 428–437. https://doi.org/10.1016/S1002-0160(15)30010-2

Kayser, M., Isselstein, J., 2005. Potassium cycling and losses in grassland systems: A review. Grass Forage Sci. 60, 213–224. https://doi.org/10.1111/j.1365-2494.2005.00478.x

Kim, J., Li, W., Philips, B.L., Grey, C.P., 2011. Phosphate adsorption on the iron oxyhydroxides goethite (α-FeOOH), akaganeite (β-FeOOH), and lepidocrocite (γ-FeOOH): A 31P NMR Study. Energy Environ. Sci. 4, 4298–4305. https://doi.org/10.1039/c1ee02093e

Kleber, M., Johnson, M.G., 2010. Advances in Understanding the Molecular Structure of Soil Organic Matter. Adv. Agron. 106, 77–142. https://doi.org/10.1016/S0065-2113(10)06003-7

Kopittke, P.M., Dalal, R.C., Finn, D., Menzies, N.W., 2017. Global changes in soil stocks of carbon, nitrogen, phosphorus, and sulphur as influenced by long-term agricultural production. Glob. Chang. Biol. 23, 2509–2519. https://doi.org/10.1111/gcb.13513

Korchagin, J., Caner, L., Bortoluzzi, E.C., 2019. Variability of amethyst mining waste: A mineralogical and geochemical approach to evaluate the potential use in agriculture. J. Clean. Prod. 210, 749–758. https://doi.org/10.1016/j.jclepro.2018.11.039

Kumar, V., Gilkes, R.J., Armitage, T.M., Bolland, M.D.A., 1994. Identification of residual P compounds in fertilized soils using density fractionation, X-ray diffraction, scanning and transmission electron microscopy. Fertil. Res. 37, 133–149. https://doi.org/10.1007/BF00748554

Le Noë, J., Roux, N., Billen, G., Gingrich, S., Erb, K.H., Krausmann, F., Thieu, V., Silvestre, M., Garnier, J., 2020. The phosphorus legacy offers opportunities for agro-ecological transition (France 1850-2075). Environ. Res. Lett. 15. https://doi.org/10.1088/1748-9326/ab82cc

Lemaire, G., Gastal, F., Chabbi, A., 2015. Grassland–Cropping Rotations: An Avenue for Agricultural Diversification to Reconcile High Production with Environmental Quality. Environ. Manage. https://doi.org/10.1007/s00267-015-0561-6

Li, M., Cozzolino, V., Mazzei, P., Drosos, M., Monda, H., Hu, Z., Piccolo, A., 2018. Effects of microbial bioeffectors and P amendements on P forms in a maize cropped soil as evaluated by 31P–NMR spectroscopy. Plant Soil 427, 87–104. https://doi.org/10.1007/s11104-017-3405-8

Liu, J., Cade-Menun, B.J., Yang, J., Hu, Y., Liu, C.W., Tremblay, J., LaForge, K., Schellenberg, M., Hamel, C., Bainard, L.D., 2018. Long-term land use affects phosphorus speciation and the composition of phosphorus cycling genes in agricultural soils. Front. Microbiol. 9, 1–14. https://doi.org/10.3389/fmicb.2018.01643

Lun, F., Liu, J., Ciais, P., Nesme, T., Chang, J., Wang, R., Goll, D., Sardans, J., Peñuelas, J., Obersteiner, M., 2018. Global and regional phosphorus budgets in agricultural systems and their implications for phosphorus-use efficiency. Earth Syst. Sci. Data 10, 1–18. https://doi.org/10.5194/essd-10-1-2018

MacDonald, G.K., Bennett, E.M., Potter, P.A., Ramankutty, N., 2011. Agronomic phosphorus imbalances across the world's croplands. Proc. Natl. Acad. Sci. 108, 3086–3091. https://doi.org/10.1073/pnas.1010808108

Mafé, S., Manzanares, J.A., Reiss, H., Thomann, J.M., Gramain, P., 1992. Model for the dissolution of calcium hydroxyapatite powder. J. Phys. Chem. 96, 861–866. https://doi.org/10.1021/j100181a062

Mai, W., Xue, X., Feng, G., Yang, R., Tian, C., 2018. Can optimization of phosphorus input lead to high productivity and high phosphorus use efficiency of cotton through maximization of root/mycorrhizal efficiency in phosphorus acquisition? F. Crop. Res. 216, 100–108. https://doi.org/10.1016/j.fcr.2017.11.017

Mangalassery, S., Kalaivanan, D., Philip, P.S., 2019. Effect of inorganic fertilisers and organic amendments on soil aggregation and biochemical characteristics in a weathered tropical soil. Soil Tillage Res. 187, 144–151. https://doi.org/10.1016/j.still.2018.12.008

Marin, F.R., Pilau, F.G., Spolador, H.F.S., Otto, R., Pedreira, C.G.S., 2016. Intensificação sustentável da agricultura brasileira Cenários para 2050. Revista de Politica agricola, n. 3. Available in: https://seer.sede.embrapa.br/index.php/RPA/article/view/1160.

McDonald, N.T., Wall, D.P., Mellander, P.E., Buckley, C., Shore, M., Shortle, G., Leach, S., Burgess, E., O'Connell, T., Jordan, P., 2019. Field scale phosphorus balances and legacy soil pressures in mixed-land use catchments. Agric. Ecosyst. Environ. 274, 14–23. https://doi.org/10.1016/j.agee.2018.12.014

Mehlich, A., 1953. Determination of P, Ca, Mg, K, Na, NH 4. Short Test Methods Used Soil Test. Div. 8.

Menezes-Blackburn, D., Giles, C., Darch, T., George, T.S., Blackwell, M., Stutter, M., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Almeida, D.S., Wearing, C., Zhang, H., Haygarth, P.M., 2018. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant Soil 427, 5–16. https://doi.org/10.1007/s11104-017-3362-2

Molina, M., Aburto, F., Calderón, R., Cazanga, M., Escudey, M., 2009. Trace Element Composition of Selected Fertilizers Used in Chile: Phosphorus Fertilizers as a Source of Long-Term Soil Contamination. Soil Sediment Contam. 18, 497–511. https://doi.org/10.1080/15320380902962320

Morel, C., Plénet, D., Mollier, A., 2021. Calibration of maize phosphorus status by plant-available soil P assessed by common and process-based approaches. Is it soil-specific or not? Eur. J. Agron. 122. https://doi.org/10.1016/j.eja.2020.126174

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. ACTA 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nascente, A.S., Li, Y., Crusciol, C.A.C., Nascente, A.S., Li, Y., Crusciol, C.A.C., 2015. Soil Aggregation, Organic Carbon Concentration, and Soil Bulk Density As Affected by Cover Crop Species in a No-Tillage System. Rev. Bras. Ciência do Solo 39, 871–879. https://doi.org/10.1590/01000683rbcs20140388

Oliveira, L.B., Soares, E.M., Jochims, F., Tiecher, T., Marques, A.R., Kuinchtner, B.C., Rheinheimer, D.S., De Quadros, F.L.F., 2015. Long-Term Effects of Phosphorus on Dynamics of an Overseeded Natural Grassland in Brazil. Rangel. Ecol. Manag. 68, 445–452. https://doi.org/10.1016/j.rama.2015.07.012

Oliveira, L.B., Tiecher, T., Quadros, F.L.F., Santos, D.R., 2011. Fósforo microbiano em solos sob pastagem natural submetida a queima e pastejo. Rev. Bras. Cienc. do Solo 35, 1509–1515. https://doi.org/10.1590/S0100-06832011000500005

Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S., 2017. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J. Genet. Eng. Biotechnol. 15, 379–391. https://doi.org/10.1016/j.jgeb.2017.06.005

Panettieri, M., Rumpel, C., Dignac, M.F., Chabbi, A., 2017. Does grassland introduction into cropping cycles affect carbon dynamics through changes of allocation of soil organic matter within aggregate fractions? Sci. Total Environ. 576, 251–263. https://doi.org/10.1016/j.scitotenv.2016.10.073

Pavinato, P.S., Rocha, G.C., Cherubin, M.R., Harris, I., Jones, D.L., Withers, P.J.A., 2020. Map of total phosphorus content in native soils of Brazil. Sci. Agric. 78, 1–5. https://doi.org/10.1590/1678-992x-2020-0077

Pavinato, P.S., Rodrigues, M., Soltangheisi, A., Sartor, L.R., Withers, P.J.A., 2017. Effects of cover crops and phosphorus sources on maize yield, phosphorus uptake, and phosphorus use efficiency. Agron. J. 109, 1039–1047. https://doi.org/10.2134/agronj2016.06.0323

Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A., Lambers, H., 2007. Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. New Phytol. 173, 181–190. https://doi.org/10.1111/j.1469-8137.2006.01897.x

Pereira, M.G., Loss, A., Beutler, S.J., Torres, J.L.R., 2010. Carbono, matéria orgânica leve e fósforo remanescente em diferentes sistemas de manejo do solo. Pesqui. Agropecu. Bras. 45, 508–514. https://doi.org/10.1590/S0100-204X2010000500010

Pinheiro, E.F.M., Pereira, M.G., Anjos, L.H.C., 2004. Aggregate distribution and soil organic matter under different tillage systems for vegetable crops in a Red Latosol from Brazil. Soil Tillage Res. 77, 79–84. https://doi.org/10.1016/j.still.2003.11.005

Poeplau, C., Zopf, D., Greiner, B., Geerts, R., Korvaar, H., Thumm, U., Don, A., Heidkamp, A., Flessa, H., 2018. Why does mineral fertilization increase soil carbon stocks in temperate grasslands? Agric. Ecosyst. Environ. 265, 144–155. https://doi.org/10.1016/j.agee.2018.06.003

Ptáček, P., 2016. Apatites and their Synthetic Analogues - Synthesis, Structure, Properties and Applications, Apatites and their Synthetic Analogues - Synthesis, Structure, Properties and Applications. InTech, Rijeka. https://doi.org/10.5772/59882

Pufahl, P.K., Groat, L.A., 2017. Sedimentary and igneous phosphate deposits: Formation and exploration: An invited paper. Econ. Geol. 112, 483–516. https://doi.org/10.2113/econgeo.112.3.483

Ramaekers, L., Remans, R., Rao, I.M., Blair, M.W., Vanderleyden, J., 2010. Strategies for improving phosphorus acquisition efficiency of crop plants. F. Crop. Res. 117, 169–176. https://doi.org/10.1016/j.fcr.2010.03.001

Raven, K.P., Loeppert, R.H., 1997. Trace Element Composition of Fertilizers and Soil Amendments. J. Environ. Qual. 26, 551–557. https://doi.org/10.2134/jeq1997.00472425002600020028x

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A., 2013. Yield Trends Are Insufficient to Double Global Crop Production by 2050. PLoS One 8. https://doi.org/10.1371/journal.pone.0066428

Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46, 205–221.

Resende, Á.V. de, Furtini Neto, A.E., Alves, V.M.C., Muniz, J.A., Curi, N., Lago, F.J. do, 2006. Resposta do milho a fontes e modos de aplicação de fósforo durante três cultivos sucessivos em solo da região do Cerrado. Ciência e Agrotecnologia 30, 458–466. https://doi.org/10.1590/s1413-70542006000300011

Rheinheimer, D. dos S., Fornari, M.R., Bastos, M.C., Fernandes, G., Santanna, M.A., Calegari, A., Canalli, L.B. dos S., Caner, L., Labanowski, J., Tiecher, T., 2019. Phosphorus distribution after three decades of different soil management and cover crops in subtropical region. Soil Tillage Res. 192, 33–41. https://doi.org/10.1016/j.still.2019.04.018

Rheinheimer, D. dos S., Somavilla, A., Oliveira, L.B. De, Tiecher; Tales, 2020. Ciclo biogeoquímico do fósforo , diagnóstico de disponibilidade e adubação fosfatada. Porto Alegre.

Rheinheimer, D.S., Anghinoni, I., 2003. Accumulation of Soil Organic Phosphorus by Soil Tillage and Cropping Systems Under Subtropical Conditions. Commun. Soil Sci. Plant Anal. 34, 2339–2354. https://doi.org/10.1081/CSS-120024068

Rheinheimer, D.S., Santos, J.C.P., Kaminski, J., Mafran, A.L., 1997. Crescimento de leguminosas forrageiras afetado pela adição de fósforo, calagem do solo e micorrizas, em condições de casa de vegetação. Ciência Rural 27, 571–576. https://doi.org/10.1590/S0103-84781997000400008

Richardson, A.E., Hocking, P.J., Simpson, R.J., George, T.S., 2009. Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci. 60, 124–143. https://doi.org/10.1071/CP07125

Rivilla, V.M., Drozdovskaya, M.N., Altwegg, K., Caselli, P., Beltrán, M.T., Fontani, F., Van Der Tak, F.F.S., Cesaroni, R., Vasyunin, A., Rubin, M., Keyser, D., Belgian, R., 2020. ALMA and ROSINA detections of phosphorus-bearing molecules: the interstellar thread between star-forming regions and comets. MNRAS 492, 1180–1198. https://doi.org/10.1093/mnras/stz3336

Robinson, J.S., Syiers, J.K., 1990. A critical evaluation of the factors influencing the dissolution of Gafsa phosphate rock. J. Soil Sci. 41, 597–605. https://doi.org/10.1111/j.1365-2389.1990.tb00229.x

Rodrigues, M., Pavinato, P.S., Withers, P.J.A., Teles, A.P.B., Herrera, W.F.B., 2016. Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. Sci. Total Environ. 542, 1050–1061. https://doi.org/10.1016/j.scitotenv.2015.08.118

Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287, 15–21. https://doi.org/10.1007/s11104-006-9056-9

Rodríguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17, 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rosolem, C.A., Li, Y., Garcia, R.A., 2016. Soil carbon as affected by cover crops under no-till under tropical climate. Soil Use Manag. 32, 495–503. https://doi.org/10.1111/sum.12309

Roy, E.D., Richards, P.D., Martinelli, L.A., Coletta, L. Della, Lins, S.R.M., Vazquez, F.F., Willig, E., Spera, S.A., VanWey, L.K., Porder, S., 2016. The phosphorus cost of agricultural intensification in the tropics. Nat. Plants 2, 2–7. https://doi.org/10.1038/NPLANTS.2016.43

Roy, E.D., Willig, E., Richards, P.D., Martinelli, L.A., Vazquez, F.F., Pegorini, L., Spera, S.A., Porder, S., 2017. Soil phosphorus sorption capacity after three decades of intensive fertilization in Mato Grosso, Brazil. Agric. Ecosyst. Environ. 249, 206–214. https://doi.org/10.1016/j.agee.2017.08.004

Rumpel, C., Crème, A., Ngo, P.T., Velásquez, G., Mora, M.L., Chabbi, A., 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. J. Soil Sci. Plant Nutr. 15, 353–371. https://doi.org/10.4067/s0718-95162015005000034

Sanaullah, M., Chabbi, A., Girardin, C., Durand, J.L., Poirier, M., Rumpel, C., 2014. Effects of drought and elevated temperature on biochemical composition of forage plants and their impact on carbon storage in grassland soil. Plant Soil 374, 767–778. https://doi.org/10.1007/s11104-013-1890-y

Sanaullah, M., Chabbi, A., Lemaire, G., Charrier, X., Rumpel, C., 2010. How does plant leaf senescence of grassland species influence decomposition kinetics and litter compounds dynamics? Nutr. Cycl. Agroecosystems 88, 159–171. https://doi.org/10.1007/s10705-009-9323-2

Sanaullah, M., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by 14C pulse labeling. Soil Biol. Biochem. 55, 132–139. https://doi.org/10.1016/j.soilbio.2012.06.004

Sarker, J.R., Singh, B.P., Cowie, A.L., Fang, Y., Collins, D., Badgery, W., Dalal, R.C., 2018. Agricultural management practices impacted carbon and nutrient concentrations in soil aggregates, with minimal influence on aggregate stability and total carbon and nutrient stocks in contrasting soils. Soil Tillage Res. 178, 209–223. https://doi.org/10.1016/j.still.2017.12.019

Schachtman, D.P., Reid, R.J., Ayling, S.M., 1998. Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiol. 116, 447–453. https://doi.org/10.1104/pp.116.2.447

Schilling, K.E., Streeter, M.T., Isenhart, T.M., Beck, W.J., Tomer, M.D., Cole, K.J., Kovar, J.L., 2018. Distribution and mass of groundwater orthophosphorus in an agricultural watershed. Sci. Total Environ. 625, 1330–1340. https://doi.org/10.1016/j.scitotenv.2018.01.035

Schoumans, O.F., Chardon, W.J., Bechmann, M.E., Gascuel-Odoux, C., Hofman, G., Kronvang, B., Rubæk, G.H., Ulén, B., Dorioz, J.M., 2014. Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: A review. Sci. Total Environ. 468–469, 1255–1266. https://doi.org/10.1016/j.scitotenv.2013.08.061

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. https://doi.org/10.1186/2193-1801-2-587

Silva, D.R.G., Marchi, G., Spehar, C.R., Guilherme, L.R.G., Rein, T.A., Soares, D.A., Ávila, F.W., 2012. Characterization and nutrient release from silicate rocks and influence on chemical changes in soil. Rev. Bras. Ciência do Solo 36, 951–962. https://doi.org/10.1590/s0100-06832012000300025

Silveira, T.C., Pegoraro, R.F., Kondo, M.K., Portugal, A.F., Resende, Á. V., 2018. Sorghum yield after liming and combinations of phosphorus sources. Rev. Bras. Eng. Agric. e Ambient. 22, 243–248. https://doi.org/10.1590/1807-1929/agriambi.v22n4p243-248

Soares, J.C., Santos, C.S., Carvalho, S.M.P., Pintado, M.M., Vasconcelos, M.W., 2019. Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. Plant Soil 1–26. https://doi.org/10.1007/s11104-019-04229-0

Soltangheisi, A., Rodrigues, M., Coelho, M.J.A., Gasperini, A.M., Sartor, L.R., Pavinato, P.S., 2018. Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res. 179, 20–28. https://doi.org/10.1016/j.still.2018.01.006

Soltangheisi, A., Teles, A.P.B., Sartor, L.R., Pavinato, P.S., 2020. Cover Cropping May Alter Legacy Phosphorus Dynamics Under Long-Term Fertilizer Addition. Front. Environ. Sci. 8, 1–12. https://doi.org/10.3389/fenvs.2020.00013

Somavilla, A., Caner, L., Bortoluzzi, E.C., Santanna, M.A., Rheinheimer, D. dos S., 2021a. P-legacy effect of soluble fertilizer added with limestone and phosphate rock on grassland soil in subtropical climate region 211. https://doi.org/10.1016/j.still.2021.105021

Somavilla, A., Caner, L., da Silva, I.C.B., Bastos, M.C., Moro, L., Schaefer, G.L., Gatiboni, L.C., dos Santos, D.R., 2021b. Chemical pattern of vegetation and topsoil of rangeland fertilized over 21 years with phosphorus sources and limestone. Soil Tillage Res. 205, 104759. https://doi.org/10.1016/j.still.2020.104759

Somavilla, A., Ramos Marques, A.C., Caner, L., de Oliveira, L.B., de Quadros, F.L.F., Chabbi, A., Tiecher, T., dos Santos, D.R., 2021c. Phosphate fertilization and liming in a trial conducted over 21 years: A survey for greater forage production and Pampa pasture conservation. Eur. J. Agron. 125. https://doi.org/10.1016/j.eja.2021.126259

Souchie, E.L., Abboud, A.C. de S., 2007. Solubilização de fosfato por microrganismos rizosféricos de genótipos de Guandu cultivados em diferentes classes de solo. Semin. Ciências Agrárias 28, 11. https://doi.org/10.5433/1679-0359.2007v28n1p11

Steen, I., 1998. Phosphorus availability in the 21st century Management of a non-renewable resource. Phosphorus & Potassium.

Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of Nitrogen Deposition on the Species Richness of Grasslands. Science (80-.). 303, 1876–1879. https://doi.org/10.1126/science.1094678

Suseela, V., Tharayil, N., 2018. Decoupling the direct and indirect effects of climate on plant litter decomposition: Accounting for stress-induced modifications in plant chemistry. Glob. Chang. Biol. 24, 1428–1451. https://doi.org/10.1111/gcb.13923

Syers, J.K., Mackay, A.D., Brown, M.W., Currie, L.D., 1986. Chemical and physical characteristics of phosphate rock materials of varying reactivity. J. Sci. Food Agric. 37, 1057–1064. https://doi.org/10.1002/jsfa.2740371102

Szilas, C., Semoka, J.M.R., Borggaard, O.K., 2007. Can local Minjingu phosphate rock replace superphosphate on acid soils in Tanzania? Nutr. Cycl. Agroecosystems 77, 257–268. https://doi.org/10.1007/s10705-006-9064-4

Taktek, S., Trépanier, M., Servin, P.M., St-Arnaud, M., Piché, Y., Fortin, J.-A., Antoun, H., 2015. Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus irregularis DAOM 197198. Soil Biol. Biochem. 90, 1–9. https://doi.org/10.1016/j.soilbio.2015.07.016

Tian, J., Boitt, G., Black, A., Wakelin, S., Chen, L., Cai, K., Condron, L., 2019. Mass balance assessment of phosphorus dynamics in a fertilizer trial with 57 years of superphosphate application under irrigated grazed pasture. Nutr. Cycl. Agroecosystems. https://doi.org/10.1007/s10705-019-09992-1

Tiecher, T., Rheinheimer, D.S., Calegari, A., 2012a. Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. Soil Tillage Res. 124, 57–67. https://doi.org/10.1016/j.still.2012.05.001

Tiecher, T., Oliveira, L.B., Rheinheimer, D.S., Quadros, F.L.F., Gatiboni, L.C., Brunetto, G., Kaminski, J., 2014. Phosphorus application and liming effects on forage production, floristic composition and soil chemical properties in the Campos biome, southern Brazil. Grass Forage Sci. 69, 567–579. https://doi.org/10.1111/gfs.12079

Tiecher, T., Rheinheimer, D.S., Kaminski, J., Calegari, A., 2012b. Forms of inorganic phosphorus in soil under different long term soil tillage systems and winter crops. Rev. Bras. Ciência do Solo 36, 271–282. https://doi.org/10.1590/s0100-06832012000100028

Tiessen, H., Stewart, J.W.B., Cole, C. V., 1984. Pathways of Phosphorus Transformations in Soils of Differing Pedogenesis1. Soil Sci. Soc. Am. J. 48, 853. https://doi.org/10.2136/sssaj1984.03615995004800040031x

Tipping, E., Somerville, C.J., Luster, J., 2016. The C:N:P:S stoichiometry of soil organic matter. Biogeochemistry 130, 117–131. https://doi.org/10.1007/s10533-016-0247-z

Toledo, M.C.M., Pereira, V.P., 2001. A variabilidade de composição da apatita associada a carbonatitos. Rev. do Inst. Geológico 22, 27–64.

Turner, B.L., Condron, L.M., 2013. Pedogenesis, nutrient dynamics, and ecosystem development: The legacy of T.W. Walker and J.K. Syers. Plant Soil 367, 1–10. https://doi.org/10.1007/s11104-013-1750-9

Tyson, J., Corkrey, R., Burkitt, L., Dougherty, W., 2020. Modelling Changes in Soil Phosphorus When Phosphorus Fertiliser Is Reduced or Ceases. Front. Environ. Sci. 8, 1–11. https://doi.org/10.3389/fenvs.2020.00093

U.S. Geological Survey, 2019. Mineral commodity summaries 2019. https://doi.org/10.3133/70202434

Vargas, R.S., Bataiolli, R., Da Costa, P.B., Lisboa, B., Passaglia, M.P., Beneduzi, A., Vargas, L.K., 2015. Microbial quality of soil from the Pampa biome in response to different grazing pressures. Genet. Mol. Biol. 38, 205–212. https://doi.org/10.1590/S1415-475738138120140230

Virto, I., Barré, P., Chenu, C., 2008. Microaggregation and organic matter storage at the silt-size scale. Geoderma 146, 326–335. https://doi.org/10.1016/j.geoderma.2008.05.021

Virto, I., Moni, C., Swanston, C., Chenu, C., 2010. Turnover of intra- and extra-aggregate organic matter at the silt-size scale. Geoderma 156, 1–10. https://doi.org/10.1016/j.geoderma.2009.12.028

Vitousek, P.M., 1984. Litterfall , Nutrient Cycling , and Nutrient Limitation in Tropical Forests Author (s): Peter M . Vitousek Published by : Ecological Society of America Stable URL : http://www.jstor.org/stable/1939481 . Litterfall , nutrient cycling , and nutrient limit.

Ecology 65, 285–298.

Vitousek, P.M., Sanford, R.L., 1986. Nutrient cycling in moist tropical forest. Annu. Rev. Ecol. Syst. Vol. 17 137–167. https://doi.org/10.1146/annurev.es.17.110186.001033

Walker, T.W., Syers, J.K., 1976. The fate of phosphorus during pedogenesis. Geoderma 15, 1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wang, D., Lv, S., Jiang, P., Li, Y., 2017. Roles , Regulation , and Agricultural Application of Plant Phosphate Transporters 8, 1–14. https://doi.org/10.3389/fpls.2017.00817

Wen, Z., Li, Hongbo, Shen, Q., Tang, X., Xiong, C., Li, Haigang, Pang, J., Ryan, M.H., Lambers, H., Shen, J., 2019. Tradeoffs among root morphology , exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. https://doi.org/10.1111/nph.15833

Withers, P.J.A., Hodgkinson, R.A., Rollett, A., Bilsborrow, P.E., Dyer, C., Dils, R., Bailey, G., Sylvester-Bradley, R., Collins, A.L., 2017. Reducing soil phosphorus fertility brings potential long-term environmental gains: A UK analysis. Environ. Res. Lett. 12, 063001. https://doi.org/10.1088/1748-9326/aa69fc

Withers, P.J.A., Neal, C., Jarvie, H.P., Doody, D.G., 2014. Agriculture and Eutrophication: Where Do We Go from Here 5853–5875. https://doi.org/10.3390/su6095853

Withers, P.J.A., Rodrigues, M., Soltangheisi, A., Carvalho, T.S. De, Nunes, R.D.S., Rosolem, C.A., Andreote, F.D., Jr, A.D.O., Coutinho, E.L.M., Pavinato, P.S., 2018. Transitions to sustainable management of phosphorus in Brazilian agriculture. Sci. Rep. 1–13. https://doi.org/10.1038/s41598-018-20887-z

Xue, H., Lan, X., Liang, H., Zhang, Q., 2019. Characteristics and environmental factors of stoichiometric homeostasis of soil microbial biomass carbon, nitrogen and phosphorus in China. Sustain. 11. https://doi.org/10.3390/su11102804

Yang, Xiaoyan, Chen, X., Yang, Xitian, 2019. Effect of organic matter on phosphorus adsorption and desorption in a black soil from Northeast China. Soil Tillage Res. 187, 85–91. https://doi.org/10.1016/j.still.2018.11.016

Yuan, Z.Y., Chen, H.Y.H., 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. Nat. Clim. Chang. 5, 465–469. https://doi.org/10.1038/nclimate2549

Zafar, M., Tiecher, T., Capoane, V., Troian, A., dos Santos, D.R., 2017. Characteristics, lability and distribution of phosphorus in suspended sediment from a subtropical catchment under diverse anthropic pressure in Southern Brazil. Ecol. Eng. 100, 28–45. https://doi.org/10.1016/j.ecoleng.2016.12.008

Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., Zhou, H., Hosseinibai, S., 2017. Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: a meta-analysis. Glob. Chang. Biol. 23, 1167–1179. https://doi.org/10.1111/gcb.13431

VITAE

André Somavilla, filho de Lucas Antonio Somavilla e Izabel Liberalesso Somavilla. Nascido em 21 de dezembro de 1992, em Pinhal Grande, Rio Grande do Sul – Brasil. Filho de Pequeno agricultor e professora de anos iniciais, aos 5 anos (1998) realizou a pré-escola na Pré Escola Municipal de Ensino Fundamental José Rubim Filho. De 1999 à 2002, entre 1ª e 4ª séries, estudou na Escola Municipal de Ensino Fundamental Olavo Bilac. Após, 2003 a 2006 (5ª à 8ª séries) retornou à Pré Escola Municipal de Ensino Fundamental José Rubim Filho para terminar o ensino fundamental. Aos 14 anos (2007) saiu da casa dos pais para cursar o ensino médio concomitantemente ao curso de Técnico Agrícola no Instituto Federal Farroupilha – *Campus* São Vicente do Sul.

Em janeiro de 2010 ingressou no curso de Agronomia na Universidade Federal de Santa Maria (UFSM) *campus* Frederico Westphalen. Logo no primeiro mês de graduação iniciou na iniciação científica como bolsista na área de agroclimatologia, onde permaneceu por um ano. Após, ingressou como bolsista no Programa de Educação Tutorial (PET) do curso de Agronomia e passou a trabalhar na área de Solos (Física do Solo). De agosto de 2012 à junho de 2013 realizou intercâmbio na Universidade de Santiago de Compostela *campus* Lugo – Espanha pelo Programa Ciência Sem Fronteira. De volta ao Brasil, em julho de 2013, transferiu-se para o campus sede da UFSM, onde continuou seus trabalhos de iniciação cientifica na área de Física do Solo e terminou a graduação em Agronomia em julho de 2015.

Entre agosto de 2015 e julho 2017, realizou o curso de mestrado em Ciência do Solo, subárea Física do solo no Programa de Pós Graduação em Ciência do Solo (PPGCS) da UFSM, sob orientação do professor Paulo Ivonir Gubiani (título da dissertação – *Modelagem do Excedente de infiltração para avaliar a funcionalidade hidrológica do solo*).

Em agosto de 2017 começou o doutorado em Ciência do Solo, subárea Química e Fertilidade do Solo, também pelo PPGCS da UFSM, sob orientação do professor Danilo Rheinheimer dos Santos, onde obteve o título de Doutor em Ciência do Solo em julho de 2021. Na UFSM, realizou o doutorado presencialmente em três anos (de agosto de 2017 à fevereiro de 2018 e março de 2019 à julho de 2021).

Em agosto de 2018 iniciou também o Doutorado na *Université de Poitiers* – França, sob orientação do professor Laurent Caner, onde obteve o título de Doutor "*Structure et évolution de la Terre et autres planètes secteur de recherche Terre solide: géodynamique des enveloppes supérieure*" em julho de 2021. Na Université de Poitiers realizou o doutorado presencialmente em um ano, de um total de três anos.